Kosin Medical Journal 2017;32:212-220.
https://doi.org/10.7180/kmj.2017.32.2.212

KMJ
Original Article

Usefulness of neutrophil gelatinase-associated lipocalin(NGAL) to confirm subclinical acute kidney injury and renal prognosis in patients following surgery

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Objectives: The neutrophil gelatinase-associated lipocalin (NGAL) level following non cardiac surgery is useful for predicting acute kidney damage. However, there is insufficient conclusive evidence as to whether NGAL can be used to predict subclinical AKI following non-cardiac surgery.

Methods: We measured serum NGAL and creatinine levels in 41 patients following non-cardiac surgery, and the increase of these variables was used to predict acute decreases in kidney function.

Results: The study included a total of 41 patients. The mean age was 64.65 ± 17.09 years. The serum creatinine concentration was increased 12 hours after surgery. The mean SD serum NGAL decreased after 4hours after surgery and continued to decrease after 12 hours after surgery. The incidence of subclinical AKI determined by the 4 hour serum NGAL level was 10(24.4%), and the incidence of serum creatinine elevation was 0(0.0%). The incidence of subclinical AKI determined by the 12 hour serum NGAL level was 4(9.8%), and the incidence of subclinical AKI determined by serum creatinine was 4(9.8%). The elevation of NGAL was more rapid than the serum creatinine 4 hours after surgery

Conclusions: We verified the usefulness of the serum NGAL level as a predictive factor for subclinical AKI after non-cardiac surgery.

Key Words: Acute kidney injury, Creatinine, NGAL, Prognostic factor, Surgery

Acute kidney injury (AKI) has multiple and various causes across the pre-, peri- and post-operative time periods.[1] Post-operative AKI occurs frequently, particularly after cardiac surgery.[2,3] Minimal changes in serum creatinine levels following surgery are related to an increased likelihood of transfer to an intensive care unit (ICU), increased length of hospitalization and high mortality.[4] Therefore, the early detection of post-operative AKI is important. AKI was defined by abrupt (within 48 hours) increase in the serum creatinine ≥ 0.3 mg/dl from baseline; a percentage increase in the

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Received: Feb. 13, 2016
Revised: Mar. 03, 2016
Accepted: May. 23, 2016

serum creatinine concentration of above 50 % or oliguria of 〈 0.5 ml/kg per hour for more than six hours by the AKIN diagnostic criteria. Typically, serum creatinine is used to identify decreases in post-operative renal function. However, this use of serum creatinine has several problems. Serum creatinine increases long after renal functional declines, and this increase occurs at different times depending on the baseline renal function.[5] An additional problem is that the defining criteria for AKI based on serum creatinine are varied.[5] Additionally, after surgery, the incidence of AKI determined using creatinine is too low to identify the risk factor for post operation AKI.

Subclinical AKI is defined as positive biomarkers for AKI that are not satisfied by the risk-injury-failure-loss-end-stage renal disease (RIFLE) criteria.[6] It is also a bad prognosis of renal function.[7]

NGAL is a biomarker of renal function. NGAL is a protein that is expressed in neutrophils and the epithelium of renal tubules, and it increases significantly during AKI.[8] The usefulness of NGAL as an indicator for AKI in post-operative cardiac surgery patients in the ICU and in contrast media has previously been demonstrated.[9,10] However, because of the low incidence of AKI, as determine using creatinine, it is not relevant to determine the usefulness of NGAL with RIFLE criteria. Therefore, we assessed renal function decreases following non-cardiac surgery according to the NGAL and serum creatinine levels using the definition of subclinical AKI.

MATERIALS AND METHODS

1. Study design

We evaluated the pre-operation, 4-hr post-operation and 12-hr post-operation serum NGAL and serum creatinine levels of 41 patients who underwent non-cardiac surgery at Seoul Paik Hospital over 1 year. We excluded patients with resistant hypertension, increment dosages of angiotensin converting enzyme inhibitors/angiotensin receptor blockers or diuretics, aspartate aminotransferase or alanine aminotransferase 〉 2 times the normal range, bilirubin 〉 2.0 mg/dl or diagnosis of AKI injury before operation, or patients with a kidney transplant.

