

The Potential Role of Novel Kidney Biomarkers in Diagnosis of Early Contrast-Induced Nephropathy Following Coronary Angiography

Nevine Sherif¹, Omar M. Sabry², Noha M. Elsheikh¹, Samia El-Shishtawy¹, H.M. Abutaleb³, Ahmed Ali Mohamed⁴, Ahmed Abdallah Emam⁵, Amal Sabry⁴, Marwa Wahdan² and Rabab Fouad²

¹Nephrology Department, Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt

²Hematology Department, Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt

³Biostatistics and Demography, Department of Environmental Research, Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt

⁴Intensive Care Department, Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt

⁵Cardiology Department, National Heart Institute (NHI), Imbaba, Giza, Egypt

Emails: dr_rabab_fouad@yahoo.com

Abstract:Background: Acute kidney injury (AKI) is a clinical syndrome that develops when a sudden kidney function impairment occurs. The reported rate of contrast-induced nephropathy (CIN) in cases following coronary angiography (CAG) ranges from 10 to 20%. The prevalence of CIN in subjects with regular kidney role is low (0–5%). Apart from determining serum creatinine (a delayed indicator of renal function but not its injury), recent biomarkers of AKI would be helpful in the diagnosis of CIN in the context of CAG. Cystatin C (Cys C), Neutrophil gelatinase-associated lipocalin (NGAL), Kidney injury molecule-1 (KIM-1), and interleukin-18 (IL-18) have been suggested as potential markers for early AKI.

Aim: This study evaluates the serum levels of the AKI indicators in cases undergoing CAG and assesses their role in the early detection of AKI associated with CIN.

Subjects & Methods: The study involved 31 cases that underwent coronary angiography (CAG) and obtained contrast agent (Ultravest), in addition to 15 sex and age-matched healthy individuals as control class. Serum concentrations of kidney biomarkers (KIM-1, NGAL, Cys C, and IL-18) were measured prior to and 6 hours post CAG using Luminex 100™ readout System.

Results: The serum concentrations of IL-18 and Cys C were greater in studied cases prior to CAG compared to controls ($p < 0.05$). Cases that underwent CAG showed a highly significant elevation in KIM-1 ($p < 0.001$) level and a significant elevation in Cys C ($p < 0.05$) level 6 h post CAG procedure. No significant differences were observed in serum levels of NGAL and IL-18 6 h post-CAG.

Conclusion: Serum Cys C and KIM-1 levels could reflect early affection of renal function in cases undergoing CAG. Thus assessment of serum Cys C and KIM-1 would provide a solid base for the early detection of CIN.

Keywords: NGAL; IL-18; KIM-1; Contrast-induced Nephropathy; Cys C; Acute kidney injury.

INTRODUCTION

Contrast-induced nephropathy (CIN) is well-determined as kidney impairment following intravenous contrast administration, and within 48-72 hours of contrast injection, it is detected by a 25% rise in serum creatinine from baseline or a 0.5 mg/dL rise in absolute value [1]. It is considered the third reason for in-hospital acute kidney failure (12%). Its incidence comes after the incidence of reduced renal perfusion (42%) and acute kidney failure after surgery (18%) [2].

CIN usually occurs within the first 2-3 days after contrast, but it can be delayed up to 5 days. Other causes of renal failure should be excluded before such a diagnosis is approached. Serum creatinine levels reach their highest point between 2 and 5 days and usually return to normal within 2 weeks [3].

Elevation in serum creatinine (s.creat) is the current gold standard for detecting renal s.creat affection, but this is problematic as the normal is affected by many variables involving age, muscle mass, gender, diet, medications, and volume status of the case. In CIN, s.creat can take several days to reach its normal level. Furthermore, up to 50% of renal function can be impaired before s.creat even starts to increase [4, 5].

Ultimately, the quest for renal biomarkers that are more sensitive than s.creat is highly demanded. Ideally, a renal biomarker should detect CIN early, even before an increase in s.creat, identify the exact site of injury within the kidney and its time of onset, differentiate between different causes of renal injury. It should also predict the renal outcome.

