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REVIEW ARTICLE

Biomarkers of kidney injury

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Abstract

Context: Acute kidney injury (AKI) represents a common serious clinical problem. Up to date mortality due to AKI, especially in intensive care units, has not been changed significantly over the past 50 years. This is partly due to a delay in initiating renal protective and appropriate therapeutic measures since until now there are no reliable early-detecting biomarkers. The gold standard, serum creatinine, displays poor specificity and sensitivity with regard to recognition of the early period of AKI.

Objective: Our objective was to review established markers versus novel urine and serum biomarkers of AKI in humans, which have progressed to clinical phase with regard to their diagnostic and prognostic value.

Materials and methods: A review was performed on the basis of literature search of *renal failure*, *acute kidney injury*, and *biomarkers* in Pubmed.

Results: Next to established biomarkers as creatinine and cystatin C, other molecules such as neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), monocyte chemotactic peptide (MCP-1), Netrin-1, and interleukin (IL)-18 are available and represent promising new markers that, however, need to be further evaluated in the clinical setting for suitability.

Discussion: In clinical settings with incipient AKI, not only the development and the implementation of more sensitive biomarkers are required for earlier treatment initiation in order to attenuate the severity of kidney injury, but also equally important remains the substantial improvement and application of refined and prophylactic therapeutic options in these situations.

Conclusion: Adequately powered clinical trials testing a row of biomarkers are warranted before they may qualify for full adoption in clinical practice.

Keywords: Acute kidney injury, acute renal failure, biomarker, NGAL, KIM-1, IL-18, NAG, Netrin-1, MCP-1

Introduction

Acute kidney injury (AKI) formally named acute renal failure (ARF) of any origin is associated with a high mortality in the critically ill patient, despite significant technical advances in therapeutics including renal replacement therapy (RRT) such as dialysis (Brar et al., 2008). From analyzed publications comprising the period from January 1970 to December 2004, determining mortality rates in patients with AKI during the past decades, one can conclude that despite technical progress in the management of AKI over the last 50 years, the mortality rates remained unchanged until now, ranging at around 50% (Ympa et al., 2005). Outstanding advances in basic research have illuminated the pathogenesis of AKI and have paved the way for successful pharmacotherapeutic approaches in animal models. However, translational research efforts in humans have yielded disappointing results so far (Nguyen and Devarajan, 2008).

Current assessments of renal function make use of serum creatinine (SCr) and blood urea nitrogen (BUN), and this remained unchanged for several decades. It is commonly accepted that these biomarkers display poor sensitivity and specificity for indicating early arising, acute changes in kidney function and do not differentiate

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between the renal function itself represented by the functional nephron number and the extent of active lesion as indicator of active kidney damage (Mori and Nakao, 2007). Therefore, new biomarkers are needed not only to aid in early diagnosis but also to serve in several other purposes in AKI as in discerning AKI subtypes like prerenal/intrinsic or postrenal AKI. This would help to identify the primary triggering site of intrarenal injury, differing between proximal tubule, distal tubule, interstitium, and the vasculature. It would also be desirable to be able to identify underlying etiologies as ischemia, toxins, sepsis in order to distinguish other forms of acute kidney disease as urinary tract infection (UTI), glomerulonephritis, and interstitial nephritis. Of equal relevance would be insights into the expected course, severity, and duration and outcome of kidney failure and by this implementing means facilitating to monitor the response to interventions.

In a first formal qualification of biomarkers, seven renal safety biomarkers have been qualified for limited use in nonclinical and clinical drug development following submission of drug toxicity studies and analyses of biomarker performance for the Food and Drug Administration (FDA) and European Medicines Agency (EMEA) by the Predictive Safety Testing Consortium's (PSTC) Nephrotoxicity Working Group. Biomarkers have been divided into clinical application of tubular markers and glomerular markers. This represents a pilot process and may provide further supportive evidence and hints of their clinical utility (Dieterle et al., 2010).

