Review article

Child Kidney Dis 2015;19:79-88
DOI: http://dx.doi.org/10.3339/chikd.2015.19.2.79

ISSN 2384-0242 (print)
ISSN 2384-0250 (online)

Neutrophil Gelatinase-Associated Lipocalin and Kidney Diseases

Hyung Eun Yim, M.D., Ph.D.

Department of Pediatrics, College of Medicine, Korea University, Seoul, 152-703, South Korea

Corresponding author:
Hyung Eun Yim, M.D., Ph.D.
Department of Pediatrics, Korea University Ansan Hospital, 123 Jeokgeum-ro, Danwon-gu, Ansan-si, Gyeonggi-do 15355, Korea
Tel: +82-31-412-5096
Fax: +82-31-405-8591
E-mail: he-yim@hanmail.net

Received: 23 September 2015
Revised: 13 October 2015
Accepted: 22 October 2015

Neutrophil gelatinase-associated lipocalin (NGAL) has emerged as one of the most promising biomarkers of renal epithelial injury. Numerous studies have presented the diagnostic and prognostic utility of urinary and plasma NGAL in patients with acute kidney injury, chronic kidney disease, renal injury after kidney transplantation, and other renal diseases. NGAL is a member of the lipocalin family that is abundantly expressed in neutrophils and monocytes/macrophages and is a mediator of the innate immune response. The biological significance of NGAL to hamper bacterial growth by sequestering iron-binding siderophores has been studied in a knock-out mouse model. Besides neutrophils, NGAL is detectable in most tissues normally encountered by microorganisms, and its expression is upregulated in epithelial cells during inflammation. A growing number of studies have supported the clinical utility of NAGL for detecting invasive bacterial infections. Several investigators including our group have reported that measuring NGAL can be used to help predict and manage urinary tract infections and acute pyelonephritis. This article summarizes the biology and pathophysiology of NGAL and reviews studies on the implications of NGAL in various renal diseases from acute kidney injury to acute pyelonephritis.

Key words : Acute renal injury, Biomarkers, Pyelonephritis

Introduction

Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin superfamily and is one of the most reliable markers of renal epithelial injury[1]. It was initially identified as a 25-kDa protein covalently bound to gelatinase from human neutrophils and has emerged as an early predictive biomarker for acute kidney injury (AKI)[2, 3]. Numerous experimental and clinical studies have shown the diagnostic and prognostic utility of urinary and plasma NGAL in AKI[4-6]. A growing body of literature suggests that NGAL can be used to predict the progression of chronic kidney disease (CKD)[7]. The renal transplant setting is another field that uses NGAL assays. NGAL is reportedly a reliable predictive biomarker for renal injury after kidney transplantation[8]. NGAL may also show promise for checking the status and treatment response of diverse renal illnesses, such as membranous nephropathy, tubulointerstitial nephritis, IgA nephropathy, and others[9-13]. Several investigators includ-

ing our group have shown that urinary and plasma NGAL levels can be used to predict and manage urinary tract infection (UTI) and acute pyelonephritis (APN)[14-18]. This review addresses the biologic and pathophysiologic features on NGAL and describes the results of experimental and clinical studies on the diagnostic and prognostic utility of NGAL in patients with AKI, CKD, and various renal diseases including APN.

NGAL from biology to pathophysiology

NGAL, also called as human neutrophil lipocalin, lipocalin-2, siderocalin, 24p3, or LCN2, is a small 178 amino acid molecule which belongs to the lipocalin superfamily[19]. The biological action of NGAL is induced by bonding with specific surface receptors, such as the 24p3R, a brain-type organic cation transporter, and the megalin multi-scavenger complex, detected largely on the brush-border surface of renal tubular epithelial cells[20]. NGAL is internalized inside the cell as a protein alone or a complex with iron-binding siderophores after interacting with these receptors[19]. In the infectious condition, bacteria synthesize siderophores that scavenge iron from the extracellular space and utilize specific transporters to recapture the siderophore-iron complex to satisfy their iron demands. NGAL inhibits growth of bacterial strains that depend on enterochelin, a bacterial catecholate siderophore[21]. Indeed, neutrophils isolated from NGAL-deficient mice demonstrate considerably less bacteriostatic activity compared to that of controls. The bacteriostatic characteristics of neutrophils in wild-type mice is eliminated by adding exogenous iron, suggesting that the principal antibacterial function of NGAL in the innate immune response is to limit this important element. Genetically deficient animals for both copies of the NGAL gene are more susceptible to certain Gram-negative bacterial infections and die more easily of sepsis than wild-type mice [22,23].

In addition, NGAL has more complex activities besides antibacterial actions, as it is a growth and differentiation factor in several cell types[24]. NGAL stimulates epithelial differentiation by targeting a stromal/interstitial/progenitor niche at the periphery of the developing kidney[25]. NGAL is found at very low levels in multiple human tissues, including bone marrow, salivary glands, lungs, liver, kidneys, gastrointestinal tract, uterus, and prostate [26]. NGAL has complex interactions with several receptors and ligands implicated in many biological responses[19,24]. The NGAL gene promoter region has binding sites for several transcription factors, such as nuclear factor-κB[26]. The discovery of NGAL in several of non-hematopoietic tissues may be explained by the binding of activated transcription factors with NGAL gene promoter region. NGAL is increased significantly in damaged epithelial cells in response to ischemia, inflammation, infection, intoxication, AKI, and neoplastic transformation[1,5,21,26]. Thus, non-renal diseases may become significant confounders when NGAL levels are interpreted in AKI.