Cystatin C (Cys C) is a non-glycosylated protein with a small molecular mass (13 kDa). Cys C is made at a stationary level by each nucleated cell. In normal conditions, the glomeruli filter it, and it is reabsorbed totally in the proximal tubule. Its serum concentration represents glomerular filtration in normal conditions and may be utilized as a functional indicator for chronic and acute variations in glomerular filtration rate (GFR) [6].

Creatinine and Cys C indicate AKI by detecting the reduced GFR. When GFR reduces, s.creat and Cys C can't be effectively eliminated in the urine, and this causes that s. creat and Cys C levels elevated in the blood [7].

This is in contrast to other biomarkers, which are produced as a direct result of tubular injury. The serum level of cystatin C is unaffected by gender, age, muscle strength, or nutritional status, but it declines in hypothyroidism and increases in hyperthyroidism [7].

IL-18 is a potent pro-inflammatory cytokine of a molecular weight of 18 kDa. It is synthesized by a large number of cells, involving macrophages, monocytes, osteoblasts, cells of the renal and intestinal epithelium. Increased IL-18 concentration occurs during AKI due to ischemia, hypoxia, and exposure to nephrotoxins in the proximal tubule cells. It can also be derived from lung injury and myocardial ischemia [8].

Kidney injury molecule-1 (KIM-1) is a transmembranous protein that is rarely determined in urine but is greatly expressed in proximal tubular epithelial cells shortly after ischemic or toxic injury. Han *et al.* claimed that urinary KIM-1 is capable of quickly detecting AKI. KIM-1 has greater of scales for “pure” ischemic insult than for other diseases that cause acute kidney failure. As the pathogenesis of CIN is multifactorial, the utilization of this indicator appears to be of restricted significance in this specific condition[9].

There are several causes to believe that KIM-1 is released into the bloodstream following renal proximal tubule damage. Tubular cell polarity is missing after proximal tubule injury. In this condition, KIM-1 can be introduced into the interstitium directly. Additionally, elevated transepithelial permeability that happens after tubular injury results in the back leak of tubular contents into the bloodstream. Moreover, The pathophysiology of kidney injury is complicated by changes in microvascular permeability. In renal microvascular endothelial cells, the actin cytoskeleton is damaged, resulting in the loss of cell-matrix adhesion junctions, cell-cell, and the separation of endothelial cells from the basement membrane, which boosts KIM-1 migration into the bloodstream[10].

Neutrophil gelatinase-associated lipocalin (NGAL) is a tiny lipocalin superfamily protein that was first discovered in the supernatant of stimulated human neutrophils in 1993 [11].

Furthermore, hepatocytes, immune cells, and renal tubular cells all synthesize and release them in various pathologic conditions. NGAL regulates cell growth and differentiation in a variety of cell types, in addition to its bacteriostatic impact through transmembranous iron shuttling. NGAL restricts apoptosis and participates in sustaining the tubular structure in renal epithelia [12]. We can consider NGAL as the “renal troponin” as it is greatly and quickly upregulated in kidneys even after ischemic or nephrotoxic damage [13] and is believed to contribute to controlling parenchymal injury [12].

This research targets to clarify the significance and clinical importance of the novel kidney biomarkers in the diagnosis of AKI and early detection of CIN in CAG cases.

SUBJECTS AND METHODS

Study population

The study was conducted on 46 subjects, divided into 2 classes:

- Class 1 involved 31 cases who underwent percutaneous CAG in the National Heart Institute (NHI), Imbaba, Giza, Egypt.
- Class 2 involved 15 sex-and age-matched healthy individuals chosen from paramedical and medical staff to serve as a control.

