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ORIGINAL ARTICLE

Infectious Complications of Acute Pancreatitis Is Associated with Peripheral Blood Phagocyte Functional Exhaustion

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Abstract

Background Infected pancreatic necrosis is one of the most severe complications of acute pancreatitis (AP). The development of secondary infection doubles the risk of death during the late stage of necrotizing pancreatitis. Phagocytes play a major role in AP pathogenesis, as well as in local and systemic complications of the disease.

Aims We aimed to investigate the relationship between quantitative and functional indices of circulating phagocyte at the time of admission and onset of infectious complications in patients with AP afterward.

Methods A post hoc analysis of 97 patients with AP was conducted. The metabolic state of peripheral blood neutrophils and monocytes was analyzed based on their phagocytic activity and generation of reactive oxygen species (ROS), which were determined by flow cytometry on admission. The clinical end point was marked by onset of infectious complications of AP.

Results On admission, baseline values and reactivity reserve of monocyte and neutrophil phagocytic activity in AP patients, who developed septic complications, were substantially decreased, whereas monocyte ROS generation was dramatically increased as compared to the group without infectious processes. ROC curve was obtained both for neutrophil and monocyte phagocytosis reactivity reserve expressed as modulation coefficient values and categorized as the risk factor of infectious complications, showing an area under curve of 0.95 ($P < 0.0001$) and 0.84 ($P < 0.0001$), respectively.

Conclusions Early (at the time of admission) detection of quantitative and functional indices of circulating phagocytes can be useful for the prediction of septic complications in SAP patients.

Keywords Acute pancreatitis · Phagocytes · Phagocytosis · Reactivity reserve

Introduction

Acute pancreatitis (AP) is a debilitating and life-threatening inflammatory disease of the pancreas with increasing incidence in many countries throughout Europe and the USA, predominantly afflicting the working age population [1, 2]. About 20–25% of patients diagnosed with severe acute pancreatitis (SAP), a rapidly progressive and potentially fatal disease, develop it in association with necrosis of the pancreatic and peripancreatic tissues [3, 4]. The course of SAP includes an early vasoactive and toxic phase followed by the late phase that is characterized by septic complications. Infected pancreatic necrosis is one of the most severe complications of AP. Patients with infected necrotizing pancreatitis (INP) have more than twice greater risk of death than patients who do not have any septic complications [5, 6]. Therefore, it is extremely important to identify the patients with increased risk of INP at the initial phase of the disease since they qualify for preventive invasive intervention. In daily practice, clinical parameters such as fever and increased serum inflammatory markers (such as C-reactive protein level) are usually used to make a decision concerning invasive intervention for suspected infected necrosis. However, early reliable predictors for the septic complications of SAP are lacking [7–9].

The late phase of SAP is associated with immune suppression or compensatory anti-inflammatory response syndrome (CARS) that follows an early inflammatory immune response syndrome (SIRS) [10–12]. Therefore, septic complications in patients with SAP can be considered as a result of the impairment of the patrolling function of innate immunity cells, especially phagocytes. Phagocytes are central players in SAP pathogenesis, orchestrating the initiation, propagation, as well as local and systemic complications of

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the disease [13, 14]. Neutrophil–lymphocyte ratio (NLR) is considered to be one of the most valuable indices for the prediction of AP patient mortality compared with other inflammation markers [15]. The evaluation of phenotypic and functional indices of these cells in the prediction of SAP severity and complications has received increasing attention in recent years. HLA-DR expression in monocytes and CD64 expression in neutrophils are suggested as markers for early prediction of local and systemic complications in SAP [16, 17]. Soluble form of monocyte endocytic/signaling CD14-receptor (presepsin), that appears after shedding of mCD14 in response to bacterial ingress, is useful in the early diagnosis of infection in a complex population of patients with SIRS (including those with SAP), sepsis, severe sepsis, and septic shock [18]. Reactive oxygen species (ROS) generation by mono- and polymorphonuclear phagocytes is also considered as a valuable marker of disease severity and septic complications in patients with SAP [19, 20]. ROS generation along with phagocytosis plays a crucial role in the elimination of infective agents by phagocytes. Alterations observed in these metabolic reactions can be associated with the impairment of phagocyte patrolling function [21, 22]. Abnormalities in monocyte signaling including those that are involved in their phagocytic function are reported in patients with severe form of AP [23]. The aim of the study was to investigate the relationship between quantitative and functional indices of circulating phagocytes at the time of admission and onset of septic complications in patients with AP afterward.

