Clinical significance of serum neutrophil gelatinase-associated lipocalin in the early diagnosis of renal function deterioration after radical nephrectomy

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Objectives: The standard metrics used to monitor the progression of acute kidney injury (AKI) include markers such as serum creatinine, blood urea nitrogen, and estimated glomerular filtration rate (eGFR). Moreover, neutrophil gelatinase-associated lipocalin (NGAL) expression has been reported to modulate oxidative stress. **Methods**: We aimed to evaluate the usefulness of serum NGAL levels for monitoring renal function after radical nephrectomy (RN). We prospectively collected data from 30 patients who underwent RN. We analyzed serum NGAL and creatinine at 6 time points: preoperative day 1, right after surgery, 6 hours after surgery, postoperative day (POD) 1, POD 3, and POD 5. We compared these measurements according to the eGFR values (classified as chronic kidney disease stage III; CKD III or not) using data obtained 3 months after surgery. **Results**: The mean age was 65.5 years (range, 45-77 years), and the male-to-female ratio was 2:1. At the last follow-up examination, there were 12 patients (40%) with CKD III. Using receiver operating characteristic analysis, we found that serum creatinine on POD 5 (area under the curve [AUC], 0.887; P = 0.000) and NGAL at 6 hours after LRN (AUC, 0.743, P = 0.026) were significant predictors of CKD III. The development of CKD III after LRN was associated with the serum creatinine level on POD 5 and the NGAL at 6 hours after surgery. **Conclusions**: Compared to serum creatinine, serum NGAL enabled earlier prediction of postoperative CKD III. Therefore, serum NGAL measured 6 hours after surgery could be a useful marker for managing patients after RN.

Key Words: Creatinine, Kidney, Nephrectomy

Radical nephrectomy has been treatment of choice for organ confined renal cancer with contralateral normal kidney, because radical nephrectomy can cure the renal cancer with minimal immediate problems associated with health-related quality of life due to end stage renal disease, hemodialysis, and etc. Recently, however, many small renal masses - incidentalloma - are diagnosed at health screening, and consequent nephron-sparing surgery is increasing and accepted due to benefit of residual renal function.[1,2] Compared with radical nephrectomy, partial

nephrectomy for a pT1a (renal mass size < 4cm) has a benefit for renal function without compromising oncological outcome. In addition, some investigators reported the partial nephrectomy has a better overall survival than radical nephrectomy due to superior renal function. According to their report, decreased renal function can make cardiovascular events, and these can cause non-cancer related death.[3-5] However, all renal cancers cannot be indications for the partial nephrectomy. The partial nephrectomy for pT1b or larger renal cancer is challenging and has high morbidity. So, radical nephrectomy still performed in many renal cancers, although it can compromise renal function. Residual renal function after radical nephrectomy in most patients decreases and mean estimated glomerular filtration rate (eGFR) decreases to about 60ml/min. Some patients can have acute kidney injury (AKI), and cannot recover from AKI, final eGFR can be decreased below 60ml/min - chronic kidney disease stage III (CKD III). However, there are limited prognostic factors for AKI such as preoperative renal function, estimated blood loss, and diabetes mellitus.[6,7] AKI is an important factor considered when we establish a management plan for patients after nephrectomy. Urologists have difficulties in predicting AKI accurately due to many unknown predisposing factors. There were argues relating to LRN in its early years that pneumoperitoneum and lateral decubitus position during laparoscopy could be a factor for AKI which can make renal parenchymal compression.[8] Usually, renal function after surgery can be monitored by serum creatinine, BUN, GFR. In addition to this, neutrophil gelatinase-associated lipocalin (NGAL) after surgery has recently emerged as a promising biomarker of AKI.[9,10] The NGAL is highly accumulated in the human kidney cortical tubules, blood and urine after ischemic injuries in the kidney[11], many studies have been reported worldwide the usefulness of serum NGAL as a marker for acute kidney injury. Since accumulation of NGAL is known to the results of renal ischemia and reperfusion, the utility of markers for AKI has been actively studied.[12,13]

In this study, we evaluated the usefulness of serum NGAL levels for monitoring renal function after laparoscopic radical nephrectomy (LRN).

MATERIAL AND METHODS

Material

We prospectively collected data from 30 patients who underwent LRN. The exclusion criteria were patients with stage III chronic kidney disease (CKD III; creatinine ≥ 1.2 mg/dl or eGFR < 60 ml/min/1.73 m^2), contralateral hydronephrosis, atrophied kidney, renal mass size > 7 cm, postoperative blood loss > 200 ml, requirement of preoperative blood transfusion, and perioperative antitumor systemic therapy such as target therapy, and another malignancy.

