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Determination of serum neutrophil gelatinase-associated lipocalin at the early stage of acute pancreatitis

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Abstract: A i m: The aim of the study was to assess the diagnostic value of serum concentrations of neutrophil gelatinase-associated lipocalin (sNGAL) for the determination of the severity of acute pancreatitis (AP) at the early stage of the disease.
M a t e r i a l s a n d M e t h o d s: The study group consisted of 65 patients (34 men and 31 women), aged 62.2 ± 16.0, admitted to the Surgery Department of the District Hospital in Sucha Beskidzka, Poland, with the diagnosis of AP according to the revised Atlanta classification (2012). sNGAL was measured with ELISA at 24, 48 and 72 hours following the onset of AP symptoms. The correlations were analyzed between sNGAL and clinical, as well as laboratory parameters, used for the assessment of AP severity.
R e s u l t s: Severe AP was associated with higher sNGAL at 24, 48 and 72 hours, while moderately severe AP was associated with higher sNGAL at 48 and 72 hours as compared to mild disease. The BISAP score ≥3 during the first 24 hours of hospital stay, and the duration of hospital stay were significantly correlated with sNGAL. Also, sNGAL positively correlated with white blood cells, C-reactive protein and fibrinogen and negatively with albumin throughout the study. The diagnostic accuracy of sNGAL for the differentiation between mild AP and more severe disease was 75%, 77% and 85% at 24, 48 and 72 hours, respectively.
C o n c l u s i o n s: Serum NGAL concentrations are associated with inflammatory markers, BISAP score and the severity of AP. sNGAL may serve as an additional prognostic biomarker in the early assessment of AP severity.

Key words: acute pancreatitis, neutrophil gelatinase-associated lipocalin, prognostic markers.

Introduction

Nearly 80% of all cases of acute pancreatitis (AP) are mild (MAP — mild acute pancreatitis). MAP requires only supportive care, and it generally resolves without any complications. However, 20% of patients develop the severe acute pancreatitis (SAP), which is a life-threatening disease with a mortality rate of 20–30% [1–4]. The mortality rate is significantly higher in older patients and in patients with BMI ≥30 [2]. At early phase of the disease, the mortality is associated with prolonged (lasting longer than 48 hours) organ failure (mainly respiratory, circulatory or renal failure) [2, 5–7]. In patients with acute kidney disease (AKI) in the course of AP the mortality rate reaches 70–80% [8–10]. An early classification of the severity of AP and initiation of adequate treatment is decisive for the prognosis as well as for the patient survival [11–12]. The "therapeutic window" in AP is usually reported to last between the 48 and 72 hours following the onset of symptoms [5]. On the other hand, despite numerous studies and significant progress in diagnostic techniques, the early determination of AP severity remains difficult. Together with clinical assessment, the multi-parameter prognostic scales such as Ranson's score, Glasgow, Acute Physiology and Chronic Health Evaluation II (APACHE II), or bedside index for severity in AP (BISAP) are used for this reason [1, 12]. Simultaneously, the diagnostic value of single laboratory markers are studied in this context. Procalcitonin (PCT) and C-reactive protein (CRP) levels are among the most recognized biomarkers [3, 10, 12–13]. Even though a significant increase in CRP concentrations is observed in SAP rather than in MAP

patients, its peak concentrations are observed relatively late, between the 48th and 72nd hour after the disease onset [3, 12]. During the first 24 hours of AP, the secretion of proteolytic enzymes from activated neutrophils, i.e. polymorphonuclear granulocyte elastase (PMN-elastase), or gelatinases is observed [3, 12, 14].

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2, is a 25kDa glycoprotein, initially found in activated human neutrophils in a form covalently bound to neutrophil gelatinase. Physiologically, NGAL is expressed in low levels in kidneys, trachea, lungs, stomach, pancreas and colon; its expression is significantly increased under inflammatory conditions and following damage to the endothelial cells [14–18]. NGAL is freely filtered by the renal glomeruli, and almost completely absorbed by the proximal tubules [19–20]. Serum NGAL concentrations depend to some extend on age, sex, and liver condition, and correlate with markers of inflammation [18, 21–24].

