The variation of selective uNGAL levels after robot-assisted partial nephrectomy: Early results of a prospective single center study

Ottavio Colamónico, Giuseppe Carato, Edmondo Ceci, Marcello Scarcia, Michele Zazzara, Mario Dassira, Angelo Porreca, Giuseppe M. Ludovico

1 Urology Department, Ospedale Generale “F. Miulli”, Acquaviva delle Fonti, Bari, Italy;
2 Specialistic Clinical Biochemistry Department, Ospedale Generale “F. Miulli”, Acquaviva delle Fonti, Bari, Italy;
3 Nuclear Medicine Department, Ospedale Generale “F. Miulli”, Acquaviva delle Fonti, Bari, Italy;
4 Urology Department, Policlinico Abano Terme, Padova, Italy.

Summary

Objectives: Acute kidney injury (AKI) secondary to nephron-sparing surgery represents a significant problem in order to preserve renal function. Since serum creatinine alone underestimates the early detection of AKI several biomarker have been investigated. Neutrophil Gelatinase-Associated Lipocalin (NGAL) is considered a good biomarker for AKI.

Materials and methods: We report our experience in 28 patients affected by localized renal cell carcinoma and submitted to robot-assisted partial nephrectomy (RAPN). In each patient selective urinary NGAL levels were dosed before surgery, then 2 and 48 hours after the procedure, through a ureteral catheter inserted into the excretory axis of the operated kidney. Moreover, we evaluated split renal function of the preserved renal parenchyma by a 99mTc-DTPA renal scintigraphy, performed before surgery and three months later.

Results: AKI was diagnosed, according to internationally criteria, in 3 patients (10.7%). The baseline selective urinary NGAL level was 20.02 ng/ml. This level significantly increased after surgery with a selective urinary NGAL level that reached 36.36 ng/ml (p < 0.0001). Moreover, a significant reduction in 99mTc-DTPA clearance of the operated kidneys after three months was detected (p < 0.0001).

Conclusions: Selective urinary NGAL assay represent a sensitive biomarker of acute kidney injury after robotic nephron sparing surgery, capable of predicting the functional outcome of the operated kidney.

Key words: Neutrophil Gelatinase-Associated Lipocalin (NGAL); Acute kidney injury (AKI); Robot-assisted partial nephrectomy (RAPN).

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Introduction

Partial nephrectomy (PN) is the treatment of choice for cT1 (< 7 cm) renal tumors because has demonstrated to offer oncological control equal to that of radical nephrectomy (RN) with superior functional outcomes (1-3).

The major goal of the nephron-sparing surgery is maximizing renal function preservation. The three main drivers of post-PN functional recovery, in order of importance, are pre-PN function, remnant vascularized nephron mass, and ischemia time (4). Nowadays robot-assisted partial nephrectomy (RAPN) is generally preferred by several worldwide centers (5-6) which can performed either with or without hilar clamping. However unclamping hilar control techniques seem to be safe and feasible approaches, with potentially superior functional outcomes, and non-inferior oncological outcomes, when compared with main artery clamping (7). The direct surgical injury and nephron loss that occurs during PN are associated with the risk of occurrence of acute kidney injury (AKI) and chronic kidney disease (CKD). Current criteria for AKI diagnosis and classification depend on serum creatinine (sCr) changes and urine output. (8) Unfortunately, the evaluation of kidney injury by sCr alone underestimates the early diagnosis of AKI, a serious complication after renal surgery, associated with prolonged hospitalization, high morbidity and mortality. The early phase of AKI is accompanied with few symptoms or may be completely asymptomatic (9). Several biomarkers have been investigated in order to identify and anticipate the diagnosis of AKI. Among the others Neutrophil Gelatinase-Associated Lipocalin (NGAL) was extensively evaluated as biomarker of AKI and predictor of CKD (10, 11). NGAL is a ubiquitous 25-KDa protein which expression increases greatly in the presence of a renal damage after ischemia reperfusion injury and nephrotoxicity (12). Nowadays NGAL is considered a biomarker for AKI that has been extensively evaluated in adult and pediatric cardiopulmonary bypass patients (13), kidney transplant patients (14), and patients in intensive care units (15). Production of NGAL is upregulated following renal injury, and consequently detectable in serum and urine hours prior to sCr increases (16). The aim of this study was to evaluate urinary NGAL (uNGAL) both as a marker for early AKI in patients undergoing RAPN for a cT1 renal cell carcinoma and as a marker able to predict the loss of function of the operated kidney.