We stated some exclusion criteria, which are; blood transfusion in the previous month, acute infection, active immunological disorder (e.g., rheumatoid arthritis, vasculitis, systemic lupus erythematosus), chronic viral infections (e.g., hepatitis B, human immunodeficiency virus, hepatitis C), utilization of medication involving antibiotics, anti-inflammatory or antifungal treatments at the period of the study, history of malignancy, or previous transplants for a good selection of our cases.

The presented study was recorded at National Heart Institute. The TBRI Ethics committee approved it (Federal wide assurance number FWA00010609). Each enrolled subject signed a written informed consent form for the use of related data.

Blood collection:

A fresh 5-ml venous blood specimen was aseptically gathered from all cases prior to CAG. Another venous specimen was drawn 6 hours post CAG. Both specimens were collected using vacutainer tubes; one tube on EDTA to perform CBC using DXH 500 hematology analyzer, Beckman Coulter, USA. In addition to, serum separator tube (SST) to perform s.creat on Olympus AU480 Chemistry Analyzer, Beckman Coulter, USA, and Luminex kidney biomarker screening assay. Specimens were permitted to clot for 30 minutes at room temperature before being centrifuged for 15 minutes at 3000 x g supernatant serum was eliminated and kept at -20 °C and thawed at the time of assay. Lab tests were carried by personnel blinded to case knowledge. Both specimens were analyzed for s.creat, NGAL, KIM1, IL-18, and Cys C. A third specimen was collected from each case 72 hours post-CAG to measure s.creat. Specimens were taken only once from the control class.

Quantification of Biomarkers

Specimen preparation for Luminex® Assay:

Serum specimens required a 2-fold dilution (specimen 75 µL of specimen + 75 µL of Calibrator Diluent RD6-52) and mixed thoroughly. Luminex® screening Assays human kidney biomarkers premixed multiplex kit (Catalogue number. LXSAH-04/ lot number 1435218, R&D Systems,

Inc., USA, was used to perform a simultaneous examination of many biomarkers. Luminex utilizes a fluorescence polystyrene bead-based method. Every bead set is covered with an antibody specific to a particular analyte. Inside the analyzer, the laser stimulates the inner stains that recognize each microsphere particle. In addition, it captures any reporter stain through the assay. In this way, this method permits the multiplexing of up to 100 unique quantitative assays within a single specimen.

Serum was analyzed for levels of kidney biomarkers using a premixed kit containing the following analytes: Cystatin C, Lipocalin-2/NGAL, IL-18, and KIM-1/TIM 1/HAVCR using Human Premixed Multi-Analyte Kit according to manufacturers' instruction (R&D Systems Inc., USA). Luminex 100™ readout System was used. The levels of each analyte were automatically calculated from standard curves using xPONENT® software.

Calculation of Results

Regular concentrations on the Certificate of Analysis were used, and 3-fold dilutions for the residual levels were calculated. The average of duplicate readings for each standard and specimen was subtracted from the average blank Median Fluorescence Intensity (MFI). Data reduction utilizing xPONENT® software generated each analyte's standard curve, generating a five-parameter logistic (5-PL) curve-fit. The normal curve level read was increased by the dilution factor of 2 after the specimens were diluted.

Statistical analysis

Data were statistically defined based on mean $\pm$ standard deviation. The time-course changes of each marker were compared utilizing the comparison paired t-test between before and after 72hrs. Mann–Whitney U-test and independent specimens t-test were utilized to test the difference among the classes as appropriate. Statistical analysis was performed using Microsoft Excel spreadsheets version 10, SPSS software for windows version 25.0 program (SPSS Inc., Chicago, IL, USA) $P < 0.05$ was considered statistically significant.

RESULTS

The information of the case was recorded for feasibility analysis at admission involving sex, age, weight, hypertension, and diabetes mellitus, as well as oral medicines, especially angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEI/ ARB). Data for both classes are revealed in (Table 1).

There was no significant difference between the control class and the cases before CAG regarding s.creat, NGAL, and KIM-1. However, Cys C and IL-18 were both significantly increased in cases before CAG when compared with the control class ($p < 0.05$) (Table 2).