Subclinical AKI was defined by a measured value that post-operative NGAL 〉 435 ng/ml or a serum creatinine increased 〉 0.3 mg/dl[11] with negative of RIFLE criteria. Age, gender, diabetes, hypertension, cerebrovascular disease, chronic liver disease, systolic blood pressure, diastolic blood pressure, height, weight, body mass index (BMI), type of surgery, duration of surgery, method of anesthesia, hemoglobin, hematocrit, albumin, cholesterol, Na, K, Cl, and the estimated glomerular filtration rate were assessed. Additionally, the method of anesthesia, the duration of anesthesia and the operation, the frequency of renal function decline based on the type of surgery and the factors related to renal function decline after surgery were evaluated. NGAL was measured in the blood using a commercial available ELISA kit (Alere healthcare ™, Sungnam, Korea), and mesurements were perfomed by a single labatory (GreenCross Lab™, Yongin, Korea), according to the manufacturere's instruction. All specimens were diluted often to obtain concentration for the optimal density. Coefficients of variation for the serum NGAL assays were 3.0%. The enzymatic reactions were quantified in an automatic microplate photometer. Foley catheter was removed in most patient right after surgery so it is hard to measure urine NGAL quickly, so we used serum NGAL for early detection of AKI. Estimated GFR was calculated using the 4-variable MDRD fomula as follows: eGFR ml/min per 1.73 m^2 = 175 × serum creatinine $(mg/dL)^{-1.154}$ × age $^{-0.203}$ × 0.742 (if female).This study was approved by the Institutional Review Board of the Seoul Paik Hospital and is registered at clinicaltrial.gov.

2. Statistical methods

Student's t-tests were performed for continuous variables, and chi-square tests were performed for categorical variables. Paired *t*-tests were performed for the NGAL and serum creatinine levels. The risk factors for subclinical AKI and progress of renal dysfunction after surgery were also tested using multiple logistic regressions. A two-sided P-value was used for all statistical analyses, and the level of statistical significance was set at $P < 0.05$.

All statistical analyses in this study were performed with R version 3.0.1 (2013-05-16, copyright 2013, The R Foundation for Statistical Computing). R core team (2012), R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-0-07-0, URL: http://www.R-project.org/.

RESULTS

1. Baseline characteristics

The study included a total of 41 patients. The mean age was 64.65 ± 17.09 years, and the sex difference was 27 (58.6%) men and 14 (41.4%) women. The prevalence of diabetes mellitus was 14 (41.4%) and of hypertension was 22 (52.7%). The BMI was 24.77 ± 4.75 kg/m2, hemoglobin was 11.34 ± 2.20 g/dL, and hematocrit was 33.11 ± 5.98 %. The serum albumin was 3.78 ± 0.47 g/dL and cholesterol was 167.10 ± 48.85 mg/dL. The amount of urine output 6 hours before operation was 633 ± 382 ml. The mean anesthesia time was 111.46 ± 55.56 min and the mean operation time was 139.51 ± 104.56 min. The anesthesia method was 1(4.5%) epidural, 8(36.4%) spinal, 25(40.9 %) general, and 7(18.2 %) localized.

The average value of eGFR in patients is around 53.61 (CKD stage 3). The 21 patients were available to compare the previous creatinine concentration and none of them was AKI on CKD. The creatinine concentration was increased compared to the previous three months in 2 patients, but there was no significant change of values. The orthopedic surgeries included foot (ostectomy, wound debridement, and PanTalar fusion ankle), knee (total knee replacement arthroplasty and lateral meniscus repair), hip (closed reduction internal fix-

