Int Urol Nephrol (2010) 42:141–150
DOI 10.1007/s11255-009-9608-z

NEPHROLOGY - REVIEW

NGAL: a biomarker of acute kidney injury and other systemic conditions

Sachin S. Soni · Dinna Cruz · Ilona Bobek · Chang Yin Chionh · Federico Nalesso · Paolo Lentini · Massimo de Cal · Valentina Corradi · Grazia Virzi · Claudio Ronco

Received: 1 April 2009 / Accepted: 22 June 2009 / Published online: 7 July 2009

Abstract Neutrophil gelatinase–associated lipocalin (NGAL) is a 25 kDa protein belonging to the lipocalin superfamily. It was initially found in activated neutrophils, however, many other cells, like kidney tubular cells, may produce NGAL in response to various insults. Recently, it has been found to have a role in iron metabolism by virtue of its binding with siderophores. It has also been found to have a role in kidney development and tubular regeneration after injury. In experimental studies, it was found to be highly expressed in response to tubular injury. In subsequent clinical studies, urine NGAL has been found to be an early predictor for acute kidney injury (AKI). Newer devices for early bedside detection of NGAL are now available. Since serum creatinine is known to be an inadequate and late marker of AKI, NGAL might soon emerge as a troponin-like early marker for AKI. Recent evidence also suggests its role as a biomarker in a variety of other renal and non-renal conditions.

Keywords Biomarker · Neutrophil gelatinase–associated lipocalin · NGAL · Acute kidney injury · AKI

S. S. Soni · D. Cruz · I. Bobek · C. Y. Chionh · F. Nalesso · P. Lentini · M. de Cal · V. Corradi · G. Virzi · C. Ronco (✉)
Nephrology Dialysis and Transplantation, San Bortolo Hospital, Via Rodolfi, 37, 36100 Vicenza, Italy
e-mail: cronco@goldnet.it

S. S. Soni
e-mail: dr_sachinsoni@yahoo.com

D. Cruz · M. de Cal · C. Ronco
International Renal Research Institute, Vicenza, Italy

S. S. Soni
Mediciti Hospitals, Hyderabad, India

S. S. Soni
Seth Nandlal Dhoot Hospital, Aurangabad, India

Introduction

Neutrophil gelatinase–associated lipocalin (NGAL) is a protein of the lipocalin family. Lipocalins are composed of eight β-strands that form a β-barrel enclosing a calyx [1]. The calyx binds and transports low-molecular weight chemicals. These proteins interact with cell-surface receptors [2, 3]. Human NGAL consists of a polypeptide chain of 178 amino acids with a molecular mass of 25 kDa. It is expressed by neutrophils and various epithelial cells [4]. Variable degrees of NGAL gene expression is demonstrated in human tissues like uterus, prostate, salivary glands, lung, trachea, stomach, colon, and kidney [5]. Until recently, NGAL was mainly of interest to structural biologists. However, our understanding about its role in human physiology and disease is rapidly increasing. The recent findings that NGAL binds siderophores (iron-chelating molecule

secreted by micro organisms) and is highly expressed in various pathological states like acute kidney injury (AKI) have prompted a large number of studies.

Mishra et al. initially in experimental [6, 7] and later in clinical setting [8] showed NGAL as a promising early biomarker for AKI. Since then, many investigators have studied its utility in AKI diagnosis and in a variety of other clinical settings. The origin of NGAL was originally proposed to be from proximal tubular epithelium [6]. However, subsequent studies found loop of Henle and collecting ducts as the major sites for NGAL synthesis in kidney [9]. The NGAL protein found in proximal tubules after ischemic injury is proposed to be derived from glomerular filtration of circulating NGAL synthesized by other organs like liver. This review discusses the role of NGAL as a biomarker in various conditions.

Physiology of NGAL

Role in iron metabolism

Siderophores are proteins produced by bacteria to scavenge iron from the extracellular space. Siderophores trap iron with high affinity and ensure continuous supply of iron necessary for survival and growth of bacteria. Enterochelin is one such siderophore produced by some strains of bacteria. The evidence for role of NGAL in iron metabolism came from crystallographic studies by Goetz et al. [10] that demonstrated enterochelin–iron complex in the NGAL calyx. NGAL is also called siderocalin as it is the first mammalian protein to bind and transport a bacterial siderophore [10].