Definition and diagnosis

AKI is defined as the abrupt (e.g. within 48 h) and sustained decrease in renal function resulting in retention of nitrogenous (urea and creatinine) and non-nitrogenous waste products as well as in dysregulation of cellular volume and electrolyte handling. In clinical practice, the diagnosis of AKI relies on a decreased glomerular filtration rate (GFR), increased serum creatinine with or without oliguria, classified in Risk-Injury-Failure-Loss-End-stage kidney disease (RIFLE) (Bellomo et al., 2004b) and Acute Kidney Injury Network (AKIN) criteria (Mehta et al., 2007). In the absence of a consensus concerning the definition, grading, and severity of AKI the acute dialysis quality initiative (ADQI) developed the RIFLE criteria (Bellomo et al., 2004b). The first three classes (risk, injury, and failure) represent the level of severity, whereas the last two (loss, end-stage kidney disease) represent the outcome criteria. For further refinement of the definition of AKI, the AKIN was created, suggesting a modified version of the RIFLE classification representing the entire spectrum of AKI known as AKIN criteria (Mehta et al., 2007). The classification for AKI is defined by three stages of increasing severity, which correspond to the RIFLE criteria for risk (stage 1), injury (stage 2), and failure (stage 3). Loss and end-stage kidney disease were removed and redefined as outcomes.

However, there are major limitations to the use of creatinine for estimating GFR. Serum creatinine does not accurately reflect GFR during the non-steady state of AKI by probably overestimating GFR. Thus, minor changes of creatinine, as typically seen earlier in AKI, do already reflect substantial declines in GFR (Bellomo et al., 2004a). To overcome these obstacles, an extensive search for more suitable laboratory markers monitoring impaired renal function is required.

Epidemiology and etiology

A multinational, multicenter, prospective epidemiological survey of AKI in 4622 consecutively admitted intensive care unit (ICU) patients (prevalence 5% to 7%) revealed that the most common contributing factor for AKI was septic shock. In 47.5% of the patients, AKI was associated with septic shock, in 34% with major surgery, in 27% it was related to cardiogenic shock, in 26% it was related to hypovolemia, and finally in 19% AKI was potentially drug-related. It should be noted that ~30% of the patients already had renal dysfunction before admission, corresponding to pre-existing significant chronic kidney disease. Overall hospital mortality for these patients was 60.3%. Dependence from chronic intermittent dialysis at discharge from hospital was 13.8% for survivors. Independent risk factors for hospital mortality included the need of vasopressors, mechanical ventilation, septic, cardiogenic shock, and hepatorenal syndrome (Nash et al., 2002; Uchino et al., 2005). Furthermore, age and pre-existing renal insufficiency were also detected as risk factors. Regarding sepsis-associated settings, in different studies AKI occurs in ~19% of patients with moderate sepsis, 23% with severe sepsis, and 51% with septic shock, when blood cultures are positive (Rangel-Frausto et al., 1995; Riedemann et al., 2003). Contrast media-induced AKI accounts for ~10% of all causes of hospital-acquired renal failure (Briguori et al., 2002). The latter mentioned condition is especially aggravated in diabetic patients with or without pre-existing renal insufficiency.

Pathophysiology

Kidney injury can be subclassified into three categories: prerenal, intrarenal, and postrenal forms. Hypoperfusion (e.g. caused by hypotension or exsiccosis) leads to prerenal azotemia, and prerenal azotemia can as well progress to renal injury. Thus, it is clinically often unclear, whether an exclusive, real prerenal circumstance is solely responsible for kidney injury or not. A primary intrarenal injury is seen in acute glomerulonephritis. Thus, the distinction between prerenal and intra renal forms is often somewhat confusing. Postrenal kidney injury is classically triggered by obstruction of the urine outflow at any level in the upper or lower urinary tract. The major pathological correlate of intrarenal AKI is acute tubular necrosis (ATN). Prerenal azotemia and ATN are considered to reflect and to pass into the same pathophysiological process and

both cause three-quarters of the entire AKI cases (Nash et al., 2002).