Measurements of serum creatinine and NGAL

We analyzed serum creatinine and NGAL levels at 6 time points; preoperative day 1, right after surgery, 6 hours after surgery, postoperative day (POD) 1, POD 3, and POD 5. We compared these measurements in the groups according to the eGFR values (eGFR < 60 ml/min/1.73 m^2 or not) using data obtained 3 months after surgery. At each time point, EDTA anticoagulated whole blood were collected to measure NGAL. We used Alere Triage NGAL test kit (Alere Inc., Walthanm, MA, USA) for serum NGAL level. Serum creatinine level was tested with ADVIA 2400 analyzer (Siemens Healthcare, Germany).

eGFR calculation

Estimated GFR (eGFR) was calculated using isotope dilution mass spectrometer (IDMS)-traceable Modification of Diet in Renal Disease (MDRD) formula. The formula is as follow; GFR (mL/min/1.73 m^2) = 175 × (Scr)$^{-1.154}$ × (Age)$^{-0.203}$ × (0.742 if female) × (1.212 if African American) where Scr is a serum creatinine level.

Correlations among serum creatinine, NGAL and eGFR

We compared the values of serum creatinine and NGAL at 6 time points to evaluate the correlations between the two markers at each time point. Correlations between the value of serum creatinine and NGAL at each time point and eGFR 3 months after surgery were calculated.

ROC analysis between serum creatinine and NGAL

To analyze the validity of test results of the serum NGAL, we compared the values of serum creatinine and NGAL at each time point and calculated the area under the curve (AUC) using ROC statistics.

Statistical analysis

To calculate the area under the curve of serum creatinine and NGAL, ROC statistics was used.

The correlations between the two results of serum creatinine and NGAL at 6 time points were analyzed with Pearson correlation statistics. All statistical analyses were performed using SPSS ver. 17.0 (SPSS Inc., Chicago, IL, USA). P-values < 0.05 were considered statistically significant.

RESULTS

Patients characteristics

The mean age of the 30 patients was 65.5 years (range, 45-77 years), and the male-to-female ratio was 2:1 (20 male patients). At the last follow-up examination, there were 12 patients (40%) with CKD III. The mean renal mass size was 4.8 cm (range, 3.5-7 cm), and the mean preoperative serum NGAL level and Cr were 81.17 ng/ml (range, 47-134 ng/ml) and 0.75 mg/dL (range, 0.22-1.2 mg/dL), respectively. The mean eGFR was 62.0 +/- 13.86 ml/min/1.73 m^2 when checked 3 months after surgery.

Table 1. Paired sample correlations between serum creatinine and NGAL

Time point	N	Pearson correlation	P
PreOD1	30	−0.019	0.919
RFS	30	−0.246	0.191
SixFS	30	−0.004	0.982
POD1	30	0.082	0.668
POD3	30	0.018	0.924
POD5	30	0.170	0.370

Abbreviations: PreOD, preoperative day; RFS, right after surgery; SixFS, 6 hours after surgery, POD, postoperative day.

Correlations between the serum creatinine and NGAL

When we analyzed the correlations between the serum creatinine and NGAL levels at 6 time points, no significant correlation was found at any time point ($P > 0.05$) (Table 1, Fig. 1, Fig. 2).

Correlations between serum creatinine and eGFR and between serum NGAL and eGFR

Creatinine values on POD 3 and 5 were well correlated with the eGFR values 3 months after surgery (Pearson correlation = −0.623, $P = 0.000$ and −0.712, $P = 0.000$, on POD3 and POD5, respectively), however, no significant correlation was found between the serum NGAL and eGFR (table 3).
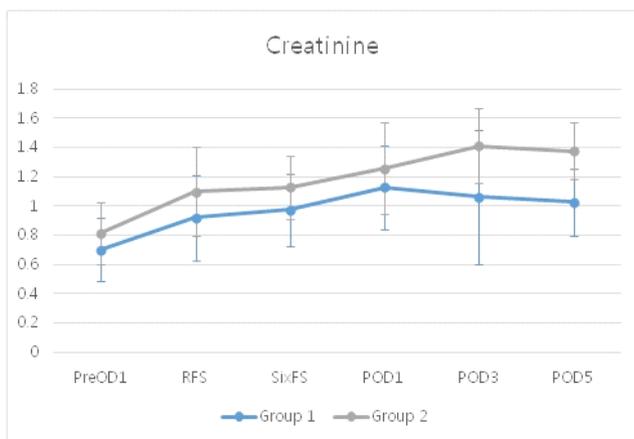

Fig. 1. Serum creatinine levels in CKD III and non−CKD III groups. Patients were grouped into 2 groups according to the eGFR values; the patients with eGFR equal to or higher than 60 mL/min/1.73 m2 were grouped into group 1 and those with eGFR lower than 60 mL/min/1.73 m2 were grouped into group 2, respectively. Abbreviations: PreOD, preoperative day; RFS, right after surgery; SixFS, 6 hours after surgery, POD, postoperative.

