Urinary biomarkers and acute kidney injury in children: the long road to clinical application

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Received: 9 February 2013 / Revised: 11 February 2013 / Accepted: 26 February 2013 / Published online: 19 March 2013
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Abstract Pediatric acute kidney injury is rising with the advances in technology available for children with chronic conditions or those who are critically ill. Serum creatinine and urine output, traditional markers of renal function, often allow only delayed and unreliable diagnosis of acute kidney injury. Biomarker development in pediatric patients with low disease prevalence is challenging (small cohorts, few analyzable events). In this issue of *Pediatric Nephrology*, Ivanisevic and colleagues report that urinary liver-type fatty-acid-binding protein (L-FABP) can be used for early identification of pediatric acute kidney injury in a small cohort undergoing cardiac surgery. Addition of the biomarker resulted in an improvement in early diagnosis compared with a clinical model (age, gender, body weight, cardiopulmonary bypass duration, and aortic clamp time). It is noteworthy that the preoperative clinical model performed excellently in predicting postsurgery pediatric acute kidney injury. Further work is needed before this or other novel biomarkers (alone or in combination) can be implemented in clinical practice. Large-scale observational studies are needed to test these biomarkers against hard clinical endpoints, independent of serial measurements of serum creatinine concentrations. Prospective randomized interventional trials using exclusively high biomarker levels to define acute kidney injury should demonstrate improved clinical outcomes.

Keywords Acute kidney injury · Biomarkers · Urinary L-FABP · Children

Introduction

Acute kidney injury (AKI) in pediatric patients is characterized clinically by rapid loss of glomerular filtration rate (GFR), resulting in a failure to excrete end products of nitrogen metabolism and to maintain fluid volume and electrolyte and acid-base homeostasis. Pediatric AKI (pAKI) is a heterogeneous disorder with various clinical presentations and an unpredictable outcome. In industrialized countries, the incidence of AKI in hospitalized children is rising, and the etiology of pAKI is dramatically changing from isolated acute renal disease to multiple organ failure. Using pediatric-modified Risk, Injury, Failure, Loss, End-Stage Kidney Disease (pRIFLE) criteria, the incidence of AKI varies between 30 % and 50 % in children undergoing cardiac surgery for congenital heart disease and may jump to extreme rates (up to 82 %) in critically ill children with multiple organ failure. Pediatric AKI is associated with greater length of hospital stay, unacceptably high in-hospital mortality rates, reduced quality of life, increased risk of incident and progressive kidney disease, and higher long-term mortality rates. The excessive morbidity of AKI in pediatric patients is related predominantly to the severity of the underlying disease. However, pAKI is no longer considered an innocent bystander merely reflecting coexisting pathologies; it is an independent risk factor for death by distant organ effects of AKI-induced inflammatory mediators, even at early stages of pAKI [1–5].

Current diagnostic markers of acute renal injury

Traditionally serial measurements of serum creatinine, estimated creatinine clearance (Schwartz formula), and urine

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output have been used as the primary test to diagnose pAKI. The differential diagnosis among the causes of pAKI relies on urinalysis (urine microscopy and fractional excretion rates of sodium and urea). These parameters are, however, not without limitations, as they are neither sensitive nor specific. They tend to represent functional change rather than being true markers of kidney injury. Serum creatinine is affected by several nonrenal factors, takes 24–48 h to rise after the initial renal insult, and cannot be used to distinguish between hemodynamically mediated changes in renal function (prerenal azotemia) as opposed to intrinsic renal failure or obstructive uropathy. Thus, recognizing pAKI is often delayed, and small increases in serum creatinine may be considered as fluctuations remaining within the normal range. Moreover, the high serum creatinine level of maternal origin at birth and its decline during the first weeks of life greatly limit creatinine as a marker of GFR in infants [6]. A possible barrier to improving clinical outcomes in critically ill pediatric patients with AKI might be potentially beneficial therapies that are not being given to these patients quickly enough, especially in light of the unreliability of serum creatinine as an early marker of GFR in acutely sick children.

