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Cytokine profile in critically ill patients and/or injured persons with secondary sepsis – influence of different pathogens

Profil citokina kod kritično obolelih bolesnika i/ili povređenih osoba sa sekundarnom sepsom – uticaj različitih patogena

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Abstract

Background/Aim. The role of the complex sepsis-related immune response has not been fully clarified and remains a subject matter of investigation. Nowadays, sepsis is considered a dynamic syndrome characterized by many, often antagonistic phenomena, ranging from hyperinflammation to anergy and immunoparalysis. The aim of the study was to determine, based on the level of pro- and anti-inflammatory mediators in critically ill patients with secondary sepsis, whether the cytokine profile differs according to the type of bacterial causative agent, as well as to assess the prognostic value regarding the outcome. The outcome measure was in-hospital mortality. **Methods**. Blood serum samples were taken from 125 critically ill patients admitted to the Surgical Intensive Care Unit with severe secondary sepsis as a consequence of peritonitis, pancreatitis, or trauma. The average age of the patients was 57.7 ± 17.3 years. Of the total number of patients, 84 (67.2%) were males, and 41 (32.8%) were females. The levels of pro-inflammatory interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-12p70, IL-17A, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, IFN-γ-inducible protein-10 (IP-10), monocyte chemoattractant protein-1(MCP-1), macrophage inflammatory protein (MIP)-1α and MIP-1β, as well as anti-inflammatory mediators IL-4, IL-10, IL-13, IL-27, IL-31, and IL-33, were determined at three time intervals – on the day of admission (the first day) and then on the third and fifth day. The type of the bacterial causative agent was determined using standard microbiological analyses. **Results**. On the third day of measurement, significant differences in the cytokine levels regarding the nature of bacteremia were determined in all pro- and anti-inflammatory cytokines, except for IL-8. In general, the lowest levels were observed in patients with polymicrobial blood cultures. On the first and fifth days of measurement, no significant differences in the cytokine levels regarding the nature of bacteremia were found. The only significant predictor of the fatal outcome on the first measurement day was IL-17A, Area Under the Receiver Operating Characteristic (ROC) Curve (AUC) of 0.665 (95% confidence interval 0.519–0.791; $p = 0.034$) in the patients with secondary sepsis as a complication of peritonitis. **Conclusion**. According to the type of bacterial causative agent, the lowest levels of cytokines have been observed in patients with the polymicrobial blood culture. The low level of IL-17A on the first day of measurement is a good predictor of a fatal outcome in patients with peritonitis as an underlying condition of secondary sepsis. On the other hand, the levels of other cytokines correlated with the outcome only on the fifth day of measurement, and they were higher in survivors than in non-survivors.

Key words:
blood culture; critical illness; cytokines; prognosis; sepsis; treatment outcome.

Apstrakt

Uvod/Cilj. Uloga kompleksnog imunskog odgovora u sepsi i dalje nije do kraja razjašnjena i ostaje predmet istraživanja. Danas se smatra da je sepsa dinamički sindrom koji karakterišu mnogi, često antagonistički fenomeni, u rasponu od hiperinflamacije do anergije i imunoparalize. Cilj rada bio je da se na osnovu nivoa pro- i anti-inflamacijskih medijatora

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kod kritično obolelih osoba sa sekundarnom sepsom utvrdi da li se citokinski profil razlikuje u odnosu na vrstu bakterijskog uzročnika, kao i da se proceni prognostička vrednost ovog nalaza u odnosu na ishod. Mera ishoda bila je hospitalni mortalitet. **Metode.** Uzorci seruma periferne krvi uzeti su od 125 kritično obolelih bolesnika primljenih u hiruršku jedinicu intenzivne nege sa potvrđenom teškom sekundarnom sepsom kao komplikacijom peritonitisa, pankreatitisa ili traume. Prosečna starost bolesnika bila je 57,7 ± 17,3 godina. Od ukupnog broja obolelih, 84 (67,2%) su bili muškarci, a 41 (32,8%) žene. Određeni su nivoi pro-inflamacijskih interleukina (IL)-1α, IL-1β, IL-6, IL-8, IL-12p70, IL-17A, faktora nekroze tumora (TNF)-α, interferona (IFN)-γ, IFN-γ-inducibilnog proteina-10 (IP-10), monocitnog hemoatraktantnog proteina (MCP)-1, inflamacijskog proteina makrofaga (MIP)-1α i MIP-1β i anti-inflamacijskih medijatora IL-4, IL-10, IL-13, IL-27, IL-31 i IL-33 u tri vremenska intervala – na dan prijema (prvi dan) a potom trećeg i petog dana. Standardnim mikrobiološkim ispitivanjima određena je vrsta bakterijskog uzročnika. **Rezultati.** Trećeg dana merenja ustanovljene su značajne razlike u nivoima citokina u odnosu na prirodu bakterijemije kod svih pro- i anti-inflamacijskih citokina, osim kod IL-8. Generalno, najniži nivoi utvrđeni su kod bolesnika sa polimikrobnom hemokulturom. Prvog i petog dana merenja nisu nađene značajne razlike u nivoima citokina u odnosu na prirodu bakterijemije. Jedini značajan prediktor fatalnog ishoda prvog dana merenja bio je IL-17A, *Area Under the Receiver Operating Characteristic* (ROC) *Curve* (AUC) 0,665 (95% interval poverenja 0,519–0,791; $p = 0.034$) kod bolesnika sa sekundarnom sepsom kao komplikacijom peritonitisa. **Zaključak.** Prema vrsti bakterijskog prouzrokovača utvrđeno je da su najniži nivoi citokina bili kod bolesnika sa polimikrobnom hemokulturom. Niska koncentracija IL-17A prvog dana merenja je dobar prediktor smrtnog ishoda kod bolesnika sa sekundarnom sepsom koja je nastala kao komplikacija peritonitisa. Nasuprot tome, nivoi ostalih citokina korelisali su sa ishodom tek petog dana merenja i bili su viši kod preživelih, u odnosu na umrle bolesnike.

Ključne reči:
bakteriološke tehnike; kritična stanja; citokini; prognoza; sepsa; lečenje, ishod.