The pathophysiology of AKI involves a complex interplay among vascular, tubular, and inflammatory factors followed by a repair process that can either restore epithelial differentiation and function to normal or result in progressive fibrotic remodeling of variable extent, ultimately leading to chronic kidney disease. Innate and acquired immunity play an important role in the injury phase, furthermore in the regulation of the inflammatory response, and in processes related to repair of the epithelial layer (Bonventre, 2010). Whether AKI is associated with ischemia–reperfusion injury, sepsis, or toxins, there is a rapid loss of proximal tubular cell cytoskeletal integrity and cell polarity (Bonventre, 2010). It is widely recognized that the S3-segment epithelial cells of the proximal tubules in the outer medulla are most susceptible for ischemia and toxic influences (see Figure 1), on the other hand this segment appears to be the source for supplying (stem) cells that organize the epithelial reconstitution in the repair phase (Humphreys and Bonventre, 2008).

Impact of AKI in the hospital

AKI is known to lead to increased length of hospital stay, increased mortality and morbidity, as well as increased costs. Mortality, length of stay, and costs in hospitalized patients with AKI were analyzed retrospectively on 19,982 adults admitted on an internal medical, surgical, neurology, as well as on obstetrics and gynecology services demonstrating the following results. Large increases in serum creatinine (SCr) concentration were relatively rarely detected (1% of patients), whereas more often, modest increases in SCr were commonly reported (13%). Modest changes in SCr were significantly positive associated with mortality, length of hospital stay, and costs. For example, an increase in SCr $\geq$ 0.5 mg/dL was associated with a 6.5-fold increase in the odds ratio for death, a 3.5-fold increase in length of hospital stay, and nearly 7500 dollars in excess, for hospital costs. Thus, AKI is associated with significantly increased mortality, length of hospital stay, and costs across a broad spectrum of conditions. Moreover, outcomes are related directly to the severity of AKI, independently if nominal or percentage changes in serum creatinine occurred (Chertow et al., 2005).

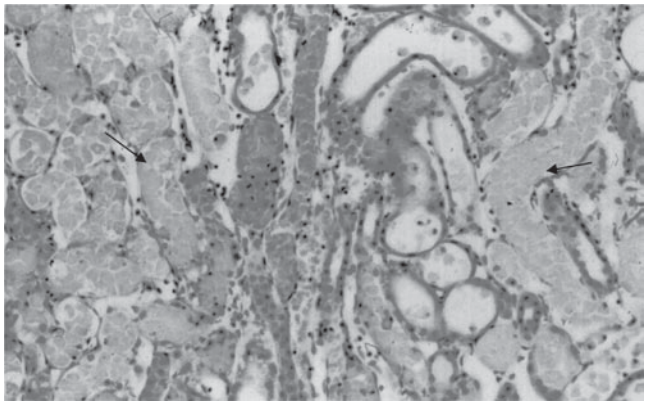

Figure 1. Inner cortex of a rat kidney shown after 24 h of reperfusion following experimental bilateral renal ischemia by clamping of both renal pedicles for 60 min. Kidneys were perfusion-fixed with paraformaldehyde and stained with hematoxylin and eosin (H&E). Slightly dilated tubules of the S3-segments of the proximal tubule are packed with detached cells in the lumen (examples are indicated by arrows), the basal membrane is denuded. Congested vasa recta (in middle) are filled blood cells (from N. Obermüller, personal data).

Characteristics and prospects of new biomarkers

The classical methods of assessing renal function and various renal diseases involve the measurement of serum BUN and creatinine, biomarkers that are not sensitive enough, especially in the setting of early diagnosis of AKI. The development of a noninvasive biomarker that could diagnose renal dysfunction early in sepsis or other circumstances and that could also monitor the response to therapy, as well as the ability to predict severity and outcome would be very valuable. It is also important to recognize that changes in serum creatinine and BUN concentrations primarily reflect functional changes in filtration capacity and are not genuine injury markers.

Besides aiding in early diagnosis and prediction, biomarkers may serve for several additional purposes in AKI. Ideally, they could be a tool for discerning prerenal, intrarenal, or postrenal AKI from each other, helping identifying AKI etiologies such as ischemia, toxins, or sepsis, and supporting the differentiation from different causes for acute kidney disease such as glomerulonephritis and interstitial nephritis or even UTI. Finally, they may be beneficial for predicting and monitoring the response to AKI interventions.

