

# The Postoperative Plasmatic Level of Neutrophil Gelatinase-associated Lipocalin Does not Reflect its Tissue Expression in Critically ill Patients with High Abdominal Pressure

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*The aim of this study was to evaluate the correlation between serum and tissue level of the Neutrophil gelatinase-associated lipocalin (NGAL), a peptide that seems to be predict the postoperative evolution of patients with high abdominal pressure. From 30 consecutive patients hospitalized in the Intensive Care Unit, we have randomly selected five cases that undergo abdominal surgical interventions, received postoperative mechanical ventilation and abdominal pressure was monitorized for at least 48 hours. In all of these cases, the plasmatic level of NGAL was measured using the Elisa method and immunohistochemical (IHC) stains with the anti-NGAL biomarker were performed in the surgically removed tissues. All of the five patients were overweight, showed high serum level of NGAL (over 950 ng/ml) and died with postoperative or septic shock. The NGAL serum level was not correlated with the abdominal pressure. No IHC positivity was observed in the examined tissues, except NGAL positivity for intravascular neutrophils. In conclusion, the postoperative high serum NGAL level may indicate unfavorable evolution. NGAL seems to be synthesized by the circulating neutrophils and its preoperative tissue expression does not reflect the serum value.*

**Key words:** NGAL, peptide, lipocalin, ELISA

Neutrophil gelatinase-associated lipocalin (NGAL), also known as Lipocalin-2, p25/24p3 lipocalin or uterocalin in mice, is a 25-kDa glycoprotein codified by the *Lipocalin 2* gene [1-5]. Its crystal structure (fig. 1) can be found on wikipedia [5].

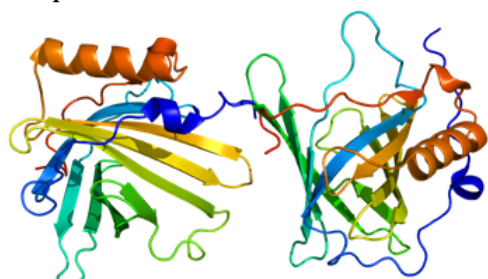


Fig. 1. The crystal structure of Lipocalin-2 (NGAL) [5]

NGAL was firstly described as being a matrix protein released from the granules of activated neutrophils [1-4]. It was later observed that NGAL can be also derived at the glomerular endothelial/mesangial cells, freely filtered through the glomerular membrane and reabsorbed by endocytosis in the proximal tubule [6]. In patients with decreased glomerular filtration rate, it is considered a biomarker of kidney injury, that increases in plasma 12-24 hours before increasing of serum creatinine concentration [2,3,6,7].

NGAL can be also expressed in epithelial cells, liver and adipose tissue, normal bronchial epithelia and epidermal keratinocytes [3,8,9] but the exact role of this protein and the stimuli responsible for its circulation are not yet understood [3].

NGAL can be measured in plasma, urine or at the mRNA level but its proteic quantitative expression can also be quantified using immunohistochemical (IHC) stains [8,10]. The urine level is a reflection of upregulation of NGAL in the distal nephron or an impaired reabsorption in the proximal tubules [6,10]. The plasma level of NGAL does not reflect the severity of acute renal failure but can be an

indicator of its increased expression in liver and lungs [6]. However, it is difficult understanding the real mechanism of its increasing levels in plasma and urine and how the daily quantification of NGAL may be useful in clinical practice.

The aim of this study was to test the relation between plasma and tissue level of NGAL in critically ill patients that died as result of postoperative or septic shock. Based on the obtained data, we have postulated a hypothesis regarding the potential NGAL-mediated mechanism of shock. As we have previously proved that high serum level of NGAL associates a decreased filtration rate and is associated with the intraabdominal pressure [2], the possible role of NGAL in inducing abdominal compartment syndrome was also explored.

## Experimental part

In the present study we have prospectively evaluated 30 randomly selected patients hospitalized in the Intensive Care Unit that underwent mechanical ventilation during November 2015 and August 2016. As a persistent acute kidney failure should be installed from at least 48 h [11], those patients that died below two days after admission, patients with chronic renal, hepatic or cardiovascular disorders, infectious diseases and diabetics were not included. The Ethical Committee approval and signed informed consent was obtained from the legal representatives. The research was performed according to the Helsinki criteria.

As the aim of the study was to examine the plasma versus tissue levels of NGAL, we have selected those patients (n=5) that underwent surgical interventions on the abdominal cavity and were then admitted to the Intensive Care Unit (table 1).

