Biomarkers of acute kidney injury

ABSTRACT

Creatinine remains the standard for laboratory diagnosis of AKI. Efforts to prevent nephrotoxicity have been harmed by the delay in the diagnosis of AKI criteria by using only the creatinine as a marker, therefore there is great interest in identifying early reliable biomarkers. Moreover, early treatment of ARF can be correlated with a better prognosis and identification of biomarkers for early diagnosis would improve the efficacy of a therapeutic strategy. Thus, it becomes imperative to find biomarkers that can stratify correctly the extent of renal damage that each patient has suffered and the risk of developing chronic kidney disease (CKD). Here, we review the main features of emerging biomarkers in nephrology.

Keywords: biological markers; creatinine; renal insufficiency.

INTRODUCTION

Acute kidney injury (AKI) is defined as a rapid decline in glomerular filtration rate (GFR), and a common problem with high incidence rates, particularly in hospital settings. AKI is responsible for 1% of all hospital admissions, complicating 7% of these hospitalizations, and its incidence increases to 40-60% in ICU inpatients. During the acute kidney injury process, many changes occur at a cellular and molecular levels, that ultimately lead to renal dysfunction and structural injury. Despite significant advances in intensive care and nephrology, the mortality rate of hospitalized patients with AKI has remained relatively constant at around 50% in recent decades. There are numerous community-acquired etiologies causing AKI, among them: ischemia, sepsis and toxins (including medications) were more common in hospitalized patients. Creatinine continues to be the standard laboratory test for AKI diagnosis. Efforts to prevent the predictable nephrotoxicity are handicapped by the delay in AKI diagnosis when using only creatinine as a marker; thus great interest in identifying reliable biomarkers earlier on. Early treatment of AKI can be correlated with a better prognosis and the identification of biomarkers for early diagnosis can improve treatment effectiveness. Finding patients who are at high risk of developing AKI can stimulate an early approach, and new biomarkers may better stratify the risk and reduce the occurrence of chronic kidney disease (CKD).
The conceptual and current AKI model identifies four components in its evolution: risk phase (normal kidney and increased risk); intermediate stage of kidney damage (functional injury); renal failure itself (with decreased glomerular filtration and renal failure) and finally, renal failure requiring renal replacement therapies, which can lead to death depending on the initial damage and the persistence of such damage. Based on serum creatinine, kidney injury diagnosis occurs only at the stage of reduction of glomerular filtration and increased serum creatinine, after a greater degree of renal injury, with a reduction of at least 30% in GFR. After an abrupt decrease in GFR, there is a delay of days until serum creatinine rises. Likewise, after glomerular filtration recovery onset, the fall in serum creatinine is also of late onset.

Creatinine is the standard serologic marker used to detect AKI. Its analysis is inexpensive and the molecule has good chemical stability in the clinical routine. However, it has marked limitations. Renal function worsening is classically detected by means of serum creatinine levels, which is then used to estimate GFR with application of different mathematical models. Creatinine clearance measurement is performed by creatinine levels in blood and in 24-hour urine test, which most used methods for determining these levels are enzymatic assays.

Serum creatinine, usually a benchmark for renal function, should be considered with caution. First, because serum creatinine concentrations vary widely according to gender, age, muscle mass, muscle metabolism, body weight, nutritional status and hydration status. Second, serum creatinine concentrations do not change until a significant amount of kidney function has already been lost, when there has already been a higher degree of renal injury with a reduction of at least 30% in GFR - which means that the kidney injury was present or occurred before serum creatinine rose. Thirdly, with low glomerular filtration rate and the amount of creatinine tubular secretion results in overestimation of renal function. Moreover, the ability of the kidneys to excrete creatinine is not very predictable in every individual, it also dependent on certain drugs interfering with the creatinine tubular transport (e.g. cimetidine, trimethoprim). And finally, during the acute phase, with marked changes in glomerular filtration rate, serum creatinine does not accurately represent kidney function until a steady state has been achieved, which may take several days. In the short term, serum creatinine and urea have low sensitivity and specificity to detect kidney disorders.

The rate of urea production is not constant and increases with a diet rich in protein and tissue injury due to hemorrhage, trauma or treatments with glucocorticoids. On the other hand, a low-protein diet and/or advanced liver disease may reduce urea without changing the GFR.

