Review

Kianoush Kashani, Wisit Cheungpasitporn and Claudio Ronco*

Biomarkers of acute kidney injury: the pathway from discovery to clinical adoption

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Abstract: Acute kidney injury (AKI) is a common complication of critical illnesses and has a significant impact on outcomes, including mortality and morbidities. Unfortunately, apart from prophylactic measures, no effective treatment for this syndrome is known. Therefore, early recognition of AKI not only can provide better opportunities for preventive interventions, but also opens many gates for research and development of effective therapeutic options. Over the last few years, several new AKI biomarkers have been discovered and validated to improve early detection, differential diagnosis, and differentiation of patients into risk groups for progressive renal failure, need for renal replacement therapy (RRT), or death. These novel AKI biomarkers complement serum creatinine (SCr) and urine output, which are the standard diagnostic tools for AKI detection. In this article, we review the available literature on characteristics of promising AKI biomarkers that are currently the focus of preclinical and clinical investigations. These biomarkers include neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), liver-type fatty acid-binding protein, interleukin 18 (IL-18), insulin-like growth factor-binding protein 7 , tissue inhibitor of metalloproteinase 2 (TIMP-2), calprotectin, urine angiotensinogen (AGT), and urine microRNA. We then describe the clinical performance of these biomarkers for diagnosis and prognostication. We also appraise each AKI biomarker’s advantages and limitations as a tool for early AKI recognition and prediction of clinical outcomes after AKI. Finally, we review the current and future states of implementation of biomarkers in the clinical practice.

Keywords: acute kidney injury; angiotensinogen (AGT); biomarkers; calprotectin; insulin-like growth factor-binding protein 7 (IGFBP7); kidney injury molecule 1 (KIM-1); microRNA; neutrophil gelatinase-associated lipocalin (NGAL); tissue inhibitor of metalloproteinase 2 (TIMP-2).

Introduction

Acute kidney injury (AKI) among acutely ill patients is common worldwide and is associated with high morbidity, mortality, prolonged hospitalization [1, 2], and long-term adverse outcomes, including chronic kidney disease (CKD) [3] and cardiovascular events [4–6]. Despite recent medical progress, the incidence of AKI continues to rise among patients who are hospitalized or admitted to an intensive care unit (ICU) [7, 8]. However, with implementation of better preventive strategies, the mortality of patients who develop AKI in the ICU appears to be on a downward trajectory [9]. Timely identification of AKI and appropriate implementation of preventive strategies are thought to be the most effective tools to improve AKI outcomes [8, 10].

Currently, the standard diagnostic tools for AKI detection are monitoring of serum creatinine concentration (SCr) and urine output, both of which are markers of renal function but not kidney injury [11]. SCr is an integrator of various intrarenal and extrarenal functions, and its concentration indicates the balance between creatinine generation and excretion [12]. SCr not only is a delayed and insensitive biomarker of changes in kidney function [13], but its concentration does not differentiate structural kidney damage and functional hemodynamic triggers and could be affected by many factors [14, 15]. In addition, patients with reduced muscle mass may not have a robust rise in SCr despite a substantial kidney injury.

