Paediatrica Indonesiana

VOLUME 56 July • 2016 NUMBER 4

Original Article

Neutrophil gelatinase-associated lipocalin as a biomarker for acute kidney injury in children after cardiac surgery

Meta Herdiana Hanindita[1], Risky Vitria Prasetyo[1], Ninik Asmaningsih Soemyarso[1], Mohammad Sjaifullah Noer[1], I Ketut Alit Utamayasa[1], Paul Tahalele[2]

Abstract

Background Acute kidney injury (AKI) is still diagnosed by measuring the estimated creatinine clearance (eCCl), despite the fact that it may not change until 50% or more of kidney function has been lost. AKI after cardiac surgery is related to prolonged intensive care, decreased quality of life, and increased long term mortality. Neutrophil gelatinase-associated lipocalin (NGAL) represents an early biomarker of AKI, which may be useful for assessing AKI in cardiac patients.

Objective To determine the validity of urinary and plasma NGAL as biomarkers for AKI in children after cardiac surgery.

Methods Subjects were children who underwent cardiac surgery in Dr. Soetomo Hospital, Surabaya, Indonesia from August 2013 to January 2014. Serial urine and blood samples were analyzed for NGAL before surgery, as well as at 2h, 4h, 12h, and 24h after surgery. The AKI was established based on pRIFLE criteria. Estimated creatinine clearance (eCCl) was calculated from the estimated glomerular filtration rate (eGFR), according to age by the traditional Schwartz formula. Serum creatinine was assayed by the Jaffe method before surgery, as well as at 12h, 24h, 48h, and 72h after surgery.

Results Of 20 subjects, 5 developed AKI. Urinary and plasma NGAL increased markedly at 2h postoperatively, as compared to eGFR which showed a rise at 12-48 h after cardiac surgery. Analysis of 2h post-operative urinary NGAL at a cut off value of 11.270ng/mL yielded an area under the curve (AUC) of 1.00 (95%CI 2.63 to 12.13), with sensitivity and specificity of 100% each for AKI. In addition, 2h post-operative plasma NGAL at a cut off value of 8.385 ng/mL yielded an AUC of 1.00 (95%CI 3.71 to 12.15) with sensitivity and specificity of 100% each for AKI.

Conclusion Urinary and plasma NGAL are valid as early biomarkers for AKI in children after cardiac surgery. **[Paediatr Indones. 2016;56:230-7. doi: 10.14238/pi56.4.2016.230-7]**.

Keywords: *NGAL, biomarker, acute kidney injury, validity, pRIFLE*

Acute kidney injury (AKI) is characterized by a sudden impairment of kidney function occurring over a period of hours to days.[1] Currently, a diagnosis of AKI is established by using the *pediatric Risk, Injury, Failure, Loss, End-stage* (pRIFLE) criteria, on the basis of the presence of increased estimated creatinine clearance (eCCl) and/or urine production. However, it should be noted that eCCl may not change until about 50% of kidney function has been lost. This delayed eCCL decrease may take 1-3 days after cardiac surgery.[2] Also the eCCl is not a very reliable marker of AKI in many patients. There are many renal and non-renal factors that influence serum creatinine independently of kidney function. For example, creatinine generation is proportional to muscle mass and is affected by age, gender, body weight, and methods of examination.[3]

Currently, there is no early biomarker for

From the Department of Child Health[1] and Department of Surgery[2], Airlangga University Medical School/Dr. Soetomo General Hospital, Surabaya, Indonesia.

Reprint requests to: Meta Herdiana Hanindita, Department of Child Health, Airlangga University Medical School/Dr. Soetomo General Hospital, Surabaya, Indonesia.Tel. +62 816507203. E-mail: kikiprasetyo1409@gmail.com.