Clinically, applicable AKI biomarkers should be predictive, noninvasive (blood serum or urine sample), and easy to be analyzed at bedside or in a standard clinical laboratory. In addition, they should be rapidly and reliably measurable using a standardized assay platform, with high sensitivity and specificity to facilitate early detection of AKI (Nguyen and Devarajan, 2008).

The discussion concerning clinical utility still remains controversial. Since currently it does not exist a specific therapy for AKI in all cases, early detection of AKI seems at some point without real consequence because of the general lack of promising measures to halt its progression. However, early detection might lead to a rethinking in order to implement potentially novel and effective therapies, thereby preventing the onset of severe AKI and significantly improving the renal and overall prognosis. Application of innovative technologies has now identified candidate molecules that are emerging as sensitive biomarkers.

Methods

A literature research using *Pubmed* focused on acute kidney injury, acute renal failure, biomarker, NGAL, KIM-1, interleukin (IL)-18, *N*-acetyl-β-D-glucosaminidase (NAG), Netrin-1, and MCP-1 was performed.

Results

The diagnosis of AKI can be accomplished by different approaches. Either by detection of increased excretion of proximal tubule proteins, indicating tubular damage, structural proteins (e.g. renal tubular epithelial antigen), as well as proximal tubular enzymes (e.g. NAG, alkaline phosphatase, and γ-glutamyltransferase) (Ozer et al., 2010) or via documentation of proximal tubular dysfunction, as assessed by decreased tubular reabsorption of freely filtered low-molecular-weight proteins (e.g. β2 microglobin, lysozyme, and cystatin C). An approach that has received the greatest attention so far has been quantified the urinary excretion of renal tubular proteins that are acutely overexpressed in response to AKI. Two important examples within this category are kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) (Mishra et al., 2003; Bonventre, 2008; Munshi et al., 2011). Novel technique is the examination of urinary excretion of injury-induced mRNA levels and associated alterations at their cognate genes (e.g. MCP-1). Important biomarker characteristics are summarized in Table 1.

Established biomarkers

Creatinine

Creatinine is the standard serum marker being used to detect AKI. Its analysis is very cheap and the molecule shows good chemical stability in clinical routine. However, it demonstrates striking limitations. Impairment of kidney function is classically detected by measurement of the serum creatinine, which is then used to estimate the GFR applying different mathematical approaches. Determination of the creatinine clearance measurement is accomplished by measurement of creatinine in blood and in 24 h collection urine. The method used is either the Jaffé method or enzymatic tests.

Serum creatinine, in general, as reference parameter for kidney function must be regarded with caution. First, serum creatinine concentrations can vary widely within age, gender, muscle mass, muscle metabolism, overall body weight, nutrition situation, and hydration status. Second, serum creatinine concentrations may not change until a significant amount of kidney function has already been lost, meaning that renal injury is already present or occurs before serum creatinine is elevated. Third, at lower rates of glomerular filtration, the amount of tubular secretion of creatinine results in overestimation of renal function. The latter capacity of the kidneys to excrete creatinine is hardly predictable for the individual; it also depends on some medication interfering with tubular creatinine transport. Finally, during acute changes in glomerular filtration, serum creatinine does not accurately depict kidney function until a steady-state equilibrium has been reached, which may require several days (Nguyen and Devarajan, 2008). In the short run, BUN and serum creatinine showed poor sensitivity and specificity for detection of renal injury (Star, 1998).

Blood urea nitrogen

The diagnosis of AKI is usually based on measurements of BUN and serum creatinine both being not very sensitive or 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. The rate of urea production is not constant increasing with a high-protein diet and with enhanced tissue breakdown due to hemorrhage, trauma, or glucocorticoid therapy, also a chronic or an abrupt absent adequate food supply or intake increases BUN. In comparison, a low-protein diet and/or advanced liver disease can lower the BUN without change in GFR, despite an already existing reduction in GFR (Proulx et al., 2005).

