REVIEW

Neutrophil Gelatinase Associated Lipocalin: An Emerging Biomarker for Acute Kidney Injury in Cardiovascular Disease

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ABSTRACT

Acute kidney injury (AKI) commonly occurs in hospitalised patients resulting in short and long-term morbidity and mortality. A subset of patients especially those with cardiovascular diseases appear particularly vulnerable. The diagnosis of AKI currently depends on changes in serum creatinine and is usually made at least 24 to 48 hours after the initial renal insult. This hinders formulation of possible early therapeutic strategies which could otherwise reduce the clinical sequelae of AKI. Neutrophil gelatinase-associated lipocalin (NGAL) is released in both serum and urine, and has shown great promise in identifying AKI as early as two to four hours after renal injury. NGAL has been demonstrated to be both specific and sensitive in a variety of renal conditions associated with AKI, compared to serum creatinine. This article discusses the emerging role of NGAL in the diagnostic and prognostic evaluation of AKI secondary to cardiovascular diseases and interventions including its benefits and pitfalls. NGAL has been shown to be useful in the diagnosis of AKI particularly for contrast induced nephropathy (CIN) after percutaneous coronary intervention (PCI) and renal dysfunction complicating acute and chronic heart failure. Larger prospective outcome studies with therapeutic interventions are warranted to further validate the role of NGAL in the diagnosis of AKI and in cardiorenal syndrome.

Keywords: Acute kidney injury, Heart failure, Ischaemic heart disease, NGAL, Percutaneous coronary intervention

INTRODUCTION

Acute kidney injury (AKI) is potentially serious and may complicate numerous cardiovascular conditions and interventions, including acute coronary syndromes, heart failure, cardio-pulmonary bypass and contrast-induced nephropathy. In these settings, AKI confers increased mortality[1] and independently predicts major adverse cardiac events (MACE) during the index hospitalisation and subsequently (Fig. 1).[1]

There is currently no specific biomarker to detect and diagnose AKI promptly, to facilitate timely effective renal protection strategies and ultimately have a positive impact on longer term outcome for patients with AKI. This review will summarise the biology and clinical efficacy of an emerging biomarker, neutrophil gelatinase-associated lipocalin (NGAL) and its potential value to assist in the management of AKI associated with common cardiovascular syndromes.

DEFINITION AND CLINICAL SIGNIFICANCE OF ACUTE KIDNEY INJURY (AKI)

AKI is largely asymptomatic, and current diagnostic standards rely on changes in serum creatinine in serial measurements as a reflection of acute reductions in glomerular filtration rate (GFR). The precision and specificity of serum creatinine in the diagnosis of AKI is notoriously limited. Firstly, its serum level can be influenced by numerous factors that are independent of GFR such as age, gender, muscle mass, metabolism, hydration status, nutrition status, medications and tubular

