

Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury

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Acute kidney injury is a frequent and serious condition. Clinically it is most important to diagnose it at an early stage, yet traditional laboratory tests are unable to do so. Neutrophil gelatinase-associated lipocalin (NGAL) has been identified in preclinical and clinical studies as a promising biomarker of acute kidney injury. It is specifically released by the damaged kidney and can be detected in the urine. Diagnostic tests that measure the urinary NGAL concentration are promising new tools in the diagnosis of acute kidney injury. Either alone or in combination with other biomarkers they will not only have an impact on medical decisions in future daily clinical routine, but they will also provide the basis for testing novel emergency therapies for a disease that is often recognized too late.

Clinical significance of acute kidney injury

Acute kidney injury (AKI) was first described in the early 19th century and at the time was named “ischuria renalis”. The term *acute kidney injury* refers to a rapidly progressive, yet potentially reversible deterioration of kidney function. In most cases, acute kidney injury is the result of a reduction in the kidneys’ blood supply. This is frequently observed in generalized infection (sepsis), blood loss or declining blood pressure during surgery or in diseases of the blood vessels that supply the kidneys (e.g. inflammation of the vascular wall). Other frequent causes are kidney-damaging drugs (e.g. i.v. contrast media) or proteins produced by the body

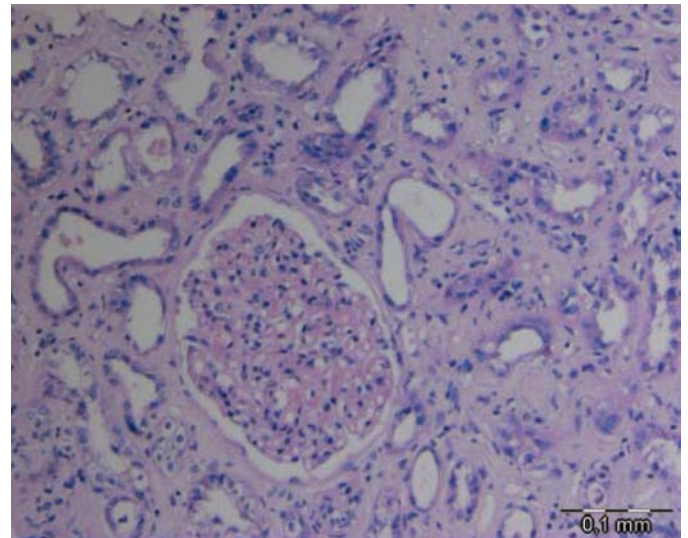


Fig. 2: Histological picture of a kidney damaged by acute tubular necrosis. Note dilated kidney tubules with regressive changes, indicating structural damage. (The photo was kindly provided by Prof. Wolfgang Schneider, Helios-Klinikum Berlin)

that in large quantities and in free form can injure the kidneys (e.g. myoglobin released from damaged muscle tissue) (see Fig. 1). Tissue damage to the kidney results in a temporary or permanent loss of function (see Fig. 2). This causes metabolic products (e.g. creatinine and urea) to accumulate in the blood, instead of being excreted by the kidney through the urine.

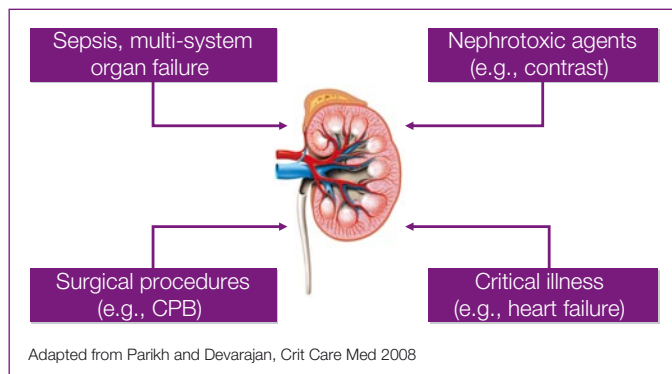


Fig. 1: Common causes of acute kidney injury

Uremia develops, in other words the body becomes poisoned with retained toxins, clinically leading to weakness, confusion and at an advanced stage to loss of consciousness. Since the kidneys also regulate the fluid, electrolyte and acid-base equilibrium of the body, these functions, too, become impaired during the course of acute kidney injury. This results in additional symptoms, including an elevation of blood pressure (hypertension), tissue swelling (edema) and shortness of breath.

Table 1:

RIFLE criteria define the degree of acute kidney injury as determined by serum creatinine, glomerular filtration rate (GFR) and urinary output criteria. The acronym RIFLE means risk, injury, failure, loss and end stage renal disease. R, I, and F are acute criteria, whereas L and E can only be assessed during the course of the disease (Bellomo et al., Crit. Care 2004; 8: R204).

