

The Rise and Fall of NGAL in Acute Kidney Injury

Johan Mårtensson^{a, d} Rinaldo Bellomo^{a–c}

^aDepartment of Intensive Care, Austin Hospital, and ^bAustralian and New Zealand Intensive Care Research Centre, and ^cDepartment of Epidemiology and Preventive Medicine, Monash University, Melbourne, Vic., Australia;

^dSection of Anaesthesia and Intensive Care Medicine, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Key Words

Neutrophil gelatinase-associated lipocalin · Acute kidney injury · Intensive care · Inflammation

Abstract

For many years, neutrophil gelatinase-associated lipocalin (NGAL) has been considered the most promising biomarker of acute kidney injury (AKI). Commercial assays and point-of-care instruments, now available in many hospitals, allow rapid NGAL measurements intended to guide the clinician in the management of patients with or at risk of AKI. However, these assays likely measure a mixture of different NGAL forms originating from different tissues. Systemic inflammation, commonly seen in critically ill patients, and several comorbidities contribute to the release of NGAL from haematopoietic and non-haematopoietic cells. The unpredictable release and complex nature of the molecule and the inability to specifically measure NGAL released by tubular cells have hampered its use as a specific marker of AKI in heterogeneous critically ill populations. In this review, we describe the nature and cellular sources of NGAL, its biological role and diagnostic ability in AKI and the increasing concerns surrounding its diagnostic and clinical value.

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Introduction

The predictive and diagnostic value of neutrophil gelatinase-associated lipocalin (NGAL) in acute kidney injury (AKI) has been assessed for more than a decade [1]. Such assessment was triggered by genomic and subsequent proteomic analyses showing that the NGAL protein was highly induced in animal kidneys and released in the urine following ischaemic or nephrotoxic insults [1, 2]. After the first promising validation study in humans [3], NGAL was for several years considered the ‘troponin’ of the kidney [4]. However, unlike myocardial infarction, AKI is rarely triggered by ischaemia. Instead, the underlying pathophysiology is characterized by a complex interaction between predisposing chronic illnesses, haemodynamic disturbances, nephrotoxic insults and inflammatory responses leading to tubular cell injury and eventually a fall in glomerular filtration rate [5]. Adding to this complexity is the fact that the NGAL molecule is produced by a number of tissues in different molecular forms [6, 7]. Indeed, the inability to distinguish the specific molecular forms produced by the kidney from other forms released by non-renal tissues has hampered its use as a reliable biomarker of AKI in the critically ill patient.

This review summarizes the current knowledge about NGAL, its cellular origin and possible role in inflammation and organ damage with focus on AKI and highlight growing concerns surrounding the diagnostic value and clinical utility of NGAL.

The Nature and Cellular Sources of NGAL in Humans

NGAL, also known as human neutrophil lipocalin or lipocalin 2, was purified from the secondary granules of human neutrophils for the first time in the early 1990s by two independent Scandinavian groups [8, 9]. Western blot analysis revealed that the NGAL molecule exists in three different molecular forms in blood and urine; as a 25-kDa monomer, as a 45-kDa disulphide-linked homodimer and as a 135-kDa heterodimer, covalently conjugated with gelatinase [matrix metalloproteinase (MMP)-9] [8, 9].

NGAL is synthesized in the bone marrow during myelopoiesis from where it is directed to and stored in the neutrophil granules [10]. In addition, Cowland and Borregaard [6] demonstrated that NGAL mRNA is expressed in several non-haematopoietic tissues, such as colon, trachea, lung and kidney epithelium. Interleukin-1 β , an inflammatory mediator, stimulates NGAL synthesis in a number of these cell lines in vitro [7, 11]. Besides, elevated plasma levels observed during conditions such as acute peritonitis, acute exacerbations of obstructive pulmonary diseases and acute bacterial infections support its role in tissue inflammation [12–14].

The nature of the NGAL molecule is complex and seems to be related to its cellular origin. Results from in vitro experiments indicate that activated neutrophils mainly release homodimeric NGAL and to a lesser extent the monomeric form [7]. In contrast, stressed kidney epithelial cells predominantly secrete monomeric NGAL apparently unable to form dimers. This is supported by elevated urinary homodimeric levels seen in patients with urinary tract infections and a relative abundance of monomeric NGAL in AKI patients [7, 15]. Although specific for tubular cells, the heterodimeric form seems to exist in very low concentrations, even during AKI [7].

NGAL-Mediated Iron Traffic

Several biological functions for NGAL have been suggested. By its ability to bind siderophores (small iron-binding molecules), NGAL is involved in the iron trans-

port to and from cells [16]. Iron is vital for bacterial proliferation and is acquired from the host by the release of siderophores. Hence, NGAL acts as a bacteriostatic agent by sequestering iron [17, 18]. While neutrophils provide the organism with a mobile source of NGAL, the production by epithelial cells may be important for the local defence against infections.

