



## New Biomarkers in Early Diagnosis of Acute Kidney Injury in Children

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### Abstract

Acute Kidney Injury (AKI) is a common condition with a high risk of mortality and morbidity, so, early diagnosis and management of AKI is very important in clinical practice. Despite significant progress in the management of AKI, it still carries high morbidity and mortality. BUN and serum creatinine are not very sensitive nor specific for the diagnosis of AKI because they are affected by many renal and non-renal factors that are independent of kidney injury or kidney function and change significantly only after significant kidney injury and with a substantial time delay. Detection of biomarkers of AKI made predominantly by the injured kidney tissue are essential for the early diagnosis of AKI. An ideal biomarker should be one that could be easily measured, with no interference with other biologic variables, and be able to clarify early phases of kidney damage. The most common biomarkers studied are Neutrophil Gelatinase-Associated Lipocalin (NGAL), Interleukin-18 (IL-18), Kidney Injury Molecule-1 (KIM-1), Cystatin-C, L type Fatty Acid-Binding Protein (L-FABP), N-Acetyl-  $\beta$ -D Glucosaminidase (NAG), netrin-1, vanin-1, and Monocyte Chemoattractant Protein-1 (MCP-1) and calprotectin.

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### Introduction

Acute Kidney Injury (AKI) is very common and its absolute incidence has increased in over the last years. AKI has been reported to complicate 1-7% of all hospital admissions and 1-25% of Intensive Care Unit (ICU) admissions<sup>1</sup>. Over the past 50 years, mortality rates of patients with AKI in ICUs have remained high approximately 50-70%. In ICU, between 5-20% of critically ill patients have at least one episode of AKI<sup>2</sup>.

Under normal conditions renal blood flow is about 5-6 *mls/g/min* with a pressure of 60-100 *mmHg* which is necessary to maintain normal renal function<sup>3</sup>. Renal blood flow is primarily governed by multiple factors involving extra-renal processes such as vascular tone, neuro-hormonal processes and vasodilator/vasoconstrictor substances among others. Failure in any of these mechanisms will lead to hypoxia of the organ which can be severe and not depending on the magnitude of these mechanisms. It also, depends on the compensatory mechanisms, such as afferent arteriolar dilatation and efferent arteriolar constriction which can regulate the supply of oxygen needs at the right time. At this time secretion of pro-inflammatory mediators due to tissue damage occurs which serve as biomarkers for early kidney injury detection. These include Neutrophil

Gelatinase-Associated Lipocalin (NGAL) and Kidney Injury Molecule-1 (KIM-1). Interleukin-18 (IL-18) secreted by inflammatory cells, such as macrophages are neutrophils that enter the kidneys during the inflammatory phase<sup>4</sup>. A number of pathophysiological mechanisms can contribute to AKI following an ischemic or toxic insult. These include (a) alterations in renal perfusion resulting from loss of autoregulation and increased renal vasoconstriction, (b) tubular dysfunction and cell death by apoptosis and necrosis, (c) desquamation of viable and dead cells contributing to intratubular obstruction, (d) metabolic alterations resulting in transport abnormalities that can lead to abnormalities of tubule glomerular balance, and (e) local production of inflammatory mediators resulting in interstitial inflammation and vascular congestion<sup>5,6</sup>. After the renal blood flow reduction, the epithelial cells cannot maintain adequate intracellular ATP for the essential metabolic processes. This ATP depletion leads to cellular damage and, if severe enough, can cause cellular death by necrosis or apoptosis. During an ischemic event, all the segments of the nephrons are potentially affected, but the proximal tubular cells are the most commonly damaged segment<sup>7</sup>. Consequently, these enzymes are

secreted by the tubular epithelial cells and are excreted in the urine as a response to the AKI; among these are N-Acetyl-b-Glucosaminidase (NAG), cytoplasmic protein lactate dehydrogenase and glycoprotein<sup>8,9</sup>. These secreted enzymes are biomarkers classified based on their role in the pathophysiology of AKI<sup>10</sup>. Detection of newer stress biomarkers such as cell cycle arrest markers measuring cellular stress even before damage or loss of function (preinjury phase) is quite promising<sup>11-14</sup>. A list of biomarkers for detecting renal injury in relation to their site of excretion is given in table 1.

