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Neutrophil gelatinase–associated lipocalin (NGAL): a promising biomarker for the early diagnosis of acute kidney injury (AKI)

Elio Antonucci[1], Giuseppe Lippi[2], Andrea Ticinesi[3-4] Federica Pigna[1], Loredana Guida[4], Ilaria Morelli[4], Antonio Nouvenne[3-4], Loris Borghi[3], Tiziana Meschi[3-4]

[1] Post-Graduate School of Emergency-Urgency Medicine, University of Parma, Parma, Italy; [2] Laboratory of Clinical Chemistry and Hematology, Department of Pathology and Laboratory Medicine, University Hospital of Parma, Parma, Italy; [3] Department of Clinical and Experimental Medicine, University of Parma, Parma, Italy; [4] Internal Medicine and Critical Subacute Care Unit, University Hospital of Parma, Parma, Italy

Summary. Acute kidney injury (AKI) is a common complication that occurs in a broad spectrum of clinical settings. Cardiac surgery-associated AKI continues to be a well-recognized complication of cardiac surgery with high morbidity and mortality. The lack of early biomarkers has for long prevented timely interventions to mitigate the effects of AKI. Serum creatinine is not a timely marker of AKI, so that it cannot be used to set potentially effective therapies to treat AKI in patients during phases when the injury is still potentially reversible. Neutrophil gelatinase–associated lipocalin (NGAL) has been identified as a promising biomarker for early detection of AKI. Several studies have shown that NGAL levels significantly increase in AKI patients 24 to 48 hours before a detectable increase of serum creatinine. Recent studies also suggest that measurements of urinary NGAL levels in patients at risk for cardiac surgery–associated AKI may facilitate its early diagnosis and allow clinicians to implement therapeutic adjustments that have the potential to reverse renal cellular damage and minimize further kidney injury.

Key words: Acute kidney injury, acute renal failure, biomarkers, NGAL

Introduction

Acute kidney injury (AKI) encompasses a broad spectrum of renal damages and often leads to acute renal failure, that is a rapid and sustained decrease in kidney function. According to the Acute Kidney Injury Network (AKIN) criteria, AKI typically leads to a serum creatinine (SCr) increase by more than 50%. Despite improvements in clinical care, AKI remains a serious problem with an increasing incidence. It occurs in approximately 5% of hospitalized patients and in more than 30% of patients in the intensive care unit (ICU) and is associated with high morbidity and mortality rates. Despite advances in the techniques and technology of cardiac surgery, AKI continues to be an important complication also in this setting.

The clinical manifestations of AKI vary from a minimal but sustained reduction in glomerular filtration rate (GFR) up to anuric renal failure (1). Depending on severity and duration, AKI can be associated with metabolic conditions such as metabolic acidosis, hyperkalemia, and changes in body fluid balance. Moreover, patients are susceptible to infectious complications and frequently develop anemia (2). In particular, renal injury is frequent after cardiac surgery because of renal hypoperfusion, reperfusion injury, hemodilution, and inflammatory response, and is a major cause of morbidity and mortality, developing in 1% to 7% of patients (3).

Limitations of serum creatinine and the importance of early detection of AKI

In the current clinical practice, the standard measure for renal function is SCr assessment. Although the production of creatinine from the skeletal muscle is directly proportional to the muscle mass and shows a relatively low intra-individual biological variability thus making it a convenient marker for kidney function, its diagnostic value for early detection of AKI is limited.

The severe consequences of AKI spotlight the need for early detection. Several studies have shown as yet that SCr is a suboptimal indicator of acute changes in kidney function (4,5). The accuracy of using SCr to measure AKI is of particular concern during acute changes in glomerular filtration because it might not be useful until steady-state equilibrium has been reached, which may occur 2 to 3 days after injury. In particular, specific problems in using SCr after cardiopulmonary bypass (CBP) surgery have been highlighted. Basically, creatinine levels may be unreliable because creatinine production is reduced when the patient becomes hypothermic as a consequence of decreased blood flow, or levels may be increased because of muscle damage during surgery. In addition, patients are fluid loaded during CPB and a varying amount of plasma water may be removed through hemofiltration (6). The sensitivity of SCr is also modest due to the presence of a vast renal reservoir, and its serum levels only increase when more than half of glomerular filtration rate is definitely lost. As such, since SCr increase (or GFR decrease) are initially masked by a compensatory improved renal function, it cannot be considered an early marker of AKI and cannot be used to timely set effective therapies to treat AKI in patients during phases when the injury is still potentially reversible.