Fig. 2. NGAL levels in CKD III and non−CKD III groups. Patients were grouped into 2 groups according to the eGFR values; the patients with eGFR equal to or higher than 60 mL/min/1.73 m2 were grouped into group 1 and those with eGFR lower than 60 mL/min/1.73 m2 were grouped into group 2, respectively. Abbreviations: PreOD, preoperative day; RFS, right after surgery; SixFS, 6 hours after surgery, POD, postoperative day.
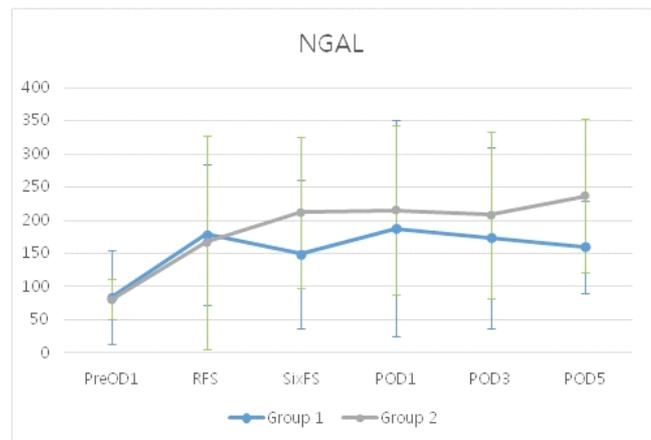
Table 2. Area under the curve of serum creatinine and NGAL in ROC statistics

	Test Result Variable(s)	AUC	P
Creatinine	PreOD1	0.650	0.169
	RFS	0.685	0.090
	SixFS	0.674	0.112
	POD1	0.632	0.228
	POD3	0.706	0.060
	POD5	0.887	0.000
NGA	PreOD1	0.583	0.446
	RFS	0.403	0.374
	SixFS	0.743	0.026
	POD1	0.676	0.108
	POD3	0.588	0.421
	POD5	0.704	0.063

Abbreviations: AUC, area under the curve; PreOD, preoperative day; RFS, right after surgery; SixFS, 6 hours after surgery, POD, postoperative day.
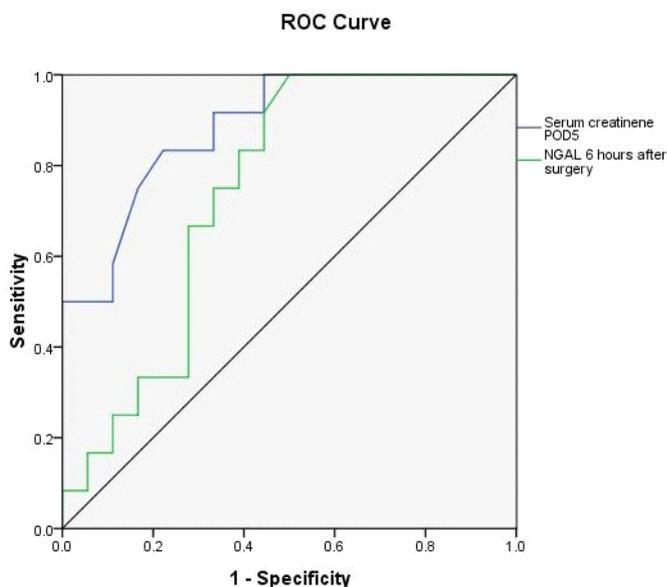

Fig. 3. Receiver operating characteristic analysis of serum creatinine and NGAL. Abbreviations: POD, postoperative day.

Area under the curve

Using receiver operating characteristic analysis, we found that serum creatinine levels on POD 5 (area under the curve [AUC], 0.887; P = 0.000) and NGAL levels at 6 hours after radical nephrectomy (AUC = 0.743, P = 0.026) were significant predictors of final CKD III (Table 2, Fig. 3).