Table 1. Baseline characteristics of patients

Variable	Value (n = 41)
Age	64.65 ± 17.09
Sex (M:F), n(%)	27:14 (58.6:41.4)
BMI (kg/㎡)	24.77 ± 4.75
DM (Y:N), n(%)	14:27 (41.4:58.6)
Hypertension	22:19 (52.7:46.3)
BUN (mg/dL)	15.46 ± 11.34
Hemoglobin (g/dL)	11.34 ± 2.20
Hematocrit (%)	33.11 ± 5.98
Albumin (g/dL)	3.78 ± 0.47
Cholesterol (mg/dL)	167.10 ± 48.85
Na (mmol/L)	138.36 ± 2.71
K (mmol/L)	4.31 ± 0.56
Cl (mmol/L)	105.56 ± 3.54
Estimated GFR	53.61 ± 29.77
Urine Output 6 hrs before operation (ml)	633 ± 382
Duration of anesthesia (min)	111.46 ± 55.56
Duration of surgery (min)	139.51 ± 104.56
Anesthesia method	
Epidural n(%)	1 (4.5)
Spinal n(%)	8 (36.4)
General n(%)	25 (40.9)
Local n(%)	7 (18.2)
Type of surgery	
CSn(%)	2 (4.8)
GSn(%)	3 (7.3)
GYNn(%)	3 (7.3)
NSn(%)	1 (2.4)
OSn(%)	26 (63.4)
PSn(%)	1 (2.4)
UROn(%)	5 (12.1)

Values are presented as mean ± standard deviation and number (%).
BMI : body mass index, DM : diabetes mellitus, BUN : blood urea nitrogen, GFR : glomerular filtration rate
CS : cardiac surgery, GS : general surgery, GYN : gynecology, NS : neurosurgery, OS : orthopedic surgery
PS : plastic surgery, URO : urology

ation with proximal femur nail antirotation, external fixation, open reduction internal fixation with intramedullary screws removal and bipolar hemiarthroplasty) and shoulder surgeries (open rotator cuff repair and arthroscopic subacromial decompression of the shoulder). The single neurosurgery case was a T12 fusion surgery. The thoracic surgery was a pulmonary resection. The general surgeries included colon resection and colostomy and hernia surgeries. The gynecology surgeries included hysterectomy, uterine fibroid removal and ovarian cyst removal. The urological surgeries included transurethral resection of the prostate, transurethral resection of bladder tumor and nephrectomy. The single plastic surgery was a skin graft surgery, the variables are shown in Table 1.

2. Changes in serum NGAL and serum creatinine levels after surgery and the incidence of subclinical AKI were determined.

Table 2. Serum NGAL, creatinine on 4, 12 hours after the operations and the incidence of subclinical acute kidney injury as judged by sesrum NGAL.

	Pre OP	Post OP 4 hr	Post OP 12 hr
NGAL (ng/mL)	268.48 ± 225.88	252.41 ± 212.53	231.82 ± 205.25
Creatinine (mg/dL)	1.55 ± 1.07	1.47 ± 1.07	1.53 ± 1.06
Subclinical AKI n(%)		10(24.4)	8(19.6)
AKI based on NGAL		10(24.4)	4(9.8)
AKI based on Cr		0(0.0)	4(9.8)

Values are presented as mean ± standard deviation and number (%).
OP : operation; NGAL : neutrophil gelatinase-associated lipocalin; AKI : acute kidney injury.

The pre-operative serum NGAL was 268.48 ± 225.88 ng/mL, the 4-hr post-operative serum NGAL was 252.41 ± 212.53 ng/mL, and the 12-hr post-operative serum NGAL was 231.82 ± 205.25 ng/mL. The pre-operative serum creatinine was 1.55 ± 1.07 mg/dL, the 4-hr post-operative serum creatinine was 1.47 ± 1.07 mg/dL, and the 12-hr post-operative serum creatinine was 1.53 ± 1.06 mg/dL (Table 2). The change in serum NGAL and serum creatinine concentration with the standard error at the time after operation is shown in Figure 1. The serum creatinine concentration was decreased 4hours after surgery and increased 12 hours after surgery. The serum NGAL concentration was decreased after 4 hours and continued to decrease after 12 hours.

3. The incidence of subclinical AKI as determined by serum NGAL

The average serum NGAL and creatinine concentration of the patients 12 hours after surgery is reduced and deterioration of the renal function of the average patients did not observed. But subclinical AKI finding risen above 450 of NGAL was observed. The incidence of subclinical AKI as determined using the 4-hr serum NGAL level was 10(24.4%), and the 12-hr serum NGAL was 4(9.8%). And the AKI findings have been observed in patients with creatinine rise of 9.8% at 12 hours after surgery. The elevation of NGAL was more rapid than the serum creatinine 4 hours after the operation (Fig. 2)(Table 2).