Regarding other kidney biomarkers at the 6-hour time point post-CAG, there was a significant increase in serum Cys C level ($p < 0.05^*$) and a highly significant elevation in serum KIM-1 level ($p \text{ value} < 0.001^{**}$), but a non-significant rise in serum IL-18 or NGAL levels ($p > 0.05$) (Table 3).

Table (1): Demographic data of CAG cases and healthy controls

Variables	Cases Mean $\pm$ SEM	Control Mean $\pm$ SEM
Age (yrs)	53.06 $\pm$ 1.70	55.31 $\pm$ 2.04

Weight (Kg)	87.69±3.11	87.78±3.29
Ultravest Dose (ml)	169.23±23.71	-
EF %	62.62±1.53	
Gender		
Men n;(%)	18 (58.1%)	10 (66.7%)
Women n;(%)	13 (41.9%)	5 (33.3%)
Smoking		
No (n;%)	19 (61.3%)	5 (33.3%)
Yes (n;%)	7 (22.6%)	2 (13.3%)
Ex-smoker (n;%)	5 (16.1%)	
Diabetes		
No (n;%)	15 (48.4%)	15 (100%)
Yes (n;%)	16 (51.6%)	0 (0%)
HTN		
No (n;%)	4 (12.9%)	15 (100%)
Yes (n; %)	27 (87.1%)	0 (0%)

Table (2): Comparison of serum creatinine and kidney biomarkers in cases' class (before CAG) and control class:

Biomarkers	Case's Class (Before CAG) (n=31) Mean ±SEM	Control (n=15) Mean ±SEM	p value
Serum Cr (mg/dL)	0.83±0.04	0.75±0.02	0.18
Cystatin C(pg/mL)	347.23±13.82	294.4±21.18	0.03*
IL-18 (pg/mL)	280.51±19.76	203.22±34.42	0.04*
NGAL (pg/mL)	41.92±3.54	44.50±4.44	0.7
KIM-1 (pg/mL)	5.12±0.09	5 ±0.16	0.48

Data are presented as mean ±IL-18 = interleukin-18; Cr =creatinine; NGAL= neutrophil gelatinase- SEM associated lipocalin; KIM-1=kidney injury molecule *p value <0.01 is highly significant, **p value < 0.05 is significant

Table (3): Comparison of serum creatinine and kidney biomarkers in case's class before and 6 hours post-CAG

Biomarkers	Before CAG Mean ±SEM	6 hr post-CAG Mean ±SEM	p-value
Serum Cr (mg/dL)	0.83±0.04	0.85±0.02	0.65
Cystatin C(pg/mL)	347.23±13.82	389.18±11.95	0.02*
IL-18 (pg/mL)	280.51±19.76	321.71±32.57	0.29
NGAL (pg/mL)	41.92±3.54	42.57±2.25	0.39
KIM-1 (pg/mL)	5.12±0.09	6.29±0.18	<0.001*

Data are presented as mean ± SEM. Cr = creatinine; IL-18 = interleukin-18, KIM-1= kidney injury molecule; NGAL= neutrophil gelatinase-associated lipocalin

* p value < 0.05 is significant, ** p value < 0.01 is highly significant

There was no significant change in s.creat levels at 6 hours post CAG, but there was a significant increase in its levels 72 hours post CAG (p-value <0.05*) (Table 4).

Three cases developed CIN after CAG. The studied biomarkers were compared in these cases, and in those who did not have been diagnosed with CIN, No significant difference between the

two classes was observed.

Table (4): Comparison of serum creatinine level in case's class before and 72 hours post-CAG

Biomarker	Before CAG Mean $\pm$ SEM	72 hr post-CAG Mean $\pm$ SEM	p-value
Serum Cr (mg/dL)	0.83 $\pm$ 0.04	0.96 $\pm$ 0.02	$p < 0.05^*$

* p value < 0.05 is significant, ** p value < 0.01 is highly significant .