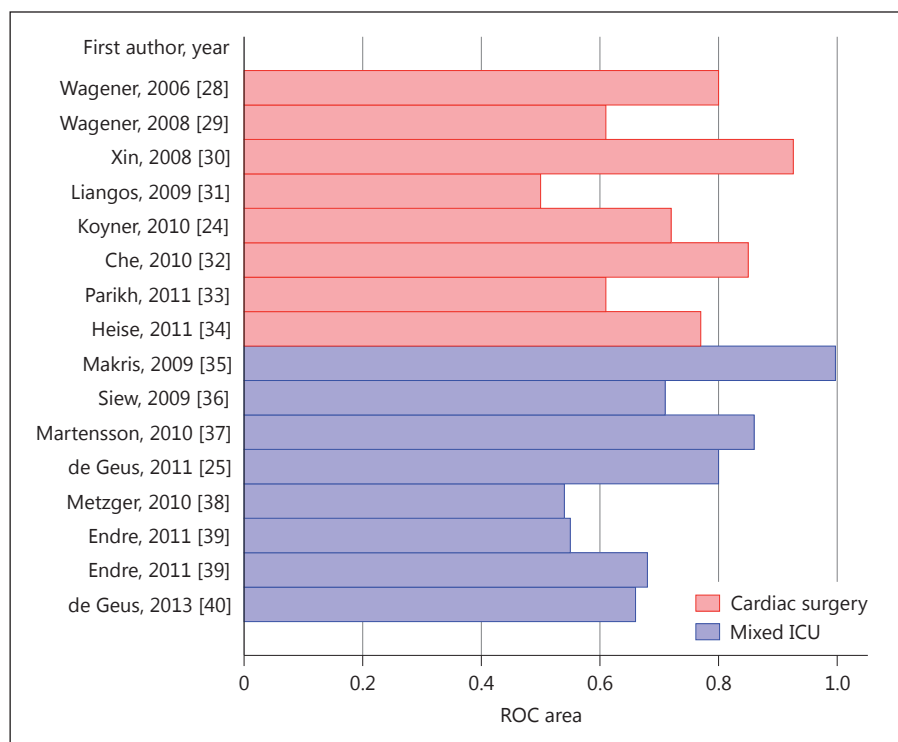
Excess extracellular iron may cause organ injury owing to its ability to catalyse the conversion of hydrogen peroxide to free oxygen radicals. Induced NGAL synthesis during cellular stress/inflammation may reduce extracellular iron-induced injury. Iron is also vital for the proliferation and differentiation of human cells. Yang et al. [19] found that NGAL promoted iron-dependent differentiation of mesenchymal progenitors into complete nephrons during the development of the kidneys. The renoprotective role of NGAL is supported by animal models of ischaemia-reperfusion-induced AKI, where intravenously administered NGAL was rapidly taken up by proximal tubular cells and reduced tubular damage and apoptosis as well as increased cell proliferation [20, 21].

NGAL in Acute Kidney Injury

During the last decade, focus has shifted and NGAL has moved from being a marker of undifferentiated systemic inflammation to become a possible marker for early detection of AKI. This was triggered by genomic analyses showing that NGAL was one of the most rapidly up-regulated genes after ischaemic AKI in animals [1, 22]. Proteomic analyses verified that the NGAL protein was induced in the kidneys following ischaemic and nephrotoxic AKI and that urinary concentration increased several-fold early on (within hours) after the insult [1, 2]. Subsequent, studies on critically ill patients have consistently shown an association with levels in plasma or urine and severity of established AKI [23–27].

The first study evaluating NGAL as a predictor of evolving AKI was conducted on children undergoing cardiac surgery. Urinary NGAL rose almost 100-fold and serum NGAL 20-fold up to 48 h before AKI was detected by creatinine. The urinary NGAL level was an almost perfect AKI predictor with an area under the receiver operating characteristics curve (ROC area) of 0.998 [3]. Since obtaining these encouraging results, the predictive performance has been tested in various clinical settings. Particularly in adult patients the results have been rather disappointing with ROC areas for urinary NGAL to predict moderately severe AKI (RIFLE R and/or AKIN stage 1)

Fig. 1. Studies in mixed ICU and cardiac surgery patients investigating the value (ROC area) of urinary NGAL to predict AKI defined by RIFLE R and/or AKIN stage 1.



ranging from 0.50 to 0.93 after cardiac surgery [24, 28–34] and from 0.54 to 0.99 in general ICU patients [25, 35–40] (fig. 1). Although the performance increases with AKI severity [25], the ROC areas for predicting the progression to severe AKI (RIFLE I and/or AKIN stage 2) was <0.70 in the most recent studies [26, 41].

