Chapter

Neutrophil Gelatinase-Associated Lipocalin as a Promising Biomarker in Acute Kidney Injury

Camila Lima, Maria de Fatima Vattimo and Etienne Macedo

Abstract

Acute kidney injury (AKI) is a common complication in several settings inside and outside hospitals. It affects millions of people around the world, and despite high levels of research funding, there is no specific treatment that changes the disease course. The basis for unfavorable outcomes related to this disease is the failure to provide early diagnosis. Currently, the diagnosis of AKI is based on serum creatinine and urine output, and both measures have several limitations, making early diagnosis difficult. In recent decades, several biomarkers of kidney injury have been proposed, with neutrophil gelatinase-associated lipocalin (NGAL) being one of most studied and promising for use in early diagnosis. Despite there being several studies on NGAL, it has not yet been applied in clinical practice; thus, furthering the understanding of the development, interpretation, and limitations of NGAL in the diagnosis of AKI is the objective of this chapter.

Keywords: acute kidney injury, biomarkers, neutrophil gelatinase-associated lipocalin

1. Introduction

Acute kidney injury (AKI) is a frequent complication in several clinical settings, including large surgeries [1], emergency departments [2, 3], and intensive care units (ICUs) [4]. The incidence of AKI has been increasing over the years, with about 2 million people being affected in 2010 [5], despite the efforts of researchers and organizations [6–8]. AKI is commonly followed by worse outcomes: prolonged length of ICU and hospital stay, need for dialysis, decreases in the glomerular filtration rate (GRF), development of chronic kidney disease (CKD), and increases in mortality [9–11].

In recent decades, therapeutic interventions aimed at reversing kidney dysfunction have had disappointing results in multiple settings; thus, the research focus has shifted from treatment to prevention and early detection by focusing on two main issues: diagnostic criteria and early diagnosis. In 2004, the Acute Dialysis Quality Initiative (ADQI) sought a more uniform definition of AKI, and the most recent consensus definition was published in 2012 by Kidney Disease: Improving Global Outcomes (KDIGO) [8]. The diagnosis of AKI is based on changes in serum creatinine (Scr) and urine output (UO), but neither marker is kidney specific. Efforts have been made to identify novel biomarkers that have high sensitivity and specificity.

The standardization of the diagnosis of AKI allowed us to compare the diagnoses made in different settings. However, the issue of early diagnosis is still a challenge. First, there are limitations regarding the use of Scr and UO. Second, determining the need for renal replacement therapy is difficult due to a lack of information about whether the AKI is transient or persistent. Last, the research advances identifying early biomarkers have thus far been inaccessible in clinical practice [12].

Neutrophil gelatinase-associated lipocalin (NGAL) [13] has been far and away the most promising biomarker to help fill this gap, and its diagnostic capabilities as a biomarker have been confirmed in a large number of clinical trials. This chapter aims to present information on the role of NGAL in the renal injury process, its expression in the kidney, confounding factors, the type of assay used, whether plasma or urine NGAL has better accuracy, the cutoff values in normal individuals, the accuracy of NGAL for diagnosing AKI, and the evaluation of other outcomes.

2. The role of NGAL

In 1993, Kjeldsen et al. [13] isolated lipocalin as a protease-resistant polypeptide covalently bound to neutrophil gelatinase, named neutrophil gelatinase-associated lipocalin (NGAL), also known as siderocalin, lipocalin 2 or oncogene 24p [14].

NGAL is a 25 kilodalton (kDa) protein covalently bound to gelatinase in neutrophil-specific granules. NGAL is expressed at very low levels in various human tissues, including the kidneys, uterus, prostate, salivary gland, trachea, lungs, stomach, and adult and fetal colon [15, 16]. The anti-inflammatory function of NGAL is demonstrated by increased NGAL expression in proliferative epithelia, inflammatory areas, and intestinal malignancies [17].

In normal kidneys, the expression of NGAL is mainly released by the thick ascending limb and the intercalated cells of the thick collecting duct. Some NGAL expression is also present in the proximal tubular epithelium, once NGAL is filtered by the glomerulus and reabsorbed by the proximal tubule in a megalin-dependent manner [17, 18]. The physiological function of NGAL in the kidneys is unknown; however, the role of NGAL in renal morphogenesis is under consideration [19]. NGAL also has a predominant role in the regulation of cell proliferation, repair processes, and tubular reepithelization. NGAL expression corresponds to an additional iron transport pathway, which increases the transcription of hemeoxygenase, an enzyme with proliferative and antiapoptotic effects that protects and preserves proximal tubular cells [20, 21].

