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Accuracy of Neutrophil Gelatinase-Associated Lipocalin in Detecting Acute Kidney Injury after Urogenital Robotic Assisted Laparoscopic Surgery under General Anesthesia

Orsolya MIHÁLY[1,*], Sorana D. BOLBOACĂ[2], Rodica RĂHĂIAN[3], Constantin BODOLEA[4], Cipriana CHIRA[4], Tudor CRISTEA[4], Ana OBLEZNIUC[4], Zoltán A. MIHÁLY[5], Ioan COMAN[5]

[1] County Urgency Clinical Hospital Cluj, ICU and Anesthesiology II Department. Cluj Napoca 1-3 Clinicilor, 400006 Cluj Napoca, Cluj, Romania.
[2] "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Department of Medical Informatics and Biostatistics, 6 Louis Pasteur, 400349 Cluj-Napoca, Cluj, Romania.
[3] County Urgency Clinical Hospital Cluj, Immunology Department. Cluj Napoca 1-3 Clinicilor, 400006 Cluj Napoca, Cluj, Romania.
[4] City Clinical Hospital Cluj, ICU and Anesthesiology Department, 11 Tabacarilor, 400139 Cluj Napoca, Cluj, Romania.
[5] City Clinical Hospital Cluj, Urology Department. 11 Tabacarilor, 400139 Cluj Napoca, Cluj, Romania.
E-mail(s): Mihaly.Maria@umfcluj.ro; sbolboaca@umfcluj.ro; rodirahaian@yahoo.com; bodolea@yahoo.com; ciprianachira@yahoo.com; oblezniuc_ana@yahoo.com; mzattika@yahoo.com; jcoman@yahoo.com

* Author to whom correspondence should be addressed; Tel.: 0040-745398005

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Abstract
The *aim* of this study was to demonstrate the accuracy of Neutrophil Gelatinase-Associated Lipocalin (NGAL) in detecting Acute Kidney Injury (AKI) after urogenital robotic surgery in general anesthesia. *Methods*: A prospective longitudinal observational study, which included patients scheduled for elective robotic surgery under general anesthesia. The serum and urine NGAL at induction, 6 hours and 12 hours were determined. Serum creatinine was measured preoperatively and daily 4 days postoperatively. AKI was defined as the absolute growth of serum creatinine by 0.3 mg/dl over baseline within 48 hours postoperatively. *Results*: 24 patients were enrolled in the study. AKI occurred in 38% of patients. Serum NGAL increased significantly at 6 hours and 12h, compared to baseline, with a higher increase in the group of patents without AKI. There were no significant results for urine NGAL. A link was observed between the values of serum NGAL, with associated significance $p<0.0001$. The correlations between urine NGAL were not significant. The predictive value of NGAL, analyzed by cross-tabulation, OR was 3 for baseline value and 5.33 for the values measured at 6 hours and 12 hours, but with no statistical significance. *Conclusions*: The modifications of the NGAL levels, measured at 6 hours and 12 hours from the induction of anesthesia, were significant with more importance at 6 hours and in patients without AKI. Serum NGAL had no predictive value for AKI, but the risk to develop AKI was 3 times higher for baseline determination and 5 times at 6 and 12 hours.

Keywords: Acute kidney injury; Biomarker; Neutrophil gelatinase associated lipocalin; General anesthesia; Robotics.

Introduction

Acute kidney injury (AKI), defined by RIFLE [1] and AKIN [2] criteria, describes minor changes in renal function, renal failure is the final stage of acute kidney injury. AKI occurs in 36% of patients admitted to intensive care [3], increases in-hospital mortality and costs in spite of new treatment and prevention methods. The cause of this failure is the delayed diagnosis. Traditional markers such as urea, creatinine and urine output, detect AKI late [4]. Increased creatinine levels, standard marker used to diagnose AKI occur in 48-72 hours from the reduction of the glomerular filtration [5]. Creatinine is not a decisive marker in the diagnosis of AKI [6]: increased serum creatinine is not specific for AKI, the cause of azotemia might be prerenal or extrarenal; serum creatinine is not specific for renal tubular injury, which is involved in the pathogenesis of AKI [6]. Increases of serum creatinine are detected later than changes in glomerular filtration because creatinine is accumulated over time. Serum creatinine is a poor marker of renal dysfunction, because the changes are not sensitive or specific to minor alterations in glomerular filtration, appears only when the kidney has lost more than 50% of functional capacity [7].