Introduction

There is a complex immune response characterized by a dysfunction of neutrophils and monocytes, the key cells of the innate immune response, activated in critically ill surgical patients with secondary sepsis, often occurring as a consequence of severe acute pancreatitis, peritonitis, or trauma [1]. In some patients, the anti-inflammatory response is prevalent. The particular problem with the treatment of critically ill patients with sepsis is the fact that a large number of them stay for a long time in the intensive care unit (ICU) with dysfunction of various organs – basically, their condition is chronically critical. Their clinical process is characterized by very persistent catabolism with malnutrition, poor wound healing, immunosuppression, and recurrent infections. Thus, a special entity has been proposed, namely, a new Persistent Inflammation, Immunosuppression, and Catabolism Syndrome – PICS [2, 3]. The study by Boomer et al. [4] has shown that the patients who died from sepsis had had biochemical, immunohistochemical, and phenotyping signs pointing to immunosuppression. According to their study, to determine the association of sepsis with changes in host innate and adaptive immunity and to examine potential mechanisms for putative immunosuppression, rapid *post-mortem* spleen and lung tissue harvest was performed at the bedsides of 40 patients who died in ICU with active severe sepsis to characterize their immune status at the time of death. Control spleens were obtained from patients who were declared brain-dead or had emergency splenectomy due to trauma; control lungs were obtained from transplant donors or lung cancer resections. Cytokine secretion assays and immunophenotyping of cell surface receptor-ligand expression profiles were performed to identify potential mechanisms of immune dysfunction. Immunohistochemical staining was performed to evaluate the loss of immune effector cells. Compared with controls, anti-CD3/anti-CD28–stimulated splenocytes from sepsis patients had significant reductions in cytokine secretion at 5 hrs: tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-6, and IL-10 ($p < 0.001$ for all). To assess the possible etiology for the markedly depressed cytokine secretion, the authors performed flow cytometric analysis and examined the expression of cell surface receptors important in cellular activation. The present study shows that splenocytes from sepsis patients had highly significant functional impairments, as evidenced by major reductions in cytokine secretion. Cytokine secretion in sepsis patients was generally less than 10% than in controls, independent of age, duration of sepsis, corticosteroid use, and nutritional status. Although differences existed between the spleen and lung, flow cytometric analysis showed increased expression of selected inhibitory receptors and ligands and expansion of suppressor cell populations in both organs. In the spleen, regulatory T cells (Treg) were increased approximately 2-fold in sepsis vs. control patients. In contrast, in the lung, no increase in Treg was detected, but there were increased cells consistent with a myeloid-derived suppressor cells (MDSC) phenotype. Expansion of suppressive cells, including Treg and MDSCs, has been reported in sepsis and provides another plausible mechanism for immunosuppression [5]. Unique differences in cellular inhibitory molecule expression existed in immune cells isolated from the lungs of sepsis patients vs. cancer patients and transplant donors. Antigen-presenting cells, i.e., dendritic cells and macrophages/monocytes, as well as tissue-specific macrophages, showed an immunosuppressive phenotype in sepsis as evidenced by decreased expression of CD86 and Human Leukocyte Antigen (HLA) – DR isotype. Immunohistochemical staining showed extensive depletion of splenic $CD4^+$, $CD8^+$, and HLA-DR-expressing cells and expression of ligands for inhibitory receptors on lung epithelial cells [4]. Evaluation of spleen tissue demonstrated a cellular loss in the periarteriolar lymphoid sheath – PALS and diminished number and size of splenic follicles in sepsis pa-

tients, as previously reported [4, 6]. These patients presented foci of bacterial infections that prolonged despite antimicrobial therapy, as well as a reactivation of latent viral infections [7, 8]. For a better understanding of the complex immune response in critically ill or injured patients with secondary sepsis, numerous pro- and anti-inflammatory mediators have been investigated, often with contradictory results. The impact of the type of bacterial causative agent on critically ill patients' immune response remains the subject of investigation. A relationship between the immune response and the survival of this patient population is still being investigated. A better insight into the immune response of critically ill patients might be gained by measuring the serum level of a larger number of inflammation mediators with predominantly pro-inflammatory features [IL-1α, IL-1β, IL-6, IL-8, IL-12p70, IL-17A, TNFα, IFN-γ, IFN-γ-inducible protein-10 (IP-10), monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α and MIP-1β] or anti-inflammatory features (IL-4, IL-10, IL-13, IL-27, IL-31, and IL-33). Nowadays, sepsis is considered a dynamic syndrome characterized by many, often antagonistic phenomena, from hyperinflammation to anergy and immunoparalysis. The former concept of a pro-inflammatory process followed by a compensatory anti-inflammatory phase does not represent a common clinical pattern. These two processes more often progress with a significant degree of synchronization, though not necessarily at the same time. The hyperinflammatory phase, known as 'cytokine storm', is characterized by an uncontrollable production of pro-inflammatory mediators that often lead to organ damage/injury and bring about multiple organ dysfunction syndrome. Such a clinical scenario may lead to a premature death within a few days, and it may occur in the case of severe acute pancreatitis. The late stage of sepsis is dominated by a state of prolonged exhaustion of the immune effector cells, which results in immunosuppression [9, 10]. A delayed death from sepsis occurs either because of progressive exhaustion of the immune cells, resulting in secondary infections, or due to inflammation-induced organ damage/injury; in addition, there is often a combination of immunosuppression and persistent inflammation. The general problem with the investigation of sepsis in critically ill and injured patients is the heterogeneity of these patients, as is the case with the immune response. Over time, this response changes and is different in various patients with sepsis syndrome. Apart from these inter-individual differences, there are also significant intra-individual ones. The immune response of a patient is influenced by many variable factors, such as the time passed from the onset of the infection until the clinical manifestation of the disease, the source of infection, pathogen virulence, a possible former immunocompromisation of the patient, or gene-determined proclivity for a certain type of the immune response [11–13]. Of particular influence on the immune response is the patient's age. The population of the critically ill is getting older, and the immune response of older patients to insult is weakened, which is called immunosenescence [14]. The immune response of this patient population is also impacted by applied therapeutic measures, including medications and dysfunctional organism-supporting measures, such as various modes of hemodialysis and mechanical pulmonary ventilation. Bearing that in mind, the use of catecholamines, inotropes, and vasopressors has a great influence on the immune response [15]. Investigations have shown that the profile of cytokines in the critically ill with sepsis is impacted by a type of bacterial causative agent [16–18]. It is important to monitor the immune response for a longer period because of all of the mentioned factors that impact it and its variability. The aim of the study was to determine, according to the levels of pro-inflammatory (IL-1α, IL-1β, IL-6, IL-8, IL-12 p70, IL-17A, TNF-α, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β) and anti-inflammatory (IL-4, IL-10, IL-13, IL-27, IL-31, and IL-33) mediators in the critically ill with secondary sepsis, whether the cytokine profile differs from the type of the bacterial causative agent, as well as to determine the influence of the cytokine profile on the outcome in this patient population. The outcome measure has been in-hospital mortality.

Methods

Patients

A total of 125 critically ill patients with secondary sepsis due to peritonitis, pancreatitis, and severe trauma, admitted to the Surgical ICU, were enrolled in a prospective study conducted in a tertiary university hospital (Military Medical Academy, Belgrade, Serbia). The study was carried out from November 2017 until October 2020 for a total duration of two years and eleven months. During the investigation period, the study encompassed critically ill patients, with ages ranging from 18 to 89 years. The average age was 57.7 ± 17.3 years. Of the total number of patients, 67.2% were males, and 32.8% were females. In concordance with the Declaration of Helsinki, the approval was obtained from the local Ethics Committee (date of issue November 29, 2017). In addition, informed consent was obtained from the patients or first-degree relatives. The study was conducted in accordance with the approved guidelines. The patients with secondary sepsis (underlying conditions being peritonitis, pancreatitis, and trauma) were enrolled if they had fulfilled current Sepsis-3 diagnostic criteria for sepsis (formerly severe sepsis) and/or septic shock [acute change in total Sequential Organ Failure Assessment (SOFA) score > 2 points, with vasopressors required to maintain mean arterial pressure (MAP) > 65 mm Hg, and serum lactate level > 2 mmol/L despite adequate volume resuscitation] [19]. The diagnostic criteria encompass any of the following variables thought to be a result of the infection: sepsis-induced hypotension, serum lactate levels greater than 2 mmol/L, urine output less than 0.5 mL/kg/hrs for more than 2 hrs despite adequate fluid resuscitation, acute lung injury with PaO_2/FiO_2 less than 250, creatinine greater than 2.0 mg/dL (34.2 micromol/L), platelet count less than 100,000, and coagulopathy with international normalized ratio – INR greater than 1.5. Moreover, critically ill patients with severe trauma (Injury Severity Score – ISS, determined using Abbreviated Injury Scale – AIS > 25 points), were enrolled after they developed secondary sepsis.

