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Myeloid-derived suppressor cells in secondary sepsis: Is there an association with lethal outcome?

Supresorske ćelije mijeloidnog porekla u sekundarnoj sepsi: postoji li povezanost sa smrtnim ishodom?

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Abstract

Background/Aim. Role of myeloid-derived suppressor cells (MDSCs) in human host response to sepsis still needs to be clarified. The aim of our study was to determine whether frequency and/or absolute numbers of the MDSCs were associated with outcome in critically ill patients with secondary sepsis and/or septic shock. **Methods.** Total of 40 critically ill patients with secondary sepsis were enrolled in a prospective study. We detected and enumerated both main subsets of MDSCs: granulocytic (G)-MDSCs and monocytic (M)-MDSCs on the Day 1 (the day of hospital admission) and the Day 5 after the. The primary end-point was hospital mortality. **Results.** Increased frequencies and absolute numbers of subpopulations corresponding to MDSCs were associated with poor outcome. As far as relative kinetics was concerned, in both survivors and non-survivors, sepsis duration from 1th to 5th day was accompanied by an increase in MDSCs values of both investigated subpopulations. In contrast to findings of stepwise multivariate logistic regression analysis of the variables on the Day 1, on the Day 5 it was determined that the Sequential Organ Failure Assessment (SOFA) score (OR 2.350; $p < 0.05$) and G-MDSCs frequencies (OR 3.575; $p < 0.05$) were independent predictors of lethal outcome. **Conclusion.** These findings suggest harmful role of MDSCs in secondary sepsis.

Key words:
myeloid cells; myeloid-derived suppressor cells; mortality; prognosis; sepsis; treatment outcome.

Apstrakt

Uvod/Cilj. Uloga supresorskih ćelija mijeloidnog porekla (MDSCs) u imunskom odgovoru bolesnika sa sepsom tek treba da bude razjašnjena kod ljudi. Cilj istraživanja je bio da se utvrdi da li kod kritično obolelih sa sekundarnom sepsom i/ili septičkim šokom postoji udruženost učestalosti i/ili apsolutnih brojeva MDSCs sa ishodom bolesti. **Metode.** U prospektivnu studiju je bilo uključeno ukupno 40 kritično obolelih pacijenata sa sekundarnom sepsom. Detektovane su i kvantifikovane obe glavne podvrste MDSCs: granulocitna (G)-MDSCs i monocitna (M)-MDSCs, po prijemu na bolničko lečenje (prvi dan) i petog dana posle prijema. Primarni ishod je bio bolnički mortalitet. **Rezultati.** Veća učestalost i apsolutni brojevi subpopulacija koje odgovaraju MDSCs bili su udruženi sa lošim ishodom. Što se relativne kinetike tiče, i kod preživelih i kod umrlih, trajanje sepse od prvog do petog dana bilo je praćeno povećanjem vrednosti MDSCs u obe ispitivane subpopulacije. Multivarijantna logistička regresiona analiza je pokazala da su, za razliku od prvog dana, petog dana *the Sequential Organ Failure Assessment* (SOFA) skor (OR 2.350; $p < 0,05$) i frekvenca G-MDSCs (OR 3.575; $p < 0,05$) bili nezavisni prediktori letalnog ishoda. **Zaključak.** Ovi nalazi ukazuju na štetnu ulogu MDSCs u sekundarnoj sepsi.

Ključne reči:
kostna srž, ćelije; kostna srž, ćelije, supresorske; mortalitet; prognoza; sepsa; lečenje, ishod.

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Introduction

Since myeloid-derived suppressor cells (MDSCs) have been first described, almost 30 years ago in the context of cancer, their roles and importance are expanding, lately rather rapidly [1]. MDSCs are heterogeneous population of cells of myeloid origin encompassing myeloid progenitor cells, immature macrophages, immature granulocytes and immature dendritic cells. One of the main features of MDSCs is potent suppression of T-cell function. In the state of activation, these cells increasingly produce arginase 1, reactive nitrogen-species and reactive oxygen species (ROS) [2, 3]. Apart from acting as regulators of adaptive immune response, MDSCs also exert their influence over cytokine production by macrophages, so innate immune response is also affected. Two main subsets of MDSCs have been identified: monocytic (M)-MDSCs and granulocytic (G)-MDSCs.

Special interest was focused on role of MDSCs in immuno-inflammatory cascade in sepsis and/or trauma [4–6]. Sepsis remains a leading cause of mortality, multiple organ dysfunction syndrome (MODS) and prolonged stay in intensive care units (ICUs) despite enormous efforts from both clinicians and researchers. More than 250,000 deaths annually in the United States can be attributed to sepsis. Incidence of sepsis is rising for the most part of the world because of ageing population. In elderly, immune function is not efficient as it used to be, this important entity is known as immunosenescence. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis 3) taskforce was well aware of how complex and intricate host response to infection is and how important it would be to know when protective and adaptive response becomes deleterious and maladaptive [7, 8]. It has been proposed that "persistent inflammation-immunosuppression catabolism syndrome (PICS)" is the predominant phenotype that has replaced late occurring multiorgan dysfunction syndrome (MODS) in surgical ICU (SICU) patients who fail to recover [9–12]. Beneficial or detrimental role of MDSCs in host response to infection is still controversial [13–17].

It is obvious that role of MDSCs in sepsis still needs to be clarified. The primary aim of the study regarding MDSCs in critically ill septic patients was to determine whether frequencies and/or absolute numbers of MDSCs were associated with outcome. The measure of outcome was hospital mortality.

