Immune profile of patients-a new approach in management of sepsis and septic shock?

ANCA BACÂREA[1], OANA COMAN[2], VLADIMIR CONSTANTIN BACÂREA[3], ANCA MEDA VĂSIEȘIU[4], IRINA SĂPLĂCAN[5], RALUCA ȘTEFANIA FODOR[6] and BIANCA LIANA GRIGORESCU[6]

Departments of [1]Pathophysiology, [2]Simulation Applied in Medicine, [3]Scientific Research Methodology and [4]Infectious Disease, University of Medicine, Pharmacy, Science and Technology 'George Emil Palade', Târgu Mureș, Mureș 540142; [5]Clinic of Anesthesiology and Intensive Care, Emergency County Hospital, Târgu Mureș, Mureș 540136; [6]Department of Anesthesiology and Intensive Therapy, University of Medicine, Pharmacy, Science and Technology 'George Emil Palade', Târgu Mureș, Mureș 540142, Romania

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Abstract. The present study was a prospective observational single center study, enrolling 102 patients with sepsis, admitted in the Intensive Care Unit of the County Emergency Clinical Hospital in Târgu Mureș (Mureș, Romania). The main goal of the present study was to compare the changes of the following parameters on day 1 compared with day 5, in sepsis compared with septic shock, as well as in survivors compared with non-survivors: Cell blood count parameters, neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and systemic inflammation index, C reactive protein (CRP), ferritin, procalcitonin (PCT), CD 3+ T cells, CD4+ T cells, CD8+ T cells, CD16+CD56+/CD3-NK cells and CD19+ B cells. The relationship between the subcategories of lymphocytes with the inflammatory markers was evaluated. The serum concentration of CRP and PCT was significantly lower on day 5 compared with day 1 and serum ferritin was significantly higher in patients with septic shock. The percentage of cytotoxic T lymphocytes was significantly decreased and the percentage of NK lymphocytes was significantly increased in patients who developed septic shock. The results indicated a negative significant correlation between the proportion of T lymphocytes and PCT concentration and a positive significant correlation between the proportion of B lymphocytes and PCT concentration.

Correspondence to: Dr Oana Coman, Department of Simulation Applied in Medicine, University of Medicine, Pharmacy, Science and Technology 'George Emil Palade', 38 Gheorghe Marinescu Street, Târgu Mureș, Mureș 540142, Romania
E-mail: oana.coman@umfst.ro

Professor Vladimir Constantin Bacârea, Department of Scientific Research Methodology, University of Medicine, Pharmacy, Science and Technology 'George Emil Palade', 38 Gheorghe Marinescu Street, Târgu Mureș, Mureș 540142, Romania
E-mail: vladimir.bacarea@umfst.ro

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Introduction

Sepsis is a serious medical condition associated with a severe systemic inflammation, termed systemic inflammatory response syndrome, and the presence of a known infection. It can evolve to septic shock, multiple organ dysfunction syndrome and mortality (1). Sepsis is regarded as the immune response of the host to fight the infection, being characterized by pro- and anti-inflammatory responses (2), resulting in hemodynamic consequences, metabolic derangement and damage to organs (3,4).

In sepsis, the behavior of the polymorphonuclear neutrophils (PMN) changes and they become resistant to apoptosis and, in addition, they induce the apoptosis of other cells, such as CD4 + lymphocytes (5-7). In an experimental study, it was demonstrated that PMN are protective at the onset of sepsis, because they control the bacteremia, but after the onset of sepsis they become harmful, as they lose their innate immune functions (8). As activated PMN are nonspecific in their function, they can harm the 'innocent bystander' cells and induce tissue injury and further organ dysfunction (5,9). The septic monocytes are resistant to apoptosis (10) and have reduced expression of the major histocompatibility antigen HLA-DR (5,11,12). One study found evidence supporting the idea that an early circulating factor in severe sepsis/shock modulates the apoptosis of CD4+ lymphocytes and monocytes (10). On the other hand, increased apoptosis induces the decrease of dendritic cells and lymphocytes: CD4+ T cells, CD8+ T cells and B cells (13). The T regulatory subcategory appears to be more resistant to apoptosis in sepsis than the other subsets of lymphocytes (5,13). One study also indicates the decrease of NK lymphocytes in a cohort of septic patients with purulent meningitis (14).