The exclusion criteria were as follows: secondary sepsis and/or septic shock with an underlying condition other than severe peritonitis, pancreatitis or trauma, malignant disease of any origin, long-term Surgical ICU stay before criteria, and a fulminant and pre-existing immunodeficiency. Out of 150 patients initially considered for enrolment, 25 were excluded.

Sampling and analysis

The vein blood samples were taken from 125 adult and critically ill patients with confirmed severe secondary sepsis as a complication of peritonitis, pancreatitis, or trauma once they fulfilled the criteria for a diagnosis of severe sepsis or septic shock (the first sample), which was repeated on the third and fifth day. The serum was extracted from the vein blood samples using a centrifuge at 1,000 revolutions for 10 min. The serum samples were frozen at -20 °C and then at -80 °C and kept until biomarker levels were determined. After the defrosting process, the biomarker levels in the serum samples were determined using the commercial flow cytometric kit (18-Plex Multiplex) using the flow cytofluorometry device (Beckman Coulter FC500). By doing so, the levels of pro-inflammatory (IL-1α, IL-1β, IL-6, IL-8, IL-12p70, IL-17A, TNF-α, IFN-γ, IP-10, MCP-1, MIP-1α, and MIP-1β) and anti-inflammatory (IL-4, IL-10, IL-13, IL-27, IL-31, and IL-33) mediators were determined at the three predefined time intervals. Detection sensitivity levels for cytokines (limit of detection – LOD) according to the manufacturer's note are the following: IL-1α < 2 pg/mL, IL-1β < 5 pg/mg, IL-6 < 5 pg/mL, IL-8 < 1 pg/mL, IL-12p70 < 3 pg/mL, IL-17A < 1 pg/mL, TNFα < 1 pg/mL, IFN-γ < 3 pg/mL, IP-10 < 3 pg/mL, MCP-1 < 2 pg/mL, MIP-1α < 2 pg/mL, MIP-1β < 5 pg/mL, IL-4 < 1 pg/mL, IL-10 < 2 pg/mL, IL-13 < 5 pg/mL, IL-27 < 5 pg/mL, IL-31 < 5 pg/mL and IL-33 < 5 pg/mL. Simultaneously, blood samples were also collected for blood culture. The type of bacterial causative agent was discovered through standard microbiological analyses. All the patients admitted to the ICU were being treated according to the latest guidelines for sepsis and septic shock treatment [20], along with adequate use of antibiotic therapy, vasoactive support, circulatory volume resuscitation, respiratory support through the application of various modes of mechanical ventilation, infection source surveillance, as well as by application of surgical treatment if deemed necessary. The outcome measure was in-hospital mortality; patients were monitored until hospital discharge (survivors) or in-hospital death (non-survivors).

Statistical analysis

Descriptive statistical methods and statistical hypotheses testing methods have been applied for the data analysis. The descriptive statistical methods included continuous variables shown as an arithmetic mean and standard deviation or a median and interquartile range (Q_1–Q_3) depending on distribution normality tested using the Shapiro-Wilk test. Frequency distributions of categorized variables are shown as absolute and relative numbers. The investigation of the hypothesis on the different significance of mean values of numerical characteristics involved the application of the Kruskal-Wallis test for independent samples, the Mann-Whitney *U* test for the test for sums of ranges, and the Friedman test and Wilcoxon test for investigating dependent samples. Chi-square and Fisher tests of precise probability were used to investigate frequency differences of categorized variables. The predictive power of all cytokines was tested by Receiver Operating Characteristic (ROC) analyses. The Area Under the ROC Curve (AUC) and 95% confidence intervals (CI), cut-off value with optimal sensitivity and specificity, and Youden index have all been calculated. Statistical hypotheses have been tested at the level of statistical significance (alpha level) of 0.05. All the analyses have been done by experts for medical statistics using the software program SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA).

Results

During the investigation period (two years and eleven months), the study encompassed 125 critically ill patients, with ages ranging from 18 to 89 years. The average age was 57.7 ± 17.3 years. Of the total number of patients, 84 (67.2%) were males, and 41 (32.8%) were females. Male gender was more common; the statistical difference was highly significant ($p < 0.001$); female patients were significantly older. According to blood culture, there were 28 (22.4%) patients with isolated Gram-positive pathogens, 29 (23.2%) of them with Gram-negative pathogens, 20 (16.0%) with polymicrobial blood culture, and 48 (38.4%) patients with negative blood culture. The overall in-hospital mortality amounted to 36.8%, and 79 (63.2%) patients survived. Demographic characteristics of the patients are shown in Table 1. Concerning the findings of the isolated blood culture, the comparison of mean cytokine values did not reveal a statistically significant difference between groups on the first day of measurement. When the mean cytokine values were compared to the nature of bacteremia on the third day of measurement, significant differences were detected between the groups for pro-inflammatory cytokines IL-1α ($p = 0.010$), IL-1β ($p = 0.009$), IL-6 ($p = 0.004$), IL-12p70 ($p = 0.011$), IL-17A ($p = 0.007$), TNF-α ($p = 0.006$), IFN-γ ($p = 0.018$), IP-10 ($p = 0.031$), MIP-1α ($p = 0.002$), MIP-1β ($p = 0.003$), MCP-1 ($p = 0.004$) and anti-inflammatory cytokines IL-4 ($p = 0.021$), IL-10 ($p = 0.008$), IL-13 ($p = 0.007$), IL-27 ($p = 0.005$), IL-31 ($p = 0.029$), IL-33 ($p = 0.006$). On the third day of measurement, mean cytokine values were statistically significantly lower in the polymicrobial group, as compared with the cytokine levels in the other investigated groups. Tables 2 and 3 present the third day of measurement's comparison of cytokines with regard to the blood culture findings. The general difference significance of the measured cytokines was tested further in order to ascertain among which blood cultures there is a difference in significance of the examined cytokine values. On the third day of measurement, statistically significantly lower values were obtained for the polymicrobial blood culture than for the

Table 1

Demographic characteristics of patients

Parameters	Values
Patients, n	125
Age (years), mean ± SD; med (min-max)	57.7 ± 17.3; 61 (18–89)
Gender, n (%)	
male	84 (67.2)
female	41 (32.8)
Sepsis-complicated primary condition, n (%)	
peritonitis	51 (40.8)
pancreatitis	33 (26.4)
trauma	41 (32.8)
Blood cultures, n (%)	
Gram-positive	28 (22.4)
Gram-negative	29 (23.2)
polymicrobial	20 (16.0)
negative	48 (38.4)
Outcome, n (%)	
non-survivors	46 (36.8)
survivors	79 (63.2)
Hospitalization length (days), mean ± SD; med (IQR)	29.9 ± 34; 22 (1–305)

n – number of patients; SD – standard deviation; med – median; min – minimum; max – maximum; IQR – interquartile range.