Materials and Methods

Patients and Controls

Ninety-seven patients with AP, who were admitted to Kyiv City Clinical Emergency Hospital, Ukraine, over a 6-year period (2013–2019), were enrolled in the pilot study. Exclusion criteria were age ≤ 18 years, pregnancy, severe heart disease, severe renal dysfunction, compromised pulmonary status, immunodeficiency. Written informed consent was obtained from all patients according to a protocol of the local ethical committee. The severity of AP was defined according to the revised Atlanta classification [24]. All patients regardless of disease severity were included in the study. Mild acute pancreatitis (MAP) was diagnosed in ten patients. Moderately severe acute pancreatitis (MSAP) [25] was defined in 68 patients: 33 of them had transitory organ failure with local and systemic complications, and 35 of them had local and systemic complications without organ failure. SAP was defined in 19 patients, 13 of which had persistent dysfunction of a single organ system and six of which had persistent multiple organ failure. Diagnosis of SAP was based on consistent clinical picture, laboratory, and radiological findings obtained during ultrasound scan and/or CT examination. Local AP complications, requiring a puncture drainage, were revealed in 38 patients, acute peripancreatic fluid collection—in six patients, postnecrotic pancreatic/peripancreatic fluid collections—in 32 patients, pleural effusion—in 30 patients. Systemic inflammatory response (SIRS) was registered in 27 patients (40%) with MSAP and in two patients (10.5%) with SAP. SIRS was identified by following criteria: temperature < 36 °C or > 38 °C; heart rate > 90 beats/min; leukocyte count $< 4 \times 10^9$ or $> 12 \times 10^9$ per L. Septic complications (infected pancreatic/peripancreatic fluid) were revealed in 22 patients (nine patients (13.2%) with MSAP and 13 patients (68.4%) with SAP) according to the imaging (the presence of extraluminal gas in the pancreatic and/or peripancreatic tissues) and bacteriological (positive bacterial culture of aspiration and drainage content of pancreatic and/or peripancreatic tissues) findings. The total mortality was 2.9%, in the group with infectious complications—20%.

Ten healthy adult men were recruited to participate in the study as a control group. Criteria of exclusion to the study were identified based on a history of somatic disease. Approval was obtained from the Ethics Committee of Kyiv City Clinical Emergency Hospital, and consent was obtained from all subjects before the commencement of the study.

Study Design

EDTA-anticoagulated blood samples were obtained from patients with AP on admission and were immediately analyzed for circulating phagocyte metabolism. No prophylactic antibiotics were used before the blood sampling. Considering circannual variability of phagocyte metabolism, healthy volunteers and patients had their blood samples taken simultaneously. The absolute number and percentage of circulating lymphocytes, monocytes, and neutrophils (mature and immature cells) were determined in peripheral blood smears by routine manual method. Alpha-amylase was measured with the use of Olympus AU-800 (Olympus).

The average length of stay in the hospital was 26.4 bed days. Septic complications were identified between the third and fifth weeks after admission.

Intracellular ROS Assay

ROS levels were determined using 2′7′-dichlorodihydrofluorescein diacetate (carboxy-H2DCFDA, Invitrogen), which is transformed into a non-fluorescent derivative (carboxy-H2DCF) by phagocyte intracellular esterases as described previously [26]. Carboxy-H2DCF is membrane impermeable and is used as an indicator of intracellular ROS generation. Totally, 200 µl of EDTA-anticoagulated

whole venous blood was incubated with 4.3 µl of PBS containing 10 µM carboxy-H2DCFDA in thermostat for 30 min at 37 °C. Over a short time period, cellular esterases hydrolyzed the acetoxymethyl ester or acetate groups, and the dye became responsive to oxidation. Erythrocytes were destroyed with lysis buffer. The cells were then placed to polystyrene tubes (Falcon, Becton–Dickinson) and analyzed by flow cytometry (excitation: 488 nm, emission: 525 nm). Based on scattering parameters, only living cells were gated and used for the analysis. Neutrophils or monocytes were gated according to forward and side scatter.

The modulation coefficient (MC) was determined after the treatment of blood samples with phorbol 12-myristate 13-acetate (PMA) in vitro and was calculated by the formula:

$$MC = ((S-B)/B) \times 100,$$

where *S*—index value after treatment with PMA in vitro; *B*—index value of untreated cells (basal value).

Phagocytosis Assay

Monocyte and granulocyte phagocytosis was determined by flow cytometry as described above [26]. *Staphylococcus aureus* Cowan I cells (collection of the Department of Microbiology and Immunology of Taras Shevchenko National University, Kyiv) were grown on general purpose solidified medium and then were heat inactivated and fluorescein isothiocyanate (FITC) labeled.