In all of the cases, the clinical and laboratory investigations were performed from the first 24 h following surgery. The abdominal pressure was invasively evaluated

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using an uretero-vesical catheter and the device AbViser (ConvaTec, Salt Lake City, USA) for measuring the pressure for at least two days after surgery. Those cases with a pressure higher than 12 mmHg in at least one of the two days were considered as having high abdominal pressure [2].

Plasma NGAL level was postoperatively measured in all subjects, in first 24 h after surgery, using the Triage NGAL kit (Alere, San Diego, CA), based on the fact that a single measured threshold for NGAL is enough to predict persistent acute kidney failure [11]. The other parameters such serum creatinine, urea, creatinine clearance, diuresis, glomerular filtration rate (GFR), number of white blood cells, and percentages of neutrophils were evaluated in at least two consecutive days. The APACHE II severity score was also determined, using as normal ranges the parameters presented in table 1.

The formalin-fixed tissues that were preoperatively removed were paraffin-embedded and immuno-histochemical (IHC) stains using the polyclonal anti-NGAL antibody (Abcam), dilution 1:25, have been performed in all of the specimens.

## Results and discussions

In critically ill patients it is difficult to predict evolution based on serum chemical or hematological biomarkers [12,13]. Although it is accepted that high NGAL plasma levels indicates increased risk of adverse outcomes for critically ill patients, with high abdominal pressure or over-resuscitated patients, the mechanism of inducing unfavorable evolution is still unknown [6,7,14].

In our study, all of the patients showed huge serum level of NGAL but only two of them (cases 2 and 4) also have increased abdominal pressure, as result of associated ileus. In the same cases the serum urea was also increased without significant disorders of the glomerular filtration

**Table 1**  
THE NORMAL RANGES OF EXAMINED PARAMETERS (BMI-BODY MASS INDEX; IAP-INTRAABDOMINAL PRESSURE; WBC-WHITE BLOOD CELLS)

Parameter	Ranges
<i>BMI</i>	<25 kg/m <sup>2</sup>
<i>PIA</i>	<12mmHg
<i>APACHE II severity score</i>	0
<i>Serum N-GAL</i>	40-100 ng/ml
<i>Serum Urea</i>	20-50 mg/dl
<i>Serum Creatinine</i>	0.6-1.2 mg/dl
<i>Creatinine Clearance</i>	Males: 97-137 ml/min Females: 88-128 ml/min
<i>Glomerular filtration rate</i>	>60 ml/min/1.73 m <sup>2</sup>
<i>Diuresis/24 h - adults</i>	1000-1600 ml
<i>WBC</i>	45-110 x10 <sup>3</sup> /μL
<i>Neutrophils</i>	54-62%
<i>Serum Amylase</i>	25-125 U/L
<i>Serum Glucose</i>	70-100 mg/dl
<i>Serum Calcium</i>	2.1-2.8 mmol/L

rate, without extremely high serum level of creatinine (table 2). As NGAL can be increased in patients with both subclinical or clinical acute kidney disease [6,15], it seems that the nephron is not the only source of NGAL and it does not reflect exclusively a renal impairment. It is necessary taking into account the fact that the level of plasma NGAL is also influenced by ischemia (increased serum NGAL is correlated to the cytokine levels), neoplasia, hypertension and systemic inflammatory diseases [6,14].

In some of the recent studies it was proved that the body mass index (BMI) seems to be correlated with the NGAL level in plasma and urine and a correlation with its mRNA and proteic levels was also postulated [8]. As in our study all of the five patients were overweight (table 2) and