DISCUSSION

CIN occurs in about 10 to 15% of hospital-acquired acute kidney failure, and infrequently it can result in permanent loss of kidney role [14]. The occurrence of CIN is 11% following outcase computed tomography, 4% after intravenous pyelography, and 9% after peripheral angiography. This problem may be progressively encountered after percutaneous CAG owing to multiple factors in cases undergoing CAG and greater amounts of contrast media (CM) utilized for complex coronary injuries [15].

In the presented study, we aimed to detect whether serum biomarkers of AKI exhibited an early rise after CAG and examined whether these biomarkers had an advantage over creatinine in the early detection of CIN.

In the current study, Cys C was significantly increased in the case class before CAG in contrast to the control class ($p < 0.05^*$).

This was in concordance with Arpegård *et al.*, who found that Cys C levels were linked to cardiovascular disease (CVD) risk after adjusting for sex, age, and traditional predisposing parameters [16].

In this respect, Joachim *et al.* stated that high Cys C concentrations suspect a greater threaten of cardiovascular issues and cardiac failure in people with coronary heart disease (CHD) [17].

Furthermore, Urbonaviciene *et al.* reported a significant correlation of Cys C with high sensitivity C-reactive protein and recommended that Cys C may be associated with mild kidney dysfunction with raised chronic low-grade inflammation [18].

Moreover, a significant elevation in IL-18 ($p < 0.05^*$) was noticed in cases before CAG compared to the control class. This was per Trøseid *et al.*, who found that IL-18 can predict cardiovascular problems in individuals with metabolic disorders, especially with elevated fasting blood glucose [19].

Additionally, Jefferis *et al.* [20] found in their prospective study between healthy middle-aged men that the standard level of circulating IL-18 levels was positively related to CHD risk. Also, Mallat *et al.* [21] proved that IL-18 is elevated in the myocardium of cases with decompensated cardiac failure of or ischemic or non-ischemic cause in contrast to cases without heart failure.

The connection between Coronary heart disease and IL-18 stems from its function in the development and rupture of atherosclerotic plaques [22]. In human atherosclerotic lesions, IL-18 is the main regulator of innate and adaptive immunity, with greater quantities in unstable plaques [23].

Many other studies found that raised IL-18 was linked to elevated CVD risk, but this observation cannot differentiate between either the IL-18 being causal in plaque rupture or ruptured plaques leak IL-18 into the circulation. Other studies stated that the association was coincidental [20].

Regarding the effect of contrast on kidney biomarkers, there was no significant change in the s.creat level in the cases 6 hours post CAG compared to its level prior to the procedure ($P > 0.05$). However, there was a significant rise in its level ($p < 0.05^*$) 72hr post-CAG.

There was a significant rise in Cys C level 6 hours post CAG compared to baseline levels. Similarly, other studies found that serum level of Cys C significantly increased 24 h in cases suffering from CIN following CAG, even before the elevation of s.creat [24, 25].

However, Cys C is an early indicator of glomerular impairment rather compared to tubular injury. Subsequently, Cys C is not known diagnostically as a specific marker for AKI [26]. Herget-Rosenthal *et al.*[27] calculated s.creat and Cys C daily in 85 cases at great risk of improving AKI. 44 cases improved AKI, and the elevation of serum Cys C forewent that of s.creat by 1-2 days. Nevertheless, Banda *et al.* [28] stated that serum Cys C at 24 h was the best indicator for detection of CIN.

In our study, the serum level of KIM-1 has significantly increased 6 hours post CAG. Our results complied with results from Han *et al.*[9] who stated that after 12 hr of heart operation, KIM-1 is a parameter of AKI in kids, although they studied KIM-1 in urine specimens but not in serum.

Wang Fenget *al.*[29] showed that urinary NGAL and KIM-1 were valuable for CIN diagnosis post CAG. NGAL could indicate CIN 3 hours post-CAG diagnosis, while KIM-1 can more sensitively indicate CIN at 12 hours post-CAG. Akdenizet *al.*[30] reported the urinary KIM-1 function in CIN and concluded that it could be utilized in early detection and beginning of treatment to decrease the risk of mortality associated with CIN induced AKI.