FITC–labeled *S. aureus* cell suspension at a concentration of 1×10^7 cells/ml in a volume of 5 µl was added to 200 µl of EDTA-anticoagulated whole blood. A tube with whole blood served only as a negative control. All blood samples were incubated in thermostat for 30 min at 37 °C. After this, phagocytosis was arrested by the addition of cold stop solution (PBS with 0.02% EDTA and 0.04% paraformaldehyde). Erythrocytes were destroyed with lysis buffer. Fluorescence of phagocytic cells with engulfed bacteria was examined by flow cytometry. Neutrophils or monocytes were gated according to forward and side scatter. The fraction of phagocytizing cells was measured as a percentage of fluorescence emitting cells (*S. aureus* engulfed cells) in the respective gate. Phagocytosis index (PhI) was calculated with the following formula:

$$[\text{Gmeanpos}/\text{Ppos}] - [\text{Gmeanneg}/\text{Pneg}],$$

where Ppos—percent of positive cells, Gmeanpos— mean channel fluorescence, Pneg—percent of positive cells in the negative control, and Gmeanneg—mean channel fluorescence of the negative control. The modulation coefficient was evaluated as mentioned above.

Statistical Analysis

Results are expressed as the mean ± SD. The Student's *t* test and Mann–Whitney *U* test were used to evaluate differences between the two groups of patients, including the patients with and without septic complications. Using receiver operating characteristic (ROC) analysis, the area under the curve (AUC) of circulating monocyte and granulocyte ROS generation, PhI, and modulation coefficients of the aforementioned functions in response to PMA in vitro have been calculated, evaluated and compared according to their prognostic performance of predicting septic complications of severe acute pancreatitis. Sensitivity and specificity were calculated and presented for optimal cutoff values of the indicated indices. A P value of < 0.05 was considered statistically significant in all cases.

Results

Quantitative Indices of Circulating Lymphocytes, Mono- and Polymorphonuclear Phagocytes in Patients with Septic Complications of Acute Pancreatitis

As mentioned above, septic complications were revealed in 22 of 87 patients with MSAP and SAP (25.3%). There were no significant differences in baseline characteristics (age, sex, onset of disease symptoms) in patients with mild disease as well as in patients with severe disease without septic complications and in patients who developed infectious complications over the period from 3 to 5 weeks after admission (Table 1). There were more women in the MSAP/SAP group with septic complications than in the group without septic complications. No significant differences were noted in average values of quantitative indices of circulating lymphocytes and phagocytes (the proportion of circulating lymphocytes, monocytes, and granulocytes, as well as the proportion of phagocytizing mono- and polymorphonuclear cells) between patients with mild disease and MSAP/SAP patients with and without infectious complications at the time of admission. Neutrophil left shift (increased proportion of immature (promyelocytes, myelocytes, and bands) neutrophils, and total WBC count 6–20% over the normal, [27]) were registered in all MSAP/SAP patients regardless of the presence of septic complications. In patients with mild disease, NLR did not exceed the reference values for this age group. In MSAP/SAP patients, this index significantly exceeded upper limit of normal, but did not differ significantly in patients with and without septic complications.

Table 1 Baseline characteristics and quantitative indices of circulating leukocytes from patients with AP

	MAP ($n=10$)	MSAP/SAP	
		No septic complications ($n=65$)	Septic complications ($n=22$)
Male sex	8 (80%)	54 (83.1%)	14 (63.6%)
Female sex	2 (20%)	11 (16.9%)	8 (36.4%)
Average age (years)	43.4 ± 14.7	44.7 ± 11.2	48.9 ± 14.8
Time since the onset of symptoms (days)	0–1	0–1	0–1
Leukocytes, $\times 10^9$/L	8.9 ± 3.4 (reference range 4.5–11.0)	12.9 ± 2.4	11.7 ± 3.1
Monocytes, %	4.5 ± 1.5 (reference range 3–9)	3.6 ± 1.6	6.2 ± 1.9
Percentage of phagocytizing monocytes	29.5 ± 11.1	32.1 ± 8.6	32.9 ± 10.3
Immature neutrophils (myelocytes, metamyelocytes and bands), %	5.5 ± 3.5 (reference range 1–3)	10.8 ± 3.9*	12.9 ± 4.7*
Segmented neutrophils, %	40.3 ± 13.8 (reference range 40–70)	70.7 ± 8.9*	82.9 ± 12.8*
Percentage of phagocytizing neutrophils	38.1 ± 12.4	29.9 ± 9.7	30.9 ± 9.1
Lymphocytes, %	21.9 ± 8.9 (reference range 20–40)	17.1 ± 8.3	14.1 ± 4.9
Neutrophil–lymphocyte ratio	2.4 ± 1.8 (reference range 1.75–2.5)	4.3 ± 1.2*	6.1 ± 1.1*