In the kidney, NGAL is synthesized in the distal nephron and is secreted into the urine through the thick ascending limb of loop of Henle and collecting ducts[24]. Due to its small molecular size, NGAL is freely filtered through renal glomeruli and can be identified in urine. NGAL is released from the distal part of the nephron immediately after AKI. Impaired proximal tubular reabsorption due to injury may additionally elevate urinary NGAL (uNGAL) concentrations[24]. NGAL is expressed in acutely damaged kidneys of experimental animal models, and uNGAL levels more accurately reflect AKI than serum creatinine (Cr). Renal ischemia-reperfusion injury in an animal model induces a massive increase of NGAL level within 3 hr of clinically significant ischemia, while serum Cr shows a milder response. Serum Cr concentrations only increase after severe bilateral renal ischemia, whereas they remain unchanged in unilateral or mild bilateral ischemia[27]. Peak levels of NGAL following AKI increase up to 1000-fold in urine (from 0.04 to 40 mg/ml) and 300-fold in blood (from 0.1 to 30 mg/ml)[4,27]. uNGAL expression levels are associated with the extent and time of renal ischemia and precede the emergence of other biomarkers, such as N-acetyl-beta-D-glucosaminidase (NAG) and beta 2-microglobulin[27]. Accordingly, these characteristics may make NGAL more reliable than Cr in the diagnosis of AKI.

NGAL as an AKI biomarker

The diagnosis of AKI is based on an increase in serum

Cr and oliguria[28]. However, serum Cr is not a reliable marker during early acute phase kidney dysfunction, as its concentrations may be not elevated until about 50% of renal function has been lost. Serum Cr and urine output do not distinguish between structural renal injury and functional hemodynamic triggers of a decrease in glomerular filtration rate (GFR)[1, 29]. NGAL may help overcome these limitations due to its ability to detect tissue damage rather than kidney dysfunction and prompt upregulation in response to AKI[1]. A large number of studies have reported NGAL as an early diagnostic biomarker for AKI in diverse clinical situations[6, 30, 31].

The kidney is very sensitive to ischemic and/or toxic injury, causing vasoconstriction, endothelial injury, and activation of the inflammatory immune response[32]. Transcriptome profiling studies have reported that NGAL is one of the most highly induced genes following ischemic or nephrotoxic AKI in an animal model, and proteomic analyses have identified NGAL as one of the most upregulated proteins[33, 34]. NGAL mRNA expression increases markedly in tubular epithelia of a rat model of acute toxin-induced renal injury 3–12 hr after a lipopolysaccharide injection. The time course for the increase in NGAL mRNA was parallel with kidney injury, and uNGAL concentrations precisely reflect alterations in kidney NGAL levels[35]. Moreover, uNGAL was one of the best predictors of gentamicin nephrotoxicity compared to other biomarkers, with considerable increases occurring within 24 hrs and before any elevations in serum Cr were apparent[36]. These animal study findings have encouraged investigators to examine NGAL as a sensitive index in human patients with AKI. Significant elevations in urine and serum NGAL have been detected by Western blotting in adults with established AKI (doubling of serum Cr) from a variety of etiologies, compared to controls[37]. Urine and serum NGAL levels are correlated with serum Cr and renal biopsies in patients with AKI who strongly express NGAL in cortical tubules, confirming that NGAL could be a sensitive indicator of established AKI in humans.

Several studies in patients who underwent cardiac surgery show that both urine and blood NGAL might be a promising AKI biomarker. Initial postoperative plasma NGAL levels in children following cardiac surgery are significantly associated with the length and severity of AKI, duration of hospital stay, and mortality[38]. uNGAL is a good predictor of early AKI in infants and young children undergoing cardiopulmonary bypass surgery[6]. Elevated uNGAL concentrations have been found as early as 1–3 hr after cardiac surgery in adult patients, whereas maximal levels of uNGAL and plasma NGAL are detected 6 hrs following surgery[30, 39, 40]. Substantial upregulation of urine and plasma NGAL have been documented 2 hr after contrast media AKI was induced in both in children and adults[31, 41]. Additional findings show significant correlations between NGAL levels and serious clinical outcomes, including deterioration of AKI, requirement for renal replacement therapy (RRT), and death. uNGAL predicts complex consequences of beginning dialysis or death during hospitalization[42]. Studies of critically ill patients have reported that NGAL is elevated in the setting of AKI. uNGAL has excellent utility for diagnosing AKI in critically ill adults in the intensive care unit (ICU)[43]. A growing number of recent studies have suggested that NGAL is a useful diagnostic and prognostic predictor in specific patients with cardio-renal syndrome, including cases of acute or chronic heart failure[44, 45].

