

## ORIGINAL RESEARCH



# Predictive value of serum and urinary neutrophil gelatinase-associated lipocalin in the early detection of acute kidney injury after major abdominal surgery

Erhan Ozyurt<sup>1,\*</sup>, Mehmet Tercan<sup>1</sup>, Orhan Aras<sup>2</sup>, Ridvan Yavuz<sup>2</sup>, Huseyin Ciyiltepe<sup>2</sup>, Tebessum Cakir<sup>2</sup>, Guzin Aykal<sup>3</sup>, Nilgun K Ozturk<sup>1</sup>

<sup>1</sup>Department of Anesthesiology and Reanimation, Antalya Training and Research Hospital, University of Health Sciences, 07100 Antalya, Turkey

<sup>2</sup>Department of Gastrointestinal Surgery, Antalya Training and Research Hospital, University of Health Sciences, 07100 Antalya, Turkey

<sup>3</sup>Department of Clinical Biochemistry, Antalya Training and Research Hospital, University of Health Sciences, 07100 Antalya, Turkey

**\*Correspondence**

erhan.ozyurt@saglik.gov.tr  
(Erhan Ozyurt)

**Abstract**

This study aimed to evaluate the predictive abilities of both serum neutrophil gelatinase-associated lipocalin (sNGAL) and urinary neutrophil gelatinase-associated lipocalin (uNGAL) in the early detection of acute kidney injury (AKI) among patients undergoing major abdominal surgery. The study commenced after obtaining approval from the local ethics committee (12 January 2023; no. 1/22). This prospective observational study was conducted between June and November 2023. Patients undergoing elective major abdominal surgery were included. Their demographic and operation data were recorded. Four hours after surgery, serum and urine samples were collected for NGAL measurements. Additionally, serum samples for creatinine (sCr) measurements were obtained from patients before surgery and at 4, 12, 24 and 48 hours after surgery. AKI was diagnosed according to the Kidney Disease Improving Global Outcomes guidelines based on changes in sCr levels over the first 48 hours postoperatively. The study included 43 patients, with AKI occurring in five individuals (11.6%). Four hours postoperatively, the sNGAL values were  $77.5 \pm 20.5$  ng/mL in the non-AKI group and  $281.3 \pm 166.4$  ng/mL in the AKI group. The uNGAL values were  $256.1 \pm 137.4$  ng/mL and  $539.7 \pm 160$  ng/mL, respectively. The areas under the curve (AUC) for sNGAL and uNGAL were 0.989 and 0.921, respectively. The optimal cut-off value for sNGAL was 121.9 ng/mL, resulting in 100% sensitivity and 94.7% specificity. For uNGAL, the optimal cut-off was 513.9 ng/mL, with a sensitivity of 80% and a specificity of 97.4%. To identify AKI after major abdominal surgery, using both sNGAL and uNGAL allows for earlier detection compared to diagnostic methods based on sCr.

**Keywords**

Acute kidney injury; Major abdominal surgery; Neutrophil gelatinase-associated lipocalin

## 1. Introduction

The pathogenesis of acute kidney injury (AKI) following major abdominal surgery is complex. AKI can occur due to factors such as fluid deficiency, neuroendocrine response to anesthesia and surgery, inflammation, urinary obstruction and increased intra-abdominal pressure [1, 2]. The incidence of AKI after major surgery varies between 11% and 50%. Morbidity and mortality rates increase in patients with AKI because of the applied treatments and prolonged hospital stays. Additionally, as a result of all these processes, hospital costs increase, thereby burdening healthcare systems [3–5].

In diagnosing AKI, classifications such as those of the Acute Kidney Injury Network or Kidney Disease Improving Global Outcomes (KDIGO) are used. These classifications assess kidney injury based on serum creatinine (sCr) levels and urine output [6–8]. However, age, gender, diet, muscle mass and

medications influence sCr levels. Additionally, in healthy individuals with sufficient renal reserve, nephron losses of up to 50% can be tolerated by the body, with no change in sCr values [9, 10]. Furthermore, sCr may remain unchanged in patients with postoperative fluid overload [3]. As a result, elevated sCr may take 24–48 hours to appear, potentially causing delayed AKI diagnosis. This delays treatment and increases morbidity and mortality rates [9].

