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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 reports in the literature 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 (UTI), 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 effective in identifying infection with 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 the most common bacterial infections in infants and children. However, reliably identifying upper tract involvement can be challenging in the clinical setting. In the article by Kim et al. [1], the authors investigated the utility of plasma neutrophil gelatinase-associated lipocalin (pNGAL) as a marker of acute pyelonephritis in children, and compared its diagnostic accuracy to that of C-reactive protein, serum white blood count, and procalcitonin. The authors also 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 for hydronephrosis or VUR. Due to the complex

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Conflict of interest statement

The authors declare there are no conflicts to declare.

interplay between urinary tract infections, VUR, and pyelonephritis, as well as the innate biologic differences between urinary NGAL (uNGAL) and pNGAL, this article deserves further comment.

Plasma and Urine NGAL

There are two forms of NGAL that have been the subject of much research in the biomarker literature: pNGAL and uNGAL. These forms have distinct mechanisms of induction and upregulation, allowing for different applications. Both pNGAL and uNGAL have been shown to have utility as early markers of acute kidney injury (AKI) [2]. However, prior to the identification of NGAL as a marker of AKI, pNGAL was 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 roles of NGAL is iron trafficking, which has implications for epithelial cell growth, differentiation and response to both injury and infection [4]. NGAL's role in iron-trafficking directly affects bacterial growth. Gram-negative bacteria are dependent upon iron for survival. In order to obtain iron, these bacteria produce a molecule called enterochelin that chelates free iron from the local environment and uses it for continued bacterial growth. NGAL competitively binds the enterochelin-iron complex and removes it through the urine, exerting a powerful bacteriostatic effect as part of the innate immune system [4]. While this effect is seen clearly 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 a result of the systemic inflammation associated with the infection. This is suggested by the data in the article by Kim et al: the ROC curves for procalcitonin, C-reactive protein, and NGAL for predicting acute pyelonephritis are quite similar in appearance, with area under the curve (AUC) of 0.855, 0.879, and 0.893, respectively. A clear understanding of the biology of both the plasma and urinary forms of NGAL helps in determining their applications as biomarkers in the setting of infection.

NGAL and Urinary Tract Infections

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. investigating the association between uNGAL and UTIs supports this hypothesis, as children with acute UTI have higher levels of uNGAL – both corrected and uncorrected for urine creatinine – compared to healthy children [6]. Mouse models further support this association. Following transurethral inoculation with strains of uropathogenic *E. 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, there are conflicting reports of the utility of NGAL as a marker of UTI within the literature, as some studies have not shown a difference in uNGAL levels between children with UTI and controls [8]. This 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 earlier cited study by Yilmaz et al. [6], all of the patients had *E. coli* as the causative agent of their UTIs, whereas bacteriology data were not reported in the latter study by Kim et al. [8], and may potentially confound the results. Despite these conflicting reports, both mouse and human data overwhelmingly support the association between UTIs with gram-negative bacteria and elevated uNGAL levels [6, 7, 9, 10].

NGAL in Vesico-ureteral Reflux and Renal Scarring

There is a well-known association between UTIs 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]. This controversy is mainly centered around the recommendation that voiding cysto-urethrograms (VCUGs) should not be routinely performed in children between 2 and 24 months 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 regard to the presence of scarring given the degree of interrelatedness between them. Accordingly, much of the work done on NGAL and VUR also comments 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; prior work has shown that uNGAL is not elevated in quiescent forms of chronic kidney disease [15]. Rat models of pyelonephritis show that NGAL gene expression is maximally upregulated two weeks following pyelonephritis, which then decreases at both four and six weeks, but does not return to baseline levels [16]. Immunostaining of rat kidneys suggests that the NGAL upregulation seen at two 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 two weeks and then decrease by week six, 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. While statistical significance is present between uNGAL levels in patients with scarring and those without, the values presented 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 supports 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

In their article, Kim et al. [1] also investigate the utility of pNGAL, procalcitonin and C-reactive protein in predicting hydronephrosis in the setting of pyelonephritis. While none of these markers had utility in diagnosing hydronephrosis in this study, others' work within the literature designed to better study NGAL in obstructive uropathy do show that NGAL has utility in this clinical setting.

Urinary tract obstruction is an area where 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]. Subsequently, in children, uNGAL has shown promise as a marker of ureteropelvic junction obstruction [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 noted hydronephrosis, may allow for identification of a population with ongoing renal damage who 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 investigate both urinary and plasma NGAL levels in patient 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 with the genitourinary system. A combination of these forms of NGAL would capture the benefit of both these markers: elevations in both pNGAL and uNGAL suggest the presence of systemic inflammation, 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 exists 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

NGAL promises to improve our understanding and management of UTI and the associated complications (Table 1). However, prior to consideration of clinical implementation in these scenarios, an understanding of the biology of NGAL within these conditions is necessary. 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 kidney damage, either due to scarring or as a result of obstruction.

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Table 1

Suggested Use of NGAL in Urinary Tract Infection, Pyelonephritis, Vesicoureteral Reflux (VUR), 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 concern for acute obstruction