The aim of the study was to assess the diagnostic value of serum concentrations of NGAL (sNGAL) for the determination of the severity of AP at early stage, i.e. the first 72 hours after the onset of symptoms of the disease. Also, the correlations were analyzed between sNGAL and clinical, as well as laboratory parameters used for the assessment of AP severity, including the inflammatory markers.

Materials and methods

The study group

The prospective study included 65 adult patients admitted with the diagnosis of AP, undergoing treatment in the Surgery Department of the District Hospital in Sucha Beskidzka between January and December 2014. AP was diagnosed according to the revised Atlanta classification (2012) [6]. Patients who were admitted later than 24 hours after the onset of symptoms of AP and those who refused to sign the informed consent for the study were excluded.

Depending on the severity of AP, patients were assigned to one of 3 groups [6]. The first group — mild acute pancreatitis (MAP) included patients in whom no organ dysfunction or local complications were observed during the hospital stay. The second group consisted of patients with moderately severe acute pancreatitis (MSAP), with transient organ failure (lasting for less than 48 hours), local complications (i.e. acute peripancreatic fluid, pancreatic pseudocyst, acute necrotic collection, walled-off necrosis), and/or exacerbation of comorbidities. Patients persistent organ failure (lasting longer than 48 hours) and ≥1 local complications were assigned to the third group — severe acute pancreatitis (SAP).

The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Bioethics Committee of the Jagiellonian University in Cracow (Poland) (approval no. KBET/247/B/2013).

Laboratory tests

Blood samples for the laboratory tests were collected at 24, 48 and 72 hours from the onset of AP symptoms. The routine laboratory tests, including the complete blood count (WBC), amylase activity, serum concentrations of urea, creatinine, calcium, C-reactive protein (CRP), albumin, and plasma concentrations of fibrinogen were performed the same day in the Medical Diagnostic Laboratory in Sucha Beskidzka, Poland. The sera for sNGAL determination were frozen within 1 hour of blood collection and stored at –70°C no longer than 6 months. The sNGAL concentrations were measured using the Human Lipocalin-2/NGAL ELISA kit (Bio-Vendor, Brno, Czech Republic) and the Automatic Micro ELISA Reader ELX 808 (BIO-TEK® Instruments Inc., Winooski, VT, USA) at the Department of Diagnostics, Chair of Clinical Biochemistry, Jagiellonian University Medical College in Cracow, Poland.

Statistical analysis

Data are reported as number of patients and percentage of the group for categories, and the mean ± standard deviation (SD) or median (lower-upper quartile) for quantitative variables, depending on the distribution (the Shapiro-Wilk test was used to assess normality). Differences between groups were tested with ANOVA or Kruskal-Wallis ANOVA. Spearman rank coefficient was calculated to assess correlations. The receiver operating characteristic (ROC) curves were used to assess the diagnostic value of sNGAL, the areas under the ROC curves (AUC) are reported with 95% confidence intervals (95% CI). All the tests were two-tailed and the results were considered significant at p-value of less than 0.05. The Statistica 10.0 package (Statsoft Inc., Tulsa, USA) was used for computations.

Results

Relationship between sNGAL and the severity of AP

The clinical characteristics of patients with MAP, MSAP and SAP together with the results of laboratory tests performed at 24 hours after the onset of AP symptoms are presented in Table 1. The differences between groups regarding age, sex, or prevalence of comorbidities were not statistically significant. AP aetiology was mostly gallstones (33 patients, 51%), alcohol (18 patients, 28%) and hypertriglyceridemia (5 patients, 8%). There were no significant differences in aetiology between MAP, MSAP and SAP patients. The most prevalent comorbidities included: hypertension (22 patients, 34%), ischemic heart disease (18 patients, 28%) and diabetes (10 patients, 15%); 7 patients (11%) were diagnosed with lung diseases and 3 (5%) with kidney diseases.