The non-consistent performance across studies in critically ill adult patients can be explained by several factors. First, comparing a biomarker of assumed parenchymal kidney injury against a reference method (i.e. creatinine) that imperfectly estimates functional kidney impairment (clinical AKI) is problematic. Due to continuing loss of muscle mass in the critically ill [42] and dilution of serum creatinine in fluid-loaded patients [43], AKI might go undetected by conventional creatinine-based criteria. Emerging evidence shows that patients with elevated NGAL in the absence of creatinine-based criteria for AKI carry an increased risk of adverse events including need for renal replacement therapy and death [44, 45]. Whether this specifically represents subclinical AKI or is simply an expression of severe systemic inflammation is yet to be determined.

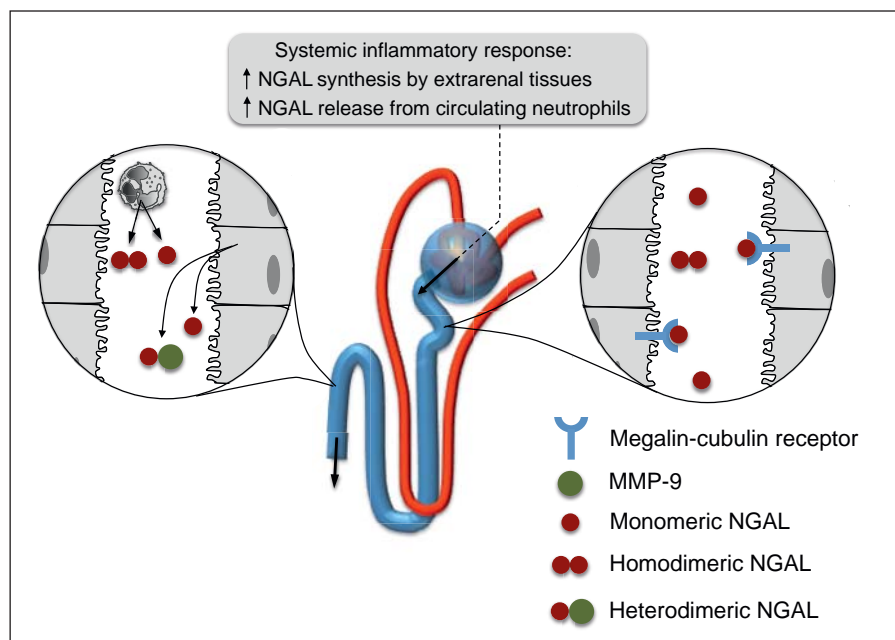
Second, the burden of comorbidities, especially chronic kidney disease (CKD), may affect the results since CKD per se is associated with elevated serum and urinary NGAL levels [46, 47]. In fact, McIlroy et al. [48] found

that NGAL only predicted AKI in patients with normal baseline renal function. The notion that comorbidities are important confounders in the studies is further supported by the fact that the predictive performance is generally better in children [49].

Third, the time from biomarker measurement to AKI diagnosis differs substantially among studies. It could be expected that predictive values would increase when NGAL is measured closer to the time of insult. This relation is not, however, seen in the studies. Notably, Kashani et al. [26] found only a limited performance (ROC area 0.69) when NGAL was used to predict severe AKI within 12 h in a large study.

Finally, existing studies are affected by the limited ability of the NGAL assays to distinguish between the various molecular forms released by different tissues. Systemic inflammation triggered by conditions such as sepsis or procedures like cardiopulmonary bypass (CPB) is strongly associated with AKI development [50]. At the same time, such inflammatory triggers will activate circulating neutrophils to release their granular contents, including NGAL. In addition, NGAL synthesis increases in non-haematopoietic cells in various tissues such as the lung [51] and the liver [18] as part of the inflammatory response. The resultant increase in plasma NGAL will in-

Fig. 2. Nature and source of NGAL in plasma and urine during AKI. Systemic inflammation induces NGAL synthesis by extra-renal tissues and the release of NGAL from neutrophils. Urinary NGAL increases due to an impaired reabsorption of the filtered load (downregulation of megalin-cubulin receptors in the proximal tubule), increased synthesis from stressed tubular cells in the distal nephron and release by infiltrating neutrophils. Neutrophils mainly release the dimeric form (and to some extent the monomeric form), whereas tubular cells mainly produce the monomeric form and to some extent NGAL conjugated with MMP-9 (heterodimeric NGAL).



crease the filtered load and hence urinary levels irrespective of any potential kidney damage. This is supported by studies showing elevated levels in plasma [52] and urine [53] after cardiac surgery in patients without postoperative AKI. The fact that NGAL levels are correlated with CPB time and that homodimeric NGAL is the predominant form in these patients' urine [53] suggests that the neutrophils, rather than the kidney tubular cells, are the main source. Similar conclusions can be drawn from general ICU populations where sepsis is associated with elevated plasma NGAL independent of degree of renal impairment [37, 54].

