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Editorial Review

A basic science view of acute kidney injury biomarkers

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ABSTRACT

Over the last decade, significant progress has been made in the identification and validation of novel biomarkers as well as refinements in the use of serum creatinine as a marker of kidney function. These advances have taken advantage of laboratory investigations, which have identified these novel molecules that serve important biological functions in the pathogenesis of acute kidney injury (AKI). As we advance and validate these markers for clinical studies in AKI, we recognize that they serve not only to improve our understanding of AKI, but they could also serve as potential targets for the treatment of AKI. This review will underscore the biological basis of specific biomarkers that will contribute to the advancement in the treatment and outcomes of AKI.

Keywords: acute renal failure, cystatin-C, IL-18, KIM-1, NGAL

INTRODUCTION

Acute kidney injury (AKI) is a complex disorder that leads to high morbidity and mortality. The most recent epidemiological study indicates that this clinical problem continues to grow unabated [1]. Treatment regimens for AKI have been unsuccessful over the years due to the incomplete understanding of the pathogenesis and biomarkers for early detection, in addition to poor clinical trial design, along with the continued use of serum creatinine as a marker of kidney function [2, 3]. To improve the precision of serum creatinine as a marker of kidney function, the acute quality dialysis initiative (ADQI) developed the risk, injury, failure, loss, end-stage kidney disease (RIFLE) criteria, the acute kidney injury network (AKIN) developed the AKIN criteria and most recently, kidney disease improving global outcomes (KDIGO) developed the KDIGO criteria that combines the two [2, 4, 5]. As a result of recent standardization of diagnostic and staging criteria for AKI, our understanding of the epidemiology of AKI has improved in a variety of settings including outpatient clinics, emergency departments, in patient wards and in intensive care units [5–7]. Despite these efforts serum creatinine continues to suffer from a number of shortcomings including assay interference, altered metabolism of creatinine in AKI, dilution during volume overload and alterations in clearance with drugs (cimetidine, organic molecules). Additional problems relate to the fact that serum creatinine is both a late and indirect reflection of kidney damage.

Over the past decade there has been an enormous expansion in the discovery and validation of unique biomarkers of kidney disease. The ideal biomarker is one that can predict and diagnose AKI, identify the location of injury, the type and etiology of injury, predict outcomes and enable the initiation and monitoring of therapeutic interventions [8]. Biomarkers report on kidney function (glomerular filtration), tubule function (reabsorption of filtered molecules) or damage/injury. Various molecules have been identified that represent non-renal molecules filtered, secreted or reabsorbed, molecules that are constitutive or upregulated or molecules released by infiltrating immune cells (Figure 1). These biomarkers are proteins or molecules that can be found in urinary exosomes and free filtered urine [9]. Table 1 lists both candidate and validated biomarkers of AKI [10]. Most recently in the 10th ADQI meeting risk assessment, diagnosis and staging, differential diagnosis, prognosis and management and novel physiological techniques were summarized [9] to guide clinicians in the eventual use of AKI biomarkers. A number of hurdles still remain before biomarkers of AKI can be implemented in clinical practice that focus on the effects of different factors on biomarker interpretation including gender and age differences, and cut-off values of each biomarker in different conditions [chronic kidney disease (CKD), cardiopulmonary bypass, sepsis etc.] [11]. Lastly, there are concerns that relate to whether

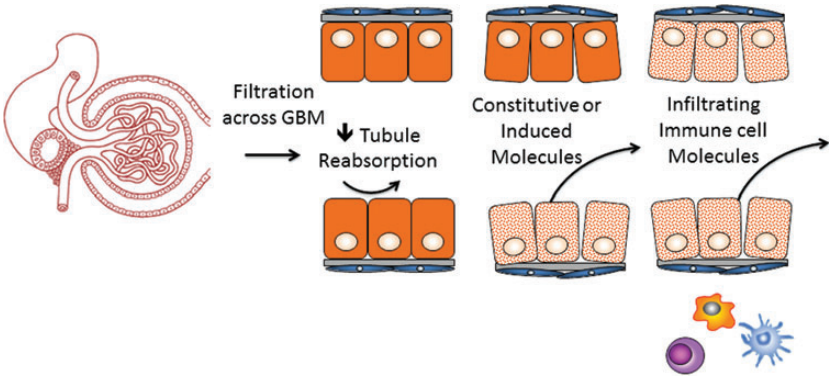

FIGURE 1: Mechanisms of urinary biomarkers in kidney injury. Biomarkers are renal and non-renal derived molecules that report on the functional status of kidney filtration and tubule injury. Markers may represent non-renal molecules filtered, secreted or reabsorbed, molecules that are constitutive or upregulated or molecules from infiltrating immune cells.