Age characteristics as well as quantitative indices of circulating leukocytes are presented as the mean ± SD

AP acute pancreatitis, *MAP* mild acute pancreatitis, *MSAP* moderately severe acute pancreatitis, *SAP* severe acute pancreatitis

*$P < 0.05$ as compared to patients with mild acute pancreatitis

Functional Indices of Circulating Mono- and Polymorphonuclear Phagocytes in SAP Patients with Septic Complications

The functional indices (PhI and ROS generation) of peripheral blood phagocytes (monocytes and neutrophils) were compared in MSAP/SAP patients who developed and did not develop infectious complications over the period from 3 to 5 weeks, and the results are presented in Table 2. In addition to the estimation of baseline level of the aforementioned phagocyte metabolic functions, we also evaluated their reactivity reserve (the remaining capacity of a cell to fulfill given metabolic activity under stress) after the treatment with PMA in vitro. PMA—protein kinase C activator—has

Table 2 Functional indices of circulating phagocytes from patients with AP

	Healthy persons ($n=10$)	MAP ($n=10$)	MSAP/SAP ($n=87$)	
			No septic complications ($n=65$)	Septic complications ($n=22$)
Monocyte PhI (spontaneous)	91.0 ± 9.9	193.4 ± 54.7*	410.2 ± 33.8*,#	231.7 ± 57.4*,^
Monocyte PhI (PMA)	71.6 ± 10.7	202.7 ± 60.3	399.9 ± 168.0	150.0 ± 51.2
Monocyte PhI (modulation coefficient)	− 17.9 ± 7.6	4.8 ± 2.2*	− 7.6 ± 12.9	− 34.7 ± 2.9*,#,^
Monocyte ROS (spontaneous), GMean	426.6 ± 86.0	689.5 ± 16.8*	611.2 ± 28.0*	971.2 ± 209.8*,#,^
Monocyte ROS (PMA), GMean	511.7 ± 121.0	770.3 ± 78.9	641.4 ± 56.7	1008.9 ± 186.9
Monocyte ROS (modulation coefficient)	16.3 ± 9.8	17.1 ± 19.3	30.3 ± 59.2	12.4 ± 6.9
Neutrophil PhI (spontaneous)	76.4 ± 7.2	183.5 ± 20.7*	477.6 ± 42.2*	224.1 ± 41.6*,^
Neutrophil PhI (PMA)	72.3 ± 8.0	220.4 ± 3.4	699.1 ± 32.6	159.0 ± 34.1
Neutrophil PhI (modulation coefficient)	− 6.0 ± 2.4	14.4 ± 12.6*	62.1 ± 14.3*,#	− 14.1 ± 2.0#,^
Neutrophil ROS (spontaneous), GMean	319.9 ± 50.7	333.8 ± 29.8	829.3 ± 74.1*,#	639.2 ± 58.6*,#,^
Neutrophil ROS (PMA), GMean	367.8 ± 66.7	490.6 ± 66.0	891.5 ± 64.2	886.3 ± 109.9
Neutrophil ROS (modulation coefficient)	18.5 ± 14.3	30.7 ± 30.7	32.3 ± 48.7	12.1 ± 37.1

AP acute pancreatitis, *MAP* mild acute pancreatitis, *MSAP* moderately severe acute pancreatitis, *SAP* severe acute pancreatitis

Data are presented as the mean ± SD. *$P < 0.05$ compared with healthy individuals, #$P < 0.05$ compared with patients with mild pancreatitis; ^$P < 0.05$ as compared to SAP patients without septic complications

the ability to stimulate macrophage oxidative metabolism and opsonophagocytosis and does not affect significantly opsonin-free phagocytosis [28]. Opsonophagocytosis is the phagocytosis mediated by opsonins such as IgG and collectins and is a component of inflammatory diseases [29]. Positive value of MC (see Materials and Methods) indicates the existence of reactivity reserve in analyzed cell population, whereas negative value evidences the maximum degree of the activation of the given function and lack of reactivity reserve. All investigated functional indices of circulating phagocytes from AP patients differed significantly from corresponding indices in healthy volunteers. Peripheral blood phagocytes from all AP patients were in activated state that was indicated by increased phagocytic activity and oxidative metabolism (baseline values). Meanwhile, metabolic states of circulating phagocytes from AP patients with distinct forms of the disease differed from each other.