AKI in Indonesia. This situation is unfortunate for cardiac surgery patients, since AKI after cardiac surgery has been associated with prolonged intensive care, diminished quality of life, dialysis dependency, and increased long term mortality.[4] The lack of early biomarkers for structural kidney injury has hampered the ability to advance promising experimental therapies for human AKI. Several studies have evaluated neutrophil gelatinase-associated lipocalin (NGAL) as an early biomarker of AKI after cardiac surgery.[5-7] However, its role as a biomarker of AKI after cardiac surgery, particularly in Indonesia, has not been well-studied. The NGAL is a protein generally expressed in very low concentrations in several human tissues, and is greatly elevated in the case of epithelial damage. In healthy kidneys, it is barely detectable in either plasma or urine. In the setting of acute tubular injury, NGAL undergoes rapid and profound up-regulation with large increases in both urine and plasma.[8]

Incidence of AKI in newborn is 8-24%, while in Dr. Soetomo Hospital, Surabaya Indonesia, the incidence of AKI in pediatric population is 11.2%. In the population of children after cardiac surgery in Hungary, the incidence of AKI is 31.9%.[9-11] Devarajan stated that NGAL has potential as a real-time marker that could indicate structural injury as early as possible.[12] A few studies in US and Europe showed that in children after cardiac surgery, NGAL had good sensitivity and specificity for diagnosing AKI earlier than eCCl.[5-7]

The aim of this study was to evaluate the validity of urinary and plasma NGAL as biomarkers for AKI in children after cardiac surgery.

Methods

This study was approved by the Ethics Committee of Dr. Soetomo Hospital, Surabaya, Indonesia. All children aged 1 month to 18 years who underwent cardiac surgery during the period of August 2013 to January 2014 and were admitted to the pediatric intensive care unitwere prospectively enrolled. Diagnosis of AKI was established by pRIFLE criteria. Exclusion criteria were pre-existing renal insufficiency or non-cardiac disease, or the use of nephrotoxic drugs before or after the study period. Written informed consent from parents or legal guardians was obtained for all patients. The minimum required sample size was 19 subjects.

Urine and blood specimens were collected 15 minutes before cardiac surgery, and then at regular intervals after the surgery. Urine was collected by urinary catheter. Urine and plasma NGAL levels were measured before and at 2, 4, 12, and 24 hours after surgery, while serum creatinine was measured before and at 12, 24, 48, and 72 hours after surgery. NGAL and creatinine were assayed using NGAL *Rapid Elisa Kit* (Thermo) and the Jaffe method, respectively.[13,14]

The pRIFLE criteria used to diagnoseAKI includes using the estimated glomerular filtration rate (eGFR) as calculated by the original Schwartz formula (eGFR =k x L/Scr, in which eGFR was the estimated GFR in milliliters per minute per 1.73 m^2, L was the height in centimeters, Scr was the serum creatinine in milligrams per deciliter, and k was an empirical constant determined by age and gender (k was 0.45 for term infants throughout the first year of life, 0.55 for children and adolescent girls, and 0.7 for adolescent boys).[15,16]

The Statistical Package for the Social Sciences software (SPSS 21.0) for *Mac* was used for analysis. To compare continuous variables, we used an independent two-sample T-test or Mann-Whitney rank sum test. To compare categorical variables, we used Chi-square or Fisher's exact test. To measure the sensitivity and specificity for urinary and serum NGAL, a conventional receiver-operating characteristic (ROC) curve was generated for urinary NGAL at 2, 4, 12, and 24 hours after cardiopulmonary bypass and for serum NGAL at 2, 4, 12, and24 hours after the procedure. Cut-off points were measured by using the ROC. We calculated the area under the curve (AUC) to ascertain the quality of NGAL as a biomarker. An AUC of 0.5 is no better than expected by chance, whereas a value of 1.0 signifies a perfect biomarker.[17] Univariate and multivariate stepwise multiple logistic regression analyses were used to assess factors that served as predictors of AKI. Potential independent predictor variables included age, sex, duration of cardiopulmonary bypass time, duration of cross-clamp, and cyanotic status. Results with P values <0.05 were considered to be statistically significant.