Methods

Patients

Total of 40 critically ill patients with secondary sepsis due to peritonitis, pancreatitis and severe trauma, admitted to SICU, were enrolled in a prospective study conducted in a tertiary university hospital (Military Medical Academy, Belgrade, Serbia). Approval in concordance with the Declaration of Helsinki was obtained from local ethics committee and informed consent from a patient or first-degree relative. The study was conducted in accordance with the approved guidelines. Sepsis patients 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 and vasopressors required to maintain mean arterial pressure (MAP) ≥ 65 mmHg and serum lactate level > 2 mmol/L despite adequate volume resuscitation) [8]. The study lasted 2 years and 1 month. The diagnostic criteria encompass any of the following variables thought to be a result of the infection: sepsis-induced hypotension, lactate levels greater than 2 mmol/L, urine output less than 0.5 mL/kg/h for more than two hours despite adequate fluid resuscitation, acute lung injury with PaO_2/FiO_2 less than 250, creatinine greater than 2.0 mg/dL (176.8 μmol/L), bilirubin greater than 2.0 mg/dL (34.2 μmol/L), platelet count less than 100,000 and coagulopathy – international normalised ratio (INR) greater than 1.5. Critically ill surgical patients with severe trauma [Injury Severity Score – ISS (determined using Abbreviated Injury Scale – AIS) > 25 points] were enrolled after they developed secondary sepsis. Regarding mechanism of injury, most frequently it was motor vehicle accident both as occupants and pedestrians. Also, fall from height and fall from standing height were present. Polytraumatized patients had predominant orthopedic, thoracic and head trauma. The exclusion criteria were as follows: secondary sepsis and/or septic shock with an underlying cause other than severe peritonitis, pancreatitis or trauma and malignant disease of any origin. A total of 25 patients were excluded out of 65 patients initially considered for enrolment.

Blood samples for MDSCs analysis were collected on admission to the SICU (Day 1) and on the Day 5 after admission. Also, samples of blood were simultaneously drawn for a blood culture. SOFA score, the Simplified Acute Physiology Score (SAPS) II and the Acute Physiology and Chronic Health Evaluation (APACHE) II score were calculated and recorded within the first 24 h after admission to the SICU (Day 1) [18–20]. SOFA score was recorded daily during SICU stay to assess severity of organ dysfunction in secondary sepsis.

The use of antibiotics, circulatory volume replacement, vasoactive support and source controlled were performed according to guidelines [21]. Various modes of mechanical ventilation and surgical procedures were performed if and when necessary in all patients. Outcome measure was hospital mortality; patients were followed until hospital discharge (survivors) or hospital death (non-survivors).

Sampling and analysis

Fresh peripheral blood samples were analyzed, frequency and absolute number of MDSCs were determined. Both main subsets of MDSCs were detected, G-MDSCs and M-MDSCs.

Three mL of venous blood were collected from the sepsis patients and 100 μL were dispensed in test tubes for staining with below listed monoclonal antibodies. After incubation for 30 min, erythrocytes were removed using the lysing buffer (EDTA, NH_4Cl, $KHCO_3$) for 20 min. The remaining nucleated cells were washed out twice in the Roswell Park Memorial Institute (RPMI) 1640 culture medium

with 5% of normal human serum, centrifuged and resuspended. Separation of peripheral blood mononuclear cells (PBMC) for the comparative analysis was performed using Lymphocyte Separation Medium (LSM) 1077. The separation process was performed by centrifugation at 1.200 × g for 20 min. The interphase layer between the plasma and the separation solution was extracted with a Pasteur pipette and washed twice in culture medium. The cell counting was done manually, in an improved Neubauer chamber, and automatically, using the Beckman Coulter ACT differ blood counter. Finally, the suspension with 1×10^6 cells/100 μL was aliquoted in 12 × 75 mm test tubes for further immunostaining.

The following antihuman monoclonal antibodies were used in different combinations for multicolor analysis of the fresh peripheral blood samples: CD15-PECy7 (Biolegend, USA), CD45-PEDyLight 594 and PECy5 (EXBIO, Czech Republic), HLA-DR-FITC (Miltenyi Biotec, Germany), CD14-PEDyLight 594 (EXBIO, Czech Republic), CD16-PECy7 (Biolegend, USA), CD11b-PE (Miltenyi Biotec, Germany), CD10-PECy5 (BD Biosciences, USA), CD3-PEDyLight 594 (EXBIO, Czech Republic), CD19-PEDyLight 594 (EXBIO, Czech Republic) and CD56-PEDyLight 594 (EXBIO, Czech Republic). The flow cytometry was performed using Beckman Coulter FC 500 flow cytometer with CXP analysis software. Given the fact that this was pilot study and we had not performed the suppressive assay yet, the acronyms M-MDSCs and G-MDSCs, refer to the phenotypically corresponding cells.

Statistical analysis

Complete statistical analysis of data was done with the statistical software package, SPSS Statistics 18. Most of the variables were presented as frequency of certain categories, while statistical significance of differences was tested with χ^2 test. In case of continuous data, variables were presented as mean value ± standard deviation (SD), median, minimal and maximal values. Kolmogorov-Smirnov test was used for evaluation of distribution of continual data. Statistical significance between groups was tested by Wilcoxon or Mann-Whitney test. Spearman's Rank Correlation analysis was used to establish the relation between parameters. Receiver operating characteristic (ROC) curves were constructed and analyzed to determine the sensitivity and specificity of variables for prediction of lethal outcome. Calculations of odds ratios (OR) and their 95% confidence intervals (CI) were done to determine the strength of the association between risk factors and outcomes. For that purpose, the most promising independent variables as single or combined risk factors were incorporated into binary logistic regression analyses. All the analyses were estimated at $p < 0.05$ level of statistical significance.

