Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

The diagnostic utility of serum Neutrophil Gelatinase Associated Lipocalin in Acute Kidney Injury at a Central Hospital in a Resource Constrained Setting

[1]Mabaya L.*, [2]Musarurwa C, [2]Gomo ZAR, [2]Nyamayaro T.

[1]Department of Biochemistry, Midlands State University Medical School, P. Bag 9055, Gweru, Zimbabwe.
[2]Department of Chemical Pathology, University of Zimbabwe Medical School Mazowe Street P. O. Box A178 Harare, Zimbabwe

ABSTRACT

The objective of the study was to determine the utility of serum NGAL as a biomarker for acute kidney injury in patients admitted into medical wards. Sixty consecutive adult patients presenting with suspected acute kidney injury at Parirenyatwa Hospital, Harare, Zimbabwe and admitted for at least three days were enrolled. Participants were enrolled if they were above 18 years but < 50 years**,** had serum urea >6.7mmol/l and/or creatinine >131µmol/l and had either oliguria or anuria. Patients were excluded if they had a history of chronic hypertension, chronic kidney disease, any malignancy or diabetes. The mean serum NGAL levels in patients were 653.3ng/ml and the mean eGFR was 9.7ml/min. There was a statistically significant correlation between NGAL and urea or creatinine (r=0.85; r= 0.46 respectively). A significant inverse correlation was observed between NGAL and eGFR (r= -0.97). There was an association between NGAL levels and dialysis requirement (OR=1.02, 95% CI (1.01 – 1.24), p=0.0008). A NGAL concentration of greater than 600ng/ml was highly predictive of RRT with an area under curve of 0.64. Indications from the study are that NGAL is a suitable biomarker for AKI because it correlates closely with renal function and allows timely identification of high risk patients to allow potentially beneficial therapies to be initiated early in the disease process. NGAL is also a suitable candidate for use in the estimation of glomerular filtration rate.

KEYWORDS: NGAL, acute kidney injury, dialysis, glomerular filtration rate, renal function

***Corresponding author**

Mabaya Lucy

Department of Biochemistry,
Midlands State University Medical School,
P. Bag 9055, Gweru, Zimbabwe.
Mail - lucymabaya@yahoo.com; mabayal@msu.ac.zw
Phone number: +263772 951 687

INTRODUCTION

The incidence of acute kidney injury (AKI) is increasing globally, affecting about 40% of all critically ill patients in whom AKI is an independent predictor of mortality and morbidity. The overall mortality rate among patients with AKI has remained high amongst hospitalised and intensive care patients despite significant advances in medical care. However, the successful translation of promising clinical treatments for AKI has been hindered by lack of early, accurate and reliable indicators of injury. [1-3]

In Zimbabwe, clinicians over the years continue to base their diagnosis of AKI on serum urea and creatinine measurements. Serum creatinine and its derived estimates of glomerular filtration rate, though considered the gold standard for diagnosis of renal failure is fraught with challenges. The wide reference range for serum creatinine makes it difficult to detect early renal disease and requires at least 50% decrease in GFR to detect any changes in the serum creatinine concentration. In addition, measurement of creatinine by the Jaffe method is subject to numerous analytical interferences such as bilirubin, certain antibiotics and ketones. Serum creatinine is also affected by muscle mass and a high protein diet. Therefore, its ability to detect early kidney injury is limited. [2, 3]

Over the last decade, intensive investigative efforts have led to the identification and characterization of several urinary and serum markers that appear to be more sensitive and specific for kidney injury. Most notable are N-acetyl-β-(D)-glucosaminidase, neutrophil gelatinase associated lipocalin, kidney injury molecule-1, interleukin-18, and liver-type fatty acid binding protein. Further assessment is needed to fully determine the clinical utility of these markers, although there is much enthusiasm that they will enhance the understanding of kidney pathophysiology and aid in the development of early targeted interventions to ameliorate injury and prevent functional decline. Among these, is neutrophil gelatinase associated lipocalin (NGAL) reported to be a highly sensitive, specific, and predictive, early biomarker for AKI in a wide range of different disease processes. [4]