These changes of leukocytes have an effect on the clinical course and outcome of patients with sepsis. Neutrophilia in association with lymphopenia are correlated with the severity of the clinical course (15-17). The need for quick indicators to predict the evolution of patients with sepsis has been

evident for many years. In line with this, different ratios were calculated from the cell blood count (CBC) with promising value for the prediction and prevention of sepsis mortality: Neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), lymphocyte-monocyte ratio (LMR) (16,18,19). These ratios are valuable also for the early prediction of neonatal sepsis (20). Systemic inflammation response index (SIRI), systemic inflammation index (SII) and the aggregate index of systemic inflammation (AISI) were found to predict the outcome in different pathologies, but especially in COVID-19 (21).

Considering these studies focused on finding early, useful and inexpensive predictors for the outcome of patients with sepsis, the main goal of the present study was to compare the changes of the following parameters on day 1 and day 5, in sepsis compared with septic shock, as well as in survivors compared with non-survivors: Cell blood count parameters, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and systemic inflammation index (SII), C reactive protein (CRP), ferritin, procalcitonin (PCT), CD 3+ T cells, CD4+ T cells, CD8+ T cells, CD16+CD56+/CD3-NK cells and CD19+ B cells. The relationship between the subcategories of lymphocytes with the inflammatory markers were also evaluated.

In Romania, these markers have not been evaluated in sepsis, and the results of the various studies are contradictory. In addition, the identification of such biomarkers as predictors of the evolution in sepsis would be of great use in a country with limited financial resources.

Materials and methods

The present study was a prospective observational single center study, enrolling 102 patients with sepsis admitted in the Intensive Care Unit (ICU) of the County Emergency Clinical Hospital from Târgu Mureș (Mureș, Romania), between July 2021 and March 2023.

The present study was approved by the Ethics Committee of the University of Medicine and Pharmacy, Science, and Technology 'G.E. Palade' from Târgu Mureș (Mureș, Romania; approval no 1425/01.07.2021) and was conducted in accordance with the Helsinki Declaration.

The current study included patients over 18 years of age, diagnosed with sepsis according to the Sepsis 3 Consensus criteria (22). Exclusion criteria were cancer with current chemotherapy or radiation therapy, treatment with corticosteroids or immunosuppressive medication, or evidence of autoimmune disorders.

Informed consent for inclusion in the study was obtained from each patient or legal guardian of patients, as well as consent for publication of obtained data.

The studied parameters were: Age, sex, body mass index (BMI), complete blood count (CBC), CRP, ferritin, PCT, T cells (CD 3+), Th cells (CD4+), Tc cells (CD8+), NK cells (CD16+CD56+/CD3-), B cells (CD19+). All these parameters were evaluated on day 1 and day 5 after admission to the ICU. The identification of leukocytes subsets was performed using a flow cytometry (BD FACSCalibur; BD Biosciences) and they were quantified as percentages (%). CBC was performed using a Sysmex XN-1000 analyzer (Sysmex Europe GmbH). For CBC and immunophenotyping venous blood samples were collected in K2 EDTA tubes. From patients' serum CRP (turbidimetry), ferritin (electrochemiluminescence immunoassay ECLIA) and PCT (chemiluminescent immunoassay, CLIA) were determined using Cobas c 501 analyzer (Roche Diagnostics).

BMI was calculated based on weight and height (kg/m^2).

The studied ratios and indexes were calculated as follows: NLR as the neutrophil to lymphocyte ratio, PLR as the platelet to lymphocyte ratio and SII as neutrophils x platelets/lymphocytes.