Neutrophil gelatinase–associated lipocalin interacts with cells by specific cell-surface receptors. Hitherto, two receptors are identified; the megalin–cubilin multiscavenger complex found on the brush-border surface of renal tubular epithelial cells [11] and the 24p3R (NGAL was originally called 24p3), an organic cation transporter [12]. NGAL is also proposed to interact with some other receptors like extracellular protein kinases, hepatocyte growth factor [13], and gelatinase B [14]. These receptors seem to have a role in cellular trafficking and endocytosis of NGAL. Endocytosis occurs as NGAL protein alone (Apo-NGAL) or NGAL complexed with iron-binding siderophores (Holo-NGAL).

The cellular effects depend on the form of NGAL endocytosed. When transported as Apo-NGAL, it captures intracellular iron and transports it outside leading to depletion of intracellular iron. On the contrary, when endocytosed as Holo-NGAL, it releases iron–siderophore complex and contributes to intracellular iron pool [12].

Role in innate immunity to bacteria

As discussed earlier, by virtue of its binding with siderophores, NGAL prevents the growth of the bacterial strains that depend on siderophores for iron supply [10]. The biologic importance of this finding is examplified in genetically modified mice, deficient for NGAL genes. These animals were more sensitive to *Escherichia coli* infection and died of sepsis more readily than wild-type mice [15, 16].

In addition to enterochelin, NGAL has been shown to bind to other siderophores like carboxymycobactin, a soluble protein secreted by mycobacteria [17]. Thus, NGAL may potentially have an important role in defending against bacterial and mycobacterial infection through iron sequestration.

Role in kidney development

In vitro studies in rats suggest that NGAL may be involved in kidney development [18]. Administration of purified NGAL to early progenitor cells in metanephric mesenchyme produced a proliferative effect followed by epithelial differentiation of these elements and generation of nephron-shape formations expressing glomerular, proximal, and distal tubular surface cellular markers [19]. NGAL participates in kidney development by virtue of its role in iron metabolism. For mechanistic details, the reader is referred to the relevant articles [18, 19].

Role as a growth factor

Several findings indirectly point to the role of NGAL as a growth factor. NGAL expression is markedly increased in stimulated epithelia. Its increased expression in colonic cells in areas of inflammation or neoplasia [20, 21] was recognized many years ago. Serum NGAL levels are increased in the patients with acute bacterial infections [22]. Elevated NGAL is also found in the sputum of subjects with asthma or

chronic obstructive airway disease [23] and in the bronchial secretions from the patients with subclinical emphysema [24]. It is postulated that interaction between inflammatory cells and the epithelial cells leads to the upregulation of NGAL in both neutrophils and epithelium [21]. The role of NGAL is also implicated in tumor growth and progression [25, 26].

It is proposed that NGAL plays an important role in renal regeneration and repair after ischemic injury [6]. It seems to support development and de-differentiation of tubular epithelia following injury. In addition, if presented before or in early stages of AKI, it may have a protective effect. An in vitro study by Mori et al. [27] in mouse models of ischemia-reperfusion injury showed that a single dose of NGAL (10 μg) protects the kidney and decreases azotemia. This effect depends on the delivery of iron by NGAL–siderophore–iron complex. This complex rescues *N*-cadherin (adhesion molecule), upregulates heme oxygenase-1 (a protective enzyme), and inhibits cell death.

Methods of NGAL measurement

A variety of methods for NGAL measurement have been used in published studies. In initial studies [6], both urine and serum NGAL estimations were carried out by Western blot technique. Subsequent clinical studies have utilized immunoblotting or research ELISA-based techniques using a commercially available monoclonal NGAL antibody (Antibodyshop, Gentofte, Denmark).

A standardized point-of-care Triage® NGAL Device (Biosite Incorporated, San Diego, CA, USA) has been devised for the measurement of plasma neutrophil gelatinase–associated lipocalin (pNGAL). The assay was found to correlate well with research ELISA in a pilot study of 40 plasma samples and 12 calibrations. Its clinical application was later validated in a study of 120 patients undergoing cardiopulmonary bypass (CPB) [28]. In 45 patients who developed AKI, the diagnosis using serum creatinine concentration was delayed by 2–3 days, while pNGAL concentrations increased threefold within 2 h. At cutoff value of 150 ng/mL for the 2-h pNGAL concentration, the area under curve (AUC) for prediction of AKI was found to be 0.96 with sensitivity of 0.84 and specificity of 0.94. The test needs only microliter quantities of plasma and quantitative results are available within 15 min. Bedside testing for plasma NGAL is potentially possible using this device.