Nevertheless, early detection of AKI is essential for immediate introduction of measures to prevent further damage and progression towards irreversible damage, as well as for establishing protocols aimed to closely monitor ongoing renal injury.

Promising biomarkers for early detection of AKI

The remarkable prevalence of both morbidity and mortality associated with AKI calls for a search for novel, early and efficient biomarkers of acute renal injury. Ideally, such biomarkers would also be useful for establishing the prognosis and identifying the physical site of injury to facilitate the differential diagnosis between AKI and other forms of acute kidney disease.

Several conventional urinary biomarkers assessed for early detection of AKI are neither specific nor sensitive. Some novel biomarkers, however, are more promising than others, and these include Neutrophil Gelatinase Associated Lipocalin (NGAL), Cystatin-C, Interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), monocyte chemotactic peptide (MCP-1), Netrin-1, Liver-type fatty acid binding proteins (L-FABP). In this review, we present an overview of NGAL, compare it with current and emerging biomarkers, and present evidence of its important role for early detection of AKI.

NGAL

Human NGAL is a small protein (i.e., 25 kDa) belonging to the lipocalin family, which was originally discovered in 2003 by a genome-wide analysis of kidney genes that are induced in response to experimental AKI in animals. It is essentially expressed by neutrophils and renal proximal tubules, while it can also be produced to a much lesser extent in prostate and epithelia of the respiratory and alimentary tracts. Human NGAL seems to be a marker of stress because it is highly expressed in cells after infections, inflammation, ischemia, or neoplastic transformation. Because of its small size and resistance to degradation, NGAL is easily detected in both blood and urine. The potential utility of NGAL as an AKI biomarker was recognized because it was one of the earliest and most rapidly induced genes in the kidney after ischemic or nephrotoxic injury in animal models (7).

CYSTATIN C

Cystatin C, a cysteine protease inhibitor, is synthesized by nucleated cells and steadily released into the blood (8). The diagnostic utility of this protein as a biomarker of AKI was assessed in patients at high risk of developing AKI. Serum cystatin C may predict AKI with good diagnostic performance (area under

the receiver operating characteristics curve [AUC], 0.97) 1 to 2 days earlier than SCr (AUC, 0.82), and an increased urine cystatin C level may reliably predict the need for dialysis (AUC, 0.75) earlier than SCr (9). In a comparative study, serum NGAL, cystatin C, creatinine, and urea values were measured after cardiac surgery in 100 adult patients in the ICU and at 24 hours postoperatively (10). For levels tested on patient admission at the ICU, serum NGAL and cystatin C showed a good independent predictive value for AKI (AUC, 0.80 and 0.83, respectively), much higher than that of SCr and urea (AUC, 0.68 and 0.60, respectively). Early postoperative measurements also showed that both serum NGAL (AUC, 0.95) and cystatin C (AUC, 0.99) were superior to SCr and urea in the prediction of cardiac surgery–associated (CSA) AKI and of prognostic value in this setting.