Table 1. Clinical characteristics of AP patients and laboratory data at admission according to severity of the disease.

	MAP (n = 46)	MSAP (n = 14)	SAP (n = 5)	p*
Age, years	59 ± 19	64 ± 16	70 ± 19	NS
Males, n (%)	25 (54)	6 (42)	3 (60)	NS
Duration of pain until admission, hours	12 (6–24)	12 (6–36)	24 (24–48)	NS
Duration of hospital stay, days	6 (5–7)	12 (10–17)	27 (13–31)	<0.001[a,b]
BISAP score ≥3 in first 24 h, n (%)	0	2 (14)	4 (80)	<0.001[c]
Comorbidities, n (%)	33 (72)	12 (86)	5 (100)	NS
Mortality, n (%)	0	0	3 (60)	–
WBC, x10^3/μL	11.1 (9.2–14.6)	12.4 (10.6–15.3)	10.4 (9.8–18.4)	NS
HCT, %	42.5 ± 4.1	43.2 ± 6.7	39.0 ± 7.1	NS
PLT, x10^3/μL	237 (196–255)	230 (210–267)	128 (121–150)	NS
Amylase, U/L	1085 (571–1722)	1038 (772–1917)	1013 (357–1909)	NS
Glucose, mmol/L	7.7 (6.4–9.8)	9.1 (7.2–12.2)	13.3 (6.3–15.7)	NS
Urea, mmol/L	5.5 (4.1–6.8)	6.7 (5.6–8.4)	13.4 (11.7–15.1)	0.002[a]
Creatinine, μmol/L	72.7 (63.4–94.8)	90.7 (67.6–113.4)	194.4 (120.0–228.0)	0.008[a]
Calcium, mmol/L	2.33 ± 0.17	2.32 ± 0.23	2.01 ± 0.34	NS
CRP, mg/L	5.9 (1.9–48.6)	24.4 (9.0–103.2)	191.1 (74.6–258.2)	0.003[a]
Albumin, g/L	40.6 ± 4.0	39.7 ± 3.9	30.7 ± 8.4	0.030[a]
Fibrinogen, g/L	2.62 (2.13–3.57)	3.19 (2.27–3.31)	4.46 (3.55–5.38)	NS
sNGAL, μg/L	104 (68–139)	226 (191–274)	329 (198–427)	0.007[a]

* p-value for overall difference between the 3 groups; superscript numbers denote significant differences in post-hoc tests: [a] — between MAP and SAP; [b] — between MAP and MSAP; [c] — in all between-groups comparisons

The BISAP score during the first 24 hours of hospital stay, and the duration of hospital stay were significantly associated with AP severity (Table 1). There were 3 deaths among SAP patients after 13–31 days of hospital stay (i.e. all are classified as late mortality). Also, SAP patients had higher concentrations of urea, creatinine, CRP, D-dimer and sNGAL, and lower concentrations of albumin at admission (Table 1, Fig. 1).

The concentrations of sNGAL on days 2 and 3 after admission were higher in SAP and MSAP patients as compared to MAP (Fig. 1, Table 1). The highest median sNGAL concentrations were detected on day 2 in all groups (Fig. 1), although the time-related differences were statistically significant only among MAP patients (p = 0.038).