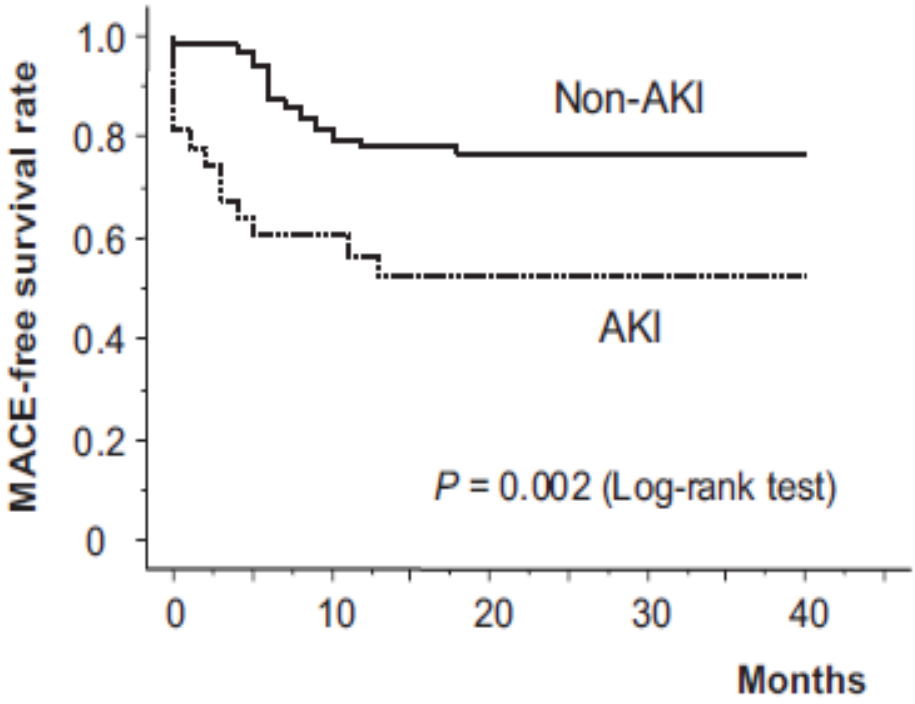

Fig. 1. Kaplan-Meier curve demonstrates the prognostic significance of acute kidney injury (AKI). AKI was associated with lower major adverse cardiac event (MACE)-free survival during the observation period after MI (*P*=0.002).[1]

secretion. Secondly, serum creatinine is an indirect reflection of GFR, has a non-linear relationship with GFR, and its rise lags behind the important structural changes that occur in the kidney during the early phases of AKI. The lack of a comparable biomarker to troponin for myocardial necrosis, to allow for prompt diagnosis of AKI, has hampered both adequate detection and implementation of potential therapies.

Previously, the field had been hampered by the lack of a consensus definition for acute renal failure, which confused the design and interpretation of clinical trials in this area. In 2001, the Acute Dialysis Quality Initiative (ADQI), comprising a team of nephrologists and intensivists, developed the RIFLE criteria (Risk, Injury, Failure, Loss, End stage renal disease) to provide a standard, comprehensive description of the elements of AKI.[2] Subsequently, the RIFLE criteria were modified by the Acute Kidney Injury Network (AKIN)[3], heralding the introduction of the term acute kidney injury (AKI) as a standard term for representing the entire spectrum of acute renal failure. The RIFLE and AKIN definitions are summarised in Tables 1-3. In essence, AKI is defined as the abrupt (within ≤48hours) increase in serum creatinine of ≥0.3mg/dL (26.4micromol/L) from baseline, a relative increase in serum creatinine ≥50%, or oliguria of <0.5ml/kg/hr for more than six hours. The criteria of an absolute increase in serum creatinine of ≥0.3mg/dL (26.4micromol/L) from baseline was derived from epidemiological data demonstrating an 80% increase in mortality risk associated with this level of serum creatinine increase.[4]

SEARCH FOR A NOVEL BIOMARKER

There is a critical need for a novel, assessable biomarker which can be measured using standardised assay platforms that allow for prompt detection, risk stratification and monitoring response to interventions.

This biomarker should be highly specific for AKI. Its levels should rise quickly within the first few hours after renal insult, to facilitate implementation of early intervention, thereby potentially preserving as much renal function as possible.

Table 1. Definition of AKI by RIFLE criteria[2].

	Serum creatinine	Reduction of GFR	Urine output
Risk	1.5x	25%	<0.5ml/kg/h x6h
Injury	2x	50%	<0.5ml/kg/h x12h
Failure	3x	75%	<0.5ml/kg/h x24h or anuria x12h
Loss	Complete loss of renal function (eg need RRT) >4 weeks		
ESRD	Complete loss of renal function (eg need RRT) >3 months		

Table 2. Definition of AKI by AKIN criteria[3].

1.	Increase serum creatinine ≥0.3mg/dl (26.4μmol/L) from baseline
2.	Increase serum creatinine ≥50% from baseline
3.	Oliguria ≤0.5ml/kg/h >6hr

Table 3. Comparison of difference in staging between the RIFLE and AKIN criteria for AKI.