Notably, large differences in the diagnostic accuracy of NGAL for AKI has been observed, with area under the curve (AUC) values fluctuating from 0.54 to 0.96 for blood NGAL and from 0.61 to 0.98 for uNGAL[29]. These variations may be due to the small number of patients enrolled in some studies or to different clinical situations. However, a large meta-analysis of data from 19 studies involving 2,538 patients showed that the diagnostic odds ratio (DOR) and AUC of NGAL to predict AKI were 18.6 and 0.815, respectively. The diagnostic accuracy of uNGAL (DOR, 18.6; AUC, 0.83) is similar to that of plasma/serum NGAL (DOR, 17.9; AUC, 0.775). Particularly, NGAL level is a better predictor in children (DOR, 25.4; AUC, 0.93) compared to that in adults (DOR, 10.6; AUC, 0.782)[42]. A recent meta-analysis confirmed the prognostic accuracy of NGAL levels. This multicenter analysis evaluated data from 10 prospective NGAL studies in 2,322 critically ill patients mostly with cardio-renal syndrome. A positive NGAL finding according to the study-specific NGAL cutoff value to predict AKI resulted in a similar risk of adverse outcome as that of a positive Cr finding. NGAL(+)/serum Cr(−) tests detected nearly 40%

more patients with AKI than serum Cr (+) alone, and these cases were at higher risk for a longer ICU stay, RRT, and death compared with those of control subjects. These results suggest that even without diagnostic increases in serum Cr, NGAL identifies likely patients with subclinical AKI, who have a greater risk of a worse prognosis[46].

NGAL and Kidney Disease

NGAL levels are clearly associated with the intensity of renal impairment, suggesting the degree of active injury underlying the chronic illness. Several studies have suggested that NGAL serves as an early biomarker reflecting tubulointerstitial injury in patients with various renal diseases. Bolignano et al[11] reported that patients with membranous nephropathy and decreased renal function have excessively high baseline NGAL levels. Patients with higher baseline NGAL levels display a substantially increased risk of worsening residual renal function within 1 yr compared with subjects with lower baseline NGAL levels. Ding et al[10] demonstrated increased occurrence of the NGAL protein in renal tubular biopsies from 70 patients with Lee grade III IgA nephropathy. uNGAL levels are significantly associated with the amount of tubular NGAL expression, proteinuria, and histological scores of mesangial proliferation and tubulointerstitial injury. uNGAL concentrations increase much more remarkably compared with uNAG values and were easily identified even in patients with Lee grade II IgA nephropathy when their NAG levels presented almost no change. Viau et al[47] evaluated 87 patients with polycystic kidney disease and GFR of 33 ± 20 ml/min/1.73 m^2. They found upregulated uNGAL levels in individuals who progressed to end-stage renal disease (ESRD). However, there was no analysis to adjust for other recognized risk factors for CKD progression. In a study of 36 patients with drug-induced chronic tubulointerstitial nephritis, uNGAL predicted worsening renal function and was the only risk factor in multivariate models[13]. Serum NGAL is potentially a specific predictor for early functional graft recovery and the need for hemodialysis after kidney transplantations from donors after cardiac death[48].

The potential of NGAL to be a congenital urologic disease biomarker has been evaluated in a few studies. A case-control study performed in children with severe congenital hydronephrosis caused by ureteropelvic junction obstruction revealed that preoperative median uNGAL concentrations are much higher in children with severe hydronephrosis than those in controls. uNGAL levels decreased considerably in patients with severe hydronephrosis 3 months later after the operation but remained more elevated than that in control children with mild non-obstructive hydronephrosis. The authors suggested that the increased uNGAL concentration was related with a worsening obstruction[49]. Notably, uNGAL values adjusted to age-matched standards were also elevated in patients with vesicoureteral reflux (VUR) without renal dysgenesis compared to those in control children[9]. The presence of renal scarring is correlated with increased NGAL level, suggesting that uNGAL may become a useful diagnostic indicator for renal scarring when monitoring patients with VUR.

NGAL as a CKD biomarker

The critical role of renal tubules has been pointed out during the early period and during progression of CKD. Tubulointerstitial injury is a powerful predictor of CKD progression to ESRD, regardless of the primary etiology of CKD. The pathogenic mechanisms responsible for progressive renal damage are tubular atrophy and hypoxia, peritubular capillary loss, and interstitial fibrosis[50]. CKD stages are classified based on the estimated GFR value and the extent of albuminuria along with the National Kidney Foundation Practice Guidelines[51]. However, albuminuria and GFR may not correctly identify accelerated deterioration of kidney function and cannot be used to monitor the progression of CKD. There appears to be a series of patients in whom loss of renal function may occur rapidly yet the proteinuria remains relatively low grade[52]. Thus, much attention has been focused on identifying markers to predict which patients with CKD are at greater risk for progressive renal decline. One current comprehensive analysis indicated that NGAL is a novel biomarker for the progression of CKD[53].