DISCUSSION

1. Confirming post-operative renal function decline

To categorize AKI, RIFLE and the AKI Network (AKIN) classification use changes in the creatinine and urine output. RIFLE classification should be applied within 7 days, and AKIN classification should be applied within 48 hours.[12,13] Studies of post-operative renal function decline used the serum creatinine concentration as the criterion for the renal function decline.[14] However, the excretion time for serum creatinine following AKI is different for chronic kidney disease patients,[5] and serum creatinine is affected by age, gender, food intake

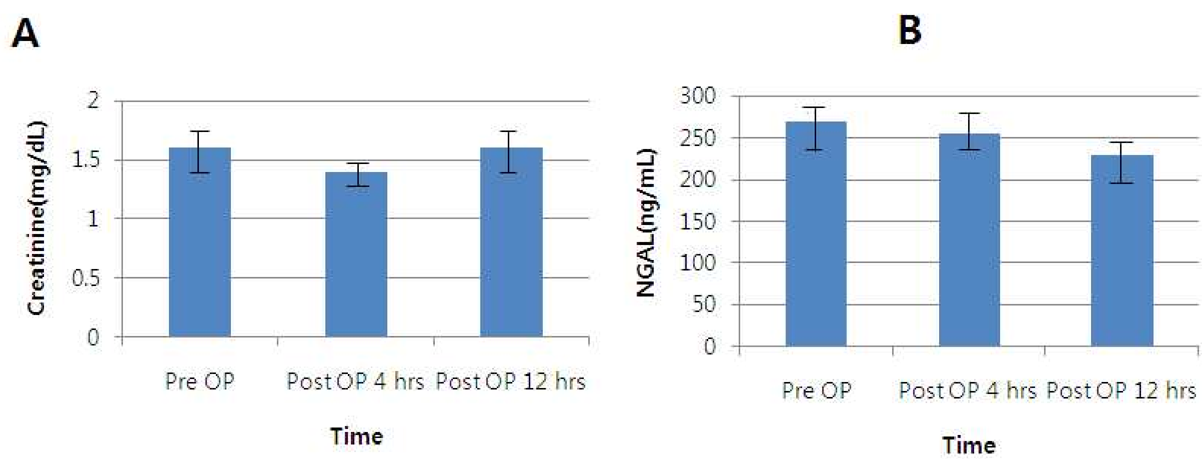

Fig. 1. Change in serum NGAL and serum creatinine after the operations with mean and standard error. (A) Serum creatinine (B) NGAL, neutrophil geletinase-associated lipocalin.

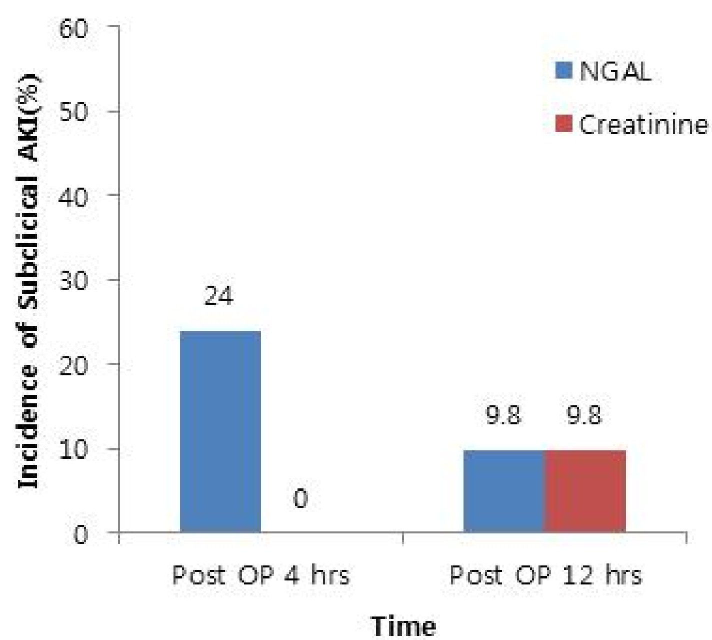

Fig. 2. Incidence of subclinical AKI with NGAL and AKI with serum creatinine after the OP.
OP : operation; NGAL : neutrophil gelatinase-associated lipocalin; AKI : acute kidney injury; CV : coefficient of variation