Table 1. Biomarkers of AKI

Functional biomarkers	Tubular enzymes	Upregulated proteins
Creatinine	Alanine aminopeptidase (AAP)	KIM-1
Cystatin C	Alkaline phosphatase (AP)	Clusterin
β2-microglobulin	α-glutathione-*S*-transferase (α-GST)	Neutrophil gelatinase-associated lipocalin (NGAL)
α1-microglobulin	γ-glutamyl transpeptidase (γΓT)	IL-18
Retinol-binding-protein (RBP)	*N*-acetyl-β-glucosaminidase (NAG)	Cysteine-rich protein (CYR-61)
Microalbumin		Osteopontin
		FABP
		Sodium/hydrogen exchanger isoform (NHE3)
		Exosomal fetuin A

urinary AKI biomarkers should be normalized to urine creatinine [12] as is done in patients with CKD. However, the underlying assumption that creatinine excretion is stable is flawed since there are dynamic changes in urine creatinine excretion during acute and recovery stages of glomerular filtration rate (GFR) during AKI. Thus, the normalization of urinary biomarkers of AKI to urine excretion of creatinine is affected by variability in urine creatinine excretion, which may on the one hand lead to an enhancement of its utility as a diagnostic tool or on the other hand lead to spurious and misleading values [12]. In clinical studies, normalization of urinary biomarkers to urinary creatinine concentration improved the prediction of AKI, but not in the diagnosis of established AKI [13]. Further guidelines will need to be developed for the clinical use of AKI biomarkers.

Whereas these issues pertain to the clinical characteristics of AKI biomarkers, this review will focus on a basic science view of the most promising AKI biomarkers.

CYSTATIN C

Initially named 'γ trace' because of its location just past the gamma band on an immunoelectropheresis gel, this low-molecular-weight protein was first discovered in the cerebrospinal fluid of healthy patients, then detected in the urine of patients with tubular diseases, and later in the serum of dialysis patients [14–16]. The protein was found to be similar to a cysteine proteinase inhibitor in the cystatin family, and renamed cystatin C [17]. In humans, cystatins are the most important endogenous inhibitor of cysteine proteinases, specifically cathepsin H, B, L and calpains [18]. Cystatin C, a 13-kDa protein, arises in all nucleated cells and is not bound to plasma proteins. Therefore, it is freely filtered by the glomerulus, and subsequently reabsorbed and degraded in the renal proximal tubule by the endocytic receptor, megalin [19]. Unlike creatinine, cystatin C is not secreted into the urine by the tubule, hence its appearance in the urine depends on AKI, reflecting its filtration at the glomerulus and reduced uptake by the damaged proximal tubules [20] (Figure 2a). In addition, because cystatin C and albumin are both reabsorbed by megalin-facilitated endocytosis in the proximal tubule [19, 21], the presence of albuminuria may competitively inhibit reabsorption and increase urinary excretion of cystatin C [22]. For similar reasons, albuminuria may increase the excretion of other biomarkers including neutrophil gelatinase-associated lipocalin (NGAL), liver fatty acid-binding protein (L-FABP), α_1-microglobulin and β_2-microglobulin [22]. The blood concentration of cystatin C depends on the individual's GFR, and the correlation between cystatin C and GFR is evident even in a range where serum creatinine cannot detect changes, GFRs of 60–90 mL/min [23]. Both particle-enhanced nephelometric and turbidometric immunoassays are the most accurate and established methods to detect cystatin C concentration in samples, but both have intra- and inter- assay variability [24].

When compared with serum cystatin C, urine cystatin C appears as an earlier and more sensitive marker in AKI. In animals exposed to cisplatin or gentamicin, urinary cystatin C rose before proximal tubule damage, supporting its value as an early biomarker [25, 26].

Although the superiority of serum cystatin C when compared with serum creatinine has been established in both animal models and clinical settings of CKD, the use of cystatin C as a biomarker in AKI continues to evolve. Currently, it is unclear if the value of cystatin C is generalizable to all forms of AKI, or specific to particular populations [27–30]. It is