Human NGAL, a member of the lipocalin superfamily, is a 25 kDa protein that is covalently bound to gelatinase in neutrophils and expressed at low concentrations in normal kidney, trachea, lungs, stomach, and colon. At these sites, NGAL plays a critical role in host innate immune response by inhibition of iron-binding molecules that are important to specific bacteria. Neutrophil gelatinase associated lipocalin expression is induced in injured epithelia, especially the kidney. The NGAL accumulates within two distinct pools, namely a systemic and a renal pool. It has been demonstrated that AKI results in increased NGAL mRNA expression in organs such as the liver and spleen, and the over-expressed NGAL protein is released into the circulation. Furthermore, any decrease in

glomerular filtration rate resulting from AKI would further decrease the clearance of NGAL, with accumulation in the blood stream. [5-7]

A number of studies have demonstrated the utility of early NGAL measurements for predicting the severity and clinical outcomes of AKI [4, 8, 9, 10, 11]. In a prospective study of children undergoing elective cardiac catheterization with contrast administration, both urine and plasma NGAL predicted contrast-induced nephropathy within 2 hours after contrast administration. Both urine and plasma NGAL were very valuable predictors of AKI in children, with an area under the curve (AUC) of the ROC of over 0.9 for the 2–6 hour urine and plasma NGAL measurements. [8] In studies on adults administered with contrast, an early rise in both urine (4 hour) and plasma (2 hour) NGAL was documented, in comparison with a much later increase in plasma cystatin C levels (8–24 hours after contrast administration), providing further support for NGAL as an early indicator of contrast nephropathy. [12, 13] However, the majority of these studies were centred on surgical patients. The present study assessed the clinical utility of NGAL in early discrimination of AKI suspects requiring dialysis from those that did not require renal replacement therapy.

MATERIALS AND METHODS

In this cross sectional study, 60 consecutive patients with suspected AKI were recruited from Parirenyatwa Hospital Emergency Department from November 2013 to April 2014. To be eligible patients had to subsequently be admitted into medical wards at the same hospital. Provisional diagnosis of AKI in these patients at presentation was based on clinical examination, renal function tests as well as findings of renal ultrasonography.

Participants

After written informed consent was sought and granted from each participant, a minimum 100µl deidentified serum samples was aliquoted and stored at -70^{o}C from the routine blood specimens collected as part of routine patient care on day of admission. These samples were only thawed once on the day of NGAL measurements. Participants were followed up three days post admission to ascertain clinical decisions regarding dialysis initiation. Medical and demographic data was abstracted from participant medical records.

Laboratory analysis

The NGAL assay was carried out using a sandwich enzyme linked immunosorbent assay (ELISA) technique using reagents provided by Quantikine R& D International USA (14). Urea and creatinine were measured on the AU680 Beckman analyser using colorimetric methods [15,16] whilst

serum electrolytes were measured on the same analyzer using direct ion selective electrodes. The glomerular filtration rate for all subjects was calculated using the MDRD equation [17].

Ethics

Ethical approval was sought and granted by the Joint Research Ethics Committee (JREC) of the University of Zimbabwe College of Health Sciences and Parirenyatwa hospital and also from the Medical Research Council of Zimbabwe.

Statistics

Statistical analysis was done using R version 2.15.1. A two sample t-test for continuous variables and Chi- squared test were used to determine if there were significant differences between AKI cases and controls in the study. Spearman's correlation coefficient test was used to determine if there were significant correlations between NGAL and the conventional biomarkers. Multiple logistic regression analysis was performed to ascertain the biomarker more predictive of disease severity and dialysis requirement. The diagnostic utility of NGAL was determined by plotting ROC (receiver operating characteristic curves) using SPSS version 17.

RESULTS

Clinical characteristics of AKI patients

The mean NGAL levels for AKI patients and health subjects were 653.3ng/ml and 75.8ng/ml respectively. There were significant differences in biomarker concentration between the two groups, $p<0.0001$ (Table I).

Table I: Characteristics of patients and respective variables

Variable	AKI Patients
Sex(F,M)	29 ; 31
Age(mean;sd)	37.2 ; 7.0
Urea(mean;sd)	33.1 ; 23.0
Creatinine(mean;sd)	977.2 ; 816.4
Sodium(mean;sd)	131. 7 ; 5.6
Potassium(mean;sd)	4.9 ; 0.9
eGFR(mean;sd)	9.7 ; 6.6
NGAL(mean;sd)	653.3 ; 219.9

Association between NGAL and conventional kidney function tests

There was a moderate statistically significant positive correlation between serum levels of NGAL and creatinine. ($r=0.46$, $p<0.0001$;

