

Early Identification of Acute Kidney Injury after Bariatric Surgery: Role of NGAL and Cystatin C

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Abstract: *Background:* The aim of our study was to evaluate plasmatic and urinary NGAL and serum cystatin C as early diagnostic markers of acute kidney injury in obese patients undergoing bariatric surgery.

Methods: For this this prospective observational study, we recruited 23 patients undergoing gastric by-pass or sleeve gastrectomy, and admitted to the Low Dependence Unit after the surgery. Plasma NGAL (pNGAL), urinary NGAL (uNGAL), serum cystatin C, serum creatinine, and serum urea were measured before surgery as well as 10 h and 24 h after surgery.

Mean values of pNGAL, uNGAL, cystatin C, creatinine, and urea concentrations of pre- and post-surgery periods were compared using Student's t test for paired data. We also evaluated the presence of correlation between modifications of NGAL and cystatin C after surgery and fluid balance, hydration (ml/kg) and diuresis using Pearson's coefficient of correlation.

Results: No patient developed AKI according to the AKIN criteria. pNGAL was significantly higher at T_{10h} than T₀ (p=0.004). There was no significant difference between uNGAL at T₀ and T_{10h} (p=0.53) and between uNGAL at T₀ and T_{24h} (p=0.31). uNGAL at T_{24h} was significantly higher in comparison to T_{10h} (p=0.024). uNGAL concentrations were normal in all patients at every time step.

Cystatin C concentration did not increase after surgery.

Serum creatinine level was significantly higher at T_{48h}, despite being still within the normal range, when compared to T₀ (p=0.038).

Conclusion: Our study shows that pNGAL can reflect mild tubular damage as its levels increase within a few hours from surgery and return to normal limits afterwards. Concerning uNGAL, there is a minimal increase at T_{24h}, when NGAL concentration in plasma has already decreased. Serum cystatin C does not show any relevant kidney changes, or at least, no more than those ones shown by pNGAL.

Keywords: Morbid obese patients, bariatric surgery, acute kidney injury, plasmatic and urinary neutrophil gelatinase-associated lipocalin (NGAL), cystatin C.

BACKGROUND

Acute Kidney Injury in Morbidly Obese Patients

Acute kidney injury (AKI) is a common clinical issue encountered in critically ill patient and is associated with high mortality, increased length of stay and health care costs. Post-operative AKI is not so uncommon in morbidly obese patients. Morbid obesity (MO) is an independent risk factor for chronic kidney disease which is, in turn, a risk factor for

AKI [1]. Obesity-related chronic renal damage presents with glomerulopathy, characterized by glomerulomegaly, mesangial expansion, and secondary focal and segmental glomerulosclerosis [2]. The result of such histological changes is glomerular hyperfiltration, expressed by a supranormal glomerular filtration rate [3]. Morbidly obese patients are oftentimes subjected to comorbidities (e.g., diabetes mellitus and hypertension), which, along with the obesity-related glomerulopathy, put them at higher risk of developing AKI.

The relationship between AKI, body mass index (BMI) and mortality in critically ill patients has not been evaluated in details. The largest epidemiologic studies of AKI in the critically ill did not include subset analyses of this patient

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population [1]. The available data on AKI in morbidly obese patients concern post-operative observational cohorts: the incidence of post-operative AKI in MO patients varies between 2.8% and 14.3% [1].

Bucaloiu and coworkers [1] have summarized the causes of AKI to which MO patients are mostly predisposed:

1. Pre-renal azotemia. Several factors can predispose MO individuals to volume depletion and the development of AKI in the critical care setting. Clinical evaluation of volume status is difficult because MO subjects often show clinical features of volume overload, such as peripheral edema or increased central venous pressure, in the setting of intravascular volume depletion. In this situation, an excessively aggressive diuresis may result in the development of AKI.

2. Rhabdomyolysis. Morbidly obese patients undergoing bariatric or general surgery are at higher risk of pressure-induced rhabdomyolysis. Risk factors associated with the development of rhabdomyolysis are high BMI, presence of comorbidities such as diabetes mellitus and arterial hypertension, pre-operative use of statins, lithotomy position during surgery and prolonged operating time [4-6]. AKI occurs in 33-50% of patients with rhabdomyolysis [7] and is the main cause of mortality among them: routine post-operative CPK measurements and attention to volume status help prevent kidney injury due to rhabdomyolysis [5].

3. Intra-abdominal hypertension and abdominal compartment syndrome (ACS). Intra-abdominal hypertension (IAH) is an independent predictor of AKI in the intensive care setting [8]. Intra-abdominal hypertension and abdominal compartment syndrome (ACS) represent common causes of AKI in the critical care setting.

IAH is defined as an intra-abdominal pressure (IAP) higher than 12 mm Hg on 3 measurements obtained 4 to 6 hours apart [9, 10]. IAH may lead to ACS, defined as IAP higher than 20 mm Hg, with or without an abdominal perfusion pressure lower than 60 mm Hg on 2 measurements obtained 1 to 6 hours apart, associated with a single- or multiple-organ system failure which was not present previously.