Methods of functional genomics and proteomics have identified new biomarkers for diagnosis of AKI [8]. These biomarkers make early and accurate diagnosis possible, have many potential applications in anesthesia, can be used to evaluate the effect of new techniques such as robotic surgery, or the effect of medication on renal function and identify patients at risk. Rapid detection of AKI is important to prevent progression to loss of function [9].

Optimal renal biomarker detects renal injury soon and allows stratification of severity in relation to prognosis.

The main characteristics of an ideal biomarker, organ specificity, allows differentiation between intrarenal, prerenal and postrenale causes of acute kidney injury and glomerular injury, being able to recognize the etiology of AKI (hypoxia, toxins, sepsis) [10]. The correlation with the histology of the biopsies, can identify pathological changes in different segments of renal tubules, while the correlation with the intensity of the lesion, gives the possibility to detect early the minor changes and the onset of severe injuries. Such markers should be detectable throughout the renal lesions with well-defined limits, to evaluate the progression or regression of the lesion. The investigations must be noninvazive and tests should be simple and fast, with high accuracy, low cost, allowing repeated measurements. One of the most commonly used biomolecule is neutrophil gelatinase-associated lipocalin (NGAL), a protein from the family of lipocalins [11].

NGAL was isolated from the supernatant of activated human neutrophils, but is expressed in other tissues too: kidney, prostate, digestive and respiratory tract epithelium [12]. It is also expressed in important quantities in adenomas, adenocarcinomas of mammary gland, urothelial carcinomas and inflamed intestinal epithelium [13]. NGAL serum concentrations above 25 μg/l predict AKI [14].

In a model of ischemia-reperfusion, purified recombinant NGAL was administered and improved the morphological and functional changes, with reduction of apoptotic cells and increased proliferation of proximal tubular cells [15]. In animal models of ischemia-reperfusion impairment, NGAL was detected in urine at 2 hours after injury [16].

NGAL is sensitive, specific and highly predictive early biomarker of AKI. Measurements of serum NGAL predict AKI after cardiopulmonary bypass [17], administration of contrast agents [18] and are sensitive but nonspecific predictor of AKI in children in septic shock [19].

The aim of this study was to demonstrate NGAL accuracy in detecting AKI after robotic urogenital surgery under general anesthesia, and to compare the role of serum NGAL with urine NGAL in detecting AKI. Furthermore, the prognostic role of serum NGAL in AKI prediction was investigated.

Material and Method

Patients Selection and Data Collection

A prospective longitudinal observational study, which included 24 patients, hospitalized in Cluj Municipal Hospital Urology Department, between May 2010 - February 2012, scheduled for uro-genital robot assisted laparoscopic surgery, was conducted.

Inclusion criteria were: no history of chronic kidney disease (RIFLE classification at most R), AKIN classification stage 1, hemodynamically and respiratory stable. The study was approved by the Ethics Committee of the "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca. All patients agreed to participate in this study by signing an informed consent form. Patients were informed of the type of surgery and anesthesia they were to receive.

The following data were collected pre- and peri-operative. Pre-operative: demographic data, comorbidities (Charlson Comorbidity Index), physiological scores (Apache II and SOFA), ASA score for anesthesia risk assessment of renal function by RIFLE and AKIN criteria. Peri-operative: intervention duration (h), haemorrhage (ml), cardiovascular (mean BP over 60 mmHg) and respiratory stability (peripheral blood oxygen saturation over 94%).

Acute kidney injury (AKI) was defined by absolute increase of serum creatinine by 0.3 mg/dl (26.4 mmol/l) over baseline levels within 48 hours postoperatively, according to the AKIN criteria. Cut-off value of urine and serum NGAL was considered 150 ng/ml [20].

