Review Article

Beyond Early Diagnosis: Prognostic Biomarkers for Monitoring Acute Kidney Injury

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The prognosis of acute kidney injury (AKI) is often adverse but varies markedly, underlying the need in clinical decision-making for biomarkers that, individually, stratify risk. At present, only a few biomarkers of AKI have the limited dual function of diagnostic and prognostic stratification, one of which is urine neutrophil gelatinase-associated lipocalin. Currently, the most feasible approach to clinically managing patients with AKI is to combine the predictive functions of several markers in order to diagnose AKI and predict short-term prognosis. Biomarkers for assessing the long-term prognosis of AKI remain to be identified. [*Hong Kong J Nephrol* 2010;12(2):45–9]

Key words: acute kidney injury, biomarker, prognosis

不同類型的急性腎損傷預後各異。只有對急性腎損傷患者的不良預後進行個體化危險分層才能真正的指導臨床決策。除了尿 NGAL，目前的生物學標誌物多數不同時具有診斷+預後分層的雙重功能，聯合檢測是目前可行的替代方法。預測急性腎損傷遠期預後的生物學標誌物還是空白。

Introduction

Acute kidney injury (AKI) is a common clinical syndrome that has been the focus of several recent studies. It is important in terms of hospital mortality, renal replacement rates, cost and duration of hospitalization, and mortality in patients with end-stage renal disease. Accordingly, current research is aimed at identifying means to reduce both the incidence of AKI (primary prevention) and its frequently adverse prognosis in patients with the disease (secondary prevention). The results of animal studies indicate that AKI progression is reversible by appropriate interventions implemented in the early stages of the disease [1,2]. This finding has prompted interest in identifying diagnostic biomarkers for early AKI. Serum cystatin C and urine neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule (KIM)-1, and interleukin (IL)-18, among others, have been shown to predict early AKI.

The pathogenesis of AKI is complex and its prognosis varies markedly depending on the underlying cause. For example, there are several critical diseases associated with AKI, and the degree of increase in serum creatinine levels has been related to serious adverse events, including high hospital mortality rates and dialysis dependence. The BEST study reported that 13.8% of surviving AKI patients still required dialysis after they were discharged from the intensive care unit (ICU), while patients with fewer complications had less impairment of renal function and a better prognosis [3]. In a study of 2,672 patients who had received coronary bypass surgery, the incidence of AKI was 8%, and only 8.7% of these patients required dialysis (based on the diagnostic standard for AKI of serum creatinine levels > 1 mg/dL over baseline) [4]. Thus, it is obvious that not all AKI patients require the same kind and intensity of treatment, and effective clinical decision-making accordingly requires stratification of AKI's adverse prognosis. In addition, despite the reversible nature of clinical AKI, defined as the restoration of normal serum creatinine, persistent injury remains a possibility, carrying with it long-term adverse outcomes [5]. Studies have shown that tubular injury, renal hemodynamics, and inflammation/oxidative stress are all involved in AKI and in subsequent renal repair processes [6]. Therefore, the identification of biomarkers reflecting the characteristics of these pathophysiological stages would provide prognostic information for AKI patients. This review describes our current understanding of urinary

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biomarkers that may predict the prognosis of patients with AKI.

Biomarkers of Short-term Prognosis

NGAL

NGAL is a classic biomarker for both the early diagnosis and short-term outcome of AKI. Although the relationship between NGAL and the course of AKI is not well understood, the protein is known to play a role in promoting growth and differentiation in renal repair processes [7,8] and in the response to focal renal oxidative stress [9]. Both serum and urinary NGAL serve as reliable biomarkers in the detection of AKI, whereas only urinary NGAL is able to predict the need for renal replacement in children with diarrhea-associated hemolytic uremic syndrome [10]. However, in children undergoing cardiac surgery, plasma NGAL levels strongly correlate with the duration and severity of AKI and with length of hospital stay [11]. It should be noted that NGAL assay may be influenced by several coexisting conditions, such as chronic disease, hypertension, systemic infections, and inflammation. While the resulting disturbances in plasma NGAL levels are small compared to the effect of AKI on the protein itself, they are sufficient to exclude heterogeneous patient populations from AKI biomarker studies. Thus, NGAL was evaluated to be a prognostic biomarker in homogeneous patient populations with strict inclusion criteria, mostly in the pediatric population (Table [12–14]). Therefore, currently, the utility of NGAL in predicting the outcomes of patients with AKI is mixed and depends on the clinical setting.