Based on data reported in the literature, the patients included in the study were divided according to the serum ferritin concentration in two groups: One with serum ferritin values <500 μg/l and one with serum ferritin values ≥500 μg/l.

Data was entered into MS Excel. Statistical, descriptive and inferential processing was performed with the GraphPad Prism 5 Demo version (Dotmatics). Means or medians with confidence intervals were calculated for descriptive statistics. The mean was calculated for data with a normal distribution, and the median was calculated for those with a non-Gaussian distribution. To establish the differences in the mean, the Student's t test or the Mann Whitney test was used, depending on the Gaussian or non-Gaussian distribution. For binary data the Chi Square test was used. The regression tests used were Pearson's or Spearman's. For receiver operating characteristic (ROC) analysis SPSS 17.0 (SPSS, Inc.) was used. $P<0.05$ was considered to indicate a statistically significant difference (23).

Results

The present study included 39 women (38.24%) and 63 men (61.76%). The mean age was 68 years (minimum 37 years old; max 90 years old). A total of 76 patients succumbed (74.51%) and 26 patients survived (25.49%). The mean BMI was 28.57±5.6 (minimum 15.60; maximum 49.40). A total of 40 patients (39.22%) evolved to septic shock.

The underlying conditions in the study group were as follows: Cardiovascular disorders (82 patients; 80.4%), renal disorders (68 patients; 66.7%), respiratory disorders (63 patients; 61.8%), neurological disorders (46 patients; 45.1%), diabetes mellitus (31 patients; 30.4%) and polytrauma (8 patients; 7.8%).

Table I shows the values of the studied parameters on day 1 and day 5. Day 1 was defined as the day on which the patient was clinically diagnosed with sepsis.

The lymphocytes count was significantly higher, and the serum concentration of CRP and PCT was significantly lower on day 5 compared with day 1.

Table II compared the studied parameters between patients with sepsis and those who developed septic shock.

Among the markers of inflammation, ferritin was significantly higher in patients with septic shock. The percentage of cytotoxic T lymphocytes was significantly decreased and the percentage of NK lymphocytes was significantly increased in patients with septic shock.

As statistically significant differences were observed in ferritin and NK lymphocytes, ROC curve analysis was

Table I. The studied parameters on day 1 and day 5.

Parameter	Day 1	Day 5	P-value
Leukocytes, x$10^3/\mu$l	14.42	13.50	0.80[a]
Neutrophils, x$10^3/\mu$l	12.02	11.69	0.98[a]
Lymphocytes, x$10^3/\mu$l	0.85	1.00	**0.02**[a]
Thrombocytes, x$10^3/\mu$l	213.00	223.00	0.67[a]
NLR	15.39	11.38	0.11[a]
PLR	272.10	214.7	0.20[a]
SII	3224.00	3868.00	0.88[a]
CRP, mg/l	179.30	131.90	**0.01**[b]
Ferritin, μg/l	592.50	446.50	0.27[a]
PCT, ng/ml	3.08	1.06	**0.01**[a]
T cells (CD3+), %	76.30	73.21	0.43[a]
Th cells (CD4+), %	63.53	65.6	0.70[a]
Tc cells (CD8+), %	30.59	30.74	0.74[a]
NK cells (CD16+56+/CD3-), %	8.00	8.45	0.74[a]
B cells (CD19+), %	12.80	10.30	0.25[a]

[a]Mann Withney; [b]Student's t-test. Bold type indicates significance. NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; SII, systemic inflammation index; CRP, C reactive protein; PCT, procalcitonin; CD, cluster of differentiation.

performed to assess the early diagnostic value of these two markers for discriminating between septic shock and sepsis, as can be seen in Figs. 1 and 2.

Analyzing the ROC curves, only ferritin is important in the early discrimination between sepsis and septic shock.

Among the 40 patients who developed septic shock, 30 patients (75%) had serum ferritin levels ≥500 μg/l (P=0.0005). The mortality rate was also significantly higher in patients with ferritin ≥500 μg/l (82.14% of these patients succumbed, P=0.028).

