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Neutrophil to lymphocyte ratio at the early phase of acute pancreatitis correlates with serum urokinase-type plasminogen activator receptor and interleukin 6 and predicts organ failure

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Abstract: B a c k g r o u n d: In early phase of acute pancreatitis (AP), systemic inflammatory response syndrome may lead to organ failure. The severe form of AP is associated with high mortality that may be prevented by timely diagnosis and treatment of the predicted severe cases. Serum interleukin 6 (IL-6) and urokinase-type plasminogen activator receptor (uPAR) have been proposed as accurate early markers of severe AP. The aim of the study was to assess whether widely available blood count indexes: neutrophil to lymphocyte (NLR), lymphocyte to monocyte (LMR) and platelet to lymphocyte ratios correlate with IL-6 and uPAR and may be utilized to predict organ complications at the early phase of AP.
M e t h o d s: The study included 95 adult patients with AP treated at the Surgical Ward Complex of Health Care Centers in Wadowice, Poland. Organ failure was diagnosed according to modified Marshall scoring system, as recommended by 2012 Atlanta classification. Blood samples for laboratory tests were collected on days 1, 2 and 3 following the onset of AP symptoms.

R e s u l t s: Patients with organ failure presented significantly lower LMR on day 1 and significantly higher NLR on days 2 and 3. Strong positive correlations between NLR and IL-6 and moderate correlations between NLR and uPAR were observed throughout the study. Day 2 and 3 NLR values significantly predicted organ failure at the early phase of AP.
C o n c l u s i o n s: Taking into account the wide availability of NLR, it may be considered as a surrogate of more expensive tests to help the early assessment of organ failure complicating AP.

Key words: urokinase-type plasminogen activator receptor, neutrophil-lymphocyte ratio, platelet--lymphocyte ratio, monocyte-lymphocyte ratio, persistent organ failure, interleukin 6.

Introduction

Acute pancreatitis (AP) is a relatively common acute digestive tract disorder, which in nearly 80% of patients progresses mildly and without complications (mild acute pancreatitis, MAP), but in about 20% of cases it develops into the severe form (severe acute pancreatitis, SAP) associated with nearly 20% mortality, reaching 50–80% in elderly patients or those with comorbidities such as chronic kidney disease, cardiovascular, autoimmunological disease or diabetes [1].

The medical advancement in imaging techniques witnessed in the last decades and the widespread availability of contrast-enhanced computed tomography, ultrasonography, and magnetic resonance have significantly facilitated the diagnostic process of AP and contributed to the timely detection of early necrotic changes [1]. According to the current Atlanta 2012 classification [2], imaging findings, beside typical symptoms and over threefold increase in pancreatic enzymes activity (amylase, lipase) are included in the diagnostic criteria for AP. The severity of AP in the revised 2012 Atlanta classifications was based on organ failure and local and systemic complications [2]. Notably, vital organ (cardiovascular, pulmonary and renal) failure persistent over 48 hours has been underscored as the main cause of early mortality in AP [2].

A significant progress has also been observed in the understanding of AP pathomechanism, in consequence of which a range of diagnostic tools for early SAP prediction has been proposed. The evaluation of laboratory markers has led to the development of prognostic scores such as Ranson, Glasgow, APACHE II or BISAP. Moreover, single biomarkers have emerged, which may help in timely prognosis of severe course of AP [1, 3–6].

A continuous and consecutive clinical observation of AP patients has highlighted the importance of the "therapeutic window" estimated at the first 48 hours from AP onset when an accurate diagnosis and immediate treatment can directly impact patients' survival. The most commonly used prognostic markers include acute phase proteins (C-reactive protein, albumin, fibrinogen), procalcitonin, total calcium

concentration, markers of renal function (urea, creatinine), coagulation parameters (D-dimers), white blood cell count and differential and recently also proinflammatory cytokine interleukin 6 (IL-6) [1, 3–6]. Recently, urokinase-type plasminogen activator receptor (uPAR) has also been suggested as an early prognostic marker of SAP [7].

Major prognostic scores — Ranson's or Glasgow-Imire's used in the first 48 hours from AP onset as well as BISAP used in the first 24 hours — include total white blood cell (WBC) count [8]. Other blood cell counts, i.e. absolute neutrophil, lymphocytes, monocytes or platelet counts and indexes based on the counts: neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) as well as lymphocyte to monocyte ratio (LMR) have not been as extensively used in day-to-day diagnostic process [1, 3–5, 9]. On the other hand, however, the review of literature on the prognostic significance of the decreased lymphocyte counts and increased NLR or PLR points to their promising diagnostic utility in SAP prognosis [3, 6, 8–12]. The study by Cho *et al.* [3] found that higher NLR or PLR values can indicate systemic inflammatory response syndrome (SIRS) and a risk of its developing into multi-organ dysfunction syndrome (MODS) [6, 13]. In 2017, Borges *et al.* [5] proposed Panc4, a new prognostic model, which comprises urea and creatinine, but also indexes: neutrophil/leukocytes, platelet/leukocytes ratio and NLR.

The aim of the study was to evaluate whether calculation of NLR, LMR and PLR on the first three days of AP can aid the prognosis of severe course of the disease, associated with vital organ failure at the early phase. Moreover, we assessed the correlations between the NLR, LMR and PLR indexes and more sophisticated laboratory markers of inflammation, including urokinase-type plasminogen activator receptor (uPAR) and IL-6 in serum over the first 72 hours from the onset of AP symptoms.