RIFLE	**AKIN**
Risk.	Stage 1
Injury	Stage 2
Failure	Stage 3
Loss, ESRD	Outcome

*Caveat in using AKIN criteria to diagnose AKI: within 48hrs, optimised volume status, urine tract obstruction to be excluded if oliguria was used as sole criterion

Table 4. Other proposed novel biomarkers for assessing AKI

Type of biomarker	Auc-roc (95% CI)	Remarks
KIM-1 Kidney injury molecule-1	0.83 (0.67-0.96)[11]	Up-regulated 24-48hr after initial insult. Not validated in sub-clinical injury[12]
IL-8 Interleukin-8	0.75 (NR)[13]	Up-regulated 4-6hr after ischaemic injury, similar when compared to urine NGAL[13]
Cyr61 Cysteine rich protein	Limited human studies	Detectable in urine 3-6hr after ischaemic injury.[14] Complicated purification process for detection
B2-microglobulin	Limited human studies	NGAL preceded its appearance and not as sensitive to dose & duration of renal ischaemia[8]
N-acetyle B-D-glucosaminidase	0.7 (0.54-0.87)[11]	

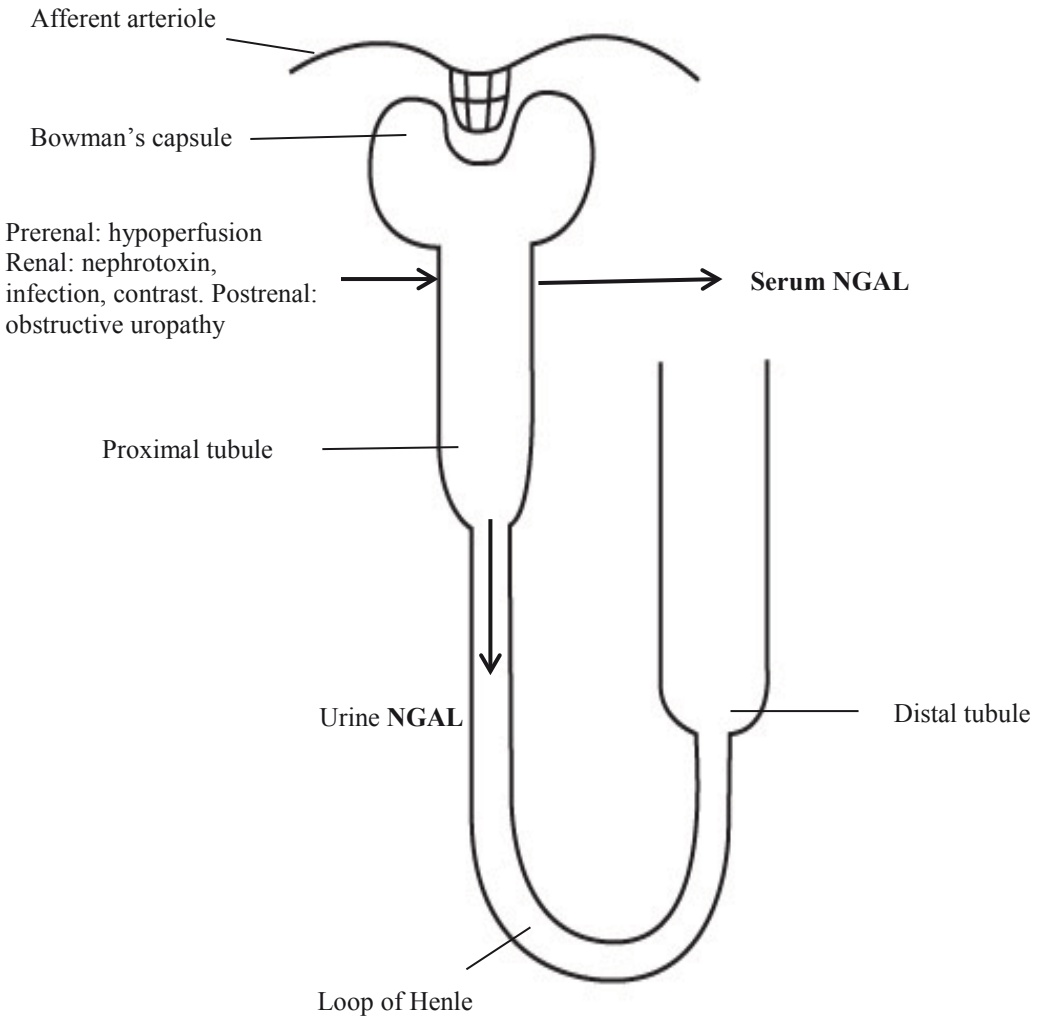

Fig. 2. Schematic of a single nephron illustrating the proximal tubular site of production for NGAL during acute kidney injury

Early recognition of AKI will also enable risk stratification of these patients and prognostication with respect to mortality, duration of hospital stay, and potential need for dialysis. Secondly, an ideal biomarker should identify the primary location of the injury (i.e. proximal tubule, distal tubule, interstitium or vasculature). Thirdly, its serum or urine level should parallel ongoing renal injury and/or recovery with a high degree of temporal specificity.

NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL)

NGAL has been identified as a promising biomarker for acute kidney injury. It is a small (25kd) covalently bound polypeptide from human neutrophils,[5] which is protease-resistant and readily detected in the urine and blood (Fig. 2). NGAL belongs to the lipocalin superfamily, secreted or cytosolic proteins with a barrel-like structure, carrying hydrophobic ligands (such as fatty acids, retinoids, pheromones) in their core pocket.[6] NGAL is expressed and secreted at extremely low levels by immune cells, kidney, liver, trachea, lungs, stomach and colon,[7] but its expression is significantly up-regulated in injured kidney epithelial cells.

Mishra J et al[8] employed a transcriptome wide interrogation strategy in murine models of AKI to identify renal genes with induced expression very early after renal ischaemia, and to determine which of these genes might serve as potential biomarkers. Marked three to four fold up-regulation of NGAL mRNA and protein levels were observed in early stages of ischaemic injury in mouse kidney. NGAL protein was expressed predominantly within the proximal tubule cells and was readily detected within the initial urine after ischaemia in both mouse

and rat models of AKI. Furthermore, the amount of NGAL in urine was proportional to the magnitude and duration of renal ischaemia. Increased urine NGAL protein could also be detected even after very mild ischaemic injury induced by five minutes of renal artery occlusion.

MEASUREMENT OF NGAL

The natural resistance of NGAL to proteases mitigates concerns for degradation in stored samples, prior to analysis. Commercially available enzyme-linked immunosorbent assays (ELISA) for human urine and serum NGAL measurement include the ARCHITECT system (Abbott Diagnostics, USA)[9] and that by BioPorto (Gentofte, Denmark),[10] respectively. NGAL has been shown to compare favourably with other promising biomarkers for AKI (summarised in Table 4).

Previous studies have assessed the utility of NGAL in both urine and serum as a biomarker in AKI (summarised in Table 5). Measurements of urinary or serum levels of NGAL each have relative advantages and disadvantages. With regard to urine, collection is non-invasive and levels may more likely reflect events in the kidney. However, collection of sufficient quantities of urine for the measurement of a biomarker can be challenging in oliguric patients and concentrations may be affected by patient's hydration status or use of diuretics. Monitoring and interpreting NGAL concentrations in serum is also complex; venesection is required and extra-renal production of NGAL in the setting of subclinical changes in renal excretion may affect serum levels independent of kidney injury. In this regard, numerous extra-renal sites including immune cells, liver, trachea, lungs, stomach and colon may be sources of NGAL in septic patients with multi-organ failure, leukocytosis, chronic inflammatory disorders and cancer. Accordingly, defining parameters for NGAL levels providing appropriate levels of sensitivity and specificity will be required.

NGAL AND CARDIOVASCULAR DISEASE

Patients with heart failure suffer from varying degrees of renal insufficiency. In 2004, a working group of investigators at National Heart, Lung and Blood Institute defined the cardio-renal syndrome as a state in which therapy to relieve heart failure symptoms is limited by further worsening renal function.[38] The complex relationship between cardiac and renal function led to a formal classification, which is summarised in Table 6.[39] Of the 105,000 patients within the Acute Decompensated Heart Failure National Registry (ADHERE),[40] an elevated serum creatinine value on admission and its deterioration after hospitalisation predicted prolonged stay, re-hospitalisation and mortality.[41]

NGAL has also been studied as a predictor of AKI in patients with acute decompensated heart failure.[10] A high serum NGAL measured on admission predicted the subsequent development of AKI in patients with preserved renal function at baseline. Furthermore, serum NGAL levels of ≥140ng/ml on admission conferred a 7.4 fold increase in risk for developing AKI. In another case-control study of 90 patients with chronic heart failure, urinary NGAL was found to be a reliable marker for tubular injury in patients with chronic heart failure. Increased urinary NGAL correlated with a deterioration in estimated GFR and worsening in both NT-proBNP levels and urinary albumin excretion.[42] In the complex biochemical, hormonal and physiological interactions of the 'cardio-renal' syndrome, the potential efficacy of NGAL as a diagnostic tool and a marker to guide therapy warrants further investigation.