Serum and Urine Determinations

6 ml of blood and respectively urine were collected preoperatively (at induction), at 6 hours and 12 hours. Each sample was divided into three Eppendorf tubes and frozen at -80ºC. Serum and urine NGAL was determined by ELISA, performed in wells loaded with a monoclonal antibody against human NGAL (NGAL Rapid ELISA Kit (KIT 037). The kit contains several tests, the determination takes about one hour. Postoperative we followed vital signs (BP, pulse, respiration), daily diuresis over 4 days postoperatively, serum creatinine was sampled daily over the first 4 days postoperatively.

Serum NGAL was detected by monoclonal antibody, conjugated with horseradish perioxidase and the reaction is developed with the color reagent. Calibrators, controls and diluted samples were incubated with the conjugate (peroxidase-conjugated antibody antiNGAL). NGAL antibody binds both the well and the antibody of the conjugate, which remains unbound and was removed by washing. Substrate containing TMB-tetrametilbenzidina, was added to each well, and peroxidase-linked antibody antiNGAL developed a color reaction. Chemical and enzymatic reaction was stopped and the color intensity was read at 450 nm in ELISA reader. Color intensity depends on the concentration of NGAL in the sample. The results of the calibrators were used to perform a calibration curve of the concentration of NGAL to be read in evidence.

Statistical Analysis

Qualitative variables were expressed percentage for calculating percent confidence interval of 95% (abbreviated as 95CI%) using an optimized method similar to that presented in [22]. Comparisons of quantitative variables were applying parametric tests when data are normally distributed and nonparametric when data are not normally distributed. More than two samples were compared by ANOVA-test (parametric) or Friedman test (Nonparametric) while paired samples were compared using Student test (parametric) or Wilcoxon test (non-parametric). Comparison between two independent samples was made with the Student test for normally distributed data, respectively Mann-Whitney test for data not normally distributed. P values <0.05 were considered statistically significant. Statistical analysis was accomplished with SPSS Version 18 and Microsoft Excel.

Results

Twenty-four patients were enrolled, 23 men and one woman. Patients enrolled were aged

between 22 and 72 years, with an average of 59.42±11.04 years and a median of 62.5 years (Q1 = 56 years and Q3 = 65 years, where Q1 and Q3 are first and third quatiles). Most patients were undergoing prostatectomy (Figure 1).

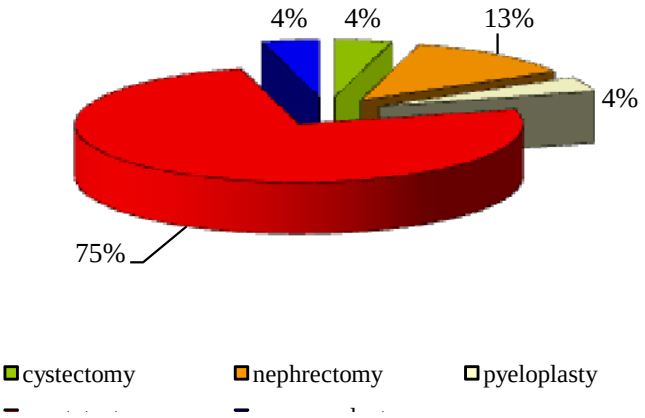

Figure 1. Distribution of performed interventions

Comorbidity score had values between 0 and 10, with a median of 3 (Q1 = 2, Q3 = 5). Apache score had values between 1 and 10, with a median of 6 (Q1 = 4, Q3 = 8). Four patients (17%, CI95% [5% -39%]) had SOFA score of 1.

The risk of anesthesia according to type of anesthesia is presented in Table 1.