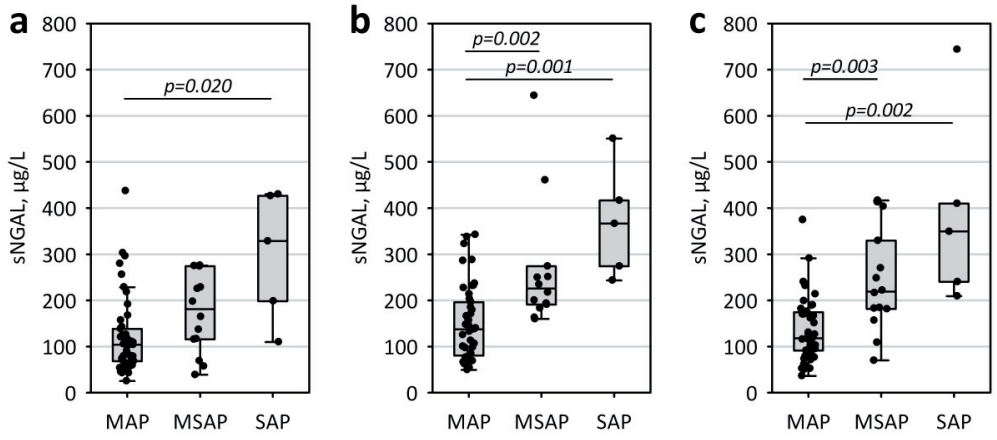

Fig. 1. The concentrations of sNGAL in patients with mild (MAP), moderately severe (MSAP) and severe acute pancreatitis (SAP) at 24 (a), 48 (b) and 72 hours (c) after the onset of AP symptoms; p-values are shown for significant differences in post-hoc tests.

The concentrations of sNGAL were significantly correlated with the duration of hospital stay and the BISAP score throughout the study (Table 2).

Table 2. Simple correlations between sNGAL and the selected variables at 24, 48 and 72 hours after onset of AP symptoms.

Variable	24 hours		48 hours		72 hours	
	R	p	R	p	R	p
Duration of hospital stay	0.38	0.002	0.54	<0.001	0.43	<0.001
BISAP score	0.39	0.002	0.47	<0.001	0.55	<0.001
WBC	0.46	<0.001	0.64	<0.001	0.58	<0.001
CRP	0.66	<0.001	0.70	<0.001	0.60	<0.001
Fibrinogen	0.51	<0.001	0.40	<0.001	0.34	0.005
Albumin	–0.43	<0.001	–0.57	<0.001	–0.60	<0.001

Relationships between sNGAL and selected markers of inflammation

Significant positive correlations were found between sNGAL and the markers of inflammation, i.e. WBC, CRP and fibrinogen. The correlations with CRP were particularly strong. Also, sNGAL negatively correlated with albumin. All the relationships were observed throughout the study (Table 2).

Diagnostic value of sNGAL in AP

When applying the upper reference limits provided by the manufacturer of the test (276 μg/L for women and 169.2 μg/L for men), 15 (23%), 23 (35%) and 21 (32%) patients had increased sNGAL at admission, on days 2 and 3 of hospital stay, respectively. However, there were no sex-related differences in sNGAL concentrations among studied patients (p-values from 0.2 at admission to 0.7 on day 2).

Table 3. The diagnostic value of sNGAL for the differentiation between MAP and more severe AP (MSAP or SAP) at the cut-off values chosen based on ROC curves analysis and the upper reference limits (URL) provided by the manufacturer of the test (i.e. 276 μg/L for women and 169.2 μg/L for men).

Time point*	Cut-off value	Sens., %	Spec., %	PPV, %	NPV, %	Acc., %
24 hours	ROC: 165 μg/L	63	80	57	84	75
	URL	42	84	53	78	72
48 hours	ROC: 183 μg/L	90	72	57	94	77
	URL	47	70	39	76	63
72 hours	ROC: 182 μg/L	84	78	62	92	80
	URL	53	76	48	80	69

Sens. — sensitivity; Spec. — specificity; PPV — predictive value of positive result; NPV — predictive value of negative result; Acc. — accuracy
* Time after the onset of AP symptoms.

In ROC curves analysis, sNGAL assessed at admission enabled the differentiation between MAP and more severe forms of AP (MSAP or SAP), although the value of AUC at admission was lower than on day 2 and 3 of hospital stay (Fig. 2). The best cut-off values were close to the upper reference limit provided by the manufacturer for men, and significantly lower than the upper reference limit for women.