Novel biomarkers of acute kidney injury in pediatric patients

Extensive research efforts over the last decade have been directed at the discovery and validation of numerous (>28) novel AKI biomarkers [7]. Most of these protein-bound biomarkers are indicators of structural renal damage rather than of decreased kidney function. An ideal AKI biomarker should be accurate, reliable, easy to measure with a standard assay, noninvasive, reproducible and sensitive, and specific with defined cutoff values. Of great importance, novel biomarkers of AKI must provide additional information that is not surmised from clinical evaluation and standard laboratory tests [8]. Urine represents an ideal body fluid for AKI biomarker assessment, as it can be obtained noninvasively and repeatedly from a spontaneously voided urine sample or from an indwelling bladder catheter. Urinary biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL), kidney-injury molecule-1 (KIM-1), interleukin-18 (I-18), and urinary liver-type fatty-acid-binding protein (L-FABP) show promise in both diagnostic and prognostic utility in adult patients with AKI arising from various causes. Utilizing the area under the receiver-operating characteristics curves (AUC-ROC) to assess the performance of these biomarkers, values >0.75 indicate good discrimination and those >0.9 excellent discrimination. However, differences in factors, such as etiology, pathogenesis, and treatment of pAKI; preexisting comorbidities; concomitant multiorgan failure; and patient age-related and size characteristics,

may prevent extrapolation from biomarker data obtained in adults [9]. Research in the field of pAKI may be difficult due to ethical considerations and low disease prevalence, which leads to low sample sizes. However, the group of children with AKI may be more suitable for biomarker development compared with adult AKI patients, as they usually do not have significant comorbid disorders, such as preexisting chronic kidney disease (CKD) or hypertension, atherosclerosis, or diabetes, which all affect kidney function. Furthermore, pediatric patients with congenital heart disease undergoing cardiac surgery have a high prevalence of well-defined renal insults and can be studied prospectively for the development of AKI [10].

Urinary liver-type fatty-acid binding protein

Urinary L-FABP is a small (14 kDa), highly conserved cytoplasmic protein that plays a key role in cellular lipid metabolism. Initially identified in hepatocytes, it is a protein that is strongly expressed in the renal proximal tubule where it mediates transport of long-chain fatty acids to the mitochondria or peroxisomes for β -oxidation. Due to its small size, it can easily leak out of necrotic cells after renal ischemia or nephrotoxic insults, leading to a rapid rise in urinary levels. Preclinical investigations demonstrate that urinary levels of L-FABP correlate histologically with the degree of renal damage [11]; its potential as a biomarker has been examined predominantly in adult patients with AKI but rarely in pediatric patients.

In this issue of *Pediatric Nephrology*, Ivanisevic and colleagues report that urinary L-FABP can be used for early identification of AKI after pediatric cardiac surgery [12]. Twenty-seven children (median age 360 days) without preexisting CKD or other major comorbidities undergoing cardiopulmonary bypass (CPB) procedures were enrolled. AKI was defined as a 50 % increase in serum creatinine concentrations within 48 h after surgery. AKI developed in 11 patients (41 % of the cohort). Mean serum creatinine change was 97 %; three AKI patients needed dialysis. Patients with transient AKI (lasting <24 h) were excluded. The 16 control patients (non-AKI group) showed a mean serum creatinine change of 14 %. Patients in both groups did not differ in age, gender, or body weight. However, children with AKI had significantly longer CPB and aorta clamping times due to much more serious and complex cardiac surgery [high Risk Adjustment in Congenital Heart Surgery (RACHS-1) scores] than children who did not develop AKI. There was one death in each patient group. Urinary L-FABP concentration was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit prior to and 2, 6, 24, and 48 h after surgery. Urinary L-FABP was normalized to urinary creatinine concentration at corresponding time points. The corrected urinary L-FABP levels were significantly

higher in the AKI group than in the non-AKI group at all time points. Peak urinary L-FABP was measured at 2 h. The AUC-ROCs were 0.867 for the 2-h and 0.867 for the 6-h urinary L-FABP level, indicating that both biomarker values were good predictors for early AKI. However, positive (PPV) and negative (NPV) predictive values were not calculated. Cutoff concentrations for AKI detection were 450 $\mu\text{g/g}$ creatinine urinary L-FABP at 2 h (sensitivity and specificity 0.500 and 0.867, respectively) and 250 $\mu\text{g/g}$ creatinine urinary L-FABP at 6 h (sensitivity and specificity 0.667 and 0.900, respectively). Addition of the biomarker resulted in a further improvement in early AKI diagnosis over a clinical model including age, gender, body weight, CPB duration, and aortic clamp time at 6 h (from 0.938 to 0.989). Of great importance, this clinical model performed excellently in predicting postoperative pAKI. Finally, urinary L-FABP correlated significantly with length of in-hospital stay.