UTILITY OF NGAL AS A DIAGNOSTIC AND PROGNOSTIC TOOL FOR AKI

NGAL abundantly accumulates within the blood, urine and renal cortical tubules in conditions associated with AKI secondary to renal ischaemia, nephrotoxins, renal parenchymal damage, haemolytic uremic syndrome (HUS) and post-transplant rejection.[35] NGAL has also proven utility in detecting AKI and in monitoring disease activity in a variety of renal conditions including systemic lupus erythematosus,[28] autosomal dominant polycystic kidney disease,[43] IgA nephropathy[44] and diabetic nephropathy.[45]

In several prospective cohort studies, urine and serum NGAL levels have been shown to rise within one to two hours after the onset of AKI whereas the rise in serum creatinine is not detected until one to two days later. This observation has been consistent across studies involving paediatric and adult populations. The results of these trials, which invariably are single centre and involve only small numbers of patients are summarised in Table 5.

Serum and urinary NGAL has been extensively

Table 5. Human studies correlating NGAL with AKI.

Clinical setting	Patients (n)	Urine/serum NGAL	Sensitivity	Specificity	AUC-ROC	Reference
Acute heart failure (adult)	91	Serum NGAL	86%	54%	0.7 (0.585-0.803)	10
Contrast (adult)	91	Urine NGAL	73%	100%	0.92 (NR)	15
		Serum NGAL	73%	100%	0.91 (NR)	
Contrast (adult)	100	Serum & urine NGAL	NR	NR	NR	16
Contrast (adult)	40	Urine NGAL	77%	71%	0.73 (0.54-0.93)	17
Contrast (adult)	91	Serum NGAL	73%	100%	0.91 (NR)	18
Contrast (adult)	60	Urine NGAL	76%	80%	NR	19
		Serum NGAL	90%	74%	NR	
Cardiac surgery (children)	71	Urine NGAL	100%	98%	0.998 (NR)	20
		Serum NGAL	70%	94%	0.906 (NR)	
Cardiac surgery (children)	196	Urine NGAL	82%	90%	0.95 (NR)	21
Cardiac surgery (adult)	81	Urine NGAL	73%	78%	0.80 (0.57-1.03)	22
Cardiac surgery (adult)	426	Urine NGAL	39.2%	78.2%	0.611 (0.544-0.679)	23
Cardiac surgery (adult)	72	Urine NGAL	49%	79%	0.69 (0.57-0.82)	24
Cardiac surgery (adult)	33	Urine NGAL	71%	73%	0.88 (NR)	25
Cardiac surgery (adult)	50	Urine NGAL	93%	78%	0.96 (0.9-1)	26
		Serum NGAL	80%	67%	0.85 (0.73-0.97)	
Cardiac surgery (adult)	100	Serum NGAL	79%	78%	0.8 (0.63-0.96)	27
Childhood-onset SLE.	35	Urine NGAL	90%	100%	0.944 (NR)	28
Critical care (children)	150	Urine NGAL	77%	72%	0.78 (0.62-0.95)	29
Critical care (children)	143	Serum NGAL	86%	39%	0.68 (0.56-0.79)	30
Critical care (adult)	31	Urine NGAL	91%	95%	0.98 (0.82-0.98)	31
Critical care (adult)	301	Serum NGAL	73%	81%	0.78 (0.65-0.9)	32
Critical care (adult)	88	Serum NGAL	82%	97%	0.92 (0.85-0.97)	32
Emergency department	635	Urine NGAL	90%	99.5%	0.95 (0.88-1.0)	34
Kidney transplant	23	Urine NGAL	90%	83%	0.90 (NR)	35
Kidney transplant	91	Urine NGAL	77%	74%	0.81 (0.7-0.92)	36
Liver transplant	59	Serum NGAL	68%	80%	0.79 (NR)	37

