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Do nature of bacteremia and origin of secondary sepsis in critically ill patients determine subset of myeloid-derived suppressor cells expansion?

Da li vrsta bakterija i poreklo sekundarne sepse kod kritično obolelih određuju tip supresorskih ćelija mijeloidnog porekla?

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Abstract

Background/Aim. Gram-positive and Gram-negative bacteria may induce different inflammatory patterns. The aim of this study was to examine the association of the myeloid-derived suppressor cells (MDSCs) with the type of infecting microorganisms (Gram positive, Gram negative, polymicrobial) and underlying cause of secondary sepsis (peritonitis, pancreatitis, trauma). **Methods.** Totally, 40 critically ill patients with secondary sepsis were enrolled in the prospective study. Two patients without documented positive blood culture were excluded. We detected and enumerated both main subsets of MDSCs: granulocytic (G)-MDSCs and monocytic (M)-MDSCs on the Days 1 and 5. Blood was simultaneously drawn for a blood culture. The patients with different underlying causes of sepsis (peritonitis, pancreatitis, trauma) were perceived as separated groups and the frequencies and absolute numbers of their G-MDSCs and M-MDSCs were compared. **Results.** Both main MDSC subpopulations were accumulated significantly in Gram-positive sepsis. Univariate logistic regression analyses of investigated variables regarding Gram-positive sepsis on the Day 5 revealed that G-MDSCs absolute number along with both M-MDSCs frequency and absolute number had statistically significant power for predicting Gram-positive sepsis. Stepwise multivariate logistic regression analyses of the variables on the Day 5 determined that M-MDSCs absolute number was independent predictor of Gram-positive sepsis [odds ratio (OR) 1.012; $p < 0.05$]. Clinical accuracy of neutrophil (Ne)/G-MDSCs (Ne/G-MDSCs) and monocyte (Mo)/M-MDSCs (Mo/M-MDSCs) ratios in predicting nature of bacteremia and outcome were investigated. Discriminative power of both Ne/G-MDSCs and Mo/M-MDSCs ratios in predicting Gram-positive blood culture was statistically significant both on the Day 1 and Day 5 [areas under curve (AUCs): 0.684 and 0.692, and 0.707 and 0.793, respectively). Ne/G-MDSCs both on the Day 1 and Day 5 were statistically significant predictors of lethal outcome (AUCs: 0.694 and 0.678, respectively). There were no statistically significant differences in G-MDSCs and M-MDSCs among different three groups of patients regarding peritonitis, pancreatitis and trauma as causes of sepsis neither on the Day 1 nor on the Day 5. **Conclusion.** Gram-positive infectious agents were powerful inducers of MDSCs generation in sepsis. Also, underlying causes of secondary sepsis might not seem to influence the MDSCs accumulation.

Key words:
gram-negative bacteria; gram-positive bacteria; critical illness; myeloid-derived suppressor cells; sepsis.

Apstrakt

Uvod/Cilj. Gram-pozitivne i Gram-negativne bakterije mogu indukovati različit imunoinflamatorni odgovor. Cilj istraživanja bio je da se utvrdi da li kod kritično obolelih bolesnika sa sekundarnom sepsom postoji povezanost učestalosti i/ili apsolutnih brojeva supresorskih ćelija mijeloidnog porekla (MDSC) sa vrstom bakterijskog prouzrokovača i poreklom sekundarne sepse. **Metode.** Prospektivnom studijom bilo je obuhvaćeno ukupno 40 kritično obolelih bole-