Interest in biomarkers used in the early diagnosis of AKI has thus increased in recent years. Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa neutrophil-derived molecule from the lipocalin family. It is secreted in small amounts from the kidneys, prostate and respiratory tract epithelial cells. After ischemic and nephrotoxic acute tubular necrosis, the NGAL level peaks at the third hour and remains elevated for approximately 24 hours. In patients who do not progress to AKI, NGAL levels begin to decrease within the

first hour after the injury [9, 11].

NGAL has been studied in the perioperative context following cardiovascular surgery [9]. However, limited data exists on the predictive capability of NGAL in patients undergoing major noncardiovascular surgery. Therefore, we aimed to test the predictive capabilities of both serum NGAL (sNGAL) and urinary NGAL (uNGAL) in identifying early-stage AKI in patients undergoing major abdominal surgery. We hypothesized that sNGAL and uNGAL measured at the 4th hour after surgery can detect AKI earlier than sCr.

## 2. Materials and methods

### 2.1 Design and setting

This prospective observational study was conducted on patients admitted to the gastrointestinal surgery clinic of our hospital for major abdominal surgery between June and November 2023.

### 2.2 Inclusion/exclusion criteria

Patients planning elective major abdominal surgery with laparotomy, classified as American Society of Anesthesiologists (ASA) I–III, aged 18 years and above, and providing written informed consent were included in the study. Patients with chronic kidney disease according to the KDIGO classification, those using nephrotoxic drugs (antibiotics, nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, *etc.*), and those with a history of kidney transplantation or nephrectomy were excluded from the study.

### 2.3 Patient data and management

Patient data recorded included age, gender, body mass index, comorbidities (such as hypertension, diabetes mellitus, cardiac disease, *etc.*), type and duration of surgery, duration of anesthesia, amount of fluids used, urine output, amount of bleeding, length of hospital stay, complications and patient outcomes. In addition to NGAL samples, routine blood samples were also collected at the fourth postoperative hour. Hypotension was defined as a 20% decrease in baseline mean arterial pressure or a systolic arterial pressure below 90 mmHg for five minutes in patients. Hypotension episodes of the patients were recorded.

All patients were operated under standard general anesthesia. The attending physician had the authority to manage perioperative patient care and postoperative intensive care. The study did not necessitate any alterations to standard care, and the attending physicians remained unaware of the NGAL measurement results throughout the patient's perioperative course, ensuring it did not impact their interpretation.

### 2.4 NGAL analysis

Four hours after surgery, samples were taken from serum and urine for NGAL analysis. Venous blood samples were drawn into 5.0-mL gel-clot activator tubes (Vacusera, Izmir, Turkey), and urine samples were collected into 10.0-mL urine tubes (Aklab Group, Erzurum, Turkey). Venous blood samples were gently inverted 5–6 times, left at room temperature for 30 minutes to allow clotting, and then centrifuged at 3000 rpm for

20 minutes at 25 °C. Hemolyzed, icteric and lipemic samples were excluded from the study. The obtained serum and urine were separated into Eppendorf tubes and stored in a –80 °C freezer until further analysis. NGAL measurement was performed using the ETI-MAX 3000 device (microelisa analyzer, DiaSorin, vercelli, Italy) with the BT-LAB Human Neutrophil Gelatinase-associated Lipocalin, NGAL ELISA Kit (Zhejiang, China).

### 2.5 AKI definition

AKI was defined by the KDIGO criteria, which require the presence of any of the following events: an increase in serum creatinine level of at least 0.3 mg/dL within 48 hours, an increase in serum creatinine of at least 1.5 times the baseline value, or a urine output of less than 0.5 mL/kg/hour for at least six hours (Table 1) [8]. We did not use the urine output parameter because it is affected by factors such as fluid balance, volume overload, diuretic use and body weight.