At admission, using the manufacturer's upper reference limits to diagnose between MAP and MSAP or SAP yielded lower sensitivity but higher specificity comparing to ROC-based cut-off value. However, on subsequent days, ROC-based values that did not differ between men and women yielded better diagnostic value.

Discussion

According to the revised Atlanta classification, two phases may be distinguished in the course of AP: an early phase — up to the first week, and a late phase thereafter [6, 12]. The severity of AP is stratified into mild, moderately severe, and severe [3]. Nearly 50% of deaths due to SAP take place in the early phase, in a consequence of multiple organ dysfunction syndrome (MODS) [3, 5]. In the present study, serum

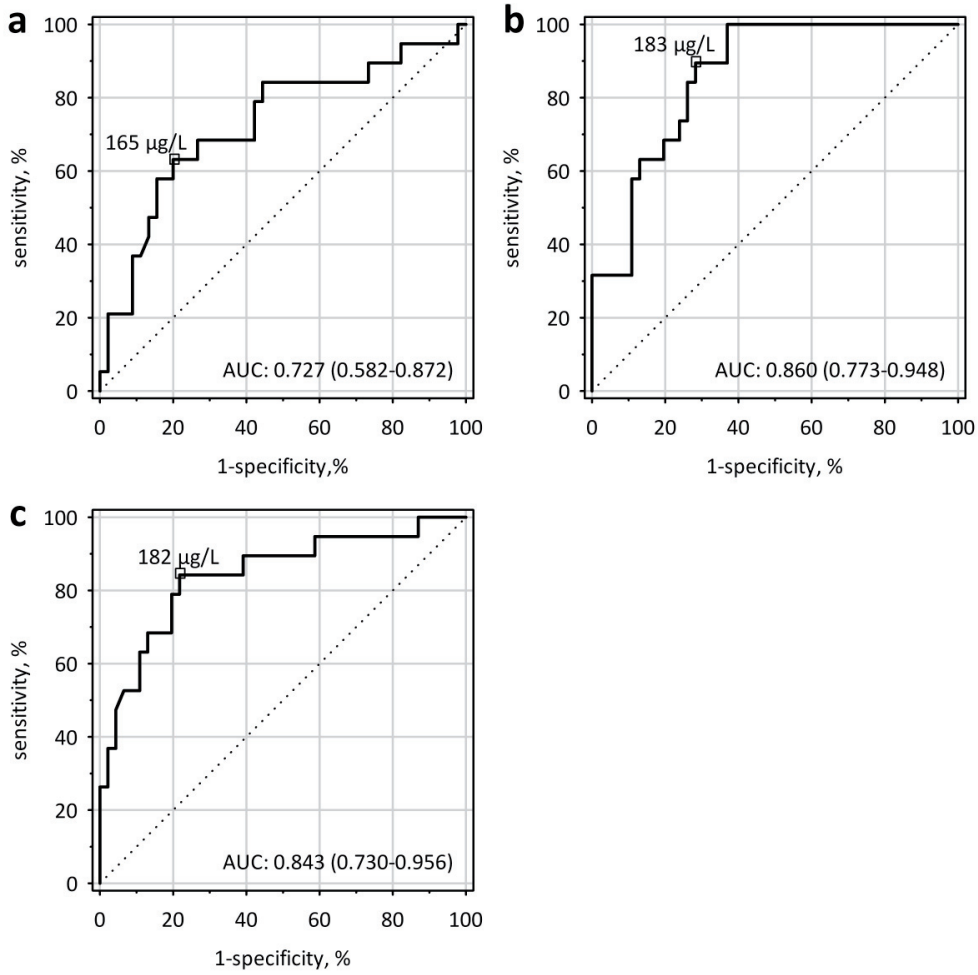

Fig. 2. ROC curves presenting the diagnostic value of sNGAL concentrations in differentiating between MAP and more severe forms of AP (MSAP or SAP) at 24 (a), 48 (b) and 72 hours (c) after the onset of AP symptoms. The selected cut-off values are highlighted. The area under curve (AUC) values are reported with 95% confidence intervals.