Biomarkers of AKI have the ability to identify the injury to the tubular system and to earlier identify patients who are going to develop AKI [8, 10]. Furthermore, biomarkers
Table 1: Characteristics of diagnostic AKI biomarkers.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Characteristics/functions</th>
<th>AUCs for AKI prediction</th>
<th>Settings (sample collections)</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGAL</td>
<td>A 25-kDa protein of the family of lipocalins with its capacity to bind iron-siderophore complexes (bacteriostatic function)</td>
<td>0.87</td>
<td>All hospitalized patients [17]</td>
<td>May be elevated in the settings of sepsis, chronic kidney disease, and urinary tract infection [18, 19]</td>
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<td></td>
<td></td>
<td>0.81</td>
<td>Emergency department [20]</td>
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<td>0.95</td>
<td>Emergency department [21]</td>
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<td></td>
<td></td>
<td>0.96</td>
<td>Pediatric cardiac surgery [22]</td>
<td>The lack of specific cutoff values [15]</td>
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<td></td>
<td></td>
<td>0.72</td>
<td>Cardiac surgery in adults [23]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0.80</td>
<td>ICU [24]</td>
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<td></td>
<td></td>
<td>0.61</td>
<td>Preeclamptic women [25]</td>
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<tr>
<td>KIM-1</td>
<td>A 38.7-kDa type I transmembrane glycoprotein with an extracellular immunoglobulin like domain topping a long mucin-like domain (tubular regeneration; mediates the phagocytosis of apoptotic cells)</td>
<td>0.71</td>
<td>Emergency department [20]</td>
<td>May be elevated in the settings of chronic proteinuria and inflammatory diseases [10, 26]</td>
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<td></td>
<td></td>
<td>0.85</td>
<td>Cardiac surgery [27]</td>
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<tr>
<td></td>
<td></td>
<td>0.77</td>
<td>ICU and others [27]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0.69</td>
<td>All hospital departments [28]</td>
<td>High cost and poor availability [15]</td>
</tr>
<tr>
<td>L-FABP</td>
<td>A 14-kDa protein from the large superfamily of lipid-binding proteins (aids in regulation of fatty acid uptake and intracellular transport)</td>
<td>0.70</td>
<td>Emergency department [20]</td>
<td>Strongly associated with anemia in nondiabetic patients [29]</td>
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<td></td>
<td></td>
<td>0.72</td>
<td>Cardiac surgery in adults [23]</td>
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<td></td>
<td></td>
<td>0.75</td>
<td>ICU [30]</td>
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<tr>
<td>IL-18</td>
<td>A 24-kDa cytokine from the IL-1 family of cytokines (regulates innate and adaptive immunity)</td>
<td>0.64</td>
<td>Emergency department [20]</td>
<td>No certain prediction of AKI in adults [10]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.82</td>
<td>Pediatric cardiac surgery [31]</td>
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<tr>
<td>[TIMP-2]</td>
<td>TIMP-2: a 21-kDa protein, endogenous inhibitor of metalloproteinase activities IGFBP7: a 29-kDa secreted protein, known to bind to and inhibit signaling through IGF-1 receptors (involved in G(1) cell cycle arrest)</td>
<td>0.59</td>
<td>ICU [32]</td>
<td>May be elevated in the setting of diabetes [34]</td>
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<td>[IGFBP7]</td>
<td></td>
<td>0.80</td>
<td>ICU [33]</td>
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<td>0.82</td>
<td>ICU [35]</td>
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<td>0.79</td>
<td>ICU [36]</td>
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<td></td>
<td></td>
<td>0.84</td>
<td>Cardiac surgery [37]</td>
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<td></td>
<td></td>
<td>0.82</td>
<td>Coronary artery bypass surgery [38]</td>
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<td>0.71</td>
<td>Coronary artery bypass surgery [39]</td>
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<td></td>
<td></td>
<td>0.84</td>
<td>High-risk surgical patients [40]</td>
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<td></td>
<td></td>
<td>0.85</td>
<td>Major surgery [41]</td>
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<tr>
<td>Calprotectin</td>
<td>A 24-kDa heterodimer composed of the 2 monomers S100A8 (10,835 Da) and S100A9 (13,242 Da); promotion of repair after AKI</td>
<td>0.97</td>
<td>Hospitalized patients [42]</td>
<td>May be elevated in the settings of urinary tract infection, rheumatoid arthritis, inflammatory bowel disease, myocardial infarction, and urethelial cancer [10, 42, 43]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.99</td>
<td>Hospitalized patients [46]</td>
<td></td>
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<tr>
<td>Urine AGT</td>
<td>A 453 amino acid–long protein with 10 N-terminal amino acids (renal renin-angiotensin system activation may contribute to the pathogenesis of AKI)</td>
<td>0.84</td>
<td>Acute decompensated heart failure [46]</td>
<td>Needs validation in other clinical settings. May be considered as a prognostic biomarker. Data for use as a diagnostic biomarker are limited</td>
</tr>
</tbody>
</table>
could assist in the evaluation of the intensity of injury, differential diagnosis, and the impact of interventions on the recovery from kidney injury [16]. Within the past 2 decades, a few novel potential biomarkers (Table 1) that are measurable in urine or plasma of patients with AKI have been identified [49], including neutrophil gelatinase-associated lipocalin (NGAL) [17, 50, 51], kidney injury molecule 1 (KIM-1) [27, 52], interleukin 18 (IL-18) [53], liver-type fatty acid-binding protein (L-FABP) [54], tissue inhibitor of metalloproteinase 2 (TIMP-2), insulin-like growth factor-binding protein 7 (IGFBP7) [33], calprotectin [42], urine angiotensinogen (AGT) [55], and urine microRNAs [47, 56].

The objective of this article is to review the literature on the new biomarkers and to summarize studies that investigate the performance of these biomarkers in diagnosis or prognostication of AKI.

### Biomarkers of AKI

#### Neutrophil gelatinase-associated lipocalin

NGAL, also known as siderocalin, lipocalin 2, or oncogene 24p, is a 25-kDa protein of the family of lipocalins. Human NGAL exists in three distinct forms: 25-kDa monomer, 45-kDa homodimer, and 135-kDa heterodimer. Heterodimeric NGAL is conjugated to gelatinase and is specific to neutrophils [57]. NGAL is also expressed at steady low levels in various cell types, such as the uterus, prostate, salivary gland, lung, trachea, stomach, colon, and kidney [58]. Its production increases with age and is higher in women than men [59, 60].

Physiologically, NGAL binds to iron-siderophore complexes. Then it exerts a bacteriostatic role of the innate immune system by sequestering iron-siderophore complexes and thereby limiting iron uptake by bacteria [61, 62]. In addition to bacteriostasis, NGAL also provides antiapoptotic effects and enhances proliferation of renal tubular cells, which establishes its potential pathways in kidney protection during AKI [63, 64].