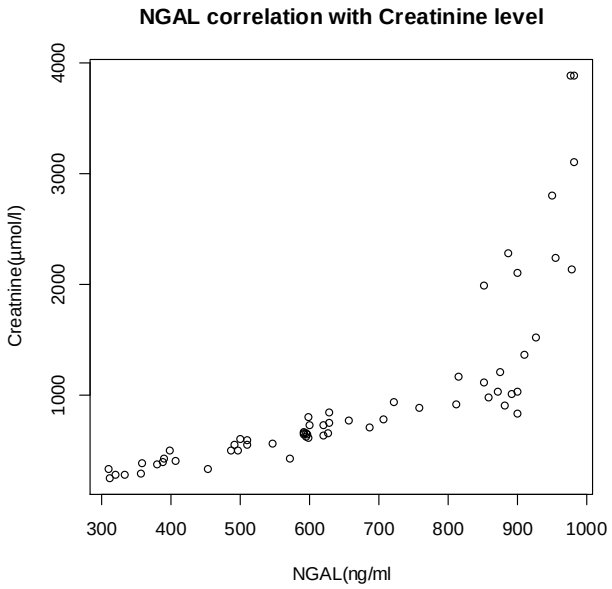

Figure I: Scattergram of serum NGAL levels and serum creatinine concentration

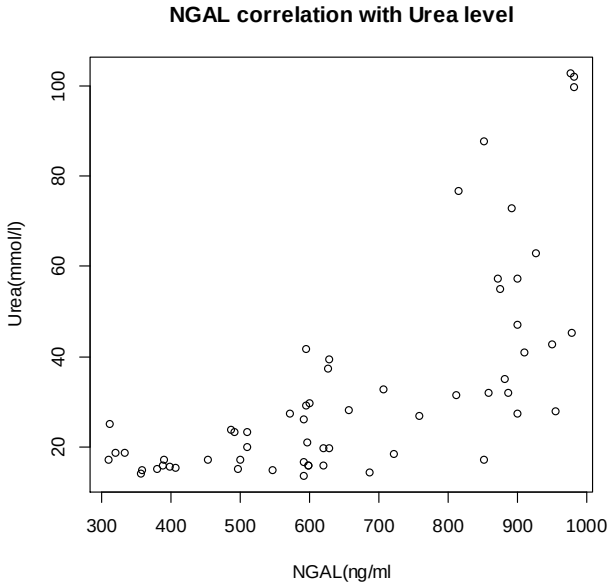

Figure II: Scattergram of serum NGAL levels and serum urea concentration

There was a highly statistically significant positive correlation between NGAL and urea (r=0.85, p<0.0001;

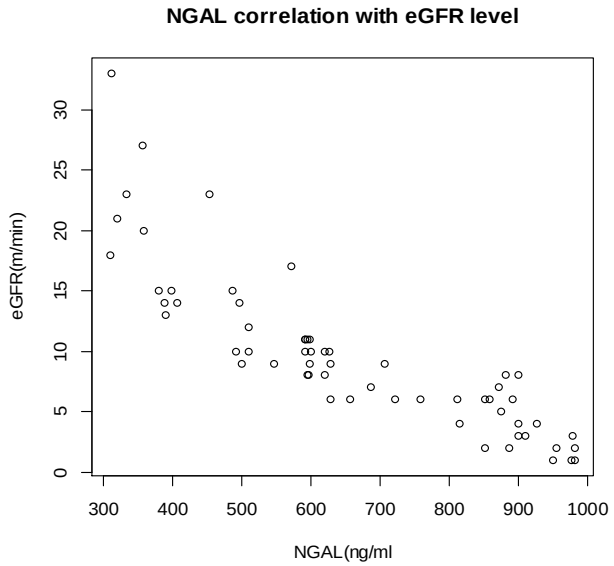

Figure III: Scattergram of serum NGAL levels and eGFR

There was a very strong negative correlation between NGAL and log transformed eGFR (r= -0.97, p<0.001; Figure III).

NGAL levels and severity of illness

The AKI patients were stratified into two groups based on dialysis requirement decision. There were no statistically significant differences between participants requiring dialysis and those not requiring dialysis in terms of sex potassium and age .However, there were statistically significant differences between the two groups in mean NGAL, urea, creatinine, sodium and eGFR levels (Table II). Dialysis patients had higher serum levels of NGAL, urea, creatinine and lower eGFR levels. (Figure IV).