Results

Forty patients (average age was 59.3 years; range: 27–86 years; 12 females, 28 males) with secondary sepsis and/or septic shock due to pancreatitis (16 patients – 40%), peritonitis (14 patients – 35%) and trauma (10 patients – 25%) as the underlying cause, were enrolled. Of the 40 patients, 20 (50%) patients developed Gram-positive bacteriemia – GPB, 8 (20%) patients developed Gram-negative bacteriemia – GNB, and 10 (25%) patients had polymicrobial bacteriemia – POLY. In 2 (5%) patients no pathogen was isolated from blood culture. ISS (determined using AIS) was calculated and recorded in all polytrauma patients (mean ± SD): 35.24 ± 4.67. The demographic and clinical data of the patients are shown in Table 1.

Table 1

Demographic and clinical data

Parameter	Values
Patients, n	40
Age (years), median (range)	59.3 (27–86)
Sex, n (%)	
male	28 (70)
female	12 (30)
Simplified Acute Physiology II (SAPS II) score, mean ± SD	57.05 ± 9.37
Acute Physiology and Chronic Health Evaluation II (APACHE II) score, mean ± SD	21.65 ± 3.360
Sequential (Sepsis) Organ Failure Assessment (SOFA) score, mean ± SD	6.850 ± 2.832
[1]Severe sepsis due to, n (%)	
pancreatitis	16 (40)
peritonitis	14 (35)
trauma	10 (25)
Blood cultures, n (%)	
Gram-positive	20 (50)
Gram-negative	8 (20)
polymicrobial	10 (25)
sterile	2 (5)
Overall hospital mortality, n (%)	20 (50)

[1] – Reason for intensive care unit (ICU) admission.
SD – standard deviation.

Detection of MDSC subsets

Both main subsets of MDSCs were detected in sepsis patients. The cells were first gated on CD45 positive events to exclude the detritus in both, the sepsis patients and the healthy controls (Figures 1A and 2A, respectively). In the next step, on HLA-DR vs. CD11b dot plot, the HLA-DR$^{-/low}$CD11b+ events were selected (Figures 1B and 2B) and further analyzed for the lineage markers (CD3, CD19 and CD56, not shown) as well as for the CD10 (not shown), CD15 (Figures 1C and 2C), CD14 (Figures 1D and 2D) and CD16 (not shown) expression. The classification of granulocytic and monocytic subsets was based on the CD15 and CD14 expression, respectively. The G-MDSC were separated from mature granulocyte population on the basis of CD10 negativity, as well as lower and inhomogeneous expression of virtually all positive markers (CD11b, CD15 and CD16). The MDSC frequency was expressed as a percentage of these cells out of all CD45 positive events.

In order to investigate whether the assumed MDSCs had altered buoyancy, we have analyzed leukocytes from fresh lysed peripheral blood samples in paralel with peripheral blood mononuclear cells obtained on density gradient centrifugation from the same patient's samples. We have found that the cells of the same phenotype retain in the mononuclear layer on density gradient (not shown). Well known immunoparalysis, decrease of HLA-DR expression on monocytes in sepsis patients, was also observed (Figures 1B and 2B).

Detection of MDSCs in healthy control represents referent value from healthy donors blood pool.

The G-MDSCs and M-MDSCs frequencies and absolute numbers are higher in nonsurvivors

Of the 40 sepsis patients there were 20 survivors (discharged from hospital) and 20 non-survivors. In both groups of patients, survivors and non-survivors, sepsis duration from 1th to 5th day was accompanied with an increase in MDSCs values of both investigated subpopulations (Figures 3 A, B, C, D).

Baseline characteristics of patients on the Day 1 and the Day 5 according to outcome are shown on Table 2.

