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Neutrophil gelatinase-associated lipocalin (NGAL) for acute kidney injury – the renal troponin?[1)]

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Abstract

Acute kidney injury (AKI) is a frequent complication of hospitalized patients and associated with significantly increased morbidity and mortality. Probably, the delay in the diagnosis of AKI resulting from currently available renal function markers, such as serum creatinine contributes to the fact that to date, no simple, safe, and effective intervention to prevent or successfully treat AKI in the general patient population has been found. At the time of diagnosis of established AKI, irreversible organ damage might already have occurred. Neutrophil gelatinase-associated lipocalin (NGAL) is a protease-resistant polypeptide, which is released from the distal nephron in response to ischemic, toxic, or inflammatory insult to the kidney. Available data suggest NGAL to be an early and predictive biomarker for AKI. Novel renal biomarkers indicating cellular/tissue damage might guide patient-tailored earlier initiation of nephroprotection or withdrawal of nephrotoxins directed at improvement of outcomes in patients developing AKI.

Keywords: acute kidney injury; acute renal failure; biomarker; neutrophil gelatinase-associated lipocalin (NGAL).

Introduction

Acute renal failure is an abrupt and persistent, but fundamentally reversible decline of the excretory renal function. It is associated with a decrease of urine production and an increase of retention parameters like serum creatinine.

Until recently more than 35 different definitions of acute renal failures had been used in literature. According to a paradigm shift, however, during the last several years the term ''acute kidney injury'' (AKI) has been used more and more frequently in place of ''acute renal failure''. This takes into account the fact that even a minimal acute decline of renal function is associated with increased morbidity and mortality (Figure 1). A creatinine increase of more than 0.3 mg/dL from the initial value within 48 h was associated with a four-fold increase of mortality in a cohort study of more than 9000 hospitalized patients [1]. Increased long-term mortality was described also in patients with a transient creatinine increase and complete recovery of renal function [2]. In response to the multitude of different definitions an international group of experts recently proposed a new classification system for AKI [3], which increasingly is being used both in research and the clinic and which a short time ago was modified once more [4] (Figure 2). Both classifications group the acute deterioration of renal function according to defined gradual increases in serum creatinine or a decrease of diuresis into three classes. This not only allows a much improved comparability of examination results, but also a stratification of the severity of AKI.

Epidemiology of acute kidney injury

Sepsis (48%), major surgeries (34%), e.g., cardiac surgeries, cardiogenic shock and nephrotoxic drugs or contrast media (19%) are the most frequent causes of severe AKI in the developed countries [5]. 5–10% of all hospitalized patients [5], 20–40% of intensive care patients [6, 7] and up to 50% of patients following cardiac surgery [8, 9] develop AKI. Every year millions of percutaneous coronary interventions are performed. Post-interventional contrast medium-induced nephropathy is a frequent complication that affects 10–20% of all patients and is associated with a significantly higher risk for renal replacement therapy, increased mortality as well as enormous costs [10, 11].

After adjusting for other relevant risk factors, patients who require extracorporeal renal replacement therapy for the treatment of severe AKI, show an approximately eight-fold increase in mortality [1]. In a considerable portion of these patients (41%), pre-existing chronic kidney disease develops or worsens with increased risk for a later end-stage renal disease and the need for chronic dialysis [12]. 5–20% of patients who are treated with renal replacement therapy require permanent replacement therapy due to irreversible

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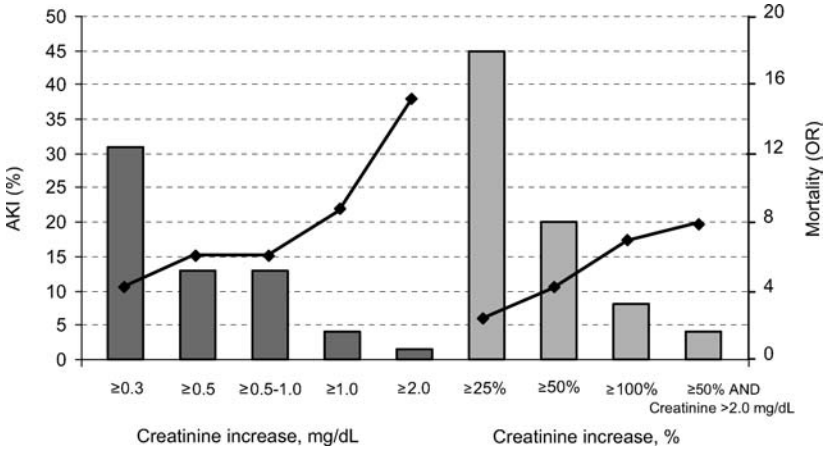

Figure 1 Incidence of acute kidney injury and associated mortality according to gradual increase in serum creatinine (modified according to [1]).

loss of renal function [12, 13]. In the USA, for example, the annual treatment costs for AKI amount to more than US$10 billion [14].