Additionally, Liao *et al.* confirmed the significance of urinary KIM-1 in detecting CIN as early as 6 hours post CAG [31]. Also, Sabbisettiet *al.*[10] reported that KIM-1 works like a blood indicator of AKI. In their study, cases who developed AKI after cardiac surgery presented significantly greater plasma KIM-1 concentrations than cases who did not develop AKI.

Since the traditional AKI definition is based on modifications in plasma creatinine, which is nonspecific and inaccurate as a predictor of proximal tubular damage, the diagnostic function of KIM-1 in comparison to creatinine is very promising. Since an elevation in plasma creatinine does not always accompany tubular injury, an increase in creatinine does not necessarily indicate injury [10].

Connolly *et al.*[32] recommended that IL-18 and KIM-1 were not useful as early CIN indicators. The median serum KIM-1 concentrations in their sample were not significantly different until 2 days after the comparison; therefore, it did not demonstrate benefit over creatinine.

This study did not find any significant increase in NGAL level 6 hours post CAG, which was in contrast to Hirsch *et al.*[33] who claimed an increase of NGAL in children's urine and serum undergoing heart catheterization utilizing contrast medium be predictive to CIN-AKI.

Also, Mishra *et al.* [34]found that in cases developing AKI after serum, urinary NGAL and heart surgery concentrations elevated after the second hour and despite dropping later, persisted higher than the original values.

Magdalena *et al.* [35]stated that serum concentrations of IL-18 and KIM were insignificantly greater in cases with CIN-AKI. NGAL, on the other hand, showed great changes in level over time (baseline vs. 24 h vs. 72 h). However, no major associations were found between the concentration of biomarkers and the reduction in eGFR or the elevation in creatinine.

Possible reasons for the inferior activity of NGAL in adults compared to children could be the age effect and the existenceof comorbidities, especially preexisting renal disorder. NGAL measurement at different times associated with the kidney insult and various sample production. Also, evaluating methods or storage can partially illustrate the variability of the findings in adults [36].

While NGAL has been considered as a useful AKI biomarker in critically ill cases, it is not used without limitations. Firstly, many coexisting diseases can affect plasma NGAL measurements,

involving systemic infections, chronic kidney disease, chronic hypertension, inflammatory conditions, anemia, hypoxia, and cancers. In order to reduce the hypoxic suffering caused by anemia, the peripheral synthesis of NGAL rises. NGAL, on the other hand, inhibits erythropoiesis and exacerbates anemia [36].

Their small number could explain the fact that there was no difference in the studied biomarkers between cases that developed CIN after CAG and the other cases in the presented study. Other limitations in our study are that cases were recruited from a single center and that overall the number of enrolled cases was small. Great multicenter reports are needed to more validate the results in heterogeneous case individuals and define cut-off values for the studied panel of kidney biomarkers. Our findings need to be verified in other cases of AKI, such as in cases of comorbid diseases like diabetes and chronic kidney disease.