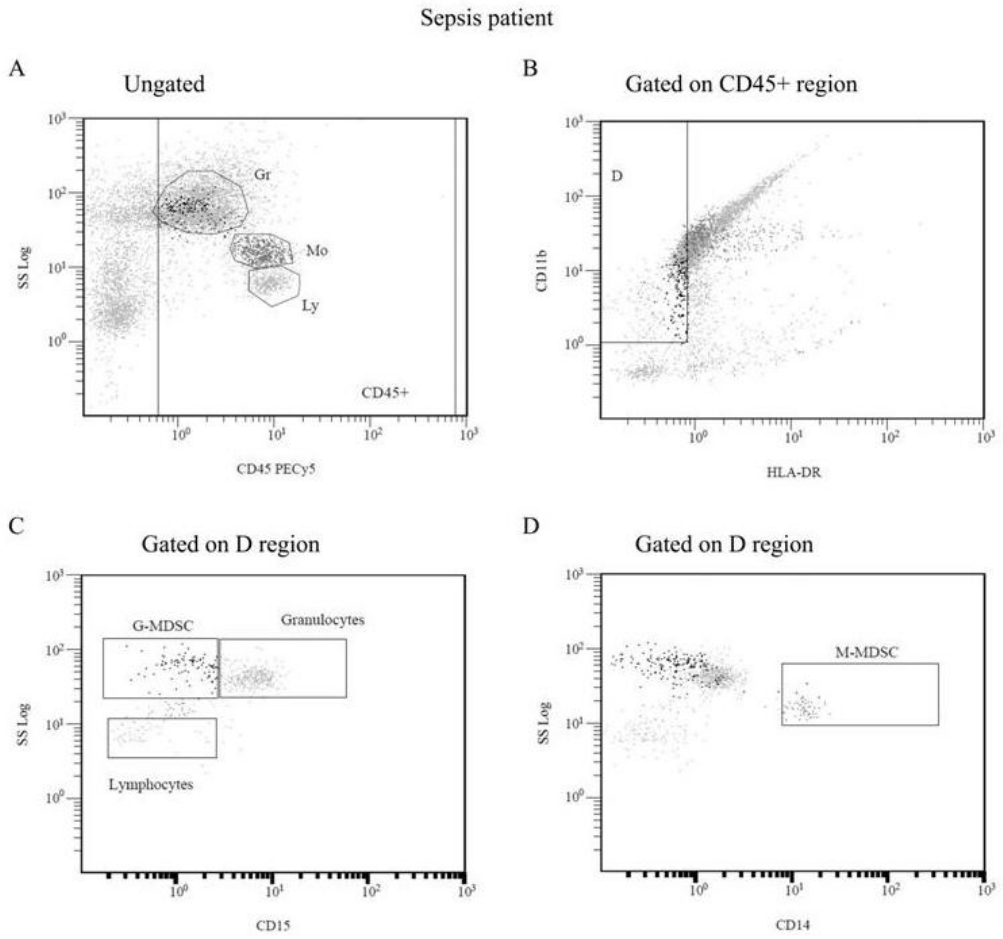

Fig. 1 – Detection of myeloid-derived suppressor cells (MDSCs) in the sepsis patients. Representative two-parameter dot plots showing identification of granulocytic (G)-MDSC in lysed peripheral blood samples. (A) The main leukocyte populations were selected based on CD45 expression. Monocytes are colored slightly darker grey for further tracking. (B) Darker grey monocytes showing low HLA-DR expression. The HLA-DR$^{-/low}$CD11b^{+} events were selected and assessed for the (C) CD15 expression, and (D) CD14 expression. The G-MDSCs are black colored for easier tracking.

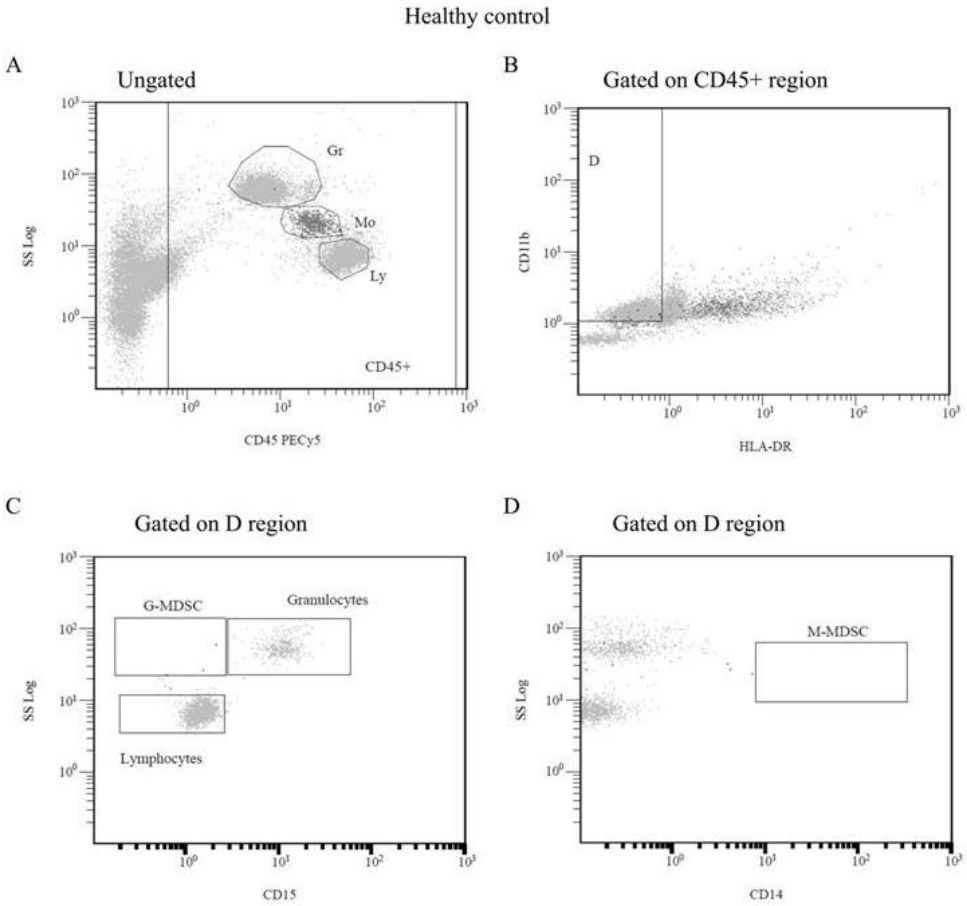

Fig. 2 – Detection of myeloid-derived suppressor cells (MDSCs) in the healthy control. Representative two-parameter dot plots showing identification of granulocytic (G)-MDSC in lysed peripheral blood samples. (A) The main leukocyte populations were selected based on CD45 expression. Monocytes are colored slightly darker grey for further tracking. (B) Darker grey monocytes showing higher HLA-DR expression than in sepsis patients. Assesment of the HLA-DR$^{-/low}$CD11b^{+} events showing „empty" (C) the G-MDSC region, as well as (D) the monocytic (M)-MDSC region in a healthy donor.

Table 2

Baseline characteristics of the patient population according to outcome on the Day 1and the Day 5