Although NGAL is represented in some human tissues, it is one of the most upregulated transcripts in the kidney early after ischemic, septic, or toxic AKI in animal models and human neonates, children, and adults, implying its role as an early marker of structural renal tubular damage [63, 65, 66]. Recent data have suggested that the thick ascending limb of the loop of Henle and the intercalated cells of the collecting duct are the primary sites of NGAL production in the kidney [64, 67, 68]. The concentration of NGAL is not elevated (~20 ng/mL at the steady state) in
the serum and urine of normal, healthy individuals [68]. NGAL is filtered by the glomerulus and is reabsorbed by the proximal tubules by a megalin-dependent pathway [69]. A decrease in tubular reabsorption after AKI may lead to a further increase in urine NGAL concentration. NGAL elevation is detectable as early as 3 h after tubular injury and peaks approximately 6 to 12 h after injury, depending on the severity of the injury [21, 70]. The elevation can persist up to 5 days after the initial injury, particularly when the injury is severe [71, 72]. Also, NGAL expression in AKI follows a dose-dependent curve with respect to the severity of kidney injury. Urine and plasma NGAL levels rise rapidly and proportionally to the severity and duration of the insult [21, 73–75]. There is also substantial evidence that increased urine NGAL can differentiate intrinsic renal damage from hemodynamic alterations due to volume depletion [12, 17, 20, 21].

The characteristics of each diagnostic biomarker of AKI are summarized in Table 1. NGAL is the most extensively investigated AKI biomarker. Many investigators have examined various patient populations and determined the performance of NGAL. Its performance has been evaluated in various settings, such as for prediction of AKI in pediatric [22] and adult cardiac surgery patients [72, 76], in critically ill patients [8, 24], in patients in the emergency department [20, 21], and in patients in the kidney transplant setting [77–79]. A recent meta-analysis by Ho et al. including 16 studies with a total of 2906 patients investigated urine NGAL as a biomarker for the prediction of AKI after cardiac surgery in adult patients. The composite area under the receiver operating characteristic curve (AUC) of urine NGAL was 0.72 [23]. A recent study of patients with preeclampsia showed that measurement of NGAL was not a suitable diagnostic test for AKI [25]. Determination of appropriate cutoff values in different settings appears to be the next important step in the clinical validation of NGAL testing [20].

Kidney injury molecule 1

KIM-1 is a 38.7-kDa type 1 transmembrane glycoprotein with an extracellular immunoglobulin-like domain topping a long mucin-like domain [80]. It has a transmembrane domain and a short intracellular domain that contains a signaling protein for tyrosine phosphorylation (KIM-1b) [15]. The extracellular domain of KIM-1 is shed from the cell surface by a metalloproteinase-dependent process [81]. KIM-1 is expressed at low levels in the normal kidney and also in other organs, but its expression is significantly upregulated in the kidney after ischemia-reperfusion injury [80] and drug-induced AKI among murine models of AKI [82, 83]. KIM-1 expression is mainly upregulated in the proximal tubule cells, especially the S3 segment both in rodents [84] and in humans [85]. Urinary KIM-1 levels linearly increase with age in healthy human individuals, and higher KIM-1 values are noted in males than in females [60]. KIM-1 is thought to participate in both kidney injury and healing processes [81]. In situ hybridization indicated KIM-1 as a marker of proliferation and regeneration in proximal tubules [86]. Also, it has been suggested that KIM-1 serves as a phosphatidylserine receptor and thereby mediates the phagocytosis of apoptotic cells presented in the postischemic kidney [87–90].

Studies in adults have suggested that urinary KIM-1 could distinguish patients with acute tubular necrosis from those without and predict adverse clinical outcomes, including dialysis requirement and mortality [91, 92]. In the setting of AKI after cardiopulmonary bypass (CPB), KIM-1 levels increase significantly at both 2 h and 24 h postoperatively [93]. KIM-1 might be useful for the detection of nephrotoxicity in preclinical and early phase 1 and 2 clinical studies [94, 95]. Therefore, KIM-1 has been approved by the US Food and Drug Administration (FDA) as an AKI biomarker for preclinical drug development [94]. Also, a lateral flow dipstick for KIM-1 has been developed, providing a simplified way of assessing KIM-1 levels [96] that yields semiquantitative results in 15 min [97].

Studies investigating KIM-1 as a biomarker of AKI have shown variable results [27, 52, 98, 99]. In patients with congestive heart failure, Damman et al. recently demonstrated that KIM-1 is an outstanding predictive marker for AKI detection [100]. Also, elevation of urinary KIM-1 levels was found to be correlated with increased risk of death or hospitalization among patients with congestive heart failure, independent of estimated glomerular filtration rate (GFR) [101]. In a study of 32 urinary biomarkers of AKI after cardiac surgery, incorporation of KIM-1 with IL-18 generated an AUC of 0.92, and the combination surpassed other biomarkers [99]. In contrast to previous research, Hazle et al. did not find KIM-1 to be a suitable prognostic marker in children. Urine KIM-1 poorly differentiated patients with either excellent or inferior outcomes and was, thus, eliminated from additional analysis [102].