Table 2

Comparison of cytokines according to blood culture findings on the third day of measurement

Cytokines pg /mL	Blood cultures				*p*-value
	G+	G-	P	N	
IL-1α	119.5 (43.4–364.0)	128.7 (71.7–504.8)	62.0 (8.5–146.3)	266.1 (97.4–500.5)	0.010*
IL-1β	258.4 (134.6–445.8)	308.8 (84.4–547.8)	80.6 (0.6–222.7)	372.0 (181.3–477.0)	0.009*
IL-4	127.6 (43.6–253.8)	80.2 (39.0–283.0)	37.7 (0.0–92.9)	186.6 (54.8–333.1)	0.021*
IL-6	458.8 (247.9–857.6)	585.2 (352.9–1020.3)	173.8 (63.1–496.1)	658.1 (374.6–1098.1)	0.004*
IL-8	186.8 (73.8–576.8)	208.8 (81.2–461.1)	109.9 (46.0–287.4)	287.8 (132.9–660.7)	0.065
IL-10	46.4 (13.7–144.3)	30.8 (9.2–98.5)	14.4 (0.0–29.4)	73.2 (20.0–156.3)	0.008*
IL-12p70	66.2 (23.1–147.2)	78.6 (35.5–155.7)	31.3 (0.0–65.1)	112.2 (41.0–182.5)	0.011*
IL-13	214.0 (46.6–647.3)	173.2 (61.8–650.6)	42.0 (0.0–108.7)	416.4 (78.9–659.0)	0.007*
IL-17A	96.2 (37.2–226.2)	69.4 (43.0–302.8)	29.2 (0.9–56.7)	140.0 (48.3–300.8)	0.007*
IL-27	146.0 (49.1–430.4)	146.2 (73.6–322.8)	43.4 (4.4–100.4)	226.9 (75.8–495.8)	0.005*
IL-31	270.8 (125.8–560.0)	285.3 (154.6–542.6)	171.2 (46.4–268.4)	345.2 (197.9–624.3)	0.029*
IL-33	195.1 (76.2–313.6)	172.0 (79.4–347.9)	64.4 (10.3–122.2)	218.6 (124.7–412.8)	0.006*
TNF-α	258.8 (82.0–691.0)	212.8 (79.0–441.2)	74.4 (0.0–158.0)	345.2 (140.4–559.3)	0.006*
IFN-γ	74.4 (41.0–137.8)	71.4 (40.7–175.9)	30.2 (0.0–71.0)	113.1 (48.9–199.8)	0.018*
IP-10	166.4 (21.5–339.3)	252.0 (37.0–288.6)	44.6 (13.0–268.2)	244.4 (65.4–524.4)	0.031*
MIP-1α	148.6 (49.9–462.4)	133.2 (72.8–852.4)	59.2 (20.1–103.3)	226.6 (112.5–1136.6)	0.002*
MIP-1β	263.6 (131.9–410.4)	281.2 (132.6–475.1)	116.6 (44.0–198.8)	323.8 (157.6–747.1)	0.003*
MCP-1	221.2 (70.9–547.8)	212.2 (72.7–710.5)	80.4 (4.2–161.8)	424.1 (147.6–897.4)	0.004*

IL – interleukin; TNF – tumor necrosis factor; IFN – interferon; IP-10 – IFN-γ-inducible protein 10; MIP – macrophage inflammatory protein; MCP – monocyte chemoattractant protein; G+ – Gram-positive blood cultures; G- – Gram-negative blood cultures; P – polymicrobial blood cultures; N – negative blood cultures.

Data are presented as median (interquartile range). Significant differences are marked by *($p < 0.05$); *p*-values were based on the Kruskal-Wallis test.

Gram-positive blood culture, and for pro-inflammatory (IL-1β, IL-6, IL-12p70, IL-17A, TNF-α, IFN-γ, MIP-1α, MIP-1β, MCP-1) and anti-inflammatory (IL-4, IL-10, IL-13, IL-27, IL-33) cytokines. Statistically significantly lower values were obtained for the polymicrobial group than for the Gram-negative and negative ones for all of the investigated pro- and anti-inflammatory cytokines, except for IL-8 (Table 3). The only biomarker distinguished with statistically significantly higher values for the negative blood culture group than for the Gram-positive one was MIP-1α ($p = 0.048$). The examined cytokines' values have not significantly differed between the Gram-positive and Gram-negative blood culture groups, nor have they been statistically significantly different between the Gram-negative and negative ones. On the fifth day of measu-

Table 3

Levels of significance of statistical difference in mean cytokine values according to blood cultures on the third day of measurement

Cytokines pg/ mL	G+/P	G-/P	P/N
IL-1α	0.054	0.034*	0.001*
IL-1β	0.011*	0.019*	0.001*
IL-4	0.011*	0.045*	0.002*
IL-6	0.014*	0.004*	0.001*
IL-8	0.237	0.157	0.006*
IL-10	0.015*	0.048*	0.001*
IL-12p70	0.024*	0.014*	0.001*
IL-13	0.015*	0.016*	0.001*
IL-17A	0.008*	0.012*	0.001*
IL-27	0.017*	0.009*	0.001*
IL-31	0.081	0.050*	0.002*
IL-33	0.010*	0.016*	0.001*
TNF-α	0.009*	0.018*	0.001*
IFN-γ	0.026*	0.024*	0.003*
IP-10	0.262	0.047*	0.005*
MIP-1α	0.048*	0.016*	< 0.001*
MIP-1β	0.006*	0.005*	0.001*
MCP-1	0.021*	0.032*	< 0.001*

Data are presented as *p*-value significant; significant differences are marked by *($p < 0.05$); *p*-values were based on the Mann-Whitney *U* tests. For abbreviations, see Table 2.