Parameters	Survivors (n = 20) mean ± SD; M; (min–max)	Non-survivors (n = 20) mean ± SD; M; (min–max)
SAPS II score 1st day	47.20 ± 11.07; 46.50; (22–65)	56.90 ± 15.52; 55.00; (23–85)
APACHE II score 1st day	14.50 ± 5.37; 15.00; (5–22)	20.80 ± 5.57; 21.00; (11–30)
SOFA score		
1st day	4.50 ± 2.87; 5.00; (0–9)	8.60 ± 3.50; 8.50; (1–14)
5th day	3.10 ± 2.53; 3.00; (0–9)	9.00 ± 4.52; 10.00; (3–14)
G-MDSCs frequencies (%)		
1st day	0.56 ± 0.61; 0.30; (0.02–1.99)	1.99 ± 2.72; 0.48; (0.02–9.35)
5th day	0.83 ± 0.82; 0.48; (0.03–2.95)	2.36 ± 2.44; 1.39; (0.37–9.00)
G-MDSCs absolute numbers		
1st day	114.28 ± 182.99; 37.14; (2.35–644.76)	180.42 ± 280.09; 55.29; (5.20–991.10)
5th day	152.17 ± 175.42; 72.24; (2.05–525.10)	268.27 ± 272.00; 178.35; (31.45–864.24)
M-MDSCs frequencies (%)		
1st day	0.44 ± 0.69; 0.25; (0.02–2.56)	0.59 ± 0.78; 0.19; (0.04–2.18)
5th day	0.55 ± 0.55; 0.53; (0.01–1.85)	0.93 ± 0.82; 0.87; (0.12–2.49)
M-MDSCs absolute numbers		
1st day	48.28 ± 45.89; 38.18; (1.67–157.95)	103.46 ± 165.89; 10.77; (1.77–533.92)
5th day	118.99 ± 158.91; 39.01; (0.68–519.85)	145.05 ± 202.22; 75.66; (3.51–689.73)

SD – standard deviation; M – median; min – minimum; max – maximum.
SAPS – Simplified Acute Physiology Score; APACHE – Acute Physiology and Chronic Health Evaluation;
SOFA – Sequential Organ Failure Assessment; G – granulocytic; MDSCs – myeloid-derived suppressor cells; M – monocytic.

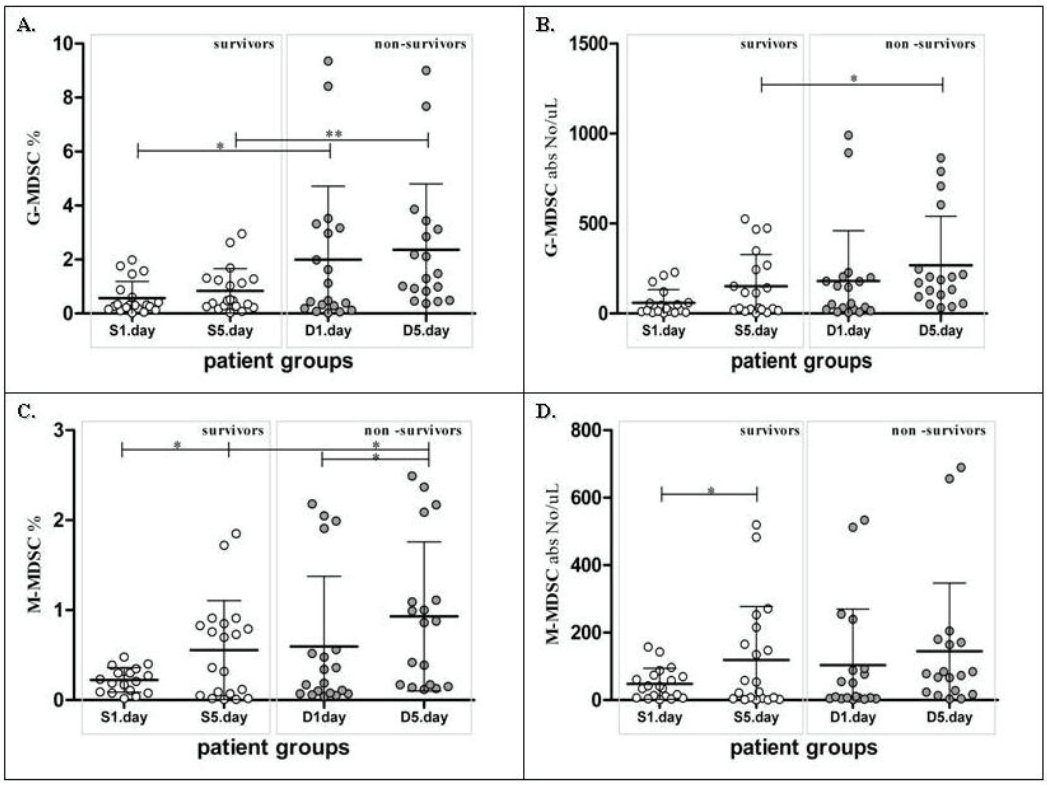

Fig. 3 – Comparison of myeloid-derived suppressor cells (MDSCs) values between survivors and non-survivors, 1th and 5th day: A) Relative number of granulocytic (G)-MDSC (%); B) Absolute number of G-MDSC (N/μL); C) Relative number of monocytic (M)-MDSC (%); D) Absolute number of M-MDSC (N/μL). S1, S5 – survivors on the Day 1 and the Day 5; D1, D5 – non-survivors on the Day 1 and the Day 5. Relative and absolute numbers given as mean ± standard deviation (Mann Whitney test, *$p < 0.05$, **$p < 0.01$).

The frequency of G-MDSCs was significantly higher in non-survivors, both on the Day 1 ($p < 0.05$) and the Day 5 ($p < 0.01$) of follow-up (Figure 3A). Absolute number of G-MDSCs was higher in non-survivors on the Day 1 (there was a trend which did not reach statistical significance) and on the Day 5 (statistically significant increase, $p < 0.05$) (Figure 3B).

Frequency of M-MDSCs was significantly higher on the Day 5, compared to the Day 1 ($p < 0.05$) in both survivors and non-survivors, but on the Day 5, frequency of M-MDSCs was also significantly higher in non-survivors compared to survivors ($p < 0.05$) (Figure 3C).

Regarding absolute number of M-MDSCs, although there was trend of higher values on the Day 5 in both survivors and non-survivors, only difference between the Day 1 and the Day 5 in survivors group reached statistical significance ($p < 0.05$) (Figure 3D).