Estimated GFR—on the basis of serum creatinine measurement

Estimation of the GFR gives an approximate measure of the number of functioning nephrons. The most common estimation equations are based upon the serum creatinine concentration: the Cockcroft–Gault equation (Cockcroft and Gault, 1976) and more often applied the Modification of Diet in Renal Disease (MDRD) study equation (Levey et al., 1999). Since all renal disorders may variably affect renal function, estimation of the GFR has no diagnostic utility. Serum creatinine and GFR estimation equations can only be used in patients with stable or chronically altered kidney dysfunction. In AKI, for example, the GFR is already initially markedly reduced but the time is too short for serum creatinine to accumulate and to rise, thus being unable to reflect the degree of renal dysfunction.

Table 1. Characteristics of AKI biomarkers.

Emerging biomarker	Measured in	Time to elevation (h)	Method of detection	Tested in scenario of AKI	Adults/children
NGAL	Plasma/urine	2–6	ELISA	CS, CN, KT, SE	Both
KIM-1	Urine	6	ELISA	CS, CN, KT, SE	Both
IL-18	Urine	4–6	ELISA	ATN, CN, CRD, CS, PA, KT, UTI	Both
NAG	Urine	6	Colorimetric assay	CS, CRD, DI, KT	Both
Netrin-1	Urine	2	ELISA	CN, CS, DI, KT, IS, SE	Adults
MCP-1	Urine	4	ELISA	CRD, IS	Adults

Abbreviations: ATN, acute tubular necrosis; CN, contrast nephropathy; CRD, chronic renal disease; CS, cardiac surgery; ELISA, enzyme-linked immunosorbent assay; KT, kidney transplantation; PA, prerenal azotemia; SE, sepsis; UTI, urinary tract infection; IS, ischemic AKI; DI, drug-induced AKI

Fractional excretion of sodium

In acute renal failure, the fractional excretion of sodium is the most accurate screening test to differentiate between prerenal and intrarenal disease origin. A value below 1% thereby suggests prerenal disease. In contrast, among patients with chronic kidney disease, an additional prerenal process may not result in a low urine sodium concentration or fractional excretion of sodium. A striking disadvantage leading to confusing results, however, is the pre-existing use of diuretics, which interferes with the result interpretation.

Cystatin C—a marker of GFR

Cystatin C is a cysteine protease inhibitor synthesized by all nucleated cells in the body. It is freely filtered by the glomerulus, reabsorbed completely, and is not secreted (Dharnidharka et al., 2002). Urinary excretion of the low-molecular-weight protein cystatin C, which is an endogenous marker of renal dysfunction, correlates with the severity of acute tubular damage. As blood levels of cystatin C are not significantly affected by age, gender, race, or overall muscle mass, it is a marker for the estimation of glomerular function for instance in cachectic patients or early AKI, where serum creatinine could underestimate the true renal function. Yet, cystatin C is, however, more a marker of GFR rather than a primary AKI biomarker, but as such, it can be used to detect AKI. In a prospective study evaluating cystatin C, the increase of cystatin C significantly preceded that of creatinine for 1 or 2 days (Herget-Rosenthal et al., 2004). Diverse studies demonstrated the superiority of serum cystatin C compared with serum creatinine, especially to detect minor alterations in GFR reduction. This was also confirmed by a meta-analysis from various studies comparing the accuracy of cystatin C and creatinine in relation to a reference standard of GFR (Dharnidharka et al., 2002). Thus, cystatin C has some advantages over serum creatinine in different settings, the costs for analysis being although considerably higher.

Proteinuria—not a marker for AKI

Employed as an essential diagnostic tool for intrinsic kidney disease, proteinuria is also a prognostic tool of considerable reliability: the severity of proteinuria (caused by even different kidney diseases) commonly predicts the risk for chronic progressive kidney disease and to a certain extent the rate at which kidney function is lost.