Table 1. Pivot desfluran general anesthesia versus sevoflurane pivot general anesthesia

	AG-D n (% [95%CI for])	AG-S n (% [95%CI for %])	Total
ASA = I	2 (13 [0-40])	2 (22 [1-65])	4
ASA = II	12 (80 [54-93])	5 (56 [23-88])	17
ASA = III	1 (7 [0-33])	2 (22% [1-65])	3
Total	15	9	24

AG-D = pivot desfluran general anesthesia, AG-S = sevoflurane pivot general anesthesia; n=Number of Patients,% = Percentage, 95%CI = 95% confidence interval

The mean duration of surgery was 5.19±1.01 hours and ranged between 4 and 7 hours. Haemorrhage ranged between 200 and 400 ml, with a median of 250 ml (Q1 = 200 ml, Q3 = 300ml).

Nine patients (38% [17-58]) presented acute kidney injury, defined as absolute increase of serum creatinine by 0.3 mg / dL above baseline within 48 hours of surgery.

Descriptive parameters of serum and urine NGAL, depending on the presence / absence of acute kidney injury are presented in Table 2. Significant test results for differences in serum and urine NGAL in patients with AKI+ versus AKI- are also presented in Table 2.

Evolution of serum and urine determinations in patients with and without AKI are shown in Figures 2 and 3.

Friedman test identified significant differences in serum NGAL evaluation when all patients were included in the analysis (n = 24, Friedman statistics = 17.828, p = 0.00013). This significant difference is observed for both groups (AKI positive - n = 9, Friedman statistics = 8.629, p = 0.013376 & AKI negative - n = 15, Friedman statistics = 9.414, p = 0.009033).

No statistically significant differences were found for urine NGAL determinations (n = 24, Statistics Friedman = 0.250, p = 0.8825). Pair comparisons of serum and urine NGAL values measurements at baseline, 6h and 12h are shown in Table 3.

Table 2. NGAL in independent samples: descriptive and inferential parameters (Mann-Whitney test)

NGAL (ng/ml)	AKI	Median	Q1	Q3	Mann-Whitney	p-value
Serum - baseline	+ [a]	200	130	380	35	0.055
	- [b]	134	89.5	237		
Serum - 6h	+	450	250	510	29	0.021
	-	170	510	250		
Serum - 12h	+	360	260	560	33	0.041
	-	180	255	255		
Urine - baseline	+	25	20	20	64	0.861
	-	30	52.5	52.5		
Urine - 6h	+	55	25	25	35	0.055
	-	20	10	48		
Urine - 12h	+	30	22	22	63	0.815
	-	29	62.5	62.5		

[a] number of patients with AKI (9 patients); [b] number of patients without AKI (15 patients, except NGALs baseline where was just 14 measurements);
Q1 = percentile 25%; Q3 = percentile 75%

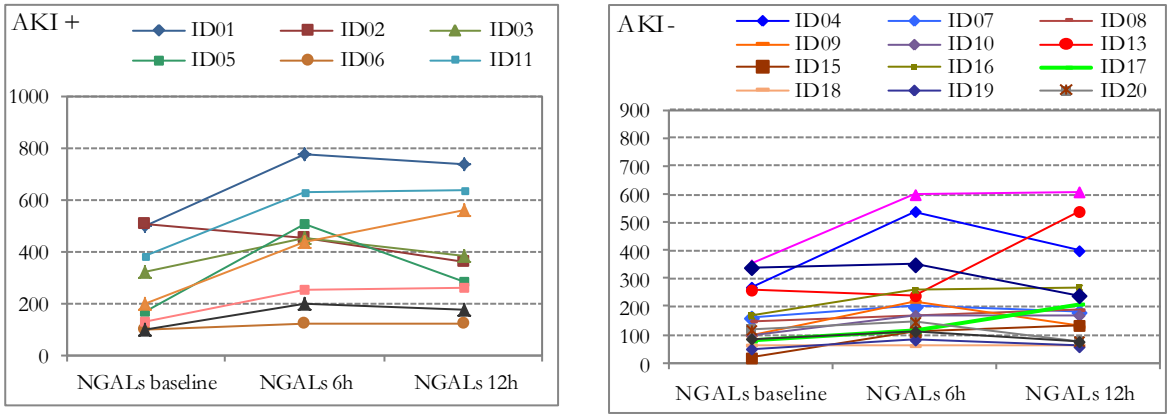

Figure 2. Serum NGAL: AKI vs non-AKI (ID = identification for patients)