Cystatin C

The cysteine protease inhibitor cystatin C is produced by nearly all human cells and released into the bloodstream. Due to its low molecular mass (13 kDa), the protein is freely filtered by the renal glomerulus and then metabolized by the proximal tubule. Increased levels of serum cystatin C indicate impaired renal function in AKI patients and, together with serum creatinine, are associated with a greater likelihood of renal replacement therapy and death [15]. In nonoliguric acute tubular necrosis (ATN), increased urinary excretion of cystatin C may predict the requirement for renal replacement therapy [16]. However, the prognostic value of cystatin C in serum is controversial. In the ICU, increases in serum cystatin C levels ≥50% are moderately predictive of the need for renal replacement therapy [17], while another ICU study showed weak predictive power of serum cystatin C with respect to hospital mortality [18].

KIM-1

KIM-1 is a type 1 transmembrane protein whose expression is markedly upregulated in the proximal tubule in the AKI rat model. Urinary KIM-1 levels are significantly higher in patients with ischemic ATN than in patients without ATN. In a multivariate model, a one-unit increase in normalized KIM-1 was associated with a greater than 12-fold risk for the presence of ATN [19]. In hospitalized patients with AKI, urinary KIM-1 can predict adverse clinical outcomes, including dialysis requirement and hospital death [20].

IL-18

The recruitment of neutrophil infiltration by IL-18 accounts for the cytokine's deleterious role in an AKI model in ischemic mice [21]. Urinary IL-18 levels have been reported to predict mortality in ICU patients [22]. In a matched case-control study involving patients who underwent cardiopulmonary bypass (CPB) surgery, urinary IL-18 levels were independently associated with AKI duration [14]. However, in studies of unmatched design, urinary IL-18 levels did not aid in the diagnosis of AKI in adult patients following CPB surgery [23,24]. This discrepancy between the diagnostic and prognostic ability of urinary IL-18 was also observed in a critically ill adult population, in which urine IL-18 did not predict AKI development in some settings but was predictive of poor clinical outcome [25].

Novel Candidate Prognostic Biomarkers

Liver fatty acid binding protein (L-FABP) is an intercellular lipid chaperone protein that selectively combines with unsaturated free fatty acids and lipid peroxides

Table. Neutrophil gelatinase-associated lipocalin as a prognostic biomarker in short-term prognosis of acute kidney injury in children

Inclusion criteria	Exclusion criteria	Definition of short-term outcomes	Reference
Children with CPB	Nephrotoxin use	AKI duration; AKI severity; hospital stay; RRT; death	12
Children in ICU with mechanical ventilation and bladder catheterization	RRT	AKI duration	13
Children with CPB	Renal insufficiency, diabetes, PVD, and nephrotoxin use	AKI duration	14

CPB = cardiopulmonary bypass; AKI = acute kidney injury; RRT = renal replacement therapy; ICU = intensive care unit; PVD = peripheral vascular disease.

under conditions of hypoxia. According to one report, both urine and serum L-FABP levels are elevated after cardiac surgery. The former was shown to increase earlier than the latter, suggesting that L-FABP is produced locally in the kidneys [26]. A cross-sectional study examining urine L-FABP levels in 92 AKI patients and 68 controls (including cardiovascular and ICU patients and volunteers) found that the diagnostic value of urine L-FABP for AKI is similar to that of NGAL, KIM-1 and N-acetyl-β-(D)-glucosaminidase, but greater than that of urine IL-18. Urine L-FABP levels could predict both the need for renal replacement and the combined endpoint of renal replacement and death [27].

Netrin-1 was shown to be involved in renal tubular epithelial cell proliferation and migration in a renal ischemia-reperfusion model [28]. The secretion of urine netrin-1 peaks 6 hours after CPB surgery. After 2, 6 and 12 hours, the AUC of urine netrin-1 in the diagnosis of AKI is 0.74, 0.86 and 0.89, respectively. Six hours postoperatively, urine netrin-1 levels reportedly correlate with the severity of AKI and the length of hospitalization [29]. That study, however, consisted of only 26 AKI patients and 34 controls and did not assess the clinical value of urine netrin-1 in AKI risk stratification.

