Neutrophil Gelatinase-associated Lipocalin: An Emerging Biomarker for Angina Renalis

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Introduction

Angina pectoris is symptomatic, and sensitive biomarkers such as troponin that are released from affected myocytes have revolutionized the early diagnosis and successful treatment of ischemic myocardial injury. In contrast, ischemic acute kidney injury (AKI), analogously referred to as *angina renalis*, is largely devoid of symptoms. Although common and serious (about one-third of intensive care patients develop AKI), the diagnosis using conventional biomarkers such as serum creatinine is unreliable and delayed [1]. Therefore, potentially effective interventions are delayed, and AKI remains an important contributor to the high mortality rate in affected critically ill subjects [2, 3]. Fortunately, recent advances have unraveled the early stress response of kidney tubule cells to ischemic injury, and have provided several novel biomarkers for AKI [4–6]. The current status of neutrophil gelatinase-associated lipocalin (NGAL), possibly the most promising AKI biomarker in intensive care and emergency medicine settings, is outlined below.

Discovery of NGAL as an AKI Biomarker

Preclinical high-throughput gene expression experiments identified *Ngal* (also known as lipocalin 2 or *lcn2*) to be one of the most upregulated genes in the kidney very early after acute injury in animal models [7]. NGAL was also found to be one of the most highly induced proteins in the kidney after ischemic or nephrotoxic AKI, and the NGAL protein was easily detected in the urine and blood in animal models [8–10]. In a translational human cross-sectional study, patients in the intensive care unit (ICU) with established AKI (doubling of serum creatinine) displayed a marked increase in urine and serum NGAL by Western blotting [11]. Urine and serum NGAL levels correlated with serum creatinine, and kidney biopsies in subjects with AKI showed intense accumulation of immunoreactive NGAL in cortical tubules, confirming NGAL as a sensitive index of established AKI in humans.

NGAL for the Early Diagnosis of AKI

An increasing number of human studies have now identified NGAL as an early noninvasive diagnostic biomarker of AKI in common intensive care and emergency department settings, preceding a rise in serum creatinine by several hours to days (**Table 1**). In prospective studies of children with normal kidney function and no co-

Table 1. NGAL for the early prediction of acute kidney injury (AKI) and its clinical outcomes

Biomarker property	Cardiopulmonary bypass (CPB)	Contrast-induced nephropathy	Kidney transplantation	Critical care or emergency setting
Time post-event	2 h post-CPB	2 h post-contrast	2–12 h post-transplant	At presentation
Time preceding AKI	2 days pre-AKI	1–2 days pre AKI	2–3 days pre-DGF	2 days pre-AKI
ROC AUC	0.61–0.99	0.91–0.92	0.90	0.78–0.95
AKI outcomes predicted	Duration, severity, death	No data available	Duration, severity	Duration, severity, death
References	[12–18, 30–35]	[22–26]	[19–21]	[27–29]

AKI, defined as a 50 % or greater increase in serum creatinine from baseline. DGF: delayed graft function, defined as dialysis requirement within the first week after transplant. ROC AUC, area under the receiver operating characteristic curve. Times shown are the earliest time points when the biomarker becomes significantly increased from baseline.

morbid conditions who underwent cardiopulmonary bypass (CPB) surgery, AKI (defined as a 50 % increase in serum creatinine) occurred 2–3 days postoperatively [12–14]. In contrast, NGAL measurements by ELISA revealed a dramatic increase in the urine and plasma, within 2–6 hours of cardiac surgery in those who subsequently developed AKI. Both urine and plasma NGAL were excellent independent predictors of AKI, with an area under the curve (AUC) of > 0.9 for the 2–6 hour urine and plasma NGAL measurements [12–14]. These findings have now been confirmed in prospective studies of adults who developed AKI after CPB surgery, in whom urinary NGAL was significantly elevated by 1–3 hours after the operation [15–17]. These early urinary NGAL measurements correlated well with bypass time and aortic cross-clamp time, whereas neither peak serum creatinine nor relative changes in serum creatinine correlated with these well known indices of intraoperative renal hypoperfusion [17]. The AUCs for the prediction of AKI were in the 0.61–0.80 range, the somewhat inferior performance perhaps reflective of confounding variables (such as old age, pre-existing kidney disease, prolonged bypass times, chronic illness, and diabetes), variations in the definition of AKI, and technical issues with the NGAL measurements [3]. A recent prospective study in adult cardiac surgical patients has also revealed a good predictive value of plasma NGAL measurements obtained on arrival in the ICU, with an AUC of 0.80 for the prediction of AKI (> 50 % increase in serum creatinine) which occurred 1–2 days later [18].