In the AKI, the fractional excretion of sodium is the most accurate screening test to differentiate between a prerenal and an intrarenal origin. A value below 1% suggests prerenal disease. In contrast, among patients with chronic kidney disease, a coexistent prerenal disease may not result in a low concentration of urinary sodium or a fractional excretion of sodium. A striking disadvantage that leads to confusing results is the previous use of diuretics, interfering with result interpretation.

**NEW BIOMARKERS**

More than 20 AKI biomarkers have already been studied and are extremely valuable, especially in ischemic injury, both experimentally and in clinical settings in which ischemia is common, as in sepsis and in cardiopulmonary bypass, among others. An ideal AKI biomarker would be one that could be easily measured without interference from other biological variables, able to detect early kidney damage and stratify its risk.

Among the most studied emerging biomarkers we list: NGAL, interleukin-18, KIM-1, cystatin-C, L-FABP, NAG, netrin-1, vanin-1 and MCP-1. Among these biomarkers, the NGAL is the one most used in clinical studies. NGAL and L-FABP are the earliest ones, and KIM-1 and IL-18 are detected later on, with better specificity. The combination of metabolic markers is promising because of its superior stability as compared to most proteins and the availability of improved
methods for validation and quantification. At this time of development of new markers, kidney function protein biomarkers should cause a greater impact on clinical practice than strategies using new metabolic markers.\textsuperscript{4,10,13}

\textbf{NGAL - NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN}

NGAL is a 25 Kdalton glycoprotein of the lipocalin family, and it is composed of eight beta strands forming a \(\beta\)-barrel in a closed cup. It is expressed at low levels in several human tissues, including lung, stomach, colon and epithelial cells located in the proximal tubule.\textsuperscript{14,15} It is considered as one of the fastest proteins formed by increased expression of genes in the early post-ischemic kidney in animal models using rats, and it is detected in the first urine sample within 2 hours after ischemia; and is increased vis-à-vis the ischemia duration. Furthermore, it was widely detected in the urine of mice with cisplatin-induced nephrotoxicity. A meta-analysis of data from 19 studies, including 2,500 patients from observational studies, was carried out to estimate the accurate diagnosis and prognosis of NGAL and its value in AKI. The population, which included adults and children, was studied in a variety of conditions: the most often investigated AKI was after cardiac surgery, followed by AKI in critically ill patients and then exposed to contrast media for coronary angiography.\textsuperscript{16-18}

NGAL was considered a useful predictor in the early phase of AKI, which worked well with urine or plasma samples. Furthermore, the NGAL level has prognostic value in clinical outcomes: such as the need for dialysis and mortality. Unfortunately, the large extrarenal production in response to systemic stress can increase its urinary excretion in the absence of AKI, and may increase in CKD - and not only in the acute stage, which can confuse its interpretation.\textsuperscript{6,11}

Among the several new kidney biomarkers recently characterized, NGAL has been the most promising. This interest has increased with the advent of rapid laboratory centers and standardized techniques for measuring NGAL in clinical practice. However, a wide range of NGAL predictive values in acute kidney diseases has been reported by observational cohort studies. Systematic reviews and meta-analysis studies carried out to clarify the NGAL predictive value for the early diagnosis of acute kidney injury involved general data and a variety of subgroups of patients with AKI. We also investigated the predictive value of NGAL in plasma/serum and urine, both in children and in adults. NGAL performance was better when the laboratory standardization was carried out with an NGAL concentration > 150 ng/ml, considered abnormal. Finally, the level of NGAL has prognostic value for clinical outcomes, such as for renal replacement therapy onset and mortality. The literature has shown different AKI definitions and several times for NGAL measurements, used to assess its true predictive value in kidney injury.\textsuperscript{10,12,18}

The performance of biomarkers for AKI is modified by the measurement methods employed and the characteristics of the patient population studied. Most of the NGAL results described in the literature have been obtained by research-based assays using the ELISA method, which is not practical in the clinical settings. The global implementation of laboratory values standardization is highly promising for a more uniform interpretation of the results. In fact, there are different urinary NGAL cutoff levels been described (over 10 mg, over 60 mg, and over 100 mg) to identify patients who will potentially develop AKI.\textsuperscript{19}