Materials and methods

In this prospective study 28 patients undergoing partial robot-assisted nephrectomy for cT1 renal cell carcinoma...
at “Francesco M utili” Hospital from June 2017 to December 2017 were enrolled. After approval from the institutional review board, we obtained written consent from all patients. The preoperative clinical tumor staging workup included computed tomography (CT) or magnetic resonance imaging (MRI) of the abdomen and pelvis and chest radiography. We included patients with a solitary renal cortical tumour of ≤ 7 cm and imaging of a normal contralateral kidney before surgery. The patient's age, gender, Charlson comorbidity index score, preoperative sCr, preoperative eGFR: estimated Glomerular Filtration Rate (eGFR), intra and postoperative data and pathologic features (tumor size, PADUA score, histologic type, and Fuhrman’s nuclear grade) were collected. We evaluated the post-operative onset of AKI according to KDIGO criteria (8). In each patient was collected serum Neutrophil Gelatinase-Associated Lipocalin (sNGAL) and selective urinary Neutrophil Gelatinase-Associated Lipocalin (uNGAL) before surgery, 2 and 48 hours after nephron-sparing surgery. In particular uNGAL was dosed on the urine collected selectively through a ureteral catheter inserted into the excretory axis of the operated kidney, before surgery. NGAL was measured into the serum and urine with a commercially available enzyme-linked immunosorbent assay kit (Human NGAL ELISA kit- KIT 036 RUO- Bioporto Diagnostics). The assay is a sandwich ELISA performed in microwells coated with a monoclonal antibody to human NGAL. Bound NGAL is detected with another monoclonal NGAL antibody labeled with biotin and the assay is developed with horseradish peroxidase (HRP)-conjugated streptavidin. The enzymatic reaction is stopped chemically, and the color intensity is read at 450 nm. Furthermore preoperatively the split renal function of the operated kidneys was measured by a 99mTc-DTPA renal scan. Sequential kidney scintigraphy is a method of choice for both static and dynamic determination of kidney function separately. Statistical Analysis: We used one-way ANOVA in order to analyze post-operative dosage variations of uNGAL and sNGAL as well as compare functional outcomes after on-clamp or off-clamp robot-assisted partial nephrectomy (RAPN). Statistical significance was considered at p < 0.05. Linear regression was applied to determine the correlation of selective urinary NGAL assay 48 hours after surgery with the variation of 99mTc-DTPA clearance three months after surgery. The analysis was performed by using the statistical MedCalc software (Version 18.2.1, MedCalc, Inc., Belgium).

**RESULTS**

Twenty-eight patients were included, mean age was 62.6 (35-88) years. Table 1 resumes baseline characteristics and main intraoperative and perioperative data (Table 1). Seventeen patients underwent off-clamp RAPN while the remaining eleven patients were submitted to on-clamp RAPN. The choice of hilar clamping during RAPN was performed according to the surgeon’s experience and the characteristics of the tumor. In patients who underwent on-clamp RAPN, the mean warm ischemia time was 12.6 minutes (Table 2).

**Table 1.**

Pre- and peri-operative characteristics of patients undergoing RAPN for cT1 RCC.