ACS is characterized clinically by multi-organ dysfunction, including oliguric or anuric AKI, low cardiac output caused by impaired venous return and increased afterload, decreased respiratory system compliance, intracranial pressure elevation, hypotension, and metabolic acidosis. The mechanisms of oliguria include increased renal vascular resistance and increased intra-parenchymal renal pressures [8-10].

Obese individuals have higher IAP than non-obese individuals: probably they are at a higher risk of developing ACS and subsequently AKI.

Carbon dioxide insufflation into the peritoneal cavity for laparoscopic surgery is associated with increased vascular resistance, decreased cardiac index and transient oliguria [11, 12]. As it is possible to develop reversible oliguric AKI in non-obese patients following laparoscopic surgery [13], we have to take into account the possibility of development of IAH and ACS also in obese patients after laparoscopic surgery.

In the study performed by Weingarten *et al.* [14] the incidence of post-operative AKI in patients undergoing bariatric surgery was 5.8%: most patients developed transient stage I injuries according to the AKIN classification. Risk factors associated with the development of AKI were increased BMI and presence of diabetes.

Yap and colleagues [15] performed a retrospective review of 11,736 patients who underwent cardiac surgery in Australia. Morbid obesity was present in 1.8% of this population and associated with increased risk of postoperative ICU admission, postoperative renal failure, and prolonged need for mechanical ventilation.

The incidence of postoperative renal failure (defined as the presence of at least 2 of the following conditions: increased serum creatinine levels >200 mmol/L, doubling of serum creatinine levels compared with preoperative values, or initiation of RRT) was significantly higher in MO patients as compared with patients of normal weight (8.4% versus 4.4%, $P = 0.006$).

Bucaloiu *et al.* [1] concluded that: firstly, post operative AKI is not uncommon in obese patients; secondly, MO patients are at higher risk of complications in the post-operative period and consequently they require intensive care admission; finally, risk factors for AKI in MO patients are similar to general population, although, as previously shown, they may often be subjected to misleading intravascular volume depletion, post-operative rhabdomyolysis and IAH/ACS.

The aim of this study was to evaluate the diagnostic performance of two novel biomarkers (plasmatic and urinary neutrophil gelatinase-associated lipocalin [pNGAL and uNGAL] and cystatin C) in obese patients undergoing bariatric surgery. We compared perioperative changes of the levels of these novel biomarkers with changes of serum creatinine in order to clarify their role for an early diagnosis of AKI.

Neutrophil Gelatinase-associated Lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) is a member of a superfamily of carrier proteins, called the lipocalins, expressed in granulocytic precursors as well as activated epithelium cells of different types in response to injuries. NGAL is expressed at very low levels in several human tissues, including kidney, trachea, lungs, stomach, and colon. Normal kidney tissue demonstrated minimal NGAL production, mainly in the distal tubular epithelia and the medullary collecting ducts, meaning that these tubules are the site of synthesis in the normal kidney. In contrast, nearly 50% of cortical tubules were NGAL-positive in ischemic or nephrotoxin-damaged kidneys. NGAL was identified as one of the most induced transcripts in the kidney after ischemic injury [16]. NGAL acts as an iron-transporting protein during nephrogenesis. This role seems to be crucial after ischemic injury in two possible ways: NGAL could carry iron recycled from damaged cells and stimulate regeneration of renal epithelial cell or, as an alternative hypothesis, NGAL could remove iron (a reactive and oxidizing ion) from the site of tissue injury, in such a way as to limit iron-mediated apoptosis of renal tubule cell death after ischemia-reperfusion injury. NGAL is induced and

released from the injured distal nephron within a few minutes after a renal insult [17].

NGAL can be measured in both plasma and urine. NGAL is one of the most investigated among novel renal biomarkers, with a promising evidence deriving from data on animal experiments and clinical studies, which overall encompass more than 3,500 cardiac surgery or critically ill patients. NGAL measured immediately at admission to ICU after surgery could identify and stratify patients with acute structural renal damage regardless of their preoperative risk and performed procedure, with an overall good predictive performance [18-20]. NGAL was also studied in septic patients. In a study enrolling adult patients with AKI, both pNGAL and uNGAL were higher in septic versus non septic patients and showed a significant association with worsening of AKI and renal replacement therapy (RRT) initiation [21].

Haase *et al.* performed a systematic review and meta-analysis [22] in order to clarify the predictive value of NGAL for an early diagnosis of AKI: NGAL levels were found to be not only a useful early predictor of AKI but also a prognostic marker for clinical outcomes, such as initiation of RRT and mortality. They concluded that uNGAL and pNGAL levels performed similarly in the early diagnosis of AKI, and both provided a notable advantage compared with serum creatinine.