In MSAP/SAP patients without septic complications, circulating monocytes demonstrated increased baseline phagocytic activity at admission, compared with patients with mild disease and healthy individuals. Treatment of these cells with PMA in vitro failed to boost their endocytosis. It is necessary to note that the response of circulating monocytes in SAP patients without septic complications to PMA in vitro was characterized by significant individual variability. Neutrophil baseline phagocytosis in MSAP/SAP group without infectious complications was also substantially amplified, compared with patients with mild pancreatitis and healthy volunteers. Nevertheless, treatment with PMA in vitro resulted in boosted endocytosis. Opsonophagocytosis, which could be activated by PMA, is more inherent for mature circulating neutrophils then less mature monocytic cells. ROS generation level was higher in these cells than in neutrophils obtained from patients with mild disease and from healthy individuals.

In MSAP/SAP patients who subsequently developed infectious complications, we observed somewhat different functional states of circulating phagocytes at admission. Baseline phagocytic activity of monocytes from these patients was reduced as compared with MSAP/SAP patients without infectious processes and did not differ significantly from those in patients with mild disease. However, in contrast to MAP patients whose monocytes responded positively to PMA in vitro, MC of this monocyte function in MSAP/SAP patients with septic complications was sharply negative indicating cell metabolic exhaustion. At the same time, oxidative metabolism indices of these cells were significantly higher than in MSAP/SAP group without septic complications. Neutrophils from the peripheral blood of MSAP/SAP patients with septic complications were also characterized by substantially lowered baseline phagocytic ability, compared with the group without infections, and by negative value of MC after stimulation with PMA in vitro.

Since monocyte phagocytic activity after the treatment with PMA in vitro in MSAP/SAP patients without septic complications was characterized by significant individual variability, it prompted us to perform more detailed analysis of baseline endocytosis values and its MC within this group of patients (Fig. 1). The research findings revealed dependence of phagocytosis MC on the baseline value of PhI. Mean baseline monocyte PhI value in one subgroup of MSAP/SAP patients without infections ($n=27$) was 2.3 times higher than in individuals with mild disease (subgroup 1). In this subgroup, we registered strongly negative value of MC (Fig. 1a). Baseline PhI value in patients from another subgroup ($n=38$) was closer to the value of patients with the mild form of the disease (subgroup 2), and MC value in this subgroup was strongly positive. Despite the differences in monocyte phagocytic function, neutrophil phagocytic activity in both subgroups did not differ significantly (Fig. 1b). We did not find out any additional distinctive features (neither clinical nor laboratory) in these subgroups, probably due to the limited patient number.

The Role of Circulating Phagocyte Functional Indices in the Prediction of Septic Complications in Patients with Severe Acute Pancreatitis

The effectiveness of the investigated functional indices of circulating phagocytes in the early recognition of patients with and without septic complications was assessed with the use of ROC analysis. The results of this analysis are summarized in Table 3. The area under the ROC curve (AUC) indicates that the values of neutrophil and monocyte phagocytic activity MCs were statistically significant predictive indicators of the development of septic complications. The largest AUC values were registered for these indices (Fig. 2a, b, respectively). The highest specificity and sensitivity were also calculated for these characteristics. The remaining metabolic indices of phagocytes were characterized by high specificity, but rather low sensitivity.

Discussion

AP is an inflammatory disease of pancreas that is accompanied by necrosis of pancreatic and peripancreatic tissue as well as remote organ failure in case of SAP. Sterile pancreatic and peripancreatic necrosis can be compartmentalized by the body, whereas infected necrosis serves as the nidus for bacteria and fungi, which can significantly aggravate the inflammatory process, as well as cause organ failure, systemic sepsis, and thereby increase the risk of a patient's death [30, 31]. Infected necrotizing pancreatitis occurs in 30–70% of SAP patients and is one of the most severe outcomes. Early evaluation of the risk of infectious