Table 4

Comparison of cytokine values at three time intervals of measurement in patients with negative blood culture

Cytokines	Time intervals of measurement			*p*	*p*-value among measurements		
pg/mL	1st day	3rd day	5th day		1st– 3rd	1st– 5th	3rd– 5th
IL-1α	137.6 (59.1–313.0)	266.1 (97.4–500.5)	287.6 (69.9–698.6)	0.050*	0.002*	0.003*	0.452
IL-1β	307.7 (85.3–533.5)	372.0 (181.3–477.0)	365.8 (126.8–527.8)	0.297	0.194	0.093	0.823
IL-4	128.9 (37.4–232.0)	186.6 (54.8–333.1)	213.1 (63.0–383.9)	0.034*	0.003*	0.006*	0.482
IL-6	617.3 (254.6–1312.8)	658.1 (374.6–1098.1)	749.2 (304.2–1133.9)	0.102	0.034*	0.309	0.933
IL-8	211.0 (84.7–494.3)	287.8 (132.9–660.7)	301.8 (134.6–747.8)	0.061	0.030*	0.041*	0.648
IL-10	40.7 (14.4–85.7)	73.2 (20.0–156.3)	74.4 (15.4–226.8)	0.023*	0.003*	0.022*	0.802
IL-12p70	79.7 (30.3–128.7)	112.2 (41.0–182.5)	106.9 (45.0–273.8)	0.040*	0.001*	0.005*	0.436
IL-13	246.9 (42.2–468.8)	416.4 (78.9–659.0)	475.6 (80.9–961.1)	0.007*	0.001*	0.003*	0.354
IL-17A	72.5 (33.1–208.3)	140.0 (48.3–300.8)	195.4 (37.9–381.0)	0.006*	0.010*	0.003*	0.347
IL-27	159.5 (51.9–299.6)	226.9 (75.8–495.8)	287.4 (68.4–726.9)	0.054	0.002*	0.006*	0.677
IL-31	272.7 (152.4–477.4)	345.2 (197.9–624.3)	405.0 (202.6–704.7)	0.004*	0.005*	0.001*	0.426
IL-33	201.7 (65.9–291.7)	218.6 (124.7–412.8)	308.1 (102.4–418.4)	0.125	0.004*	0.028*	0.707
TNF-α	252.4 (107.6–448.9)	345.2 (140.4–559.3)	422.7 (114.7–825.1)	0.138	0.020*	0.029*	0.350
IFN-γ	77.9 (31.8–136.3)	113.1 (48.9–199.8)	122.1 (52.2–308.4)	0.187	0.002*	0.023*	0.861
IP-10	174.6 (46.2–256.3)	244.4 (65.4–524.4)	307.8 (100.0–737.4)	0.031*	0.039*	0.021*	0.712
MIP-1α	146.7 (58.6–305.0)	226.6 (112.5–1136.6)	510.6 (80.4–1148.0)	0.005*	0.001*	0.004*	0.667
MIP-1β	233.4 (137.3–423.8)	323.8 (157.6–747.1)	429.1 (182.8–600.4)	0.003*	0.001*	0.010*	0.563
MCP-1	263.6 (111.0–594.0)	424.1 (147.6–897.4)	601.6 (122.6–909.0)	0.099	0.005*	0.019*	1.00

Data are presented as median (interquartile range). Significant differences are marked by *($p < 0.05$). For abbreviations, see Table 2.

rement, the cytokines did not statistically significantly differ according to the isolated blood culture (Table 4). An absence of a statistically significant difference was ascertained at the three measurement intervals in patients with Gram-positive blood culture, except for MCP-1. The patients with Gram-positive blood culture present median values that are statistically significantly different at the monitored intervals for MCP-1 ($p = 0.047$), and they were statistically significantly higher on the third measurement day than on the first day, whereas the difference was not statistically significant between other intervals. None of the cytokines compared at the three time intervals in the patients with Gram-negative and polymicrobial blood cultures has presented a statistically significant difference. The cytokine values comparison at the

three measurement intervals in the patients with negative blood culture has shown the presence of a statistically significant difference for pro-inflammatory [IL-1α (p = 0.050), IL-12p70 (p = 0.040), IL-17A (p = 0.006), IP-10 (p = 0.031), MIP-1α (p = 0.005), MIP-1β (p = 0.003)] and anti-inflammatory [IL-4 (p = 0.034), IL-10 (p = 0.023), IL-13 (p = 0.007), IL-31 (p = 0.004)] cytokines. The patients with negative blood cultures presented statistically significantly higher values of the mentioned cytokines on the third and fifth day of measurement, compared to the first day, whereas the difference was not statistically significant between the third and the fifth day of measurement. Table 4 shows the cytokine values comparison at the three time intervals of measurement in the patients with negative blood cultures. At the first two time intervals of measurement, the statistical analysis determined no significant difference by comparing mean values of cytokines according to the outcome (survivor, non-survivor). The statistically significant difference appears only on the fifth day. At that time interval of measurement, the cytokine values were statistically significantly higher in survivors than in non-survivors – IL-1α, IL-1β, IL-8, IL-12p70, IL-17A, IFN-γ, IP 10, MIP-1α, MIP-1β, IL-4, IL-13, IL-27, IL-31, IL-33 (Table 5). The values of the investigated cytokines in non-survivors did not have statistically significant differences at the three time intervals. The comparison of the cytokine values in survivors at the three time intervals showed significant differences in biomarkers given in Table 6. The general tendency was that the levels of biomarkers increased at the monitored time intervals of measurement from the first until the fifth day. On the first day of measurement in the patients with secondary sepsis as a complication of peritonitis, the only important predictor of the fatal outcome was IL-17A, AUC of 0.665 (95% CI 0.519–0.791; p = 0.034). The levels of IL-17A on the first day of measurement, lower than the cut-off values (43.20 pg/mL), were moderate predictors of the fatal outcome in this group of patients (Figure 1). All the examined biomarkers on the third day of measurement became significant predictors of the polymicrobial blood culture. On the third day of measurement, the values of AUC for most cytokines were 0.7–0.8, which makes them quite discriminatorily powerful. The most powerful predictor of the polymicrobial blood culture was MIP-1β, AUC amounting to 0.772 (95% CI 0.684–0.845; p < 0.001). The MIP-1β levels lower than

Table 5

Cytokine values on the fifth day of measurement against the outcome

Cytokines pg/ mL	Outcome		*p*-value
	non-survivors	survivors	
IL-1α	89.5 (36.1–300.2)	352.9 (67.0–697.7)	0.025*
IL-1β	176.4 (69.0–375.2)	382.7 (99.5–714.5)	0.028*
IL-4	101.8 (0.0–217.4)	207.0 (47.2–443.6)	0.043*
IL-6	536.1 (164.5–1138.7)	693.8 (249.6–1020.3)	0.684
IL-8	124.6 (55.5–488.5)	403.0 (108.4–846.8)	0.021*
IL-10	30.3 (0.0–124.2)	65.8 (13.0–65.8)	0.181
IL-12p70	45.8 (2.8–109.1)	112.9 (38.9–379.7)	0.012*
IL-13	89.0 (7.6–526.7)	475.6 (52.6–990.3)	0.032*
IL-17A	51.6 (13.6–238.3)	195.4 (37.1–450.8)	0.017*
IL-27	89.0 (13.0–322.1)	277.8 (62.7–676.3)	0.022*
IL-31	154.2 (83.8–487.5)	471.0 (226.0–904.1)	0.002*
IL-33	103.2 (28.9–350.6)	301.2 (80.2–531.4)	0.012*
TNF-α	154.2 (24.2–523.9)	422.7 (75.4–860.0)	0.055
IFN-γ	58.8 (10.9–121.0)	122.2 (33.0–257.8)	0.038*
IP-10	88.4 (19.5–490.6)	332.9 (96.6–717.1)	0.014*
MIP-1α	98.2 (50.8–554.8)	237.0 (76.4–1127.8)	0.042*
MIP-1β	151.4 (97.5–439.9)	446.4 (159.1–637.3)	0.026*
MCP-1	159.6 (67.3–735.0)	501.3 (83.4–884.5)	0.325

Data are presented as median (interquartile range). Significant differences are marked by *(p < 0.05).
For abbreviations, see Table 2.