Table II: Characteristics of Dialysis patients (AKI + D) and none Dialysis requiring (AKI–) patients

Variable	Clinical outcome		P-value
	AKI + D	AKI -D	
Sex(F,M)	18,12	11,19	0.1211
Age	38.1	36.3	0.3161
Urea(mean)	45.0	21.2	<0.0001***
Creatinine(mean)	1449.5	520.6	<0.0001 ***
Sodium(mean)	129.7	133.9	0.0025**
Potassium(mean)	5.0	4.7	0.3471
eGFR(mean)	5.0	14.4	<0.0001***
NGAL(mean)	826.5	480.1	<0.0001***

Dialysis patients had higher serum levels of NGAL, urea, creatinine and lower eGFR levels. (Figure VI).

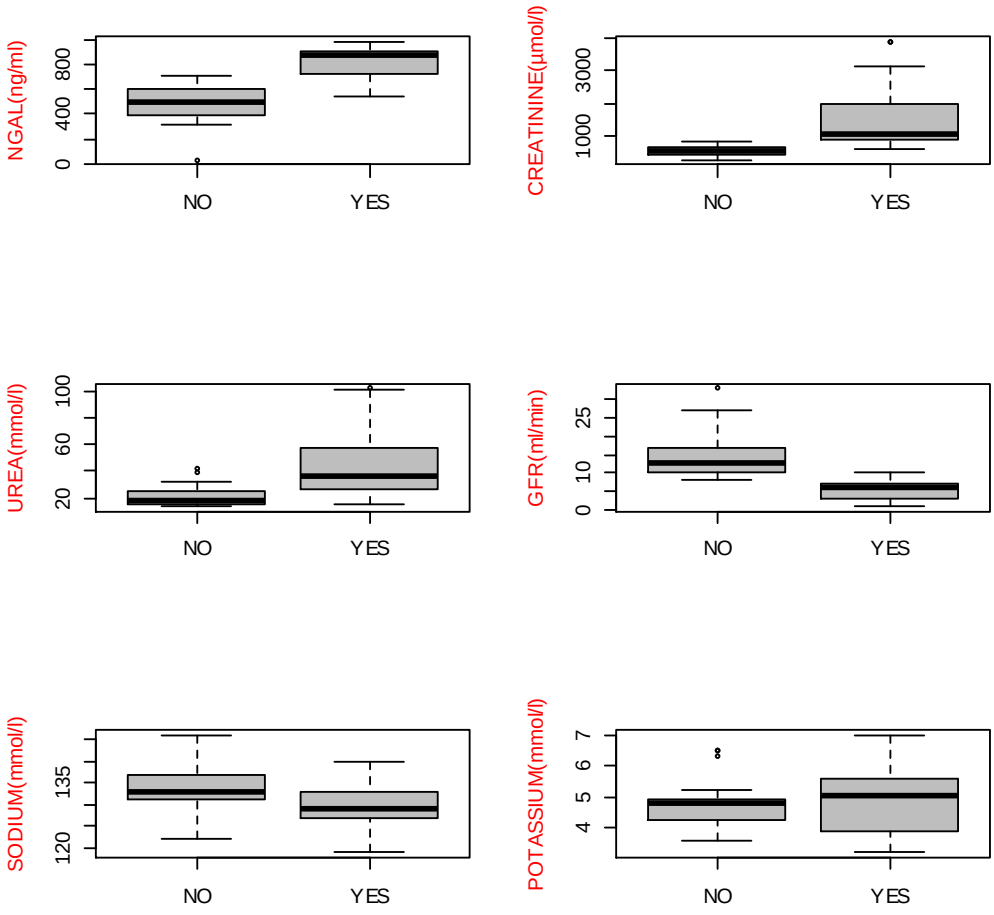

Figure IV: Boxplots-By dialysis requirement

NGAL and prediction of clinical outcome

Univariate logistic regression was done to determine association between each predicting variable (age, sex, urea, creatinine and NGAL) and the clinical outcome of severe AKI requiring dialysis or none severe AKI. Results showed that urea (OR=1.11, 95% CI (1.05 – 1.19) p=0.0013), creatinine (OR=1.01, 95% CI (0.65 – 3.72) p=0.0007) and NGAL levels (OR=1.02, 95% CI (1.01 – 1.24) p=0.0008) in patients are the only variables significantly associated with clinical outcome (Table III).