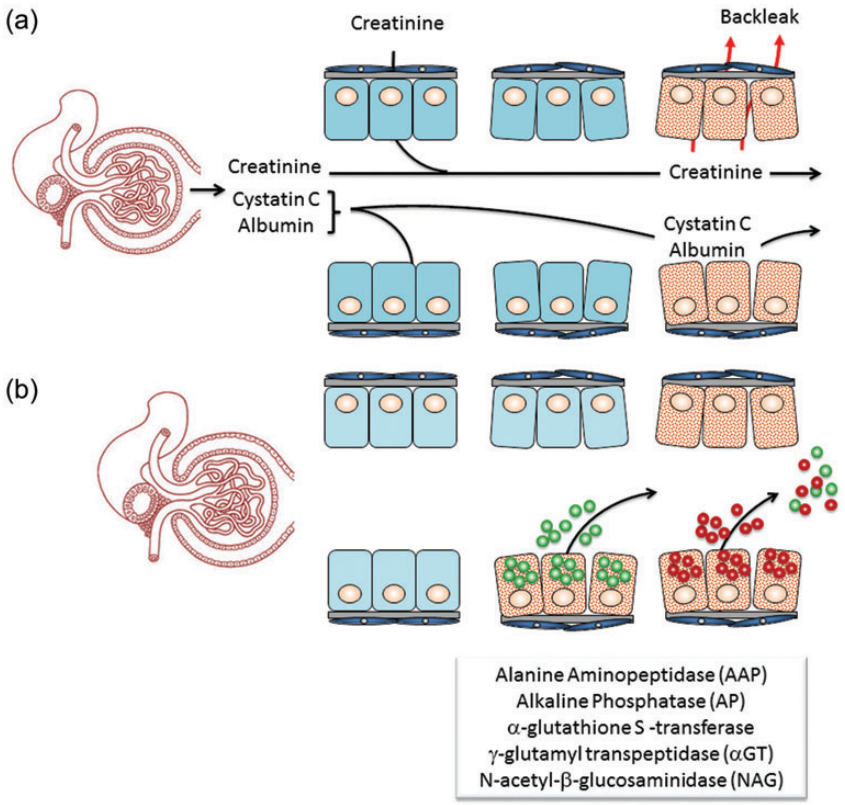

FIGURE 2: Functional biomarkers and enzymatic injury biomarkers. (**a**) Functional biomarkers. Creatinine is freely filtered and a small amount is secreted into the tubular lumen. During AKI the increase in serum creatinine is due to a decrease in glomerular filtration rate and backleak through damaged proximal tubule cells. Cystatin C is freely filtered and reabsorbed by the proximal tubule. During AKI, reabsorption by the proximal tubule may be diminished due to damage to the epithelium, which augments its appearance in the urine. The reduced filtration rate causes the rise in cystatin C following AKI. A small amount of albumin passes through the filtration barrier and ischemic damage to glomeruli likely enhances albumin leak. Normally, albumin is reabsorbed by the proximal tubule; however, with damage to the proximal tubule, reabsorptive mechanisms are diminished, increasing the appearance of albumin in the urine. (**b**) Enzymatic injury biomarkers. Alanine aminopeptidase (AAP), alkaline phosphatase (AP). γ-glutamyl transpeptidase (gGT) and *N*-acetyl-β-glucosaminidase (NAG) are present in the epithelial cells and are released into the urine following cellular injury.

important to recognize that the analysis of cystatin C is affected by diabetes, large doses of corticosteroids, hyperthyroidism, inflammation, hyperbilirubinemia, rheumatoid factor and hypertriglyceridemia [23, 24]. Cystatin C has not been validated for use in children <2 years old.

MICROALBUMIN

Microalbuminuria is urinary albumin that is below the threshold of detection by the conventional urinary dipstick (30–300 mg/L). It is widely recognized as a critical diagnostic tool in the development and progression of renal disease, signaling altered glomerular structure and function [31]. This paradigm may be relevant for AKI as well. Urinary albumin levels are increased in both glomerular and tubular diseases, but recently it was found that gene expression of albumin is increased in AKI [32] making urinary albumin a more sensitive marker than previously thought.

The use of urinary albumin as a marker for AKI was shown in a rat model of toxic AKI and was thought to be due to impaired proximal tubular function, as urinary levels of beta2-microglobulin were also high in these animals (Figure 2a). The albuminuria preceded changes in the urinalysis, urinary NAG levels and serum creatinine [33]. In an animal model of septic AKI, urine albumin to creatinine ratios rose within the first 24 h after lipopolysaccharide (LPS) administration; a response that was prevented by a therapeutic intervention [34]. In patients receiving cancer chemotherapeutic agents such as cisplatin, ifosfamide, methotrexate [35, 36] and antibiotics such as aminoglycosides, tubular dysfunction was detected by urinary albumin excretion [37]. In both the translational research investigating biomarker end-points (TRIBE) consortium and smaller studies, urine albumin predicted the infants and children that would develop AKI after cardiopulmonary bypass surgery [38, 39]. The limitations of using albuminuria as a biomarker for AKI include the following: (i) non-specific site of injury, (ii) ability to separate CKD from AKI and (iii) albuminuria can be non-pathologic, occurring in the setting of vigorous exercise, hematuria, urinary tract infections, dehydration, fever and poor glycemic control and (iv) albumin can degrade with storage [40, 41]. The benefits of using albuminuria in the setting of AKI are: it is inexpensive, is

obtained in readily available body fluid and can be quantified in a high through-put manner.

N-ACETYL-β-D-GLUCOSAMINIDASE

Within the kidney, *N*-acetyl-β-D-glucosamininidase (NAG) originates from the lysosomes of the proximal tubule cells and can be measured in the urine using a colorimetric assay. Increased urinary concentration of NAG is a sensitive marker for proximal tubule injury with loss of lysosomal integrity [42] (Figure 2b). NAG's large size (~140 kDa) precludes renal filtration and therefore high urinary levels are unlikely to originate from a non-renal source. Administration of proximal tubule toxins such as gentamicin and mercury in rats causes a significant increase in concentrations of urinary NAG [43, 44]. Not only does NAG appear to correlate well with histologic evidence of proximal tubule injury, it may also reflect effective treatment of renal tubular injury. In animal models of nephrotoxicity, urinary NAG returned to baseline with antioxidant therapy [44, 45].

Urinary NAG performs reasonably well in the clinical setting as well. In humans, Westhyzen *et al.* [46] reported urinary NAG concentrations were sufficiently sensitive to detect AKI in critically ill adults, preceding serum creatinine by 12 h to 4 days. Critically ill patients admitted for AKI had urinary NAG levels that correlated with poorer outcomes [47]. There are limitations of urinary NAG as a biomarker for AKI. Since urinary NAG is a particularly sensitive marker of tubular injury, its ability to indicate damage in a specific tubular segment may be overshadowed by the low threshold for the release of tubular enzymes in response to any tubular injury and its prognostic value needs to be assessed. Urinary NAG is also inhibited by urea [48], industrial solvents and heavy metals [49]. There have been reports in the literature of false-positive values for urinary NAG including in the setting of rheumatoid arthritis [50], impaired glucose tolerance [51] and hyperthyroidism [52]. Particularly important for research purposes, NAG tends to degrade most appreciably over time when compared with other biomarkers even when stored at −80°C. This degradation is not improved with alkalinization or protease inhibition [53].