As a whole the incidence of AKI, uniformly defined, is said to be rising further in recent years because of the changed aging structure of the patient population and an increase of co-morbidities of hospitalized and surgically treated patients [13, 15].

Complications of acute kidney injury

Before the introduction of renal replacement therapy, patients with AKI died of hyperkalemia, organ edema, particularly pulmonary edema, or bleeding caused by dysfunctional platelets, while today medium- to long-term complications are primarily moving into the foreground.

Frequently, patients with AKI remain in the intensive care unit (ICU) or the hospital for longer periods of time and in addition show increased susceptibility to infections, chronic kidney disease and an increased rate of re-admission to the hospital.

AKI damages non-renal organs by pathogenic mechanisms including humoral, cellular and sympathetic activation pathways which so far are not yet completely understood [16]. In animal experiments isolated bilateral renal ischemia with subsequent reperfusion resulted in damage to the lungs and various areas of the brain. These studies demonstrated distinct pulmonary-vascular congestion with endothelial leakage and intra-alveolar hemorrhage [17] as well as a cerebrally increased apoptosis rate and massive inflammation processes [18].

Therefore, it is increasingly acknowledged that these patients die not with, but rather of AKI and its complications.

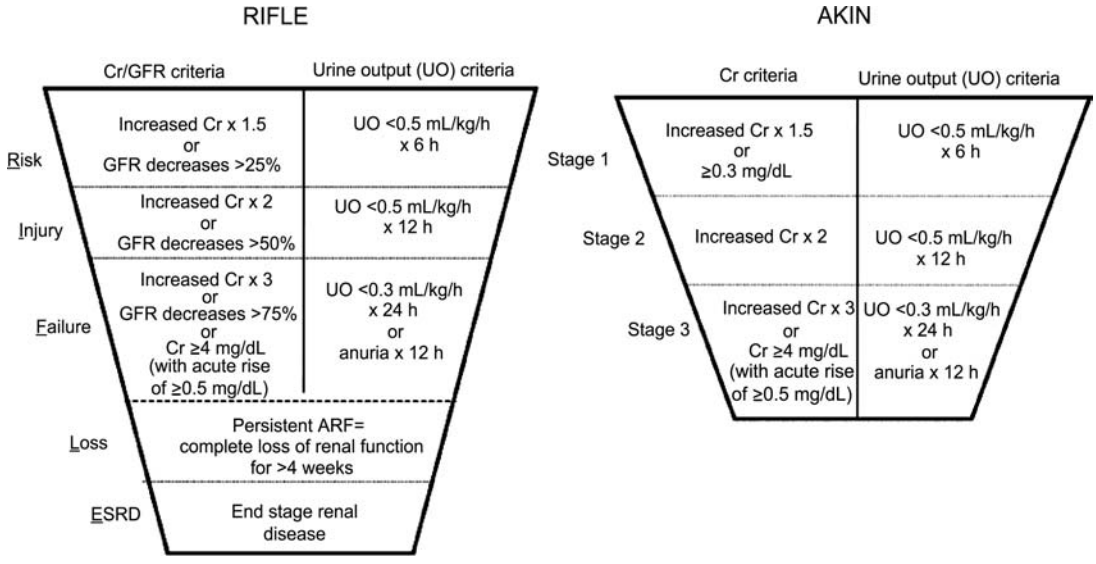

Figure 2 The ''RIFLE'' (R-risk, I-injury, F-failure, L-loss of function, E-end-stage renal disease [3]) and the ''AKI Network'' (AKI, acute kidney injury [4]) classification of acute kidney injury.