There are some limitations to the value of NGAL as a predictor of acute kidney disease and its severity. NGAL levels seem to be more sensitive and specific in predicting AKI in studies with homogeneous patients with a single acute illness, easily identifiable and predictable nephrotoxic insults - such as ECC or intravenous contrast use. NGAL appears to be less sensitive and specific in studies with multifactorial causes of AKI. It is also unclear whether NGAL levels can differentiate potentially reversible causes of AKI; e.g. to distinguish prerenal azotemia from a more serious kidney disorder. NGAL levels seem to predict AKI in children with better accuracy than in adults - which make up the vast majority of patients with AKI. Plasma NGAL levels are also higher in patients with underlying CKD and, in most clinical trials, NGAL excludes CKD patients.
from the analysis. This exclusion is a matter of confusion, because CKD is an important risk factor for AKI, particularly in intensive care settings. In a prospective study with over 25,000 patients with acute kidney injury, more than 30% had underlying CKD.\textsuperscript{20,21}

The basal levels of plasma NGAL are higher in patients with malignancies and systemic bacterial infections, and these can be confounding. The levels of urinary NGAL may also be elevated in urinary tract infections in models using NGAL to diagnose early infections of the urinary tract, in the absence of AKI. Finally, most investigations with NGAL used laboratory studies based on enzyme immunoassays (ELISAs) with variable and potentially long response time.\textsuperscript{20}

**KiM-1 hUman - KIDney I njURY M olecule-1**

The human KIM-1 is a type-one transmembrane glycoprotein with an immunoglobulin domain and mucin that is not detectable in normal kidney tissue or in the urine, but it is expressed at very high levels in dedifferentiated cells of the kidney proximal tubular epithelium in human and rodent kidneys after ischemic or toxic damage. KIM-1 (Kim-1 as depicted in rodents, and KIM-1 in humans) was found markedly increased after 24-48h in the proximal tubule of the post-ischemic rat kidney. A soluble form of human KIM-1 can be detected in the urine of patients with acute tubular necrosis (ATN) and can serve as a useful biomarker in kidney proximal tubular damage, facilitating the early diagnosis and serving as a differential diagnosis of renal injury.\textsuperscript{22} In addition, high urinary expression of KIM-1 was prospectively evaluated in a cohort of 201 hospitalized patients with acute kidney injury and was also associated with adverse clinical outcome (death and need for dialysis) in patients with acute kidney injury. Although the KIM-1 gene or protein expression is undetectable in normal kidneys, after injury, the mRNA (messenger ribonucleic acid) KIM-1 is rapidly synthesized and the protein 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. There are a number of features that could make it an attractive biomarker of kidney damage, such as: lack of KIM-1 expression in normal kidneys, its marked increased expression and insertion into the apical membrane of the proximal tubule, and its persistence in the epithelial cell until the cell’s full recovery.\textsuperscript{4,10,11}

**INTERleukin-18**

Interleukin-18 (IL-18) is a proinflammatory cytokine that is constitutively expressed in the interspersed cells of the distal convoluted tubule and the collecting tubule in the healthy human kidney. Furthermore, these cells contain three major components required for the release of this active and pro-inflammatory cytokine, the so-called pro-IL-18: the P2X7 and caspase-1 intracellular cysteine protease, which converts the IL-18 pro-form into its active form, which then exits the tubular cell into the lumen and increases its urinary levels in AKI.\textsuperscript{21} In a study in humans with various kidney diseases, urinary levels of IL-18 were significantly higher and had high sensitivity and specificity for the diagnosis of acute tubular necrosis (ATN), compared with urinary tract infection, CKD and normal renal function among healthy subjects and their control counterparts. IL-18 can serve as a marker for proximal tubular damage in ATN. In addition, it was significantly elevated before the increase in serum creatinine in patients with acute respiratory failure/acute respiratory distress syndrome who developed AKI, predicting mortality during mechanical ventilation.

Early IL-18 concentrations in the urine are correlated with the acute kidney injury severity, as well as mortality. However, in a prospective analysis, IL-18 showed no ability to predict the subsequent development of AKI. 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 endotoxemia, inflammatory diseases and autoimmune diseases. IL-18 levels increase in a number of pathophysiological states such as inflammatory arthritis, inflammatory bowel disease, systemic lupus erythematosus, psoriasis, hepatitis, and multiple sclerosis. Thus, this cytokine seems to be a candidate biomarker in defining
AKI, but its pro-inflammatory properties and its high levels in inflammatory diseases may limit its use vis-à-vis its sensitivity and specificity.\textsuperscript{4,10,11}