However, in the setting of early AKI detection, proteinuria does function as a suitable biomarker. This is strengthened by several points: classical AKI is not accompanied by proteinuria/albuminuria; therefore, proteinuria would also not even be a marker in the course of recovering from AKI. It is clear that depending on the underlying or associated disease of an AKI (e.g. systemic infection) proteinuria/albuminuria can occur in this setting as a consequence of glomerular impairment. It should also be taken into account that measured changes in (pathological) proteinuria must always be interpreted in the light of pre-existing proteinuria, which is the case with diabetes and hypertension, and which is often not known. Finally, to really determine the actual amount of proteinuria in a given clinical case, collection of small urine samples are not representative and exact for determining acute and slight changes in protein excretion. Vice versa, and clinically more important proteinuria and edema display serious issues in glomerulonephritis with or without decrease of GFR.

Importantly, massive proteinuria (e.g. >10 g/day) as seen in the nephrotic syndrome may aggravate intravasal volume depletion, promote hypotension, and thereby initiate AKI, or worsen pre-existing chronic renal failure.

Emerging biomarkers

Neutrophil gelatinase-associated lipocalin

NGAL is a protein of the lipocalin family and is composed of 8 β-strands that form a β-barrel enclosing calyx (Flower et al., 2000); it is expressed by neutrophils and epithelial cells including those of the proximal tubule. NGAL was identified as one of the fastest up-regulated genes in the early phase of the post-ischemic mouse kidney (Supavekin et al., 2003) being detected in the very first urine sample within 2 h following ischemia and displaying increased levels correlating with the duration of ischemia. Moreover, NGAL was amply detectable in the urine of mice with cisplatin-induced nephrotoxicity (Mishra et al., 2003). So far, NGAL has been investigated across a range of different clinical settings of AKI. However, with accumulating evidence, conflicting observations raised some concerns about the robustness of NGAL as a biomarker. Therefore a meta-analysis of data from 19 studies including 2500 patients of observational studies was performed to estimate the diagnostic and prognostic accuracy of NGA and its value in AKI. The population included both adults and children, studied in a range of setting: most frequently investigated AKI after cardiac surgery, followed by AKI in critically ill patients and after exposure to contrast media for coronary angiography. In conclusion, NGAL was found to be a useful early predictor of AKI, with urine or plasma/serum NGAL levels functioning as well. Additionally, NGAL level had prognostic value for clinical endpoints, such as initiation of dialysis and mortality (Haase et al., 2009). Unfortunately, substantial extrarenal NGAL generation in response to systemic stress can increase urinary NGAL excretion in the absence of AKI as well, and this may also arise from chronic and not just acute, renal disease (Haase et al., 2009).

Human KIM-1

Human KIM is a type 1 transmembrane glycoprotein with an immunoglobulin and mucin domain that is not detectable in normal kidney tissue or urine, but is expressed at very high levels in dedifferentiated proximal tubule epithelial cells in human and rodent kidneys after ischemic or toxic injury. The KIM-1 (designated as Kim-1 in rodents, KIM-1 in humans) was found to be markedly

up-regulated after 24–48 h in the proximal tubule of the post-ischemic rat kidney (Ichimura et al., 1998). The A soluble form of human KIM-1 can be detected in the urine of patients with ATN and may serve as a useful biomarker for renal proximal tubule injury facilitating the early diagnosis of the disease and serving as a diagnostic discriminator (Han et al., 2002). Furthermore, high urinary KIM-1 expression was evaluated prospectively in a cohort of 201 hospitalized patients with AKI and was also associated with adverse clinical outcome (death and need for dialysis) in patients with AKI (Liangos et al., 2007). Although KIM-1 gene or protein expression is undetectable in the normal kidney, following injury KIM-1 mRNA is rapidly synthetized and protein is generated and localized at very high levels in the apical membrane of proximal tubule. In human ischemic and toxic AKI, it is found in all three segments of the proximal tubule. There are a number of characteristics that might make it attractive as a biomarker of kidney injury: absence of KIM-1 expression in the normal kidney, its marked up-regulation and insertion into the apical membrane of the proximal tubule, its persistence in the epithelial cell until the cell has completely recovered, and the *ex vivo* room temperature stability of the ectodomain (Bonventre, 2009).