The need for novel renal biomarkers

In spite of the general recognition of AKI as a prognosis-determining disease of epidemic magnitude, all therapy attempts in the clinical practice have so far failed. While in animal models new nephroprotective attempts in part seemed promising, translational efforts up to now have often been disappointing. Reasons for this could be the incomplete pathophysiologic understanding of AKI, but also a delay in therapeutic measures that are initiated at a point in time at which irreversible organ damage may already be present.

Limitations of established renal function parameters

In clinical practice the diagnosis of AKI is not based on a marker for acute renal structural damage, but on functional markers of glomerular filtration rate, such as an increase in serum creatinine concentration and a decrease in diuresis.

Serum creatinine is a retrospective, non-sensitive and misleading marker for AKI. The marker is retrospective, because serum creatinine must accumulate to show kidney injury, non-sensitive, because 50% of the glomerular filtration rate must first have been lost before an increase of serum creatinine occurs, which can take several days [19], and misleading, because small, but clinically relevant increases – in spite of their known prognostic validity – are often overlooked or underestimated. Since creatinine formation is proportional to muscle mass, serum creatinine values of many chronically ill persons of malnutritioned and older patients or persons ill with muscular dystrophy are within the normal range, even though AKI is developing. Furthermore, an infusion of massive amounts of fluids made necessary by cardiocirculatory shock complicates the establishment of a diagnosis. A single measurement of serum creatinine cannot with certainty differentiate between AKI, normal renal function, chronic kidney disease or prerenal azotemia with dehydration. The sensitivity of diuresis, a further marker for the assessment of kidney function, is impaired by the use of diuretics and its specificity of dehydration conditions.

As a result the clinical diagnosis is delayed by 24–48 h, a window of time that currently cannot be used for nephroprotective measures. Unfortunately, methods for the precise evaluation of renal function, like renal function scintigraphy are cost- and labor-intensive and do not allow intensive monitoring of critically ill patients, since these examinations mostly can be performed only outside the ICU. In clinical practice they therefore do not represent a reasonable alternative for the early diagnosis of AKI.

While the last few decades saw the development of more and more sensitive and specific cardiologic biomarkers for the early diagnosis of acute myocardial infarction, and with early therapies an improved prognosis for patients, for nearly 50 years the diagnosis of AKI has been unchanged based on serum creatinine (Table 1). Not least for this reason the ''Acute Dialysis Quality Initiative'', the ''Acute Kidney Injury Network'' and the ''American Society of Nephrology'' have made the search for new, predictive biomarkers for the early diagnosis of AKI their priority [20, 21].

Requirements for new renal biomarkers

An ideal biomarker should fulfill several criteria: 1) The protein must generate from damaged cells. 2) The protein concentration in the body fluid must be proportionally dose-dependent to the damaging event. 3) The biomarker should be expressed early after the occurrence of the organ damage, when such damage is still potentially reversible. 4) The biomarker concentration should drop quickly following the end of the acute injury episode. 5) The biomarker should represent a significant component in the pathophysiology of the disease.

During the last 5–10 years intensive efforts have been made to develop novel renal biomarkers. One marker that appears to be fulfilling a number of the significant criteria named above is the neutrophil gelatinase-associated lipocalin (NGAL, syn. lipocalin-2, siderocalin).

NGAL

In the search for novel renal biomarkers, NGAL, in a genome-wide screening, was identified as the gene with the earliest and highest rise of mRNA and protein concentration in renal tissue, in urine and plasma following renal ischemia in a mouse model [22, 23].

NGAL was first described in neutrophilis as a protein with a size of 25 kDa and covalently bound to gelatinase [24]. NGAL belongs to the family of lipocalins and possesses a calyx molecule structure ideally suited for binding lipophile molecules or as a transporter for iron and numerous ligands and metabolites. From the perspective of a medical laboratory the protease resistance described facilitates the stability of the molecule and hence its identification in urine.

NGAL is secreted from distal tubular cells into the urine or it presumably arrives in plasma via a tubular ''back leak'', is filtrated glomerularly and by means of endocytosis is partially reabsorbed in the proximal tubule via megalin and other receptors [25]. It regulates the intrarenal iron metabolism and acts to stimulate proliferation and epitheliarization [26]. By binding of bacterial iron-siderophore complexes NGAL carries out bacteriostatic effects [25, 27], reduces pro-apoptotic processes and in this way appears to limit damage to

Table 1 Cardiac vs. renal acute biomarkers.