NR- not reported
AUC-ROC - area under the receiver- operating characteristic curve; CI 95%

studied in patients undergoing cardiopulmonary bypass. A recent prospective cohort trial of 81 adults undergoing cardiac surgery compared urinary NGAL against serum creatinine.[22] In this study, 16 patients (≈20%) developed AKI after surgery. The mean urinary NGAL concentrations in patients who subsequently developed AKI were significantly higher one hour after surgery compared with patients who did not develop AKI (4195±6520 vs 1068±2129 ng/ml; *P* <0.01) Notably, serum creatinine peaked only at postoperative day four in the AKI patient group whereas mean urinary NGAL concentrations continued to increase and remain significantly higher than baseline at three and 18 hours after cardiac surgery. In contrast, in patients without AKI, urinary NGAL decreased rapidly after cardiac surgery. This result may have clinical relevance because increases in postoperative serum creatinine, of as little as 0.3mg/dl within the first 48 hours, is associated with increased ICU and hospital duration stay, morbidity and mortality after cardiac surgery.[46,47]

Contrast induced nephropathy (CIN) after interventional procedures has a reported incidence ranging from three to 20% and is recognised as a significant cause of mortality and morbidity[48,49] (Fig. 3). Current diagnosis uses serum creatinine levels, which do not elevate until 24 to 48 hours after the procedure. In comparison, urine and plasma NGAL may prove to be useful in detecting CIN much earlier. In a prospective trial of 91 children with congenital heart disease undergoing elective cardiac catheterisation and angiography, 12% (11 patients) developed contrast-induced nephropathy (CIN).[15] In patients with CIN, there was a significant rise in both urinary and serum NGAL within two hours of contrast administration compared to patients without CIN (urine NGAL, 135±32 vs 11.6±2 ng/ml, *P* <0.001; serum NGAL, 151±34 vs 36±4, *P* <0.001). The diagnostic rise in serum creatinine was not evident until six to 24 hours after the procedure. Notably, the serum and urine NGAL level remained consistently low in patients who did not develop CIN. Using a cut-off value of 100ng/ml, the combined sensitivity and specificity of both two-hour urine and serum NGAL was 73% and 100%, respectively.

Urinary NGAL has been evaluated as a risk stratification tool in the emergency room setting. A large prospective cohort study of 635 patients[34] in New York City showed that a single measurement of urinary NGAL distinguished AKI from normal renal function, pre-renal azotemia and chronic kidney disease and predicted inpatient outcomes. Patients with AKI had significantly higher urinary NGAL levels compared to other groups (416±387 µg/g creatinine; *P*=0.001). Using a cut-off value of 130 µg/g creatinine, the sensitivity and specificity of NGAL for detecting AKI were 0.9 (95% CI, 0.73 to 0.98) and 0.995 (CI, 0.99 to 1.00), respectively. In a multiple logistic regression analysis, urinary NGAL level was also found to be highly predictive of clinical outcomes (odds ratio 24.71 [CI, 7.69 to 79.42]).

A recent meta-analysis of 19 studies, involving 2538 patients by Haase et al[50] using custom-made data collection sheets completed by each author concluded that both serum and urine NGAL were similarly useful for the early prediction of AKI. In addition, NGAL level also had prognostic value in the prediction of renal replacement therapy initiation and in-hospital mortality. The clinical settings studying AKI were diverse and included adults and children who were critically ill, post-cardiac surgery, post-contrast exposure and post-renal transplant. However, the result of this meta-analysis was limited by the use of serum creatinine in the diagnosis of AKI.

LIMITATIONS OF NGAL

The variability of serum NGAL measurements in patients with chronic co-morbidities represents an important limitation to widespread clinical application. Furthermore, both urine and serum NGAL levels correlate with the severity of renal insufficiency (defined by GFR or level of proteinuria) in patients with chronic kidney disease. Furthermore, the relative increase in NGAL levels is less when such individuals suffer acute insults, compared to those with normal baseline renal function. Accordingly, the majority of the published studies exploring the utility of NGAL to screen for AKI have excluded patients with chronic kidney disease (CKD). Such data could be very useful, as this represents a particularly vulnerable population that may be of particular risk for AKI.