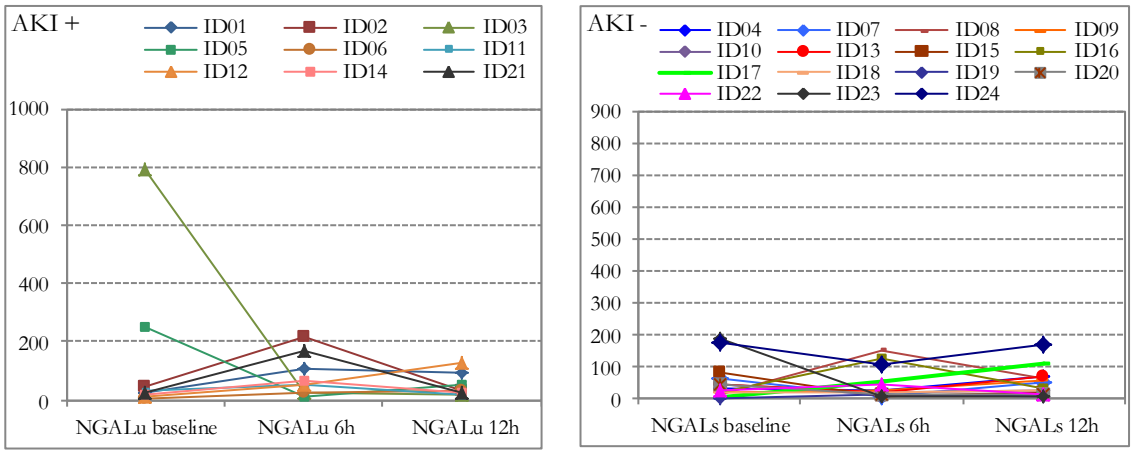

Figure 3. Urine NGAL: AKI vs non-AKI (ID = identification for patients)

Table 3. Serum and urine NGAL - paired comparisons Wilcoxon test

AKI	Wilcoxon test	NGALs1-NGALs0	NGALs2-NGALs0	NGALs2-NGALs1	NGALu1-NGALu0	NGALu2-NGALu0	NGALu2-NGALu1
Positive	Statistics	-2.429[a]	-1.955[a]	-1.122[b]	-0.653[a]	-0.296[b]	-1.009[b]
	p-value	0.015	0.051	0.262	0.514	0.767	0.313
Negative	Statistics	-3.153[a]	-2.323[a]	-0.660[b]	-0.569[b]	-0.170[a]	-1.023[a]
	p-value	0.002	0.020	0.509	0.570	0.865	0.307

NGALs0 = NGAL serum baseline; NGALs1 = NGAL serum 6h; NGALs2 = NGAL serum 12h;
NGALu0 = NGAL urinar baseline; NGALu1 = NGAL urinar 6h; NGALu2 = NGAL urinar 12h;
a = negative ranks; b = positive ranks;

Correlation matrix obtained for NGAL is presented in Table 4. Correlations between NGALs0-NGALu1 respectively NGALs2-NGALu1 was observed only in patients without AKI. Negative correlation NGALs1- NGALu1 was found only in AKI positive patients.

Table 4. Serum and urine NGAL - paired comparisons Wilcoxon test

	NGALs 0	NGALs 1	NGALs 2	NGALu 0	NGALu 1	NGALu 2
NGALs 0		0.924	0.864	0.342	0.474	0.325
NGALs 1	< 0.0001		0.901	0.302	0.438	0.287
NGALs 2	< 0.0001	< 0.0001		0.172	0.482	0.385
NGALu 0	0.102	0.152	0.422		-0.345	-0.179
NGALu 1	0.019	0.032	0.017	0.098		0.401
NGALu 2	0.121	0.175	0.063	0.403	0.052	

s = serum; u = urine; 0 = baseline; 1 = 6h; 2 = 12h

The predictive value of NGAL was analyzed by cross-tabulation, as shown in Table 5. Seventy four percent (95%CI [52-91]) of patients had values over cut-off at 6 hours and 12 hours.