Table 6

Comparison of cytokine values in survivors at three time intervals of measurement

Cytokines pg/mL	Time intervals of measurement			*p*	*p*-value among measurements		
	1st day	3rd day	5th day		1st–3rd	1st–5th	3rd–5th
IL-1α	126.4 (59.1–348.0)	138.4 (75.0–465.8)	352.9 (67.0–697.7)	0.036*	0.057	< 0.001*	0.290
IL-17A	69.7 (35.6–257.0)	97.9 (40.6–271.1)	195.4 (37.1–450.8)	0.019*	0.156	0.003*	0.050*
IL-31	268.2 (161.2–551.0)	305.3 (178.0–566.8)	471.0 (226.0–904.1)	0.007*	0.091	0.001*	0.088
IP-10	175.8 (48.8–359.7)	195.5 (42.7–507.4)	332.9 (96.6–717.1)	0.004*	0.231	0.002*	0.138
MIP-1α	120.3 (60.2–353.1)	176.3 (73.6–847.6)	237.0 (76.4–1127.8)	0.044*	0.006*	0.003*	0.726
MIP-1β	227.8 (116.3–471.8)	291.0 (129.1–517.0)	446.4 (159.1–637.3)	0.028*	0.030*	0.015*	0.869

Data are presented as median (interquartile range). Significant differences are marked by *(p < 0.05).
For other abbreviations, see Table 2.

the cut-off values (215.4 pg/mL) were good predictors of the polymicrobial blood culture. The AUC values for cytokines as predictors of polymicrobial blood culture on the third day of measurement are given in Table 7. On the third day of measurement, all the biomarkers became significant predictors of the negative blood culture. The values of AUC for these biomarkers were in the range of 0.6–0.7. The most powerful predictor of the negative blood culture was MIP-1α, whose levels, higher than the cut-off values (176.3 pg/mL), were good predictors of the negative blood culture. The AUC values for cytokines as predictors of negative blood culture on the third day of measurement are given in Table 8. On the third day of measurement, AUC analysis did not reveal statistical significance in the prediction of either the Gram-positive or Gram-negative blood cultures. As for the patients with Gram-positive, polymicrobial, and negative blood cultures, the third day of measurement revealed that cytokines were statistically insignificant for discrimination between the non-survivors and survivors. As for the patients with Gram-negative blood cultures, all the biomarkers were statistically significant predictors of the fatal outcome, except for IL-6. The values of AUC for all other biomarkers on the third day of measurement were in the range of 0.7–0.8. The most powerful predictor for the fatal outcome became MIP-1α, AUC amounting to 0.796. The values of the MIP-1α on the third day of measurement, less than the cut-off values

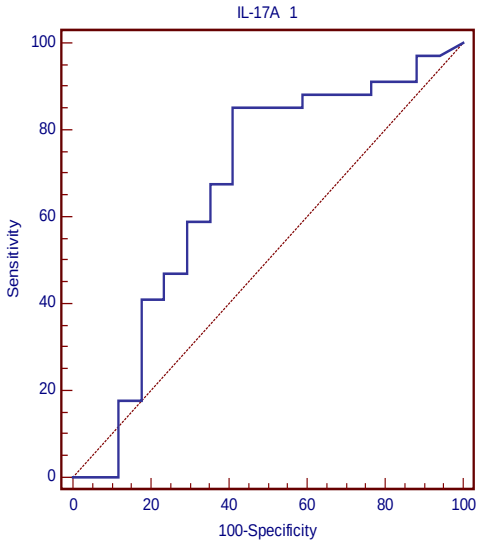

Fig. 1 – Receiver operating characteristic curve for interleukin (IL)-17A in prediction of peritonitis-related fatal outcome on the first day of measurement.

Table 7

The AUC values for cytokines as predictors of polymicrobial blood cultures on the third day of measurement

Cytokines pg/mL	AUC	*p*-value	95% confidence interval		Cut-off value	Sensitivity %	Specificity %	Youden index
			lower bound	upper bound				
IL-1α	0.733	< 0.001*	0.642	0.811	82.7	68.7	72.7	0.41
IL-1β	0.752	< 0.001*	0.663	0.828	248.9	87.5	58.6	0.46
IL-4	0.730	< 0.001*	0.639	0.808	96.9	81.2	60.6	0.42
IL-6	0.760	< 0.001*	0.672	0.835	205.0	62.5	86.9	0.49
IL-8	0.669	0.011*	0.575	0.754	155.9	68.7	63.6	0.32
IL-10	0.734	< 0.001*	0.643	0.812	30.4	81.2	64.6	0.46
IL-12p70	0.739	< 0.001*	0.649	0.817	70.0	87.5	56.6	0.44
IL-13	0.752	< 0.001*	0.663	0.828	114.2	81.2	63.6	0.45
IL-17A	0.759	< 0.001*	0.670	0.834	65.1	87.5	59.6	0.47
IL-27	0.757	< 0.001*	0.668	0.832	104.2	81.2	68.7	0.50
IL-31	0.711	< 0.001*	0.620	0.792	200.3	75.0	71.7	0.47
IL-33	0.755	< 0.001*	0.666	0.831	165.9	87.5	61.6	0.49
TNF-α	0.754	< 0.001*	0.665	0.830	159.6	81.2	67.7	0.49
IFN-γ	0.727	< 0.001*	0.636	0.806	59.3	75.0	67.7	0.43
IP-10	0.684	0.004*	0.591	0.768	68.9	68.7	71.7	0.40
MIP-1α	0.751	< 0.001*	0.661	0.827	110.5	81.2	70.7	0.52
MIP-1β	0.772	< 0.001*	0.684	0.845	215.4	87.5	65.7	0.53
MCP-1	0.748	< 0.001*	0.658	0.824	167.5	81.2	63.6	0.45

AUC – Area Under ROC Curve; ROC – Receiver Operating Characteristic. For other abbreviations, see Table 2. Significant differences are marked by *($p < 0.05$).