### 2.6 Study endpoints

The primary endpoint of this study was to compare the efficacy of sNGAL and uNGAL as predictors of diagnosing AKI in patients who underwent major abdominal surgery. The secondary endpoint of the study was to determine the threshold values of both sNGAL and uNGAL for the detection of AKI.

### 2.7 Statistical analysis

All patients meeting the inclusion criteria during the specified period were included in the study. All data are expressed as mean (standard deviation; SD), mean (95% confidence interval; CI), or number (percentage). After testing the normal distribution of the data, the Mann-Whitney U test was used for the comparison of non-normally distributed numerical variables, and Fisher's exact test was used for categorical variables in comparing patients who developed AKI with those who did not. The *t*-test was used to compare normally distributed numerical data, and the chi-square test was used to compare categorical variables. Receiver operating characteristic (ROC) curve analysis was conducted to determine the sensitivity and specificity of biomarkers (sNGAL, uNGAL) in predicting AKI. The optimal cut-off values for each biomarker were determined at the points on the ROC curve where the maximum balance of specificity and sensitivity was reached. A *p*-value of < 0.05 was considered statistically significant. The SPSS 17.0 (SPSS Inc., Chicago, IL, USA) statistical package was used for all statistical analyses.

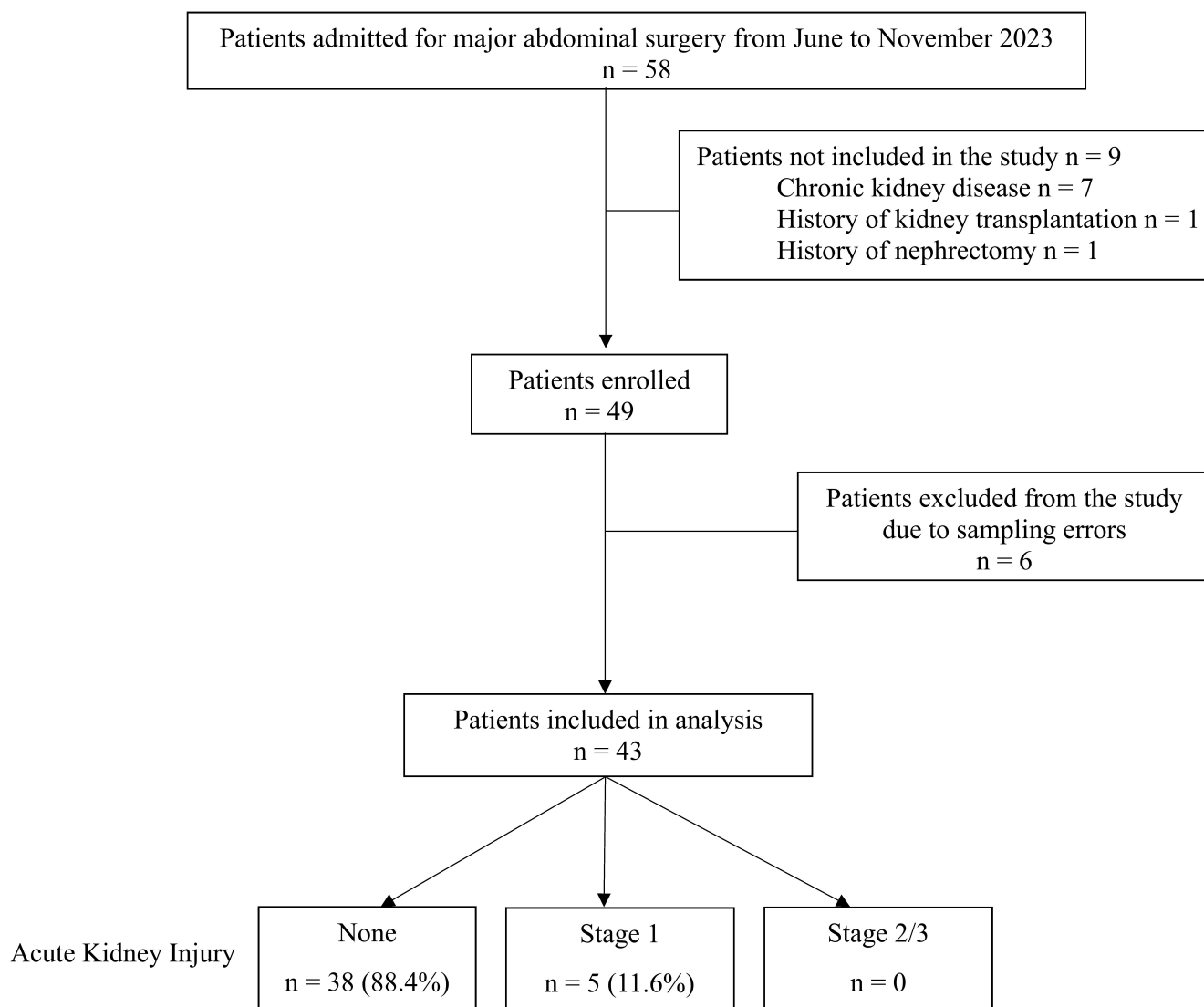
## 3. Results

Fifty-eight patients who underwent major abdominal surgery with laparotomy were evaluated. Seven patients with chronic kidney disease, one with a history of kidney transplantation, one with a history of nephrectomy, and six due to sampling errors were excluded. Ultimately, 43 patients were analyzed (Fig. 1). The average age of all patients was 60.3 ± 12.2 years. Five patients (11.6%) developed AKI (AKI group), and 38 (88.4%) did not (non-AKI group). There were no differences

