Neutrophil gelatinase-associated lipocalin: utility in urologic conditions

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Abstract Neutrophil gelatinase-associated lipocalin (NGAL) is best known as a non-invasive early marker of acute kidney injury (AKI). However, recent published reports have described additional utility of both plasma NGAL (pNGAL) and urine NGAL (uNGAL) in various pathologic conditions within the pediatric urinary tract, including urinary tract infection, vesicoureteral reflux (VUR), renal scarring, and obstructive uropathy. These two forms of NGAL have different applications related to their mechanisms of upregulation: pNGAL can serve as a marker of systemic inflammatory conditions, whereas uNGAL is specific for insults to the renal epithelium. Therefore, pNGAL has good predictive accuracy in systemic inflammation associated with pyelonephritis and renal damage, while uNGAL is an effective marker for identifying infection within the genitourinary environment as well as subclinical renal damage as a result of scarring or obstruction. Continued work should focus on the effect of trending NGAL values in patients with pyelonephritis, VUR, and hydronephrosis, to determine if longitudinal NGAL patterns have value in predicting adverse outcomes.

Keywords Biomarkers · Urinary tract infections · Vesico-ureteral reflux · Pyelonephritis · Renal scar

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections in infants and children, but reliably identifying upper tract involvement can be challenging in the clinical setting. In a study recently published in *Pediatric Nephrology*, Kim et al. [1] investigated the utility of plasma neutrophil gelatinase-associated lipocalin (pNGAL) as a marker of acute pyelonephritis in children, also comparing its diagnostic accuracy to that of C-reactive protein, serum white blood count, and procalcitonin. In addition, these authors performed a receiver operating characteristic (ROC) analysis for these markers as predictors of hydronephrosis and vesicoureteral reflux (VUR). They found that by multivariable analysis pNGAL alone was a predictor of acute pyelonephritis, but not of hydronephrosis or VUR. Due to the complex interplay between urinary tract infections, VUR, and pyelonephritis, as well as the innate biologic differences between urinary NGAL (uNGAL) and pNGAL, we feel that this article deserves further comment.

pNGAL and uNGAL

Both pNGAL and uNGAL, the two forms of NGAL, have been the subject of much research in the literature on biomarkers. These forms have distinct mechanisms of induction and upregulation, allowing for different applications in clinical settings. Both pNGAL and uNGAL have been shown to have utility as early markers of acute kidney injury (AKI) [2]. However, even prior to the identification of NGAL as a marker of AKI, pNGAL had been shown to be secreted by neutrophils as a result of systemic inflammation [3]. uNGAL, on the other hand, is specific for insults to the genitourinary epithelium. Within the genitourinary tract, one of the main biologic

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roles of NGAL is iron trafficking, which has implications for epithelial cell growth, differentiation, and response to both injury and infection [4]. The role of NGAL in iron-trafficcking directly affects bacterial growth. Gram-negative bacteria require iron for growth and survival, and they obtain iron by producing a molecule called enterochelin that chelates free iron from the local environment and mediates its uptake by the bacterial for continued growth. NGAL competitively binds the enterochelin–iron complex and removes it through the urine, thereby exerting a powerful bacteriostatic effect as part of the innate immune system [4]. While this effect can be clearly seen in the urine, it is not as apparent in plasma given the confounding effect of systemic inflammation associated with Gram-negative bacteremia. Therefore, within the setting of infection, uNGAL levels are reflective of a localized infection within the genitourinary system, while pNGAL levels are more likely to be a result of the systemic inflammation associated with the infection. This difference is suggested by the data published in the article by Kim et al. [1]: the ROC curves for procalcitonin, C-reactive protein, and NGAL for predicting acute pyelonephritis are quite similar in appearance, with an area under the curve of 0.855, 0.879, and 0.893, respectively. A clear understanding of the biology of both the plasma and urinary forms of NGAL is required to determine their applications as biomarkers in the setting of infection.