Results

Twenty children were included in the study. Acute kidney injury occurred in 5/20 children (25%) within a 3-day period. In these 5 children, the eGFR remained static at the first 4 hours after cardiac surgery, and the eGFR decline was evident only at 12 hours after surgery (**Figure 1**). The range of eGFR rise was 18.18–189.31 (median 35.62) mL/minute/1.73 m^2. Therefore, the diagnosis of AKI by the currently accepted practices of eCCl could only be made in days after the inciting event. We classified children into with and without AKI groups, based on their eCCL, according to age. The characteristics of subjects are shown in **Table 1**. No differences were noted between the two groups with respect to age, sex, or duration of cross-clamp (**Table 1**).

Significant differences were noted in duration of cardiopulmonary by pass (CPB) (P=0.033) and cyanotic status (P=0.038) between the two groups. Children who developed AKI had significantly longer CPB and more frequently found in children with cyanotic congenital heart diseases (40% *vs.* 80%, respectively).

Preoperative mean urinary NGAL levels were not significantly different in patients with AKI compared to those without AKI [mean 1.37 (SD 0.64) *vs.* 0.73 (SD 32)ng/mL, respectively (P=0.052)]. However, urinary NGAL level peaked immediately after cardiac surgery by more than 20-fold and remained significantly higher in the AKI group at 2, 4, and 12 hours after surgery (P=0.001, P=0.001, and P=0.002, respectively).

Similarly, pre-operative plasma NGAL levels were not significantly different in patients with and without AKI [mean 1.46 (SD 0.69) *vs.* 1.7 (SD 0.79) ng/mL, respectively (P=0.54)]. However, plasma NGAL level peaked immediately after cardiac surgery by more than 15-fold and remained significantly higher in the AKI group at 2, 4, and 12 hours after surgery (P=0.001, P=0.001, and P=0.018, respectively).

The eGFR levels between the two groups are shown in **Figure 1**. Before surgery, median eGFR level in patients with no AKI was 172.17 mL/min/1.73m^2, while that in patients with AKI was

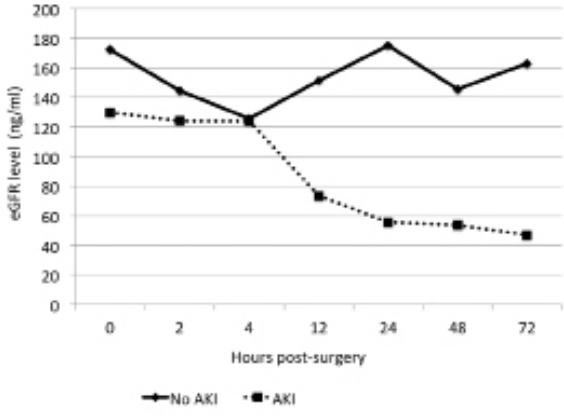

Figure 1. eGFR levels between the AKI and non-AKI groups

Table 1. Characteristics of subjects

Characteristics	No AKI (n=15)	AKI (n=5)
Median age (range), months	24 (11-204)	30 (14-120)
Gender, n		
Male	7	1
Female	8	4
Median clinical outcome (range), minutes		
Duration of cardiopulmonary bypass	94 (34-152)	121(67-258)
Duration of cross-clamp	41(0-59)	58 (0-75)
Diagnosis, n		
Acyanotic congenital heart disease	9	1
Atrial septal defect	4	0
Double outlet right ventricle	0	1
Patent ductus arteriosus	1	0
Ventricular septal defect	4	0
Cyanotic congenital heart disease	6	4
Total anomalous pulmonary venous drainage	2	0
Tetralogy of Fallot	4	3
Tricuspid atresia	0	1

129.86 mL/minutes/1.73m^2. These preoperative eGFR levels were not significantly different between the two groups. The eGFR level rose significantly 12 hours after cardiac surgery, peaked at 24 hours after surgery and remained significantly greater at 48, and 72 hours after surgery (P=0.001, P=0.001, and P=0.001, respectively).