| Sex          | Male: 15 (53.5%) | Female: 13 (46.5%) |
|--------------|------------------|--------------------|
| Age          | 62.6 years (35-88) |
| RCC histotype| Clear cell RCC: 24 (85.7%) | Chromophobe RCC: 3 (10.7%) | Papillary RCC: 1 (3.6%) |
| Fuhrman grade| I: 15 (53.5%) | II: 13 (46.5%) |
| pT1          | a: 22 (78.5%) | b: 6 (21.5%) |
| Mean tumor diameter (cm) | 3.1 (1.2-6.5) |
| PADUA score  | 6-7: 15 (53.6%) | ≥ 8: 10 (35.7%) | ≥ 10: 3 (10.7%) |
| Surgical technique | Off-clamp RAPN: 17 (60.7%) | On-clamp RAPN: 11 (39.3%) |
| Medium Warm Ischemia Time (min) | 12.6 (7-22) |
| Post-operative AKI | 3 (10.7%) |
| Charlson Comorbidity Index | 2.32 (0-6) |
| Preoperative eGFR (ml/min/1.73 m²) | 87.03 (34-115) |
| Preoperative sCr (mg/dl) | 1.07 (0.63-1.73) |
| Preoperative uNGAL (ng/ml) | 20.02 (36.8-4.9) |
| Preoperative operated kidney CI | 37.45 (58.7-14.2) |
| 99mTc-DTPA (ml/min) | RCC = renal cell carcinoma; RAPN: Robot-Assisted Partial Nephrectomy; AKI = Acute Kidney Injury; eGFR = estimated glomerular filtration rate; sCr = serum creatinine; uNGAL = urinary Neutrophil Gelatinase-Associated Lipocalin; CI = clearance; 99mTc-DTPA = Technetium-99m-diethylenetriaminepentaacetic acid. |
The sNGAL baseline level was 169.5 ng/ml and did not change postoperatively (p = 0.13). The selective uNGAL baseline level was 20.02 ng/ml and showed a not negligible increase at 2 and 48 hours after surgery (Figure 1). Variation after 48 hours after surgery was statistically significant (p < 0.0001). However no statistically significant differences emerged between patients subjected to on-clamp vs off-clamp RAPN (66.17 ng/ml vs 50.02 ng/ml; p = 0.242). According to KDIGO criteria for AKI, we detected postoperative AKI in 3 of 28 patients, using sCr dosage 48 hours after surgery. Comparing the pre-operative creatinine with the 48-hours postoperative one, no statistically significant difference was found (p = 0.0669). Before comparing patients undergoing on-clamp and off-clamp robot-assisted partial nephrectomy (Table 2), we evaluated the homogeneity of the two groups through ANOVA. They were homogeneous for eGFR pre-intervention (p = 0.88), preoperative sCr (p = 0.218), maximum tumor size (p = 0.83), PADUA score (p = 0.07), preoperative uNGAL (p = 0.07), Charlson Comorbidity Index (p = 0.13), age (p = 0.09) and pre-operative selective 99m TC-DTPA clearance (p = 0.831). Evaluating postoperative functional outcome of the operated kidneys at three months we highlighted a statistically significant reduction in 99mTC-DTPA clearance of the operated kidneys 3 months after surgery (p < 0.0001), with a mean functional loss of the operated kidneys by 8.2% (mean change in 99mTC-DTPA clearance of -3.081 ml/min). Stratifying the cohort according to the execution of clamping technique (on-clamp vs off-clamp RAPN), there was no statistically significant differences between the two groups (p = 0.414) as far as the variation of 99mTC-DTPA clearance of the operated kidneys. Finally, we evaluated the correlation between the variation in 99mTC-DTPA clearance 3 months after surgery with the selective dosage of uNGAL at 48 hours from surgery (Figure 2), finding a statistically significant association between the two variables (R² = 0.2391, p = 0.0083). This correlation has not been highlighted by comparing the variation in 99mTC-DTPA clearance 3 months after surgery with sCr dosage 48 hours postoperatively (R² = 0.12, p = 0.0669).