Table 5. Predictive analysis of serum NGAL

Serum NGAL	Value	AKI positive	AKI negative	p-value[a]	OR [95%CI]
baseline	≥150	6	6	0.206	3.000 [0.5326; 19.898]
	<150	3	9		
6h	≥150	8	9	0.1487	5.3333 [0.5234; 54.3464]
	<150	1	6		
12h	≥150	8	9	0.1487	5.3333 [0.5234; 54.3464]
	<150	1	6		

[a] = Fisher exact test; OR = odds ratio; 95%CI = 95% confidence interval

Discussion

Renal function, assessed in terms of serum NGAL variations, changes in urogenital robotic assisted laparoscopic surgery under general anesthesia. The incidence of acute kidney injury, defined by increase of serum creatinine by 0.3 mg / dl within 48 hours, was 38%. The incidence of AKI in other studies are presented in Table 6.

Table 6. The incidence of AKI

Pacients studied	Incidence of AKI (%)
Intensive care [22]	36
Bone marrow transplant [23]	33.5
Burns [24]	33.7
Liver Transplant [25]	35.9
Septic [26]	37.45

Depending on the presence or absence of acute kidney injury, patients were distributed into two groups AKI+ and AKI-.

Accuracy of NGAL in the Diagnosis of Acute Kidney Injury

NGALs identified renal function changes in laparoscopic assisted robotic surgery. Friedman test performed on the whole group, showed a statistically significant change ($p = 0.00013$) of NGALs. The statistical significance is maintained when analyzed in groups AKI +/- (0.0133 vs. 0.009).

There were no differences between the basal values in the 2 groups: without acute kidney injury (AKI-) and patients who experienced acute renal injury (AKI +). The independent sample analysis by Mann-Whitney showed no statistically significant differences ($p> 0.05$) between baseline values of serum NGAL in the groups.

Serum NGAL reaches a peak value up to 6 hours, after it begines to decline. The statistical significance of the p value is more important at 6 hours comparative to 12 hours (0.021 vs. 0.041). Increase of serum NGAL in 6-12 hours interval is not significant ($p> 0.05$). In other studies NGALs changes were detected at 2 hours from the injury [27]. For an early diagnosis of AKI in robotic surgery, based upon NGAL changes, further studies are necessary, to determine NGAL at shorter intervals from injury, at 2-4 hours.

A significant increase of NGALs was also detected by comparing the pair NGAL values with Wilcoxon test. NGALs changes were important at 6 hours compared with baseline values and in the AKI- group (AKI- 0.002 vs AKI+ 0.015).

Renal function modification, assessed by changes in serum NGAL, is maintained at 12 hours compared with baseline, with lower significance than the values recorded at 6 hours. The p-value was lower in AKI- group compared with AKI+ (AKI- 0.02 vs AKI + 0.05).

NGALs detects more significant changes in NGAL- patients, both at 6 hours and 12 hours, but the median value is higher in the AKI+ patients, at 6 hours (AKI+ 450 vs. AKI- 170) and 12 hours (AKI+ 360 vs. AKI- 180).

The importance of the data obtained was highlighted by the significant correlation between basal NGALs at 6 hours and 12 hours, with a statistical significance of $p <0.0001$.

Comparing the Role of Serum NGAL and Urine NGAL in Detecting Acute Kidney Injury after Robotic Assisted Laparoscopic Surgery under General Anesthesia

Urine NGAL (NGALu) has no statistical important role to detect AKI in robotic assisted laparoscopic interventions, under general anesthesia, for urological pathology.

No significant changes were identified in NGALu determinations by the Friedman test, when all patients were included.

There were no statistically significant differences of urine NGAL (NGALu) on independent samples by Mann-Whitney test. P-value was above 0.05 at baseline and 12 hours.