Table III: Univariate Logistic Analysis by dialysis requirement status

Variable	Odds	95% CI	p-value
Age	1.04	0.64 – 4.80	0.319
Sex(M)	0.39	0.32 - 4.02	0.0732
Urea	1.11	1.05 – 1.19	0.0013
Creatinine	1.01	0.65 – 3.72	0.0007
NGAL	1.02	1.01 – 1.24	0.0008

The best models for dialysis initiation were creatinine and NGAL. Fitting these two factors into the multiple logistic regression NGAL had statistically significant predictor of dialysis (OR=1.02, 95% CI (0.98 - 1.13) p = 0.009; Table IV) as compared to creatinine (OR=1.00, 95% CI (0.32 - 4.02) p=0.989).

Table IV: Multiple Logistic regressions by dialysis requirement status

Variable	Odds	95% CI	p-value
Main effects			
NGAL	1.02	0.98 - 1.13	0.009
Creatinine	1.00	0.32 - 4.02	0.989

Diagnostic Accuracy of NGAL for AKI severity

The diagnostic accuracy of NGAL was evaluated by calculating the area under the curve (AUC). The eGFR was used as the state variable and the value of the state variable was chosen at 10ml/min indicating the true category to which a subject belongs (AKI+ D or AKI-D).

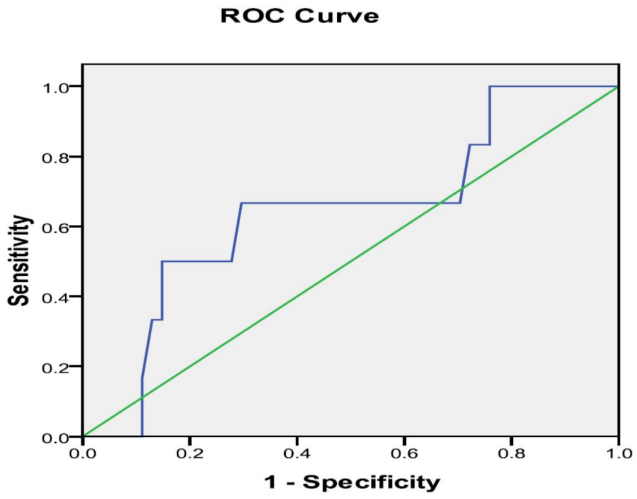

Figure V: ROC curve indicating area under curve for NGAL

Figure V indicates an area under curve for NGAL of 0.64 (95% CI 0.41- 0.88).The value demonstrates that NGAL is an acceptable biomarker for the prediction of AKI severity.

DISCUSSION

In this study of patients presenting to the emergency department at Parirenyatwa Hospital with acute renal dysfunction, NGAL demonstrated to be a valuable predictor of AKI and disease severity.

The study revealed a high concentration of NGAL in AKI patients. The mean level of NGAL in patients was 653.3ng/ml. This study provides substantial support to previous findings. Studies in children who underwent elective cardiac surgery revealed a tenfold or more increase in urine and plasma NGAL by ELISA method in those who developed AKI. [18] Similar results were obtained in adults who developed AKI after cardiac surgery with contrast administration. [19]

Of the 60 AKI patients, 51.7% had greater severity of kidney dysfunction and needed the assistance of dialysis whilst the condition of the other 48.3% patients did not warrant such a measure. Higher levels of NGAL (greater than 600ng/ml) on admission were highly predictive of need for renal replacement therapy with 90.3% of the patients requiring dialysis.

In this regard, a number of studies have demonstrated the utility of NGAL measurements for predicting the severity and clinical outcomes of AKI. [20] In children with severe sepsis, plasma NGAL measurements predicted the severity of AKI and dialysis requirement with high sensitivity (AUC-ROC: 0.72). [21] In adult patients with out of hospital cardiac arrest, NGAL was able to predict AKI, 30 day survival and good neurological outcome with AUC-ROC of 0.81, 0.72 and 0.72 respectively at 95% CI. [22] Studies conducted in the cardiac surgery setting have identified NGAL as a good clinical outcome and mortality marker with an overall ROC curve area of 0.71. [23]

In addition to these studies, this study has demonstrated that plasma NGAL levels have a relatively good predictive value for AKI severity at patient presentation with AUC- ROC of 0.64 with 95% CI of (0.41- 0.88). However, the slight differences between this study and those studies could be due to the fact that the current study was conducted in a clinical setting in which the actual timing of the renal insult was unknown. In addition, differences in sample sizes could have contributed to the differences in predictive power of NGAL. Previous studies have also identified age as a contributing factor to NGAL's performance as an AKI biomarker with better predictive ability in children than adults [24] which was not part of the investigation in this study. Lastly, the diagnostic accuracy of NGAL is determined by the definition of AKI employed as well as the severity or stage of AKI. [25] In the present study, the baseline creatinine values for AKI patients were

not known and AKI was defined by standard laboratory parameters whereas previous studies defined AKI using the RIFLE criteria which depended on baseline creatinine values.