KIDNEY INJURY MOLECULE-1

Studies examining repair after ischemia–reperfusion injury (IRI) identified rat and human cDNAs for a type I membrane glycoprotein that contains both a novel six-cysteine immunoglobulin-like domain and a mucin domain in its extracellular portion. This molecule was named kidney injury molecule-1 (KIM-1)/T cell immunoglobulin and mucin domain containing protein-1 (Kim-1/TIM-1) [54]. Kim-1 mRNA levels increased more than any other gene after kidney injury, and the ectodomain of Kim-1 is shed from cells *in vitro*, as well as *in vivo* in the urine from rodents after proximal tubular injury [55] (Figure 3). Kim-1/TIM1 is also expressed in immune cells where it is thought to activate T-helper2 (Th2), Th1 and Th17 differentiation as well as activating receptor in B cells, dendritic cells and natural killer cells [56]. *In situ* hybridization and immunohistochemistry demonstrated that Kim-1 was expressed in proliferating and regenerating proximal tubules. After injury, Kim-1 expressed in epithelial cells was responsible for phagocytosis in cultured primary rat tubule epithelial cells by recognizing apoptotic cells, phosphatidylserine and oxidized lipoproteins [57]. The findings of this study suggest that Kim-1 is capable of facilitating remodeling of injured epithelia.

Urinary Kim-1 protein concentration was significantly increased within 12 h after an initial ischemic renal insult, when compared with urine samples from patients with other forms of acute and chronic renal failure [58]. Kim-1 expression was also found in proximal tubule epithelial cells in human kidney biopsy sections from patients with acute tubular necrosis (ATN). There are two spliced variants, Kim-1a which is the major form expressed in the liver and Kim-1b, which is the predominant form in the kidneys. Both isoforms have identical extracellular domain but differ on their cytoplasmic domains [55]. By a metalloproteinase-dependent process, Kim-1 sheds its ectodomain appearing in the urine of patients with ATN. In nephrotoxic models of AKI (folic acid and cisplatin) upregulation of Kim-1 expression precedes the rise in serum creatinine and suggests that this protein may serve as a general biomarker for tubular injury [59]. Although initial clinical studies suggested that the rise of urinary Kim-1 could be delayed when compared with other novel biomarkers, more recent studies suggest that Kim-1 elevations do occur within hours of renal injury [10, 60, 61]. In fact, extensive analysis of studies in patients with AKI conducted between 2002 and 2009 demonstrated that Kim-1 is an early biomarker of AKI within 24 h after a kidney insult [62]. The development of sensitive and reproducible quantitative microbead-based KIM-1

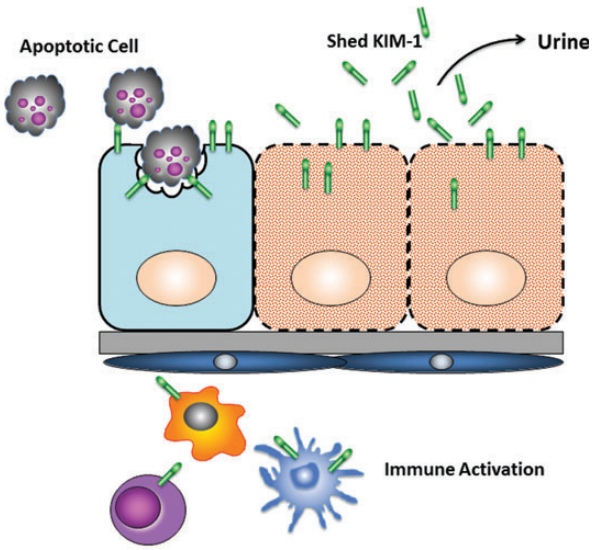

FIGURE 3: KIM-1. It is expressed in proximal tubule cells and is thought to promote apoptotic and necrotic cell clearance. Upon injury, KIM-1 is upregulated and shed into the urine and extracellular space. It is thought to activate immune cells in injury-induced immune response.

ELISA [63] tests should further facilitate their use in mice and humans, which will help to demonstrate the utility of urinary Kim-1 as a kidney biomarker of AKI. Kim-1 has been approved by the US Food and Drug Administration as an AKI biomarker for preclinical drug development [64].

NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN

NGAL is a novel 25-kDa protein associated with gelatinase from human neutrophils with similar motifs to other known lipocalins [65]. The tertiary structure of this protein contains an α-helix and a β-barrel surrounding a hydrophobic core that binds small lipophilic ligands [66, 67]. NGAL exists as a 25-kDa monomer, 45-kDa homodimer and conjugated to gelatinase as a 135-kDa heterodimeric form [65]. The monomeric form and to some extent the heterodimeric form are the predominant forms produced by tubular epithelial cells, whereas the homodimeric form is specific to neutrophils [68]. NGAL protein levels are very low in various biological fluids in the steady-state level. The serum concentration of NGAL is ∼20 ng/mL, which is probably derived from neutrophils and from limited expression in the liver, spleen and kidneys [69]. Renal clearance is a major regulator of this steady state, because circulating NGAL undergoes glomerular filtration due to its low molecular weight and positive charge. Filtered NGAL is captured by the proximal tubule, where it is degraded to a 14-kDa fragment in lysosomes. Endocytosis of NGAL from the apical membrane is considered the most likely pathway for NGAL traffic because it appeared in the urine when the apical megalin receptor was deleted [70] (Figure 4). Similar to serum, the normal concentration of urinary NGAL is also ∼20 ng/mL at steady state. The origin of this protein is not clear, but could be derived from serum NGAL that bypasses capture in the proximal tubule, from neutrophils or from bladder epithelia. An interest in NGAL as a clinical biomarker came after it was found that NGAL was markedly upregulated in the kidney tissue in the mouse models of renal IRI and cisplatin, and these changes were accompanied by the presence of urinary NGAL which preceded the rise in serum creatinine [71, 72]. Many clinical studies have shown that increases in urinary and plasma NGAL are powerful and independent predictors of AKI when compared with serum creatinine [73–75]. To further investigate the quantitative relationships between the expression of NGAL in the kidneys and the amount of NGAL protein in the urine, Paragas *et al.* [76] created a bioluminescent mouse by placing Luciferase2-mCherry reporters in the lipocalin2 locus. Luciferase2 expression, designated as 'kidney NGAL', was proportional to the dose of ischemia or the dose of LPS. In addition, kidney luminescence paralleled the amount of NGAL protein appearing in the urine in a dose-responsive fashion. The proximal tubule, [71] thick ascending limb and collecting ducts [76] appear to be sources of urinary NGAL. NGAL was identified in mouse proximal tubules after kidney IRI through immunohistochemistry and NGAL mRNA in human proximal tubule cells following ATP depletion [71]. Somewhat surprising was the identification of NGAL in macula densa, distal convoluted and intercalated cells as well as the thick ascending limb and collecting ducts following kidney ischemia in NGAL reporter mouse; however, the protein secretion from these nephron segments has not been well studied [76]. Differences between these studies may relate to the different methods used and their sensitivities. In addition, some of the proximal tubule NGAL identified by immunohistochemistry may be detecting filtered NGAL reabsorbed by the proximal tubule.

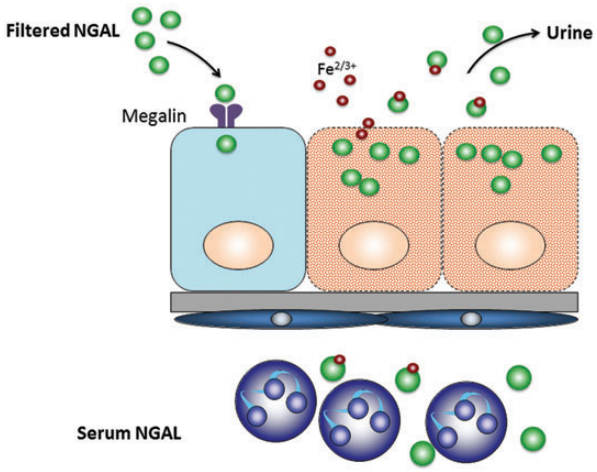

FIGURE 4: NGAL. It is produced by neutrophils and is expressed to a limited degree in the liver, spleen and kidney. Several functions have been described including inhibiting bacterial growth, scavenging iron and inducing epithelial cell growth. A small amount of NGAL is filtered and taken up by the proximal tubule through megalin. Upon injury, NGAL (a stress response protein) is upregulated and released into the urine and plasma. Its protective effect when infused may be related to its ability to scavenge iron as depicted or through its ability to induce cell growth.

Intravenous administration of purified recombinant NGAL protected mouse kidneys from IRI [77]. Quantitative differences in the production of NGAL in different nephron segments may protect kidneys to varying degrees. When compared with proximal tubules collecting ducts were not apoptotic [76]. The mechanism of protection by NGAL is not known. NGAL forms a complex with iron-binding siderophores and for this reason has also been called siderocalcin. NGAL released by nephron segment may chelate labile Fe released from damaged tubules and prevent the formation of hydroxyl radicals and superoxide (Figure 4). Using a mouse model of AKI, a single dose of NGAL dramatically protected the kidneys, and blockade of the siderophore with gallium inhibited the rescue from ischemia [78]. Furthermore, the Ngal:siderophore:Fe complex upregulated heme oxygenase-1, preserved N-cadherin and inhibited cell death. NGAL is intensively upregulated in models of sepsis, suggesting that the release of NGAL into the urinary system is a major response of the kidney to systemic infections as well as local urogenital infections. The available data support the concept that the urinary pool of NGAL derives from tubular cells of thick ascending limb of the loop of Henle (TALH) as well as from collecting duct.