Table 2 summarizes the mean urinary and plasma NGAL levels as well as serum creatinine levels taken preoperative and postoperatively. **Figure 2** shows the differences in NGAL values taken at various times. For urinary NGAL, the AUC was 1.0 at 2 hours after CPB (95%CI 2.63 to 12.13) (**Figure 3**) and 1.0 at 4 hours after surgery (not shown). For plasma NGAL, the AUC was 1.0 at 2 hours after CPB (95%CI 3.71 to 12.15) (**Figure 4**). **Table 3** lists the derived sensitivities, specificities, and predictive values (positive predictive value (PPV) and negative predictive value (NPV) at various cut-off concentrations.

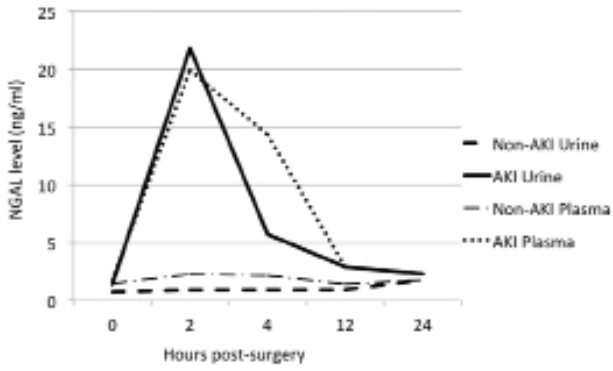

Figure 2. Urinary and plasma NGAL levels

For urinary NGAL, a cut-off point of 11.270 ng/ml yielded good sensitivity and specificity at 2h after surgery. For plasma NGAL,the best sensitivity and specificity was at cut-off point of 8.835 ng/ml at 2h after surgery.

Table 2. NGAL and creatinine levels of subjects

Variables	Time	Without AKI (n=15)	AKI (n=5)	P value
Mean urinary NGAL (SD), ng/mL	Pre-op	0.73 (0.54)	1.37 (0.64)	0.052
	2h	1.32 (1.09)	24.17 (8.10)	0.001*
	4h	1.07 (0.84)	13.76 (15.23)	0.001*
	12h	0.98 (0.37)	8.91 (14)	0.002*
	24h	1.71 (1.14)	8.2 (14.06)	0.126
Mean plasma NGAL (SD), ng/mL	Pre-op	1.7 (0.79)	1.46 (0.69)	0.540
	2h	2.31 (0.92)	18.25 (3.4)	0.001*
	4h	2.19 (0.83)	14.89 (13.46)	0.001*
	12h	1.83 (1.08)	9.53 (14.56)	0.018*
	24h	2.24 (1.68)	8.6 (14.86)	0.541
Mean serum creatinine (SD), mg/dL	Pre-op	0.32 (0.16)	0.39 (0.16)	0.383
	2h	0.36 (0.16)	0.45 (0.17)	0.299
	4h	0.38 (0.17)	0.71 (0.48)	0.149
	12h	0.34 (0.15)	0.89 (0.48)	0.001*
	24h	0.32 (0.18)	1.08 (0.44)	0.001*
	48h	0.35 (0.17)	1.21 (0.44)	0.001*
	72h	0.34 (0.13)	1.28 (0.50)	0.001*

*statistically significant by independent two-sample T-test

Table 3. Sensitivity, specificity, PPV, NPV, Kappa, McNemar, LR, and AUC

Parameter	Cut-off, ng/mL	Sens, %	Spec, %	PPV, %	NPV, %	Kappa (P value)	McNemar	LR	AUC
Urinary NGAL 2h	11.270	100	100	100	100	1.00 (<0.001)	1	22.5	1.00
Urinary NGAL 4h	11.205	40	100	40	60	0.50 (0.01)	1	6.3	1.00
Plasma NGAL 2h	8.835	100	100	100	100	1.00 (<0.001)	1	22.5	1.00
Plasma NGAL 4h	9.115	60	100	100	88.3	0.69 (0.001)	0.50	10.2	1.00