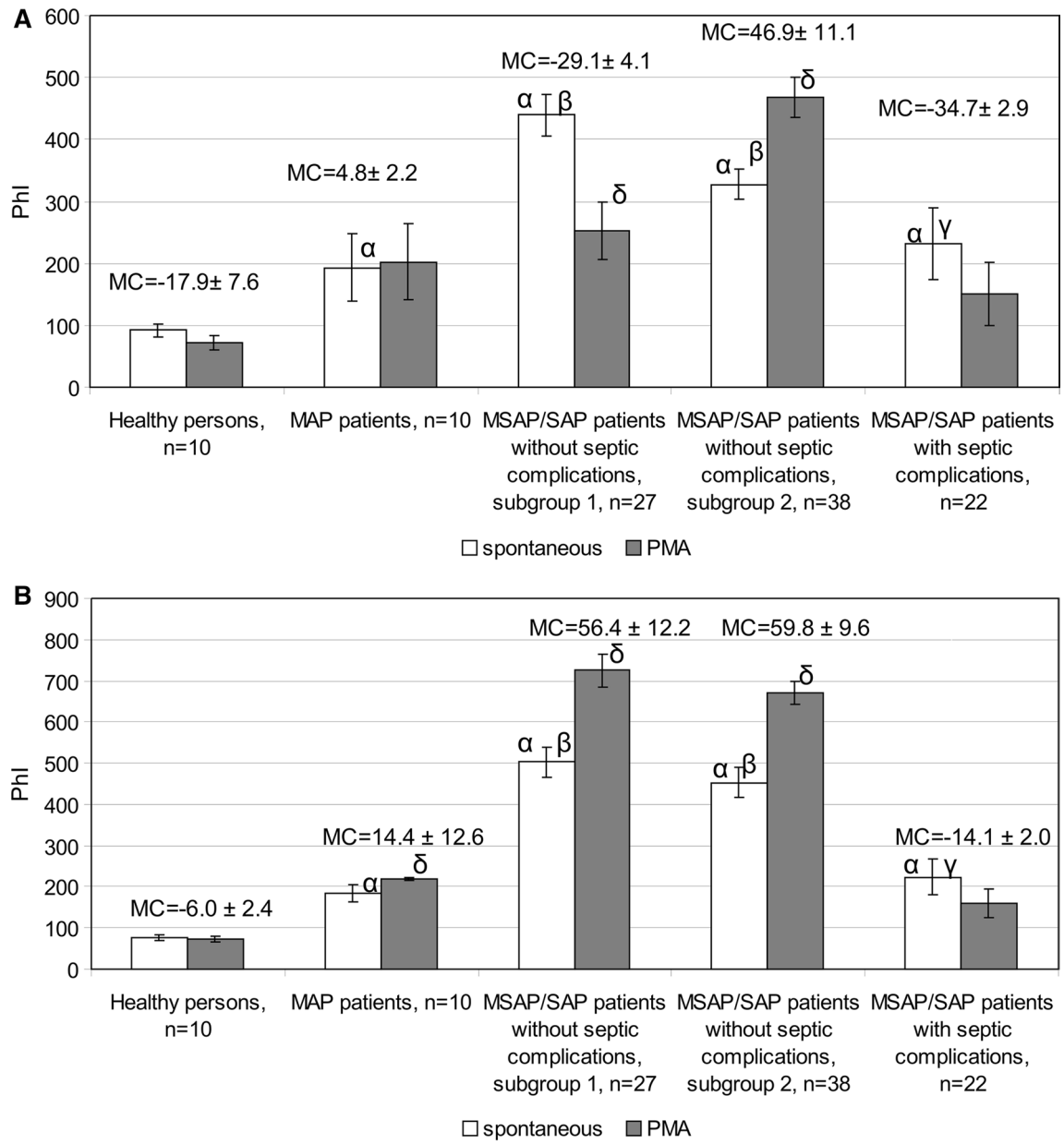

Fig. 1 Phagocytic activity of circulating monocytes (**a**) and neutrophils (**b**) from patients with MSAP/SAP. Data are presented as the mean ± SD. α—$P < 0.05$ as compared to corresponding index of healthy volunteers; β—$P < 0.05$ as compared to corresponding index of patients with MAP; γ—$P < 0.05$ as compared to corresponding index of MSAP/SAP patients without septic complications; δ—$P < 0.05$ as compared to corresponding unstimulated probe

complications in patients with SAP is important to timely differentiate therapeutic approaches because patients with infected necrotizing pancreatitis often need invasive intervention. Poor assessment of prognosis of septic complications in SAP patients necessitates the search for early reliable prognostic markers of pancreatic infections.

Monocytes and granulocytes together with tissue macrophages are professional phagocytes that maintain sterility throughout the body and defend tissues from pathogen invasion and bacterial translocation that is characteristic for severe form of pancreatitis. Phagocytes are capable of eliminating microorganisms by multiple mechanisms. Key events in antiinfective immune responses mediated by phagocytes are phagocytosis followed by oxygen-dependent killing of engulfed microorganism. Phagocyte endocytosis also plays a crucial role in a scavenger function that allows the body to maintain tissue homeostasis [32]. At the initial stages of AP, phagocytes are important players of disease progression.