Materials and Methods

The study group included patients with AP diagnosis, treated at the Surgical Ward Complex of Health Care Centers in Wadowice from March 2014 until December 2015. The patients were included provided they were admitted within the first 24 hours from the onset of AP symptoms. The study group included solely adult patients with AP who provided their written voluntary consent to participate in the study. The exclusion criteria included liver, kidney disease, chronic pancreatitis or cancer at the moment of recruitment. AP diagnosis and the classification of AP severity were based on the revised 2012 Atlanta classification [2], according to which two out of the three listed criteria must be met: typical abdominal pain, at least a threefold increase in pancreatic enzymes' activity and imaging findings characteristic of AP (in computed tomography, ultrasonography or magnetic resonance imaging). Mild (MAP), moderately severe (MSAP) and severe (SAP) acute pancreatitis were distinguished based on the course of the disease.

Organ failure during the first week of AP was assessed according to the modified Marshall scoring system (MMSS) [2]. The Bioethics Committee of the Beskidy Medical Chamber approved the study protocol (approval number 2014/02/06/1 issued on 6 February 2014).

Blood samples for laboratory tests were collected from the patients thrice: within the first 24 hours from the onset of AP symptoms (day 1), and then on the following two days (day 2 and day 3). The routine tests (complete blood counts, serum amylase, alanine and aspartate transaminase, and lactate dehydrogenase activities, serum albumin, total protein, total calcium, glucose, urea, creatinine, bilirubin, procalcitonin and C-reactive protein concentrations, and plasma D-dimer concentrations) were conducted in the Diagnostic Laboratory in Wadowice. Complete blood counts were performed with automated hematologic analyzer Sysmex XN (Sysmex Corporation, Cobe, Japan). Based on the absolute counts of neutrophils, lymphocytes, monocytes and platelets, NLR, LMR, and PLR indexes were calculated. For the assessment of routine biochemical and immunochemical tests, Cobas E411 (Roche Diagnostics, Mannheim, Germany) and Vitros 5600 (Ortho Clinical Diagnostics, Raritan, NJ, USA) were used, whereas the coagulation tests were conducted with Coag XL (Diagno, Budapest, Hungary).

Moreover, the aliquots of the sera were used for the measurements of IL-6 and uPAR concentrations. IL-6 was measured in the Diagnostics Department of University Hospital in Krakow by automatized electrochemiluminescence assay (ECLIA) on the Cobas 8000 analyzer (Roche Diagnostics, Manheim, Germany). The measurements of uPAR were conducted at the Department of Diagnostics, Chair of Clinical Biochemistry at Jagiellonian University in Krakow using the Quantikine Human uPAR Immunoassay reagent kits (R&D Systems, Minneapolis, USA). The intraassay precision for the test was ≤7.5% and the interassay precision ≤5.6%, the minimum detectable dose of uPAR was 0.033 ng/mL, while the concentrations of uPAR in healthy volunteers were between 1.195 and 4.414 ng/mL as provided by the manufacturer of the kit.

Statistical analysis

Data on categories were reported as the number of patients (percentage of the appropriate group). As most of the quantitative variables were non-normally distributed (according to Shapiro-Wilk's test), we reported the median [lower; upper quartile], while the mean and standard deviation (SD) were provided for age. Contingency tables were analyzed with chi-squared test. The differences between the groups were assessed with Mann-Whitney's test. The correlations were assessed with Spearman's rank coefficient. The tests were two-tailed and the p-values ≤0.05

indicated statistically significant results. We used Statistica 12 (StatSoft, Tulsa, OK, USA) for computations.

Results

Comparison of NLR, LMR and PLR values between patients with and without organ failure

The study group included 95 patients: 65 (68%) men and 30 (32%) women. Mean (SD) age of the study group was 48 (17) years. During the first week of AP, organ failure with ≥2 points in MMSS was diagnosed in nine patients. The baseline clinical characteristics of the study patients who developed organ failure and those without organ failure were presented in Table 1.

Table 1. Clinical characteristics of the study group according to the score in modified Marshall scoring system (MMSS).

Characteristic	MMSS ≥2 (n = 9)	MMSS <2 (n = 86)	p-value
Male sex, n (%)	7 (78)	58 (67)	0.5
Mean age (SD), years	57 (20)	47 (16)	0.2
Pre-existing comorbidities, n (%)	4 (44)	38 (44)	0.9
Cardiac diseases, n (%)	4 (44)	26 (30)	0.4
Diabetes, n (%)	1 (11)	7 (8)	0.8
Etiology:			
Biliary, n (%)	3 (33)	24 (28)	0.9
Alcoholic, n (%)	3 (33)	26 (30)	
Hypertriglyceridemia, n (%)	0	5 (6)	
Other/idiopathic, n (%)	3 (33)	31 (36)	
Pancreatic or peripancreatic necrosis, n (%)	2 (22)	10 (12)	0.4
Median Ranson score [Q1; Q3], points	6 [5; 7]	3 [2; 4]	<0.001
Median BISAP [Q1; Q3], points	3 [2; 3]	2 [2; 2]	0.022
Median duration of hospital stay [Q1; Q3], days	15 [10; 27]	12 [8; 15]	0.5
SIRS in first 24 hours, n (%)	8 (89)	66 (77)	0.4
Early/late mortality, n (%)	1 (11)/2 (22)	0/1 (1)	<0.001

Table 1. Cont.

Characteristic		MMSS ≥2 (n = 9)	MMSS <2 (n = 86)	p-value
Therapeutic ERCP, n (%)		2 (22)	3 (3)	0.016
Surgery, n (%)		4 (44)	4 (5)	<0.001
Transfer to ICU, n (%)		5 (56)	2 (2)	<0.001
Final severity	MAP, n (%)	0	29 (34)	<0.001
	MSAP, n (%)	4 (44)	54 (63)	
	SAP, n (%)	5 (56)	3 (3)	

ERCP — endoscopic retrograde cholangiopancreatography; ICU — intensive care unit; MAP — mild acute pancreatitis; MSAP — moderately severe acute pancreatitis; n — number of patients; SAP — severe acute pancreatitis; SIRS — systemic inflammatory response syndrome; SD — standard deviation; Q1 — lower quartile; Q3 — upper quartile.