The prospective case–control study by Ivanisevic et al. [12] corroborates and extends the pilot study by Portilla et al. [13], demonstrating for the first time that urinary excretion of L-FABP in pediatric patients undergoing cardiac surgery is significantly increased within the first hours and precedes the rise of serum creatinine not seen until 24–72 h after cardiac surgery. Forty children with congenital heart disease were enrolled in their analysis, and AKI was defined as a serum creatinine rise of 50 % over baseline levels over a 72-h period. AKI (change in mean serum creatinine 190 %) occurred in 21 patients who did not have preexisting renal insufficiency, comorbid disorders, or concomitant nephrotoxic drug use. None of these patients had AKI severe enough to initiate renal replacement therapy (RRT) or that they died. Compared with patients without AKI, the AKI group tended to be younger (2.7 vs 4.3 years) and had significantly longer CPB times. Levels of serum and urinary L-FABP were determined using ELISA kits before and 4 and 24 h after cardiac surgery and factored for urinary creatinine excretion. Urinary L-FABP was increased about 94- and 45-fold at 4 and 12 h, respectively, after cardiac surgery. Urinary biomarker concentration did not rise significantly in children who did not develop AKI. The area under the urinary L-FABP ROC curve was 0.81 at 4 h postsurgery. A cutoff value of 486 ng/mg creatinine yielded a sensitivity of 0.714 and a specificity of 0.684. Univariate logistic regression analysis showed that both bypass time and urinary L-FABP concentration at 4 h were significant independent risk indicators for AKI. Measurements of L-FABP in serum samples of a subgroup of the cohort (eight patients of each group) showed that there was a significant increase in serum L-FABP at 12 h post cardiac surgery in children with but not those without AKI. These findings suggest that increased urinary L-FABP levels at 4-h post cardiac surgery in pAKI patients represented an increased

shedding of L-FABP proximal tubular cells rather than just reflecting increased glomerular filtration of high serum L-FABP concentrations. Finally, Krawczeski et al. [14] investigated the temporal pattern and predictive value (alone or in combination) of four urinary biomarkers (NGAL, IL-18, L-FABP, and KIM-1) for cardiac-surgery-associated pAKI. Three hundred and ninety pediatric patients with cardiac diseases necessitating cardiopulmonary bypass surgery were enrolled; 220 (56 %) were analyzed. None of these patients had severe preexisting kidney disease. AKI was defined as an at least 50 % increase in serum creatinine concentration from baseline within 48 h after CPB. AKI occurred in 27 % of patients in the cohort. None of the patients had AKI requiring RRT, and one of the AKI patients died. Urine NGAL significantly increased in AKI patients at 2 h after CPB initiation; IL-18 and L-FABP increased at 6 h, and KIM-1 increased at 12 h. Addition of the 2-h NGAL value to the clinical model increased the AUC from 0.72 to 0.91. At 6 h, NGAL, IL-18 and L-FABP each improved the AUC, from 0.72 to 0.91, 0.84, and 0.77, respectively. The added predictive ability of the biomarkers was supported by net reclassification improvement and integrated discrimination improvement. Biomarker combinations further improved AKI prediction. The authors concluded that these biomarkers, particularly in combination, may help establish AKI timing.

Unsolved problems of biomarkers in pediatric patients

The ultimate goals of the ideal AKI marker are early AKI detection, differential diagnosis of AKI, prognosis, response to therapy, and recovery of renal function. However, the heterogeneity of AKI causes and patient cohorts make it unlikely that a single biomarker will achieve these aims. Despite differences in the age of cardiac patients, pAKI severity, optimal time point for urine collection, and definition of the cutoff concentration of the biomarker, published data—however sparse—indicate that urinary L-FABP levels represent a sensitive and predictive early biomarker of pAKI after cardiac surgery. The prognostic potential of this novel biomarker remains unknown, because the number of patients with pAKI requiring dialysis or dying was extremely low. Ivanisevic et al. [12] surprisingly found comparable urinary L-FABP excretion rates in the non-AKI patient group and in the group of patients with transient AKI (defined by resolution within 24 h). If these data are reliable, there are serious doubts about the usefulness of urinary L-FABP levels in the differential diagnosis of intrinsic pAKI and prerenal azotemia. A recent systematic review of studies assessing the performance of urinary L-FABP in patients found that only seven cohorts (six adult AKI, one pAKI, predominantly after cardiac surgery) focused on diagnosis, four reports on dialysis requirements [adult intensive care