A urine NGAL assay ARCHITECT® analyzer (Abbott Diagnostics, Illinois, USA) is also available for clinical application. In a pilot study with 136 urine samples and six calibration standards, urinary neutrophil gelatinase–associated lipocalin (uNGAL) concentrations by the ARCHITECT® analyzer correlated well with research ELISA. Its clinical application was later validated in a study of 196 patients undergoing CPB [29]. The diagnosis of AKI using serum creatinine concentration was delayed by 2–3 days after CPB, while uNGAL concentrations increased by 15-fold within 2 h and by 25-fold at 4 and 6 h after CPB. At cutoff value of 100 g/L for the 2-h urine NGAL concentration, the AUC for prediction of AKI was 0.95 with sensitivity of 0.82 and specificity of 0.90 [29]. The test is found to be easy for application with no manual pre-treatment steps [30]. The test needs only 150 μL of urine and quantitative results are available within 35 min.

NGAL as a biomarker

NGAL is found to be a promising biomarker in many conditions as shown in Table 1. It has been found as a promising biomarker for early diagnosis, for predicting disease severity, for therapeutic monitoring, and for predicting clinical outcomes. The following section discusses the potential role of NGAL in a variety of renal and non-renal conditions.

Acute kidney injury

The last decade has witnessed many advances in the treatment of AKI. However, morbidity and mortality have not improved significantly [31–33]. Serum creatinine is an insensitive and late marker [34] for AKI. It is important to diagnose AKI as early as possible to facilitate effective interventions [35]. This had led to an aggressive search for newer biomarkers for AKI. NGAL appears to be the most promising molecule among the many newer molecules [36].

As mentioned earlier, Mishra et al. [6] first proposed NGAL as a novel early biomarker. In mouse models of renal ischemia-reperfusion injury subjected to 30 min of bilateral renal artery

Table 1 Potential uses of NGAL as a biomarker

1. Biomarker for early detection of AKI
A: Timing of kidney injury known
a. After cardiac surgery
b. After contrast procedures
B: Timing of kidney injury unknown
a. In critically ill patients
b. After multiple trauma
2. Predictor of outcome of AKI
3. As a marker of disease activity and severity
a. Pediatric lupus nephritis
b. IgA nephropathy
c. Idiopathic glomerulonephritis
4. As a marker for cyst volume progression in ADPKD
5. Biomarker for
a. Detection and therapeutic response of brain tumor
b. Inflammatory bowel disease
c. Breast tumors

occlusion, NGAL was found to be one of the seven genes which were highly upregulated. NGAL was easily detected in the urine within 2 h following ischemia. In the same study, separate sets of mice were subjected to 5, 10, and 20 min of ischemia. Importantly, uNGAL was detected even in these mice, appearing after 6 h in mice with 5 min ischemia and after 4 h in mice with 10 and 20 min of ischemia. Thus, uNGAL was found to be a very sensitive marker of ischemic AKI and its levels correlated to the dose and duration of renal ischemia. Additionally, uNGAL was detectable after 1 day of cisplatin administration in a mouse model of nephrotoxic AKI, suggesting its sensitivity in other models of tubular injury [6].

Many clinical studies followed these important experimental observations (Table 2). In a prospective study of 71 children undergoing CPB, pNGAL and uNGAL levels were markedly increased within 2 h in patients who developed AKI [8]. The diagnosis of AKI by serum creatinine criteria was possible only after 1–3 days. uNGAL at 2 h after CPB was the most powerful independent predictor of AKI with area under curve (AUC) of 0.998. A cutoff value of 50 μ/L was 100% sensitive and 98% specific in predicting AKI. Similarly, at cutoff value of 25 μg/L pNGAL was 70% sensitive and 94% specific in predicting AKI with AUC of 0.91. In another prospective study of 91 children with congenital heart disease, both urine and plasma NGAL predicted contrast-induced nephropathy within 2 h of elective cardiac catheterization [37]. The AUC for prediction of contrast nephropathy (at 2 h) was found to be 0.92 for uNGAL and 0.91 for pNGAL.

Many other studies followed this novel observation. Zapitelli et al. [38] studied uNGAL in 140 mechanically ventilated patients. uNGAL levels increased significantly (>6 times) 2 days earlier than 50% increase in serum creatinine levels. uNGAL levels also correlated with severity of AKI. Serum NGAL was also studied in 143 critically ill children with systemic inflammatory response syndrome (SIRS) or septic shock within 24 h of admission to ICU [39]. It was found to be highly sensitive but non-specific (sensitivity 84%, specificity 39%) biomarker of AKI. Makris et al. [40] have found uNGAL as an early marker of AKI in critically ill polytrauma patients.