Koukoulaki *et al.* [23] studied the diagnostic performance of uNGAL in 23 patients with severe obesity who underwent biliopancreatic bypass surgery. In this study, AKI was observed in 2 patients in the immediate postoperative period; one patient required RRT with hemodialysis. In both patients, uNGAL level had increased within the first postoperative hour, before the values of serum creatinine increased. Despite being a small study, it shows that uNGAL can be a potential biomarker of AKI in obese patient.

Cystatin C

Creatinine production changes significantly according to body muscle mass and dietetic factors. It is filtered by the glomeruli, and partly secreted by the renal tubules. This tubular secretion approximately contributes to 20% of the total creatinine excretion by the kidney, and it can increase as GFR decreases. All of these factors explain why serum creatinine concentration may not be a good parameter for an accurate determination of GFR, especially at lower rates.

Cystatin C is produced by nucleated body cells at a constant rate. Cystatin C production is a stable process that is not influenced by renal conditions, increased protein catabolism, or dietetic factors. Moreover, it does not change with age or muscle mass like creatinine does. Its biochemical characteristics allow for free filtration in the renal glomerulus, and subsequent metabolism and reabsorption by the proximal tubule. For these reasons, serum Cystatin C has been suggested to be an ideal endogenous marker of GFR.

GFR can change rapidly in critically ill patients because of renal hypoperfusion secondary to shock or the use of nephrotoxic agents. Despite this, it is not uncommon to see changes in serum creatinine for up to several days until the

stabilization phase is reached. Villa and coworkers [24] analyzed the role of serum cystatin C as a marker of renal function in fifty critically ill patients. They found a poor diagnostic utility of serum creatinine (AUC= 0.694) compared to a previous study [25], maybe because of the aforementioned reasons. However, the diagnostic utility of cystatin C seen in their study (AUC = 0.927) was high and similar to that previously reported by other investigators. The fact that most of their patients (76%) with acute renal dysfunction had high serum cystatin C levels at the time of creatinine clearance evaluation demonstrates that cystatin C is a good marker for application in real time, and suggests that serum cystatin C is a marker of GFR better than serum creatinine in unstable, critically ill patients.

Methods

This was a prospective, observational study of 23 patients subjected to bariatric surgery and admitted to the low dependence unit (LDU) for post-operative monitoring, from July to September 2012.

Patients were enrolled the day before surgery and an informed written consent was obtained from each patient. This study was approved by the Ethics Committee.

All patients were operated at Nuovo S.Chiera Hospital, AOUP, University of Pisa, in Italy. All patients were transferred to the LDU for a period of 24 hours after surgery.

Multiple data elements were collected for each patient and included gender, age, BMI, and type of surgery.

Study Design

Plasma NGAL (pNGAL), urinary NGAL (uNGAL), serum cystatin C, serum creatinine, and serum urea were determined before surgery (T₀: measured in the operating room, after anesthesia induction), 10 h, and 24h after surgery (T_{10h}, T_{24h}: measured in the LDU).

For each patient, upon discharge from the LDU, multiple data from the medical record were collected and included:

1. Intra-operative and post-operative fluid balance (first 24 hours after surgery).
2. Hydration (expressed as ml/kg, calculated from total fluid administered intra-operatively and post-operatively during the first 24 hours after surgery).
3. Intra-operative and post-operative diuresis (first 24 hours after surgery).
4. PaO₂/FiO₂ ratio calculated at LDU admission and 24 h after surgery.
5. Post-operative serum CPK and myoglobin measured during the first post-operative day in order to verify as to whether patients had developed post-operative rhabdomyolysis, which is a potential cause of AKI.

Serum creatinine and urea, measured 48 hours after surgery, were collected from the medical record. At this time the development of AKI through the AKIN classification was evaluated for each patient.

Table 1. Patient characteristics.

N	23
Male sex (%)	6 (26)
Body Weight (kg)	140.6 ± 29.97
Body Mass Index (BMI)	50.6 ± 10.8
Type of surgery: Gastric By-pass (%)	21 (91.3)
Type of surgery: Sleeve Gastrectomy (%)	2 (8.7)
Intra-operative fluid balance (ml)	-913.5 ± 900
Post-operative fluid balance, first 24 hours after surgery (ml)	634 ± 788
Total fluid balance (intra-operative + post-operative) (ml)	-300 ± 1024
Hydration (ml/kg)	40 ± 12
Intra- operative diuresis (ml)	382 ± 223
Post-operative diuresis (ml)	135 ± 36
Diuresis pro kg (ml/kg)	0.95 ± 0.3
PaO ₂ /FiO ₂ rate (at LDU admission)	261 ± 77
PaO ₂ /FiO ₂ rate (24 hours after surgery)	262 ± 72

Statistical Analysis

Plasma NGAL, uNGAL, cystatin C, creatinine, and urea concentrations were expressed as mean ± standard deviation and their values, before and after surgery, were compared using the Student's t test for paired data. Using Pearson's coefficient of correlation, we evaluated the presence of significant correlation between:

1. Difference of NGAL's concentration between T₀ and T_{24h} and total fluid balance (intra-operative fluid balance and post-operative fluid balance in the first 24 h after surgery) in order to verify a correlation between presence of negative fluid balance and increase in pNGAL and/or uNGAL.
2. Difference of NGAL's concentration between T₀ and T_{24h} and hydration (ml/kg) in order to verify as to whether elevated hydration can be protective for kidneys.
3. Difference of NGAL's concentration between T₀ and T_{24h} and diuresis in order to assess if an eventual increase of NGAL is correlated to diuresis reduction.