Classification of acute kidney injury subject to RIFLE criteria	RIFLE category	Criteria	
Acute classification	Risk	Acute increase of serum creatinine, greater than 1.5 fold or 25% decline of GFR	Urine output < 0.5 ml/kg/h for at least 6 hours
	Injury	Acute increase of serum creatinine, greater than twofold or 50% decline of GFR	Urine output < 0.5 ml/kg/h for at least 12 h
	Failure	Acute increase of serum creatinine, greater than threefold or 75% decline of GFR	Urine output < 0.3 ml/kg/h for at least 24 h or anuria for at least 12 h
Classification of the outcome	Loss	Persistent kidney injury for > 4 weeks	
	ESRD (End stage renal disease)	Dialysis-requiring renal failure for > 3 months	

When these symptoms are pronounced and conservative therapy regimens fail, end-stage renal disease ensues, which requires renal replacement therapy (e.g. hemodialysis and/or hemofiltration) (see Fig. 3).

Despite these serious and potentially life-threatening effects of acute kidney injury, the current tools for diagnosing this disease at an early stage are limited. In clinical routine, acute kidney injury is identified by a rise in the serum concentration of creatinine, a metabolic product in the blood that is normally filtered through the kidneys and excreted in the urine (see Table 1). An increase of serum creatinine indicates deterioration of kidney function. By its very nature, however, this increase is only apparent hours to days after the actual kidney damage has occurred, since the metabolic product creatinine initially has to accumulate in the blood. Alternatively, the diagnosis of acute kidney injury can be established by detecting a reduced urine production. However, in clinical practice this is difficult, since it requires the positioning of a urinary catheter and/or close monitoring. Unlike in other organ systems – for example, the heart, where acute tissue damage can be detected within minutes (e.g. by measuring cardiac troponine levels in the blood) – analogous tests have not been available for the kidney in everyday clinical routine. Therefore, the opportunity of an early diagnosis of acute kidney injury is often missed. Yet an early detection of injury would be very desirable, since medical measures could then be initiated timely to contain the damage.

The potential significance of an early diagnosis of acute kidney injury

The incidence of acute kidney injury is estimated at 7 % of all in-hospital patients. Notably, its occurrence is associated with a particularly poor prognosis. For instance, of critically ill patients, approximately 6 % develop acute kidney injury requiring renal replacement therapy. The mortality rate in these patients (despite effective dialysis) is approximately 60 %. This is attributed to the fact that acute kidney injury as such constitutes a major risk factor for complications in other organ systems. Thus, an early detection of acute kidney injury would substantially improve the clinical risk assessment of a given patient and hence may be a critical factor determining medical decisions during the course of the disease (e.g. the decision to transfer a patient into an intensive care setting at an early stage). Furthermore, animal studies have shown that several different pharmacotherapeutic approaches are effective in the treatment of acute kidney injury – yet only if therapy is initiated early on. Consequently a laboratory test that could detect acute kidney injury at an early stage would not only guide the medical management of acutely ill patients, but it would also provide the grounds for novel therapeutic approaches that require an early diagnosis.

Table 2:

Test characteristics of urinary NGAL and serum creatinine for the detection of acute kidney injury in patients admitted to an emergency room (Nickolas et al., <i>Ann. Intern. Med.</i> 2008; 148: 810). AUC: Area under the receiver-operating characteristic curve).				
Marker	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)
Urinary NGAL	85 µg/g creatinine	0.93 (0.78–0.99)	0.98 (0.97–0.99)	0.948 (0.881–1.0)
	130 µg/g creatinine	0.90 (0.73–0.98)	0.995 (0.99–1.0)	
Serum creatinine	124 µmol/l	0.93 (0.73–0.99)	0.75 (0.71–0.78)	0.921 (0.865–0.978)
	221 µmol/l	0.77 (0.58–0.90)	0.93 (0.90–0.95)	

Identification of NGAL as a biomarker of acute kidney injury

The search for novel biomarkers of acute kidney injury has been significantly accelerated by the introduction of novel experimental approaches allowing genome-wide analysis of gene expression by means of microarray analysis. In animal models, the expression of a gene, which encodes the protein NGAL, was massively activated after experimentally induced acute kidney damage. In analogy, proteome analyses showed that NGAL was one of the earliest and most highly induced proteins in the damaged kidney. Interestingly, the protein was secreted from kidney cells and could be detected in the blood and urine. It soon became apparent that this finding was highly specific and reproducible in a number of independent animal models of kidney disease. The induction of the expression of NGAL in the damaged kidney correlated closely with the degree of injury and displayed up to 1000-fold elevations as compared to intact kidneys. These findings from animal experiments were observed to an almost identical degree in diseases of the human kidney. In an early study of patients with acute kidney injury who were in an intensive care unit, the plasma NGAL concentration increased by approximately 10-fold and at the same time the increases in urinary NGAL levels were greater than 100-fold, when compared to healthy individuals.