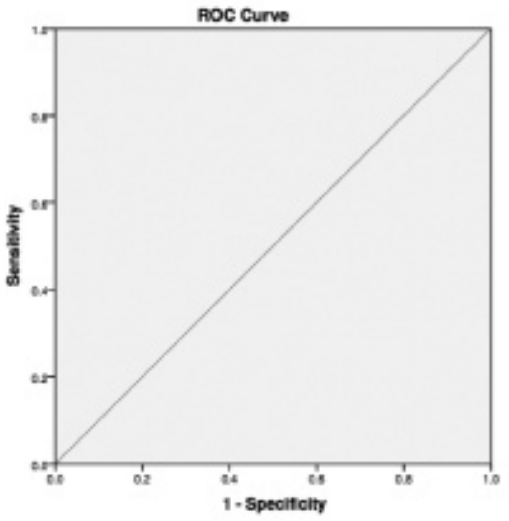

Figure 3. ROC curve for urinary NGAL 2h after cardiac surgery

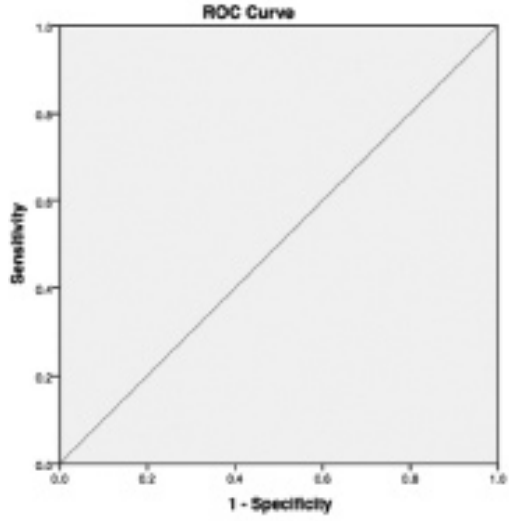

Figure 4. ROC curve for serum NGAL 2h after cardiac surgery

Discussion

Diagnosing AKI after cardiac surgery needs to be done as early as possible to avoid worsening renal injury, which can increase morbidity and mortality.[4]

The prevalence of AKI after cardiac surgery in this study was 5/20, similar to other studies in the US that showed a ranged of prevalences from 17% to 51% as reported by Parikh *et al*., Mishra *et al*., Dent *et al*.,and Bennet *et al*.[2,5-7] In Pakistan, the prevalence of AKI after cardiac surgery in children was lower, at 13%.[18] In addition, Fadel *et al*. reported that 47.5% of children developed AKI after cardiac surgery in Egypt.[19]

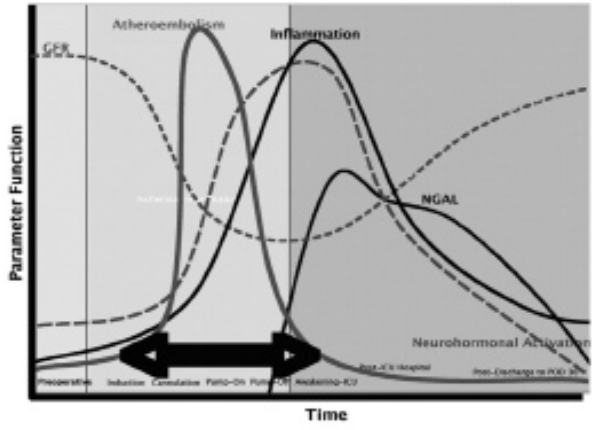

Figure 5. The likely time course of four major mechanisms of kidney injury associated with cardiac surgery[30]

Table 4. Studies in urinary and plasma NGAL as a biomarker for AKI after cardiac surgery in children

No	Study	NGAL	Cut-off, ng/mL	Hour post-op	Sensitivity, %	Specificity, %	AUC
1	Mishra (2005)[5]	Urine	25	2	100	98	0.998
				4	100	96	1.000
		Plasma	25	2	70	94	0.906
2	Dent (2007)[6]	Plasma	150	2	84	94	0.980
3	Bennet (2008)[7]	Urine	100	2	82	90	0.950
4	Zheng (2012)[17]	Urine	54	4	79.3	89.7	0.857
5	Fadel (2012)[16]	Plasma	100	2	100	90.7	-
6	Munir (2013)[15]	Urine	87	4	90.9	98.7	0.910
7	This study	Urine	11.270	2	100	100	1.000
			11.205	4	40	100	1.000
		Plasma	8.385	2	100	100	1.000
			9.115		60	100	1.000