and muscle percentage.[15] Therefore, the use of serum creatinine to confirm post-operative AKI has many drawbacks. However, because NGAL levels increase more rapidly following decreases in renal function, NGAL has the advantage of quickly indicating AKI.[16] Previously, it was confirmed that NGAL is advantageous for the diagnosis of AKI compared to serum creatinine following cardiac surgery, during ICU care, and in contrast nephropathy.[8,9] However until now, studies of the diagnosis of AKI based on NGAL in non-cardiac surgery patients have been unsuccessful. We propose this lack of success may be due to the low incidence with which AKI is identified by decreases in serum creatinine and urine output. Our study identified acute kidney dysfunction based on the increase of the NGAL and serum creatinine levels. Furthermore, we used the definition of subclinical AKI. We confirmed that NGAL verified post-operative acute kidney dysfunction more rapidly than serum creatinine. The incidence of subclinical AKI was high 4 hours after surgery and was more rapid than serum creatinine.

2. Possible causes of post-operative renal function decline

Post-operative renal dysfunction has many causes, with or without AKI, including a pre-operative lack of fluid, nephrotoxin use, inadequate cardiac function, decreases in renal perfusion during surgery, vasoactive agent use, inflammation, post-operative lack of fluid, and infection.[3]

The current known risk factors for post-operative renal dysfunction are diabetes, age greater than 56 years, male gender, intraperitoneal surgery and hypoalbuminemia.[17,18]

We also assessed the risk factors for AKI in the incidence of subclinical acute AKI, including the elevation of creatinine concentration at 6months and 12 months; however, we did not find any factors for these risks.

3. Subclinical AKI and prognosis

The criteria for AKI based on NGAL typically use a cutoff value of 435 ng/mL, although many studies have used different values.[19] NGAL values of 100-270 ng/mL have been predicted to have the highest sensitivity and specificity for AKI.[20] However, this range is too wide to be applied to diagnosis AKI. Urine NGAL can be tested when the patient urinates after surgery; therefore, serum NGAL is easier for a clinician to use to confirm AKI compared to urine NGAL. In our study, the range of the pre-operative serum NGAL values was 268.48 ± 225.88 ng/mL and the 4-hr post-operative serum NGAL range was 252.41 ± 212.53 ng/mL.

Therefore, we defined kidney function decline with the increase of NGAL and confirmed that the advantage of using NGAL to identify AKI following non-cardiac surgery was because it is more rapid than using serum creatinine. With this definition, there was a study regarding renal prognosis in subclinical AKI after bariatric surgery using urine NGAL Approximately 15%-20% of patients have acute tubular damage without creatinine-based consensus criteria. In this case, the incidence of dialysis and mortality was higher than who did not have acute tubular damage.[21] The problem with serum creatinine that it could not be used to diagnose tubular damage.

Plasma NGAL is also associated with kidney function in uremic patients before and after kidney transplantation. In one case, there was a case of nephroureterectomy due to ureter cancer. In that case, there could be an error of NGAL level because of difference of the amount of NGAL after nephrectomy. In our study, for the patient with subclinical AKI, we tested the serum creatinine 6 and 12 months after surgery. NGAL may related to the long-term prognosis of kidney function because of 〉 50% of subclinical AKI patient who diagnosis with NGAL showed creatinine elevation with 12 months.

We used subclinical AKI criteria by detecting serum NGAL for early prediction of AKI on patients after surgery. Early prediction of AKI using RIFLE, AKIN criteria was difficult because of the difficulty of measuring urine output in patients with removed Foley catheter or without Foley catheter after surgery. Also serum creatinine based RIFLE, AKIN criteria is required at least 12~24 hours to diagnose AKI. So we used subclinical AKI to early detection and treatment of AKI in the real clinical situation.

4. Limitations of the study

We did not compare AKI patients who were diagnosed with the NGAL to AKI patients who were diagnosed according to the existing diagnostic criterion, which is based on the creatinine level. Moreover, the number of patients in this study was low.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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