SOHAG MEDICAL JOURNAL
Vol. 23 No.2 Apr 2019

Renal biomarkers

Mohamed A. Kassem
Department of Pediatrics, Faculty of Medicine, Sohag University, Sohag, Egypt

Abstract

Acute kidney injury is common in critically ill children admitted to intensive care unit. The etiology of acute kidney injury is multifactorial and the incidence varies between 1 and 41% (Krishnamurthy, 2013) probably due to the different definitions used in clinical studies.

Unfortunately, Serum Creatinine (the main acute kidney injury biomarker used in the clinical setting) is a late marker of reduced glomerular filtration rate, which limits ability to detect acute kidney injury early and to initiate clinical therapeutic studies. Therefore, Diagnostic specificity and sensitivity of new biomarkers are currently weighed against creatinine-based criteria (Waikar et al., 2009).

Several proteins and biochemical markers emerged as sensitive and specific biomarkers capable of the early detection of acute tubular injury (Devarajan , 2011). The most famous of these biomarkers is neutrophil gelatinase-associated lipocalin (NGAL).

Key words: Neutrophil gelatinase-associated lipocalin, Acute kidney injury,Creatinine.

INTRODUCTION

Pediatric AKI is largely asymptomatic and often occurs in the wake of other underlying conditions such as cardiac surgical procedures, critical illness, and nephrotoxin use. Making the diagnosis in the estimated 5% of all hospitalized children and ~30% of critically ill neonates and children who suffer from AKI and its consequences currently depends on serial measurements of functional biomarkers such as serum creatinine. This approach is flawed due to several reasons. [Devarajan, 2010][Devarajan, 2013] First, non-renal factors such as age, gender, diet, muscle mass, and medications can influence serum creatinine concentration independent of changes in kidney structure or function. Serum creatinine in a neonate is merely reflective of maternal creatinine for approximately the first 10 days of life. GFR is normally low in infants, and physiological maturation of renal function occurs until 2 years of age. Children with chronic conditions such as congenital heart disease display low muscle mass, resulting in falsely lowered serum creatinine. Second, a previously healthy kidney displays a significant functional reserve, such that >50% of kidney function can be lost due to an acute damaging insult without any change in serum creatinine. This is especially true in the pediatric population, where the lack of chronic comorbid conditions generally results in pristine kidneys with maximal functional reserve. Third, a rise in serum creatinine concentration accompanies any condition that leads to transient renal hypoperfusion. These episodes of "pre-renal azotemia" are common in children with gastrointestinal illness and must be differentiated from the more ominous forms of "intrinsic AKI" with accompanying structural damage. Fourth, there is typically a lag period of hours to days after an acute injurious event before serum creatinine rises. During this time, structural

damage is known to occur to the kidney tubules. Accumulated experimental data have identified several interventions that can prevent and/or treat AKI during this "sub-clinical" phase, before the serum creatinine rises. The paucity of an early biomarker of structural AKI has hampered our ability to translate these promising interventions to human AKI in a timely manner. The search for biomarkers for the early diagnosis of AKI and its outcomes is an area of intense contemporary research that has yielded several promising candidates. A select few that are especially pertinent to pediatric AKI are summarized in the following.

NGAL [Neutrophil gelatinase-associated lipocalin]

The most extensively studied and promising biomarker in pediatric AKI is NGAL. [Ciccia&Devarajan 2017] Preclinical gene expression analyses reported in >150 distinct studies performed in AKI models from several species ranging from rodents to humans have consistently revealed the NGAL gene to be one of the most dramatically upregulated genes in the kidney soon after an ischemic or a nephrotoxic insult.[Supavekin et al2003][Devarajan, 2015]

The NGAL protein is also highly induced in regenerating and recovering kidney tubule cells. [Mishra et al., 2003] NGAL binds iron; chelation of toxic iron is an important mechanism that protects the kidney tubules from worsening injury. Thus, the biologic role of NGAL in AKI is one of enhanced tubule cell proliferation and recovery [Mishra et al., 2004].

The induced NGAL protein is rapidly secreted into both the urine and the plasma in animal models of AKI, and a decade of translational studies in human beings have now established NGAL as a biomarker to predict AKI and its adverse outcomes independent of serum creatinine.