On the first day of observation, the WBC counts of patients with AP were in the wide range from $5.2 \times 10^3/\mu L$ to $32.8 \times 10^3/\mu L$. There were no statistically significant differences in the total WBC counts between patients with MMSS ≥2 points and those without organ failure (Table 2). The average neutrophil counts and percentages were higher in patients with high MMSS, while the lymphocyte counts were lower, but the differences were also statistically non-significant (Table 2). The median monocyte count in high MMSS group was twice as high as in low MMSS patients, however, in both groups the variability of monocyte counts was high and thus the difference was non-significant (Table 2). The platelet counts in both groups were comparable (Table 2). The values of NLR and PLR observed on day 1 of the study did not differ significantly between the groups, however, LMR was significantly lower among the patients with MMSS ≥2 points.

Table 2. The results of laboratory tests in the study group according to the score in modified Marshall scoring system (MMSS). Data are shown as median [Q1; Q3].

Characteristic	MMSS ≥2 (n = 9)	MMSS <2 (n = 86)	p-value
WBC, $\times 10^3/\mu L$	13.7 [9.7; 21.9]	12.4 [10.3; 15.6]	0.3
Neutrophils, $\times 10^3/\mu L$	15.4 [8.6; 24.2]	10.3 [6.9; 14.7]	0.3
Neutrophils, %	89.5 [78.3; 92.5]	83.5 [77.9; 86.7]	0.2
Lymphocytes, $\times 10^3/\mu L$	0.77 [0.46; 1.38]	1.16 [0.66; 1.61]	0.3
Lymphocytes, %	2.85 [2.6; 14.6]	9.3 [4.9; 13.7]	0.2

Table 2. Cont.

Characteristic	MMSS ≥2 (n = 9)	MMSS <2 (n = 86)	p-value
NLR	31.17 [16.77; 34.75]	8.61 [5.59; 18.49]	0.2
Monocytes, $\times 10^3/\mu L$	1.71 [0.57; 2.0]	0.84 [0.62; 1.07]	0.3
Monocytes, %	5.2 [4.2; 9.8]	6.2 [5.3; 7.9]	0.6
LMR	0.47 [0.28; 0.57]	1.44 [0.78; 1.95]	0.033
Hemoglobin, g/dL	14.9 [14.1; 15.4]	15.4 [14; 16.7]	0.6
RBC, $\times 10^6/\mu L$	4.68 [4.56; 4.90]	4.90 [4.46; 5.29]	0.5
Hematocrit, %	43 [41.7; 46]	43.7 [40.6; 46.5]	0.9
Platelets, $\times 10^3/\mu L$	189 [165; 278]	204 [168.5; 261]	0.9
PLR	278.3 [138.4; 969.6]	171.5 [111.2; 253.2]	0.3
Serum amylase, U/L	492 [331; 1996]	473 [171; 1812]	0.7
Albumin, g/L	33 [26; 26]	37 [33; 41]	0.1
Total protein, g/L	69 [61; 77]	65 [63; 77]	0.8
Total calcium, mmol/L	2.05 [1.85; 2.28]	2.14 [1.98; 2.28]	0.4
Glucose, mmol/L	7.72 [6.94; 9.16]	7.44 [6.16; 9.0]	0.5
Urea, mmol/L	8.66 [5.83; 13]	4.33 [3.33; 5.83]	<0.001
Creatinine, µmol/L	166.1 [86.6; 184.7]	68.1 [58.3; 84.8]	<0.001
Bilirubin, µmol/L	30.9 [19.8; 49.5]	24.9 [13.8; 39.6]	0.3
AST, U/L	118 [82; 166]	91 [46; 202]	0.2
ALT, U/L	77 [54; 92]	107 [49; 243]	0.8
LDH, U/L	854 [698; 1281]	619 [498.5; 813.5]	0.07
CRP, mg/L	36.6 [14,8; 338]	25.4 [8.7; 178]	0.4
D-dimer, mg/L	3076.6 [1499.4; 4888.6]	1788.4 [883.7; 2917]	0.1
Procalcitonin, ng/mL	1.68 [0.29; 17]	0.14 [0.05; 0.36]	0.001
uPAR, ng/mL	6.73 [3.98; 7.76]	3.80 [2.90; 4.92]	0.010
Interleukin 6, pg/mL	724.7 [62.7; 1713]	72.75 [23.93; 145.5]	0.013

ALT — alanine aminotransferase; AST — aspartate aminotransferase; CRP — C-reactive protein; IL-6 — interleukin 6; LDH — lactate dehydrogenase; LMR — lymphocyte to monocyte ratio; NLR — neutrophil to lymphocyte ratio; PLR — platelet to lymphocyte ratio; RBC — red blood cells; uPAR — urokinase-type plasminogen activator receptor; WBC — white blood cells.