NGAL and UTIs

The upregulation of NGAL in the setting of Gram-negative bacteriuria occurs as a result of the interaction of the bacteria and toll-like receptors at the alpha-intercalated cells of the kidney, leading to NGAL secretion into the urinary space [5]. This mechanism suggests that uNGAL may have utility as a marker of UTI. Indeed, early work by Yilmaz et al. [6] investigating the association between uNGAL and UTI supports this hypothesis, as children with acute UTI were found to have higher levels of uNGAL—both corrected and uncorrected for urine creatinine—than healthy children. Mouse models further support this association. Following transurethral inoculation with strains of uropathogenic *Escherichia coli*, NGAL knock-out mice have a higher bacterial burden for a significantly longer period of time compared to wild-type mice, highlighting the importance of NGAL in local bacteriostasis [7]. However, the relevant body of literature also contains conflicting reports on the utility of NGAL as a marker of UTI, with some studies not showing a difference in uNGAL levels between children with UTI and controls [8]. These conflicting results may be related in part to the specificity of uNGAL for Gram-negative bacteriuria or due to misclassification bias given that the UTI group in the study in which no difference was reported included patients

with asymptomatic bacteriuria in the UTI group [8]. In the study by Yilmaz et al. [6], all of the patients had *E. coli* as the causative agent of their UTI, whereas bacteriology data were not reported in the latter study by Kim et al. [8], and this lack of data may potentially confound the results. Despite these conflicting reports, both mouse and human data overwhelmingly support the association between UTI with Gram-negative bacteria and elevated uNGAL levels [6, 7, 9, 10].

NGAL in VUR and renal scarring

There is a well-known association between UTI and the presence of VUR in children [11]. However, there is disagreement regarding the management of patients with UTI in regards to screening for VUR. Much of this debate began with the publication of the American Academy of Pediatrics Guideline in 2011 [12, 13]. The controversy is mainly centered around the recommendation that voiding cystourethrograms (VCUGs) should not be routinely performed in children between 2 and 24 months of age with first time febrile UTIs [12]. Advocates of this approach are concerned about the invasive nature of VCUGs and the lack of an effective treatment for lower grades of VUR. However, proponents of the routine use of VCUGs cite the outcomes of reflux nephropathy and renal scarring, which have potential to cause significant morbidity, as well as the potential for delay of surgical intervention for patients with high grade VUR [14]. Given the invasiveness of VCUGs, and the potential for morbidity with untreated VUR, a non-invasive marker of VUR would have significant utility in the management of patients with first time UTIs.

It is difficult to comment upon the utility of NGAL in VUR without considering the presence of scarring given the degree of interrelatedness between them. Accordingly, much of the work done on NGAL and VUR also bears on renal scarring. NGAL is a potential candidate marker for VUR, and subsequent scarring, due to its specificity for renal tubular damage. However, the presupposition of this theory is that the damage associated with renal scarring is ongoing, and prior work has shown that uNGAL is not elevated in quiescent forms of chronic kidney disease [15]. Rat models of pyelonephritis show that the upregulation of NGAL gene expression peaks at 2 weeks following pyelonephritis, after which it decreases at both 4 and 6 weeks, but does not return to baseline levels [16]. Immunostaining of rat kidneys suggests that the NGAL upregulation seen at 2 weeks is an inflammatory response to the presence of the bacteria, but that the NGAL upregulation at 6 weeks, which is localized to the renal tubule, is in response to tubular injury rather than infection. Urine NGAL levels correspond to these transcriptome profiling results: uNGAL levels peak at 2 weeks

and then decrease by week 6, although not to baseline levels [17]. The differences in these uNGAL values, while of mechanistic interest, likely have little utility in clinical practice when examined at a single point in time. The clinical data in the literature agrees with this notion. While the difference between uNGAL levels in patients with scarring and those without is statistically significant, the values presented to date are all within the normal range of NGAL for age [18, 19]. Further, in a separate cross-sectional analysis of children with pyelonephritis, while there was a statistical difference in uNGAL levels between children who developed renal scarring and those that did not, the mean uNGAL values of patients who developed scarring (9.8 ± 4.5 ng/ml) and those who did not (7.2 ± 3.8 ng/ml) are still within the normal range of uNGAL for age [19, 20]. These results are again interesting from a mechanistic standpoint, but the value of using a single NGAL as a prognostic marker for the development of scarring is limited.

Despite the lack of utility of a single uNGAL value in this setting, following longitudinal uNGAL levels in children with known recurrent UTIs may provide a non-invasive method to monitor for progression of renal scars. As the data support the notion that uNGAL does not return to normal levels following the development of scarring as a result of pyelonephritis, then trending these values over time may have increased value compared to a single uNGAL measurement in the setting of an acute infection in determining the risk of scarring.