Table 3 Results of ROC analysis for the evaluation of the significance of circulating phagocyte functional indices for the prediction of infectious complications in patients with MSAP/SAP

Index	AUC	*P* value	Cutoff value	Sensitivity (%)	Specificity (%)
Monocyte PhI (spontaneous)	0.67	0.12	≤184.0	22.8	100
Monocyte PhI (PMA)	0.77	0.0005	≤338.6	22.0	100
Monocyte PhI MC	0.84	<0.0001	≤−24.8	76.2	83.6
Monocyte ROS (spontaneous)	0.61	0.50	>1008.9	22.0	100
Monocyte ROS (PMA),	0.61	0.30	>992.4	33.3	100
Monocyte ROS MC	0.66	0.05	>2.9	0.0	100
Neutrophil PhI (spontaneous)	0.68	0.02	≤278.3	44.4	100
Neutrophil PhI (PMA)	0.64	0.05	≥308.2	22.0	100
Neutrophil PhI MC	0.95	<0.0001	≤−8.2	88.6	90.8
Neutrophil ROS (spontaneous)	0.62	0.30	≤832.8	22.2	100
Neutrophil ROS (PMA)	0.68	0.03	≤849.2	11.1	100
Neutrophil ROS MC	0.59	0.50	≤7.18	44.4	100

The discrepancy between the efficacy of phagocytosis (efferocytosis) of apoptotic and necroptotic pancreatic cells by resident and recruited from the circulation pancreatic phagocytes and growing pancreatic cell death rate is associated with the release of damage-associated molecular patterns (DAMPs) and is one of the trigger mechanisms of the pancreatic inflammation [33, 34]. Recruited circulating neutrophils and monocytes produce cytokines, chemokines, and ROS and thereby amplify the migration of leukocytes to the pancreas, and latter—to remote organs. Recruited neutrophils contribute to activation of trypsinogen and disease progression. Pro-inflammatory mediators produced by recruited phagocytes determine the disease course, but, at the same time, maintain tissue sterility. Successively, CARS develops. CARS can promote bacterial superinfection of necrotic pancreatic tissue and subsequent development of septic complications [35]. Numerous recent studies reported the alteration and/or impairment of phagocyte functions in patients with SAP, as well as the significance of these alterations in the prediction of septic complications [12–14, 19–22]. Some of the methods for determining these alterations are not used routinely in the clinical settings due to their complexity.

In this study, we examined metabolic state of circulating phagocytes as an early predictor of septic complications in MSAP/SAP. Monocyte and neutrophil functional states were characterized by phagocytic activity and intracellular oxidative metabolism (ROS generation), as these metabolic reactions are necessary to maintain tissue sterility. The aforementioned phagocyte metabolic processes were evaluated by flow cytometry. This method is simple, relatively inexpensive and requires a little volume of a patient's blood. In addition to the evaluation of basal level of phagocytic activity and ROS generation, we estimated reactivity reserves of these functions by the treatment of blood samples with PMA in vitro. We performed retrospective data analysis by dividing 87 patients with MSAP/SAP into two groups. The patients, who developed infectious complications over the period from 3 to 5 weeks after admission, were assigned to one group ($n=22$), and the patients, who had no infectious complications, were allocated to the other group ($n=65$). Some phagocyte quantitative characteristics (NLR, neutrophil left shift) that are routinely used in clinical practice for the prognostication of infectious complications in patients with severe form of AP did not differ significantly in MSAP/SAP patients with and without subsequent infections. Unlike quantitative phagocyte parameters, the difference in functional characteristics of these cells was found out at admission between patients with MSAP/SAP, who later developed septic complications, and patients without infections. The research findings showed that monocyte and neutrophil baseline phagocytic activity at admission was decreased in MSAP/SAP patients with septic complications as compared to those who had none. Our observations correlate with the data of other research groups. Cardinale et al. reported that profound impairment of phagocyte-mediated immune responses can predispose the host to septic complications [36]. Zang et al. [37] observed strong correlation between AP severity and down-regulated phagocytosis receptor expression by circulating monocytes. Phagocytosis in monocytes and neutrophils is associated with NFκB-mediated cell signaling [38, 39]. Therefore, our results also correlate with the data of Oiva et al., according to which the proportion of NFκB -positive granulocytes is decreased in AP patients compared with healthy individuals, and it tended to be lower in response to *E. coli* or *S. aureus* in vitro. Oiva et al. [40] also reported that monocytes from AP patients are characterized by the impaired NFκB signaling. The authors suppose that such functional state of phagocytes may increase susceptibility of AP patients to secondary infections. Distinctive feature of our research is the evaluation of reactivity reserve of the investigated phagocyte functions in addition to the examination of baseline values. Our findings revealed sharply reduced phagocytosis reactivity reserve in both circulating monocytes and granulocytes as well as moderately