NGAL has been evaluated as a biomarker of AKI in kidney transplantation. Protocol biopsies of kidneys obtained 1 hour after vascular anastomosis revealed a significant correlation between NGAL staining intensity and the subsequent development of delayed graft function [19]. In a prospective multicenter study, NGAL levels in urine samples collected on the day of transplant identified those who subsequently developed delayed graft function (which typically occurred 2–4 days later), with an AUC of 0.9 [20]. Plasma NGAL measurements have also been correlated with delayed graft function following kidney transplantation from donors after cardiac death [21].

Several investigators have examined the role of NGAL as a predictive biomarker of nephrotoxicity following contrast administration [22–26]. In a prospective study

of children undergoing elective cardiac catheterization with contrast administration, both urine and plasma NGAL predicted contrast-induced nephropathy (defined as a 50 % increase in serum creatinine from baseline) within 2 hours after contrast administration, with an AUC of 0.91 – 0.92 [26]. In studies of adults administered contrast, an early rise in both urine (4 hours) and plasma (2 hours) NGAL were documented, in comparison with a much later increase in plasma cystatin C levels (8 – 24 hours after contrast administration), providing further support for NGAL as an early biomarker of contrast nephropathy [23, 24].

Urine and plasma NGAL measurements represent early biomarkers of AKI in the pediatric intensive care setting, being able to predict this complication about 2 days prior to the rise in serum creatinine, with high sensitivity and AUCs of 0.68 – 0.78 [27, 28]. In patients seen in the emergency department for a myriad of reasons, a single measurement of urine NGAL at the time of initial presentation predicted AKI with an outstanding AUC of 0.95 [29]. Thus, NGAL is a useful early AKI marker that predicts development of AKI even in heterogeneous groups of patients with multiple co-morbidities and with unknown timing of kidney injury.

The results described thus far have been obtained using research-based assays, which are not practical in the clinical setting. In this regard, a major advance has been the development of a standardized point-of-care kit for the clinical measurement of plasma NGAL (Triage® NGAL Device, Biosite Incorporated). In children undergoing cardiac surgery, the 2-hour plasma NGAL measurement measured by the Triage® Device showed an AUC of 0.96, sensitivity of 0.84, and specificity of 0.94 for prediction of AKI using a cut-off value of 150 ng/ml [30]. The assay is facile with quantitative results available in 15 minutes, and requires only microliter quantities of whole blood or plasma. In addition, a urine NGAL immunoassay has been developed for a standardized clinical platform (Architect® analyzer, Abbott Diagnostics). In children undergoing cardiac surgery, the 2-hour urine NGAL measurement by Architect® analyzer showed an AUC of 0.95, sensitivity of 0.79, and specificity of 0.92 for prediction of AKI using a cut-off value of 150 mg/ml [31]. This assay is also easy to perform with no manual pretreatment steps, a first result available within 35 minutes, and requires only 150 microliters of urine. Both assays are currently undergoing multicenter validation in adult populations.

NGAL for Monitoring the Response to AKI Therapy

Because of its high predictive properties for AKI, NGAL is also emerging as an early biomarker in interventional trials. For example, a reduction in urine NGAL has been employed as an outcome variable in clinical trials demonstrating the improved efficacy of a modern hydroxyethyl starch (HES) preparation over albumin or gelatin in maintaining renal function in elderly cardiac surgery patients [32, 33]. Similarly, the response of urine NGAL was attenuated in adult cardiac surgery patients who experienced a lower incidence of AKI after sodium bicarbonate therapy when compared to sodium chloride [34]. In addition, adults who developed AKI after aprotinin use during cardiac surgery displayed a dramatic rise in urine NGAL in the immediate postoperative period, attesting to the potential use of NGAL for the prediction of nephrotoxic AKI [35]. The use of NGAL as a trigger to initiate and monitor novel therapies, and as a safety biomarker when using potentially nephrotoxic agents, is expected to increase in the near future.