Hypoxia-inducible factor-α (HIF-α) is a key cellular transcription factor that, as its name suggests, responds to tissue hypoxia. It plays an important role in the development of AKI and in recovery from the disease. Vascular endothelial growth factor-A and angiopoietin-2 are important factors expressed downstream of HIF-α. Different HIF-α isoforms have been related to different adverse prognoses of AKI, including dialysis, inhospital mortality, and mechanical ventilation. In addition, angiopoietin-2 was shown to positively correlate with inhospital mortality, the combined endpoint of dialysis and inhospital mortality, and the combined endpoint of mechanical ventilation, dialysis and inhospital mortality [30]. Given that HIF-α levels do not represent the extent of injury, downstream factors may better reflect the prognosis of patients with AKI. In addition, the relationship between HIF-α, vascular endothelial growth factor-A and angiopoietin-2 has yet to be adequately explained [30]; therefore, it remains unclear as to whether the HIF-α system can be used as a biomarker with prognostic predictive power.

Advanced oxidation protein products (AOPP) are dityrosine-containing protein cross-linking products that have been used as indicators of free iron and uremia-related inflammation/oxidative stress [31,32]. AOPP have been shown to accelerate the progression of renal disease [33]. Among ICU patients, AOPP levels in patients with severe AKI (RIFLE level F) are higher than in patients with less severe forms of the disease (RIFLE levels R and I), but AOPP levels cannot be used to identify patients suffering from AKI [34] and is of little prognostic value.

Evaluating the Long-term Prognosis: New Challenges

According to the 2004 Acute Dialysis Quality Initiative, renal function following AKI is evaluated based on the serum creatinine value. The second Acute Dialysis Quality Initiative conference recommended a follow-up period of 60–90 days in the assessment of patients with AKI [6]. Consistent with this "acute injury" definition, several short-term predictors based on serum creatinine value have been used as endpoints in a number of studies of AKI biomarkers and disease prognosis, including the RIFLE or AKIN classification, renal function recovery time, inhospital mortality, renal replacement therapy, and length of hospitalization. The problem remains that serum creatinine levels do not reflect the subclinical pathological changes that occur in the kidneys during AKI. Animal studies suggest that renal function does not recover completely after AKI and that sodium metabolic disorders, hypertension, and subsequent renal disease may ensue [35]. Epidemiological data have shown that even if serum creatinine levels return to normal after injury, the long-term risk of chronic renal disease, renal failure and death is still higher in AKI patients than in those without AKI [5,36]. Therefore, viable prognostic biomarkers should also include indicators of chronic renal disease secondary to AKI. For example, albuminuria is a traditional indicator of impaired glomerular filtration function, which in turn is a recognized marker of progressive chronic renal disease. In recent years, some researchers have concluded that albuminuria is the result of renal tubular injury [37,38]. Since tubulointerstitial damage is the primary type of damage associated with AKI, albuminuria may be useful in predicting the long-term prognosis of these patients.

Possible Future Directions

Since not all biomarkers fulfill the bifunctional role of diagnosis and prognosis, some studies have attempted to measure several specific "complementary" biomarkers in a combined approach. A recent nested case-control study conducted on a group of 122 patients who underwent CPB surgery detected urine KIM-1 and IL-18 simultaneously and found that the former marker had an early diagnostic value while the latter marker predicted AKI progression. The two biomarkers were statistically combined in a multiple logistic regression analysis to predict the progression of AKI. The areas under the ROC were 0.833 and 0.902 at 6 and 12 hours, respectively [24]. Although this method has not been widely used and is not convenient for clinical practice, the results suggest that combining indicators with different functions or strengths is beneficial until biomarkers with "dual function" have been identified. Furthermore, commercial

multimarker assay kits are needed for simple and rapid determinations of AKI, as well as those easy kits in emergency and cancer medicine settings [39,40].

At present, there are no studies on the long-term prognostic stratification of AKI. Clinically, urine albumin and traditional markers of tubulointerstitial injury such as β2-microglobulin and N-acetyl-β-(D)-glucosaminidase are typically selected as markers of chronic kidney injury secondary to AKI. This strategy is empirically based because the relationship between these markers and long-term adverse prognosis is not necessarily the same in acute as in chronic renal disease. Instead, biomarkers that are specific to renal repair mechanisms may better predict the long-term prognosis of AKI and further investigations to identify such markers are certainly warranted.

Conclusion

Urine NGAL appears to be not only diagnostic of AKI but also indicative of the disease's short-term prognosis; however, the accuracy of this biomarker must still be confirmed in different AKI settings. The remaining biomarkers identified to date lack the dual function of diagnostic and prognostic stratification. The most feasible choice is therefore to combine the predictive functions of several markers to diagnose AKI and to predict the short-term prognosis of these patients. The lack of convenient multimarker assay kits for clinical practice is problematic, while biomarkers that predict the long-term prognosis of AKI patients are as yet unknown.

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