#### NGAL

Neutrophil Gelatinase-Associated Lipocalin (NGAL) NGAL is a 21-kDa protein of the lipocalin superfamily. NGAL is a critical component of innate immunity to bacterial infections and is expressed by immune cells, hepatocytes, and renal tubular cells in various disease states<sup>15</sup>. NGAL is a small secreted polypeptide protease resistant and so, may be easily detected in urine. The origin of NGAL is proximal tubular cells and is detected in the urine at early stage of AKI. It is reabsorbed almost totally in the proximal tubule and its elevated levels may be an indication of proximal tubular damage. Production of NGAL can be increased up to 1000 times in Henle's loop and distal tubule when AKI is occurring<sup>16</sup>. NGAL is identified as being one of the seven genes whose expression was up-regulated more than tenfold within the first few hours after ischemic renal injury in a mouse model<sup>17,18</sup>. NGAL levels seem to be more sensitive and specific in predicting AKI in studies with homogeneous patients

with a single acute illness. NGAL levels seem to predict AKI in children with better accuracy than in adults (which make up the vast majority of patients with AKI)<sup>19</sup>. The basal levels of plasma NGAL are higher in patients with malignancies and systemic bacterial infections, and these can be confusing. The levels of urinary NGAL may also be elevated in urinary tract infections. Urinary NGAL can be used to diagnose early infections of the urinary tract in the absence of AKI<sup>20</sup>. NGAL is also, an early predictive biomarker of Contrast-Induced Nephropathy (CIN) in children. A significant elevation of NGAL concentrations in urine and plasma was noted within 2 hr after cardiac catheterization. In contrast, detection of CIN by an increase in serum creatinine was only possible 6 to 24 hr after cardiac catheterization. By multivariate analysis, the 2-hr NGAL concentrations in the urine and plasma, but not patient demographics or contrast volume, were found to be powerful independent predictors of CIN<sup>21</sup>.

#### KIM-1

Human-Kidney Injury Molecule-1 (KIM-1) is a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain. KIM-1 messenger RNA and protein are expressed at a low level in normal kidney but are increased dramatically in post ischemic kidney<sup>22</sup>. KIM-1 is a 38.7 kDa transmembrane type 1 glycoprotein with a similar domain as extracellular immunoglobulin similar to mucin. It is expressed in low levels in the kidney and other organs but, its expression is accentuated in pre-renal kidney injury and after its reperfusion<sup>23</sup>. The *KIM-1* gene or protein ex-

Table 1. List of biomarkers for detecting renal injury

Proximal tubule	Glomerulus	Preinjury biomarkers
Albumin	Albumin	IGFBP-7
IL-18	Total protein	TIMP-2
Cystatin C (urinary)	Cystatin C (urinary)	DKK1-4 (DKK-3) * (serum, urinary)
KIM-1	$\alpha$ -1 microglobulin	Hemojuvelin (HJV) (urinary)
$\alpha$ -1 microglobulin	$\alpha$ -2 microglobulin	Micro-RNAs (U) (urinary)
$\alpha$ -2 microglobulin	Loop of henle	Wnt (serum, urinary)
NGAL	Osteopontin	Others
HGF	NHE-3	Cytochrome-C (urinary)
Netrin-1	Distal tubules	Epidermal growth factor (urinary)
Osteopontin	GST- $\mu/\pi$	Malondialdehyde (urinary)
NHE-3	NGAL	
Cyr61	Osteopontin	
GST- $\alpha$ (urinary)	Clusterin	
Clusterin	H-FABP	
Exosomal fetuin-A	Calbindin D-28	
Calprotectin		
NAG	Collecting duct	
l-FABP	Calbindin D-28	
RBP		

DKK-3-Dickkopf-3, \* DKK-3 is used most commonly; NHE-3-Na<sup>+</sup>/H<sup>+</sup> exchanger isoform 3; NAG-N-Acetyl-  $\beta$ -d-Glucosaminidase; NGAL-Neutrophil Gelatinase-Associated Lipocalin; RBP-Retinol Binding Protein; Cyr 61-Cysteine-rich 61; IL-18- Interleukin 18; GST- $f_i$ , Glutathione S-Transferase- $f_i$ ; HGF-Hepatocyte Growth Factor; l-FABP- l-type Fatty Acid-Binding Protein; IGFBP-7-Insulin-like Growth Factor-Binding Protein-7; TIMP-2-Tissue Inhibitor of Metalloproteinase 2; [IGFBP-7] [TIMP-2] are always used together and are marketed as such; H-FABP, heart fatty acid-binding protein.

pression is undetectable in normal kidneys after injury and the related mRNA (messenger ribonucleic acid) of KIM-1 is rapidly synthesized and the excess protein production generated is found in high levels at the apical membrane of the proximal tubule. In humans with ischemic and toxic AKI, KIM-1 is found in all three segments of the proximal tubules KIM-1<sup>24</sup>. It is also a sensitive biomarker of tubular injury in other renal diseases besides. Renal KIM-1 expression was significantly increased in human kidney tissue in patients with focal glomerulosclerosis, immunoglobulin A nephropathy, membranoproliferative glomerulonephritis, membranous glomerulonephritis, acute rejection, chronic allograft nephropathy, systemic lupus erythematosus, diabetic nephropathy, hypertension and Wegener's granulomatosis compared with normal kidney tissue<sup>25</sup>. Urinary KIM-1 has shown to be very sensitive and specific marker of proximal tubular kidney injury and can also, distinguish ischemic acute tubular necrosis from pre-renal azotemia<sup>12</sup>.