Comparing the analyzed parameters between survivors and non-survivors on admission, no significant difference were obtained, as can be seen in Table III.

Table IV evaluated the correlations between the subcategories of leukocytes and the inflammation markers.

A negative significant correlation was observed between the percentage of T lymphocytes and PCT concentration, and a positive significant correlation between the percentage of B and PCT concentration.

Discussion

The present study focused on finding early, useful and inexpensive predictors for patients with sepsis. For this purpose, the studied parameters were compared in a cohort of patients admitted in ICU on day 1 and day 5, in sepsis compared with septic shock, as well as in survivors compared with non-survivors. Briefly, the results showed that the serum concentration of CRP and PCT were significantly lower on day 5 compared with day 1 and serum ferritin was significantly higher in patients with septic shock. The percentage of cytotoxic T lymphocytes was significantly decreased and the percentage of NK lymphocytes was significantly increased in patients who developed septic shock.

The non-survivors were older than the survivors, even if not significantly, probably due to associated chronic diseases (e.g. diabetes mellitus, chronic obstructive pulmonary disease) and altered immune response, which is similar the results of other studies (24,25).

Despite its involvement in the pathogenesis of many diseases (26,27), increasing BMI appears to offer an advantage in the survival of patients with sepsis, as the BMI was higher in survivors compared with non-survivors. This phenomenon has been described as the obesity paradox (25,28,29).

The mortality rate in the present study group was much higher, compared with the values reported in other studies (30,31). This could be considered as a consequence of an immune-paralysis due to an immunosuppressive state that exposes patients to a secondary sepsis with bacteria, viruses or fungi and might progress with uncontrolled inflammatory response (32). These results might suggest the need to improve the management of sepsis and septic shock according to the patient immunologic profile.

Neutrophilia and lymphopenia are known hematological changes in sepsis. The increase in neutrophils is due to the release of immature neutrophils and delayed apoptosis of circulating neutrophils (33). In the present study the neutrophil count at admission was similar between survivors and non-survivors, in accord with other findings (24,34).

Furthermore, it is considered that some neutrophil subsets can suppress the immune function of T cells through several mechanisms: Depletion of L-arginine, release of reactive oxygen species and interferon γ-induced programmed cell death ligand 1 and apoptosis of T lymphocytes (33). This last mechanism is especially important in sepsis (33,35). On day 5, compared with day 1, an increase was observed in the number of lymphocytes, as well as a decrease in inflammatory markers, among which CRP and PCT decreased significantly, probably because of the compensatory anti-inflammatory response syndrome, or immune-paralysis (36). Ferritin also decreased, although not significantly. The number of T helper, cytotoxic, NK, and B lymphocytes varied very little between day 1 and 5, as it is known that both their number and their function need several weeks to recover, in those who survive (33,36,37). Sepsis modifies both the naive T-cell pool, as well as the memory T cells, increasing the risk of secondary infections (38). When the patients with septic shock were compared with those with sepsis it was observed that the number of CD8 cells was significantly lower in septic shock and the number of NK cells was significantly higher. The reported results regarding the changes of CD8 cells in septic shock are controversial. As in the present results, the CD8 cells decrease in septic shock is the finding of one study (39), but according to another study, the percentage of CD8+T lymphocytes in the septic shock group was slightly higher than that in the sepsis group (40).

The number and function of B cells is also affected in sepsis. According to some studies, although the number of B cells decreases, the proportion of B cells in total lymphocytes appears to increase and the circulating B cell number is reduced in septic shock patients (41,42). In the present study, the percentage of B cells was lower in day 5 compared with

Table II. The studied parameters in septic shock vs. sepsis.