DISCUSSION

Since NGAL is highly accumulated in the cortical tubules of human kidney, blood and urine after ischemic injuries in the kidney,[11] many studies have been reported worldwide the usefulness of serum NGAL as a marker for acute kidney injury.[14]

The RIFLE classification, the classification system for acute kidney injury (AKI) was established in 2004 by the members of the Acute Dialysis Quality Initiative (ADQI).[15] The Acute Kidney Injury Network (AKIN) group proposed a new criteria for AKI which is AKIN classification in 2007.[16] Although there is difference in detection rate depending on the criteria applied for the AKI, such injury according to the criteria by either RIFLE or AKIN is associated with increased hospital mortality.[17]

Urine NGAL does not seem to be a useful marker for detecting renal injury in healthy patients treated with partial nephrectomy or in patients with normal contralateral kidney and relatively good preoperative renal function.[18,19] The known prognostic factors of residual renal function after partial nephrectomy are warm ischemic time, preserved renal volume, diabetes mellitus, and age.[20-22] Among these, modifiable factor by surgeon is warm ischemia time.

In the many studies, warm ischemia time within 20-25 minutes does not influence to the residual renal function immediately or permanently.[21,23,24] So, in case of renal function prediction after partial nephrectomy, the feasibility of serum NGAL still seems to need more validation studies.[25,26] The known factors for affecting residual renal function after nephrectomy are preoperative renal function, age, diabetes mellitus, which is debatable.[7]

When performing radical nephrectomy, there is no vessel clamp and de-clamp, the reperfusion step cannot be present as well. So, ischemic damage may be excluded from the affecting factors of residual renal function. However, the possibility of contralateral kidney compression from the lateral decubitus position during radical nephrectomy can affect to the residual renal function, especially, during laparoscopy, pneumoperitoneum also can make renal compression.[8] There were several studies for impact of these factors - contralateral renal compression under laparoscopy, and most studies could not find any association between contralateral renal compression and renal function.[7,26] But, the previous study used serum creatinine as the standard renal function test, in this study, we used NGAL which is affected by ischemia, oxidative stress more accurately. And we compared serial serum creatinine and NGAL levels. We could not find any significant correlation between the serum Cr and NGAL levels at 6 time points (Table 2). In table 3, serum Cr was significantly correlated with eGFR on POD 3 and POD 5, respectively (r = -0.623 and -0.712, P = 0.000, respectively). But no significant correlation was found between serum NGAL and eGFR at any time points.

From the view point of patient management, early detection of kidney injury is critical issue

Table 3. Paired sample correlations between serum creatinine and eGFR and between serum NGAL and eGFR

	Pairs	N	Pearson correlation	P
Cr and eGFR	PreOD1	30	−0.292	0.118
	RFS	30	−0.208	0.269
	SixFS	30	−0.280	0.134
	POD1	30	−0.198	0.295
	POD3	30	−0.623	0.000
	POD5	30	−0.712	0.000
NGAL and eGFR	PreOD1	30	−0.062	0.744
	RFS	30	0.084	0.661
	SixFS	30	−0.193	0.306
	POD1	30	−0.147	0.439
	POD3	30	−0.079	0.680
	POD5	30	−0.275	0.141

Abbreviations: AUC, area under the curve; PreOD, preoperative day; RFS, right after surgery; SixFS, 6 hours after surgery, POD, postoperative day.

because proper modification of patient management is believed to guarantee the better prognosis for patients. In our study, we intended to evaluate whether NGAL could be an early marker for predicting the prognosis of patients 3 months after LRN. We calculated eGFR 3 months after LRN and compared it with the values of serum creatinine and NGAL at 6 time points. Serum creatinine level on POD 5 and NGAL at 6 hours after surgery were statistically significant and the area under the ROC curve were 0.887 and 0.743, respectively (Table 1, Fig. 1). Although the AUC of the serum creatinine on POD 5 is superior to that of NGAL at 6 hours after surgery, NGAL is preferred as an early marker because it can give more chances to the physicians to manipulate the management plan for a better outcome after 3 month follow-up.

AKI was reported to associates with increased long-term mortality risk, independent of residual kidney function, for patients who survive 90 days after discharge and long-term mortality risk was highest among the most severe cases of AKI.[26] By the results of our study, serum NGAL at postoperative 6 hours can reflect the probability of AKI and CKD III earlier than serum creatinine, and we can manage patient cautiously by optimizing fluid hydration, monitoring urine output, and avoiding nephrotoxic antibiotics and analgesics. After all, we believe that serum NGAL could give us information about renal function preservation after LRN earlier than serum creatinine.

In conclusion, the development of CKD III at 12 month after LRN was associated with the serum creatinine level on POD 5 and the NGAL level at 6 hours after surgery. Although, serum NGAL levels after LRN for overall prediction of CKD III is not superior to serum creatinine, serum NGAL levels enabled earlier prediction of postoperative CKD III, thus facilitating better management of

patients, including fluid hydration as well as antibiotic and analgesic administration. Therefore, serum NGAL levels measured 6 hours after surgery could be a useful marker for managing patients after LRN.

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