Period	Acute myocardial infarction	Acute kidney injury
1960s	LDH	Serum creatinine
1970s	CK, myoglobin	Serum creatinine
1980s	CK-MB	Serum creatinine
1990s	Troponin T	Serum creatinine
2000s	Troponin I	Serum creatinine
	⇩	⇩
	Multiple therapies 50% reduction in mortality	Supportive therapy high mortality

Requirements for new renal biomarkers.

the proximal tubule [26]. Furthermore, it exhibits effects on angiogenesis and modulates growth of neoplasiae of a different genesis [28]. In healthy persons, no NGAL or only traces of NGAL are detectable in various tissues or organs with activated epithelial tissue like the kidneys, lungs, stomach and intestine [29].

Depending on the degree and duration of the renal insult, NGAL is detectable in urine and plasma within only a few hours after ischemic, septic or toxic renal injury [23, 27]. Even progressive chronic kidney diseases, e.g., in diabetes mellitus, systemic lupus erythematosus and IgA-nephropathy, are accompanied by an NGAL increase [30–32].

While originally demonstrated in an animal model, the results were also confirmed in translational, prospective studies with adults, children and newborns [23, 25, 33, 34].

In a cross-sectional study that included patients manifesting AKI, NGAL showed an up to 100-fold increase of urine concentration when compared to a healthy control group [25]. Kidney biopsies performed on these patients showed massive concentration of NGAL in 50% of the cortical kidney tubules, primarily in the distal tubules [25].

Predictive value of NGAL in clinical studies

In a pilot study with 71 children following cardiac surgery to correct a congenital heart defect, NGAL proved to be of very high predictive value as early as two hours after the surgery with an area under the curve (AUC) of the receiver-operating characteristic of 0.99 (urine-NGAL) or 0.90 (serum-NGAL) for AKI whose clinical diagnosis was not established until 24–48 h later [33]. In larger follow-up studies that again included a pediatric patient collective without significant preexisting diseases these predictive values were confirmed (urine-NGAL AUC 0.96 [35], plasma-NGAL AUC 0.96 [36]).

In a typical cardiac surgery patient collective for coronary revascularization and heart valve surgery, good, although lower predictive values for NGAL were found in the prediction of a postoperative AKI with an AUC of 0.74 [37] or 0.80 [19]. Possible reasons could include co-morbidities, different measurement times of NGAL and different specimen preparation or measuring techniques. In these cardiac surgery studies NGAL concentration was proportional to the degree of severity and duration of AKI [35, 38] and in multivariate regression analyses it was the strongest independent risk factor for AKI. Additionally, NGAL proved to be of good prognostic value in the prediction of renal replacement therapy or a prolonged stay in the ICU or in hospital.

Several prospective studies investigated the role of NGAL as predictor of AKI following percutaneous coronary intervention with the use of contrast media [39, 40]. NGAL in urine or plasma was a highly reliable predictor of contrast medium-induced nephropathy in children (AUC 0.92 or 0.91) within two to four hours following the application of the contrast medium [39].

The value of NGAL was also examined with critically ill patients in the ICU. In spite of the unknown beginning of AKI in this setting, an NGAL increase in a cohort of 301 intensive care patients with an AUC of 0.78 indicated AKI 48 h before serum creatinine [41]. After excluding patients manifesting AKI at the time of ICU admission, a study found an AUC of 0.96 for AKI that developed in the next few days [42]. In a cohort of septic patients with AKI, NGAL concentrations in urine or plasma were found to be higher than in a comparison group without AKI [43]. NGAL predicted renal replacement therapy with an AUC of 0.79 [42] or 0.82 [41].

A single measurement of NGAL in 635 patients admitted to an emergency department contributed to the certain differentiation between AKI and normal kidney function, chronic kidney disease and prerenal azotemia [44]. A positive NGAL test anticipated prolonged hospitalization. The measurement of NGAL in these patients made it possible to establish a prognosis for other clinical end points, i.e., initiation of extracorporeal renal replacement therapy, admission to the ICU and in-hospital mortality [44].

A meta-analysis was performed based on clinical studies investigating the predictive value of NGAL for AKI of various etiology [45]. The authors of 26 identified prospective observational studies were asked to recalculate the predictive value of NGAL for a uniform AKI definition and by using a standardized time of NGAL measurement in relation to the renal insult (24–48 h before AKI was diagnosed by serum creatinine increase). The standardization of these important influence factors on the predicted value of NGAL [46] served to reduce heterogeneity in this meta-analysis.