4. Hydration (ml/kg) and post-operative PaO₂/FiO₂ rate (calculated 24 hours after surgery) in order to verify as to whether an elevated hydration can determine a reduction of PaO₂/FiO₂ rate.

RESULTS

Table 1 shows patient characteristics; Table 2 shows biomarker concentrations measured at time points T₀, T_{10h}, T_{24h} and T_{48h}.

Plasmatic NGAL (Fig. 1)

- pNGAL concentration was significantly higher at T_{10h} compared to that at T₀ (p=0.004)
- There was no significant difference between pNGAL concentration at T₀ and T_{24h} (p=0.076)
- pNGAL was significantly lower at T_{24h} compared to the concentration at T_{10h} (p=0.004)

Plasma NGAL increased within 10 hours after surgery, then decreased over the following hours. Mean pNGAL concentration at T_{10h} was higher than normal levels.

Urinary NGAL (Fig. 2)

- There was no significant difference between uNGAL at T₀ and T_{10h} (p=0.53)
- There was no significant difference between uNGAL at T₀ and T_{24h} (p=0.31)
- uNGAL at T_{24h} was significantly higher compared to T_{10h} (p=0.024)

There was a minimal increase of uNGAL concentrations at T_{24h} compared to T_{10h}; however, uNGAL concentrations were normal in all patients at every time point.

Serum Cystatin C (Fig. 3)

- There was no significant difference between serum cystatin C at T₀ and T_{24h} (p=0.95).
- There was no significant difference between serum cystatin C at T_{10h} and T_{24h} (p=0.81).
- Mean cystatin C at T_{10h} was significantly lower compared to T₀ (p=0.003).

As a result, cystatin C concentrations did not increase after surgery.

Table 2. Concentrations of biomarkers (expressed as mean ± standard deviation) at time points T₀, T_{10h}, T_{24h}, T_{48h}.

	T ₀	T _{10h}	T _{24h}	T _{48h}
pNGAL (ng/ml)	73.71 ± 17.82	181.75 ± 89.3	111.78 ± 70.71	
uNGAL (ng/ml)	12.63 ± 19.46	6.1 ± 3.04	17.03 ± 25.57	
Cystatin C (mg/l)	0.80 ± 0.11	0.67 ± 0.10	0.76 ± 0.17	
Creatinine (mg/dl)	0.72 ± 0.17	0.72 ± 0.15	0.72 ± 0.17	0.76 ± 0.23
Urea (mg/dl)	27.61 ± 5.38	26.75 ± 6.40	22.62 ± 7.29	22.3 ± 7.37

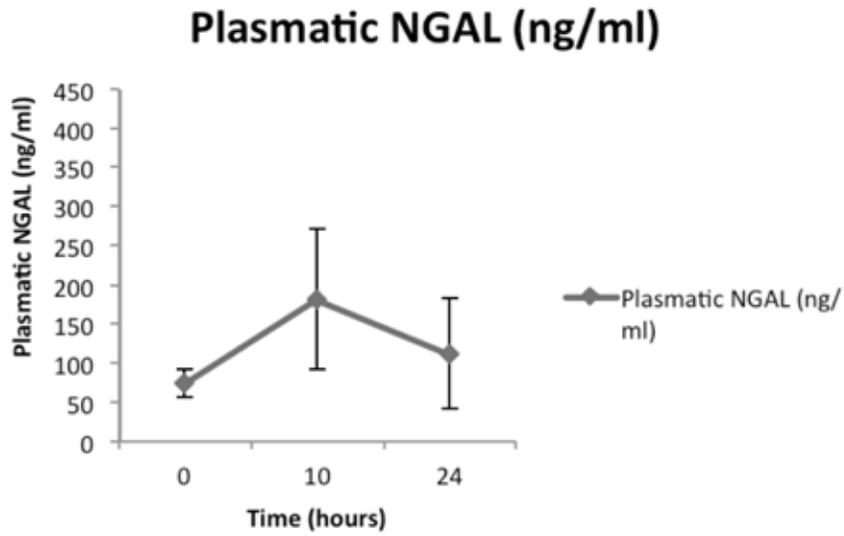


Fig. (1). Plasmatic NGAL concentrations (mean \pm standard deviation) at time points: T₀; T_{10h}; T_{24h}.