Based on these findings, NGAL might be a more specific AKI marker in patient populations where the 'noise' from severe systemic inflammation and multi-organ damage is less pronounced. In fact, this was shown in a 635-patient emergency department cohort where only 6% were admitted to the ICU [55]. The authors found that urinary NGAL distinguished sustained AKI (RIFLE R for at least 3 days despite fluid resuscitation) from non-AKI with or without concomitant stable CKD with high sensitivity (90.0%) and specificity (99.5%). The prevalence and hence the possible confounding effect of sepsis was low in this study, which likely contributed to the excellent predictive values. A similar study investigating septic patients on emergency department admission found plasma NGAL to be a sensitive (sensitivity 0.96) but non-specific (specificity 0.51) AKI predictor [56].

Under normal conditions, filtered NGAL is almost completely reabsorbed by the proximal tubules via megalin-cubulin receptor-mediated endocytosis, resulting in minimal urinary NGAL levels [21, 57]. The total concentration of urinary NGAL in AKI probably represents a mixture of different molecular forms of NGAL with different cellular origins (fig. 2). Monomeric NGAL increase either due to an induced synthesis in the tubular cells or as an effect of impaired reabsorption of the filtered load produced by extra-renal tissues [21, 58]. Furthermore, infiltration of neutrophils in the kidney has been observed in both animal models and in biopsy specimens from patients with AKI [59, 60]. Dimeric NGAL in urine might emanate from these infiltrating neutrophils, but glomerular filtration of dimeric NGAL released from activated neutrophils in the circulation, e.g. in septic patients, may also contribute.

The different forms of NGAL (monomeric, dimeric, heterodimeric) expose different epitopes and the configuration of antibodies will have an impact on the clinical performance of the assay [53]. Notably, most studies use commercial NGAL assays such as the BioPorto (Gentofte, Denmark) and R&D Systems (Minneapolis, Minn., USA) assays. Unfortunately, these assays are unable to specifically measure NGAL secreted by stressed or injured tubular cells. Although a dimer-specific assay with the potential to quantify neutrophil-derived NGAL was recently proposed, efforts to amplify the monomeric, tubular cell-specific signal have been less successful [15, 61].

The fervent introduction of presumed tissue-specific biomarkers has pervaded the field of intensive care medicine for many years. These biomarkers have traditionally been discovered and validated in patients with an isolated organ injury. Indeed, biomarkers of acute ischaemic myocardial injury (troponins), heart failure (brain natriuretic peptide), traumatic brain injury (S-100) and ischaemic brain injury (neuron-specific enolase) are of added value in the decision process and their use may even affect outcomes in selected patient populations [62–65]. However, ICU patients at risk of AKI suffer from a strong inflammatory response with stress/injuries in several organs, are exposed to repeated invasive procedures, fluid therapy and vasopressor support, blood transfusion and multiple other biological modifiers.

Therefore, it is not surprising that tissue-specific biomarker signals drown in the sea of such confounders. Indeed, ‘biomarkeraemia’ is frequently seen in ICU patients and is likely a signal of overall illness severity rather than specific organ damage [65–67].

Now, NGAL can be added to the existing list of biomarkers of single organ injury that appear to perform well in specific populations but show a major loss of diagnostic accuracy when used in the ICU, where they likely represent yet another biological expression of illness severity.

Since the rise of NGAL as a potential biomarker of early AKI, beginning for more than a decade ago, numerous studies have investigated its role in various clinical situations. However, the idea of NGAL as a troponin for the kidney has fallen as the understanding of the molecule’s complex nature has emerged. NGAL levels increase unpredictably during evolving AKI but also during other chronic and acute inflammatory conditions frequently encountered in the ICU, such as in sepsis, after CPB surgery, in CKDs and during urinary tract infections and acute exacerbations of obstructive pulmonary diseases. Therefore, adding NGAL to the clinical evaluation of critically ill patients with or at risk of AKI has to wait until assays measuring kidney-specific NGAL are available. Simultaneously, the ability of dimeric, neutrophil-derived NGAL to detect severe sepsis, the most common trigger of AKI in the ICU, needs to be further explored. Until such studies are completed, NGAL measurement, which had shown such promise early on, should be confined to research protocols and to the assessment of selected emergency department patients.

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