Liver-type fatty acid-binding protein

L-FABP, also known as fatty acid-binding protein 1 (FABP1), is a 14-kDa protein from the large superfamily of lipid-binding proteins [103]. L-FABP is encoded by the FABP1 gene.
in humans. It pertains to a family of carrier proteins for fatty acids and aids in regulation of fatty acid uptake and intracellular transport [104, 105]. L-FABP is expressed not only in the liver but also in the stomach, intestine, lung, and kidney [106]. L-FABP binds and transports fatty acids to the mitochondria and peroxisomes to generate energy via β-oxidation [107]. L-FABP also plays a cell-protective role by mitigating H₂O₂-induced oxidative stress [108]. In the kidney, L-FABP is located in the proximal tubule and is excreted into the tubular lumen along with bound toxic peroxisomal products [109, 110]. Increased L-FABP expression and urinary excretion prior to the increase in SCr have been described in several animal models of AKI, including ischemia-reperfusion and cisplatin AKI models [111, 112].

In pediatric patients, urinary L-FABP was found to be a sensitive early biomarker of AKI after CPB surgery [54, 113]. Higher urinary L-FABP levels are associated with worse outcomes or the need for renal replacement therapy (RRT) in patients with accelerated deterioration of renal function [114].

Susantitaphong et al. recently conducted a systematic review evaluating the performance of urinary L-FABP in AKI. The estimated sensitivity and specificity of urinary L-FABP were 75% and 78% for the AKI diagnosis, 69% and 43% for prediction of the need for dialysis, and 93% and 79% for in-hospital mortality, respectively [115]. In another systematic review, which included six studies with approximately 1700 patients, the AUC of urinary L-FABP for AKI prediction after cardiac surgery was 0.72 (range, 0.52–0.85) [23, 54]. Patients with elevated L-FABP levels measured at the time of ICU admission had a higher risk of AKI development within the first week of admission [30]. In a recent study of critically ill patients, urinary L-FABP improved the predictive capability of a clinical prediction model for AKI progression, dialysis, or death within 7 days among patients with early AKI [116]. In addition, among patients undergoing major surgery, Zeng et al. described the use of NGAL and L-FABP as a promising combination that may improve the diagnostic performance of AKI detection, although the investigators found this panel of biomarkers to be a poor predictor of renal recovery after AKI [117]. In summary, urinary L-FABP appears to be a promising biomarker for both diagnosis and prediction of AKI and its outcomes among critically ill patients [118].

**Interleukin 18**

IL-18, also known as interferon-gamma inducing factor, is a 24-kDa cytokine from the IL-1 family of cytokines, which regulates innate and adaptive immunity [119, 120]. IL-18 is synthesized by multiple tissues, including monocytes, macrophages, proximal tubular epithelial cells, and the intercalated cells of the collecting ducts, as an inactive precursor [121]. It resides intracellularly until it is processed into an active form by caspase 1 [122]. Cleaved IL-18 exerts a proinflammatory effect by signal transduction through the IL-18 receptor/IL-18 receptor accessory protein heterodimer [123]. IL-18 levels are enhanced in numerous endogenous inflammatory processes, such as sepsis [124], and multiple studies indicated IL-18 as both mediator and biomarker of AKI [125, 126]. Its level rises approximately 6 h after the ischemic injury, 24 to 48 h before the AKI diagnosis, and peaks about 12 h later at values up to 25 times normal levels [125].

Interestingly, IL-18-deficient mice are protected from ischemia/reperfusion-induced AKI [126]. Likewise, caspase-deficient mice, which cannot cleave IL-18, or using caspase inhibitors are also found to have less severe AKI ischemia/reperfusion and glycerol exposure [127, 128]. Overall, only a few clinical studies have examined the use of IL-18 as an AKI biomarker [53]. These investigations showed reasonable results in pediatric patients with AKI after cardiac surgery [31, 129]. Nevertheless, other studies failed to indicate strong predictive ability of IL-18 for AKI among the ICU or emergency department population [20, 32]. A recent systematic review denoted that these inconsistent results may be due to the lack of definite agreement on the suitable cutoff level of IL-18 for AKI prediction [53].

**Insulin-like growth factor-binding protein 7 and tissue inhibitor of metalloproteinase 2**

Cell cycle arrest in G₁ phase may be a cellular mechanism to emerge from circumstances when dormant DNA breakage can happen [130]. Renal epithelial cells have been shown to undergo G₁ cell cycle arrest in the setting of ischemic or septic AKI [131, 132]. The cyclin-dependent kinase inhibitor p21 halts cell cycle succession from G₁ to S phase. p21-deficient mice are more sensitive to cisplatin-induced AKI, develop a more severe injury, and have a greater mortality, implying that cell cycle arrest is critical in restricting the consequences of AKI [133]. Studies have indicated that both IGFBP7 and TIMP-2 are involved in G₁ cell cycle arrest during the very early phases of cellular injury [33, 35].

TIMP-2, a 21-kDa protein, is a member of the TIMP family. TIMP-2 is an endogenous inhibitor of metalloproteinase activities. IGFBP7, a 29-kDa secreted protein, is known to bind and inhibit signaling via insulin-like
growth factor 1 receptors. In a healthy patient population, the product of TIMP-2 and IGFBP7 ([TIMP-2]×[IGFBP7]) was not significantly different between men and women but showed a small reverse correlation with age [134].