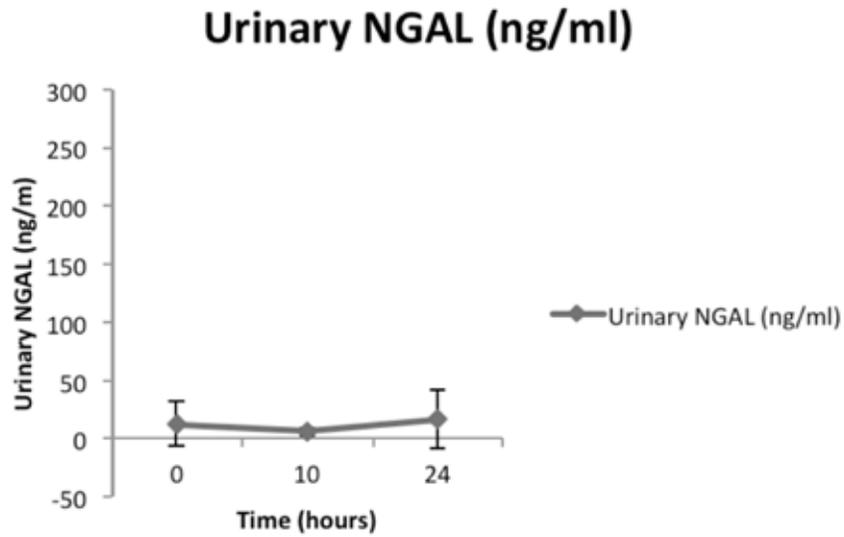


Fig. (2). Urinary NGAL concentrations (mean \pm standard deviation) at time points: T₀; T_{10h}; T_{24h}.

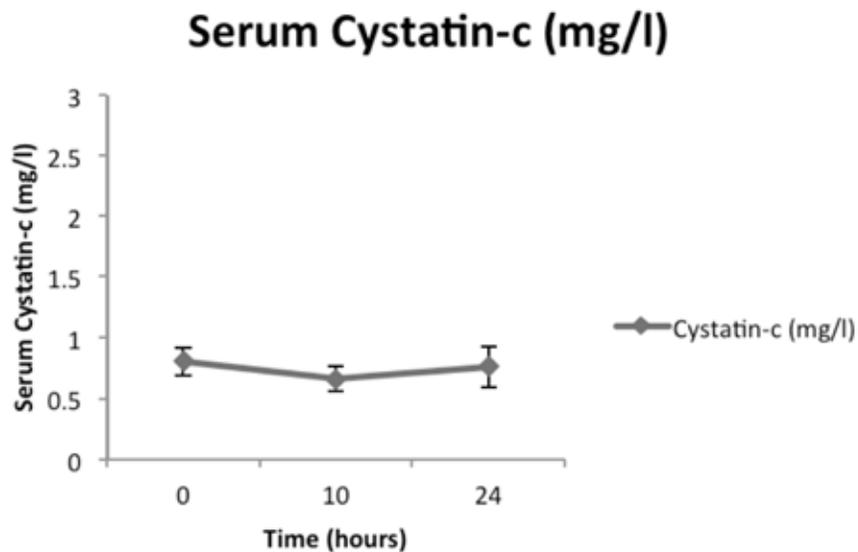


Fig. (3). Serum cystatin C concentrations (mean \pm standard deviation) at time points: T₀; T_{10h}; T_{24h}.

Serum creatinine (mg/dl)

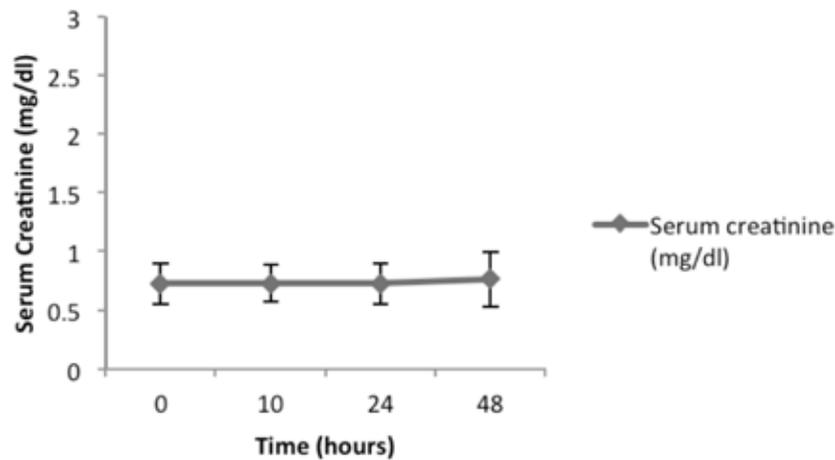


Fig. (4). Serum creatinine concentrations (mean± standard deviation) at time points: T₀; T_{10h}; T_{24h}.