On study day 2 and 3, lymphocyte counts differed significantly between patients with MMSS ≥2 points and <2 points (Fig. 1). The differences between the groups in WBC and neutrophil counts became significant on day 3 of the study (Fig. 1). On days 2 and 3, the difference in NLR between the patients with MMSS above

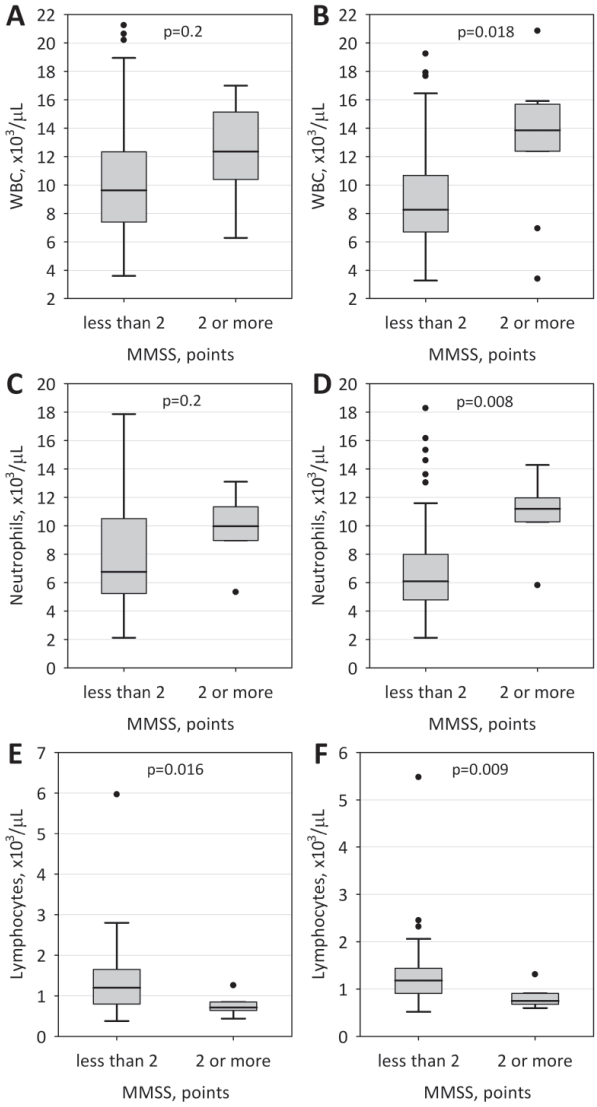

Fig. 1. WBC (A, B), neutrophil (C, D) and lymphocyte (E, F) counts on day 2 (A, C, E) and day 3 (B, D, F) of the study among patients with acute pancreatitis with and without organ failure associated with two or more points in modified Marshall scoring system (MMSS). Data are shown as median, interquartile range (box), non-outlier range (whiskers) and outliers (dots).

and below 2 points was highly significant (Fig. 2). As on day 1, we observed no differences between the groups in day 2 and 3 monocyte and platelet counts ($p > 0.4$ in all cases). Moreover, day 2 and 3 LMR and PLR values did not differ between the groups (Fig. 2).

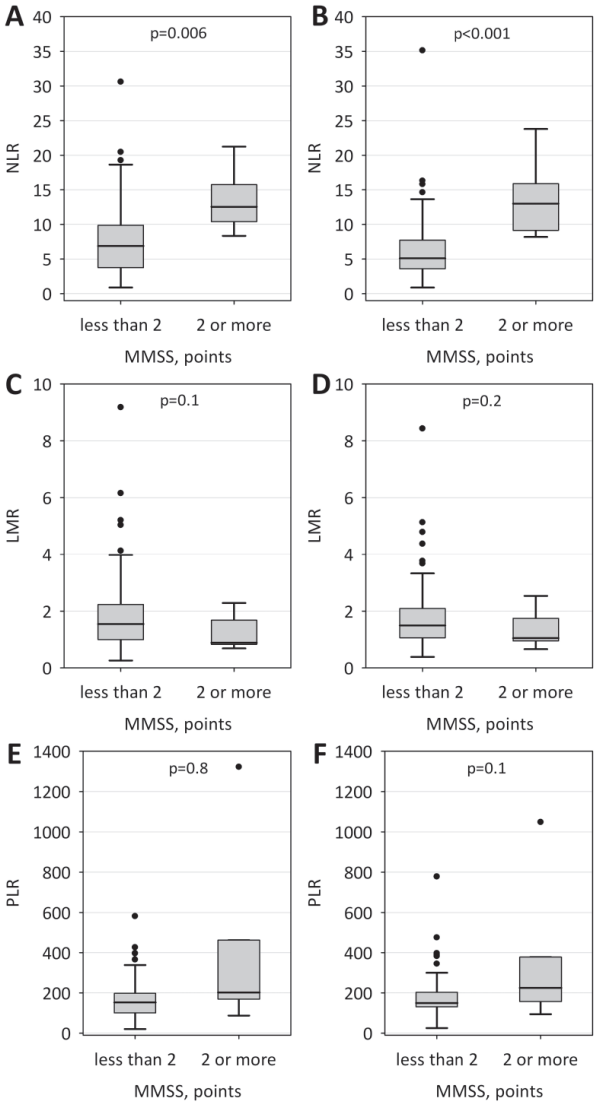

Fig. 2. NLR (A, B), LMR (C, D) and PLR (E, F) indexes on day 2 (A, C, E) and day 3 (B, D, F) of the study among patients with acute pancreatitis with and without organ failure associated with two or more points in modified Marshall scoring system (MMSS). Data are shown as median, interquartile range (box), non-outlier range (whiskers) and outliers (dots).

In logistic regression analysis, NLR values assessed on day 2 and 3, as well as WBC, neutrophil and lymphocyte count on day 3 of the study significantly predicted organ failure in the early phase of AP (Table 3).

Table 3. Statistically significant predictors of organ failure in the first week of acute pancreatitis associated with two or more points in modified Marshall scoring system. Odds ratios (OR) in simple logistic regression analysis per 1 unit increase in the value of predictor variable are given with 95% confidence intervals (CI).