#### **Cystatin C**

Cystatin C (Cys-C) is a cysteine protease inhibitor synthesized by all nucleated cells in the body. It is freely filtered by the glomerulus, fully reabsorbed and not secreted at all so, it is an endogenous marker of renal dysfunction. The urinary excretion of low molecular weight cystatin C protein correlates with the severity of acute tubular injury<sup>26</sup>. Cys-C is a 13 kDa proteinase inhibitor which plays an important role in intra-cellular catabolism of proteins and peptides<sup>27</sup>. Over the past decade, serum Cys-C has been extensively studied and found to be a sensitive serum marker of Glomerular Filtration Rate (GFR) and stronger predictor than serum creatinine in detecting the risk of death and cardiovascular events in older patients. Its serum concentration appears to be independent of sex, age and muscle mass<sup>28</sup>. Serum Cys-C can be a useful biomarker of AKI prediction; its urinary excretion indicates tubular damage, and it needs a moderate diagnostic facility<sup>29</sup>. Its concentrations are elevated in acute and chronic kidney disease, and in contrary to creatinine does not depend on height, weight, age, sex, nutritional status, and inflammatory processes<sup>30</sup>. However, Cys-C is more of a GFR marker instead of a biomarker indicator of primary AKI. So far other factors such as thyroid dysfunction, obesity, use of corticosteroids and inflammation can interfere in its serum levels<sup>31</sup>.

#### **IL-18**

Interleukin-18 (IL-18) is a proinflammatory cytokine constitutively expressed in the interspersed cells of the distal convoluted tubule and the collecting tubule in the healthy human kidney<sup>32</sup>. It is initially synthesized as a signal-free inactive precursor and remains in the intracellular space until it is excised by caspase-1. It is secreted predominantly by monocytes and macrophages<sup>33</sup>. It is an inflammatory mediator produced in response to ischemia of several organs, including the

heart, brain, and kidneys. Urinary levels of IL-18 were significantly higher and had high sensitivity and specificity in detection of Acute Tubular Necrosis (ATN). Urinary IL-8 also, raise in urinary tract infection, Chronic Kidney Disease (CKD) and even normal renal function among some healthy subjects. In this way IL-18 can serve as a marker for proximal tubular damage in ATN<sup>34</sup>. Early increase of IL-18 concentrations in the urine correlates with AKI severity, as well as mortality. Considering IL-18 as a proinflammatory cytokine that plays an important role in sepsis, its concentrations can also be influenced by a number of coexisting variables such as, inflammatory diseases and autoimmune diseases. Serum IL-18 level increases in inflammatory arthritis, inflammatory bowel disease, systemic lupus erythematosus, psoriasis hepatitis, and multiple sclerosis<sup>35</sup>. Urinary IL-18 level is elevated in patients with AKI and delayed graft function compared with normal subjects and patients with pre-renal azotemia, Urinary Tract Infection (UTI), chronic renal insufficiency and nephrotic syndrome<sup>36</sup>.

#### **NAG**

N-Acetyl-β-D-Glucosaminidase N, -Acetyl-β-D-Glucosaminidase (NAG) is a lysosomal enzyme predominantly found in proximal tubules, so increased activity of this enzyme in urine suggests tubular cell injury and, therefore, it can serve as a specific urinary marker of tubular cells damage<sup>37</sup>. Increased activity of urinary N-acetyl-β-D-glucosaminidase (NAG) can serve as an early indicator of damage to the tubular epithelium<sup>38</sup>. The increase in urinary NAG activity indicates damage to the tubular cells, although it may also reflect an increased lysosomal activity without any cell damage. Increased urinary excretion of NAG was reported in acute kidney diseases due to various etiologies induced by toxic agents, after cardiac surgery and after kidney transplantation and diabetic nephropathy, hyperthyroidism and rheumatic diseases<sup>39</sup>.

#### **Calprotectin**

Calprotectin is an immunomodulatory protein, regarded as an inflammatory factor, and has a protective role in oxidative processes of inflammation<sup>40,41</sup>. It is mostly derived from neutrophils and a few amounts are secreted by monocyte and macrophage. Tubular epithelial cells of the collecting system also, secrete in response to inflammation; such as renal tissue injuries. It has also been reported in long-lasting urinary tract obstructions, UTI (secreted from epithelial cells or leukocytes in the urine), rheumatoid arthritis, inflammatory bowel diseases, CKD, and urethra and bladder carcinomas<sup>14,42</sup>. Detecting calprotectin in urine before its detection or its elevation in serum is much more helpful in clinical practice. Urinary calprotectin also differentiates α between pre-renal and intrinsic acute renal allograft failure so, urinary calprotectin is a promising biomarker to differentiate pre-renal and intrinsic acute renal allograft failure<sup>43</sup>.