In a recently published study, a single measurement of uNGAL in emergency department is found to be highly sensitive and specific (sensitivity 90%, specificity 99%) in diagnosing AKI in 635 adult patients [41]. uNGAL level also helped to distinguish patients with AKI from other morbid conditions with elevated creatinine, like pre-renal azotemia and CKD. Many other studies have found NGAL as a reliable early biomarker for AKI [42–44].

In addition to being an early biomarker for AKI, uNGAL has been found to be a predictor of severity of AKI. In a study of 196 children undergoing CPB at a single center, 2-h post-operative uNGAL levels predicted requirement of renal replacement therapy, duration of hospital stay and mortality [29].

Renal transplantation

Mishra et al. [45] studied protocol biopsy specimens within 1 h of reperfusion in 13 cadaveric and 12 living-related renal allografts for NGAL expression by immunohistochemistry. The staining intensity for NGAL was found to correlate well with cold ischemia time, peak post-transplant creatinine, and requirement of dialysis. In another study involving 33 cases, uNGAL was found to be an early predictor of delayed graft function (DGF) defined as need for dialysis within the first week after transplantation [46]. uNGAL on the day of transplant surgery was predictor of DGF with AUC of 0.9. Importantly, creatinine level peaked only after 2–4 days. In a

Table 2 Major studies reporting use of uNGAL as an early biomarker in AKI

Author	Year	Study population	Serum NGAL evaluated	Method of detection	Cutoff	Major findings
Mishra et al. [8]	2005	71 children undergoing cardiac surgery	Yes	Western blot and research ELISA	>50 μg/L	2 h post-operative uNGAL had 100% sensitivity and 98% specificity for predicting AKI (AUC 0.998)
Wagener et al. [42]	2006	81 adults undergoing cardiac surgery	No	Immunoblotting	213 ng/mL	18 h post-operative uNGAL had 73% sensitivity and 78% specificity for predicting AKI (AUC 0.8)
Bachorzewaska-Gazewaska et al. [66]	2006	35 adults undergoing percutaneous coronary interventions (PCI)	Yes	ELISA	Not reported	Significant elevation of serum NGAL at 2 and 4 h and uNGAL at 4 and 12 h after PCI. Both serum and uNGAL had good correlation with serum creatinine.
Zapitelli et al. [38]	2007	140 critically ill children	No	ELISA	Various	uNGAL was a useful predictor of development of AKI
Nickolas et al. [41]	2008	635 adults in emergency department	No	Immunoblotting	130 μg/g creatinine	A single measurement in emergency department was useful in distinguishing patients with AKI from patients with pre-renal azotemia, chronic kidney disease, or normal renal function. uNGAL had 90% sensitivity and 99% specificity in predicting AKI (AUC 0.948)
Benett et al. [29]	2008	196 children undergoing CPB	No	ARCHITECT® analyser	100 ng/mL	2 h post-operative uNGAL had 82% sensitivity and 90% specificity in predicting AKI (AUC 0.95)
Xin et al. [44]	2008	33 adults undergoing cardiac surgery	Yes	ELISA	250 μg/mmol creatinine	uNGAL at 2 h after surgery with suggested cutoff had 81% sensitivity and 78% specificity in predicting AKI. Serum NGAL levels were not found to be useful in predicting AKI

AKI acute kidney injury, *AUC* area under curve, *uNGAL* urinary neutrophil gelatinase–associated lipocalin

study by Kusaka et al. [47] in cadaveric transplant patients, fall in NGAL levels occurred earlier than fall in serum creatinine or improvement in urine output during recovery from DGF.

Thrombotic microangiopathy

In pediatric patients with diarrhea-associated hemolytic uremic syndrome, Trachtman et al. [48] used peak uNGAL levels within 5 days of hospitalization to divide patients into two groups (<200 and ≥200 ng/mL). Patients with higher uNGAL levels required dialysis more frequently. uNGAL served as an adjunctive early predictor of severity of the microangiopathic disease in this study. Increased uNGAL level was postulated to be secondary to associated tubular damage in HUS.

Chronic kidney disease

Polycystic kidney disease

The uNGAL levels are shown to correlate well with residual renal function, serum creatinine, and cyst enlargement in patients with autosomal dominant polycystic kidney disease [49]. In an experimental setting, addition of NGAL to the cells from PKD1 mutant mice suppressed the cyst development [50].

Glomerular diseases

Brunner et al. [51] in a cohort of 35 cases of childhood-onset systemic lupus erythematosus (SLE) found strong correlation of uNGAL levels with renal disease activity. In addition, uNGAL levels correlated with the activity and chronicity score on renal histology. In a similar study, Suzuki et al. found strong correlation of uNGAL levels with SLE disease activity [52]. However, plasma NGAL levels did not show such correlation in this study.