To date, most of the prospective trials evaluating the use of NGAL in screening for AKI are relatively small. Larger, randomised patient trials are needed in order to validate NGAL as a predictor of AKI severity and should include hard clinical endpoints such as cardiovascular morbidity, dialysis requirement and

Table 6. Cardiorenal syndrome (CRS) classification[39]

Type 1 (Acute cardiorenal syndrome	Acute heart failure resulting in acute kidney injury
Type 2 (Chronic cardio-renal syndrome)	Chronic cardiac dysfunction (example chronic congestive heart failure) causes progressive chronic kidney disease
Type 3 (Acute reno-cardiac syndrome)	Abrupt and primary worsening of kidney function, (example, to renal ischemia or glomerulonephritis) causes acute cardiac dysfunction, which may be manifested as heart failure
Type 4 (Chronic renocardiac syndrome)	Primary chronic kidney disease contributes to cardiac dysfunction, which may be manifested as coronary artery disease, heart failure or arrhythmia
Type 5 (secondary cardiorenal syndrome)	Acute or chronic systemic disorders (eg. Sepsis or diabetes mellitus) that cause both cardiac and renal disorders

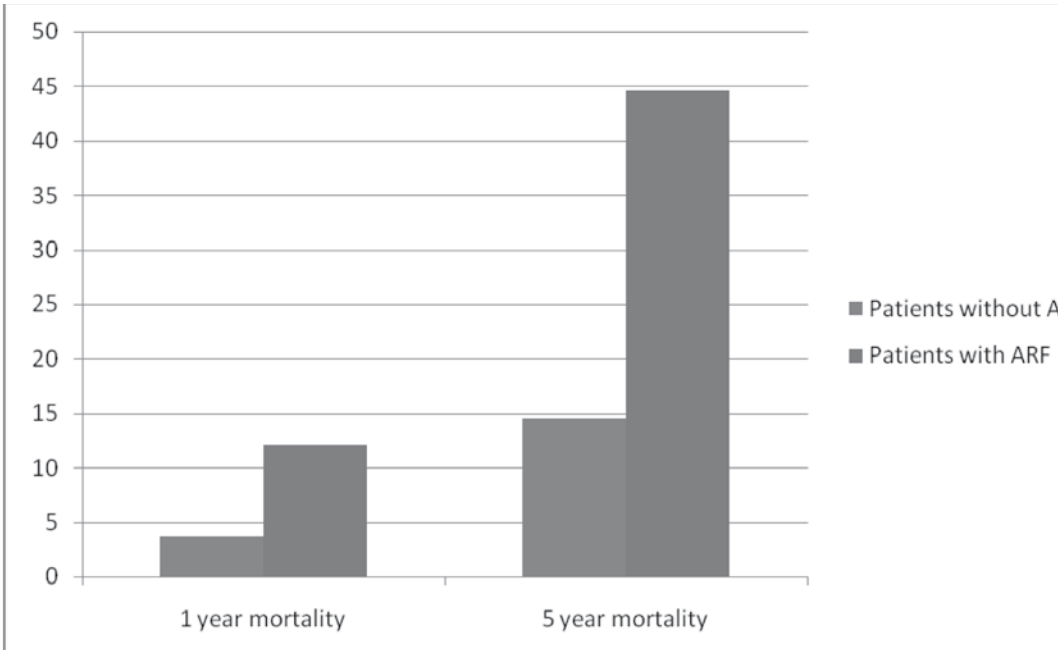

Fig. 3. One year and five year mortality rates associated with acute kidney injury after percutaneous coronary intervention[48]

mortality and should include patients with the wide range of chronic conditions that may confound the interpretation of NGAL levels, but will be common in any inpatient population.

CONCLUSION

Measurements of serum and urinary NGAL appear promising as a highly sensitive and specific marker for AKI in heterogeneous patient groups. NGAL has several characteristics of an ideal biomarker that may facilitate early diagnosis of AKI, compared to serum creatinine, the existing gold standard. Resistance to protease-mediated degradation and the availability of commercial assays further adds to its practical value. However, there is a need for multi-centre validation studies to determine the threshold values of both serum and urine NGAL in various pathophysiological conditions and to test the reliability and precision of NGAL to diagnose AKI. Incorporation of NGAL assays into the protocols of large, prospective outcome trials, with randomisation to differing strategies to prevent and treat AKI would establish its value as a marker for guiding therapy and reducing long-term cardiovascular morbidity.

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