In current practice, AKI is typically diagnosed by measuring serum eCCl, which may not change until 50% or more of kidney function has been lost. Unfortunately, eCCl is a delayed and unreliable indicator of AKI. In the case of AKI after cardiac surgery, it takes 1-3 days for eCCl to increase. eCCl is also influenced by several non-renal factors such as age, gender, muscle mass, and method of examination.[2,3]

The NGAL is one of the most strikingly upregulated genes and overexpressed proteins in the kidney after ischemia. NGAL is easily detected in urine early after ischemia.[5] This study was a prospective cohort of a population of children aged 1 month to 18 years who underwent cardiac surgery.

We recruited pediatric subjects in whom the only obvious etiology for AKI would be the result of CPB. These patients comprise an ideal and important population for the study of AKI biomarkers because they do not exhibit common comorbid variables that complicate similar studies in adults, such as hypertension. Hence, pediatric subjects are the ideal population in which to investigate AKI after cardiac surgery.[20] The exclusion criteria in our study were pre-existing renal insufficiency or the use of nephrotoxic drugs. We studied a homogeneous population of children with possibly no major confounding variables, in whom the only obvious renal insult would be the result of an ischemia-reperfusion injury after cardiac surgery.

We measured urinary and serum NGAL before surgery, and 2, 4, 12, and 24 hours after surgery, because a pre-experimental study showed that NGAL levels above 12h after surgery did not differ greatly. We also measured eCCl every 12h until the third day after surgery. Our pre-experimental study showed that eCCl level started to increase after 12h.

Gender and age of subjects were similar between the two groups. This was in line with some previous studies.[6,7,12,18,20] However, CPB duration was significantly longer in the AKI group than in the non-AKI group(P=0.033). In agreement with this finding,Boldt *et al*. observed that post-operative levels of NGAL were related to the duration of CPB.[21] A few previous studies also reported that duration of CPB was a highly significant factor in AKI after cardiac surgery. Many studies have supported the hypothesis that duration bears a negative impact on postoperative renal function.[2,5-7,18,19,20,22,23] Sethi *et al*. reported that duration of CPB >90 minutes could cause AKI in adults.[24] Similarly, Zheng *et al*. reported that AKI was significantly more common in patients with duration of CPB >90 minutes.[20] Although the mechanism is unknown, the release of proinflammatory mediators, coagulation products, oxidation products, and the adhesion of blood cells to the vascular endothelium reportedly increases with prolonged CPB time.[25]

We found no significant difference between the AKI and non-AKI groups in cross-clamp duration (P=0.344). In contrast, Parikh *et al*. and Munir *et al*. reported duration of cross-clamp to be a significant factor in AKI after cardiac surgery.[2,13] Nevertheless, our result was in agreement with that of Shiekh *et al*., who reported no significant differences in cross-clamp durationbetween groups.[26] Durandy *et al*. reported that duration of cross-clamp >90 minutes was a risk factor of AKI after cardiac surgery.[27] In our study, the duration of cross-clamp varied between 0-75 minutes. Cross-clamp is associated with increased renal vascular resistance and decreased renal blood flow.[28]

We also noted that a significantly higher percentage of children with AKI had cyanotic congenital heart disease, than the non-AKI group (P=0.038). Post-operative AKI is associated with ischemic renal tubular damage. Cyanotic glomerulopathy has been shown to be associated with elevated hematocrit. The tubular apparatus, the most vulnerable to ischemic damage, is provided by the capillaries of the peritubular plexus, the second capillary bed in series after the glomerular capillaries. To maintain normal blood flow in the peritubular capillaries, as there is increased resistance to flow of viscous blood in patients with elevated hematocrit, a higher intravascular pressure is required, which will result in proteinuria. It seems obvious that patients who need chronic compensatory mechanisms to maintain peritubular supply in the preoperative situation are more sensitive to hypoxic tubular damage during cardiopulmonary bypass perfusion or low cardiac output postoperatively.[29]