Bolignano et al[7] observed that serum and uNGAL concentrations are directly related with a doubling of serum Cr or initiation of RRT during a median follow-up of 18.5 months,

regardless of age and baseline GFR in a study in 96 patients focusing on non-advanced CKD with stable renal function. The authors suggested that NGAL may be helpful as a surrogate parameter of residual renal function under these particular conditions. A study of 158 elderly patients with pre-dialysis CKD and relatively low-level proteinuria reported that uNGAL and conventional risk factors improve the prediction of renal disease progression. The uNGAL-to-Cr ratio (uNGAL/Cr) is related with the primary endpoint of all-cause mortality or the beginning of RRT within 2 yr[54]. These data suggest that the uNGAL/Cr ratio may help detect a subset of patients that show a rapid decrease in estimated GFR, but in whom proteinuria remains relatively low[52]. Albuminuria is most commonly caused by significant glomerular leakage that exceeds tubular reabsorptive competence, whereas uNGAL, which is released particularly from damaged renal tubular cells, is positively correlated with the extent of tubular atrophy and interstitial fibrosis in kidney biopsies[54, 55]. Consequently, the uNGAL/Cr ratio and total proteinuria or albuminuria could potentially offer significant prognostic information of tubular capability thereby avoiding invasive procedures. Plasma NGAL concentrations are negatively correlated with GFR in children with CKD caused by renal dysplasia, obstructive uropathy, and glomerular and cystic diseases. NGAL outperforms Cr as a predictor of kidney failure when renal function diminishes to < 30 ml/min[56].

However, conflicting reports question whether uNGAL is an independent risk factor for predicting renal decline after adjusting for conventional risk factors of CKD progression. The Atherosclerosis Risk in Communities study results demonstrated that the relationship between increased baseline uNGAL levels and stage 3 CKD disappeared after controlling for urinary Cr and albumin concentration in a small cohort[57]. Peters et al[12] reported that uNGAL was a predictable risk factor of ESRD in a univariate analysis but not after adjusting for other factors including serum Cr in 65 patients with IgA nephropathy. A recent cohort study of 3,386 patients with stages 2–4 CKD showed that uNGAL is positively correlated with CKD severity and is a risk factor for progression of CKD in the first 2 yr after measurement. Nevertheless, this novel indicator only very modestly predicted outcome events[58].

NGAL in APN

Iron is required for pathogenic microorganisms to survive in human or animal hosts. The quantity of iron available to a microorganism may be very restricted because most iron in host tissues is bound by heme, ferritin, transferrin, or lactoferrin. Therefore, many potential pathogens, such as Escherichia coli, produce siderophores under iron limited conditions and the high-affinity iron-binding ability of siderophores allows them to detach iron from host iron-binding proteins and carry it into the bacterial cell through specific receptors[59]. Most pathogenic E. coli strains secrete phenolate iron chelators, such as enterobactin or enterochelin and aerobactin[60]. The genes for producing aerobactin can be plasmid-encoded, and such plasmids in E. coli have been linked with potent extra-intestinal infections[22, 60].

The significance of the capability of NGAL to limit bacterial growth by separating iron siderophores has been studied in a knock-out mouse model[23] NGAL is protective against infections caused by E. coli injected directly into the peritoneum. Toll-like receptors on immune cells induce transcription, translation, and secretion of NGAL when exposed to invading bacteria. NGAL then inhibits bacterial growth by isolating the iron-laden siderophores. NGAL-deficient mice consistently show increased susceptibility to bacterial infection[22]. Wu et al[61] demonstrated that intratracheal instillation of E. coli in mice induces abundant NGAL expression in the respiratory tract epithelial cells and that limited of NGAL expression increases morbidity and mortality of the infected mice. These findings suggest that NGAL is vital for defense of airways against infection by E. coli. Kidney NGAL mRNA and protein levels increased rapidly after bacterial injection in an experimental rat UTI model and then decreased quickly but were followed by persistently high levels for 6-weeks after the injection. uNGAL concentrations are also elevated during the early phases of infection and continue at high levels after the infection and subsequent extensive fibrosis develops, suggesting the potential value of NGAL as an APN and renal scarring marker[62].

Indeed, human data show the clinical utility of NAGL for detecting invasive bacterial infections. Serum NGAL concentration increase remarkably < 24 hrs after critically

ill children with septic shock are admitted to the ICU compared with healthy controls and subjects with systemic inflammatory respiratory syndrome[63]. Serum NGAL levels > 155 μg/l have been suggested as predictive of acute bacterial infections in a group of patients. Serum NGAL level is more sensitive and specific than C-reactive protein (CRP) to discriminate viral and bacterial infections[64]. Additionally, NGAL is better predictable for monitoring antibacterial treatments than serum CRP. NGAL and CRP levels were significantly upregulated at admission of 92 children hospitalized with symptoms and signs of acute infections compared to those with viral infections. However, 83% of the children with bacterial infections still demonstrated increased CRP concentrations after 25–48 hr, whereas 11% had high NGAL levels[65]. NGAL does not increase in healthy term newborns at birth, but neutrophils from newborns, even premature babies, rapidly produce NGAL upon bacterial or fungal stimulation[66].