In a cohort of 70 cases of IgA nephropathy, Ding et al. [53] compared levels of uNGAL, urinary *N*-acetyl-β-D-glucosaminidase (uNAG) and urinary creatinine with 40 healthy controls. uNGAL levels were significantly elevated in IgA nephropathy patients compared with controls. uNGAL/creatinine ratio showed strong correlation with histological and clinical disease activity, while uNAG levels lacked such correlation.

In proteinuric patients with idiopathic glomerulonephritis, Bolignano et al. [54, 55] found significant correlation of uNGAL with serum creatinine; daily proteinuria and residual renal function over 1 year follow-up. The authors have also published their observations regarding change of uNGAL levels in 15 such patients after anti-inflammatory therapy with intravenous immunoglobulin [56]. An impressive decrease in uNGAL level was observed 1 h after the infusion. The authors hypothesized that uNGAL can serve as biomarker to assess treatment response and predict disease severity.

Other systemic conditions

Poniatowski et al. have found serum and urine NGAL as sensitive early markers of renal impairment in patients with chronic heart failure [57]. In another study, uNGAL levels were found to be significantly elevated in patients with chronic heart failure. Its level correlated directly with urine albumin excretion rate and inversely with estimated glomerular filtration rate [58]. Thus, uNGAL can be a potential biomarker for the cardio-renal syndrome.

Urinary NGAL was also found to be elevated in premature and low birth weight infants, and it correlated significantly with birth weight and gestational age [59]. A recently published study by Smith et al. [60] suggests uNGAL along with other urine metalloproteinases as the promising biomarkers for the presence of brain tumor. They propose that uNGAL level can be useful for therapeutic follow-up and early non-invasive detection of recurrence. Similarly, it can potentially serve as a marker to assess severity of inflammatory bowel diseases [61]. Fernandez et al. [62] found uNGAL–MMP9 complex as a useful biomarker for breast cancer.

Comparison of serum NGAL and uNGAL as biomarkers

The rapidly evolving knowledge about NGAL kinetics in AKI suggest that NGAL gene upregulation occurs in two major areas in early phase of AKI, namely, kidney and other systemic organs like lungs and liver [63]. It is postulated that the NGAL synthesized in the distal tubules of the kidneys is efficiently excreted in urine and that synthesized in other organs constitute the systemic pool [9, 63, 64]. Both serum and uNGAL have

Table 3 Major studies comparing NGAL with other biomarkers

Author	Year	Study population	Other biomarker	Major findings
Clinical setting: cardiac surgery				
Koyner et al. [67]	2008	72 Adults	Cystatin C	Both urinary Cystatin C and NGAL were superior to creatinine for early diagnosis of AKI
Portilla et al. [68]	2008	40 Children	Liver fatty acid binding protein (L-FABP)	Both urinary L-FABP and NGAL were early predictors of AKI. NGAL had superior area under curve (AUC) with 100% sensitivity and specificity compared with L-FABP (71% sensitivity and 68% specificity)
Xin et al. [44]	2008	33 Adults	Urinary IL-18	Both urinary IL-18 and NGAL were early predictors of AKI. Serum NGAL was not useful for early diagnosis of AKI.
Haase-Fielitz et al. [69]	2008	100 Adults	Serum cystatin C	Both serum cystatin C and plasma NGAL were early predictors of AKI
Clinical setting: contrast administration				
Bachorzewska-Gajewska et al. [43]	2007	100 Adults	Serum cystatin C	Elevation of urinary NGAL (2 h), serum NGAL (4 h), and serum cystatin C (8 h) occured earlier than serum creatinine. NGAL performed better than serum cystatin C as early biomarker
Ling et al. [70]	2008	150 Adults	Urinary IL-18	Both urinary IL-18 and NGAL were early predictors of AKI
Clinical setting: kidney transplantation				
Parikh et al. [46]	2006	53 Patients	Urinary IL-18	Both urinary IL-18 and NGAL were early predictors of delayed graft function

NGAL neutrophil gelatinase–associated lipocalin

been found to be reliable predictors of AKI [8]. uNGAL measurement is probably more reflective of local renal injury and is non-invasive in nature reducing the need for frequent blood sampling and handling of blood lines in critically ill patients. Urine sample is also relatively free of interfering proteins [29]. On the other hand, serum NGAL measurement is clearly useful in anuric patients and does not need correction for creatinine concentration unlike urine sample. Whether one measurement is useful over the other can be known after wider clinical application of these tests. It is also possible that together they provide complementary information.