Changes in post-operative renal function in cyanotic patients compared to controls may have been influenced by cyanotic glomerulopathy, degree of cyanosis, or cardiopulmonary bypass time.[29] Our study showed significantly increased urinary NGAL levels in the AKI group at 2h (P=0.001), 4h (P=0.001), and 12h (P=0.001) after cardiac surgery, compared to the

non-AKI group. The urinary NGAL level peaked at 2h after cardiac surgery, in agreement with previous studies.[5,7] Urinary NGAL level that had the best sensitivity and specificity at 2h after cardiac surgery, with a cut-off point of 11.270 ng/mL (sensitivity 100%, specificity 100%, PPV 100%, NPV 100%, and AUC 1.0).

Bellomo *et al*. reported the time course of AKI after cardiac surgery (**Figure 5**).[26]

In cardiac surgery, the atheroembolism process increasesduring the pump-on phase. The ischemia-reperfusion injury occurs starting from induction phase to the pump-off phase. The NGAL (urinary or plasma) rises as a marker of the inflammation process when there is a tubular damage.[26] A comparison of studies in urinary and plasma NGAL as a biomarker for AKI after cardiac surgery in children is shown in **Table 4**.

Differences of cut-off points found between our study and others may have been due to the use of different kits with different values. We used the NGAL kit from *Thermo*.

In conclusion, both urinary and plasma NGAL levels are valid as early biomarkers for AKI in children after cardiac surgery.

Conflict of Interest

None declared.

References

1. Thadhani R, Pascual M, Bonventre JV. Acute renal failure. N Engl J Med.1996;334:1448-60.
2. Parikh CR, Devarajan P, Zappitelli M, Sint K, Thiessen-Philbrook H, Li S, *et al*. Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery. J Am Soc Nephrol. 2011;22:1737-47.
3. Devarajan P. Proteomics for biomarker discovery in acute kidney injury. Semin Nephrol.2007;27:637–51.
4. Lok CE, Austin PC, Wanh H, Tu JV. Impact of renal insufficiency on short- and long-term outcomes after cardiac surgery. Am Heart J. 2004;148:430-8.
5. 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.2005;365:1231-8.
6. Dent CL, Ma Q. Dastrala S, Bennet M, Mitsnefes MM, Barasch J, *et al*. Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury,morbidity and mortality after pediatric cardiac surgery: a prospective uncontrolled cohort study. Crit Care.2007;11:127.
7. Bennett M, Dent CL, Ma Q, Dastrala S, Grenier F, Workman R, *et al*. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. Clin J Am Soc Nephrol.2008;3:665-73.
8. Mori K, Lee HT, Rapoport D, Drexler IR, Foster K, Yang J, *et al*. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J Clin Invest.2005;115:610-21.
9. Askenazi DJ, Ambalavanan N, Goldstein SL. Acute kidney injury in critically ill newborns: what do we know? What do we need to learn? Pediatr Nephrol. 2009;24:265–74.
10. Wennas, Supriyo AR, Prasetyo RV, Sumyarso NA, Noer MS. Mortality of acute kidney injury in children at nephrology ward Dr.Soetomo Hospital, Surabaya. Paediatr Indones. 2011;51:235.
11. Toth R, Breuer T, Cserep Z, Lex D, Fazekas L, Sapi E, *et al*. Acute kidney injury is associated with higher morbidity and resource utilization in pediatric patients undergoing heart surgery. Ann Thorac Surg.2012;93:1984-90.
12. Devarajan P. Novel biomarkers for the early prediction of acute kidney injury. Cancer Therapy.2005;3:477-88.
13. Thermo Fisher Scientific [homepage on the internet]. NGAL Rapid ELISA Kit Product Information. [cited 2016 January 1]. Available from: https://www.thermofisher.com/order/catalog/product/KIT037.
14. Peake M, Whiting M. Measurement of serum creatinine – current status and future goals. Clin Biochem Rev. 2006;27:173–84.
15. Akcan-Arikan A, Zappitelli M, Loftis LL, Washburn KK, Jefferson LS, Goldstein SL. Modified RIFLE criteria in critically ill children with acute kidney injury. Kidney Int. 2007;71:1028-35.
16. Alatas H. Pemeriksaan laboratorium pada penyakit ginjal. Dalam: Alatas H, Tambunan T, Trihono PP, Pardede SO, editors. 2nd edition. Jakarta: Balai Penerbit IDAI; 2002. p.51-72.
17. Dahlan MS. Penelitian diagnostik: dasar-dasar teoretis dan aplikasi dengan program SPSS dan Stata. Jakarta: Salemba Medika; 2009. p.23.
18. Munir MU, Khan DA, Khan FA, Shahab Naqvi SM. Rapid detection of acute kidney injury by urinary neutrophil gelatinase-associated lipocalin after cardiopulmonary bypass