Table 8

The AUC values for cytokines as predictors of negative blood cultures on the third day of measurement

Cytokines pg/mL	AUC	*p*-value	95% confidence interval		Cut-off value	Sensitivity %	Specificity %	Youden index
			lower bound	upper bound				
IL-1α	0.635	0.014*	0.540	0.722	189.2	65.1	66.7	0.32
IL-1β	0.610	0.046*	0.515	0.700	292.4	60.5	62.5	0.23
IL-4	0.605	0.057	0.510	0.695	133.0	62.8	62.5	0.25
IL-6	0.626	0.022*	0.531	0.714	350.8	81.4	41.7	0.23
IL-8	0.622	0.026*	0.527	0.711	233.8	62.8	59.7	0.23
IL-10	0.632	0.016*	0.537	0.720	61.4	58.1	68.1	0.26
IL-12p70	0.620	0.029*	0.525	0.709	82.4	65.1	63.9	0.29
IL-13	0.623	0.025*	0.528	0.711	257.4	67.4	63.9	0.31
IL-17A	0.610	0.046*	0.515	0.700	97.9	58.1	62.5	0.21
IL-27	0.633	0.015*	0.538	0.721	169.1	67.4	65.3	0.33
IL-31	0.615	0.038*	0.519	0.704	163.4	90.7	31.9	0.23
IL-33	0.627	0.020*	0.532	0.716	123.1	76.7	47.2	0.24
TNF-α	0.616	0.036*	0.520	0.705	221.7	69.8	56.9	0.27
IFN-γ	0.618	0.032*	0.523	0.707	100.6	62.8	66.7	0.30
IP-10	0.614	0.039*	0.518	0.703	34.1	88.4	33.3	0.22
MIP-1α	0.669	0.002*	0.576	0.754	176.3	69.8	65.3	0.35
MIP-1β	0.631	0.017*	0.536	0.719	397.0	48.8	79.2	0.28
MCP-1	0.649	0.006*	0.555	0.736	116.9	81.4	45.8	0.27

Significant differences are marked by *($p < 0.05$).
For abbreviations, see Tables 2 and 7.

Table 9

Fatal outcome prediction on the third day of measurement of cytokines in patients with Gram-negative blood culture

Cytokines pg/mL	AUC	*p*-value	95% confidence interval		Cut-off value	Sensitivity %	Specificity %	Youden index
			lower bound	upper bound				
IL-1α	0.782	0.002*	0.587	0.914	102.0	71.4	85.7	0.57
IL-1β	0.776	0.003*	0.579	0.910	241.5	66.7	85.7	0.52
IL-4	0.762	0.006*	0.564	0.901	60.7	76.2	71.4	0.48
IL-6	0.551	0.694	0.353	0.738	1020.3	85.7	42.9	0.29
IL-8	0.728	0.026*	0.528	0.877	114.0	81.0	71.4	0.52
IL-10	0.748	0.012*	0.549	0.891	28.3	71.4	85.7	0.57
IL-12p70	0.776	0.003*	0.579	0.910	68.5	66.7	85.7	0.52
IL-13	0.782	0.002*	0.587	0.914	89.7	71.4	85.7	0.57
IL-17A	0.772	0.004*	0.575	0.908	60.4	66.7	85.7	0.52
IL-27	0.755	0.009*	0.557	0.896	107.3	76.2	71.4	0.48
IL-31	0.762	0.006*	0.564	0.901	183.2	85.7	71.4	0.57
IL-33	0.789	0.001*	0.594	0.919	123.1	71.4	85.7	0.57
TNF-α	0.755	0.009*	0.557	0.896	139.6	76.2	71.4	0.48
IFN-γ	0.776	0.003*	0.579	0.910	61.2	66.7	85.7	0.52
IP-10	0.762	0.006*	0.564	0.901	30.4	90.5	57.1	0.48
MIP-1α	0.796	< 0.001*	0.602	0.923	79.0	85.7	71.4	0.57
MIP-1β	0.755	0.009*	0.557	0.896	215.0	81.0	71.4	0.52
MCP-1	0.759	0.007*	0.560	0.898	111.8	71.4	85.7	0.57

Significant differences are marked by *($p < 0.05$).
For abbreviations, see Tables 2 and 7.

(79.0 pg/mL), were good predictors of the fatal outcome in the patients with Gram-negative blood culture. The prediction of the fatal outcome on the third day of measurement of cytokines in the patients with Gram-negative blood culture is given in Table 9.

Discussion

The progress in the therapeutic measures of support for organ systems in the critically ill with sepsis and/or trauma has led to improvements in their thirty-day survival rate [21]. That has shown changes in the immunoinflammatory response in this category of patients. In the past, the pro-inflammatory response was considered a generator for premature mortality (for the first couple of days), and the compensatory anti-inflammatory response would induce organ damages/injuries, immunosuppression, and mortality after a few weeks [22, 23]. New observations have revealed that the long-term and simultaneous pro-inflammatory and anti-inflammatory response, the heart of which is a dysfunctional,

innate, and suppressed immunity, culminates in persistent organ damage/injury and fatal outcome [24, 25]. Immune dysfunction has a significant role in the delayed, late death of a critically ill patient. Regarding the described immune response in the critically ill with sepsis, there are various data in the literature – that is why this represents a topical focus of research. The importance of the issue is found in the fact that the new definition of sepsis (Sepsis-3) from 2016 includes the term 'dysregulation of immune response' [20], which defines sepsis as a life-threatening dysfunction of organs caused by a dysregulation of the host's response to infection. This problem is complex because there is a subgroup of patients with sepsis dominated by the pro-inflammatory immune response and a larger number of patients in whom the dysregulation of immune response is manifested through immunosuppression. In order to discriminate these two categories of patients, it is necessary to perform monitoring of immune phenotypes for every single patient, which would help to reach a desired individual therapeutic approach to immunomodulation [26, 27]. So far, the role of the complex immune response in sepsis has not been fully clarified and represents a subject matter of research with often contradictory results. The mentioned 18 mediators in the previous investigations have not been evaluated simultaneously, and their mutual relationship in conditions of chronic critical disease is yet to be researched. Our prospective and observational study has focused on the simultaneous measurement of pro- and anti-inflammatory cytokines in well-defined populations of critically ill patients with severe secondary sepsis as a complication of peritonitis, pancreatitis, or trauma to minimize heterogeneous differences accentuated in sepsis. Simultaneous assessment of a larger number of cytokines in sepsis at different time intervals may identify complex cytokine patterns [28], which reflect the immune response of critically ill patients. The appearance of multiplex testing has made it possible to study a wider immuno-inflammatory response, and this new technique has been proposed as a potential diagnostic implement for sepsis, owing to its possibility to characterize specific subgroups of patients with sepsis [19]. The patterns of pro- and anti-inflammatory cytokines in patients with sepsis have been widely researched in previous studies [28–31]. Although the international guidelines for severe sepsis and septic shock treatment do not take into account the type of the pathogenic causative agent, *in vitro* data suggest that there are numerous differences in the cytokine profiles and mortality rates between subclasses, such as the Gram-negative bacteria (GNB) and Gram-positive bacteria (GPB) [32]. Several studies have demonstrated the significant influence of the type of bacterial causative agent on the cytokine profile in the critically ill patients [18, 33]. However, although there is a considerable percentage of microbiologically undocumented infections [34] in the literature available for our research, we have not found a study on critically ill patients in ICU that would report on the cytokine profile in the negative blood cultures. Only one study has had information on the cytokine measurement in the negative blood cultures in patients with sepsis in the emergency department [35]. The aim of this prospective observational study was to investigate whether the levels of the inflammatory mediators in plasma differ between the sepsis-affected patients with bacteremia and those without bacteremia during the preliminary hospitalization phase. In total, 80 patients were divided into two main subgroups, according to whether bacteremia could have been discovered. The samples of plasma in this study were collected within 24 hrs (mostly within 3 hrs) from the time of hospitalization, and they were measured only at that interval. The authors have come to the conclusion that bacteremia was related to the higher levels of the inflammatory mediators, as opposed to our results, which reveal that the inflammatory mediators were significantly lower in the polymicrobial blood culture compared to the Gram-negative, Gram-positive, or negative ones on the third day of measurement. At other measurement intervals, the cytokine profile differences were not significant regarding blood culture. The investigated cytokines have proven to be good predictors for the polymicrobial and negative blood cultures, whereas the discrimination of the Gram-negative and Gram-positive blood cultures has not produced any of the cytokines as a good predictor. Feezor et al. [36] have determined that considerably higher levels of pro-inflammatory cytokines in 52 patients with sepsis result from the GPB compared to the Gram-negative ones. Baseline levels of TNF-α, IL-1Ra, IL-8, IL-10, IL-18BP, procalcitonin, and protein C in plasma were not significantly different between septic patients with Gram-positive and Gram-negative infections. In contrast, plasma IL-1β, IL-6, and IL-18 concentrations were significantly higher among patients with sepsis due to GPB than patients with sepsis due to GNB despite no significant differences in the magnitude of the physiologic response (Acute Physiology And Chronic Health Evaluation – APACHE II score), the degree of organ injury (Multiple Organ Dysfunction Score), or other pro-inflammatory cytokines. These findings suggest that the patterns of plasma cytokine appearance may differ between patients with sepsis due to GNB and GPB [36]. On the other hand, researchers of another study have determined that the GNB induce more pro- and anti-inflammatory cytokines compared to the GPB [18]. The aim of their study was to determine whether the early cytokine profile can discriminate between GPB and GNB, respectively, in critically ill patients with severe abdominal sepsis. Blood samples were obtained from 165 adult patients with confirmed severe abdominal sepsis. Levels of the pro-inflammatory mediators TNF-α, IL-8, IL-12, and IFN-γ and the anti-inflammatory mediators IL-1Ra, IL-4, IL-10, and Transforming growth factor (TGF)-β1 were determined and correlated with the nature of the bacteria isolated from the blood culture. The cytokine profile in their study indicated that the TNF-α levels were 2-fold, IL-8 levels were 3.3-fold, IFN-γ were 13-fold, IL-1Ra were 1.05-fold, IL-4 were 1.4-fold, and IL-10 were 1.83-fold higher in the GNB group compared with the GPB group [18]. We have expected to find a larger difference in the immuno-inflammatory response between the Gram-negative and Gram-positive bacterial infections, but unlike the preceding studies [18, 36] significantly larger differences in the inflammatory mediators have not been detected. Mortality may be impacted by the type of an infec-