We would like to comment on our experience with urinary and plasma NGAL in APN and febrile UTIs in children. Febrile UTI has long been recognized as one of the most common bacterial infections in children, with the potential consequence of renal scarring[67]. Collecting urine and interpreting results are difficult in infants and young children, although instructions for diagnosing a UTI according to urinalysis and urine culture have been documented[68]. Distinguishing APN from a lower UTI is challenging in this patient group, as urinary tract symptoms can be nonspecific. A dimercaptosuccinic acid renal scan is very sensitive for evaluating renal damage but it is costly and not readily available, and exposes the subjects to radiation[69]. Thus, a more practical method to detect renal parenchymal lesions is needed for treating UTIs in a timely manner. The significance of an indicator of renal parenchymal involvement in a patient with a UTI is great because longer antibiotic therapy is required for parenchymal damage. Acute, mainly tubulointerstitial kidney injury is present in both AKI and APN, and it is diffuse in AKI and restricted to a section of renal parenchyma in APN[14]. uNGAL values in adults with established AKI have been used to successfully distinguish intrinsic AKI from prerenal AKI[5]. Similarly, urine and plasma NGAL levels can be used to detect children with UTIs and distinguish APN from a lower UTI in children with a febrile UTI[16-18].

Yilmaz et al[15] firstly report the diagnostic utility of NGAL in a study of 60 children with UTIs and 29 healthy controls. They demonstrated that uNGAL levels increased significantly higher in a UTI group than in controls. Using the best uNGAL cutoff of 20 ng/ml for diagnosing UTI, sensitivity and specificity were 97% and 76%, respectively (AUC, 0.979). However, they did not compare NGAL concentrations between patients with APN and those with lower UTIs. We evaluated three novel biomarkers, including NGAL, kidney injury molecule (KIM)-1, and cystatin C in a cohort of 73 children with febrile UTIs and 56 controls. Patients with a history of renal disease or accompanying AKI were excluded. Among these markers, uNGAL and uNGAL/Cr were the most promising to diagnose and manage febrile UTIs and APN in children. The uNGAL/Cr ratio was more elevated in the UTI group than that in the control subjects and in patients with APN than in those with lower UTIs. The best uNGAL cutoff values for predicting UTI and APN were 23.95 ng/ml (sensitivity, 82.4%; specificity, 83.6%; AUC, 0.89) and 73 ng/ml (sensitivity, 75%; specificity, 73.7%; AUC, 0.8), respectively. The optimal uNGAL/Cr ratio cutoff values for predicting UTI and APN were 276.5 ng/mg Cr (sensitivity, 76%; specificity, 90%; AUC, 0.9) and 390 ng/mg Cr (sensitivity, 90.3%; specificity, 63.2%; AUC, 0.78), respectively. The uNGAL/Cr ratios in the APN and UTI groups decreased after antibiotic treatment compared to those before treatment. The uNGAL/Cr ratio was positively correlated with serum levels of white blood cells (WBC), CRP, cystatin C, and the uKIM-1/Cr ratio. Although uKIM-1 and uKIM-1/Cr levels were greater in the UTI and APN groups than those in the controls, they did not discriminate between APN and lower UTIs in children with febrile UTIs. Serum cystatin C concentrations also increased in patients with APN compared to those with lower UTIs, but persistently high levels after treatment did not reflect clinical improvement after the acute phase of UTI[17]. Additionally, we assessed the predictive value of plasma NGAL in children with symptomatic UTIs[16, 18]. In a preliminary study of 47 infants with febrile UTIs, plasma NGAL levels were higher in the APN group than those of the non-APN group during the acute phase of febrile UTI. Plasma NGAL level decreased during the next 3–4 days after initiating antibiotic treatment[16]. It appeared to be a helpful predictor for

diagnosing APN in infants with acute febrile UTI. Next, we confirmed that plasma NGAL is a sensitive and specific biomarker for identifying APN and monitoring the treatment response in children with UTIs. A total of 123 children were enrolled (53 APN and 70 lower UTI). After adjusting for age and sex, plasma NGAL levels were higher in the APN group than those in the lower UTI group. NGAL levels were significantly correlated with serum levels of WBCs, CRP, and Cr, as well as fever duration. A multivariate analysis revealed that log-transformed plasma NGAL was the only independent predictor of APN. A receiver operating curve analysis showed a good diagnostic profile for NGAL to detect APN (AUC, 0.864) with an optimal best cut-off value of 102.5 ng/ml. Plasma NGAL had sensitivity of 89.1% and specificity of 71.0% for diagnosing APN. The positive and negative predictive values of this cut-off value were 68.3% and 90.7%, respectively. NGAL levels in both two groups decreased after treatment compared to levels before treatment[18]. None of our studies showed that urinary or plasma NGAL has definite predictive value for the presence of VUR or renal scarring. Conflicting results suggest that uNGAL levels are significantly higher in patients with VUR compared to those in controls. uNGAL also increases more in patients with renal scarring than in those without scarring[9]. The relatively small number of enrolled patients with VUR or renal scarring in our studies may have contributed to these different findings. The acute infectious condition in our studies may be an additional explanation for the negative results.