**TABLE 1. Modified KDIGO staging of AKI by sCr criteria.**

Stage	Serum creatinine
1	1.5–1.9 times baseline or $\geq 0.3$ mg/dL ( $\geq 26.5$ mmol/L) increase
2	2.0–2.9 times baseline
3	3 times baseline or $\geq 4.0$ mg/dL ( $\geq 353.6$ mmol/L) increase or initiation of RRT

*KDIGO: Kidney Disease Improving Global Outcomes; AKI: acute kidney injury; sCr: serum creatinine; RRT: Renal replacement therapy.*



**FIGURE 1. Flow diagram of the patients showing reasons for exclusion.** Acute kidney injury was defined according to KDIGO classification.

between the groups in terms of demographic or surgical data. Only the length of hospital stay was longer in the AKI group ( $6.8 \pm 3.2$  vs.  $10.2 \pm 2.6$ ,  $p = 0.01$ ; Table 2).

The grade of five patients who developed AKI was KDIGO stage 1. None of the patients with AKI required renal replacement therapy (RRT) or developed AKI stages 2 or 3 according to the KDIGO guidelines. No difference existed between the groups in sCr values in the pre-operative period or at 4, 12 or 48 hours postoperatively. However, the sCr values measured at the 24th postoperative hour were higher in the group that developed AKI ( $p = 0.001$ ; Table 3). No complications or

deaths occurred during the study period.

At 4 hours postoperatively, the sNGAL values were  $77.5 \pm 20.5$  ng/mL in the non-AKI group and  $281.3 \pm 166.4$  ng/mL in the AKI group, whereas the uNGAL values were  $256.1 \pm 137.4$  ng/mL and  $539.7 \pm 160$  ng/mL, respectively (Table 3). The areas under the curve for sNGAL and uNGAL were 0.989 and 0.921, respectively. The optimal cut-off value for sNGAL was 121.9 ng/mL, resulting in 100% sensitivity and 94.7% specificity. For uNGAL, the optimal cut-off value was 513.9 ng/mL, with a sensitivity of 80% and specificity of 97.4% (Table 4; Fig. 2).