tious agent. Some authors have reported increased mortality with GPB, while other researchers report significantly higher mortality rates with GNB [37]. Zahar et al. [38] have not demonstrated a relationship between the bacteria and mortality. Our study's prediction of the fatal outcome has singled out only the Gram-negative blood culture, wherein all the cytokines, except for IL-6, were significant predictors for the fatal outcome, with AUC values in the range of 0.7–0.8 at the third measurement interval. The most powerful predictor for the fatal outcome became MIP-1α, with values lower than 79 pg/mL in non-survivors compared to survivors. MIP-1α is an inflammatory chemokine produced by cells during infection or inflammation. It belongs to the CC chemokine family, which displays potent chemotactic properties. This protein was called MIP-1α because of its biological function of inducing an inflammatory response characterized by neutrophil infiltration. It performs various functions, such as recruiting inflammatory cells, wound healing, inhibition of stem cells, and maintaining effector immune response [39]. Most mature hematopoietic cells can induce the synthesis of MIP-1α. Monocytes, T lymphocytes, B lymphocytes, neutrophils, dendritic cells, and natural killer cells are known to secrete MIP-1α. Under normal conditions, synthesis of MIP-1α occurs at very low levels. However, upon stimulation of receptive cells with endotoxins such as lipopolysaccharide or proinflammatory cytokines, cellular signaling events are activated, and this activation induces increased production of MIP-1α. Elevated circulating MIP-1α levels are also found in patients with septic shock [40]. Identification of critically ill patients with higher death risk is important [41]. The total in-hospital mortality in our research amounted to 36.8%. Afuwape et al. [42] have analyzed the influence of the cytokine response (TNF-α, IL-1α) on the survival of patients with generalized peritonitis for six months in a pilot study during which they concluded that the TNF-α lower levels and the IL-1α higher levels are related to survival. As the only significant predictor of fatal outcome on the first day of measurement, our study revealed IL-17A, with lower values in non-survivors compared to survivors in the case of secondary sepsis occurring as a complication of peritonitis. On the other hand, the levels of other cytokines correlated with the outcome only on the fifth day of measurement and were higher in the survivors compared to non-survivors. From the clinical point of view, the fifth day of measurement is quite late for the outcome prediction. The studies investigating the role of IL-17A on animal models of sepsis have published that IL-17A causes considerable pathology and that a significantly improved survival included the elimination of this cytokine [43]. Later studies have published opposite results [44], and the contemporary literature comprises numerous studies that demonstrate mixed blockade effects of IL-17A in sepsis [45]. In a study carried out on humans, done by Ahmed Ali et al. [46], the increased levels of IL-17A in the serum predict the development of sepsis and mortality in patients with polytrauma. On the first day of measurement, the patients with secondary sepsis as a complication of pancreatitis or trauma did not present any of the examined cytokines as having a discriminatory power with regard to the prediction of the fatal outcome. Despite the high prevalence of negative blood culture, previous studies have not reported on cytokine profiles in critically ill septic patients in the ICU. We have expected to find a much weaker production capacity of cytokines in a negative blood culture. These are some of the questions solicited by our research: Why would we expect a lot of cytokines where bacteria are low in number, and what is hiding in the negative blood cultures? What is exactly the cytokine production capacity like in negative sepsis? Although this study cannot answer all the questions, its findings offer insight into some of these possibilities. The assumed reasons include exposure to antibiotics prior to being admitted to the ICU, as well as a possible presence of slow-growing or fastidious bacteria. Molecular techniques based on the polymerization chain reaction (PCR) may improve discovery rates of pathogens, and many patients with clinical sepsis are truly PCR-positive but negative in blood culture [47]. Our present study has several limitations – this is an observational study carried out within one institution on a relatively small sample size (125 patients). That is why the tendencies and patterns discovered herein should be confirmed in a larger patient population through a multicentric study. We cannot use our results to generalize about other groups of critically ill and traumatized patients. The overall applicability of our results to other forms of sepsis is unclear. Our findings represent preliminary results, and further investigations are justified with a larger number of patients and other subpopulations of septic patients.

Conclusion

On the third day of measurement, we demonstrated statistically significant differences in cytokine values according to the type of bacteremia, with the lowest levels of cytokines in the polymicrobial blood culture. IL-17A is a good predictor of the outcome of secondary sepsis occurring as a complication of peritonitis. The low level of IL-17A in these patients predicts a fatal outcome. On the other hand, only on the fifth day of measurement did the levels of other cytokines correlate with the outcome, and they were higher in survivors compared to non-survivors.

Conflict of interest

The authors report no conflict of interest.

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