Interleukin-18

IL-18 is a proinflammatory cytokine that is constitutively expressed in the intercalated cell of the late distal convoluted tubule, the connecting tubule, and the collecting duct of the healthy human kidney. Moreover, these cells contain three major components required for the release of the active proinflammatory cytokine IL-18, namely pro-IL-18, P2X7, and the intracellular cyteine protease caspase-1 (Gauer et al., 2007), which converts the proform of IL-18 to its active form, which then exits the cell and may enter the urine, for example, in AKI (Melnikov et al., 2001). In a first cross-sectional study in humans with various renal diseases, urine levels of IL-18 were significantly greater and had a high sensitivity and specificity for the diagnosis of ATN in comparison with PA, UTI, chronic kidney disease, and normal renal function in healthy control subjects. IL-18 may serve as a marker for proximal tubular injury in ATN (Parikh et al., 2004). Furthermore, urinary IL-18 was significantly up-regulated prior to the increase in serum creatinine in patients with acute respiratory distress syndrome who developed AKI, predicting mortality at the time of mechanical ventilation (Parikh et al., 2005). Early urine IL-18 measurements correlated with the severity of AKI as well as mortality, however, in prospective analysis IL-18 demonstrated no ability to predict the subsequent development of AKI. Considering IL-18 being a proinflammatory cytokine that plays an important role in sepsis, Il-18 measurements may also be influenced by a number of coexisting variables, such as endotoxemia, inflammatory, and autoimmune diseases. Plasma IL-18 levels are known to be increased in various pathophysiological states, such as inflammatory arthritis, inflammatory bowel diseases, systemic lupus erythematosus, psoriasis, hepatitis, and multiple sclerosis. Thus, this cytokine seems to be a candidate biomarker in the setting of AKI, its proinflammatory properties, and its up-regulation in inflammatory disease may limit its application in terms of sensitivity and specificity.

N-Acetyl-β-D-glucosaminidase

NAG is a lysosomal enzyme, found predominantly in proximal tubules so that increased activity of this enzyme in the urine suggests injury to tubular cells and therefore can serve as a specific urinary marker for the tubular cells. Due to its relatively high molecular weight, filtration of the enzyme is precluded by glomeruli. In the course of active kidney disease, urinary NAG levels remain persistently elevated. The increase in urinary NAG activity indicates damage to tubular cells, although it can also reflect increased lysosomal activity without cellular damage (Liangos et al., 2007). Increased urinary NAG excretion has been reported in acute renal disease of varying etiology, induced by toxic agents, after cardiac surgery and after renal transplantation (Bernard et al., 1987; Price, 1992). However, the use of NAG remains limited by the fact, that urinary excretion of the enzyme is also elevated in glomerular diseases such as diabetic nephropathy (Marchewka et al., 2001).

Recently emerging potential biomarkers

Netrin-1

Netrin-1, a laminin-related neuronal guidance molecule, is not or barely expressed in tubular epithelial cells of normal kidneys. However, it is highly expressed and excreted in the urine after AKI in animals. In a recent study, Netrin-1 levels rose 2 h after cardiopulmonary bypass operation (CPB) and peaked at 6 h, remaining elevated up to 48 h. Furthermore, a correlation with duration and severity of AKI and hospital stay was found. Therefore, the authors postulated Netrin-1 being a new early predictive biomarker of AKI after CPB (Ramesh et al., 2010a). In a mouse model, urinary Netrin-1 levels increased markedly within 3 h of ischemia–reperfusion, reaching a peak level at 6 h, with a decrease thereafter, returning to near baseline values by 72 h. Interestingly, serum creatinine did not increase significantly until 24 h of reperfusion. Similarly, in cisplatin-, folic acid-, and lipopolysaccharide-treated mice, urine Netrin-1 excretion increased as early as 1 h and reached a peak level at 6 h after injection. Here too, serum creatinine only rose significantly after 6, 24, and 72 h, respectively. In comparison, NGAL excretion in folic acid- and lipopolysaccharide-treated mice urine samples could only be detected 24 h after drug administration. Moreover, urinary Netrin-1 excretion increased dramatically in 13 patients with AKI, whereas no changes were detected in six healthy volunteer urine samples. The authors concluded therefore that urinary Netrin-1 is a promising early up-regulated biomarker for detection of renal injury (Reeves et al., 2008). In order to test Netrin-1 in human urine samples, Netrin-1 levels

were analyzed by sandwich enzyme-linked immunosorbent assay. The authors came to the conclusion that Netrin-1 can serve as universal biomarker for AKI by demonstrating increased levels in patients with various forms of AKI. In detail, significantly higher levels were found in urine samples from patients with ischemic AKI, radiocontrast-induced AKI, sepsis-induced AKI, and drug-induced AKI in comparison with healthy controls (Ramesh et al., 2010b).