In addition to its appearance in the urine in its free form, NGAL is readily excreted complexed with matrix metalloproteinase-9 (MMP-9) [79]. MMP-2 and 9 are endopeptidases

that degrade extracellular matrix and are involved in ischemic organ injuries. Both expressions of MMP-2 and MMP-9 increase following kidney IRI [80]. Whereas mice deficient in MMP-2 were protected from acute IRI, mice deficient of MMP-9 were not [81]. However, when mice deficient in MMP-9 were followed for 2 weeks post-IRI, they were protected from microvascular loss following kidney IRI [81]. Because MMP-9-NGAL preserves enzymatic activity of MMP-9 [79], the role of preserved MMP-9 activity by the complex in AKI will need to be examined.

NGAL has been one of the most widely studied biomarkers in AKI. A recent extensive meta-analysis of data from 19 studies including >2500 patients, serum and urine NGAL levels were found to be not only diagnostic of AKI, but also predicted clinical outcomes such as the need for initiation of dialysis and mortality [75].

INTERLEUKIN-18

Interleukin 18 (IL-18), a member of the IL-1 cytokine superfamily and known as an interferon-γ (IFN-γ)-inducing factor, regulates innate and adaptive immunity [82]. IL-18 was derived from a liver cDNA library from animals injected with heat killed *Propionibacterium acnes* and challenged with LPS [83]. The murine IL-18 precursor is a polypeptide of 192 amino acids lacking a conventional signal peptide and is cleaved by caspase-1 forming a mature protein of 157 amino acids. The murine and human IL-18 are 65% homologous [84]. The precursor IL-18 (pro-IL-18) is a 24-kD inactive molecule and is cleaved after Asp35 by caspase -1, an endoprotease IL-1β-converting enzyme (ICE; caspase-1) to generate the biologically active 18-kD molecule [85, 86]. IL-18 is produced by mononuclear cells, macrophages and non-immune cells including proximal tubule cells (Figure 5). IL-18 mRNA is expressed in human peripheral blood mononuclear cells, murine splenic macrophages and non-immune cells [87, 88]. IL-18 binds to IL-18R complex, a heterodimer containing an α and β chain. The α chain is the IL-1Rrp, the chain responsible for binding of IL-18 and the β chain and AcP (accessory protein) is the chain responsible for signal transduction [89–91]. IL-18R is expressed on hematopoietic cells (macrophages, neutrophils, natural killer cells) as well as endothelial cells and smooth muscle cells [82].

IL-18 levels in the kidneys more than doubles following AKI and the conversion of IL-18 precursor to the mature form requires caspase-1 as this conversion is not observed in caspase-1-deficient mice [92]. IL-18 blocking antibodies decreased injury to a similar degree as seen in caspase-deficient mice. These results demonstrate that IL-18 is an important mediator of acute ischemic AKI. The source of IL-18 responsible for kidney injury is thought to be the proximal tubule [88] and not macrophages [93], neutrophils or CD4 T cells [92, 94]. In an obstructive model of kidney injury, the deleterious effect of IL-18 appears to be due to its activation of epithelial FasL expression, increase active caspase-8 and caspase 3 expression and effects that are blocked by IL-18 neutralization [95].

In a cross-sectional study urine IL-18 levels increased in patients with ATN compared with other kidney diseases (prerenal azotemia, urinary tract infection, CKD and nephrotic syndrome) and had a sensitivity and specificity of >90% for the diagnosis of AKI [96]. In the intensive care unit in patients with acute respiratory distress syndrome, urine IL-18 levels of >100 pg/mL were associated with an increased odds of AKI of 6.5 (95% confidence interval: 2.1–20.4) and increased predicted mortality [97]. In a recent meta-analysis of 23 studies and 4512 patients, urinary IL-18 was found to be a predictive

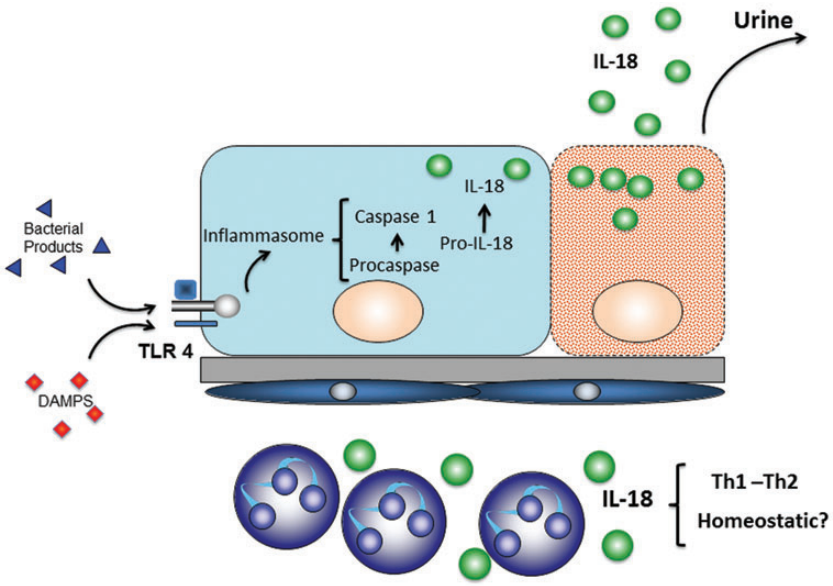

FIGURE 5: IL-18. It is produced by immune cells and by active epithelial cells. Following activation of toll like receptor 4 (TLR4), activation of inflammasome leads to cleavage of pro-caspase 1 to caspase-1. This in turn cleaves pro-IL-18 into the active IL-18 molecule. IL-18 has proinflammatory properties or may have homeostatic properties.

biomarker of AKI in various settings including cardiac surgery patients, intensive care unit or coronary care units as well as in children, adolescents and adults [98].