IGFBP7 and TIMP-2 were identified as better AKI prediction biomarkers among 340 potential candidate biomarkers [33]. Urine [TIMP-2]×[IGFBP7] was superior to other biomarkers (plasma NGAL and cystatin C and also urinary NGAL, KIM-1, IL-18, pi-glutathione S-transferase, and L-FABP) and to TIMP-2 and IGFBP7 alone in forecasting AKI stage 2 or 3, with an AUC of 0.8. Also, follow-up studies ascertained an AUC of 0.82 [35] and 0.79 [36] for the prediction of AKI stages 2 and 3, respectively. These findings indicated that, in the ICU setting, [TIMP-2]×[IGFBP7] is a fair to excellent biomarker in predicting moderate to severe AKI within 12 h. This test was found to perform better mainly among those who are categorized at high risk for AKI. Application of this test among lower-risk patients resulted in suboptimal performance of the test. For example, Bell et al. evaluated the predictive performance of [TIMP-2]×[IGFBP7], analyzed by NephroCheck, a commercial point-of-care kit (Astute Medical, Inc), in 94 patients with urine collected within 48 h of ICU admission. The investigators failed to show that urine [TIMP-2]×[IGFBP7] was useful for predicting AKI [34]. In addition, they found an independent association between diabetes and elevated [TIMP-2]×[IGFBP7] levels. In long-term follow-up of the original validation study (i.e., Sapphire study), Koyner et al. showed that urine [TIMP-2]×[IGFBP7] levels at the time of ICU admission were predictive of a composite long-term outcome of death or RRT requirement during the next 9 months in patients who developed AKI [135]. [TIMP-2]×[IGFBP7] was also investigated in other settings, including cardiac surgery patients [37–39] and major surgery patients [40, 41], showing AUCs between 0.7 and 0.85 for AKI prediction. Nevertheless, the performance data of [TIMP-2]×[IGFBP7] in different patient populations outside the ICU/perioperative setting is still lacking.

**Calprotectin**

Calprotectin is a 24-kDa heterodimer composed of the two monomers S100A8 (10,835 Da) and S100A9 (13,242 Da) [136]. It was initially recognized as an antimicrobial protein in the cytoplasm of neutrophil granulocytes. Intracellular calprotectin’s principal function is to associate with the cytoskeleton, whereas when secreted by activated immune cells, it serves as a danger-associated molecular pattern protein [137]. No singular receptor uses calprotectin signal transduction, but S100A8 and S100A9 have been noted to be endogenous activators of Toll-like receptor 4 [138].

Epithelial cells of renal collecting duct were shown to produce S100A8 and S100A9 in a model of AKI in response to unilateral ureteral obstruction [139]. S100A8 and S100A9 are also induced in response to ischemic reperfusion injury in mice [140] when infiltrating kidney neutrophils are the principal source of S100A8/9 in the ischemic kidney. S100A9-knockout mice, which lack active calprotectin, showed increased renal fibrosis in response to ischemia-reperfusion injury when the initial renal injury is comparable to that of the wild-type mice [140].

Ebbing et al. [43] examined time-dependent variations of calprotectin levels in patients undergoing nephron-sparing surgery for kidney tumors, which resulted in iatrogenic renal ischemic reperfusion injury from transient clamping of the renal artery. Urine calprotectin levels began to increase significantly at the end of surgery (approximately 2 h after ischemia) and reached maximal levels in 48 h after surgery, with a 69-fold increment above baseline. Moreover, calprotectin levels were still significantly elevated on the fifth postoperative day [43].

Studies have also demonstrated an elevation of calprotectin levels in a few medical conditions, including rheumatoid arthritis [141], inflammatory bowel disease [142], myocardial infarction [143], urothelial carcinoma [43], prostate cancer [144], and others [145–147]. In addition, since calprotectin is predominantly derived from neutrophils and monocytes, pyuria can substantially increase urine calprotectin. Thus, elevated urine calprotectin levels need to be cautiously interpreted [10].

Studies investigating the diagnostic accuracy of calprotectin in differentiating prerenal from intrinsic AKI have shown particularly high precision, with an AUC ranging from 0.92 to 0.97 [42, 44, 45]. A recent multicenter study was conducted to examine the diagnostic accuracy of calprotectin in the differentiation of prerenal and intrinsic acute allograft kidney injury. Notably, urine calprotectin levels of patients with intrinsic AKI were 36-fold greater than those of patients with prerenal allograft kidney injury, yielding an AUC of 0.94 [148].

**Urine angiotensinogen**

AGT is a 453-amino-acid-long protein with 10 N-terminal amino acids that are cleavable by renin, leading to the formation of angiotensin I [55, 149]. Angiotensin I is further converted to angiotensin II by angiotensin-converting enzyme and exerts its robust biologic effects [55, 149].
Studies in animal models of AKI render data in support of the notion that kidney renin-angiotensin system (RAS) activation contributes to the pathogenesis of AKI [150–153]. An increase in urine AGT is now considered as one of the most promising biomarkers of AKI progression in patients with acute decompensated heart failure [46, 55, 154, 155].