Serum Urea (mg/dl)

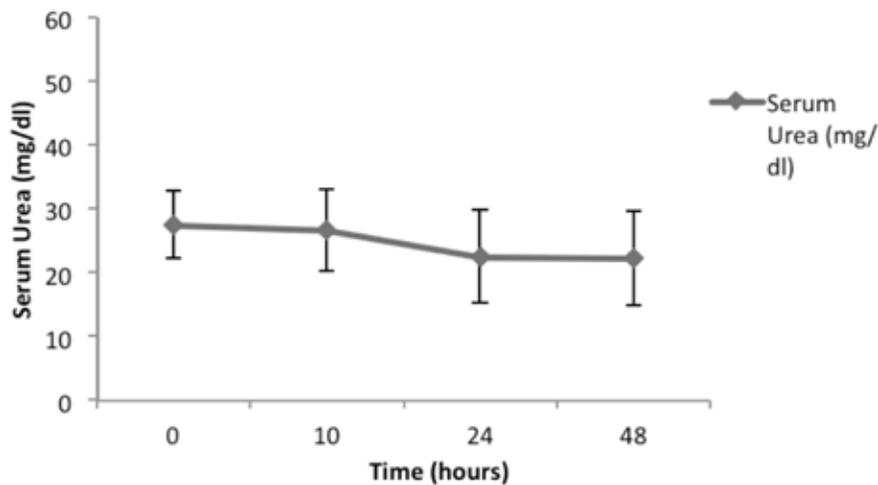


Fig. (5). Serum urea concentrations (mean± standard deviation) at time points: T₀; T_{10h}; T_{24h}.

Serum Creatinine (Fig. 4)

- No patient developed acute kidney injury after surgery according to the AKIN criteria.
- There was no significant difference between serum creatinine at T₀ and T_{10h} (p=0.53), between T_{10h} and T_{24h} (p=0.83), between T₀ and T_{24h} (p=0.68).
- Serum creatinine at T_{48h} was significantly higher compared to T₀ (p=0.038).

Serum Urea (Fig. 5)

- There was no significant difference between serum urea at T₀ and T_{10h} (p=0.6)
- Serum urea at T_{24h} was significantly lower compared to T_{10h} (p=0.001)
- Serum urea at T_{48h} was significantly lower compared to T₀ (p=0.002)

Therefore, there was no increase of serum urea after surgery.

Correlation between pNGAL and uNGAL Courses, Hydration, Intra-operative Fluid Balance and Diuresis

- There was no significant correlation between pNGAL course (difference between its concentration at T₀ and T_{24h}) and total fluid balance (r=-0.14; p=0.6), as well as hydration (r=-0.094; p=0.7).
- There was no significant correlation between uNGAL course (difference between its concentration at T₀ and T_{24h}) and hydration (r=-0.24; p=0.37).
- There was a moderate negative correlation between uNGAL course (difference between its concentration at T₀ and T_{24h}) and total fluid balance (r=-0.457; p=0.054) (Fig. 6).

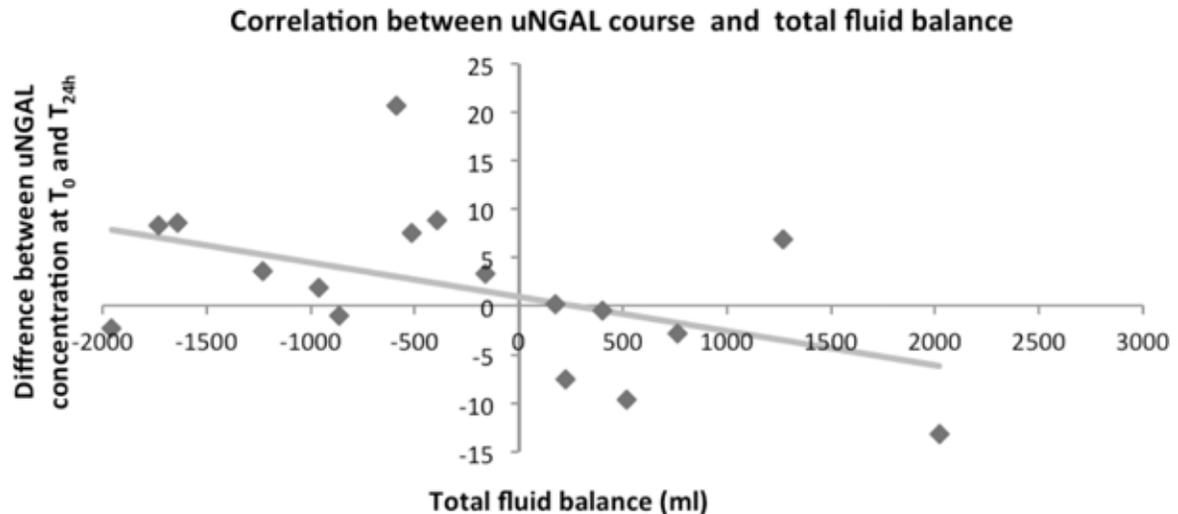


Fig. (6). Analysis of correlation between uNGAL course and total fluid balance.