unit (ICU) patients], and five studies on mortality (critically ill adult patients) [15]. Within the limitations of this meta-analysis (paucity and low quality of data, different clinical settings, and variable definitions of AKI), the authors concluded that urinary L-FABP may be a promising biomarker for early detection of AKI and prediction of in-hospital death but not of dialysis requirement.

Available studies reporting on the performance of urinary L-FABP in pediatric or adult patients have highlighted a number of limitations of this and other biomarkers for early detection and prediction of AKI, precluding its clinical application in various clinical settings. Most pediatric studies (all reports of urinary L-FABP) come from single-center studies and homogeneous patient populations (postsurgery AKI). The single-center studies of Mishra et al. [16] and Bennett et al. [17] showed excellent performance for NGAL under ideal circumstances (children without comorbidities after CPB surgery). However, the first multicenter prospective study analyzing the use of a novel biomarker to diagnose post-bypass-associated severe AKI in 311 children found that urinary NGAL did not perform as well in this study as in previous single-center reports [18]. The reasons for this discrepancy are probably multifactorial. Due to the nature of single-center studies, patient characteristics, management of the study population, sample selection, storage, and testing are more likely to be more homogeneous than would occur in a multicenter trial. Results on biomarker performance are less robust when we search for validation in heterogenic adult populations. The performance of urinary or plasma biomarkers in pAKI is affected by the severity of acute illness precipitating pAKI. Septic pediatric patients have higher urinary NGAL and IL-18 levels than nonseptic patients, indicating that the association of these biomarkers with pAKI in septic patients might reflect more the association between severity of underlying disease and AKI rather than true renal damage [19–21]. There is published work suggesting that circulating L-FABP is a marker of liver injury in critically ill adult patients with sepsis [22] and in children with post-cardiac-surgery AKI [13]. Second, most pediatric cases do not have severe kidney injuries, based on the rapid reversibility of serum creatinine and the small number of pAKI patients requiring RRT. Most existing studies have been insufficiently powered to establish a cutoff value predictive for pAKI, especially with severe injuries. Studies evaluating urinary L-FABP report a wide range of cutoffs used in pediatric and adult patients. We do not know how to formally define at what point in time urinary L-FABP should be measured for early detection of AKI or prediction of pAKI prognosis [15]. Optimal timing of biomarker measurements remains unknown, and many studies use serial measurements through the early postoperative phase (up to 12–24 h) to define the typical pattern of biomarker increase and decrease. However, such

repeated measurements of single or multiple biomarkers are expensive in comparison with the daily measurements of serum creatinine. A recently published study evaluated the reference values for urinary NGAL in healthy children (age 1 day–248 months) and found that the distribution of urinary NGAL values in pediatric patients approximated a log-normal distribution, with values higher in neonates than in children [23]. Most studies report higher values for biomarkers in patients with AKI but with a substantial overlap between AKI and non-AKI patients, hampering discrimination in individual cases. Only the study by Portilla and colleagues reported true positive (38 %), false positive (15 %), false negative (15 %), and true negative values (32 %) for urinary L-FABP tests in pediatric patients [13]. However, the percentage of false positive or false negative tests ranged from 4 % to 51 % and 0 % to 14 %, respectively, in adult patients with post-cardiac-surgery AKI. Third, most studies assessing pAKI excluded patients with CKD. Plasma NGAL levels are also increased in CKD [24], and urinary L-FABP level has shown some value in predicting CKD progression [25], blurring the differential diagnosis between CKD and AKI. Moreover, baseline serum creatinine concentration of a given critically ill patient is not always available, and it is unclear whether an increased serum creatinine at ICU admission is due to AKI or CKD. The major challenge in the emergency room is to distinguish fluid-responsive from non-fluid-responsive AKI. Discrimination of these conditions using current biomarkers remains largely impossible in the individual patient [26]. Finally, a simple (and the most commonly reported) way to determine whether and to what extent a novel biomarker adds to the predictive power of the clinical model is to compare whether the AUC from the predictive model including the biomarker is significantly higher than the AUC of the clinical model alone. However, it has been shown that evaluating improvements in AUC is not very sensitive. The net reclassification improvement (NRI) is a statistical method specifically aimed at addressing this added value. However, this method is used only in a few analyses.