AGT is plentiful in plasma. Even with a generous supply of exogenous angiotensin-converting enzyme, an active angiotensin II–metabolizing enzyme, AGT concentrations do not decline [156]. Whether circulating plasma AGT is a source of urine AGT or originally kidney descended, is still unclear [157]. In healthy kidneys, circulating AGT should not be filtered into the urine in any significant amounts because its molecular size is 65 kDa [158]. Furthermore, efficient tubular reabsorption or degradation of any filtered AGT could preclude it from appearing in the urine. The evidence that AGT is detectable in urine samples from humans and animals without kidney disease supports the point that urine AGT arises principally from local kidney sources [158, 159]. Moreover, renal angiotensin II concentrations are much larger than those detected in the circulation [157–159]. One plausible mechanism of enhanced intrarenal angiotensin II could be transcriptional upregulation of AGT mRNA in the proximal renal tubule [160]. Studies in murine models of AKI have revealed that circulating AGT can stimulate kidney RAS when the glomerular barrier is altered [161]. In a transgenic mouse model of podocyte-selective injury, increased renal angiotensin II content and notably increased tubular AGT, as well as urine AGT proteins, were attributed to increased glomerular passage of circulating AGT. This happened without an increment in renal renin activity. These investigations explicitly supported the dependency of kidney angiotensin II production on filtered AGT.

In the setting of acute decompensated heart failure, Yang et al. demonstrated that urine AGT was able to predict AKI, with an AUC of 0.84, and outperformed urine NGAL, with an AUC of 0.78 [46]. Moriyama et al. recently assessed the ability of urine AGT to predict AKI after CPB surgery and found no significant differences in urinary AGT concentrations between the AKI and non-AKI groups at any time point [162].

Nevertheless, urine AGT measurement may provide additional information for the prediction of severe AKI and other adverse outcomes, other than early AKI detection. Previous observational studies have suggested that urine AGT can predict AKI progression to more severe stages or death after cardiac surgery [154, 155]. Recently, Chen et al. reported their findings of a prospective study investigating the potential use of urinary AGT in combination with 3 AKI biomarkers, including NGAL, IL-18, and KIM-1, in the prediction of AKI progression. In the adjusted model, patients with the highest tertile of urine AGT had a 10.8-fold greater risk of AKI progression compared with those with the lowest urine AGT tertile. In addition, urine AGT outperformed the other 3 biomarkers, with an AUC of 0.78 for AKI progression and 0.85 for AKI progression with death [55].

**Urine microRNA**

A new area of study involves evaluation of the value of microRNAs, endogenous and noncoding RNA molecules containing 18 to 22 nucleotides, in AKI [16]. These short strands of RNA regulate gene expression by inhibiting protein translation. In cardiac surgery populations, it has been shown that both urine and plasma miR-21 concentrations, which orchestrated a microRNA-controlled apoptosis of renal tubular epithelial cells and promoted cellular proliferation in response to renal ischemia-reperfusion injury [163], may be helpful in detection of AKI, with an AUC of 0.68 for urine and 0.80 for plasma. In addition, urine and plasma miR-21 can predict AKI progression, with an AUC of 0.81 and 0.83, respectively [47]. Moreover, a recent pilot study showed that other sets of microRNAs, including miR-101-3p, miR-127-3p, miR-210-3p, miR-126-3p, miR-26b-5p, miR-29a-3p, miR-146a-5p, miR-27a-3p, miR-93-3p, and miR-10a-5p, were altered several days prior to the increase in SCr, indicating their potential as prognostic AKI biomarkers among ICU patients [48]. Lorenzen et al. recently demonstrated that elevated levels of plasma miR-210, a microRNA upregulated by hypoxia-inducible factor, was an independent predictor of mortality, with a hazard ratio (HR) 1.69 in patients with AKI requiring RRT [56].

**Biomarkers of AKI outcome prognostication**

Although early recognition of the declining GFR is critical, some biomarkers may independently provide insights into AKI progression regardless of GFR changes [149]. Biomarkers have been used to risk stratify for severe AKI, the need for RRT, or death. The predictive abilities of biomarkers for prognosis of AKI and its outcomes are summarized in Table 2. In a subset study from the Acute Respiratory Disease Study Network trial, after adjustment for Acute Physiology and Chronic Health Evaluation (APACHE) III,
scores, a urine IL-18 level >100 pg/mL not only predicted AKI in the next 24 h but also was associated with increased odds of mortality by 1.6-fold [165]. In addition, urine KIM-1 (>2.37 ng/mL) was recently found to be positively correlated with AKI progression in hospitalized patients, with an AUC of 0.70 [28].

In patients who had stage 1 AKI after cardiac surgery, a plasma NGAL level >323 ng/mL and urine IL-18 level >185 pg/mL showed an odds ratio (OR) of 7.7 (95% CI, 2.6–22.5) for AKI progression [164]. Urine AGT to creatinine ratio has also been shown to predict progression to a higher stage of AKI and death [155]. In patients with cirrhosis, IL-18, KIM-1, L-FABP, and microalbuminuria have been shown to predict the composite end point of AKI progression and death [166]. Also, in patients enrolled in the biomarker substudy of the Acute Renal Failure Trial Network clinical trial [167], increased plasma IL-8 and tumor necrosis factor receptor 1 (TNFR1) were associated with delayed renal recovery after AKI. Furthermore, higher levels of IL-6, IL-8, IL-10, IL-18, TNFR1, macrophage migration inhibitory factor, and death receptor 5 were associated with greater mortality. In addition, TIMP-2 and IGFBP7, 2 recently FDA-approved biomarkers [168], have been found to be helpful in predicting prognosis in critically ill patients. A [TIMP-2]×[IGFBP7] level >2.0 was associated with a HR of 2.16 to predict the composite end point of need for RRT or death at 9 months [135].