Cardiac surgery-associated acute kidney injury (CS-AKI) is a common cause of AKI in children, with a reported incidence of 20- 60%. [Jefferies &Devarajan 2016] However, the increase in serum creatinine typically occurs only 1–3 days after cardiopulmonary bypass (CPB). As first highlighted by Mishra et al,[Mishra et al2005] a dramatic increase in both urine and plasma NGAL is detected within 2–6 hours of CPB in children destined for AKI, with a predictive area under the receiver operating characteristic curve (AUC) of >0.9. These findings have now been confirmed in >7500 patients, [Haase-Fielitzet al2014] [Zhou et al2016] with measurements obtained within 4–6 hours after initiation of CPB, yielding an overall predictive pooled AUC of 0.86. The predictive performance for CSAKI was similar for both urine and plasma NGAL. Subgroup analyses revealed that NGAL displays the highest predictive accuracy for CS-AKI in children (AUC 0.89 versus 0.83 in adults) and in subjects without pre-existing renal insufficiency (AUC 0.87 versus 0.81 with pre-existing renal insufficiency).

Critical illness, including sepsis, is the most common cause of AKI worldwide, with a reported incidence of 30–50%. The ability of NGAL to predict AKI in this heterogeneous population has been confirmed in >8500 critically ill patients,[Haase-Fielitz et al2014] with measurements obtained within 6 hours of clinical presentation yielding an overall predictive AUC of 0.8.11[Basu et al2014] In a recent meta-analysis28[Zhou et al2016] specifically examining the value of NGAL for the prediction of AKI in patients with sepsis, the specificity of plasma NGAL for predicting AKI was inferior to that

of urine NGAL in sepsis, although the sensitivities and pooled AUCs were similar and promisingly high (0.8–0.9). Contrast-induced acute kidney injury (CI-AKI) is a common cause of AKI, although less common in children compared to adults. However, the increase in serum creatinine occurs only 1–3 days after contrast administration.

As first described by Hirsch et al, [Hirsch et al2007] both urine and plasma NGAL concentrations increase within 2–6 hours of contrast administration, with a predictive AUC of >0.9. A recent metaanalysis30 of 1520 patients has confirmed the high diagnostic accuracy of NGAL in the early detection of CI-AKI, with measurements obtained 2–24 hours after contrast administration yielding a pooled AUC of 0.93.

An increase in serum creatinine occurs in both true structural (intrinsic) AKI and functional volume-responsive pre-renal azotemia or even in chronic kidney disease. It is critical to make these distinctions in the acute setting, since the medical management of each is different and mismanagement is deleterious. [Devarajan, 2014] Nickolas et al [Nickolas et al2008] first showed that measurement of urinary NGAL at the time of initial patient encounter can differentiate those who subsequently develop intrinsic AKI from those who would follow a more benign course of pre-renal azotemia, with an AUC of 0.95. These findings have now been confirmed in studies involving >2000 patients, [Nickolas et al2012] [Soto et al2013] confirming the diagnostic accuracy of NGAL in the early prediction of intrinsic AKI, with AUCs in the 0.81–0.87 range. AKI with structural nephron damage portends a number of adverse outcomes, including worsening severity, increased length of hospital stay, and death. Initial single-center studies of cardiac surgical patients by Bennett et al [Bennett et al2008] and Dent et al [Dent et al2007] identified the correlation of early NGAL measurements in the urine and plasma, respectively, with severity and duration of AKI, length of stay, dialysis requirement, and death. In a meta-analysis of 2000 patients with predominantly cardiorenal syndrome, [Haaseet al2009] early NGAL measurements predicted dialysis and death with a pooled AUC of 0.78 and 0.75, respectively.

NGAL identifies structural kidney injury in the absence of an increase in serum creatinine. In a multicenter pooled analysis of 10 prospective studies involving >2300 patients with predominantly cardiorenal syndrome, ~ 20% of patients displayed increased NGAL concentrations but no increase in serum creatinine [Haaseet al2011]. This previously undetectable condition (termed subclinical AKI) was associated with an almost threefold increased risk of mortality or dialysis requirement and a doubling of median length of hospital stay. This “added value” of NGAL measurements for the prediction of AKI and its adverse consequences over and above clinical and functional scores has now been repeatedly demonstrated in several large studies involving cardiac surgical patients.[Krawczeski et al2011][Parikh et al2011]