In total the data of 2538 patients from eight countries were analyzed. Approximately 20% of patients developed AKI. Table 2 summarizes several performance characteristics of the diagnostic value of NGAL. Sensitivity and specificity of NGAL were $>75\%$ both in total as well as in typical subgroups of patients. NGAL's predictive value for AKI was AUC 0.80 and for subsequent initiation of renal replacement therapy AUC 0.78 (Figure 3).

The cut-off value of NGAL varies considerably according to the etiology of AKI, but could probably be estimated at >150 ng/mL (Table 2), as was last determined with the help of standardized clinical laboratory platforms (Triage, Biosite/Inverness or ARCHITECT, Abbott). This corresponds to the normal values of healthy persons (<100 ng/mL [24]), but requires confirmation in larger patient cohorts.

The predictive value of urine NGAL appears to be minimally better than that of plasma NGAL. There is an advantage for the standardized laboratory platforms (AUC 0.83) in comparison to assays which were performed by means of commercially available antibodies (AUC 0.73). Whether this constitutes a robust effect requires further investigations.

This meta-analysis can certainly not answer all questions, e.g., those for a sharp cut-off value of NGAL for AKI, and it shows limitations. The results of the study are based on single center studies that used a creatinine-based endpoint. There was no standardization for specimen processing or storage. No data exist for an NGAL analysis in freshly collected specimens. In patients in the ICU the optimal time for NGAL determination might have been missed. The influence of co-morbidities on the predictive value of NGAL must be left to further studies.

Table 2 Performance characteristics of NGAL in the prediction of AKI (with kind permission of Elsevier Publ.) [45].

NGAL for AKI early diagnosis	Sensitivity (95% CI)	Specificity (95% CI)	DOR (95% CI)	AUC-ROC (95% CI)	Cut-off value (ng/mL)
Total (487/2538 patients)	76.4 (70.4–81.6)	85.1 (76.6–90.9)	18.6 (9.0–38.1)	0.815 (0.732–0.892)	>190.2 (122.8–257.2)
After cardiac surgeries (307/1204 patients)	75.5 (70.2–82.4)	75.1 (65.2–86.3)	13.1 (5.7–34.8)	0.775 (0.669–0.867)	>273.6 (145.0–289.2)
In critically ill patients (123/602 patients)	76.4 (59.9–87.5)	75.5 (52.2–89.7)	10.0 (3.0–33.1)	0.728 (0.615–0.834)	>155.0 (150.8–169.0)
After administration of contrast medium (34/191 patients)	77.8 (62.8–88.0)	96.3 (74.4–99.6)	92.0 (10.7–794.1)	0.894 (0.826–0.950)	>100.0 (80.0–100.0)
Urine NGAL (n=14)[a]	77.8 (70.9–83.5)	84.3 (72.8–91.3)	18.6 (7.2–48.4)	0.837 (0.762–0.906)	>193.2 (123.7–405.7)
Plasma NGAL (n=9)[a]	73.4 (62.3–82.2)	86.6 (72.0–94.3)	17.9 (6.0–53.7)	0.775 (0.679–0.869)	>179.2 (153.9–199.3)
Assay with commercial Ab (n=14)	76.9 (69 4 83 1)	83.4 (72 0 90 8)	16.7 (7 1 39 7)	0.732 (0 656 0 830)	>246.4 (88 5 277 2)
Standardized laboratory platform (n=5)	75.4 (63.8–84.2)	89.3 (81.9–93.9)	25.5 (8.9–72.8)	0.830 (0.741–0.918)	>150.6 (145.0–155.0)

[a]Contains studies with NGAL measurements in urine as well as in plasma. AKI, acute kidney injury; DOR, diagnostic odds ratio; AUC-ROC, area under the curve for the receiver operating characteristic; Ab, Antibodies.