Univariate logistic regression analyses were performed in order to determine whether associations of each individual variable with lethal outcome existed. Standardized regression coefficient (β) and OR with 95% CI were calculated for each variable. Forward stepwise multivariate logistic regression model was performed in order to determine the independent predictors of lethal outcome, without the effect of possible confounders. In Table 3 univariate OR of variables for predicting lethal outcome in patient population, on the Day 1 and the Day 5 are shown.

Univariate logistic regression analyses of investigated variables regarding lethal outcome on the Day 1 revealed that all three severity scores (SAPS II, SOFA, APACHE II) along with G-MDSCs frequencies had statistically significant power for predicting lethal outcome. When stepwise multivariate logistic regression analyses of the same variables on the Day 1 were performed, it was demonstrated that none of the investigated variables was independent predictor of lethal outcome.

Univariate logistic regression analyses of investigated variables regarding lethal outcome on the Day 5 revealed that SOFA score along with G-MDSCs frequencies had statistically significant power for predicting lethal outcome. In contrast to findings of stepwise multivariate logistic regression analyses of variables on the Day 1, on the Day 5 it was determined that SOFA score and G-MDSCs frequencies were independent predictors of lethal outcome which is shown in Table 4.

ROC curves were constructed to assess predictive values of investigated variables regarding lethal outcome. On the Day 1 neither frequencies nor absolute numbers of G-MDSCs and M-MDSCs were significant in discriminating between survivors and non-survivors.

Table 3

Univariate odds ratios (ORs) of variables for predicting lethal outcome in the patient population on the Day 1 and the Day 5

Variables	Standard β value	Odds ratio	95% confidence interval		*p*-value
			lower bound	upper bound	
SAPS II score 1st day	0.059	1.061	1.001	1.124	0.045*
SOFA score					
1st day	0.411	1.508	1.147	1.982	0.003**
5th day	0.40	1.504	1.167	1.938	0.002**
APACHE II score 1st day	0.216	1.241	1.068	1.443	0.005**
G-MDSCs frequencies					
1st day	0.671	1.956	0.958	3.997	0.040*
5th day	0.821	2.272	1.075	4.800	0.032*
G-MDSCs absolute numbers					
1st day	0.001	1.001	0.998	1.004	0.387
5th day	0.002	1.002	0.999	1.006	0.135
M-MDSCs frequencies					
1st day	0.292	1.339	0.552	3.252	0.519
5th day	0.807	2.242	0.820	6.131	0.116
M-MDSCs absolute numbers					
1st day	0.005	1.005	0.998	1.012	0.199
5th day	0.001	1.001	0.997	1.004	0.651

Significant differences are marked by *($p < 0.05$) or **($p < 0.01$).
For abbreviations see under Table 2.

Table 4

Independent predictors of lethal outcome by multivariate logistic regression analysis on the Day 5

Variables	Standard β value	Odds ratio	95% confidence interval		*p*-value
			lower bound	upper bound	
SOFA score	0.854	2.350	0.929	5.941	0.042*
G-MDSCs frequencies	1.274	3.575	1.098	11.639	0.030*

Significant differences are marked by *($p < 0.05$) or **($p < 0.01$).
For abbreviations see under Table 2.

Table 5

Clinical accuracy of variables in predicting lethal outcome in the patient population on the Day 5

Variables	AUC ROC	*p*-value	95% confidence interval		Cut-off value	Sensitivity (%)	Specificity (%)	Youden index
			lower bound	upper bound				
SOFA score	0.861	0.000**	0.748	0.975	6.50	67.0	90.0	0.56
G-MDSCs frequencies	0.758	0.007**	0.607	0.909	0.36	100.0	40.0	0.40
G-MDSCs absolute numbers	0.692	0.040*	0.519	0.864	30.75	100.0	50.0	0.50
M-MDSCs frequencies	0.699	0.037*	0.530	0.867	0.86	56.0	80.0	0.35

Significant differences are marked by *($p < 0.05$) or **($p < 0.01$).
AUC ROC – area under curve; ROC – receiver operating characteristic; for other abbreviations see under Table 2.

Table 6

Spearman's rho correlations between variables and lethal outcome in the patient population on the Day 5

Variables	G-MDSCs frequencies	G-MDSCs absolute numbers	M-MDSCs frequencies	M-MDSCs absolute numbers
Lethal outcome	0.447; $p = 0.005$	0.332; $p = 0.042$	0.344; $p = 0.035$	0.168; $p = 0.313$
G-MDSCs frequencies		0.818; $p = 0.000$	0.484; $p = 0.002$	0.389; $p = 0.016$
G-MDSCs absolute numbers			0.663; $p = 0.000$	0.749; $p = 0.000$
M-MDSCs frequencies				0.899; $p = 0.000$

G – granulocytic; MDSCs – myeloid-derived suppressor cells; M – monocytic.

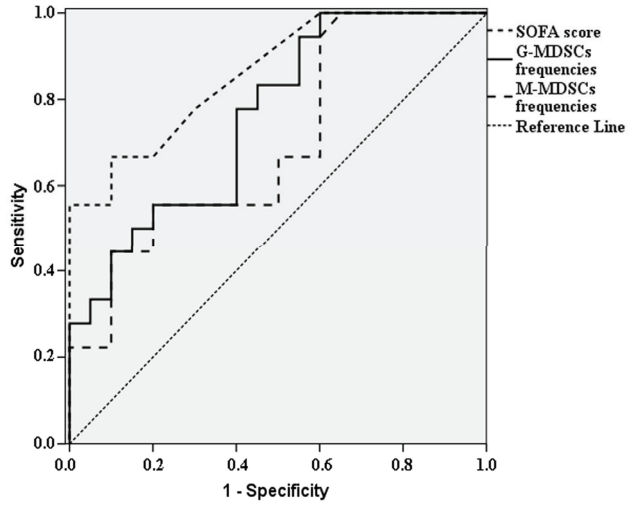

Fig. 4 – Receiver operating characteristic (ROC) curves for SOFA score, G-MDSCs and M-MDSCs frequencies in patient population on the Day 5 and the lethal outcome. For abbreviation see under Table 2.