**TABLE 2. Comparison of study groups in terms of demographic and operational data.**

	No AKI (n = 38)	AKI (n = 5)	p-value
	Mean ± SD or n (%)	Mean ± SD or n (%)	
Age (yr)	59.7 ± 12.7	65.0 ± 5.8	0.369
Female/Male (n)	15/23	2/3	0.982
ASA II/III (n)	30/8	4/1	0.957
Body mass index (kg/m <sup>2</sup> )	25.5 ± 3.6	25.5 ± 2.0	0.981
Presence of comorbidity	25 (65.8)	3 (60.0)	0.798
Hypertension	14 (36.8)	2 (40.0)	0.891
Diabetes mellitus	9 (23.7)	0 (0.0)	0.221
Cardiac disease	3 (7.9)	1 (20.0)	0.381
Type of surgery			
Colorectal	21 (55.3)	1 (20.0)	0.078
Upper Gastrointestinal	8 (21.1)	1 (20.0)	
Pancreatic	5 (13.2)	3 (60.0)	
Other	4 (10.5)	0 (0.0)	
Hypotension during surgery	14 (36.8)	2 (40.0)	0.891
Amount of fluids (mL)	3447.4 ± 1265.7	4060.0 ± 1947.6	0.661
Amount of blood loss (mL)	271.1 ± 257.0	440.0 ± 343.5	0.163
Blood transfusion (n)	5	2	0.126
Urine output (mL)	482.9 ± 413.2	580.0 ± 416.2	0.543
Duration of surgery (min)	185.3 ± 60.5	194.0 ± 53.2	0.704
Duration of anesthesia (min)	214.0 ± 68.5	222.0 ± 60.6	0.676
Hospital stay (d)	6.8 ± 3.2	10.2 ± 2.6	0.010

ASA: American Society of Anesthesiologists classification system; AKI: acute kidney injury; SD: standard deviation.

**TABLE 3. Levels of serum creatinine, serum NGAL, and urinary NGAL in study participants with or without AKI.**

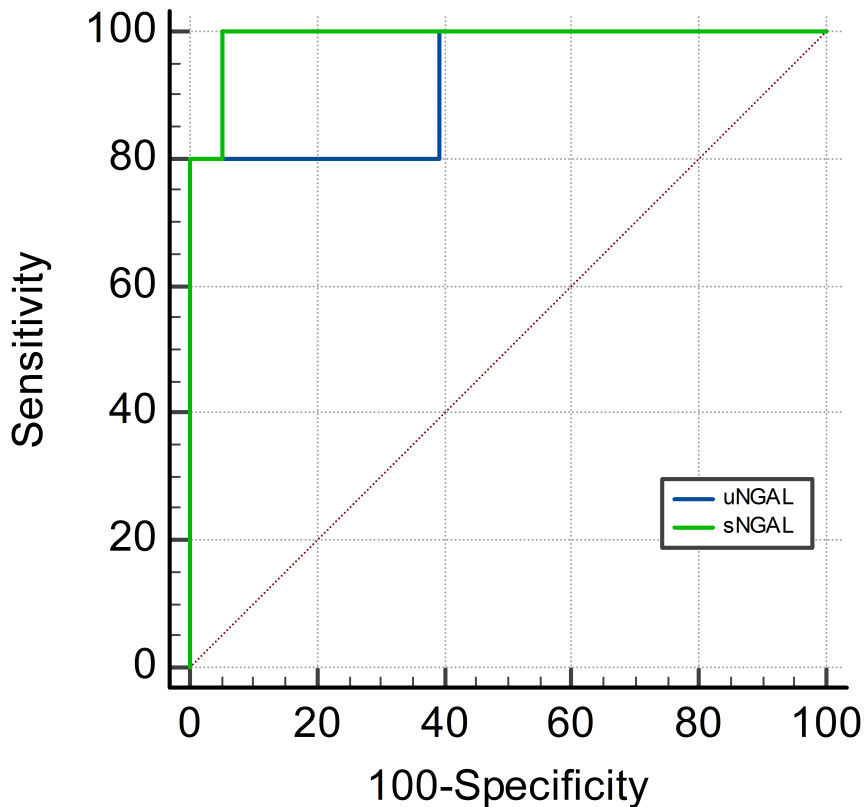
	No AKI (n = 38)	AKI (n = 5)	p-value
	Mean (95% CI Lower–Upper)	Mean (95% CI Lower–Upper)	
Serum NGAL ng/mL			
4 h postoperatively	77.5 (70.7–84.2)	281.3 (74.6–487.9)	<0.001
Urine NGAL ng/mL			
4 h postoperatively	256.1 (210.9–301.3)	539.7 (341.1–738.3)	<0.001
Serum Creatinine mg/dL			
Preoperatively	0.94 (0.89–0.99)	0.98 (0.75–1.20)	0.612
4 h postoperatively	0.82 (0.76–0.88)	0.86 (0.61–1.11)	0.644
12 h postoperatively	0.80 (0.78–0.88)	0.90 (0.61–1.10)	0.939
24 h postoperatively	0.80 (0.79–0.89)	1.30 (1.05–1.57)	0.001
48 h postoperatively	0.80 (0.78–0.90)	1.00 (0.70–1.32)	0.071
C-reactive protein mg/L			
4 h postoperatively	44.2 (31.6–56.8)	67.7 (1.1–134.3)	0.382
WBC 10 <sup>3</sup> /mm <sup>3</sup>			
4 h postoperatively	12.8 (11.5–14.2)	12.5 (5.9–19.0)	0.901