Taken together, our studies indicate that uNGAL, uNGAL/Cr, and plasma NGAL have promise for aiding in the diagnostic assessment and therapeutic monitoring of UTI in children without AKI. Measuring these markers could be incorporated into current algorithms, although it is unclear whether they can replace urinalysis and/or urine culture as primary UTI diagnostic tests. Higher NGAL levels in urine and plasma can help physicians manage children with suspected UTI without AKI. Early detection of APN based on NGAL level may facilitate cost-effective treatment, particularly in infants and young children. The routine practice of imaging studies to localize febrile UTI would not be necessary for children with low NGAL levels. Persistently high NGAL levels can be related with insufficient treatment or a follow-up dimercaptosuccinic acid renal scan may be required to rule out potential renal scarring.

Conclusions

Collectively, NGAL is a promising kidney disease biomarker. However, translating its use into routine clinical practice is incomplete. Reference ranges with best cutoff values, ethnicity, adjustments for age and sex, and ruling AKI conditions or other significant confounders in or out should be considered in diverse clinical settings. Further investigations with a large number of enrolled patients for a longer observational period are required to confirm previous findings in various kidney diseases.

Disclosure

None

References

1. Singer E, Markó L, Paragas N, Barasch J, Dragun D, Müller DN, et al. Neutrophil gelatinase-associated lipocalin: pathophysiology and clinical applications. Acta Physiol (Oxf) 2013;207: 663-72.
2. Kjeldsen L, Johnsen AH, Sengeløv H, Borregaard N. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem 1993; 268:10425-32.
3. Lisowska-Myjak B. Serum and urinary biomarkers of acute kidney injury. Blood Purif 2010;29: 357-65.
4. Schmidt-Ott KM, Mori K, Kalandadze A, Li JY, Paragas N, Nicholas T, et al. Neutrophil gelatinase-associated lipocalin-mediated iron traffic in kidney epithelia. Curr Opin Nephrol Hypertens. 2006;15:442-9.
5. Singer E, Elger A, Elitok S, Kettritz R, Nickolas TL, Barasch J, et al. Urinary neutrophil gelatinase-associated lipocalin distinguishes pre-renal from intrinsic renal failure and predicts outcomes. Kidney Int. 2011;80:405-14.
6. Zheng J, Xiao Y, Yao Y, Xu G, Li C, Zhang Q, et al. Comparison of urinary biomarkers for early detection of acute kidney injury after cardiopulmonary bypass surgery in infants and young children. Pediatr Cardiol. 2013;34:880-6.
7. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, Fazio MR, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. Clin J Am Soc

Nephrol 2009;4:337-44.