NGAL is protease resistant and remarkably stable in urine and blood. Short-term storage of samples at 4°C for up to 24 hours and long-term storage at -80°C for up to 5 years result in no clinically significant loss in the NGAL signal. [Schuhet al2016] There are currently three clinical analytic platforms for NGAL measurement in patient samples, with results available within 15–30 minutes. These include a point-of-care immunoassay for plasma NGAL (Alere Triage® NGAL test), a

urine immunoassay developed for an exclusive platform (ARCHITECT; Abbott Diagnostics), and a particle-enhanced turbidimetric immunoassay for urine and plasma NGAL that can be run on a large variety of standard automated clinical chemistry analyzers (NGAL TestTM; BioPorto Diagnostics). All of these three tests are CE (ConformitéEuropéene [European Conformity]) marked and launched for clinical diagnostic use worldwide, but are currently pending US Food and Drug Administration (FDA) approval for diagnostic use in the USA.

Kidney injury molecule-1 (KIM-1)

Preclinical studies have also identified the KIM-1 gene to be induced in the proximal tubule cells of ischemic rat kidneys. [Ciccia&Devarajan 2017] In experimental AKI, KIM-1 protein regulates phagocytosis of damaged cells and thereby limits injury.[Yang et al2015] An extracellular domain of KIM-1 can be detected by enzyme linked immunosorbent assays and is useful as a urinary biomarker in patients with AKI. In a study of 40 children undergoing CPB, urinary KIM-1 levels were markedly increased in the children who developed AKI, with an AUC of 0.83 at 12 hours post-CPB for predicting AKI, indicating that it is a delayed biomarker of AKI compared to NGAL.[Han et al2008] Subsequent pediatric single center studies have yielded conflicting results. A prospective multi-center study of 311 children undergoing cardiac surgery confirmed the delay in upregulation of urinary KIM-1 in AKI patients and showed that KIM-1 was not significantly associated with AKI after adjusting for other injury biomarkers. [Parikh et al2013] Elucidation of the role of KIM-1 as an AKI biomarker awaits additional confirmatory studies. No clinical laboratory assays for urine KIM-1 are currently available.

Interleukin-18

Animal studies have shown that IL-18 is induced in then proximal tubule and detectable in the urine following ischemic AKI. [Ciccia&Devarajan 2017] IL-18 represents a pro-inflammatory cytokine that might worsen the degree of AKI. In children undergoing CPB, urinary IL-18 is an early, predictive biomarker, with levels increased at 6-hour post-CPB and an AUC of 0.75 for predicting AKI.[Parikh et al2006] Subsequent pediatric studies have confirmed that urine IL-18 obtained 6- to 12-hour post-CPB moderately predicts AKI, with AUCs in the 0.72–0.82 range.[Krawczeski et al2011] Clinical assays for IL-18 measurement are not available at the present time. Liver-type fatty acid-binding protein (L-FABP) L-FABP is an anti-oxidant, renoprotective molecule induced in the proximal tubule early after experimental AKI. In children undergoing CPB, urinary L-FABP increases within 4 hours of initiating CPB in those destined for AKI, with a predictive AUC of 0.81.47 Follow-up single-center studies in children [Parikh et al2011] indicated that urine L-FABP levels obtained 6 hours post-CPB are predictive of AKI, with AUCs in the 0.75–0.78 range. However, a prospective multi-center study of 311 children undergoing cardiac surgery indicated that the increase in L-FABP was not significantly associated with AKI after adjusting for other injury biomarkers.[Parikh et al2013] Confirmation of the role of L-FABP as a potential AKI biomarker awaits additional confirmatory studies, and no clinical laboratory assays are currently available.

Markers of cell-cycle arrest

Targeted proteomic approached have identified markers of cell-cycle arrest,

such as tissue inhibitor of metalloproteinases- 2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7), to be induced in tubule cells after AKI. [Ciccia&Devarajan 2017]

It is postulated that the resultant cell-cycle arrest limits proliferation of damaged tubule cells. The product of these TIMP-2 and IGFBP7 can be measured in the urine using a point-of-care kit that has been approved by the FDA. In a small study of children undergoing CPB, the urinary TIMP-1/ IGFBP7 product was increased at 4 hours post-CPB in children who developed AKI, with an AUC of 0.85 for predicting AKI. [Portilla et al2008] The corresponding AUC for urinary NGAL was reported to be comparably good at 0.87. Although promising, additional studies are required to elucidate cell-cycle markers as AKI biomarkers in children.

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