Predictor variable	Day 2		Day 3	
	OR (95% CI)	p-value	OR (95% CI)	p-value
WBC, per 1 $\times 10^3/\mu L$	–	–	1.28 (1.07–1.54)	0.007
Neutrophils, per 1 $\times 10^3/\mu L$	–	–	1.30 (1.04–1.62)	0.017
Lymphocytes, per 1 $\times 10^3/\mu L$	–	–	0.01 (0.0002–0.65)	0.028
NLR, per 1	1.15 (1.01–1.31)	0.028	1.18 (1.03–1.35)	0.016

Comparison of other laboratory markers between patients with and without organ failure

Among studied laboratory markers, urea, creatinine, procalcitonin, uPAR and IL-6 concentrations were significantly higher among patients with organ failure (MMSS ≥2 points) than among those without the complication (Table 2). These differences remained statistically significant throughout the study. Moreover, on day 2 and 3, patients with MMSS ≥2 points presented higher concentrations of bilirubin (median 38.9 versus 18.6 μmol/L on day 2; $p = 0.029$, and 35.3 versus 15.1 μmol/L on day 3; $p = 0.019$, respectively), and higher amylase activity in sera (median 590 versus 141 U/L on day 2; $p = 0.032$, and 184 versus 76 U/L on day 3; $p = 0.041$, respectively).

Correlations of CBC values and NLR, LMR and PLR indexes with IL-6, uPAR and other laboratory markers

In the whole studied group of patients at the early phase of AP, we observed significant correlations between the studied CBC values as well as the NLR, LMR, and PLR indexes, and the results of laboratory tests associated with organ failure and inflammation (Table 4).

WBC (on day 3), neutrophil count (on days 1 and 3), and NLR (on days 2 and 3) correlated positively with urea concentrations, while negative correlations were observed between urea and lymphocyte count (on day 2) and LMR (on day 2) (Table 4). Monocyte count correlated positively with serum creatinine (throughout

the study), and there was a negative correlation between platelet count and creatinine on day 3. Serum bilirubin correlated positively with NLR (on day 2), and negatively with lymphocyte and platelet counts (on days 2 and 3). Serum lactate dehydrogenase activity on days 2 and 3 was positively correlated with WBC, neutrophil, monocyte counts and NLR and negatively with lymphocyte, platelet counts and LMR (Table 4).

Table 4. Statistically significant correlations of white blood cell counts, differential counts, neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR) and platelet to lymphocyte ratio (PLR) with selected laboratory markers during early stage of acute pancreatitis.

Variable	Day 1		Day 2		Day 3	
	R	p-value	R	p-value	R	p-value
White blood cell count						
Urea	–	–	–	–	0.24	0.02
LDH	–	–	–	–	0.30	0.01
CRP	0.21	0.04	0.40	<0.001	0.50	<0.001
Procalcitonin	0.29	0.01	0.37	<0.001	0.52	<0.001
D-Dimer	–	–	0.34	0.001	0.46	<0.001
IL-6	0.37	<0.001	0.50	<0.001	0.59	<0.001
Ranson score	–	–	0.41	<0.001	–	–
Absolute neutrophil count						
Urea	0.42	0.004	–	–	0.30	0.006
LDH	–	–	0.32	0.006	0.27	0.02
CRP	–	–	0.48	<0.001	0.55	<0.001
Procalcitonin	0.33	0.03	0.52	<0.001	0.61	<0.001
D-Dimer	–	–	0.45	<0.001	0.48	<0.001
uPAR	–	–	–	–	0.26	0.03
IL-6	0.42	0.006	0.63	<0.001	0.63	<0.001
Ranson score	–	–	0.45	<0.001	–	–
NLR						
Urea	–	–	0.37	0.001	0.33	0.003
Bilirubin	–	–	0.23	0.04	–	–
LDH	–	–	0.39	<0.001	0.44	<0.001
CRP	–	–	0.51	<0.001	0.57	<0.001
Procalcitonin	0.51	<0.001	0.61	<0.001	0.68	<0.001
D-Dimer	–	–	0.40	<0.001	0.47	<0.001

Table 4. Cont.

Variable	Day 1		Day 2		Day 3	
	R	p-value	R	p-value	R	p-value
uPAR	0.36	0.03	0.34	0.002	0.40	<0.001
IL-6	0.53	<0.001	0.53	<0.001	0.64	<0.001
Ranson score	–	–	0.29	0.008	–	–
Absolute lymphocytes count						
Urea	–	–	−0.28	0.01	–	–
Bilirubin	–	–	−0.37	<0.001	−0.26	0.02
LDH	–	–	−0.25	0.03	−0.37	0.001
CRP	–	–	−0.28	0.01	−0.26	0.01
Procalcitonin	−0.40	0.007	−0.38	<0.001	−0.41	<0.001
uPAR	−0.34	0.03	−0.42	<0.001	−0.38	<0.001
IL-6	−0.29	0.06	−0.27	0.006	−0.29	0.009
Absolute monocyte count						
Creatinine	0.37	0.01	0.24	0.03	0.29	0.01
LDH	–	–	–	–	0.40	<0.001
CRP	–	–	–	–	0.45	<0.001
Procalcitonin	0.38	0.01	–	–	0.41	<0.001
D-Dimer	–	–	–	–	0.46	<0.001
uPAR	–	–	–	–	0.27	0.02
IL-6	–	–	–	–	0.53	<0.001
LMR						
Urea	–	–	−0.26	0.02	–	–
LDH	–	–	−0.28	0.01	−0.28	0.02
CRP	–	–	−0.33	0.003	−0.29	0.01
Procalcitonin	−0.52	<0.001	−0.33	0.004	−0.37	0.001
D-Dimer	–	–	−0.32	0.005	–	–
uPAR	−0.35	0.04	−0.26	0.02	–	–
IL-6	−0.50	0.001	−0.30	0.009	−0.32	0.005
Platelet count						
Bilirubin	–	–	−0.46	<0.001	−0.41	<0.001
LDH	–	–	−0.32	0.006	–	–

Table 4. Cont.