In clinical studies NGAL appears to react timely to nephroprotective or nephrotoxic effects. In a randomized, double-blind and placebo-controlled study that included 100 patients after cardiac surgery, a perioperative sodium bicarbonate infusion decreased the incidence of AKI that developed during the first five days after surgery by 40%. NGAL demonstrated this therapeutic effect of bicarbonate as early as on the day of the surgery [47]. By analogy, the use of

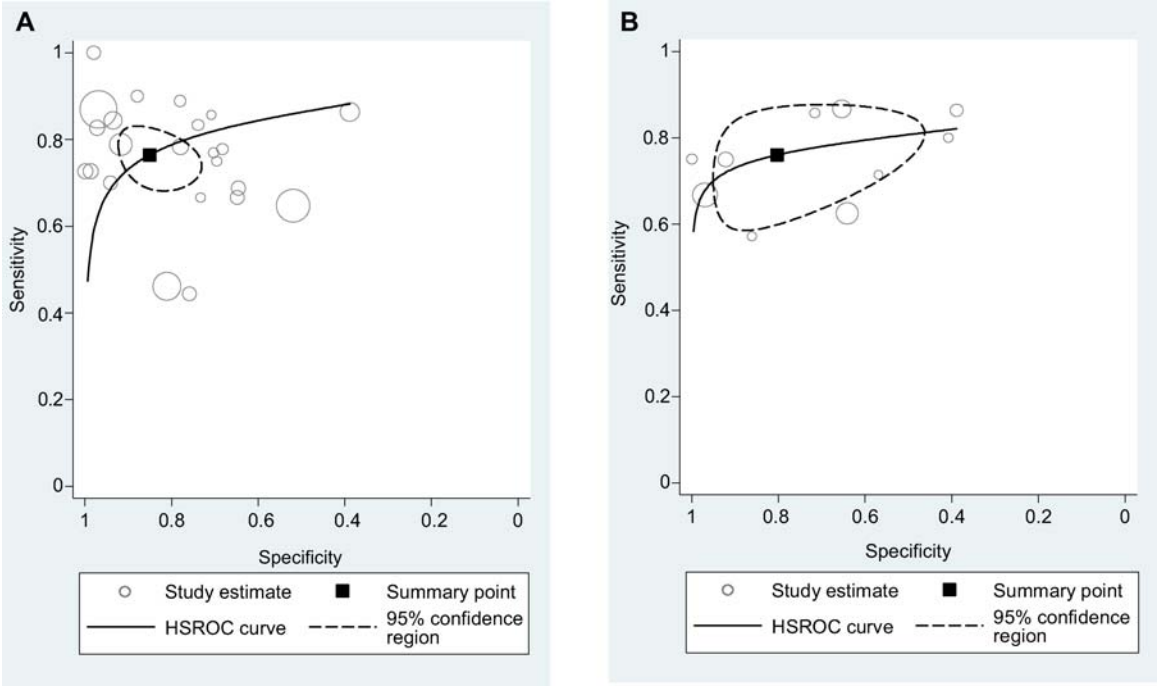

Figure 3 Predictive value of NGAL for (A) acute kidney injury and (B) renal replacement therapy in a hierarchical summary ROC (HSROC) analysis [45].
ROC, receiver operating characteristic. (With kind permission of Elsevier Publ.)

aprotinin, a nephrotoxic antifibrinolytic agent [48], was associated with an early increase of NGAL values in a similar patient cohort [49].

NGAL analysis

Currently two automated laboratory-diagnostic systems for NGAL measurements are available in Germany. Biosite/Inverness Medical introduced the Triage NGAL test in March 2009 and Abbott Diagnostics introduced the ARCHITECT NGAL test in December 2009.

Triage NGAL is a ''Point-of-care'' fluorescence immunoassay for the quantitative determination of NGAL from EDTA-anticoagulated specimens of whole blood or plasma. According to information from the manufacturer no relevant interferences are detectable between plasma NGAL and hemoglobin (to 5 mg/mL), lipids, rheumatoid factors, bilirubin (to 0.15 mg/mL) or drugs like ceftriaxon, furosemid, heparin or contrast media.

The ARCHITECT Urin-NGAL is a chemiluminescence-microparticle-immunoassay for the quantitative determination of NGAL in human urine.

According to the manufacturers the measuring ranges of the Triage or the ARCHITECT NGAL test are from 60 to 1300 ng/mL or to 1500 ng/mL, respectively. Each specimen volume consists of a few hundred microliters and the measuring time of both systems is $\sim$15 min.

NGAL: the renal troponin?

Table 3 contains a comparison of selected properties and characteristic values of NGAL and cardiac troponin. Cardiac troponin appears to show higher organ specificity and more favorable predictive properties, which might be attributed in part to the perfected chemical analysis. To what extent the analysis methods for NGAL can be improved further remains to be seen.