In assessing the correlation between serum NGAL and conventional renal function tests, a closer relationship was established between NGAL and eGFR (r= -0.97) than with creatinine (r= 0.46). This implies that NGAL levels are a true reflection of the glomerular filtration rate which is the cornerstone for assessment of renal function. This is consistent with findings of a study done by Mitsnets and colleagues [26] who reported that plasma NGAL correlated better with eGFR than with serum creatinine and cystacin C.

The study has made positive revelations. Firstly, it has demonstrated that plasma NGAL remained diagnostic even when the timing of injury was unknown, making it a useful indicator of kidney injury for many clinical presentations. In addition, NGAL has proved to be a good prognostic marker because admission levels were predictive of need for RRT. This research is one of the very few studies that have examined the predictive value of NGAL in plasma. The majority of biomarkers for AKI so far are measured in urine [4]. The advantages of using urine samples are that the sampling procedure is non-invasive and the self-testing kits can be easily made available. However, specimen collection can be difficult in patients with oliguria or anuria and quantitation of the 24 hr urine output.

Although the data obtained from the research was of great significance, it was a single centre study and in future will certainly need to be validated in larger settings to get a better representation of the general population. In addition to NGAL, simultaneous examination of other urinary biomarkers as predictors of AKI may be informative [27]. Lastly, our study relied on a single measurement of NGAL due to financial constraints. Follow up measurements of NGAL in renal failure patients could evaluate the performance of NGAL in monitoring response to AKI intervention.

CONCLUSION

The information obtained from the research makes an important contribution to the current body of literature on NGAL and AKI. From our findings, it can be concluded that NGAL is a useful marker for the diagnosis of AKI. It is also an early predictor of need for renal replacement therapy and correlates with AKI severity. Timely identification of AKI patients and those who may require dialysis will allow early targeted therapy of high risk patients before progression to irreversible kidney damage (chronic renal failure).

ACKNOWLEDGEMENTS

I thank the Lord almighty with whom all things are possible. I express my sincere gratitude to my supervisor Mr. T. Nyamayaro for his wisdom and guidance throughout the study, and the whole Chemical Pathology Department team at the University of Zimbabwe, Medical School for their assistance and guidance. Special thanks also go to my husband Chamaona Mabaya for being my pillar of strength, and my parents for their support and encouragement. This study would not have been possible without them all.

REFERENCES

1. Nisula S, Kaukonen KM, Vaara ST. Incidence, risk factors and 90-day mortality of patients with acutekidney injury in Finnish intensive careunits: the finnaki study. Intensive CareMed. 2013; 39:420–428.

2. Kerr M, Bedford M, Matthews B, O Donoghue D. The economic impact of acute kidney injury inEngland. Nephrol Dial Transpl. 2014; 29:1362–1368.

3. Kellum JA, Lameire N, for the KDIGOAKI Guideline Work Group Diagnosis, evaluation, and managementof acute kidney injury: a KDIGO summary (Part 1). Crit Care 2013; 17:204.

4. Devarajan, Preview: Neutrophil gelatinase-associated lipocalin: A troponin-like biomarker for human acute kidney injury. Nephrology. 2010; 15: 419–428.

5. Xu S, Venge P. Lipocalins as biomarkers of disease. Biochem Biophys Acta. 2000; 1482:298–307.

6. Schmidt-Ott KM, Mori K, Kalandadze A, Li JY, Paragas N, Nicholas T, et al. NGAL-mediated iron traffic in kidney epithelia. Curr Opin Nephrol Hypertens. 2006; 15:442–449.

7. Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P, Barasch J. Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol. 2007; 18:407–413.

8. Peggy Sekula, Oemer-Necmi Goek, Lydia Quaye, Clara Barrios. A Metabolome-Wide Association Study of Kidney Function and Disease in the General Population J. Am. Soc. Nephrol. 2016; 27: 1175-1188.