In contrast to the Day 1, on the Day 5 all investigated variables were good predictors of lethal outcome apart from M-MDSCs absolute numbers [area under curve (AUC) 0.597; $p = 0.306$]. Frequencies and absolute numbers higher than cut-off values were predictors of lethal outcome. In Table 5 and Figure 4, clinical accuracy of variables in predicting lethal outcome in patient population on the Day 5 is shown.

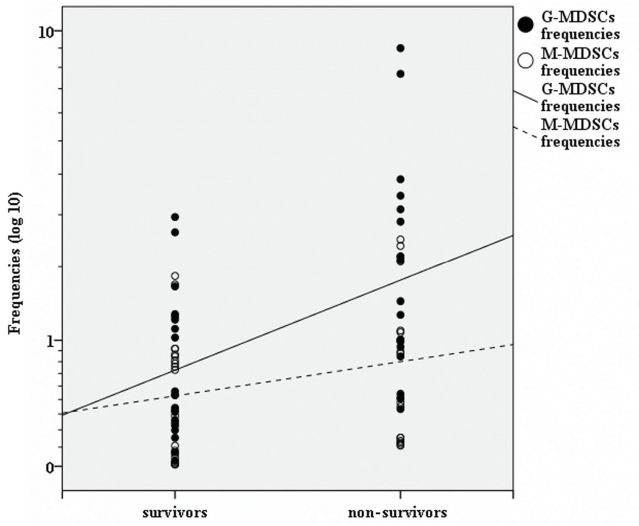

Fig. 5 – Scattergram on $\log_{10}$ scales of G-MDSCs and M-MDSCs frequencies versus lethal outcome in the patient population on the Day 5. G – granulocytic; MDSCs – myeloid-derived suppressor cells; M – monocytic.

The Spearman's rho test of correlation between frequencies and absolute numbers of G-MDSCs and M-MDSCs, on one hand, and lethal outcome, on the other hand, was performed to assess strength of association. On the Day 1 neither frequencies nor absolute numbers of G-MDSCs and M-MDSCs correlated significantly with lethal outcome. In contrast to the Day 1, on the Day 5, apart from M-MDSCs absolute numbers, there were significantly positive correlations between investigated variables and lethal outcome (Table 6). The strongest correlation was between G-MDSCs frequencies and lethal outcome (Figure 5).

There was no statistically significant association of gender, age, cause of secondary sepsis or nature of blood culture with outcome.

Discussion

The role of MDSCs has been extensively studied in a cancer field, but investigations regarding their function in sepsis are still sparse with previously contradictory results. While some studies demonstrated their deleterious effects [5], the others showed that the MDSCs expansion and activation could actually protect the sepsis host [6, 22]. In the present study, which included 40 patients with sepsis and/or septic shock secondary to pancreatitis, peritonitis and trauma, we detected and enumerated MDSCs on the Day 1 (the day of SICU admission and fulfillment of current sepsis and/or septic shock criteria) and on the Day 5 after the admission . These two specific time points were chosen because animal studies have shown dynamic change in MDSCs function during sepsis. In one study, although both MDSCs harvested at the Day 3 and the Day 10 were able to inhibit T cell proliferation, only MDSCs harvested at the Day 10 were also able to decrease peritoneal release of cytokines, enhance bacterial clearance and improve rate of survival [16]. In another study, authors demonstrated, on animal sepsis model, that early (Day 3) MDSCs adoptive transfer from septic into naive mice led to increased proinflammatory cytokine profile, decreased peritoneal bacterial growth with high early mortality rate. Contrary to that, transfer of late (Day 12) MDSCs effect was completely opposite [17]. To the best of our knowledge, this has not been investigated in humans yet. So, the Day 1 corresponds to early MDSCs in animal sepsis model. The Day 5 was chosen bearing in mind that survival of critically ill patients with secondary sepsis and/or septic shock on the day 10 or 12 is rather uncertain. Previously, we have emphasized that sample handling is of great importance during flow cytometric detection of MDSCs in the study with melanoma patients and indicated several reasons why we decided to analyze fresh, lysed peripheral blood samples [23]. However, Sagiv et al. [24], showed remarkable ability of mature neutrophils to change their density from 'normal' high, to low-density neutrophils and *vice versa* in the peripheral blood of tumor-bearing mice and human lung cancer patients. If this could be the truth for MDSCs as well, then analysis of fresh lysed samples might have the advantage in preserving the possible "high-density'' MDSCs. Altered buoyancy of our targeted cells is, however, in accordance with many studies that showed immunosuppressive capacity of a low density granulocyte-like cells [25–28].

As mentioned, it is still not definitely clarified whether MDSCs are friends or foes in sepsis and what determines whether they carry benefit or harm to the sepsis patients. De-