- There was no significant correlation between plasmatic and urinary NGAL course (difference between their concentration at T₀ and T_{24h}) and post-operative diuresis (pNGAL/diuresis $r=0.36$, $p=0.24$; uNGAL/diuresis $r=0.3$, $p=0.3$).

Correlation between Hydration (ml/kg) and Post-operative PaO₂/FiO₂ Rate

There was a moderate negative correlation between hydration and post-operative PaO₂/FiO₂ rate (calculated 24 hours after surgery). The coefficient of correlation between these two parameters was $r=-0.395$, with a p value of 0.26: this result shows that there was a moderate correlation between hydration and PaO₂/FiO₂ rate but this correlation was not statistically significant.

DISCUSSION

No patients developed AKI according to the AKIN criteria. Plasma NGAL increased significantly ($p<0.01$) within 10 h after surgery: at this time point its mean value was higher than the normal level (T_{10h}: 181.75 ± 89.3 ng/ml). Afterwards, pNGAL concentration decreased significantly ($p<0.01$) within 24 hours after surgery (T_{24h}: 111.78 ± 70.71 ng/ml). Plasma NGAL course could be explained in these terms: the surgery and the negative intra-operative fluid balance induced a mild ischemic tubular damage, shown by the increase of pNGAL at T_{10h}. Subsequently, the restoration of positive fluid balance improved kidney's perfusion and the resolution of tubular damage, which was shown by the decrease of pNGAL at T_{24h}.

As shown in the introduction, frequent causes of kidney injury in these patients are: pre-renal azotemia, rhabdomyolysis, and intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS).

1. **Pre-renal azotemia.** Intra-operative fluid balance was often negative (-913.5 ± 900 ml) in the enrolled patients, with a relatively large variability in fluid balance. In order to properly evaluate this aspect, standardized protocol of peri-operative hydration

should be used. It is more difficult to personalize hydration in obese patients since they have a unique body composition and because of the lack of reliability of physical examination for the assessment of volume status.

2. **Rhabdomyolysis.** In our study this complication developed in one female patient, with a BMI=85, who underwent open gastric by-pass and addomoplasty. The surgery lasted around 5 hours. Serum myoglobin was 1024 ng/ml in the first post-operative day. Serum CPK day was 1222 UI/l in the second post-operative. Plasma NGAL was persistently higher (186 ng/ml) at T_{24h}. Furthermore, serum cystatin C and creatinine showed an increase after surgery, with their levels being 0.2 mg/l and 0.25 mg/dl respectively, at T_{24h} compared to their values at T₀.
3. **Intra-abdominal Hypertension (IAH) and Abdominal Compartment Syndrome (ACS).** Nearly all the patients enrolled for this study underwent laparoscopic bariatric surgery, they were then at higher risk of developing IAH/ACS.

The uNGAL course was different from that of pNGAL. Urinary NGAL concentrations were almost stable and within the normal range at time points T_{10h} and T_{24h}.

This discrepancy could be explained in two possible ways. According to one hypothesis, there could be a delay in the appearance of this biomarker in the urine, due to the transport of NGAL, produced by tubules, to the blood instead of urine. In fact, experimental studies have found that NGAL in plasma predominantly originate from the injured thick ascending tubules and the collecting ducts via back-leak from injured renal tissue [26]. Based on another hypothesis, the high concentration of NGAL in plasma could be due to a production from other activated epithelia during the surgery [27], such as gastric epithelium. If pNGAL were produced in tissues other than the kidney and then filtered, the proximal renal tubules would completely reabsorb NGAL with not even minimal uNGAL levels [26].

In contrast, the decrease in the glomerular filtration rate seen in AKI would decrease NGAL clearance, resulting in a decrease in uNGAL and increased pNGAL [28].

Due to the aforementioned reason, it is difficult to determine the source of plasmatic and urinary NGAL, but the kidney is likely to be the major source of NGAL [28].

Urinary NGAL concentration could be slightly modified by changes in urine output. In fact, statistical analysis showed a correlation between the uNGAL course (difference between its concentration at T_0 and T_{24h}) and total fluid balance ($r=-0.457$; $p=0.054$). This result suggests the presence of a moderate negative correlation between the increase of uNGAL at time point T_{24h} and total fluid balance. The more negative the fluid balance, the more uNGAL increases at T_{24h} ; negative fluid balance is characterized by a reduction of urine output and, as a result, uNGAL was higher in those cases.