NGAL and urinary tract obstruction

Kim et al. [1] also investigated the utility of pNGAL, procalcitonin and C-reactive protein in predicting hydronephrosis in the setting of pyelonephritis. While these authors found that none of these markers had utility in diagnosing hydronephrosis, published studies by other groups which were better designed to study NGAL in obstructive uropathy do show that NGAL has utility in this clinical setting.

Urinary tract obstruction is a medical condition for which a biomarker has the potential to improve clinical care as serum creatinine lacks both sensitivity and specificity for unilateral obstruction, which can delay diagnosis. As NGAL is a marker of tubular injury, it may have clinical use as a marker of renal damage due to a variety of obstructive conditions. Early work in adults suggests that uNGAL is superior to serum creatinine in identifying both bilateral and unilateral obstruction [21]. In subsequent studies, uNGAL has shown promise as a marker of ureteropelvic junction obstruction in children [22] and in patients with renal dysfunction due to hydronephrosis [23]. Given the specificity of NGAL for tubular damage, it may have utility in distinguishing children with benign, self-resolving hydronephrosis from those with persistent or progressive hydronephrosis who might require intervention. Similar to following children with VUR, longitudinal assessments of NGAL levels in patients with prenatally diagnosed hydronephrosis, or otherwise incidentally detected hydronephrosis, may allow for the identification of a patient population characterized by ongoing renal damage that will disproportionately benefit from intervention.

NGAL and pyelonephritis

There are several other studies, in addition to the one published by Kim et al. [1], that have investigated both urinary and plasma NGAL levels in patients with pyelonephritis. While a study in adults found that uNGAL did not differentiate upper and lower tract infection, despite a significant difference in uNGAL between patients with UTI and controls, a pediatric study did report a difference in pNGAL levels between lower tract UTI and pyelonephritis [24, 25]. These results are consistent with the utility of pNGAL as a marker of inflammation and bacterial infection, while uNGAL is specific for localized infection within the genitourinary system. A combination of these forms of NGAL would capture the benefit of both these markers, with elevations in both pNGAL and uNGAL suggesting the presence of systemic inflammation,

Table 1 Suggested use of neutrophil gelatinase-associated lipocalin in urinary tract infection, pyelonephritis, vesicoureteral reflux, and hydronephrosis

Condition	Type of NGAL	Recommended use
Urinary tract infection	Urine	Single measurement in acute setting to identify Gram-negative urinary tract infection
Pyelonephritis	Plasma or plasma/urine combination	Single measurement in acute setting to differentiate upper tract infection and systemic inflammation from lower tract infection
VUR/prediction of scarring	Urine	Longitudinal measurement to monitor for development or worsening of scarring
Hydronephrosis	Urine or plasma	Longitudinal measurement to monitor for development of renal damage, or single measurement when there is concern for acute obstruction

NGAL, Neutrophil gelatinase-associated lipocalin; VUR, vesicoureteral reflux

with a UTI as the most likely source of inflammation. Conversely, a low pNGAL in the setting of an elevated uNGAL is indicative of lower tract infection that has not led to systemic inflammation.

The penultimate test of the utility of NGAL in identifying pyelonephritis will be to determine if knowledge of the NGAL value changes patient management and, most importantly, clinical outcomes. While knowledge of the presence of upper versus lower tract involvement may have long-term implications for the development of scarring, there may be limited clinical utility in this distinction at the point of care. Limited data exist to suggest differing antimicrobial management is warranted when there is renal parenchymal involvement, and the decision to admit a patient for observation is typically made based on clinical status. Future work on biomarkers of pyelonephritis should also focus on the clinical impact of NGAL in this setting.

Conclusion

Neutrophil gelatinase-associated lipocalin has the potential to improve our understanding and management of UTI and its associated complications (Table 1). However, prior to consideration of its clinical implementation in these scenarios, an understanding of the biology of NGAL within these medical conditions is necessary. Continued work should focus on the effect of trending NGAL values in patients with pyelonephritis, VUR, and hydronephrosis in order to determine if longitudinal NGAL patterns have diagnostic value in predicting kidney damage, either due to scarring or as a result of obstruction.

Compliance with ethical standards

Conflict of interest statement PD is a co-inventor on patents of NGAL as a biomarker of kidney injury.

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