Variable	Day 1		Day 2		Day 3	
	R	p-value	R	p-value	R	p-value
Creatinine	–	–	–	–	–0.23	0.03
CRP	–0.20	0.06	–	–	–	–
PLR						
Procalcitonin	–	–	–	–	0.24	0.03
uPAR	–	–	0.23	0.04	–	–
IL-6	–	–	–	–	0.25	0.02

ALT — alanine aminotransferase; AST — aspartate; CRP — C-reactive protein; IL-6 — interleukin 6; LDH — lactate dehydrogenase; LMR — lymphocyte to monocyte ratio; NLR — neutrophil to lymphocyte ratio; PLR — platelet to lymphocyte ratio; uPAR — urokinase-type plasminogen activator receptor; WBC — white blood cells.

The studied laboratory markers of inflammation correlated positively with WBC, neutrophil count, NLR, monocyte count, and PLR, and negatively with lymphocyte count, and LMR (Table 4). Total WBC counts and NLR values were significantly associated with CRP, procalcitonin and IL-6 throughout the study, and with D-dimer on days 2 and 3. Moreover, NLR positively correlated with serum uPAR during the entire observation period. Neutrophil counts significantly correlated with procalcitonin and IL-6 throughout the study, with CRP and D-dimer on days 2 and 3 and with uPAR on day 3. Lymphocyte counts were negatively associated with uPAR, IL-6 and procalcitonin on days 1 to 3, and with CRP on days 2 and 3. Only day 3 monocyte counts correlated with the studied inflammatory markers, except for correlation with procalcitonin on day 1, while more significant correlations were observed for LMR (Table 4). In case of platelet counts, only a weak negative correlation with CRP was observed on day 1, while PLR positively correlated with uPAR (on day 2), procalcitonin and IL-6 (on day 3) (Table 4).

Moreover, significant correlations were observed between Ranson's score and WBC, neutrophil, count and NLR (Table 4).

Discussion

The increase in peripheral blood neutrophil counts and the decrease in lymphocyte counts is commonly observed in patients with SIRS developing in the course of various diseases, including AP, severe septic complications, bacteremia, as a consequence of extensive surgical injury or after a cardiothoracic surgery [1, 8, 11, 13, 14]. It has been proposed previously that monitoring the values of NLR can be more useful than total WBC counts in differentiating between the mild and severe AP [14]. In the course

of AP neutrophils propagate inflammation and tissue injury via the activation of a wide range of mediators and inflammatory markers i.e. proinflammatory cytokines (IL-6, IL-8, tumor necrosis factor a), proteolytic enzymes (elastase, myeloperoxidase, collagenase) and reactive oxygen species. In turn, lymphopenia in the first 24 hours can be an independent marker of progression of inflammation, necrosis and septic complications and it remains a negative prognostic factor in this group of patients [1, 8, 11]. Shen *et al.* [11] revealed that reduced lymphocyte count within 48 hours from AP onset reflects the dysfunction of the immune system and might be an early and effective predictor of pancreatic necrosis [11].

The present study showed that in patients with vital organ failure in the early phase of AP (MMSS ≥2 points in the first week of AP), lower absolute lymphocyte counts were observed on days 2 and 3 from the onset of AP symptoms in comparison to patients without organ failure. The lymphocyte counts observed in patients with organ failure were, in fact, below the lower reference limit of 1.0 to 5.0 $\times 10^3/\mu L$ already on day 1 of AP. Simultaneously, in the whole studied group, we observed absolute neutrophilia and increased NLR index as compared to the previously reported reference values (1.8 to 8.0 $\times 10^3/\mu L$ and 0.78 to 3.53, respectively) [15, 16]. NLR on days 2 and 3 from AP onset significantly predicted vital organ failure developing in the first week of the disease.

The widely available automated 5-diff hematological analyzers can provide 28 diagnostic parameters which in the case of WBC differentiate their 5 main populations i.e. neutrophils, lymphocytes, monocytes, eosinophils, and basophils. The wide panel of red blood cell indexes may be used for the evaluation of anemia. The extended flagging system enables the identification of almost 30 various abnormalities regarding the CBC result [16]. The recommendations established in 1992 by the National Committee for Clinical Laboratory Standards [currently functioning under the name of Clinical and Laboratory Standards Institute (CLSI)] advocated for the use of absolute cell counts instead of percentages of white blood cells subpopulations, in consequence of wide availability of automated blood cell counters. This was a significant shift in the interpretation of CBC results that also enabled the use of indexes such as NLR, LMR, or PLR in the routine medical diagnostics [16]. At present, the values of NLR, PLR, and LMR indexes can be easily obtained following ordering an automated CBC with WBC differential. Their availability in everyday clinical practice depends solely on the laboratory's decision to include them in the diagnostic panel by implementing the simple formulas for their mathematical computation. This wide availability encouraged us to analyze the indexes in association with more sophisticated laboratory markers such as IL-6 and uPAR with respect to early prediction of organ failure in AP.