A multicenter analysis [28] of 10 prospective studies has examined the hypothesis that increased NGAL levels identify patients with subclinical acute kidney injury and therefore worse prognosis, in the absence of diagnostic changes in serum creatinine. The findings of such a study have shown that NGAL positive patients are at a greater risk of adverse outcomes (i.e., death and need for renal replacement therapy) both in the presence or absence of an increase in serum creatinine. Patients with NGAL positivity/creatinine negativity have acute tubular damage without loss of excretory function: these patients might be classified as having AKI, even though they do not fulfill current AKI diagnosis criteria. For this reason, we have to take NGAL positivity into account, because it implies a worse prognosis also when there is no increase of serum creatinine.

Concentrations of serum cystatin C were stable and within the normal range at time points T_{10h} and T_{24h} ; serum cystatin C did not seem to be as effective as pNGAL in showing mild kidney change.

Cystatin C is described as a valid biomarker of glomerular filtration rate; it may be more sensitive compared to serum creatinine in the detection of early and mild changes of kidney function [29]. In a cohort of 85 critically ill patients at risk for AKI increases in cystatin C preceded those of serum creatinine, fulfilling RIFLE-R criteria approximately 1.5 days prior to serum creatinine [30].

Moreover, cystatin C is a urinary biomarker of tubular damage: there is virtually no detection of cystatin C in the urine in the normal kidney, because it is completely reabsorbed by the proximal tubular cells. Indeed, high urine cystatin C may indicate tubular epithelial damage and has been proposed as an additional biomarker for AKI [31].

In critically ill obese patients it would be interesting to assess the role of urinary cystatin C as a biomarker of tubular damage.

The biomarkers object of our study were compared to serum creatinine. It was stable at time points T_{10h} and T_{24h} ; afterwards, it increased significantly at T_{48h} ($p < 0.05$). The mean value at T_0 was 0.72 ± 0.17 mg/dl and that at T_{48h} was

0.76 ± 0.23 mg/dl. The late increase of serum creatinine could be due to a mild kidney impairment, which was initially shown by the increase of pNGAL (T_{10h}). Serum creatinine does not reflect kidney function during acute changes until a steady state has been reached, which can take several days [32]. It does not mirror then the changes of kidney function in its pathway of development.

We noticed that serum creatinine concentrations were particularly low in some patients: in 30% (7 patients) concentrations were between 0.5-0.6 mg/dl at time point T_0 . This result can be important: low serum creatinine concentrations could be a sign of obesity-related glomerulopathy, which is characterized by glomerular hyperfiltration [3].

CONCLUSION

Plasma NGAL can reveal mild tubular damage if its values increase within a few hours from surgery; with the subsequent restoration of a positive fluid balance, its concentrations return within normal limits.

An appropriate timing of pNGAL levels should be defined. Nevertheless, we can conclude that a normal concentration is safe 24 hours after surgery because NGAL accurately reflects tubular changes: the attenuation of renal injury is accompanied by an early decrease in NGAL concentration [33].

The uNGAL course was different from that of pNGAL: there was a minimal increase at T_{24h} , when NGAL concentrations in plasma had already decreased. This discrepancy could be due to a delay in the appearance of this biomarker in the urine in obese patients: this unexpected result deserves accurate research in future studies.

We also found that uNGAL increase was correlated to negative fluid balance: we should take into account the influence of fluid balance in the interpretation of uNGAL concentration. The variability of urine output can change its value, especially when its concentration is near normal limits.

Serum cystatin C concentrations were stable in all measurements; this result shows that serum cystatin C did not reveal minimal kidney change, differently from pNGAL. In critically ill, obese patients it would be interesting to evaluate the diagnostic role of urinary cystatin C and uNGAL, and compare the performance of these biomarkers for the early diagnosis of AKI [34, 35].

The very low concentrations of serum creatinine found in some of the enrolled patients require attention: given the low serum creatinine in normal conditions, creatinine concentration may remain within the normal range for the adult population, when kidney damage has already occurred. Consequently, it appears to be more important to consider relative variations of serum creatinine in obese adult patients, with the application of RIFLE/AKIN criteria, instead of absolute values.

We acknowledge two limitations in this study: firstly, as bariatric patients only were recruited and subsequently

admitted to the low dependent unit for post-operative monitoring, we analyzed data of patients for which LDU admission had been previously established on the basis of their co-morbidities and clinical history. Secondly, if we had enrolled a bigger number of patients, the results could have been more conclusive.

In the future, further studies should deeply investigate the incidence, causes, and characteristics of AKI in bariatric patients, as well as the role of new biomarkers in its early diagnosis.

ABBREVIATIONS

ACS	=	Abdominal compartment syndrome
AUC	=	Area under curve
AKI	=	Acute kidney injury
BMI	=	Body mass index
IAH	=	Intra-abdominal hypertension
IAP	=	Intra-abdominal pressure
ICU	=	Intensive care unit
LDU	=	Low dependence unit
MO	=	Morbid obese
NGAL	=	Neutrophil gelatinase-associated lipocalin
pNGAL	=	Plasmatic Neutrophil gelatinase-associated lipocalin
uNGAL	=	Urinary Neutrophil gelatinase-associated lipocalin

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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