**DISCUSSION**

Urological patients represent a population at risk of AKI which can affect long-term renal function (17). The risk of occurrence of AKI in patients undergoing partial/radical nephrectomy and nephroureterectomy is about 43.1% (18). Currently, AKI is defined according to KDIGO criteria based on sCr changes and urine output, which arise after renal damage (8). AKI observed in renal surgery patients is largely related to direct renal damage. In particular, after a partial nephrectomy, AKI is caused by direct removal of renal parenchyma and damage of the remaining tissue from hyperfiltration or ischemia (19, 20). AKI does not act exclusively on the renal parenchyma but also systemically through the release of inflammatory cytokines (21). The post-operative onset of AKI leads to an increase in post-surgical complications, a lengthening of hospitalization time, an increase in the postoperative mortality rate and a significant increase in health care expenditure with an additional risk of CKD (9, 22, 23). Since traditional definitions of AKI seem to be not very sensitive until the healthy nephrons are reduced by 50%, a growing interest towards biomarkers able to evaluate even slight worsening of renal function represents an expanding research area (24, 25).

Neutrophil gelatinase-associated lipocalin (NGAL) represents an acute renal injury marker that the latest Acute Dialysis Quality Initiative (ADQI) guidelines recommend use in patients with suspected AKI (26). Baseline reference values of sNGAL are 86.3 ng/ml in men and 88.9 ng/ml in women while uNGAL has a reference value of 5.7-17.7 ng/ml, but they may increase > 10-fold in serum and > 100-fold in urine following an acute injury. Abassi has shown that the severity of acute renal injury after nephron sparing surgery is quantitatively correlated to the urinary dosing of NGAL (28). In contrast, Spremkle highlighted that changes in urinary NGAL dosage of patients undergoing partial nephrectomy are comparable to those of patients undergoing thoracic surgery (29).
In addition, Kyo Chul Koo examining 176 patients who underwent partial open and laparoscopic nephrectomy, had not shown that uNGAL could represent a predictive marker of both postoperative AKI and CKD 6 months after surgery (30). To the best of our knowledge the current report represents the first prospective study that evaluates postoperative AKI and the functional outcome after 3 months from surgery, adopting selective uNGAL assay. First of all, we showed a statistically significant increase in the selective dosage of uNGAL after 48 hours from surgery. This result was not confirmed for the serum NGAL assay after 48 hours from surgery. Probably sNGAL dosage is significantly affected by postoperative blood loss (not considered in this study), as well as could be influenced by the patient’s hydration status. For this reason, we decided to focus our interest on the uNGAL. Comparing selective uNGAL assay 48 hours after surgery between patients undergoing on-clamp and off-clamp RAPN, we did not find a statistically significant difference. In fact, it seems that hilar clamping does not influence the increase in selective uNGAL dosage and therefore the severity of the acute post-operative renal injury. On the other hand, the diagnosis of AKI using the KDIGO criteria allowed to diagnose postoperative renal injury in only 3 patients, suggesting a lack of sensitivity of the diagnostic criteria worldwide used for AKI. Later we highlighted a worsening of renal function of the operated kidneys 3 months after surgery, through a sequential renal scintigraphy performed before and 3 months after surgery. This result should be explained not as a failure of nephron sparing surgery but as an effect of the high sensitivity of renal scintigraphy in detecting even small changes in renal function compared to the use of sCr. Indeed, the curvilinear relationship between serum creatinine and eGFR may lead to the lack of detection of early stages of AKI or CKD (31). The choice of the third post-operative month for the execution of the control scintigraphy, follows the KDIGO recommendations (8). Comparing patients undergoing on-clamp and off-clamp RAPN, there are no differences in losses of $^{99m}$Tc-DTPA clearance 3 months after surgery. This result could be due either to the short post-operative follow-up period but also to the limited warm ischemia time of on-clamp procedures (the mean warm ischemia time was 12.6 minutes). As we know, warm ischemia time is an important, modifiable predictor of postoperative renal function. In particular, warm ischemia time should not exceed 25 minutes, to avoid a short and long-term reduction in renal function (32). The statistically significant association between the reduction in $^{99m}$Tc-DTPA clearance 3 months after surgery with the selective dosage of uNGAL at 48 hours from surgery, highlighted a direct relationship between these two predictors. The limitations of this report should be acknowledged. First, the small number of patients as well as the short time of postoperative follow-up (3 months) limited the power of the analyses. Second, the normalized uNGAL (the ratio of urine NGAL to urine creatinine) was not used in this study; moreover, the postoperative urine collection for uNGAL was performed selectively through a ureteral catheter inserted into the excretory axis of the operated kidney, unlike previous studies where urine was collected from the urethral catheter (28-30). Further studies are needed to understand the clinical use of the uNGAL although it seems clear the importance of implementing the definition of AKI with the introduction of new biomarkers. The importance of reno-protective surgery is not in question in this report but we tried to demonstrate the lack of sensitivity of traditional methods. Our findings support the concept that the development of reno-protective techniques can prevent the onset of small changes in postoperative renal function, not detected with the sCr dosage, but which may impact on the functional outcome of the operated kidney.