We observed significantly lower LMR values on day 1. The result is in line with the study by Li *et al.* [9], which showed that LMR index has a minor prognostic

value in SAP prediction. LMR index, however, has not been yet carefully studied in AP, and the most abundant research on its prognostic value concerns primarily colorectal cancer [17], pancreatic cancer [18–20], hematological malignancies [21] and breast cancer [22]. The mentioned studies pointed to correlations between the LMR values and the degree of systemic inflammation, as well as progression-free and overall survival. The significantly higher NLR values on days 2 and 3 in patients with complicated course of AP are also in accordance with the previous observations [3, 8, 12, 14]. The work by Cho *et al.* [3] as the only one highlights clearly higher NLR and PLR indexes values in patients with biliary etiology of SAP [3]. In 2011, Azab *et al.* [14] found that the NLR >4.7 allows for an accurate prognosis of SAP and the necessity to transfer patients to intensive care unit [6, 12, 14]. Our study did not confirm the observations regarding PLR, including those of Kaplan *et al.* [6] who showed significantly higher values of PLR index in patients with severe or complicated course of AP [6].

In our study, NLR values strongly positively correlated with procalcitonin and IL-6 concentrations and also positively correlated with serum uPAR during the whole observation time. Moreover, it correlated with several markers of organ dysfunction (urea, bilirubin, lactate dehydrogenase) and the values of Ranson's score. A statistically significant inverse correlation was observed between LMR and both procalcitonin and IL-6 on all days of the study as well as with uPAR on days 1 and 2. Our previous studies on diagnostic usefulness of IL-6 in the early phase of AP demonstrated that it is useful for prediction of severe course of AP [23]. IL-6 is one of the cytokines which first reaches the inflammation site and is produced by a wide array of cells: monocytes, T-cells, B-cells, neutrophils, fibroblasts and also pancreatic acinar cells [23, 24]. In prediction of SAP, it has been shown to complement the SIRS criteria [24]. As IL-6 is not available in most routine diagnostic laboratories, NLR may be considered as a surrogate marker in the early phase of AP.

uPAR, in turn, is a glycoprotein expressed on various cells, including monocytes, macrophages, neutrophils, endothelial cells and some cancer cells [25]. uPAR and its urokinase-type plasminogen activator ligand play an important role in the immunological response linked with migration, adhesion, angiogenesis, fibrinolysis, and cell proliferation [26]. The increase in serum concentrations of the soluble form of the receptor has been linked with the activation of the immunological system and reflects the severity of inflammatory diseases. In the present study, we observed significantly higher concentrations of uPAR among the patients with vital organ failure starting from day 1 from the onset of AP symptoms. Significant positive correlations were observed between uPAR and NLR as well as negative correlations with absolute lymphocyte counts on each study day. Moreover, LMR negatively correlated with uPAR on two first days of the study. Previously, uPAR has been associated with the severity of AP as well as other acute states [7, 27], thus its correlation with NLR

can additionally justify the need for the evaluation of NLR values in the daily clinical practice.

Conclusions

Since the computation of indexes: NLR, LMR and PLR is easily attainable based on routine CBC with WBC differential, incorporating them into routine diagnostics has been suggested. Constantly increased values of NLR observed in the first three days following the onset of AP in the patients who developed early organ failure and the correlations of NLR with more sophisticated inflammatory markers (including uPAR and IL-6) point to the potential usefulness of this index in the early prognosis of the severe course of AP.

Conflict of interest

None declared.

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References

1. *Qi X., Yang F., Huang H., Du Y., Chen Y., Wang M., et al.*: A reduced lymphocyte ratio as an early marker for predicting acute pancreatitis. Scientific Rep. 2017; 7: 44087. doi: 10.1038/srep44087.
2. *Banks P.A., Bollen T.L., Dervenis C., Gooszen H.G., Sarr M.G., Tsiotos G.C., et al.*: Classification of acute pancreatitis — 2012. Revision of the Atlanta classification and definitions by international consensus. Gut. 2013; 62: 102–111.
3. *Cho S.K., Jung S., Lee K.J., Kim J.W.:* Neutrophil to lymphocyte ratio and platelet to lymphocyte ratio can predict the severity of gallstone pancreatitis. BMC Gastroenterology. 2018; 18. doi: 10.1186/s1276-018-0748-4.
4. *Goral V., Berekatoglu N., Mete N.*: Correlation of disease activity, IL-6 and CRP levels and leukocytes/lymphocyte ratio among patients with acute pancreatitis. J Gastroint Dig Syst. 2012. doi: 10.4172/2161-069X.1000112.
5. *Borges T.A., Franzon O., Perizio Mello A.L.*: Analysis of new inflammatory markers in acute pancreatitis and confection of new prognostic definition model: Panc4. EC Gastroenterol Dig System. 2017: 240–246.
6. *Kaplan M., Ates I., Oztas E., Yuksel M., Akpinar M.Y., Coskun O., et al.*: A new marker to determine prognosis of acute pancreatitis: PLR and NLR combination. J Med Biochem. 2018; 37. doi: 10.1515.jomb-2017-0039.
7. *Lipinski M., Rydzewska-Rosołowska A., Rydzewski A., Cicha M., Rydzewska G.*: Soluble urokinase-type activator receptor (suPAR) patients with acute pancreatitis (AP) — Progress in prediction of AP severity. Pancreatology. 2017; 17: 24–29.