The combination of clinical models with biomarkers renders a robust ability to predict outcomes. Pike et al. showed that combining plasma IL-8 with a 4-variable clinical model consisting of age, mean arterial blood pressure, mechanical ventilation, and bilirubin value enhanced the prediction of recovery of kidney function and death compared with the clinical model alone [169]. Likewise, the addition of a positive furosemide stress test to an elevation of NGAL >150 ng/mL or [TIMP-2]×[IGFBP7] >0.3 improved the predictive ability for AKI progression, need for RRT, and death compared with either biomarker alone [170].

### Biomarker limitations

Biomarkers have some important limitations that need to be acknowledged (Table 1). As mentioned earlier, none of the reported biomarkers are entirely specific for AKI. NGAL, IL-18, and calprotectin levels are known to be elevated in urinary tract infections and sepsis, regardless of AKI. Moreover, NGAL, KIM-1, and IL-18 levels are also elevated in patients with CKD [10]. TIMP-2 and IGFBP7 have been tested mostly in the ICU setting and their association

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Settings</th>
<th>Outcomes</th>
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<tbody>
<tr>
<td>Plasma NGAL (&gt;323 ng/mL) [164]</td>
<td>Cardiac surgery</td>
<td>OR 7.7 (95% CI, 2.6–22.5) for AKI progression</td>
</tr>
<tr>
<td>Urine NGAL [55]</td>
<td>Acute cardiorenal syndrome</td>
<td>OR 4.7 (95% CI, 1.7–13.4) for AKI progression</td>
</tr>
<tr>
<td>Urine NGAL [116]</td>
<td>ICU</td>
<td>AUC 0.65 for AKI progression, dialysis, or death</td>
</tr>
<tr>
<td>Urine KIM-1 [116]</td>
<td>ICU</td>
<td>AUC 0.62 for AKI progression, dialysis, or death</td>
</tr>
<tr>
<td>Urine L-FABP [115, 116]</td>
<td>Cardiac surgery</td>
<td>AUC 0.70 for AKI progression</td>
</tr>
<tr>
<td>Urine L-FABP + clinical model [116]</td>
<td>ICU</td>
<td>AUC 0.77 for nonrecovery of kidney function</td>
</tr>
<tr>
<td>Urine IL-18 &gt;100 pg/mL [165]</td>
<td>ICU</td>
<td>HR 1.6 (95% CI, 0.8–2.9) for death</td>
</tr>
<tr>
<td>Urine IL-18 &gt;185 pg/mL [164]</td>
<td>Cardiac surgery</td>
<td>OR 3.0 (95% CI, 1.3–7.3) for AKI progression</td>
</tr>
<tr>
<td>Urine IL-18 &gt;200 pg/mL [165]</td>
<td>ICU</td>
<td>HR 2.32 (95% CI, 1.2–4.4) for death</td>
</tr>
<tr>
<td>Urine IL-18 &gt;500 pg/mL [165]</td>
<td>ICU</td>
<td>HR 5.1 (95% CI, 2.0–13.1) for death</td>
</tr>
<tr>
<td>Urine IL-18 [55]</td>
<td>Acute cardiorenal syndrome</td>
<td>OR 3.6 (95% CI, 1.4–9.5) for AKI progression</td>
</tr>
<tr>
<td>Urine AGT [55]</td>
<td>Acute cardiorenal syndrome</td>
<td>OR 10.8 (95% CI, 3.4–34.7) for AKI progression</td>
</tr>
<tr>
<td>Urine AGT/urine Cr [155]</td>
<td>Cardiac surgery</td>
<td>AUC 0.81 to predict of stage 3 AKI or death</td>
</tr>
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<tr>
<td>Urine microRNAs</td>
<td>ICU</td>
<td>HR 1.69 (95% CI, 1.001–2.681) for death</td>
</tr>
<tr>
<td>miR-210 [56]</td>
<td>Cardiac surgery</td>
<td>AUC 0.81 (95% CI, 0.72–0.91) for AKI progression</td>
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<tr>
<td>miR-21 [47]</td>
<td>Cardiac surgery</td>
<td>AUC 0.81 (95% CI, 0.72–0.91) for AKI progression</td>
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</tbody>
</table>

AGT, angiotensinogen; AKI, acute kidney injury; AUC, area under the curve; Cr, creatinine; HR, hazard ratio; ICU, intensive care unit; IGFBP7, insulin-like growth factor-binding protein 7; IL-18, interleukin 18; KIM-1, kidney injury molecule 1; L-FABP, liver-type fatty acid-binding protein; NGAL, neutrophil gelatinase-associated lipocalin; OR, odds ratio; TIMP-2, tissue inhibitor of metalloproteinase 2.
with diabetes has been reported [34]. After AKI occurs, biomarker levels remain elevated for a period of time. This makes assessment of AKI timing rather challenging [164]. Moreover, in clinical trials, the use of biomarkers to select patients for enrollment in a study may lead to a greater screening failure rate and necessitate a higher number of patients to be screened for enrollment. Also, enrollment based on biomarker levels may restrict the generalizability of findings. Finally, there is a scarcity of data from longitudinal and interventional studies to validate the use of any of the AKI biomarkers for predicting patient-centered outcomes in large-scale trials [171].