surgery. J Coll Phy Surg Pak.2013;23:103-6.

19. Fadel F, Abdel Rahman AM, Mohamed MF, Habib SA, Ibrahim MH, Sleem ZS, *et al.* Plasma neutrophil gelatinase-associated lipocalin as an early biomarker for prediction of acute kidney injury after cardio-pulmonary bypass in pediatric cardiac surgery. Arch Med Sci. 2012;8:250-5.
20. Zheng J, Xian Y, Yao Y, Xu G, Li C, Zhang Q, *et al*. Comparison of urinary biomarkers for early detection of acute kidney injury after cardiopulmonary bypass surgery in infants and young children. Pediatr Cardiol.2013;34:880-6.
21. Boldt J, Brenner T, Lehmann A, Suttner SW, Kumle B, Isgro F. Is kidney function altered by the duration of cardiopulmonary bypass? Ann Thorac Surg.2003;75:906–12.
22. Chiravuri SD, Riegger LQ, Christensen R, Butler RR, Malviya S, Tait AR, *et al*. Factors associated with acute kidney injury or failure in children undergoing cardiopulmonary bypass: a case-controlled study. Paediatr Anaesth.2011;21:880-6.
23. Alcaraz AJ, Gil-Ruiz M, Castillo A, López J, Romero C, Fernández S, *et al.* Postoperative neutrophil gelatinase-associated lipocalin predicts acute kidney injury after pediatric cardiac surgery. Pediatr Crit Care Med. 2014;15:121-30.
24. Sethi SK, Goyal D, Yadav DK, Shukla U, Kajala PL, Gupta VK, *et al*. Predictors of acute kidney injury post-cardiopulmonary bypass in children. Clin Exp Nephrol. 2011;15:529-34.
25. Conlon PJ, Stafford-Smith M, White WD, Newman MF, King S, Winn MP, *et al*. Acute renal failure following cardiac surgery. Nephrol Dial Transplant.1999;14:1158–62.
26. Shiekh IA, Bhat MA, Bhat AW, Ommid M, Gani T, Nisa G. Renal function in patients undergoing cardiopulmonary bypass for open cardiac surgical procedures. Sri Lankan J Anaesthesiol.2013;21:20-26.
27. Durandy YD. Pediatric cardiac surgery: effect of a miniaturized bypass circuit in reducing homologous blood transfusion. J Thorac Cardiovasc Surg.2009;138:1454-5.
28. Nagelhout JJ, Plaus KL. Vascular surgery. In: Nagelhout JJ, Plaus KL, editors. Nurse Anesthesia. 5th ed. Maryland: Elsevier;2010.p. 568-70.
29. Dittrich S, Kurschat K, Dahnert I, Vogel M, Muller C, Alexi-Meskishvili V, *et al*. Renal function after cardiopulmonary bypass surgery in cyanotic congenital heart disease. Int J Cardiol.2000;28:173-9.
30. Bellomo R, Auriemma S, Fabbri A, D'Onofrio A, Katz N, McCullough PA, *et al*. The pathophysiology of cardiac surgery-associated acute kidney injury. Int J Artif Organs. 2008;31:166-78.