REFERENCES

1. Murphy SW, Barrett BJ, Parfrey PS. Contrast nephropathy. *Journal of the American Society of Nephrology: JASN*. 2000; 11(1):177-82.
2. McCullough PA. Contrast-induced acute kidney injury. *Journal of the American College of Cardiology*. 2008; 51(15):1419-28.
3. Devarajan P. Emerging urinary biomarkers in the diagnosis of acute kidney injury. *Expert opinion on medical diagnostics*. 2008; 2(4):387-98.
4. Murty MSN, Sharma UK, Pandey VB, Kankare SB. Serum cystatin C as a marker of renal function in detection of early acute kidney injury. *Indian journal of nephrology*. 2013; 23(3):180-3.
5. Wu I, Parikh CR. Screening for kidney diseases: older measures versus novel biomarkers. *Clinical Journal of the American Society of Nephrology: CJASN*. 2008; 3(6):1895-901.
6. Soto K, Coelho S, Rodrigues B, Martins H, Frade F, Lopes S, et al. Cystatin C as a marker of acute kidney injury in the emergency department. *Clinical journal of the American Society of Nephrology: CJASN*. 2010;5(10):1745-54.
7. Benzer M, Alpay H, Baykan O, Erdem A, Demir IH. Serum NGAL, cystatin C and urinary NAG measurements for early diagnosis of contrast-induced nephropathy in children. *Ren Fail*. 2016;38(1):27-34.
8. Rosner MH, Okusa MD. Acute kidney injury associated with cardiac surgery. *Clinical journal of the American Society of Nephrology: CJASN*. 2006;1(1):19-32.
9. Han WK, Waikar SS, Johnson A, Betensky RA, Dent CL, Devarajan P, et al. Urinary biomarkers in the early diagnosis of acute kidney injury. *Kidney international*. 2008;73(7):863-9.
10. Sabbisetti VS, Waikar SS, Antoine DJ, Smiles A, Wang C, Ravisankar A, et al. Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. *Journal of the American Society of Nephrology : JASN*. 2014;25(10):2177-86.
11. Bachorzewska-Gajewska H, Poniatowski B, Dobrzycki S. NGAL (neutrophil gelatinase-associated lipocalin) and L-FABP after percutaneous coronary interventions due to unstable angina in cases with normal serum creatinine. *Advances in medical sciences*. 2009;54(2):221-4.
12. Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P, et al. Dual action of neutrophil gelatinase-associated lipocalin. *Journal of the American Society of*

- Nephrology : JASN. 2007;18(2):407-13.
13. Devarajan P. Neutrophil gelatinase-associated lipocalin (NGAL): a new marker of kidney disease. *Scandinavian journal of clinical and laboratory investigation Supplementum*. 2008; 241:89-94.
 14. Maliborski A, Zukowski P, Nowicki G, Bogusławska R. Contrast-induced nephropathy--a review of current literature and guidelines. *Medical science monitor : international medical journal of experimental and clinical research*. 2011;17(9):Ra199-204.
 15. Devarajan P. Biomarkers for the early detection of acute kidney injury. *Current opinion in pediatrics*. 2011; 23(2):194-200.
 16. Arpegård J, Viktorin A, Chang Z, de Faire U, Magnusson PK, Svensson P. Comparison of heritability of Cystatin C- and creatinine-based estimates of kidney function and their relation to heritability of cardiovascular disease. *Journal of the American Heart Association*. 2015;4(1):e001467.
 17. Ix JH, Shlipak MG, Chertow GM, Whooley MA. Association of Cystatin C with Mortality, Cardiovascular Events, and Incident Heart Failure Among Persons With Coronary Heart Disease. *Circulation*. 2007;115(2):173-9.
 18. Urbonaviciene G, Shi GP, Urbonavicius S, Henneberg EW, Lindholt JS. Higher cystatin C level predicts long-term mortality in cases with peripheral arterial disease. *Atherosclerosis*. 2011;216(2):440-5.
 19. Trøseid M, Seljeflot I, Hjerkin EM, Arnesen H. Interleukin-18 is a strong predictor of cardiovascular events in elderly men with the metabolic syndrome: synergistic effect of inflammation and hyperglycemia. *Diabetes care*. 2009;32(3):486-92.
 20. Jefferis BJ, Papacosta O, Owen CG, Wannamethee SG, Humphries SE, Woodward M, et al. Interleukin 18 and coronary heart disease: prospective study and systematic review. *Atherosclerosis*. 2011;217(1):227-33.
 21. Mallat Z, Heymes C, Corbaz A, Logeart D, Alouani S, Cohen-Solal A, et al. Evidence for altered interleukin 18 (IL)-18 pathway in human heart failure. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2004;18(14):1752-4.
 22. Gerdes N, Sukhova GK, Libby P, Reynolds RS, Young JL, Schönbeck U. Expression of interleukin (IL)- 18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for atherogenesis. *The Journal of experimental medicine*. 2002;195(2):245-57.
 23. Mallat Z, Corbaz A, Scoazec A, Besnard S, Lesèche G, Chvatchko Y, et al. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. *Circulation*. 2001;104(14):1598-603.
 24. Briguori C, Visconti G, Rivera NV, Focaccio A, Golia B, Giannone R, et al. Cystatin C and contrast induced acute kidney injury. *Circulation*. 2010;121(19):2117-22.
 25. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, Malyszko JS, Pawlak K, Mysliwiec M, et al. Could neutrophil-gelatinase-associated lipocalin and cystatin C predict the development of contrast-induced nephropathy after percutaneous coronary interventions in cases with stable angina and normal serum creatinine values? *Kidney & blood pressure research*. 2007;30(6):408- 15.
 26. Coca SG, Yalavarthy R, Concato J, Parikh CR. Biomarkers for the diagnosis and risk stratification of acute kidney injury: a systematic review. *Kidney international*. 2008;73(9):1008-16.