Table 3:

Urinary NGAL is a better predictor of a poor clinical course than serum creatinine (defined by admission to the intensive care setting, nephrology consultation, dialysis initiation, or mortality) (<i>Multivariate regression analysis, Nickolas et al., Ann. Intern. Med.</i> 2008; 148:810).	
Marker	Odds ratio (95% CI)
Urinary NGAL > 130 µg/g	24.70 (7.69 – 79.42)
Serum creatinine > 221 µmol/l	6.03 (2.25 – 16.14)

An important early clinical study focused on pediatric patients who had undergone cardiopulmonary bypass surgery, since these patients are known to show a high incidence of acute kidney injury. In fact, in this study, 28% of the patients developed acute kidney damage (defined by a 50% rise in serum creatinine concentration) following surgery, yet this diagnosis was derived from serum creatinine measurements and could only be established one to three days after the initial damage had occurred. By contrast, NGAL values in the urine or the plasma of these patients were elevated as early as 2 to 6 hours after surgery with urinary NGAL levels displaying superior test characteristics when compared with blood NGAL. Importantly, increased NGAL levels constituted an independent predictor of acute kidney injury, which was diagnosed later in the clinical course based on an increased serum creatinine concentration.

Similar findings were reported in adult patients who developed acute kidney injury after cardiac surgery, who also displayed significant increases in urinary NGAL concentrations only one to three hours after surgery. Further studies focused on other types of kidney injury. For instance, an increase of the urinary NGAL concentration could predict a rejection of kidney transplants and kidney damage induced by contrast media. While these initial data were obtained on relatively small groups of patients, they have now been validated on larger patient cohorts as well (Table 2 and Table 3).

One study conducted on 635 patients admitted to an urban emergency department in New York demonstrated significantly increased urinary NGAL levels in patients who developed acute kidney injury in the course of their disease, as compared to patients without structural kidney damage. The sensitivity and specificity indicators of this test were superior to those of serum creatinine measurements (Table 2). In addition, an elevated urinary NGAL level was an independent predictor of a poor clinical outcome (Table 3). These data are currently being validated in a multicenter cohort study.



Fig. 3: Acute kidney injury is treated by renal replacement therapy. The equipment shown on the right is used for veno-venous hemofiltration (CVVH) thereby replacing or supporting the function of the acutely damaged kidney. (The image was kindly provided by Dr. Saban Elitok, Helios-Klinikum Berlin)

NGAL compared to other biomarkers of acute kidney injury

Besides NGAL, several other proteins have been identified as potential biomarkers of acute kidney injury in recent years. These include kidney injury molecule –1 (Kim-1), N-acetyl- β -glucosaminidase (NAG), α -1-microglobulin, α -1-acid glycoprotein, liver-type fatty acid binding protein 1 (L-FABP) and interleukin-18 (IL-18). For each of these markers, preclinical as well as clinical data are available. However, there are only few comparative studies studying these markers in parallel (for one example see Table 4).

One potential future strategy may be the introduction of a multi-marker panel made up of combinations of several biomarkers, which may outperform the individual test characteristics of each single marker.

Table 4:

AUC (area under the receiver-operating characteristic curve) as a measure of test performance for different biomarkers of acute kidney injury. An AUC of 1.00 would signify a perfect test with a sensitivity and specificity of 100%. NGAL: neutrophil gelatinase-associated lipocalin; NAG: N-acetyl- β -glucosaminidase; α -1 MG: α -1-microglobulin; α -1-AG: α -1-acid glycoprotein (Nickolas et al., *Ann. Intern. Med.* 2008; 148: 810).

Marker	Urinary NGAL	Urinary NAG	Urinary- α -1-MG	Urinary- α -1-AG	Serum creatinine
AUC	0.948	0.713	0.887	0.832	0.921

Open questions

Despite the enthusiasm for this new generation of diagnostics, it should be noted that current clinical data on NGAL (and other biomarkers) have limitations. One major inherent problem of the diagnostic process in acute kidney injury is the frequent lack of a diagnostic “gold standard”, i. e. a renal biopsy with histopathological confirmation of structural kidney damage. In acute kidney injury, this test is often not performed because of the risk of the biopsy procedure. Thus, in most studies the diagnosis of acute kidney injury is based on clinical information and traditional laboratory tests.

Another potential issue is the lack of standardization in most studies to date. Several different techniques have been used to measure NGAL as a marker of kidney injury, including enzyme-linked immunosorbent assay (ELISA)-based kits from various providers as well as semi-quantitative western blot-based assays from research laboratories. These methodological differences make it difficult to compare the results of the published studies and to define cutoff levels of NGAL for the detection of acute kidney injury.

Abbott Diagnostics is currently developing a urine NGAL assay for the ARCHITECT analyzer. These efforts will resolve many of the problems of the currently available studies in the near future and will ensure comparability of the results from different clinical centers.

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