In another study of 72 adults undergoing elective cardiac surgery, serum cystatin C and NGAL were not useful predictors of AKI within 6 hours after surgery (11). In marked contrast, both urine cystatin C and NGAL were elevated in the 34 patients who later developed AKI, compared with those with no injury. The urine NGAL levels at the time of ICU arrival and the urine cystatin C levels 6 hours after ICU admission were the most useful for predicting AKI. This suggested that urine cystatin C and NGAL may be superior to conventional plasma markers in the early diagnosis of CSA-AKI, and that urine NGAL was an earlier marker than cystatin C for CSA-AKI. Cystatin C is mainly a biomarker of renal clearance, and serum concentrations only increase as the GFR begins to decrease. Conversely, NGAL is rapidly induced in kidney tubule cells in response to ischemic injury, and its early appearance in both serum and urine is independent from the GFR, but is highly predictive of a subsequent decline in GFR. As such, cystatin C levels are also affected by thyroid function and inflammation (12). Therefore, although cystatin C appears to be be a much better marker for AKI than SCr ,urine NGAL seems however superior to cystatin C for earlier detection of AKI.

KIM 1 (Kidney Injury Molecule 1)

Another promising biomarker is KIM-1, a type-1 trans-membrane glycoprotein that is highly expressed in proximal tubule cells after ischemic and nephrotoxic injury (12). In a study of 40 children undergoing cardiac surgery, urine KIM-1 levels peaked 12 hours after injury (AUC, 0.83) in patients with AKI (13), and predicted the need for dialysis or mortality in hospitalized patients (AUC, 0.61) (14). KIM-1 seems to be more specific for ischemic or nephrotoxic kidney injury than NGAL, and is not significantly affected by chronic kidney disease or urinary tract infections. Thus, KIM-1 represents a sensitive and specific marker for AKI after CPB. Because NGAL and KIM-1 are increased in urine early after injury, they may serve as temporally sequential biomarkers for early detection of AKI, with NGAL being most sensitive at the earliest time points and KIM-1 adding significant specificity at slightly later time points, although its diagnostic performance for early detection of AKI needs to be confirmed in larger investigations (12).

INTERLEUKIN-18

Recent research has shown that IL-18, a proinflammatory cytokine, is highly up-regulated and suitably detectable in urine after ischemic AKI in animal models. Both urine IL-18 and NGAL were early, predictive, sequential AKI biomarkers in children undergoing cardiac surgery and independently associated with duration of AKI (15). In patients who developed AKI 2 to 3 days after surgery, urine NGAL peaked at 25-fold within 2 hours and declined 6 hours after surgery. In a further investigation (16), serum and urine NGAL as well as urine IL-18 were assessed in patients undergoing cardiac surgery at different time points. Interestingly, postoperative AKI onset in 9 out of the 33 cases (i.e., 27%), but the diagnosis with SCr could be made only 12-48 hours after cardiac surgery. The concentration of IL-18 as well as that of urine (but not serum) NGAL was significantly increased in the AKI group post-surgery, the peak being reached 2 to 4 hours after surgery. Logistic regression analysis also showed that urine NGAL corrected for urine creatinine 2 h after surgery was the most powerful biomarker of AKI. It was hence concluded that although both urine NGAL and urine IL-18 may be good predictors of CSA-AKI, urine NGAL enables earlier detection of renal damage.