Parameter	Septic shock	Sepsis	P-value
Leukocytes, x$10^3/\mu$l	16.22±1.61	16.68±1.34	0.83[a]
Neutrophils, x$10^3/\mu$l	14.26±1.49	14.23±1.22	0.98[a]
Lymphocytes, x$10^3/\mu$l	0.88±0.085	1.22±0.16	0.12[a]
Thrombocytes, x$10^3/\mu$l	239.70±24.46	239.10±16.41	0.98[a]
NLR	18.32±1.70	16.63±1.43	0.45[a]
PLR	326.20±34.60	320.90±35.31	0.91[a]
SII	4415±551.80	4003±472.20	0.57[a]
CRP, mg/l	182.00±20.18	177.5±13.74	0.84[b]
Ferritin, μg/l	1436±235.50	811.60±137.80	**0.01[a]**
PCT, ng/ml	25.92±17.82	8.80±2.77	0.19[a]
T cells (CD3+), %	63.76±2.68	83.11±11.85	0.23[a]
Th cells (CD4+), %	65.65±2.61	62.38±1.93	0.31[b]
Tc cells (CD8+), %	26.41±1.87	32.86±1.83	**0.02[a]**
NK cells (CD16+56+/CD3-), %	13.61±2.18	9.22±0.87	**0.03[a]**
B cells (CD19+), %	20.07±2.46	16.05±1.92	0.20[a]

[a]Mann Withney, [b]Student's t-test. Bold type indicates significance. NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; SII, systemic inflammation index; CRP, C reactive protein; PCT, procalcitonin; CD, cluster of differentiation.

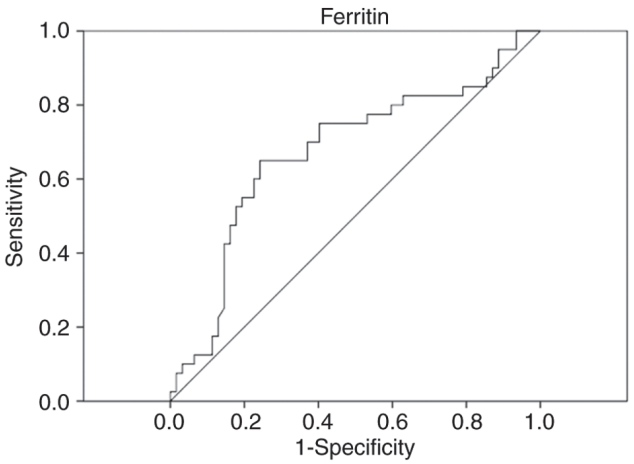

Figure 1. Receiver operating characteristic curve for ferritin and septic shock (area under the curve: 0.67; P=0.003).

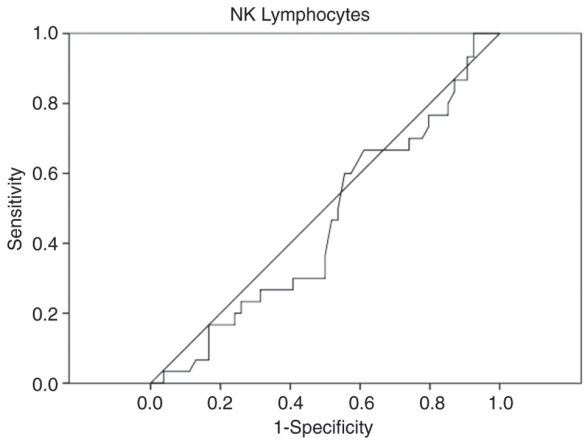

Figure 2. Receiver operating characteristic curve for NK lymphocytes and septic shock (area under the curve: 0.45; P=0.49). NK, natural killer lymphocytes.

day 1, a consequence of apoptosis, but was not significantly changed when sepsis was compared with septic shock.