**CONCLUSIONS**

Selective uNGAL assay represent a sensitive biomarker in detecting postoperative AKI in patients submitted to RAPN for a cT1 renal cell carcinoma. Furthermore, selective uNGAL assay may be consider a predictive biomarker of CKD after nephron-sparing surgery. In our opinion KDIGO criteria for AKI should be implemented with the clinical use of biomarkers such as uNGAL.

**REFERENCES**

1. Lee JH, You CH, Min GE, et al. Comparison of the surgical outcome and renal function between radical and nephron-sparing surgery for renal cell carcinomas. Korean J Urol. 2007; 671.
2. Van Poppel H, Da Pozzo L, Albrecht W A prospective, randomised EORTC intergroup phase 3 study comparing the oncologic outcome of elective nephron-sparing surgery and radical nephrectomy for low-stage renal cell carcinoma. Eur Urol. 2011; 59:543-52.
3. Huang, WC, Elkin EB, Levey AS, et al. Partial nephrectomy versus radical nephrectomy in patients with small renal tumors is there a difference in mortality and cardiovascular outcomes? J Urol. 2009; 181:55-61.
4. Thompson RH, Lane BR, Lohse CM, et al. Renal function after partial nephrectomy: effect of warm ischemia relative to quantity and quality of preserved kidney. Urology. 2012; 793660.
5. Vartolomei MD, Matei DV, Renne G, et al. Long-term oncologic and functional outcomes after robot-assisted partial nephrectomy in elderly patients. Minerva Urol Nefrol. 2019; 71:13-37.
6. Castellucci R, Primiceri G, Castellan P, et al. Trifecta and Pentafecta Rates After Robotic Assisted Partial Nephrectomy: Comparative Study of Patients with Renal Masses < 4 and ≥ 4 cm. J Laparoendosc Adv Surg Tech A. 2018; 28:799803.
7. Cacciamani GE, Medina LG, Gill TS, et al. Impact of Renal Hilar Control on Outcomes of Robotic Partial Nephrectomy: Systematic Review and Cumulative Meta-analysis. Eur Urol Focus. 2018; pii: 524054569(18)300130.
8. KDIGO AKI Working Group. KDIGO clinical practice guideline for acute kidney injury. Kidney Int Suppl. 2012; 21138.
9. Schmid M, Ravi P, Abd-El-Barr AE, et al. Chronic kidney disease and perioperative outcomes in urological oncological surgery. Int J Urol. 2014; 21:12451252.
10. Rysz J, Guba-Brzózka A, Franczyk B, et al. Novel Biomarkers in the Diagnosis of Chronic Kidney Disease and the Prediction of Its Outcome. Int J Mol Sci. 2017; 18 pii: E1702.
11. Antonelli A, Allinovi M, Cocci A, et al. AGILE Group. The Predictive Role of Biomarkers for the Detection of Acute Kidney Injury.
After Partial or Radical Nephrectomy: A Systematic Review of the Literature. Eur Urol Focus. 2018; pii: S2405-4569(18)30293-1.

12. Mishra J, Ma Q, Prada A, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol. 2003; 14:2334-43.

13. Bennett M, Dent CL, Ma Q, 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.

14. Parikh CR, Jani A, Mishra J, et al. Urine NGAL and IL-18 are predictive biomarkers for delayed graft function following kidney transplantation. Am J Transplant. 2006; 6:1639-45.

15. Cruz DN, de Cal M, Garzotto F, et al. Plasma neutrophil gelatinase-associated lipocalin is an early biomarker for acute kidney injury in an adult ICU population. Intensive Care Med. 2010; 36:444-51.