***$\alpha$ -1 microglobulin and  $\beta$ -2 microglobulin ( $\beta$ 2M)***

Both of these molecules rise in serum and urine in response to glomerular or tubular lesions leading to a considerable reduction in the GFR. Their main advantage is their low cost; despite this it depends on the urinary pH as they are degraded in lower pHs, decreasing their beneficently if the pH is less than 5.5<sup>44</sup>. Increased urinary  $\beta$ 2M excretion has been observed to be an early marker of tubular injury in a number of settings including nephrotoxic substance exposure, cardiac surgery, and renal transplantation, preceding the rise in serum creatinine by as many as 4-5 days<sup>45,46</sup>. Unfortunately, the utility of  $\beta$ 2M as a biomarker has been limited by its instability in urine, with rapid degradation occurring at room temperature and in urine with a pH lower than 6.0<sup>47</sup>. Urinary  $\beta$ 2M is a potential biomarker of tubular injury in renal allografts<sup>48</sup>.  $\alpha$ -1 microglobulin has been found to be a sensitive biomarker for proximal tubular dysfunction even in the early phase of injury when no histologic damage is detectable. Urinary  $\alpha$ -1 has been proposed to be a useful marker of tubular dysfunction even in low-gestational-age preterm infants, a population at higher risk of AKI. A number of other conditions have been associated with altered plasma/serum levels, including liver disease, HIV, and mood disorders and therefore urinary specificity and sensitivity for AKI may be suboptimal in these settings<sup>49</sup>.

***Monocyte chemotactic peptide-1 (MCP-1)***

MCP-1 has been reported as a potent chemokine produced by kidney cells and it acts as a mediator of acute ischemic and toxic kidney injury. The MCP-1 protein and its corresponding mRNA increase in intrarenal lesions in larger amounts than the NGAL. In pre-renal and post-renal injuries NGAL expression of the *MCP-1* gene increases comparatively. In contrast, uremia *per se* induced the *NGAL* gene in the absence of renal injury, but not of the MCP-1, showing better MCP-1 specificity for the AKI diagnosis<sup>20</sup>.

***Vanin-1***

Vanin-1 is an epithelial ectoenzyme with pantotheine activity that responds to oxidative stress and converted of pantotheine to pantothenic acid (vitamin B5) and cysteamine. Yoshida *et al* discovered increased levels of kidney vanin-1 mRNA in rats with ischemia-reperfusion type of lesion<sup>50</sup>. It has been found that elevated urinary concentration of vanin-1 occurs before conventional markers with nephrotoxin-induced lesions. Therefore, it appears that urinary vanin-1 may be a potential biomarker for early detection of AKI<sup>51</sup>.

***Netrin-1***

Netrin-1 is one of the most related kidney injury biomarkers expressed in tubular epithelial cells of normal kidneys. Netrin-1 levels increased 2 hr after extracorporeal circulation and peaked at 6 hr and remained elevated until 48 hr<sup>52</sup>. Significantly higher levels were

found in urine samples from patients with ischemic AKI induced by radiocontrast agents, sepsis and drugs. Therefore, netrin-1 is a urinary biomarker that rises early on for the detection of renal injury and can also serve as a universal biomarker of AKI<sup>53</sup>.

***Alkaline phosphatase and gamma-glutamyl transferase (GGT)***

Alkaline phosphatase is an endogenous metalloenzyme found in the serum and multiple organs, including kidneys, liver, bone and intestines. It has shown efficacy in sepsis-induced AKI with alkaline phosphates<sup>54</sup>. GGT is an enzyme located in the cell membrane found in the proximal renal tubules, liver, pancreas and intestines. Urinary GGT is an indicator of tubular damage as it can express changes in renal function state<sup>55</sup>.

***YKL-40***

YKL-40 is a glycoprotein involve in inflammation, cellular protection and repair. It is synthesized by renal macrophages and contributes to tissue remodeling and scarring by limiting cellular apoptosis; it promotes cellular repair after ischemic renal injury, becoming a good marker of AKI recovery stage<sup>56,57</sup>.

**Conclusion**

Numerous biomarkers have been used for early detection of AKI but unfortunately, none of the biomarkers have been truly specific for AKI. Early detection and intervention in AKI decrease the ongoing damage and reduce the chance of complications and mortality. It seems that due to the etiological diversity of these markers using a panel of biomarkers for diagnosing AKI may be a better strategy than using a single biomarker assay.

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