lano et al. [5] showed, on experimental animal sepsis model, that the Gr-1$^+$CD11b$^+$ MDSCs accumulate in bone marrow and peripheral lymphoid organs in mice during polymicrobial sepsis, and contribute to the T cell suppression seen after sepsis, as well as to the polarization from a Th1 towards Th2 immune response. Based on Delano et al. [5] findings, it was expected to connote the MDSC population as detrimental to the septic host. Surprisingly, blockages of the MDSCs expansion by using gemcitabine or anti-Gr-1 antibodies, with an aim to improve survival in septic mice, have led to unexpected, significantly worsened outcomes. This worsening in septic mice survival is partially explained by nonselective action of gemcitabine and anti-Gr-1 antibodies, but still, the beneficial effect of blocking MDSCs has not been reached [4]. The aforementioned evidences could lead towards opinion that the MDSCs accumulation is beneficial to the septic host. But, as emphasized by Cuenca et al. [4] and Delano et al. [5], the function and role of MDSCs in sepsis cannot be simplified to this point. There is still complex and intertwined relationship between impact of MDSCs on sepsis severity and survival, on one hand, and kinetics of their accumulation in sepsis, on the other hand. In that regard, we found that non-survivors had significantly higher frequencies of G-MDSCs both on the Day 1, and the Day 5, but on the fifth day difference was more pronounced, statistically highly significantly. On the Day 5, G-MDSCs frequencies were independent predictors of lethal outcome, determined by stepwise multivariate logistic regression analysis; this was confirmed by ROC curve analysis, which revealed good discriminative power regarding outcome, and by the Spearman's rho test showing the strongest positive correlation between G-MDSCs frequencies and lethal outcome in comparison with other investigated variables. Similarly, in the animal model of sepsis, Cuenca et al. [4] found no changes in either splenocyte or peripheral lymph node CD11b$^+$GR-1$^+$ numbers in the first twenty-four hours after sepsis. They found first expansion of the CD11b$^+$GR-1$^+$ cells in the spleen and peripheral lymph nodes only after 3–5 days, with continuous increase in their concentrations for the next 10–14 days. All of these results, regarding the increase in MDSCs, are consistent with the theory that the host immune response to sepsis is characterized by an initial hyperinflammatory phase which evolves over several days into a more protracted immunosuppressive phase [12]. In support of this notion that MDSCs contribute to the secondary, immunosuppressive phase of sepsis, are also the findings of Brudecki et al. [17]. These investigators clearly showed that GR-1$^+$ CD11b$^+$ cells from late sepsis are endowed with immunosuppressive capabilities. Namely, they showed that adoptive transfer of GR-1$^+$ CD11b$^+$ cells from the bone marrow of the day 12 septic mice into naive mice, immediately after induction of sepsis by cecal ligation and puncture, significantly improved early sepsis survival. In addition, IL-10 and TGF-β levels were significantly higher in mice that received GR-1$^+$ CD11b$^+$ cells from the day 12 septic mice than in mice which received saline or cells from the day 3 septic mice. Dramatic expansion of the GR-1$^+$ CD11b$^+$ cells in late sepsis was also documented in this study [17]. Predictive value of many components of immune response in sepsis, regarding disease severity and outcome, has been investigated; future large sample studies are required to explore MDSCs in this regard [29, 30].

As already mentioned, MDSCs are heterogeneous group of immature myeloid cells, poor phagocytes, which can prevent overactivation of the immune system by producing IL-10 or TGF-β [31]. But, protracted presence of these cells can lead to persistent inflammation (*via* NO, myeloperoxidase and ROS) and induce immunosuppression (by T-cell proliferation, anti-inflammatory mediators elaboration or defective presentation of antigens) [32].

A year ago, two very important studies regarding MDSCs in patients with sepsis and/or septic shock were published, emphasizing and reiterating the importance and novelty of this subject. Mathias et al. [33] focused their attention on patients with PICS, the predominant clinical phenotype in the ICU population, for which current interventions are ineffective. They noted that pivotal for the immune response in chronic sepsis (as well as in cancer) was the expansion of MDSCs, aimed at preserving innate immunity. Their hypothesis was that after sepsis in humans, MDSCs would be persistently increased, functionally immunosuppressive and associated with adverse clinical outcome. They enrolled 74 patients with sepsis and/or septic shock and 18 healthy controls. Blood was obtained at set intervals out to 28 days, MDSCs were phenotyped. They also performed functional and genome-wide expression analyses. This study design allowed them to assess role of MDSCs after sepsis. They found circulating MDSCs to be persistently increased, functionally immunosuppressive and associated with adverse long-term outcome consistent with PICS. These results are similar to our findings that higher values of MDSCs are associated with adverse outcome.

Uhel et al. [34] performed peripheral blood transcriptomic analysis in 29 patients with sepsis and 15 healthy donors, and in a second cohort of 94 patients with sepsis, 11 severity-matched ICU patients and 67 healthy donors, they performed functional analysis in order to clarify phenotype, suppressive activity, origin and clinical impact of MDSCs in patients with sepsis. Their results showed that MDSCs were major players in sepsis-induced immunosuppression. They concluded that CD14^{pos}HLA-DR$^{low/neg}$ M-MDSCs and CD15^{pos} G-MDSCs strongly contributed to T-cell dysfunction in patients with sepsis. Our findings generally go in the same direction. In both studies, authors stated that role of MDSCs in host response to sepsis was still not well-defined and needed to be clarified in large trials [35]. Multicentre large trials of this sort are difficult to conduct due to complexity of the design. Nevertheless, in the future, in our opinion, effort will be made because these important cells are potential target for future immunomodulating therapies. In this regard, it should be noted that MDSCs are phenotypically plastic which allows them a diverse functionality in response to their environmental conditions [36–38].

Main limitation of our study is sample size. Significant number of critically ill patients with secondary sepsis due to diffuse peritonitis had to be excluded because of malignant disease. Larger trial is essential for possible confirmation of our findings.

Conclusion

The role of MDSCs in different clinical settings, especially in sepsis, where the proinflammatory and antiinflammatory responses are simultaneously initiated, is not completely elucidated yet. In this study, we demonstrate that subpopulations corresponding to MDSCs can be phenotypically identified in the whole blood samples of sepsis patients and that their increased frequencies and absolute numbers are associated with poor outcome. As far as relative kinetics is concerned, we found that, in both survivors and nonsurvivors, sepsis duration from 1th to 5th day was accompanied by an increase in MDSCs values of both investigated subpopulations. These findings suggest that there is harmful role of MDSCs in sepsis and that larger trials are warranted in future research of these intriguing cells.

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