Several biological functions for NGAL have been suggested; in the kidney, NGAL release is associated with ischemic or nephrotoxic insults. Additionally, a decrease in tubular reabsorption after AKI may lead to a further increase in urinary NGAL concentration, resulting acquiring a status of the "troponin" of the kidneys [22–24].

KEY POINT: The role of NGAL remains unclear, but its release mainly from the distal tubule has been associated with an increase in kidney injury.

3. Confounding factors affecting NGAL

The conditions that can interfere with the performance, sensitivity, and specificity of NGAL, already identified as a biomarker, are sepsis, chronic obstructive pulmonary disease, and cardiac dysfunction, and the presence of these conditions may act as confounding factors for NGAL measurements. The predictive performance of NGAL seems to also be influenced by age (higher predictive value

in children than in older patients), sex (higher predictive value in female patients than in male patients), urinary tract infection, and impaired renal function (higher predictive value in patients with chronic kidney disease) [25–27].

Sepsis will be more thoroughly addressed in the next chapter.

KEY POINT. Controlling for confounding factors in clinical trials is vital to maintain the internal validity of a study.

4. Types of NGAL assays

The commercialization of NGAL as the gold standard for the diagnosis of AKI is somewhat controversial [28]. There are many types of NGAL assays available on the market that use different nonautomated ELISA platforms, which makes it difficult for comparisons to be made among studies from around the world.

The first kit for the quantitative and automated determination of NGAL by the ELISA method was developed by Abbott Laboratories (Abbott Park, IL, USA) for the urinary evaluation of NGAL, with a cutoff value of 141 μg/L (95% CI 125–158 μg/L). An EDTA plasma blood test was created by the Triage Meter platform (Biosite-Inverness Medical, Waltham, USA) with a cutoff value of 163 μ/L (CI 109–221 μ/L) [29].

Bioporto (Bioporto Diagnostics A/S, Gentfte, Denmark) developed a new particle-enhanced turbidimetric immunoassay (PETIA) that has the advantages of flexibility (adaptation for clinical use in different analyzers), automation (closer to clinical practice), and applicability in different biological matrices (urine and plasma) [30].

The chemiluminescence test is an alternative to assess NGAL, and it is commonly used to analyze studies with small animals since it is possible to do the analysis with few substrates [31].

Because there are three known molecular forms of NGAL, the assay of interest should differentiate the 25 kDa NGAL monomer produced by the monocyte tubular epithelial cells from other forms of NGAL: 45 kDa NGAL, from the homodimer predominantly secreted by neutrophils, and the 145 kDa NGAL/matrix metalloproteinase-9 (MMP9) covalently complexed heterodimer [32, 33].

According to Mårtensson et al. [34] and Cai et al. [33], the combination of two ELISAs, may improve the diagnostic accuracy of NGAL, one to determine the monomeric form and the other to determine the homodimeric form.

The confounding factor of sepsis is described herein and is dependent on the assay method, as well as whether the chosen kit is less sensitive to 25 kDa NGAL expressed by tubular epithelial cells. The test can also measure the 45 kDa homodimer predominantly secreted by neutrophils, which are common in sepsis, and the increase in neutrophils increases homodimeric NGAL expression and results in false positives.

KEY POINT: Choose a method closer to those used in clinical practice and a test more accurate for measuring the monomeric form of NGAL expressed by tubular epithelial cells after injury.

5. Plasma or urine NGAL measurement and normalization of urine values

The consensus is clear that NGAL measured in urine has better performance [35], because the release and increase in NGAL will occur first in urine. However, the collection of urine depends on the urine output, which is sometimes not available. Some benefits of plasma NGAL are that it is available at any time and is more accurate in anuric or oliguric patients.

The issue about the normalization of urinary NGAL by correction for the urinary creatinine level is debatable, but it has been used to correct for urine output in cases of oliguria or pollakiuria, avoiding inaccurate concentration or dilution measurements of the biomarkers. The fact is that the creatinine release time is different from the biomarker release time, and the normalized level will be affected by this difference and will not represent a real physiological value [36].

KEY POINT: Urinary NGAL is released earlier than plasma NGAL, and the accuracy of the normalization of urinary NGAL by creatinine is debatable.

6. Cutoff values of NGAL in normal individuals

The determination of normal NGAL levels in healthy adults has been inadequately described in the literature. However, some studies have been performed, such as that of Cullen et al. [26], which analyzed urine by the Abbot-Architec assay in 174 healthy people (100 men and 74 women aged between 19 and 88 years). The value of the immunoassay result was normalized by urinary creatinine, and the cut-off value was 107 μ/mmol (13 μ/mmol). There was a higher concentration of creatinine-normalized urinary NGAL in women, in the elderly and in patients with leukocyturia.