8. Hollmen ME, Kyllönen LE, Inkinen KA, Lalla ML, Salmela KT. Urine neutrophil gelatinase-associated lipocalin is a marker of graft recovery after kidney transplantation. Kidney Int 2011;79:89-98.
9. Ichino M, Kusaka M, Kuroyanagi Y, Mori T, MorookaM, Sasaki H, et al. Urinary neutrophil-gelatinase associated lipocalin is a potential noninvasive marker for renal scarring in patients with vesicoureteral reflux. J Urol 2010;183:2001-7.
10. Ding H, He Y, Li K, Yang J, Li X, Lu R, et al. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is an early biomarker for renal tubulointerstitial injury in IgA nephropathy. Clin Immunol 2007;123:227-34.
11. Bolignano D, Coppolino G, Lacquaniti A, Nicocia G, Buemi M. Pathological and prognostic value of urinary neutrophil gelatinase-associated lipocalin (NGAL) in macroproteinuric patients with worsening renal function. Kidney Blood Press Res 2008;31:274-9.
12. Peters HP, Waanders F, Meijer E, van den Brand J, Steenbergen EJ, van Goor H, et al. High urinary excretion of kidney injury molecule-1 is an independent predictor of end-stage renal disease in patients with IgA nephropathy. Nephrol Dial Transplant 2011;26:3581-8.
13. Wu Y, Su T, Yang L, Zhu SN, Li XM. Urinary neutrophil gelatinase-associated lipocalin: A potential biomarker for predicting rapid progression of drug-induced chronic tubulointerstitial nephritis. Am J Med Sci 2010;339:537-42.
14. Piccoli GB, Ferraresi M, Aroasio E, Gonella S, De Pascale A, Veltri A. The search for perfect biomarkers in acute kidney damage: the case of NGAL, from AKI to acute pyelonephritis: back to the clinic? Nephrol Dial Transplant 2012;27:3665-6.
15. Yilmaz A, Sevketoglu E, Gedikbasi A, Karyagar S, Kiyak A, Mulazimoglu M, et al. Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin. Pediatr Nephrol 2009;24:2387-92.
16. Seo WH, Nam SW, Lee EH, Je BK, Yim HE, Choi BM. A rapid plasma neutrophil gelatinase-associated lipocalin assay for diagnosis of acute pyelonephritis in infants with acute febrile urinary tract infections: a preliminary study. Eur J Pediatr 2014;173:229-32.
17. Yim HE, Yim H, Bae ES, Woo SU, Yoo KH. Predictive value of urinary and serum biomarkers in young children with febrile urinary tract infections. Pediatr Nephrol 2014;29:2181-9.
18. Sim JH, Yim HE, Choi BM, Lee JH, Yoo KH. Plasma neutrophil gelatinase-associated lipocalin predicts acute pyelonephritis in children with urinary tract infections. Pediatr Res 2015;78:48-55.
19. Borregaard N, Cowland JB. Neutrophil gelatinase-associated lipocalin, a siderophore-binding eukaryotic protein. Biometals 2006;19:211-5.
20. Devireddy LR, Gazin C, Zhu X, Green MR. A cell surface receptor for lipocalin 24p3 selectively mediates apoptosis and iron uptake. Cell 2005;123:1293-305.
21. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell 2002;10:1033-43.
22. Berger T, Togawa A, Duncan GS, Elia AJ, You-Ten A, Wakeham A, et al. Lipocalin 2-deficientmice exhibit increased sensitivity to Escherichia coli infection but not to ischemia-reperfusion injury. Proc Natl Acad Sci USA 2006;103:1834-9.
23. Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature 2004;432:917–21.
24. Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P, et al. Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol 2007;18:407-13.
25. Yang J, Blum A, Novak T, Levinson R, Lai E, Barasch J. An epithelial precursor is regulated by the ureteric bud and by the renal stroma. Dev Biol 2002;246: 296–310.
26. Cowland JB, Borregaard N. Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase associated lipocalin from humans. Genomics 1997; 45:17–23.
27. Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol 2003;14:2534-43.
28. Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, et al. Acute Kidney Injury Network. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit Care 2007;11:R31.
29. Clerico A, Galli C, Fortunato A, Ronco C. Neutrophil gelatinase-associated lipocalin (NGAL) as biomarker of acute kidney injury: a review of the laboratory characteristics and clinical evidences. Clin Chem Lab Med 2012;50:1505-17.
30. Wagener G, Gubitosa G, Wang S, Borregaard N, Kim M, Lee HT. Urinary neutrophil gelatinase-associated lipocalin and acute kidney injury after cardiac surgery. Am J Kidney Dis 2008;52:425-33.
31. Hirsch R, Dent C, Pfriem H, Allen J, Beekman RH 3rd, Ma Q, et al. NGAL is an early predictive biomarker of contrast-induced nephropathy in children. Pediatr Nephrol 2007;22:2089-95.
32. Lattanzio MR, Kopyt NP. Acute kidney injury: new concepts in definition, diagnosis, pathophysiology, and treatment. J Am Osteopath Assoc 2009;109:13-9.
33. Supavekin S, Zhang W, Kucherlapati R, Kaskel FJ, Moore LC, Devarajan P. Differential gene expression following early renal ischemia-reperfusion. Kidney Int 2003;63:1714-24.
34. Devarajan P, Mishra J, Supavekin S, Patterson LT, Potter SS. Gene expression in early ischemic renal injury: clues towards pathogenesis, biomarker discovery, and novel therapeutics. Mol Genet Metab 2003;80:365-76.
35. Han M, Li Y, Liu M, Li Y, Cong B. Renal neutrophil gelatinase associated lipocalin expression in lipopolysaccharide-induced acute kidney injury in the rat. BMC Nephrol 2012;13:25
36. Hoffmann D, Fuchs TC, Henzler T, Matheis KA, Herget T, Dekant

W, et al. Evaluation of a urinary kidney biomarker panel in rat models of acute and subchronic nephrotoxicity. Toxicology 2010;277:49–58.