**NAG - N-ACETYL-\(\beta\)-D-GLUCOSAMINIDASE**

N-Acetyl-\(\beta\)-D-glucosaminidase (NAG) is a lysosomal enzyme predominantly found in proximal tubules, so that the increased activity of this enzyme in urine suggests tubular cell injury and, therefore, it can serve as a specific urinary marker for these tubular cells. Due to its high molecular weight, filtration of the enzyme is prevented in the glomeruli. During the active renal disease, NAG levels remain persistently elevated. The increase in urinary NAG activity indicates damage to the tubular cells, although it may also reflect an increased lysosomal activity without cell damage.\textsuperscript{23} Increased urinary excretion of NAG was reported in acute kidney disease of various etiologies, induced by toxic agents, after cardiac surgery and after kidney transplantation.\textsuperscript{24} However, the use of NAG remains limited by the fact that the urinary excretion of the enzyme is also high in diseases such as diabetic nephropathy, hyperthyroidism and rheumatic diseases.\textsuperscript{25,26}

**NETRIN-1**

Netrin-1 is one of the most recent kidney injury biomarkers, a laminina-related molecule little expressed in tubular epithelial cells of normal kidneys. However, it is highly expressed and excreted in the urine after AKI in animais.\textsuperscript{27} Netrin-1 levels increased 2 hours after extracorporeal circulation and peaked at 6h and remained elevated until 48h. Furthermore, we found a correlation with the acute kidney injury duration and severity, and hospital stay.\textsuperscript{28} In a murine model, there was a significant increase in urinary levels of netrin-1 within 3 hours of ischemia followed by reperfusion, peaking at 6 hours, followed by a decrease, returning to near basal values by 72 hours. Interestingly, serum creatinine was not significantly increased 24 hours after reperfusion. In rats treated with cisplatin, folic acid and lipopolysaccharide, increased urinary excretion of netrin-1 happened early at 1h and peaked at 6h after injection. In these rats, serum creatinine only increased significantly 6, 24, and 72 hours after drug injection.

In contrast, NGAL excretion in urine samples of rats treated with folic acid and lipopolysaccharides can only be detected 24 hours after drug administration. Furthermore, the urinary excretion of netrin-1 dramatically increased in 13 patients with acute kidney injury, whereas no changes were detected in urine samples from six healthy volunteers. Significantly higher levels were found in urine samples from patients with ischemic AKI induced by radiocontrast agents, sepsis and drugs compared with healthy controls. Therefore netrin-1 is a promising urinary biomarker that rises early on for the detection of renal injury and can also serve as a universal biomarker for AKI.\textsuperscript{29,30}

**MCP-1 - MONOCYTE CHEMOTACTIC PEPTIDE 1**

Several years ago, the monocyte chemotactic peptide-1 (MCP-1) mRNA was found to have increased expression due to ischemia-reperfusion injury. Thus, the MCP-1 has been reported as a biomarker for mononuclear inflammatory processes that occur following ischemia-induced AKI. In additional studies, 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. Therefore, the MCP-1 protein and MCP-1 mRNA were evaluated against NGAL in a murine model by inducing intrarenal, prerenal and post renal injury. This represents a new approach to quantification of mRNA levels and the corresponding modifications of the histone proteins in their associated genes. In the murine model, the MCP-1 protein and its corresponding mRNA increased in intrarenal lesions in larger amounts than the NGAL. In prerenal and post renal injuries, NGAL expression of the MCP-1 gene increased 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. In conclusion, urinary MCP-1 may be a useful.\textsuperscript{11,31}

**FABPs - FATTY ACIDS BINDING PROTEINS**

Fatty acids binding proteins (FABPs) are a family of small cytosolic proteins that facilitate beta-oxidation by binding and transporting long chain fatty acids. In addition, selective binding to
lipid peroxidation products limits the subsequent cell toxicity caused, and this protective role sparked interest in FABPs as potential markers of cell damage. There are currently nine specific FABPs found in each specific tissue. The liver type, or L-FABP (or FABP-1) is a 14-kda protein synthesized by the liver and localized in the liver, in the intestine and in the proximal renal tubule epithelium, a fatty-acid-dependent cell type in primary metabolism. In preclinical research, the antioxidant role of L-FABP was demonstrated by exposing liver cells to in vitro oxidative stress. Transfected cells showed increased expression of L-FABP, which exhibited a significant decrease in the generation of reactive species. L-FABP expression was shown to be protective of renal tubulointerstitial damage and prevented the buildup of lipid peroxidation products after ureteral obstruction. Clinical trials using L-FABP have been small and mostly cross-sectional. Prospective studies including multiple causes of renal disease are needed to truly assess L-FABP diagnosis and prognosis capacity.4,10,32