Implications of NGAL

With the help of a predictive renal biomarker a paradigm shift can be initiated in current clinical practice. Early information about an acute structural kidney injury should direct the physician's attention to the timely avoidance of a manifest and possibly irreversible loss of renal function.

NGAL-positive patients could benefit from the earlier initiation of hemodynamic monitoring and adjusted hemodynamic target values, e.g., for the mean arterial pressure or the cardiac output, or from optimized intravascular hydration. An early stop of nephrotoxins like non-steroidal antiphlogistic agents, ACE inhibitors or gentamycin and the sparingly or delayed use of contrast media would be further conceivable measures.

Depending on confirmation in large, multi-centric studies a measurement of NGAL immediately after admission into the ICU could identify those patients who already have early-stage structural kidney injury and stratify them for developing AKI. The time of the measurement of NGAL during ICU stay requires further studies. However, it is conceivable that NGAL measurement would be useful within six hours following any potential renal insult, in order to detect relevant subclinical renal injury early before any loss of renal function and to adjust treatment accordingly.

In patients with increased NGAL an early start of extracorporeal renal replacement therapy could be considered prior to the development of organ edema and uremia. Any potential benefit of an early start of renal replacement therapy in NGAL-positive patients should be investigated in large, prospective studies before definitive recommendation can be made.

Additionally, prompt identification of AKI in patients admitted to an emergency department could guide further clinical decisions. In patients showing no increase in serum creatinine, an NGAL-positive finding could contribute to the decision on the patient's admission to the hospital or to the ICU for hemodynamic monitoring. On the other hand, a negative NGAL finding or an NGAL concentration below the cut-off value for AKI would be a criterion for deciding whether to discharge the patient from the hospital. An increased serum creatinine value in the presence of an NGAL finding could be interpreted as chronic kidney disease.

After establishing a uniform cut-off value of NGAL predicting AKI new or already known drugs with potential nephroprotective properties, e.g., natriuretic peptide [51] or sodium carbonate [47], could be used earlier.

Table 3 Biologic properties and performance characteristics of NGAL vs. cardiac troponin.

	NGAL	Cardiac troponin
Occurence	Activated epithelia (kidney, lung, liver) and neutrophil	Myocardium
AUC	>0.73 (>0.83 for new assays)	>0.85 (sTNI: >0.95 [50])
Sensitivity	70–80% (children $>90\%$)	$>70\%$ (sTNI: $>90\%$ [50])
Specificity	70–80% (children $>90\%$)	$>90\%$ (sTNI: $>90\%$ [50])
Cut-off value	>150 ng/mL?	Trop T: >0.03, (sTNI: >0.04)
Increase	Acute kidney injury, sepsis, progressive chronic kidney disease, tumors	Myocardial cell injury (not to be equated with reduced coronary perfusion)
Intervention	Requires studies	Cardiac catheter, lysis

sTNI, sensitive troponin I.

Nevertheless, the possibilities for the use of NGAL as described still need to be investigated in randomized studies.

Limitations of NGAL

Data on the predictive value of NGAL for AKI are not consistent, as yet no definite cut-off value could be established. In existing studies different definitions of AKI were often used, which complicates comparisons of sensitivity and specificity of NGAL for AKI between the studies and which can represent a confounding variable regarding the AKI definition. The slightly reduced predictive value of NGAL for AKI in adults as compared to children could be related to concurrent underlying chronic diseases of older patients. On the other hand, the limitations of serum creatinine as a renal marker possibly do not allow higher predictive values for NGAL in the prediction of creatinine-based endpoints of studies. Additional confounding factors could include the measuring technique, urinary tract infections and co-morbidities associated with a non-renal release of NGAL. These and other limitations of NGAL or the clinical benefit of an NGAL measurement and the clinical decisions based on such measurement should be investigated in large multi-centric studies. A favorable precondition for such studies may be seen in the biological plausibility of the discovery of NGAL as a renal biomarker, the existing results of numerous studies and a meta-analysis with a good predictive value for NGAL in the prediction of AKI.

Conflict of interest

M.H. has received lecture fees from Biosite/Inverness and Abbott Diagnostics. Both companies are developing NGAL as a marker for the early diagnosis of kidney injury and had no part in the writing/editing of this manuscript.

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