Comparison of NGAL with other newer biomarkers of AKI

In addition to NGAL, many other molecules have been studied and found useful as early biomarkers of AKI when compared with serum creatinine. Very few studies have directly compared NGAL with other molecules (Table 3). Performance of NGAL has been comparable or better than other biomarkers.

Future directions

NGAL appears to have successfully cleared the initial hurdles of biomarker development at the experimental stage and in initial clinical studies. Newer commercial tools for its measurement will definitely help to enhance clinical application in broader population. It will likely emerge as a powerful biomarker in AKI panel akin to troponins in cardiac panel [65].

Conclusion

Urinary NGAL is rapidly emerging as a very promising early biomarker in a variety of renal and

non-renal conditions. The horizons for its applications are rapidly expanding. It is likely to be used as a powerful predictive tool for many disorders, especially AKI in future. Early detection of AKI will provide a much-needed window of opportunity for quick and effective interventions. The easy and rapid detection of uNGAL using newer devices increase the feasibility of its widespread clinical application.

Acknowledgments Dr. Sachin S. Soni is supported by a fellowship grant from international Society of Nephrology.

References

1. Flower DR, North AC, Sansom CE (2000) The lipocalin protein family: structural and sequence overview. Biochim Biophys Acta 1482:9–24
2. Akerstrom B, Flower DR, Salier JP (2000) Lipocalins: unity in diversity. Biochim Biophys Acta 1482:1–8
3. Flower DR (1996) The lipocalin protein family: structure and function. Biochem J 318(Pt 1):1–14
4. Uttenthal O (2005) NGAL: a marker molecule for the distressed kidney? Clin Lab Internat 29:39–41
5. Cowland JB, Borregaard N (1997) Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. Genomics 45:17–23
6. Mishra J, Ma Q, Prada A et al (2003) Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol 14:2534–2543
7. Mishra J, Mori K, Ma Q et al (2004) Neutrophil gelatinase-associated lipocalin: a novel early urinary biomarker for cisplatin nephrotoxicity. Am J Nephrol 24:307–315
8. Mishra J, Dent C, Tarabishi R et al (2005) Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 365:1231–1238
9. Schmidt-Ott KM, Mori K, Kalandadze A et al (2006) Neutrophil gelatinase-associated lipocalin-mediated iron traffic in kidney epithelia. Curr Opin Nephrol Hypertens 15:442–449
10. Goetz DH, Holmes MA, Borregaard N et al (2002) The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell 10:1033–1043
11. Hvidberg V, Jacobsen C, Strong RK et al (2005) The endocytic receptor megalin binds the iron transporting neutrophil-gelatinase-associated lipocalin with high affinity and mediates its cellular uptake. FEBS Lett 579:773–777
12. Devireddy LR, Gazin C, Zhu X et al (2005) A cell-surface receptor for lipocalin 24p3 selectively mediates apoptosis and iron uptake. Cell 123:1293–1305
13. Kjeldsen L, Johnsen AH, Sengelov H et al (1993) Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem 268:10425–10432
14. Yan L, Borregaard N, Kjeldsen L et al (2001) The high molecular weight urinary matrix metalloproteinase (MMP) activity is a complex of gelatinase B/MMP-9 and neutrophil gelatinase-associated lipocalin (NGAL). Modulation of MMP-9 activity by NGAL. J Biol Chem 276:37258–37265
15. Berger T, Togawa A, Duncan GS et al (2006) Lipocalin 2-deficient mice exhibit increased sensitivity to *Escherichia coli* infection but not to ischemia-reperfusion injury. Proc Natl Acad Sci U S A 103:1834–1839
16. Flo TH, Smith KD, Sato S et al (2004) Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature 432:917–921
17. Holmes MA, Paulsene W, Jide X et al (2005) Siderocalin (Lcn 2) also binds carboxymycobactins, potentially defending against mycobacterial infections through iron sequestration. Structure 13:29–41
18. Gwira JA, Wei F, Ishibe S et al (2005) Expression of neutrophil gelatinase-associated lipocalin regulates epithelial morphogenesis in vitro. J Biol Chem 280:7875–7882
19. Yang J, Goetz D, Li JY et al (2002) An iron delivery pathway mediated by a lipocalin. Mol Cell 10:1045–1056
20. Nielsen BS, Borregaard N, Bundgaard JR et al (1996) Induction of NGAL synthesis in epithelial cells of human colorectal neoplasia and inflammatory bowel diseases. Gut 38:414–420
21. Carlson M, Raab Y, Seveus L et al (2002) Human neutrophil lipocalin is a unique marker of neutrophil inflammation in ulcerative colitis and proctitis. Gut 50:501–506
22. Xu SY, Pauksen K, Venge P (1995) Serum measurements of human neutrophil lipocalin (HNL) discriminate between acute bacterial and viral infections. Scand J Clin Lab Invest 55:125–131
23. Keatings VM, Barnes PJ (1997) Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. Am J Respir Crit Care Med 155:449–453
24. Betsuyaku T, Nishimura M, Takeyabu K et al (1999) Neutrophil granule proteins in bronchoalveolar lavage fluid from subjects with subclinical emphysema. Am J Respir Crit Care Med 159:1985–1991
25. Friedl A, Stoesz SP, Buckley P et al (1999) Neutrophil gelatinase-associated lipocalin in normal and neoplastic human tissues. Cell type-specific pattern of expression. Histochem J 31:433–441
26. Stoesz SP, Friedl A, Haag JD et al (1998) Heterogeneous expression of the lipocalin NGAL in primary breast cancers. Int J Cancer 79:565–572
27. Mori K, Lee HT, Rapoport D et al (2005) Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J Clin Invest 115:610–621
28. Dent CL, Ma Q, Dastrala S et al (2007) Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: a prospective uncontrolled cohort study. Crit Care 11:R127