NGAL for the Prediction of AKI Outcomes

Recent studies have demonstrated the utility of early NGAL measurements for predicting clinical outcomes of AKI in various common clinical settings (**Table 1**). In children undergoing cardiac surgery, the 2 hour post-operative plasma NGAL levels measured by the Triage® device strongly correlated with duration and severity of AKI, and length of hospital stay. In addition, the 12 hour plasma NGAL strongly correlated with mortality [30]. Similarly, the 2 hour urine NGAL levels measured by the Architect® analyzer highly correlated with duration and severity of AKI, length of hospital stay, dialysis requirement, and death [31]. In a multicenter study of children with diarrhea-associated hemolytic uremic syndrome, urine NGAL obtained early during the hospitalization predicted the severity of AKI and dialysis requirement with high sensitivity [36]. Early urine NGAL levels were also predictive of duration of AKI (AUC 0.79) in a heterogeneous cohort of critically ill subjects [27]. In adults undergoing CPB, those who subsequently required renal replacement therapy were found to have the highest urine NGAL values upon arrival in the ICU [16]. In adult kidney transplant patients undergoing either protocol biopsies or clinically indicated biopsies, urine NGAL measurements were found to be predictive of tubulitis or other tubular pathologies [37], raising the possibility of NGAL representing a non-invasive screening tool for the detection of tubulo-interstitial disease in the early months following kidney transplantation.

Limitations of NGAL as an AKI Biomarker

Clearly, NGAL represents a novel predictive biomarker for AKI and its outcomes. However, the majority of studies published thus far have involved relatively small numbers of subjects from single centers, in which NGAL appears to be most sensitive and specific in homogeneous patient populations with predictable forms of AKI. Plasma NGAL measurements may be influenced by a number of coexisting variables such as chronic kidney disease, chronic hypertension, systemic infections, inflammatory conditions, and malignancies [38–43]. In the chronic kidney disease population, plasma NGAL levels correlate with the severity of renal impairment [38, 42, 43]. However, the increase in plasma NGAL in these situations is generally much less than that typically encountered in AKI.

There is an emerging literature suggesting that urine NGAL is also a marker of chronic kidney disease and its severity [44]. In subjects with chronic kidney disease due to glomerulonephritides, urine NGAL levels were elevated and significantly correlated with serum creatinine, glomerular filtration rate (GFR) and proteinuria [45]. In patients with autosomal dominant polycystic kidney disease, urine NGAL measurements correlated with residual GFR and severity of cystic disease [40]. Urine NGAL has also been shown to represent an early biomarker for the degree of chronic injury in patients with IgA nephropathy [46], lupus nephritis [47, 48], and congestive heart failure [49], and may be increased in urinary tract infections. However, the levels of urine NGAL in these situations are significantly blunted compared to those typically measured in intrinsic AKI. This was most recently demonstrated in a study examining unselected patients presenting to an emergency room, in whom a single measurement of urine NGAL at the time of initial presentation reliably distinguished intrinsic AKI from prerenal azotemia and from chronic kidney disease [29].

Conclusion

NGAL as an AKI biomarker appears to have successfully passed through the preclinical, assay development, and initial clinical testing stages of the biomarker development process. It has now entered the prospective screening stage, facilitated by the development of commercial tools for the measurement of NGAL on large populations across different laboratories. The current status of NGAL as an AKI biomarker is shown in **Table 1**. But will any single biomarker such as NGAL suffice in AKI? In addition to early diagnosis and prediction, it would be desirable to identify biomarkers capable of discerning AKI subtypes, identifying etiologies, predicting clinical outcomes, allowing for risk stratification, and monitoring the response to interventions. In order to obtain all of this desired information, a panel of validated biomarkers may be needed. Other AKI panel candidates may include interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), cystatin C, and liver-type fatty acid binding protein (L-FABP), to name a few [1–6].

The availability of such a panel of AKI biomarkers could revolutionize renal and critical care. However, we must remain cognizant of the technical and fiscal issues surrounding the identification, validation, commercial development, and acceptance of multi-marker panels. Deriving from the recent cardiology literature, a clinically useful biomarker should (a) be easily measurable at a reasonable cost with short turnaround times; (b) provide information that is not already available from clinical assessment; and (c) aid in medical decision making [50]. In this respect, troponin as a stand-alone biomarker provides excellent diagnostic and prognostic information in acute coronary syndromes and acute decompensated heart failure. If the current prospective multicenter studies of NGAL measurements with standardized laboratory platforms provide promising results, we may already have a troponin-like biomarker for *angina renalis*.

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