Monocyte chemotactic peptide-1

Several years ago, a monocyte chemotactic peptide-1 (MCP-1) mRNA has been found to be up-regulated in ischemia–reperfusion injury. MCP-1 therefore has been sought to be a biomarker for the mononuclear inflammatory processes that occurs after ischemia-induced AKI (Rice et al., 2002).

In further studies, this MCP-1 was found to be a potent chemokine produced by renal cells and acting as mediator in acute ischemic and toxic kidney injury. Therefore, MCP-1 protein and MCP-1 mRNA was examined in comparison with NGAL in a mouse model, by inducing intrarenal, prerenal, and postrenal injury. This represents a new approach quantifying mRNA levels and corresponding histone modifications at their cognate genes. Furthermore, a complementary study was conducted in a clinical setting with the following findings. In the mouse model, MCP-1 protein and its mRNA increased in intrarenal injury to a greater extent than NGAL. In prerenal and postrenal injury, NGAL and MCP-1 gene expression increased comparably. In contrast, uremia *per se* already induced the NGAL gene in the absence of renal injury, but not MCP-1, arguing for a better specificity of MCP-1 for AKI. Clinical assessments supported the potential utility of MCP-1 as a biomarker, by observing increases in urinary MCP-1 protein and mRNA levels without overlap in the absolute urine in patients with AKI. In conclusion, urinary MCP-1 may be a useful biomarker of AKI possibly providing complementary information to that deriving from NGAL analysis (Munshi et al., 2011).

Conclusion

The existing panel of emerging biomarkers currently still displays some limitations, having been validated mostly in animal models of AKI or in a limited number of clinical studies investigating selected patient collectives; therefore, their transition to clinical application still needs further validations.

There are high demands for ideal properties and roles of biomarkers in AKI, which should be organ-specific (i.e. kidney-specific), rapidly, inexpensive and reliably measurable, noninvasive, sensitive, not elevated in chronic kidney disease, easily measured and have reproducible results in easily accessible samples like plasma or urine, highly sensitive and specific for AKI, capable of an early detection, able to give insight into etiology, nature, and duration AKI, in the sense of a predictor of AKI severity and reversibility and therefore helpful in monitoring course and the response to interventions. Thus, the challenge consists in having a marker of true renal injury, unaffected by other biological variables, being in parallel a typically marker of renal function. A so-called ideal biomarker for AKI will probably not be available easily in the near future. One main question in this issue is whether the biomarker of choice should arise/will be produced by the kidney or should it originate from the circulation and then be filtered/and processed by the kidney. Chronic kidney disease will probably affect biomarker levels in the blood under both circumstances. In our view, an AKI biomarker should derive from the kidney itself, rapidly indicating injury, and being easily detected in the peripheral blood.

Urine and blood are both well-accessible; however, if low urine output exists, the validity of a small urine sample taken regarding the levels of distinct biomarker levels remains questionable. The future may lie in the application of a combined urine and plasma panel, a cocktail of various biomarkers, to increase discrimination and to giving answers regarding the underlying disease origins in order to better treat different etiologies of AKI as well as regarding prognosis and severity. It still remains a challenge to evaluate an evolving marker of renal injury with an insufficient marker of renal function and renal injury such as creatinine, since other options are lacking.

On top, it remains to raise the critical question regarding the current consequence and clinical usefulness. Actually, besides avoiding toxic influences, the treatment of AKI consists of re-establishing fluid and electrolyte balance, if needed, also RRT. The aim is to get specific insights in disease pathogenesis in clinical setting and at the same time develop preventive therapies, provided the availability of valid, early responsive biomarkers.

Declaration of interest

The authors have no conflict of interests.

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