29. Bennett M, Dent CL, Ma Q et al (2008) Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. Clin J Am Soc Nephrol 3:665–673
30. Devarajan P (2008) Neutrophil gelatinase-associated lipocalin (NGAL): a new marker of kidney disease. Scand J Clin Lab Invest Suppl 241:89–94
31. Uchino S, Kellum JA, Bellomo R et al (2005) Acute renal failure in critically ill patients: a multinational, multicenter study. JAMA 294:813–818
32. Kohli HS, Bhat A, Aravindan AN et al (2007) Predictors of mortality in elderly patients with acute renal failure in a developing country. Int Urol Nephrol 39:339–344
33. Lima RS, Marques CN, Silva Junior GB et al (2008) Comparison between early and delayed acute kidney injury secondary to infectious disease in the intensive care unit. Int Urol Nephrol 40:731–739
34. Devarajan P (2007) Emerging biomarkers of acute kidney injury. Contrib Nephrol 156:203–212
35. Ronco C (2007) N-GAL: diagnosing AKI as soon as possible. Crit Care 11:173
36. Ronco C (2008) NGAL: an emerging biomarker of acute kidney injury. Int J Artif Organs 31:199–200
37. Hirsch R, Dent C, Pfriem H et al (2007) NGAL is an early predictive biomarker of contrast-induced nephropathy in children. Pediatr Nephrol 22:2089–2095
38. Zappitelli M, Washburn KK, Arikan AA et al (2007) Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: a prospective cohort study. Crit Care 11:R84
39. Wheeler DS, Devarajan P, Ma Q et al (2008) Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. Crit Care Med 36:1297–1303
40. Makris K, Markou N, Evodia E et al (2009) Urinary neutrophil gelatinase-associated lipocalin (NGAL) as an early marker of acute kidney injury in critically ill multiple trauma patients. Clin Chem Lab Med 47:79–82
41. Nickolas TL, O'Rourke MJ, Yang J et al (2008) Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. Ann Intern Med 148:810–819
42. Wagener G, Jan M, Kim M et al (2006) Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. Anesthesiology 105:485–491
43. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E et al (2007) Could neutrophil-gelatinase-associated lipocalin and cystatin C predict the development of contrast-induced nephropathy after percutaneous coronary interventions in patients with stable angina and normal serum creatinine values? Kidney Blood Press Res 30:408–415
44. Xin C, Yulong X, Yu C et al (2008) Urine neutrophil gelatinase-associated lipocalin and interleukin-18 predict acute kidney injury after cardiac surgery. Ren Fail 30:904–913
45. Mishra J, Ma Q, Kelly C et al (2006) Kidney NGAL is a novel early marker of acute injury following transplantation. Pediatr Nephrol 21:856–863
46. Parikh CR, Jani A, Mishra J et al (2006) Urine NGAL and IL-18 are predictive biomarkers for delayed graft function following kidney transplantation. Am J Transpl 6:1639–1645
47. Kusaka M, Kuroyanagi Y, Mori T et al (2008) Serum neutrophil gelatinase-associated lipocalin as a predictor of organ recovery from delayed graft function after kidney transplantation from donors after cardiac death. Cell Transpl 17:129–134
48. Trachtman H, Christen E, Cnaan A et al (2006) Urinary neutrophil gelatinase-associated lipocalcin in D+HUS: a novel marker of renal injury. Pediatr Nephrol 21:989–994
49. Bolignano D, Coppolino G, Campo S et al (2007) Neutrophil gelatinase-associated lipocalin in patients with autosomal-dominant polycystic kidney disease. Am J Nephrol 27:373–378
50. Wei F, Karihaloo A, Yu Z et al (2008) Neutrophil gelatinase-associated lipocalin suppresses cyst growth by Pkd1 null cells in vitro and in vivo. Kidney Int 74:1310–1318
51. Brunner HI, Mueller M, Rutherford C et al (2006) Urinary neutrophil gelatinase-associated lipocalin as a biomarker of nephritis in childhood-onset systemic lupus erythematosus. Arthritis Rheum 54:2577–2584
52. Suzuki M, Wiers KM, Klein-Gitelman MS et al (2008) Neutrophil gelatinase-associated lipocalin as a biomarker of disease activity in pediatric lupus nephritis. Pediatr Nephrol 23:403–412
53. Ding H, He Y, Li K et al (2007) Urinary neutrophil gelatinase-associated lipocalin (NGAL) is an early biomarker for renal tubulointerstitial injury in IgA nephropathy. Clin Immunol 123:227–234
54. Bolignano D, Coppolino G, Campo S et al (2008) Urinary neutrophil gelatinase-associated lipocalin (NGAL) is associated with severity of renal disease in proteinuric patients. Nephrol Dial Transpl 23:414–416
55. Bolignano D, Coppolino G, Lacquaniti A et al (2008) Pathological and prognostic value of urinary neutrophil gelatinase-associated lipocalin in macroproteinuric patients with worsening renal function. Kidney Blood Press Res 31:274–279
56. Bolignano D, Coppolino G, Aloisi C et al (2008) Effect of a single intravenous immunoglobulin infusion on neutrophil gelatinase-associated lipocalin levels in proteinuric patients with normal renal function. J Investig Med 56:997–1003
57. Poniatowski B, Malyszko J, Bachorzewska-Gajewska H et al (2009) Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in patients with chronic heart failure and coronary artery disease. Kidney Blood Press Res 32:77–80
58. Damman K, van Veldhuisen DJ, Navis G et al (2008) Urinary neutrophil gelatinase associated lipocalin (NGAL), a marker of tubular damage, is increased in patients with chronic heart failure. Eur J Heart Fail 10:997–1000
59. Lavery AP, Meinzen-Derr JK, Anderson E et al (2008) Urinary NGAL in premature infants. Pediatr Res 64:423–428
60. Smith ER, Zurakowski D, Saad A et al (2008) Urinary biomarkers predict brain tumor presence and response to therapy. Clin Cancer Res 14:2378–2386