9. Jay L. Kogner, Amit X. Gorg, Steven G. Coca et al. Biomarkers Predict Progression of Acute Kidney Injury after Cardiac Surgery. J. Am.Soc. Nephrol. 2012; 23: 905-914.

10. Devarajan, Prasad. NGAL for the detection of acute kidney injury in the emergency room. Biomarkers in medicine. 2014; 8.2: 217-219.

11. Antonucci, E, Lippi, G, Ticinesi, A, Pigna, F, Guida, L, Morelli, I, Nouvenne, A, Borghi, L. and Meschi, T. Neutrophil gelatinase–associated lipocalin (NGAL): a promising biomarker for the early diagnosis of acute kidney injury (AKI). Acta Bio Medica Atenei Parmensis. 2014; 85(3): 289-294.

12. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E. Kidney Blood Press Res.. 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? 2007: 408-415.

13. Akrawinthawong, Krittapoom. Subclinical and clinical contrast-induced acute kidney injury: data from a novel blood marker for determining the risk of developing contrast-induced nephropathy (ENCINO), a prospective study. Renal failure. 2015; 37.2: 187-191.

14. K. Mori and K. Nakao, Neutrophil gelatinase- associated lipocalin as a real time indicator of active kidney damage. Kidney International. 2007; 71: 967-970.

15. Talke H. and Schubert G.E. Klinische Wochenschrift. 1965; 43: 174.

16. Burtis, Carl A., and David E. Bruns. Tietz fundamentals of clinical chemistry and molecular diagnostics. Elsevier Health Sciences. 2014; 345-7.

17. Earley A, Miskulin D, Lamb EJ, Levey AS, Uhlig K. Estimating equations for glomerular filtration rate in the era of creatinine standardization: a systematic review. Annals of Internal Medicine. 2012; 156(11):785-95.

18. Benzer M, Alpay H, Baykan Ö, Erdem A, Demir IH. Serum NGAL, cystatin C and urinary NAG measurements for early diagnosis of contrast-induced nephropathy in children. Renal Failure. 2015; 18:1-8.

19. Singer E, Markó L, Paragas N, Barasch J, Dragun D, Müller DN, Budde K, Schmidt-Ott KM. Neutrophil gelatinase-associated lipocalin: pathophysiology and clinical applications. Acta Physiologica. 2013; 207(4):663-72.

20. Basu RK, Wang Y, Wong HR, Chawla LS, Wheeler DS, Goldstein SL. Incorporation of biomarkers with the renal angina index for prediction of severe AKI in critically ill children. Clinical Journal of the American Society of Nephrology. 2014; 9(4):654-62.

21. Trachtman H, Christen E, Cnaan A. Urinary neutrophil gelatinase associated lipocalin in D + HUS: a novel marker of renal injury. Pediatr Nephrol. 2006; 21:989-994.

22. Park SO, Ahn JY, Lee YH, Kim YJ, Min YH, Ahn HC, Sohn YD, Park SM, Oh YT, Shin DH. Plasma neutrophil gelatinase-associated lipocalin as an early predicting biomarker of acute kidney injury and clinical outcomes after recovery of spontaneous circulation in out-of-hospital cardiac arrest patients. Resuscitation. 2016; 101:84-90.

23. Wagener G, Jan M, Kim M. Association between increases in urinary neutrophil gelatinase associated lipocalin and acute renal dysfunction after adult cardiac surgery. Anesthesiology. 2006; 105:485-49.

24. Devarian P. Neutrophil gelatinase –associated lipocalin: a promising biomarker for acute kidney injury. Biomark Med. 2010; 4(2): 265-80.

25. Haase-Fielitz A, Bellomo R, Devarajan P. The predictive performance of plasma neutrophil gelatinase-associated lipocalin (NGAL) increases with grade of acute kidney injury. Nephrol Dial Transplant. 2009; 24(11):3349–3354.

26. Mitsnefes M, Kathman T, Mishra J, Kartal j, Khoury P, Nickolas T. Serum Neutrophil gelatinase associated lipocalin as a marker of renal function in children with chronic kidney disease. Pediatric Nephrol. 2007; 22:101-8.

27. Kohei J, Ishida H, Kazunari T, Tsuchiya K, Nitta K. Neutrophil gelatinase-associated lipocalin is a sensitive biomarker for the early diagnosis of acute rejection after living-donor kidney transplantation. International Urology and Nephrology. 2013; 45(4):1159-67.