**Clinical application of AKI biomarkers**

**AKI from a clinical syndrome to a molecular diagnosis**

After the introduction of the RIFLE criteria for diagnosis of AKI, modified definition criteria by the AKI Network and Kidney Disease Improving Global Outcomes were proposed in the literature [172–174]. All three definitions rely on a relative or absolute increase in SCr and a weight-based decrease in urine output. Due to inherent limitations of SCr level (delayed diagnosis and dependence on muscle mass) and urine output measurements, these definitions have not been able to improve the outcomes of patients with AKI significantly. Therefore, the need for the development of new biomarkers for prediction and diagnosis of AKI among patients at high risk has been very palpable. The emergence of the novel biomarkers not only provided a clearer path to more accurate and timely AKI diagnosis and risk stratification, but it has also opened the door to better understand the pathophysiology of AKI and its consequences and to reach a better differential diagnosis of this deadly syndrome [175]. For example, discovery of cell cycle arrest biomarkers of AKI has led to new investigations and growing knowledge regarding the role of cell cycle arrest in development of AKI, impact of cell senescence in AKI on CKD progression, and advent of new concepts such as acute kidney stress, subclinical AKI, and acute kidney disease. Acute kidney stress, kidney attack, and subclinical AKI are the new terms related to AKI diagnosis on a biochemical and molecular level. The term acute kidney stress is defined as the preinjury phase that leads to AKI [176, 177]. At the initial stage of critical illness, stressed cells are not injured, but continuation of the injurious processes leads to tubular cell injury. Subclinical AKI occurs when biochemical or anatomical evidence of injury exists but the classic criteria of AKI are not met [175, 178–180].

**Clinical adoption of AKI biomarkers**

The Acute Dialysis Quality Initiative investigators proposed a conceptual model of AKI development which suggested that AKI is a spectrum of the process that starts with damage in high-risk individuals and progresses with decreased GFR, kidney failure, and death (Figure 1) [181]. Identification of at-risk patients and early recognition of AKI appear to be critical to avoid further progression of the AKI process to worse outcomes, including more severe AKI, death, increased cost, development of CKD, and end-stage renal diseases [182–184]. Recently, development of intelligent electronic health record alerts and novel early biomarkers of AKI has provided such opportunity to potentially affect the outcomes of patients with AKI by earlier recognition and intervention [185]. This is where alliance among clinical providers and laboratory medicine experts provides tools to achieve such objectives by providing earlier, more accurate biomarkers of AKI. In the most efficient case scenario, high-risk patients could be automatically identified by electronic health records and preventive measures could be initiated after protocolized measurement of AKI biomarkers confirmed the risk category of the critically ill patient [186, 187]. Teams comprising providers, allied health staff, and laboratory medicine and information technology experts need to be built to achieve these goals (Figure 1).

Cost-effectiveness analysis is one of the first steps in the clinical implementation of AKI biomarkers. Health technology assessment is a multidisciplinary process that could provide an appropriate platform for such analysis [188, 189]. The cost of research and development, along with the clinical application of such biomarkers, could be justified by the downstream savings achieved by avoiding the need for long-term dialysis.

Clinical implementation of AKI biomarkers requires a multidisciplinary team approach. Protocolized measurement of the biomarkers after clinical or electronic identification of high-risk patients, followed by early engagement of appropriate providers and escalation of care, seems the most suitable approach to clinical implementation of AKI biomarkers. Clinical prediction models for risk stratification of patients in the hospital or ICU or novel approaches via the advent of electronic surveillance tools can identify such high-risk patients. After measurement of a battery
of biomarkers, clinicians can evaluate patients with the entire spectrum of the syndrome.

**Conclusions**

During the past decade, multiple AKI biomarkers have been studied and viewed as promising, including urine NGAL, KIM-1, IL-18, L-FABP, calprotectin, AGT, microRNAs, and the combination of TIMP-2 and IGFBP7. Nevertheless, each of these biomarkers has its own advantages and shortcomings. Although no new biomarker has been universally accepted for routine use in clinical practice, some of the biomarkers are locally available for clinical use (e.g. NGAL in Europe, L-FABP in Japan, TIMP-2 × IGFBP7 in the USA) [190]. Additionally, KIM-1 is approved by the FDA for preclinical drug development [94]. Several AKI biomarkers have been validated in ongoing clinical trials, and incorporation of novel AKI biomarkers has the potential to increase statistical power, decrease the sample size, and lower the cost of AKI trials [171]. Although the development of AKI biomarkers is a long-term investment, it is the path toward successful development of therapeutic options for AKI [171].

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