In terms of survival, in the present study, none of the analyzed parameters was significantly changed. In one study, the results indicate that the percentages of CD4+ lymphocytes and CD19+ lymphocytes were lower in the non-survivor group, the percentage of NK lymphocytes was higher in the non-survivor group and there was no difference in the percentage of CD8+ lymphocytes between the non-survivor and survivor groups (43). The present study obtained just a mild decrease of CD19+ and a mild increase of NK cells in non-survivors. Considering the low number of patients included in the aforementioned study (43), further testing, using a larger cohort of patients is needed to evaluate these results. As many studies use a healthy control group, the present study found limited information regarding the dynamics of changes in lymphocyte subsets in sepsis/septic shock.

In the present study, PLR, NLR and SII did not prove useful for early indication of unfavorable evolution, since significant differences between day 1 and day 5, between sepsis and septic shock and between survivors and non-survivors were not obtained. One study indicates NLR is higher in non-survivors, but PLR values did not differ significantly between survivors and non-survivors (24). However, the results of the present study indicated a higher value of NLR in non-survivors vs. survivors (19.29 vs. 15.65). In a cohort of 194 patients with sepsis, both NLR and PLR were significantly higher in the non-survival group than in the survival

Table III. The studied parameters in survivors vs. non-survivors.

Parameter	Survivors	Non-survivors	P-value
Age (years)	64.57	69.33	0.08[b]
Sex M/F (no, %)	18, 28.57%/8, 20.51%	45, 71.43%/31, 79.49%	0.18[c]
BMI (kg/m^2)	29.33	28.31	0.55[a]
Leukocytes, x10^3/μl	16.13	18.67	0.34[a]
Neutrophils, x10^3/μl	13.99	16.54	0.33[a]
Lymphocytes, x10^3/μl	1.11	1.15	0.82[a]
Thrombocytes, x10^3/μl	233.3	246.9	0.73[a]
NLR	15.65	19.29	0.40[a]
PLR	263.2	285.9	0.71[a]
SII	3595.00	5416.00	0.34[a]
CRP, mg/l	157.7	130.8	0.26[b]
Ferritin, μg/l	742.10	817.10	0.71[a]
PCT, ng/ml	4.75	9.81	0.29[a]
T cells (CD3+), %	72.18	71.77	0.91[a]
Th cells (CD4+), %	65.25	66.63	0.72[a]
Tc cells (CD8+), %	30.62	29.74	0.79[a]
NK cells (CD16+56+/CD3-), %	6.91	8.67	0.24[a]
B cells (CD19+), %	16.27	15.92	0.92[a]

[a]Mann Withney, [b]Student's t-test, [c]Chi Square. Bold type indicates significance. NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; SII, systemic inflammation index; CRP, C reactive protein; PCT, procalcitonin; CD, cluster of differentiation.

Table IV. Correlations between leukocyte subcategories and inflammation markers.

Parameter	CRP (mg/l)	Ferritin (μg/l)	PCT (ng/ml)
Neutrophils, x10^3/μl	ρ=0.10	ρ=0.12	ρ=0.17
	(-0.10-0.30)	(-0.08-0.31)	(-0.10-0.43)
	[a]P=0.33	[a]P=0.22	[a]P=0.19
T cells (CD3+) %	ρ=-0.09	ρ=-0.16	ρ=-0.32
	(-0.31-0.12)	(-0.37-0.04)	(-0.57-0.02)
	[a]P=0.38	[a]P=0.11	**[a]P=0.03**
Th cells (CD4+) %	r=0.007	ρ=0.09	ρ=-0.01
	(-0.21-0.22)	(-0.11-0.30)	(-0.32-0.28)
	P[b]=0.94	[a]P=0.36	[a]P=0.91
Tc cells (CD8+) %	ρ=-0.08	ρ=-0.17	ρ=-0.09
	(-0.29-0.14)	(-0.37-0.04)	(-0.38-0.22)
	[a]P=0.46	[a]P=0.09	[a]P=0.55
NK cells (CD16+56+/CD3-) %	ρ=-0.10	ρ=0.04	ρ=-0.25
	(-0.32-0.11)	(-0.16-0.26)	(-0.51-0.05)
	[a]P=0.32	[a]P=0.65	[a]P=0.09
B cells (CD19+) %	ρ=0.02	ρ=0.08	ρ=0.36
	(-0.20-0.24)	(-0.13-0.29)	(0.06-0.60)
	[a]P=0.84	[a]P=0.44	**[a]P=0.01**