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snika sa sekundarnom sepsom. Dva bolesnika bez dokazanog prisustva bakterija u sistemskoj cirkulaciji bila su isključena iz daljih analiza. Detektovane su i kvantifikovane obe glavne podvrste MDSC: granulocitne (G)-MDSC i monocitne (M)-MDSC) 1. i 5. dana. Istovremeno je uzimana i krv za određivanje hemokultura. **Rezultati.** Utvrdili smo da su obe glavne podvrste koje odgovaraju MDSCs bile značajno akumulirane u Gram-pozitivnoj sepsi. Univarijantna logistička regresiona analiza ispitivanih varijabli pokazala je da su 5. dana apsolutni broj G-MDSC, kao i učestalost i apsolutni broj M-MDSC bili značajni prediktori Gram-pozitivne sepse. Multivarijantna logistička regresiona analiza pokazala je da je 5. dana apsolutni broj M-MDSC bio nezavisni prediktor Gram-pozitivne sepse [*odds ratio* (OR) 1,012; $p < 0,05$]. Odnosi neutrofili (N)/G-MDSC i monociti (M)/M-MDSC bili su značajni prediktori Gram-pozitivne sepse u oba termina [*area under curve* (AUC) 0,684 i 0,692, odnosno 0,707 i 0,793]. Takođe, N/G-MDSC odnos je u oba termina bio značajan prediktor smrtnog ishoda (AUC 0,694, odnosno 0,678). Posmatrajući bolesnike sa različitim poreklom sekundarne sepse (peritonitis, pankreatitis, trauma) kao zasebne grupe, i poređenjem učestalosti i apsolutnog broja G-MDSC i M-MDSC, nisu utvrđene statistički značajne razlike ni prvog ni petog dana. **Zaključak.** Gram-pozitivne bakterije su snažni induktori akumulacije MDSC u sepsi. Takođe, izgleda da poreklo sepse ne utiče na akumulaciju MDSC.

Ključne reči:
gram-negativne bakerije; gram-pozitivne bakterije; kritična stanja; kostna srž, ćelije, supresorske; sepsa.

Introduction

Sepsis is a principal cause of death in critical care units worldwide and consumes considerable healthcare resources. There is evidence suggesting that there are different mechanisms of clinical manifestations of Gram-positive and Gram-negative sepsis to the extent that they may represent different disease entities [1]. Some microbial challenges may elicit levels of mediators that damage both the infecting microorganism and the host. Lipoteichoic acid (LTA) of Gram-positive bacteria as well as lipopolysaccharide (LPS) of Gram-negative bacteria elicit different response from the host. Furthermore, Gram-positive and Gram-negative bacteria may induce different inflammatory patterns. But, it is not physiologically or clinically apparent because of the fact that signs of systemic inflammatory response syndrome and routine laboratory markers of infection are nonspecific [1–3].

Myeloid-derived suppressor cells (MDSCs), with its two main subsets being monocytic (M-MDSCs) and granulocytic (G-MDSCs) are important regulators of intricate and complex immuno-inflammatory response to various insults such as bacteria [4].

The aim of this study were to examine the association of the MDSCs with the type of infecting microorganism (Gram positive, Gram negative, polymicrobial) and underlying cause of secondary sepsis (peritonitis, pancreatitis, trauma).

Methods

Totally, 40 critically ill patients with secondary sepsis due to peritonitis, pancreatitis and severe trauma, admitted to a surgical intensive care unit (SICU), were enrolled in prospective study conducted in a tertiary university hospital (Military Medical Academy, Belgrade, Serbia). Approval in concordance with Declaration of Helsinki was obtained from local Ethics Committee and informed consent from a patient or first-degree relative. Detailed description of the study population is reported elsewhere [5]. Blood samples for MDSCs analysis were collected on admission (the Day 1) and on the Day 5. These two specific time points were chosen because dynamic changes in MDSCs function during sepsis were expected. Blood was simultaneously drawn for a blood culture. The Sequential Organ Failure Assessment (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 hours after admission to the SICU (the Day 1). SOFA score was recorded daily during SICU stay to assess severity of organ dysfunction in secondary sepsis [6–8]. The use of antibiotics, circulatory volume replacement and vasoactive support were performed according to guidelines [9]. Various modes of mechanical ventilation and surgical procedures were performed if and when necessary in all patients.

Detailed description of demographic and clinical data of examined patients was presented in our previous study [5].