27. Herget-Rosenthal S, Marggraf G, Hüsing J, Göring F, Pietruck F, Janssen O, et al. Early detection of acute renal failure by serum cystatin C. *Kidney international*. 2004;66(3):1115-22.
28. Banda J, Duarte R, Dix-Peek T, Dickens C, Manga P, Naicker S. Biomarkers for Diagnosis and Prediction of Outcomes in Contrast-Induced Nephropathy. *International Journal of Nephrology*. 2020;2020:8568139.
29. Feng Wang QZ, Cheng Peng, Junhui Li, ChangyouXuan and Niansong Wang. <significance-of combined-detection-of-cysc-ngal-and-kim1-in-contrastinduced-nephropathy-after-coronary angiography.pdf>. *European Journal of Experimental Biology*. 2014;4(3):157-61.
30. Akdeniz D, Celik HT, Kazanci F, Yilmaz H, Yalcin S, Bilgic MA, et al. Is Kidney Injury Molecule 1 a Valuable Tool for the Early Diagnosis of Contrast-Induced Nephropathy? *J Investig Med*. 2015;63(8):930-4.
31. Liao B, Nian W, Xi A, Zheng M. Evaluation of a Diagnostic Test of Serum Neutrophil Gelatinase Associated Lipocalin (NGAL) and Urine KIM-1 in Contrast-Induced Nephropathy (CIN). *Medical science monitor: international medical journal of experimental and clinical research*. 2019; 25:565-70.
32. Connolly M, Kinnin M, McEneaney D, Menown I, Kurth M, Lamont J, et al. Prediction of contrast induced acute kidney injury using novel biomarkers following contrast coronary angiography. *QJM: monthly journal of the Association of Physicians*. 2018;111(2):103-10.
33. Hirsch R, Dent C, Pfriem H, Allen J, Beekman RH, 3rd, Ma Q, et al. NGAL is an early predictive biomarker of contrast-induced nephropathy in children. *Pediatric nephrology (Berlin, Germany)*. 2007;22(12):2089-95.
34. Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. *Lancet (London, England)*. 2005;365(9466):1231-8.
35. Zdziechowska M, Gluba-Brzozka A, Poliwczak AR, Franczyk B, Kidawa M, Zielinska M, et al. Serum NGAL, KIM-1, IL-18, L-FABP: new biomarkers in the diagnostics of acute kidney injury (AKI) following invasive cardiology procedures. *IntUrolNephrol*. 2020;52(11):2135-43.
36. Bolignano D, Coppolino G, Donato V, Lacquaniti A, Bono C, Buemi M. Neutrophil gelatinase associated lipocalin (NGAL): a new piece of the anemia puzzle? *Medical science monitor: international medical journal of experimental and clinical research*. 2010; 16(6):Ra131-5.