LIVER FATTY ACID-BINDING PROTEIN

L-FABP is a 15-kDa protein that belongs to the family of the fatty acid-binding proteins (FABP). To date, nine FABP protein-coding genes have been identified in the human genome. L-FABP is not only expressed in the liver, but also in the intestine, pancreas, kidneys, lungs and stomach. This 15-kDa protein also belongs to the family of lipocalins, and compriseds an anti-parallel β-barrel containing a ligand-binding pocket, and a helical N terminus that is involved in the regulation of fatty acid (FA) transfer from membranes via electrostatic interactions [99]. The gene promoter of L-FABP also contains several response elements including the presence of a peroxisome proliferator response element that is important for the regulation of FABP1. Treatment with hypercholesterolemia and hyperlipidemic drugs (statins and fibrates) up-regulates FABP1 expression in the liver and kidney tissue [100].

Although the kidney in rodents does not synthesize a significant amount of L-FABP, recent work suggests that L-FABP under normal conditions resides in the lysosomal compartment of the proximal tubule, and can also be reabsorbed from the glomerular filtrate via megalin, a multi-ligand proximal tubule endocytic receptor. On the other hand, the amount of L-FABP present in the human kidney is considerably much higher than the amount of L-FABP present in mouse kidney, and its expression is restricted to the proximal convoluted and straight tubules [77]. In more recent studies, the creation of a human L-FABP chromosomal transgenic mouse, as well as the development of an ELISA method to measure urinary human L-FABP, were developed to identify the localization and the role of the human protein in various models of AKI [100, 101]. Human kidney L-FABP is expressed in the proximal tubule. L-FABP was found to traffic from the cytoplasm of proximal tubule to the tubular lumen in human L-FABP transgenic mice subjected to IRI, a result that suggested that increased urinary L-FABP after IRI could be a useful biomarker of acute ischemic injury [102] (Figure 6). In a cisplatin model of AKI, there was an increase in urinary excretion of human L-FABP detected within the first 24 h of cisplatin injection. In addition, the authors demonstrated that fibrate pretreatment prevented cisplatin-mediated shedding of urinary L-FABP. The increased shedding of urinary L-FABP before a rise in serum creatinine suggested that human L-FABP could be considered as an early biomarker of cisplatin-mediated AKI [103].

In clinical studies, urine L-FABP was assessed in 40 pediatric patients prior to and following cardiopulmonary bypass surgery. Elevated urinary L-FABP levels 4 h after surgery were an independent risk indicator of AKI post-cardiac surgery, and appears to be a sensitive and predictive early biomarker of AKI after cardiac surgery [103]. Subsequent studies have confirmed this initial observation in adult patients having cardiac surgery as well as patients admitted to the ICU with sepsis [104, 105]. L-FABP has been approved as a diagnostic test for human AKI in Japan.

A recent study conducted by the TRIBE in Acute Kidney Injury consortium in adult and children patients undergoing cardiac surgery validated the use of several of the biomarkers and identified a strong signal for risk of AKI when five AKI biomarkers (NGAL in urine and serum, and urine IL-18, KIM-1 and L-FABP) were considered in aggregate [106].

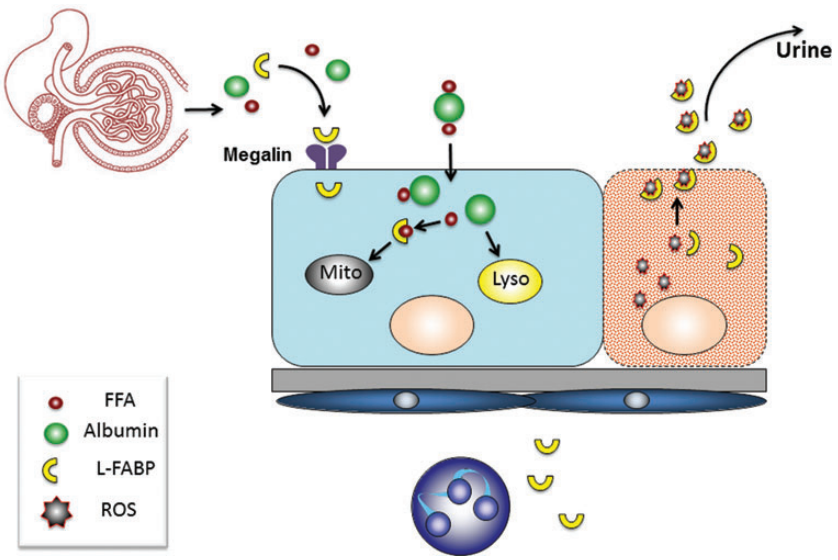

FIGURE 6: L-FABP. They are bound to serum albumin and are reabsorbed into the proximal tubule bound to serum albumin. Filtered L-FABP is taken up by the proximal tubule and acts as a carrier protein and transports free fatty acids to mitochondria and peroxisomes for metabolism. Upon stress and ischemia–reperfusion there is an upregulation of L-FABP, which binds lipid hydro-peroxides and other reactive oxygen—which together are released into the urine.