NGALu at 6 hours seems to have a more significant role in the detection of kidney function alteration, there was a p value close to 0.05 (0.055) and Spearman correlation matrix analysis, revealed a correlation between NGALu at 6 hours with NGALs values, both basal, at 6 hours and 12 hours ($p <0.05$).

In robot assisted laparoscopic surgery for urological pathology, urine NGAL values reach a peak before 6 hours and the values were in decline at our measurements. In the literature there are studies that determine changes of NGALu at 2 hours from injury, such as cardiopulmonary bypass [28] or contrast nephropathy [29]. The discrepancy between the literature data and those obtained in our study is probably due to the fact that most interventions were robotic assisted laparoscopic prostatectomies, in which the hydration is restricted and urine samples were not relevant.

Renal function modification was detected by variations of uNGAL. Median values were higher in patients AKI +, but the difference is not significant ($p>0.05$).

There were no significant modifications of NGALu, at 6 and 12 hours, when compared to baseline, with Wilcoxon test. Studies that have investigated the role of NGALu in early detection of AKI, demonstrated his accuracy in diagnosis. Data from studies are represented in Table 7 [30].

Table 7. Statistical characteristics of urine NGAL

Domain studied	Cut-off	Sensitivity (%)	Specificity (%)	AUC-ROC
Cardiovascular surgery	>150ng/ml	73	74	77
Contrast nephropathy	>100ng/ml	90	99	97
Intensive Care	>0.2ng/mg cr	78	67	79

Further studies are needed to investigate the accuracy of NGALu for detecting AKI in urologic robotic surgery for various pathologies separately, such as prostatectomy, nephrectomy or cystectomy.

There are discussions regarding the normal value of NGALu. NGALu value (95th percentile) of 107 ng/ml was determined by a study in Ireland [31], conducted on 174 healthy volunteers. There was a difference in NGALu value reported to urine creatinine in age groups under 40 years and 60-88 years old. Whether NGAL should be normalized to creatinine is still an area of debate [32].

Determining the Role of NGALs in the Prognosis of Acute Kidney Injury

NGALs values were not predictive for the occurrence of AKI in robotic assisted laparoscopic surgery for urogenital diseases. Predictive value of NGALs was analyzed by cross-tabulation. Cut-off value was considered 150ng/ml. The result was a higher risk, but without statistical significance, $p > 0.05$. The risc was higher for NGALs at 6 and 12 hours (OR 5.33 for NGAL 6 and 12 hours vs OR 3 for baseline).

In literature, studies on the predictive value of NGALs/u in cardiovascular surgery, with AUC-ROC method (area under curve-receiver operator curve) had significant results. Cut-off value of 150ng/ml, measured at 6 hours AUC-ROC (95% CI) was 0.8 (0.63-0.96), with sensitivity of 79% and specificity of 78% [33].

Limit of the study was that AKI definition used was based on creatinine changes. In further studies, more sensitive AKI definitions should be evaluated, using markers others than serum creatinine and urine output.

Conclusions

Renal function changes in robotic assisted laparoscopic surgery for urogenital disorders in general anesthesia. The incidence of acute kidney injury was 38%.

The modifications of the NGAL levels, measured at 6 hours and 12 hours from the induction of anesthesia, were significant with more importance at 6 hours and in patients without acute kidney injury.

NGALu does not have a statistical significance in early detection of AKI in robotic assisted laparoscopic interventions for urological disorders. No significant changes were identified in urine NGAL measurements.

NGALs has no predictive value for AKI, but the risk to develop AKI is 3 times higher for NGALs baseline and 5 times higher for NGALs at 6 hours and 12 hours, with a cut-off of 150 ng/ml.

Earlier diagnosis and intervention should significantly reduce the morbidity and mortality associated with AKI. The NGAL tests are now becoming available in the clinical practice, and will change the diagnosis and management of AKI.

Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not adequately supported by the data.

Ethical Issues

The study has been approved by the Ethical Committee of the "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca.

Conflict of Interest

The authors declare that they have no conflict of interest.

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