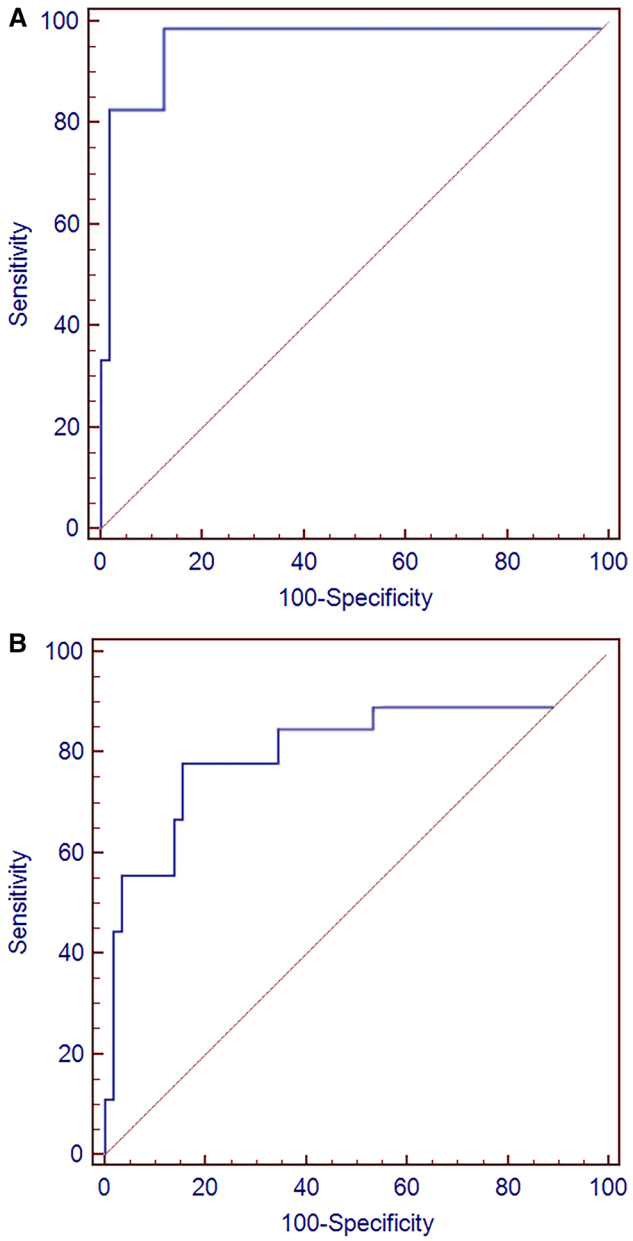

Fig. 2 ROC curve for phagocytosis activity MC of circulating neutrophils in MSAP/SAP patients, measured at admission (**a**). Area under the curve (AUC) = 0.95; the cutoff value of MC is − 8.2. ROC curve for phagocytosis activity MC of circulating monocytes, measured at admission (**b**). Area under the curve (AUC) = 0.86; the cutoff value of MC is − 24.8

reduced reactivity reserve of oxidative metabolism in these cells.

ROC analysis was performed to evaluate the prognostic value of metabolic characteristics of circulating phagocytes for early (at the time of admission) identification of the patients at risk of developing infectious complications. The results of this analysis revealed that decreased reactivity reserve of phagocytosis (characterized by MC) in monocytes and neutrophils is strongly associated with the risk of septic complication in the disease course. Monocyte phagocytosis MC seems less valuable for the prediction of septic complications in AP than phagocytic activity MC of circulating neutrophils. The heterogeneity of monocyte phagocytosis indices in patients, who did not develop infectious complications, can be one of the reasons for it. The heterogeneity of monocyte phagocytosis indices can be caused by the polymorphism in some monocyte phagocytosis-associated receptors that is characteristic for patients with AP [41, 42]. It would be interesting to investigate whether there is an association between monocyte phagocytosis indices and endocytosis-associated receptor polymorphism in these cells.

In conclusion, CARS always occurs in AP patients who might develop septic complications. Lack of phagocytosis reactivity reserve at admission, which indicates exhaustion of this function in circulating phagocytes, reflects the impairment of the patrolling function of innate immunity cells and might be indicative of the likelihood of septic complication in AP patients. In our study, predictive power of the suggested phagocytosis indices can be influenced by a small number of patients with septic complications. In order to prove the results of this pilot study, it is necessary to extend the study population in a follow-up research.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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