16. Koyner JL, Vaidya VS, Bennett MR, et al. Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. Clin J Am Soc Nephrol. 2010; 5:2154-65.

17. Cho A, Lee JE, Kwon GY, et al. Post-operative acute kidney injury in patients with renal cell carcinoma is a potent risk factor for new-onset chronic kidney disease after radical nephrectomy. Nephrol Dial Transplant. 2011; 26:3496-501.

18. Caddeo G, Williams ST, McIntyre CW, Selby NM. Acute kidney injury in urology patients: incidence, causes and outcomes. Nephrourol Mon. 2013; 5:955-61.

19. Brenner BM, Lawler EV, Mackenzie HS. The hyperfiltration theory: a paradigm shift in nephrology. Kidney Int. 1996; 49:1774-1777.

20. Lane BR, Babineau DC, Poggio ED, et al. Factors predicting renal functional outcome after partial nephrectomy J Urol. 2008; 180:2363-8.

21. Hohe TS, Douglas IS, Klein CL, et al. Acute renal failure after bilateral nephrectomy is associated with cytokine-mediated pulmonary injury. J Am Soc Nephrol. 2007; 18:155-164.

22. Chertow GM, Soroho SH, Paganini EP, et al. Mortality after acute renal failure: models for prognostic stratification and risk adjustment. Kidney Int. 2006; 70:1120-1126.

23. Lameire NH, Bagga A, Cruz D, et al. Acute kidney injury: an increasing global concern. Lancet. 2013; 382:170-9.

24. Slocum JL, Hwang M, Pennathur S. Marking renal injury: can we move beyond serum creatinine? Trans Res. 2012; 159:277-80.

25. Hostetter TH, Olson JL, Renneke HG, et al. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. J Am Soc Nephrol. 2001; 12:1315-1325.

26. Haase M, Bellomo R, Devarajan P, et al. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis. 2009; 54:1012-1024.

27. Schmid M, Dalela D, Tabbaz R, et al. Novel biomarkers of acute kidney injury: Evaluation and evidence in urologic surgery. World J Nephrol. 2015; 4:160-168.

28. Abassi Z, Shalabi A, Sohotnik R, et al. Urinary NGAL and KIM-1: biomarkers for assessment of acute ischemic kidney injury following nephron sparing surgery. J Urol. 2013; 189:1559-1566.

29. Sprengle PC, Wren J, Maschino AG, et al. Urine neutrophil gelatinase associated lipocalin as a marker of acute kidney injury after kidney surgery. J Urol. 2013; 190:159-64.

30. Kyo CK, Jung HH, Hye SL, et al. Accuracy of Urinary Neutrophil Gelatinase-Associated Lipocalin in Quantifying Acute Kidney Injury after Partial Nephrectomy in Patients with Normal Contralateral Kidney. PLoS One. 2015; 10:e0133675.

31. Steubl D, Bloch M, Herbst V, et al. Plasma uromodulin correlates with kidney function and identifies early stages in chronic kidney disease patients. Medicine. 2016, 95, e3011.

32. Volpe A, Blute ML, Ficarra V, et al. Renal Ischemia and Function After Partial Nephrectomy: A Collaborative Review of the Literature Eur Urol. 2013; 68:61-74.

Correspondence
Ottavio Colamonico, MD
ottaviocolamonico@gmail.com
Giuseppe Cardo
Marcello Scarica
Michele Zazzara
Giuseppe M. Ludovico
Urology Department, Ospedale Generale “F. Miulli”
Via Enrico Toti n. 2 Acquaviva delle Fonti (BA) (Italy)

Edmondo Ceci
Specialistic Clinical Biochemistry Department,
Ospedale Generale “F. Miulli”, Acquaviva delle Fonti, Bari (Italy)

Mari Dassira
Nuclear Medicine Department, Ospedale Generale “F. Miulli”,
Acquaviva delle Fonti, Bari, (Italy)

Angelo Porreca
Urology Department, Policlinico Abano Terme, Padova (Italy)