The study reported by Stejkal et al. [37] analyzed BioVendor's NGAL assay using the serum of 136 healthy, nonobese individuals (53 men and 83 women). The authors reported median NGAL values (78.8 μg/L for men and 80 μg/L for women). Pernnemans et al. [27] analyzed the NGAL ELISA (RD System Europe, Abingdon, UK) and other urinary biomarkers in 338 healthy individuals (199 women and 139 men, aged 0 to 95 years). They reported that the NGAL reference range of the 21–95 years age group was 73.88–211.16 μg/L in women and 149.26–182.58 μg/L in men, and there was higher expression in elderly individuals.

An NGAL PETIA (Bioporto Diagnostics A/S, Gentfte, Denmark) [36] was evaluated for 200 healthy nonobese individuals (137 men and 63 women, with a mean age of 39 years (SD 11.2)). They proposed reference plasma concentrations from 38.7–157.6 ng/ml for women and 24.4–142.5 ng/ml for men and proposed reference urine concentrations of <9–54.5 ng/ml for both sexes. The authors reported that the mean values in men were higher than in women, 78.9 ng/ml vs. 73.8 ng/ml, respectively; there was a significant difference in NGAL in relation to age.

In addition to the variability of the chosen immunoassay, the unit of measurement in the interpretation of the NGAL studies should be considered—the most commonly found are ng/ml, μg/L, ng/dl, mg/ml and μg/mmol.

KEY POINT: The median cutoff value for urinary NGAL in healthy men was between 78.8 and 182.58 μg/L, and the median plasma value was between 24.4 and 142.5 ng/ml in men. The broad variability of the results difficult to interpret.

7. NGAL to predict AKI

NGAL is the most widely investigated AKI biomarker. Its performance for predicting AKI has been evaluated in various settings, such as in pediatric and adult cardiac surgery patients, in critically ill patients, and in patients in the emergency room, as well in kidney transplant and other settings [38, 39].

Numerous studies have demonstrated the ability of NGAL to diagnose AKI. For example, the study reported by Constantin et al. [40], that evaluated the plasma NGAL of 88 patients at ICU admission, found a sensitivity of 82%, specificity of 97% and AUC of 0.92 to cut-off value of 155 mmol/L to predictor of AKI.

A multicenter study, reported by Di Somma et al. [41], with 665 patients admitted to the emergency department, assessed plasma NGAL in several points after admission. Serial evaluation of NGAL at times zero and six hours provided a high negative predictive value (NPV) (98%) to rule out the diagnosis of AKI within six hours of the arrival of patients to the emergency department. The NGAL value at admission could demonstrated a strong predictive value for in-hospital mortality of the patient, with a cut-off value of 400 ng/ml.

In the meta-analysis by Haase et al. [42]—with 19 studies, totaling 2538 patients, of whom 487 (19.2%) developed AKI—NGAL was demonstrated to have diagnostic and prognostic value for AKI, with an OR of 18.6 (95% CI 9–38.1) and AUC of 0.81 (95% CI 0.73–0.89). The cut-off value ranged from 100 to 270 ng/ml, but a value of 150 ng/dl was suggested for the diagnosis of AKI.

In another recent meta-analysis by Zhou et al. [39]—with 24 studies, a total of 4066 patients from 9 countries, including studies with serum and urinary NGAL—the sensitivity for the diagnosis of AKI was 0.68 (95% CI, 65–0.70), and the specificity was 0.79 (95% CI 0.77–0.80).

In the study by Singer et al. [38], urinary NGAL was useful for classifying and stratifying patients with established AKI: the level of NGAL>104 μg/L indicated intrinsic AKI (odds ratio of 5.97), while the level of NGAL <47 μg/L indicated unlikely intrinsic AKI (odds ratio of 0.2). In the logistic regression analysis, NGAL was able to predict the worsening of the RIFLE class, the need for RRT and in-hospital mortality. The performance of NGAL to evaluate other outcomes will be discussed in the next chapter.

KEY POINT: Despite the good results of NGAL for predicting AKI, the variability of the cutoff value is still a challenge for applying NGAL in clinical practice.