NGAL: neutrophil gelatinase-associated lipocalin; AKI: acute kidney injury; WBC: white blood cell; CI: confidence interval.

**TABLE 4. Receiver operating characteristic curve analysis for serum and urinary NGAL.**

	AUC	Sig.	95% CI Lower–Upper	Sensitivity %	Specificity %	Cut-off ng/mL
Serum NGAL	0.989	<0.001	0.963–1	100	94.7	121.9
Urine NGAL	0.921	0.002	0.777–1	80	97.4	513.9

NGAL: neutrophil gelatinase-associated lipocalin; AUC: areas under the curve; CI: confidence interval.



**FIGURE 2. Receiver operating characteristic curve of serum and urinary neutrophil gelatinase-associated lipocalin (NGAL) levels at postoperative 4th hour in patients with acute kidney injury.**

#### 4. Discussion

Although many biomarkers have been used to detect AKI during the postoperative period, NGAL has been the most promising and extensively studied [3]. Our study concluded that both sNGAL and uNGAL values at 4 hours postoperatively are reliable biomarkers for detecting AKI.

Consistent with the literature, we found an AKI incidence of 11.6% [12]. The grade of all patients with AKI was KDIGO stage 1. Patients with AKI received hemodynamic monitoring, volume status regulation, and avoidance of nephrotoxic agents. There was no clinical worsening in the AKI patients and sCr values decreased at the 48th hour. Furthermore, no patients required RRT, and no patients had risk stratification during discharge. The failure to detect Stage 2/3 patients can be explained by the small sample size and absence of complications during or after surgery. In a study conducted on patients undergoing major gastrointestinal surgery, STARSurg Collaborative found the rate of KDIGO stage 1 patients to be 9.2%. Additionally, they identified the rate of major adverse kidney events to be 18.6% and the mortality rate to be 13.9%

during the one-year follow-up of these patients [12]. Similarly, Brown *et al.* [13], in their study on patients undergoing cardiac surgery, found the adjusted hazard ratio for patients with transient AKI lasting 1–2 days to be 1.46. As shown, although there may be no progression in the severity of AKI or only a transient elevation in sCr, this condition is still associated with poor patient outcomes, including an increased risk of long-term mortality.