NGAL concentrations measured early in the course of AP was associated with the disease severity. Already at 24 hours following the onset of symptoms of AP, sNGAL was significantly higher in SAP than in MAP, and enabled the differentiation between MAP and more severe disease. Also, sNGAL at 24 hours was positively correlated with BISAP score and the duration of hospital stay. There is an emerging literature showing the associations between serum, plasma, urine and NGAL concentrations and prognosis of SAP [3, 11, 25–26].

Increased concentrations of NGAL in serum and in urine have been reported in the course of various infectious and non-infectious diseases [14, 17, 24, 27–28]. The increased expression of NGAL has been shown in acute and chronic inflammatory states, e.g. in acute renal failure, chronic kidney disease, chronic obstructive pulmonary disease, sepsis, and acute pancreatitis [9, 14–15, 17, 24, 28–29]. The observed overexpression of NGAL indicates activation of neutrophils, and may be considered a symptom of the development of acute systemic inflammation in response to the presence of harmful stimuli, and a symptom of the development of complications [3, 11, 17–18, 28]. In the present study, a number of correlations between sNGAL and acute phase proteins, i.e. CRP, albumin and fibrinogen, were observed. In AP, premature activation of pancreatic enzymes, microcirculatory disorders, local gathering of inflammatory cells, i.e. macrophages and neutrophils, as well as the excessive inflammatory response with production of cytokines and inflammatory mediators initiate a series of processes which, within a short period of time, may lead to the development of irreversible changes and death, even in patients for whom the prognosis was initially good. The rapid, dynamic changes in the course of AP make it difficult to create a unified regimen for the assessment and treatment of AP patients [9]. At present, the most widely used routine diagnostic procedure for the prediction of the severity of AP is, next to imaging examinations and the multifactor prognostic scoring systems, measurement of serum CRP and PCT [8, 12–13]. However, neutrophils and proteolytic enzymes secreted after the activation of neutrophils (neutrophil elastase, gelatinase), play the key role in the inflammatory cascade in the course of AP [3, 12]. Our earlier studies [30] point towards the diagnostic value of PMN elastase in the early-phase of AP. PMN elastase was significantly increased in AP patients starting from the first day of hospital stay, being one of the early markers of the developing inflammatory state. In turn, Helanova *et al.* [21] show the diagnostic value of NGAL for the evaluation of the intensity of the systemic inflammatory response syndrome (SIRS), and for the early prognosis organ dysfunction, mainly acute respiratory distress syndrome, and acute kidney injury [12, 20].

Although the present study shows some usefulness of sNGAL in the early prediction of AP severity, the diagnostic value of sNGAL is too low to support the its use as a single predictor. Also, its diagnostic accuracy at 24 hours of AP is lower than at 48 and 72 hours. After 72 hours, the diagnostic sensitivity (84%) and specificity (78%) for the cut-off value 182 μg/l are similar to other markers of inflammation, e.g. CRP [12]. Also, our data support the need for establishing the appropriate cut-off or decision values, as the use of manufacturer-provided values resulted in lower diagnostic utility.

The low number of patients included and the one-center design are obvious limitations of our study. At present, the determination of NGAL in serum is connected with methodological limitations, that limit its use as a routine laboratory test.

However, also in the light of other reports [25–26], our data indicate the usefulness of sNGAL as an additional marker for the stratification of the severity of AP.

Conclusions

The measurement of sNGAL during the early phase of AP enables the differentiation between mild and more severe forms of the disease with acceptable diagnostic accuracy. Also, sNGAL concentrations in AP patients are correlated with inflammatory markers, BISAP score, and the duration of hospital treatment. The present study indicates that sNGAL may serve as an additional prognostic biomarker in the early assessment of AP severity.

Conflict of interest

No declared.

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