Road to clinical application

Even promising urinary biomarkers for early detection of pAKI and prediction of worse events must clear major hurdles on the road to clinical application. The diagnostic and prognostic characteristics of novel biomarkers are generally compared against serum-creatinine-based measurements of pAKI as the existing gold standard. Undisputedly, a serum-creatinine-based diagnosis of pAKI is in itself imperfect, potentially contributing to apparent limitations of novel biomarkers and necessitating other means of evaluating the accuracy of a new test. In fact, a new biomarker with perfect sensitivity and specificity when

compared with serum creatinine would simply replicate inaccuracies of serum creatinine, although perhaps a little earlier. Moreover, technical issues involving measurement and interpretation of novel biomarkers are unsettled, including whether urine biomarker levels should be corrected or normalized for urine creatinine excretion to account for fluctuations of urine concentrations. Even if one accepts the need for correction of urinary biomarker levels, such normalizations assume that a constant amount of urine creatinine is excreted ensconced in varying quantities of urine volume. More information must be gathered regarding the accuracy of biomarker measurements in the presence of interfering disorders (systemic inflammation, acute severe infection, malignancy, CKD, or liver cirrhosis) and stability of the various biomarkers for measurements in routine clinical storage, including a number of freeze–thaw cycles.

There is emerging evidence, at least in pediatric postcardiac-surgery AKI, that perioperative AKI risk assessment may improve our ability to detect, treat, and improve outcome of pediatric patients with postoperative AKI. Combining preoperative patient characteristics (age, previous heart surgery, GFR, proteinuria, measures of cardiac dysfunction) with intraoperative surgical factors (CPB time, duration of aortic clamping, complexity of cardiac surgery), and postoperative renal features (oliguria) can help the clinician to predict AKI, its severity, and its risk of mortality. In the Translational Research Investigating Biomarker Endpoints in AKI (TRIBE-AKI) multicenter cardiac study, a clinical risk model that included age, gender, surgical severity score and cardiopulmonary bypass time, preoperative estimated GFR percentile, and study site, predicted the development of postoperative AKI with an AUC=0.77 and predicted Acute Kidney Injury Network (AKIN) stage 2 or worse with an AUC=0.85. This level of prediction is consistent with many AUCs found in the evaluation of novel biomarkers in children. Thus, it is undisputed that clinical information is useful [27]. It is worth noting that preoperative clinical risk assessment was good to excellent in studies testing the performance of urinary L-FABP in pediatric patients with post-cardiac-surgery AKI [12–14]. Importantly, addition of biomarkers resulted in only modest improvement (net reclassification improvement at 2 h: IL-18 +14 %, L-FABP +17 %, and KIM-1 –3 %), except for NGAL (+87 %), in early AKI diagnosis over the clinical model (age, CPB time) [14]. Whether a combination of biomarkers is best used or perhaps is optimal as part of a larger risk-scoring system requires further investigation. Putting too much emphasis on performance of urinary biomarkers may distract from thorough clinical judgment.

Finally, biomarkers are not an end in themselves but, rather, a means to an end. Avoiding AKI remains the cornerstone of management. Currently, all interventions to reduce intrinsic AKI severity or hasten resolution of intrinsic

AKI have generally been disappointing. It is critical to know the timing of the renal insult. However, a significant proportion of cases admitted to the ICU already have raised serum creatinine levels. The mainstay of conservative treatment is RRT. There are no prospective randomized trials demonstrating that early initiation of RRT results in lower morbidity and mortality rates.

We are in an unprecedented era for nephrologists/intensivists to evaluate pediatric patients with AKI. At present, novel biomarkers such as NGAL, KIM-1, IL-18, and L-FABP remain experimental. It is highly likely that other novel biomarkers alone or in combination will be introduced over the next few years [28]. Further work is needed before such promising novel biomarkers can be implemented in clinical practice. Large-scale observational studies are vital to test these biomarkers against hard clinical endpoints independent of serial measurements of serum creatinine concentrations. In addition, prospective randomized intervention trials using high levels of biomarkers to define AKI should demonstrate improved clinical outcomes.

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