Parameter	Case 1	Case 2	Case 3	Case 4	Case 5
<i>Age (years)/sex</i>	63/M	66/F	65/M	73/M	82/M
<i>BMI (Kg/m<sup>2</sup>)</i>	29.41	37.83	33.66	25.95	24.49
<i>Preoperative diagnosis</i>	Recto-sigmoidian cancer. Peritonitis	Ileus. Appendicitis. Grade II obesity	Septic shock. Hepatitis. Grade I obesity	Inguino-scrotal hernia with incarceration and ileus. Hydrocele	Gastric cancer
<i>IAP (mmHg)</i>	9	36	7	15	9
<i>Postoperative histopatologic diagnostic</i>	Recto-sigmoidian carcinoma pT4N2 stage	Appendicitis. Peritonitis	Ischemic necrosis of the liver. Hepatitis.	Hydrocele. Acute ischemic colitis. Peritonitis	Gastric carcinoma pT2N3 stage
<i>Serum N-GAL (ng/ml)</i>	1300	1300	955	1300	1300
<i>Serum Urea (mg/dl)</i>	50.78	126.24	40.96	76.18	21.49
<i>Serum Creatinine (mg/dl)</i>	3.02	4.71	1.01	0.54	0.87
<i>Creatinine Clearance (ml/min)</i>	30.10	81.8	83.28	129.2	70.60
<i>Glomerular filtration rate (ml/min/1.73/m<sup>2</sup>)</i>	22	52	59	100	32
<i>Diuresis/24 h</i>	200	200	200	1600	800
<i>WBC x10<sup>3</sup>/μL</i>	48	13.8	22.2	66	28.19
<i>Neutrophils (%)</i>	86.7	81.7	86.4	93	89.5
<i>Serum amilase (U/L)</i>	34	-	52	-	-
<i>Serum glucose (mg/dl)</i>	-	142	-	98	119
<i>Serum calcium (mmol/L)</i>	1.12	1.04	1.07	1.08	1.07
<i>APACHE II score</i>	33	32	22	27	28

**Table 2**  
THE CLINICOPATHOLOGICAL FEATURES OF THE POSTOPERATIVELY EVALUATED CRITICALLY ILL PATIENTS (F-FEMALE; BMI-BODY MASS INDEX; M-MALE; IAP- INTRAABDOMINAL PRESSURE; WBC-WHITE BLOOD CELLS)

showed progressive renal failure, without chronic renal disorders, we can suppose that high levels of NGAL might occur as a result of a combined mechanism that involve the glomeruli, IAP and the amount of adipose tissue. Taking into account that NGAL is an adipokine, it might be synthesised not only by renal tubes but also by the adipose tissue that play an important role in metabolic homeostasis [8,9].

On the other hand, the only significant high intra-abdominal pressure was seen in the case 2, that showed not only high serum urea level but also high serum glucose (table 2). In line to our data, in patients with gestational diabetes mellitus, NGAL increased level in plasma proved to be an indicator of risk for development of insulin resistance and post-gestational development of type 2 diabetes mellitus [8]. Because most of the patients with postoperative shock show acute ischemic pancreatitis and high levels of serum amylases [16], a supplementary increased plasma level of NGAL could increase the severity of insulin resistance and can explain the high APACHE score and fulminant evolution of the five cases included in this study (table 2). In critically ill patients, extremely high plasma levels of NGAL can be a possible indicator of installing of a severe acute pancreatitis [17].

The increased plasma level of NGAL was also induced in our patients by the high number of neutrophils. As IHC expression of NGAL was observed in the intravascular neutrophils only (fig. 1), we can suppose that it might mark the immature neutrophils that are released in the systemic circulation in patients with septic shock [11]. It was proved that, in patients with septic shock, independently from other associated factors, a plasmatic NGAL level higher than 257 ng/ml is an independent predictor (with 68% sensitivity and 75% specificity) of non-recovered acute renal failure [15,18]. In our previously published data, the plasmatic value was restricted to 450 ng/mL [2]. For the urinary level, it was suggested that a value of NGAL higher than 348.2 mg/dl (sensitivity 0.84 and specificity 0.69) is a potential indicator of higher risk of mortality [15,19].

Except the intravascular neutrophils, no other examined tissues or cells (gastric, appendix and colorectal mucosa, gastric and colorectal carcinoma, testis epididymis, liver parenchyma) were IHC marked by NGAL. The tissue negativity, despite the postoperative huge serum levels of NGAL proved that the tissue level is not an indicator of postoperative evolution but the number of NGAL positive intravascular neutrophils may be correlated with the severity of septic shock. No literature data have been founded to reveal these aspects.

## Conclusions

Based on our results and data from literature, we conclude that, after laparotomy, the tissue IHC expression of NGAL does not indicate the postoperative evolution and is not correlated with the plasma level of NGAL. The postoperative plasma level of NGAL, that is counted in first 24 h after surgery, might be used as an indicator of high risk of installing fulminant postoperative or septic shock. In patients with high intra-abdominal pressure, the plasma NGAL reflects the early phase of acute kidney injury but, in critically ill patients with normal IAP, it can be related on sepsis, pancreatic defficiency/acute pancreatitis or multiorgan failure syndrome.

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