Cystatin C

Cystatin 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. The urinary excretion of low molecular weight cystatin C protein, which is an endogenous marker of renal dysfunction correlates with the severity of acute tubular injury. As blood levels of cystatin C are not significantly affected by age, gender, race, or muscle mass in general, it is a marker for estimating the glomerular function in cachectic patients or early AKI, in which serum creatinine could underestimate the true renal function. However, cystatin C is more of a GFR marker instead of a biomarker for primary acute kidney injury.

Prospective studies show that the increase of cystatin C significantly precedes the increase in creatinine in one or two days. Several studies have demonstrated the superiority of cystatin C as compared to serum creatinine, particularly for detecting small changes in the GFR reduction. This was also confirmed by a metanalysis from several studies that compared the accuracy of cystatin C and creatinine vis-à-vis a GFR reference standard. The costs for analysis are still considered high, which limits its use in clinical practice, and factors such as thyroid dysfunction, obesity, use of corticosteroids and inflammation can interfere in its serum levels.11,33

Vanin-1

Vanin-1 is an epithelial ectoenzyme with pantetheine activity that is anchored to glicosilphosphatidilinositol; it participates in the response to oxidative stress in vivo and catalyzes the conversion of pantetheine to pantothenic acid (vitamin B5) and cysteamine. vanin-1 is highly expressed in normal kidney tissues of humans and rodents.34,35 In vanin-1 rats (_/_), the lack of cysteamine is associated with an increased activity of glutamylcystein synthetase, leading to elevation of tissue levels of endogenous glutathione (5-L-glutamyl-L-cysteinilglicine)34,36,37 which exerts an important function in tissue protection against the effects of oxidative stress by scavenging free radicals from exogenous or endogenous compounds. As a result, vanin-1 rats (_/_) are resistant to colitis induced by 2,4,6-trinitrobenzene sulphonic acid.38

Yoshida et al. discovered the existence of increased levels of kidney vanin-1 mRNA in rats with ischemia-reperfusion type of lesion.39 In addition to this one, another recent study showed increased levels of renal vanin-1 in rats with streptozotocin-induced diabetic nephropathy and in patients with diabetic nephropathy.40 It has been found that elevated urinary concentration of vanin-1 occurs before conventional markers in rats with nephrotoxin-induced lesions.35 Therefore, it appears that urinary vanin-1 may be a potential biomarker for early detection of AKI. To address this issue, it has been found that the urinary vanin-1 was detected prior to elevation of serum creatinine and urinary biomarkers NAG, NGAL and Kim-1 in two well established animal models of drug-induced AKI.41,42

Conclusion

Although there have been significant advances in the identification of AKI clinical biomarkers, the field is still developing. With the use of biomarkers
that may lead to better patient care by avoiding nephrotoxins, by suitably modifying drug dosage, because it provides more attention to fluid and electrolyte balance, the biomarkers will possibly facilitate therapeutic interventions that, so far failed to show any benefit, due to late detection with monitoring based solely on creatinine. Despite the many advances in our knowledge regarding biomarkers, many are the features which still need to be determined. Although Kim-1 seems to be a poor biomarker for early diagnosis of AKI and for monitoring recovery after kidney injury, it seems to be a good biomarker for established AKI.

Many questions remain unexplored with respect to IL-18, cystatin C and NAG. In addition, some studies have shown mixed results with respect to cystatin C as a biomarker of early AKI. Whether NGAL and L-FABP have been promising in their ability to monitor a kidney protection intervention remains to be determined. The sensitivity and specificity of each biomarker are variables of the same and in different clinical situations. These discrepancies may be due to the lack of guidelines for cutoff values and standardization of the test method, the time of measurements and sample storage protocols. Some studies have demonstrated a high variability observed when the same biomarker is used to diagnose AKI in the same clinical context. The studies show that, due to the etiological diversity, a panel of biomarkers for diagnosing AKI may be a better strategy than using a single biomarker. Cost-benefit analyzes are also needed to establish whether a panel of biomarkers can reduce the extra costs that AKI poses to healthcare in each country.

REFERENCES