8. *Suppiah A., Malde D., Arab T., Hamed M., Allgar V., Smith A.M., et al.*: The prognostic value of the neutrophil-lymphocyte ratio (NLR) in acute pancreatitis: identification of an optimal NLR. J Gastrointest Surg. 2013; 17: 675–681. doi: 10.1007/s11605-012-2121-1.
9. *Li Y., Zhao Y., Feng L., Guo R.*: Comparison of the prognostic values of inflammation markers in patients with acute pancreatitis: a retrospective cohort study. BMJ Open. 2017; 7: e013206. doi: 10.1136/bmjopen-2016-013206.
10. *Naskalski J.W., Maziarz B., Kuśnierz-Cabala B., Dumnicka P., Panek J.*: Monocyte and neutrophil direct counts correlate with C-reactive protein concentration in patients with acute pancreatitis. Central Eur J Med. 2006. doi. 10.2478/s11536-007-008-4.
11. *Shen X., Sun J., Ke L., Zou L., Li B., Tong Z., et al.*: Reduced lymphocyte count as an early marker for predicting infected pancreatic necrosis. BMC Gastroenterology. 2015; 15. doi: 10.1186/s12876-015-0375-2.
12. *Zhang Y., Wu W., Dong L., Yang C., Fan P., Wu H.*: Neutrophil to lymphocyte ratio predicts persistent organ failure and in-hospital mortality in an Asian Chinese population of acute pancreatitis. Medicine. 2016; 95. doi: 10.1097/MD0000000000004746.
13. *Gurol G., Ciftci I.H., Terzi H.A., Atasoy A.R., Ozbek A., Koroglu M.*: Are there standardized cutoff values for neutrophil-lymphocyte ratios in bacteriemia or sepsis? J Microbiol Biotechnol. 2015; 25 (4): 521–525. doi: 10.4014/jmb.1408.08060.
14. *Azab B., Jaglall N., Atallah J.P., Lamet A., Raja-Surya V., Farah B., et al.*: Neutrophil-lymphocyte ratio as a predictor of adverse outcome of acute pancreatitis. Panceratology. 2011; 11 (4): 445–452.
15. *Forget P., Khalifa C., Defour J.P., Latinne D., van Pel M.C.*: What is the normal value of the neutrophil-to-lymphocyte ratio? BMC Res Notes. 2017; 10. doi: 10.1186/s13104-016-2335-5.
16. *Kabata J., Hellman A., Podolak-Dawidziak M., Solnica B., Sacha T., Giannopoulos K., et al.*: Badania laboratoryjne i morfologiczne. Interna Szczeklika. Wyd. Medycyna Praktyczna. 2018; 1681–1687.
17. *Shibutani M., Maeda K., Nagahara H., Iseki Y., Ikeya T., Hirakawa K.*: Prognostic significance of the preoperative lymphotyce-to-monocyte ratio in patients with colorectal cancer. Oncology Letters. 2017; 13: 1000–1006. doi: 10.3892/ol.2016.5487.
18. *Li W., Tao L., Zhang L., Xiu D.*: Prognostic role of lymphocyte to monocyte ratio for patients with pancreatic cancer: a systemic review and meta-analysis. Onco Targets Ther. 2017; 10: 3391–3397.
19. *Sierzega M., Lenart M., Rutkowska M., Surman M., Mytar B., Matyja A., et al.*: Preoperative neutrophil-lymphocyte and lymphocyte-monocyte ratios reflect immune cell population rearrangement in resectable pancreatic cancer. Ann Surg Oncol. 2017; 24: 808–815. doi: 10.1245/s10434-016-5634-0.
20. *Piciucchi M., Stigliano S., Archiburgi L., Zerboni G., Signoretti M., Barucca V., et al.*: The neutrophil/lymphocyte ratio at diagnosis is significantly associated with survival in metastatic pancreatic cancer. Int J Mol Sci. 2017; 18: 730. doi: 103390/ijms18040730.
21. *Gu L., Li H., Chen L., Ma X., Li X., Gao Y., et al.*: Prognostic role of lymphocyte to monocyte ratio for patients with cancer: evidence from a systemic review and meta-analysis. Oncotarget. 2016; 7 (22): 31926–31942. doi: 10.18632/0ncotarget.7876.
22. *Hu R.J., Liu Q., Ma J.Y., Zhou J., Liu G.*: Preoperative lymphocyte-to-monocyte ratio predicts breast cancer outcome: A meta-analysis. Clin Chem Acta. 2018; 484: 1–6. doi: 10.1016/j.cca.2018.05.031.
23. *Kolber W., Dumnicka P., Maraj M., Kuśnierz-Cabala B., Ceranowicz P., Pędziwiatr M., et al.*: Does the automatic measurement of interleukin 6 allow for predict of complications during the first 48 h of acute pancreatitis? Int J Mol Sci. 2018; 19: 1820. doi. 10.3390/ijms19061820.
24. *Jain S., Midha S., Mahapatra S.J., Gupta S., Sharma M.K., Nayak B., et al.*: Intereukin-6 significantly improves predictive value of systemic inflammatory response syndrome for predicting severe acute pancreatitis. Pancreatology. 2018; 1–7. doi. 10.1016/j.pan.2018.05.002.

25. *Kolber W., Kuśnierz-Cabala B., Dumnicka P., Maraj M., Mazur-Laskowska M., Pędziwiatr M., et al.*: Serum urokinase-type plasminogen activator receptor does not outperform C-reactive protein and procalcitonin as an early marker of severity of acute pancreatitis. J Clin Med. 2018; 7: 305. doi: 10.3390/jcm7100305.
26. *Khater W.S., Salah-Eldeen N.N., Khater M.S., Saleh A.N.*: Role of uPAR and lactatic acid in diagnosing sepsis and predicting mortality in elderly patients. Eur J Micr Immunol. 2016; 3: 178–185. doi: 10.1556/1886.2016.00011.
27. *Geboers D., de Bear F., Tuip-de Boer A., van der Poll T., Horn J., Cremer O., et al.*: Plasma suPAR as a prognostic biological marker for ICU mortality in ARDS patients. Int Care Med. 2015; 41: 1281–1290.