NGAL is primarily derived from renal tubular epithelial cells, and its secretion increases during an acute phase response to stress after exposure to ischemia, inflammation or toxins. sNGAL and uNGAL levels have a much faster increase following an injury compared to traditional markers like sCr [9, 11]. This allows the possibility of earlier intervention and potentially improved outcomes for patients. Therefore, NGAL has become a prominent biomarker in recent years for the early detection of AKI. In a recent meta-analysis involving 38,725 patients comparing biomarkers predicting AKI, NGAL was found to be the best-performing biomarker [3]. NGAL is effective in detecting AKI after major surgeries [10, 14, 15].

However, Cullen *et al.* [16] report that uNGAL has a poor predictive role after abdominal surgery. Furthermore, similar to our study, Gombert *et al.* [14] reported that both types of NGAL could be used in the detection of AKI after cardiovascular surgery, with uNGAL being more effective. In our study, both sNGAL and uNGAL values at 4 hours postoperatively and sCr values at 24 hours postoperatively were higher in the AKI group. The different results obtained in studies may be due to the precise timing of NGAL measurement being unknown.

Taking samples at the right time for NGAL measurement enhances its predictive value. NGAL measurements can be made from the 2nd hour to the 5th postoperative day [17]. Because of the increase in NGAL values in patients 3 hours after surgery, we decided to perform measurements at the 4th postoperative hour [14, 18]. On the other hand, Fanning *et al.* [15] stated that uNGAL measured 24 hours after cardiac surgery had a better predictive value than sNGAL. However, NGAL tends to peak 4–6 hours postoperatively, followed by a decrease in blood levels [9]. Therefore, in this study, we may have obtained better predictive values for both sNGAL and uNGAL because the samples were collected at the 4th hour postoperatively.

The cut-off values for sNGAL and uNGAL are reported to be 62–426 ng/mL and 20–460 ng/mL, respectively [9, 19]. However, in our study, the threshold value for sNGAL was 122 ng/mL, whereas that for uNGAL was 514 ng/mL. These different values can be explained by the absence of a specific threshold for NGAL, studies being conducted in very different patient groups, and the lack of consensus on the timing of serum or urinary NGAL measurements.

Whether the elevation of NGAL during the postoperative period is related to kidney damage or an inflammatory response is unclear [9, 15, 16, 18]. After major surgery, inflammation and infection triggered by tumor necrosis factor and lipopolysaccharide stimulate NGAL release from neutrophils [20]. Therefore, NGAL values measured in the early postoperative period are thought to indicate both inflammation and renal injury. We found an increase in the NGAL values in all patients. However, NGAL levels above the threshold were only detected in the AKI group. Additionally, there was no difference in the levels of inflammation markers such as C-reactive protein and white blood cell in the postoperative period between patients who developed AKI and those who did not. Therefore, higher NGAL levels may be associated with AKI rather than inflammation.

The strength of our study is that it is the first to test the predictive value of both sNGAL and uNGAL for AKI in patients undergoing major abdominal surgery. But, this study has several limitations. Firstly, the fact that it was prospective, single-center and conducted on elective surgical patients may hinder the generalization of the results. Secondly, the number of cases was small and this may have prevented us from identifying stage 2/3 patients, but this study represented a niche of the population.

## 5. Conclusions

Using both sNGAL and uNGAL for AKI detection following major abdominal surgery allows for earlier diagnosis compared

to sCr-based methods. Larger studies are necessary to establish the optimal measurement times and cut-off values for NGAL in AKI detection.

## AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

## AUTHOR CONTRIBUTIONS

EO, MT, OA and RY—designed the research study. EO, OA, RY, HC and TC—performed the research. GA—performed the NGAL analysis. EO, MT, OA, RY and NKO—analyzed the data; wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the institutional review board (IRB) of Antalya Training and Research Hospital, University of Health Sciences (IRB No.: 12 January 2023; no.: 1/22). Clinical Trial Registration: [clinicaltrials.gov](https://clinicaltrials.gov) (NCT05721638). Written consent was obtained from the patients.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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