61. Manfredi MA, Zurakowski D, Rufo PA et al (2008) Increased incidence of urinary matrix metalloproteinases as predictors of disease in pediatric patients with inflammatory bowel disease. Inflamm Bowel Dis 14:1091–1096
62. Fernandez CA, Yan L, Louis G et al (2005) The matrix metalloproteinase-9/neutrophil gelatinase-associated lipocalin complex plays a role in breast tumor growth and is present in the urine of breast cancer patients. Clin Cancer Res 11:5390–5395
63. Schmidt-Ott KM, Mori K, Li JY et al (2007) Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol 18:407–413
64. Liu Q, Nilsen-Hamilton M (1995) Identification of a new acute phase protein. J Biol Chem 270:22565–22570
65. Devarajan P (2008) Neutrophil gelatinase-associated lipocalin—an emerging troponin for kidney injury. Nephrol Dial Transplant 23:3737–3743
66. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E et al (2006) Neutrophil-gelatinase-associated lipocalin and renal function after percutaneous coronary interventions. Am J Nephrol 26:287–292
67. Koyner JL, Bennett MR, Worcester EM et al (2008) Urinary cystatin C as an early biomarker of acute kidney injury following adult cardiothoracic surgery. Kidney Int 74:1059–1069
68. Portilla D, Dent C, Sugaya T et al (2008) Liver fatty acid-binding protein as a biomarker of acute kidney injury after cardiac surgery. Kidney Int 73:465–472
69. Haase-Fielitz A, Bellomo R, Devarajan P (2009) Novel and conventional serum biomarkers predicting acute kidney injury in adult cardiac surgery-A prospective cohort study. Crit Care Med 37:553–560
70. Ling W, Zhaohui N, Ben H et al (2008) Urinary IL-18 and NGAL as early predictive biomarkers in contrast-induced nephropathy after coronary angiography. Nephron Clin Pract 108:176–181