Clinical utility of NGAL

The potential of NGAL to predict CSA-AKI is promising. Early postoperative measurement of serum NGAL in adult patients undergoing cardiac surgery has been shown of good value for identifying patients developing AKI after surgery and had excellent prognostic value for the defined end points, that is, the need for renal replacement therapy and in-hospital mortality (12,15,16). In a study of 196 children undergoing cardiac surgery with CPB (17), urine NGAL levels respectively increased by 15 and 25 folds within 2 hours and at 4-6 hours after CPB. The authors also showed the superiority of NGAL over SCr as a biomarker for AKI, because 2-hour urine NGAL levels were related to several key parameters, including the severity and duration of AKI, length of hospital stay, dialysis requirement, and death. Nickolas and colleagues (18) showed that urine NGAL levels identified the presence of AKI in a broad patient sample with different mechanisms of injury. In a cohort study of 635 patients, the prognostic utility of urine NGAL was compared with conventional and novel biomarkers in predicting AKI and its comorbid conditions. The sensitivity and specificity of a single measurement of urine NGAL were 0.90 and 0.99, respectively, and urine NGAL levels were highly predictive of clinical outcomes, including nephrology consultation, dialysis, and ICU admission. NGAL was also shown to be a useful early marker that helps to distinguish AKI from normal function, prerenal azotemia, and chronic kidney disease and predicts poor inpatient outcomes (18). This study helped to establish the validity of NGAL by demonstrating that urine NGAL levels remained highly diagnostic even when the timing of injury was unknown, thus making NGAL a potentially diagnostic tool in renal disease for several clinical presentations. The study had some limitations, however, because the patients came from a single center. Therefore, it would be important to validate these findings in other groups of patients from multiple sites. A systematic review and meta-analysis of available data from 19 studies including more than over 2,500 patients, showed an AUC of NGAL of 0.82 across all AKI settings, with 85% specificity and 76% sensitivity (19). Urinary NGAL also performed better for AKI prediction than serum NGAL (i.e., AUC 0.84 versus 0.77) (19). The potential superiority of urinary over serum NGAL has also been recently confirmed some clinical studies showing that stress and acute leukocytes variations (especially of neutrophils), have a dramatic influence on serum but not on urine NGAL, thereby decreasing the specificity of the former for detection of acute renal injury (20,21).

Discussion

According to current scientific evidence, we recommend that baseline NGAL measurements should be determined in all individuals undergoing cardiac surgery, whereas sequential measurements should be limited to high-risk patients. It is recommended that serial testing of NGAL should be carried out in patients with strong risk factors for AKI, although they do not uniformly result in cardiac surgery-associated acute kidney injury (CSA-AKI). The further occurrence of AKI cannot be predicted from the presence of these factors or, as noted above, from monitoring SCr. However, if serial NGAL testing is performed, the early diagnosis of CSA-AKI can be made and early therapeutic adjustments established to minimize irreversible kidney injury and shorten the time of postoperative recovery. We also endorse that urinary measurement should be preferred over serum testing, since the former is less sensitive to extra-renal sources of variations, has thereby a greater specificity for AKI and displays a much greater increased after renal injury (e.g., more than 10,000-fold in urine versus ~100-fold in serum). Novel, fully automated immunoassay designed to be implemented on a variety of clinical chemistry instruments are also available, that would allow a widespread diffusion of NGAL measurement in most clinical laboratories (22).

High-risk patients with concomitant raised values of NGAL should be managed through close monitoring in the ICU and avoidance of nephrotoxic agents (eg, radiocontrast agents, nonsteroidal anti-inflammatory drugs, and aminoglycosides), and a nephrology consultation should be considered.

Conclusions

AKI is a frequent and serious complication after cardiac surgery. The need for biomarkers for AKI has

been emphasized in recent years through advances in technology and a better understanding of the importance of early detection. At present, the greatest clinical challenges in AKI are in the ICU setting, where biomarkers may be essential for an early diagnosis of AKI and enabling the most appropriate management strategies, which should be started before irreversible renal damage occurs. Biomarkers may also provide a timely assessment of nature and severity of AKI (i.e., for predicting the need of dialysis). During the course of AKI, biomarkers offer relatively non invasive opportunity to monitor the effectiveness of treatment and assess the overall prognosis. The latter concept is supported by several studies suggesting that SCr may be poorly sensitive and late marker, so that clinical intervention may have little effect by the time kidney dysfunction is detected through an increase of SCr. Conversely, reliable data substantiate the highly predictive nature of NGAL for detection and assessment of CSA-AKI, in both children and adults (19). Serial testing of NGAL in selected patients at high-risk for CSA-AKI may facilitate its early diagnosis and allow clinicians to implement adequate therapeutic adjustments to potentially reverse renal cellular damage that has already occurred and minimize further kidney injury.

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Correspondance:
Antonio Nouvenne M.D.
University of Parma
Department of Clinical and Experimental Medicine
Via A. Gramsci 14 - 43126 Parma
Phone: +39 0521 703626
Fax: +39 0521 940993
E-mail: antonio.nouvenne@alice.it