Other biomarkers

Netrins belong to the laminin-related family, initially described as axon guidance molecules [107] later noted for their role in the development of many organs [108]. In mammals, there are three secreted netrins (netrin-1, -3 and -4) [107]. Within the kidneys, netrins are normally located in the peritubular capillaries of the kidneys, but during IRI netrin-1 is highly induced within the tubular epithelial cells [109]. Netrins mediate their effects through two known receptors, deleted in colon cancer and UNC5H [110]. Netrin-1 inhibits leukocyte migration during sepsis, mitogenesis and chemoattraction of endothelial cells, with a role in angiogenesis, cell migration, tissue morphogenesis, tumor progression and growth and regulation of inflammation. Netrin regulates inflammation and inflammatory cell migration during AKI through the suppression of COX-2 induction of PGE2 and thromboxane A2 production [111]. Netrin-1 provides some protection from IRI, potentially by the suppression of oxidation and inhibition of neuropeptide Y expression [112]. Netrin-1 has been evaluated as a urinary biomarker in multiple animal models of AKI, including IR, cisplatin, LPS and folic acid administration. In IRI, netrin-1 increased gradually in the urine over the hours subsequent to the injury, peaked at 6 h and by 24 h had returned to baseline. This pattern was consistent in the group receiving cisplatin and LPS, but in the folic acid group the urinary levels of netrin-1 peaked at 3 h and did not return to baseline. This increased urinary excretion of netrin-1 always preceded increases of serum creatinine or urea BUN [113]. Although a relatively new biomarker in both basic and clinical research, urinary netrin-1 seems to perform well in the detection of AKI. Netrin-1 identifies AKI early and found to return to baseline within 24 h [113]. Netrin-1 performs well as a biomarker for AKI across many types of injury and can be measured in urine by western blots and commercially available ELISA. *MicroRNAs* are endogenous single-stranded molecules of ∼22 non-coding nucleotides that induced gain or loss of function and contribute to specific diseases have been identified in the urine of patients with AKI [114]. Recent studies have identified urine *insulin-like growth factor-binding protein 7 (IGFBP7)* and *tissue inhibitor of metalloproteinases-2 (TIMP-2)*, both inducers of G_1 cell cycle arrest as important new biomarkers [115]. These proteins are expressed in epithelial cells and act in an autocrine and paracrine manner to arrest cell cycle in AKI. Further testing will be necessary to determine their significance as a biomarker of AKI.

CONCLUSIONS

There have been major advances in the discovery and validation of biomarkers of AKI in a variety of clinical settings and it is likely that these findings will provide information on diagnosis, etiology and prognosis of AKI. Further advances have been made to identify new biomarkers in the future as well as using newer discovery tools. Isolation of *urinary exosomes* that contain epithelial membranes and intracellular fluids released into the urine may be recovered and analyzed [116]. Through proteomic analysis of urinary exosomes including two-dimensional electrophoresis, liquid chromatography and capillary electrophoresis all of them coupled to mass spectrometry, novel proteins may be identified. *Metabolomics* refers to the study of the metabolite pool that exists within a cell, tissue or biofluid under a particular set of conditions [117], whereas *metabonomics* has been defined as the quantitative measurement of the dynamic metabolic response of living systems to pathophysiological stimuli or genetic modification [118]. Studying the metabolome will allow understanding of changes in phenotype and function [119–121]. The use of current biomarkers or newer biomarkers in the future will be combined with the use of creatinine as a functional biomarker, which together will enhance the ability of the RIFLE, AKIN or KDIGO criteria to define AKI (Figure 7).

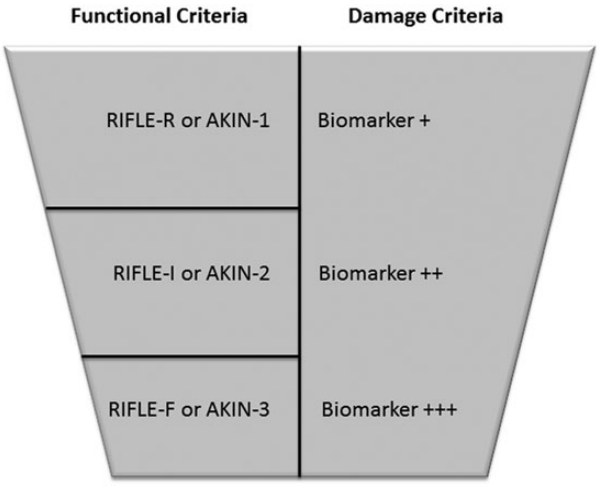

FIGURE 7: Use of biomarkers in diagnosis of AKI. Diagnosis of AKI will be facilitated in the future using RIFLE/AKIN (or KDIGO) criterion combined with biomarker criterion. Increasing biomarker severity associated with increasing kidney damage is denoted by +/++/+++. AKIN, acute kidney injury network; RIFLE, acute dialysis quality initiative [122].

CONFLICT OF INTEREST STATEMENT

None declared.

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