[a]Spearman test, [b]Pearson test. Bold type indicates significance. CRP, C reactive protein; PCT, procalcitonin; CD, cluster of differentiation.

group (44). The results of another study are opposite, as NLR was reduced in the non-survivor group (34). One meta-analysis indicates different changes of NLR and the outcomes in heterogeneous cohorts of critically ill adults with sepsis and highlights the need to evaluate NLR in future stratification models (45).

SII was evaluated in cohort of 209 patients with sepsis and the results showed that it was significantly lower in patients with

sepsis compared with those with septic shock (46). The results of the present study were similar, even if not significant. An important difference in SII value can be observed comparing the group of survivors vs. that of non-survivors (3,595.00 vs. 5,416.00). As SII is little investigated, further studies are necessary and SII will be evaluated in a larger cohort of patients.

Inflammatory markers are used to diagnose and monitor the evolution of patients with sepsis as well as the treatment and for prognosis (47-49). Among the three evaluated inflammatory markers, ferritin was significantly increased in patients with septic shock, compared with those with sepsis, as well as the percentage of NK cells, probably because of the macrophage activation syndrome (MAS) complicating sepsis, but no significant difference between survivors and non-survivors was found. When the patients we compared according to the ferritin threshold of 500 μg/l, ferritin levels were significantly higher in patients with septic shock and in non-survivors, similar to the results of one study (50). According to the results of one study, high-level serum ferritin is an independent prognostic marker for the prediction of mortality in patients with sepsis (51).

PCT increased in patients with septic shock, even if not significantly. The pathogenesis of MAS is not fully understood and it is associated with increased activation of macrophages and NK cells (52). Other studies indicate that both CRP and PCT have poor predictive value referred to 30-day all-cause mortality in patients admitted with sepsis or septic shock (53) and that PCT and CRP threshold values or their kinetics cannot predict ventilator-associated pneumonia survival or septic shock development (54). A study on the administration of antibiotics in patients with COVID-19 indicates that procalcitonin remains useful for associated bacterial infection (55).

The present study tested possible correlation between the three tested inflammatory markers and the changes of lymphocytes subsets and found that PCT decreases with decreasing proportion of T cells and increases with the increasing proportion of B cells, probably related to the pathogenic phases of sepsis and the functional abnormalities of T and B cells subsets during sepsis.

The present study has some limitations. The limited number of patients made it difficult to draw final conclusions. As it was a single center study, there was some bias regarding the overview of the pathology. In the future, the authors hope to increase the study group and continue the evaluation of the tested parameters on a larger cohort of patients.

In conclusion, the serum concentration of CRP and PCT was significantly lower on day 5 compared with day 1 and serum ferritin was significantly higher in patients with septic shock. The percentage of cytotoxic T lymphocytes was significantly decreased and the percentage of NK lymphocytes was significantly increased in patients who developed septic shock. The results indicated a negative significant correlation between the proportion of T lymphocytes and PCT concentration, and a positive significant correlation between the proportion of B and PCT concentration. Regarding the value of the present study in clinical practice, among the parameters tested, ferritin is important in predicting early evolution towards septic shock.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

AB wrote the draft of the manuscript and contributed to conception and design, acquisition of data, analysis and interpretation of data; OC was responsible for investigation and read and corrected the manuscript; VB was responsible for the study design and performed the statistical analysis; AV read and corrected the manuscript; IS, RF and BG were responsible for investigation and read and corrected the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the University of Medicine and Pharmacy, Science, and Technology 'G.E. Palade' from Târgu Mureș (Mureș, Romania; approval no 1425/01.07.2021) and was conducted in accordance with the Helsinki Declaration.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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