8. The early timing diagnosis by NGAL versus standard serum creatinine

The study by Bennett et al. [43] clearly indicated that urinary NGAL is a powerful early biomarker of AKI after cardiopulmonary bypass that preceded the increase in serum creatinine by 2–3 days. Studies have shown that elevation of NGAL is detectable after 3 hours and peaks approximately 6–12 hours after injury. The elevation can persist up to 5 days according to the severity of injury [44–46]. In addition to Benett's study, other studies in general have failed to reach conclusions about the early timing diagnosis of NGAL, which is the main finding required to reach a therapeutic window and better evaluate future medication targets in AKI.

KEY POINT: If you perform a study or analyze a biomarker, remember to compare the pattern of biomarker early timing diagnosis with serum creatinine.

9. Evaluation of other outcomes by NGAL

Several studies have assessed the diagnostic value of NGAL to predict AKI, but only a few have analyzed the early diagnosis in hours/days and compared it with serum creatinine, as seen in the last chapter. Still fewer studies have evaluated the predictive performance of NGAL in other outcomes, such as the need for RRT, recovery of renal function, progression to end stage renal disease (ESRD) and mortality, which will be discussed in this chapter.

In the meta-analysis by Hall et al. [47], for 91 kidney transplant patients, the incidence of need for RRT was 4.3%, and NGAL, in this scenario, had an OR of 12.9 and AUC of 0.78. In the same study, NGAL and urinary IL18 were predictors of the need for RRT up to 1 week after transplantation. NGAL presented a good AUC of

0.81 (95% CI 0.70–0.92) 6 hours after transplantation and was also a predictor of graft recovery for up to 3 months.

In the study conducted by Constantin et al. [40], the cutoff value of NGAL to assess the need for RRT was 330 mmol/L. The value of urinary NGAL (Architect, Abbot Park, IL) was correlated with the need for dialysis (r: 0.48 P: 0.01), presenting an AUC of 0.86 2 hours after cardiopulmonary bypass in children [43].

A recent meta-analysis by Klein et al. analyzed 12 studies to predict the need for RRT and found an AUC of 0.70 (95% CI 0.63–0.80) for NGAL [48].

Bhavsar et al. [49] concluded that higher levels of NGAL (measured by the Luminex assay) were associated with stage 3 CKD incidence. Some researchers have discussed whether the association of NGAL level is not exclusively related to the increase in neutrophils already described by Tian et al. [50] and maintain that further studies would be needed to elucidate this issue.

In the meta-analysis by Haase et al. [42], the incidence of mortality was 5.4%, and NGAL, in this scenario, showed an OR of 8.8 and AUC of 0.70.

In the Ariza study [51], PNGAL and UNGAL were demonstrated to be strong predictors of prognosis, and UNGAL was significantly predictive of the MELD score using the 28-day mortality score AUC of 0.88 (0.83–0.92).

In the study by Bennett et al. [43], the value of urinary NGAL (Architect, Abbot Park, IL) was also correlated with mortality (r: 0.53 p 0.01), with an AUC of 0.91, 2 hours after cardiopulmonary bypass in children.

The study by Dent et al. [52], using PNGAL (Biosite Inc., San Diego, USA) in 120 children undergoing cardiopulmonary bypass (CBP) and a cut-off value of 150 ng/ml and AKI prediction, found an AUC of 0.96 2 hours after CBP. PNGAL was also strongly correlated with the duration of AKI ($r = 0.57$, $p < 0.001$) and hospital stay time ($r = 0.44$, $p < 0.001$), and PNGAL at 12 hours was correlated with mortality ($r = 0.48$, p: 0.004).

In the study by Daniels et al. [53], PNGAL (Alere Inc., Waltham, USA) was measured in 1393 adult patients with cardiovascular disease (CVD) who were followed for 11 years. Of these, 436 did not survive, and 169 died from CVD. PNGAL was a predictor of CVD mortality, with a risk ratio of 1.33% and a risk ratio of 1.19% for all causes of mortality.

KEY POINT: Evaluating outcomes by NGAL beyond the limitation of only the diagnosis of AKI is important to know how more than one parameter evaluates the outcome and prognosis, and it could help physicians by indicating an early need for RRT, for example.

10. Conclusion

This brief review, based on accumulated evidence, discussed the role and value of NGAL in the diagnosis and prognosis of AKI. Studies' findings suggest that induction of NGAL plays an important role in kidney function preservation, reducing apoptosis, and enhancing proliferative responses. In kidney injury, rapid and massive upregulated synthesis of NGAL occurs in the distal tubule, which quickly increases the concentration of NGAL in urine [54]. In addition, other important considerations have been provided as "key point" to help researchers move the NGAL analysis to clinical practice as soon possible.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this chapter.

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