37. Mori K, Lee HT, Rapoport D, Drexler IR, Foster K, Yang J, et al. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J Clin Invest 2005;115:610-21.
38. Dent CL, Ma Q, Dastrala S, Bennett M, Mitsnefes MM, Barasch J, et al. Plasma NGAL predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: a prospective uncontrolled cohort study. Crit Care 2007;11:R127.
39. Bennett M, Dent CL, Ma Q, Dastrala S, Grenier F, Workman R, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. Clin J Am Soc Nephrol 2008;3:665-73.
40. Koyner JL, Garg AX, Coca SG, Sint K, Thiessen-Philbrook H, Patel UD, et al. Biomarkers predict progression of acute kidney injury after cardiac surgery. J Am Soc Nephrol 2012;23:905-14.
41. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, Malyszko JS, Dobrzycki S. Neutrophilgelatinase-associated lipocalin and renal function after percutaneous coronary interventions. Am J Nephrol 2006;26:287-92.
42. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis 2009;54:1012-24.
43. Bagshaw SM, Bennett M, Haase M, Haase-Fielitz A, Egi M, Morimatsu H, et al. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. Intensive Care Med 2010;36:452-61.
44. Ronco C, McCullough P, Anker SD, Anand I, Aspromonte N, Bagshaw SM, et al. Cardio-renal syndromes: report from the consensus conference of the acute dialysis quality initiative. Eur Heart J 2010;31:703-11.
45. Damman K, van Veldhuisen DJ, Navis G, Voors AA, HillegeHL. Urinary neutrophil gelatinase associated lipocalin (NGAL),a marker of tubular damage, is increased in patients with chronic heart failure. Eur J Heart Fail 2008;10:997-1000.
46. Haase M, Devarajan P, Haase-Fielitz A, Bellomo R, Cruz DN, Wagener G, et al. The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies. J Am Coll Cardiol 2011;57:1752-61.
47. Viau A, El Karoui K, Laouari D, Burtin M, Nguyen C, Mori K, et al. Lipocalin 2 is essential for chronic kidney disease progression in mice and humans. J Clin Invest 2010; 120:4065-76.
48. Kusaka M, Kuroyanagi Y, Mori T, Nagaoka K, Sasaki H, Maruyama T, et al. Serumneutrophil gelatinase-associated lipocalin as a predictor of organ recovery from delayed graft function after kidney transplantation applying donation after cardiac death. Cell Transplant 2008;17:129-34.
49. Wasilewska A, Taranta-Janusz K, Dębek W, Zoch-Zwierz W, Kuroczycka-Saniutycz E. KIM-1 and NGAL: new markers of obstructive nephropathy. Pediatr Nephrol 2011;26:579-86.
50. Eddy AA, Neilson EG. Chronic kidney disease progression. J Am Soc Nephrol 2006;17:2964-6.
51. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med 2003;139:137-47.
52. Clark WF, Macnab JJ, Sontrop JM, Jain AK, Moist L, Salvadori M, et al. Dipstick proteinuria as a screening strategy to identify rapid renal decline. J Am Soc Nephrol 2011;22:1729-36.
53. Fassett RG, Venuthurupalli SK, Gobe GC, Coombes JS, Cooper MA, Hoy WE. Biomarkers in chronic kidney disease: a review. Kidney Int 2011;80:806-21.
54. Smith ER, Lee D, Cai MM, Tomlinson LA, Ford ML, McMahon LP, et al. Urinary neutrophil gelatinase-associated lipocalin may aidprediction of renal decline in patients with non-proteinuric Stages 3 and 4 chronic kidney disease (CKD). Nephrol Dial Transplant 2013;28:1569-79.
55. Nickolas TL, Forster CS, Sise ME, Barasch N, Solá-Del Valle D, Viltard M, et al. NGAL (Lcn2) monomer is associated with tubulointerstitial damage in chronic kidney disease. Kidney Int 2012;82: 718-22.
56. Mitsnefes MM, Kathman TS, Mishra J, Kartal J, Khoury PR, Nickolas TL, et al. Serum neutrophil gelatinaseassociatedlipocalin as a marker of renal function in children with chronickidney disease. Pediatr Nephrol 2007;22:101-8.
57. Bhavsar NA, Kottgen A, Coresh J, Astor BC. Neutrophil gelatinaseassociatedlipocalin (NGAL) and kidney injury molecule 1 (KIM-1) as predictors of incident CKD stage 3: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Kidney Dis 2012;60:233-40.
58. Liu KD, Yang W, Anderson AH, Feldman HI, Demirjian S, Hamano T, et al. Urine neutrophil gelatinase–associated lipocalin levels do not improve risk prediction of progressivechronic kidney disease. Kidney Int 2013;83:909-14.
59. Braun V, Braun M. Active transport of iron and siderophore antibiotics. Curr Opin Microbiol. 2002;5:194-201.
60. Johnson JR, Lockman HA, Owens K, Jelacic S, Tarr PI. High-frequency secondary mutations after suicide-driven allelic exchange mutagenesis in extraintestinal pathogenic Escherichia coli. J Bacteriol 2003:185:5301-5.
61. Wu H, Santoni-Rugiu E, Ralfkiaer E, Porse BT, Moser C, Høiby N, et al. Lipocalin 2 is protective against E. coli pneumonia. Respir Res 2010;11:96.
62. Ichino M, Kuroyanagi Y, KusakaM, Mori T, Ishikawa K, Shiroki R, et al. Increased urinary neutrophil gelatinase associated lipocalin levels in a rat model of upper urinary tract infection. J Urol 2009; 181:2326-31.
63. Wheeler DS, Devarajan P, Ma Q, Harmon K, Monaco M, Cvijanovich N, et al. Serum neutrophil gelatinase-associated lipocalin (NGAL) as amarker of acute kidney injury in critically ill

children with septic shock. Crit Care Med 2008;36:1297-303.

64. Xu S, Pauksen K, Venge P. Serum measurements of humanneutrophil lipocalin (HNL) discriminate between acute bacterial and viral infections. Scand J Clin Lab Invest 1995;55:125-31.
65. Fjaertoft G, Foucard T, Xu S, Venge P. Human neutrophil lipocalin (HNL) as a diagnostic tool in children with acute infections: a study of the kinetics. Acta Paediatr 2005;94:661-6.
66. Björkqvist M, Källman J, Fjaertoft G, Xu S, Venge P, Schollin J. Human neutrophil lipocalin: normal levels and use as a marker for invasive infection in the newborn. Acta Paediatr 2004;93:534-9.
67. Montini G, Tullus K, Hewitt IK. Febrile urinary tract infections in children. N Engl J Med 2011; 365:239-50.
68. Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management, Roberts KB. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. Pediatrics 2011; 128:595–610.
69. Rushton HG. The evaluation of acute pyelonephritis and renal scarring with technetium 99m-dimercaptosuccinic acid renal scintigraphy: evolving concepts and future directions. Pediatr Nephrol 1997;11:108–20.