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. MDSCs analysis is described elsewhere [5].

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 the 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 analyses were 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 bacteremia nature and outcome. Calculations of odds ratios (OR) and their 95% confidence intervals (CI) were done to determine the strength of the association between variables and nature of bacteremia. For that purpose, the most promising independent variables, as single or combined, were incorporated into binary logistic regression analyses.

All the analyses were estimated at $p < 0.05$ level of statistical significance.

Results

Demografic and clinical data of 40 patients are shown in Table 1. Two patients with sterile blood cultures were excluded from further analysis.

Baseline characteristics of the patient population according to nature of bacteremia on the Day 1 and Day 5 are shown in Table 2.

Both main MDSC subpopulations accumulate significantly in Gram-positive sepsis

We compared frequencies and absolute numbers of G-MDSCs and M-MDSCs in sepsis patients according to the nature of bacteremia (Gram-positive, Gram-negative and Polymicrobial groups) (Figure 1).

Table 1

Demographic and clinical data of critically ill patients with secondary sepsis

Parameter	Values
Age (years), mean (range)	59.3 (27–86)
Sex, n (%)	
male	28 (70)
female	12 (30)
Scores	
Simplified Acute Physiology Score II (SAPS II), mean ± SD	57.05 ± 9.37
Acute Physiology and Chronic Health Evaluation II (APACHE II), mean ± SD	21.65 ± 3.360
Sequential (Sepsis) Organ Failure Assessment (SOFA), mean ± SD	6.850 ± 2.832
Reason for ICU admission due to severe sepsis, 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)

ICU – Intensive Care Unit; SD – standard deviation.

Table 2

Presence of MDSCs subpopulations in patients with secondary sepsis according to nature of bacteremia on the Day 1 and Day 5

Parameters	Gram-positive bacteremia (n = 20) mean ± SD; M (min-max)	Gram-negative bacteremia (n = 8) mean ± SD; M (min-max)	Polymicrobial bacteremia (n = 10) mean ± SD; M (min-max)
G-MDSCs			
frequencies (%)			
Day 1	2.00 ± 2.72; 0.88 (0.02–9.35)	0.56 ± 0.77; 0.20 (0.02–1.99)	0.58 ± 0.50; 0.37 (0.19–1.58)
Day 5	1.69 ± 1.12; 1.39 (0.17–3.86)	2.55 ± 3.61; 0.81 (0.25–9.00)	0.49 ± 0.35; 0.45 (0.03–1.13)
absolute number			
Day 1	237.42 ± 306.16; 153.44 (5.20–991.10)	57.92 ± 68.11; 31.12 (2.35–178.50)	72.09 ± 80.53; 48,12 (5.92–229.10)
Day 5	273.91 ± 236.53; 194.58 (12.56–864.24)	205.34 ± 282.57; 71.67 (19.40–708.30)	75.12 ± 97.00; 34.85 (2.05–267.81)
M-MDSCs			
frequencies (%)			
Day 1	0.66 ± 0.83; 0.30 (0.04–2.56)	0.19 ± 0.15; 0.19 (0.02–0.39)	0.58 ± 0.82; 0.21 (0.04–2.18)
Day 5	0.94 ± 0.69; 0.84 (0.13–2.49)	0.63 ± 0.93; 0.19 (0.01–2.17)	0.39 ± 0.41; 0.13 (0.01–0.99)
absolute number			
Day 1	106.57 ± 153.82; 55.12 (4.81–533.92)	29.02 ± 28.35; 21.21 (1.67–74.61)	66.97 ± 98.00; 13.96 (3.52–255.06)
Day 5	200.51 ± 216.24; 109.76 (3.51–689.73)	49.46 ± 73.44; 14.45 (0.89–170.78)	58.47 ± 76.61; 15.39 (0.68–215.67)

MDSCs – meloid derived suppressor cels; G – granulocytic; M – monocytic;
SD – standard deviation; M – Median; min – minimum; max – maximum.

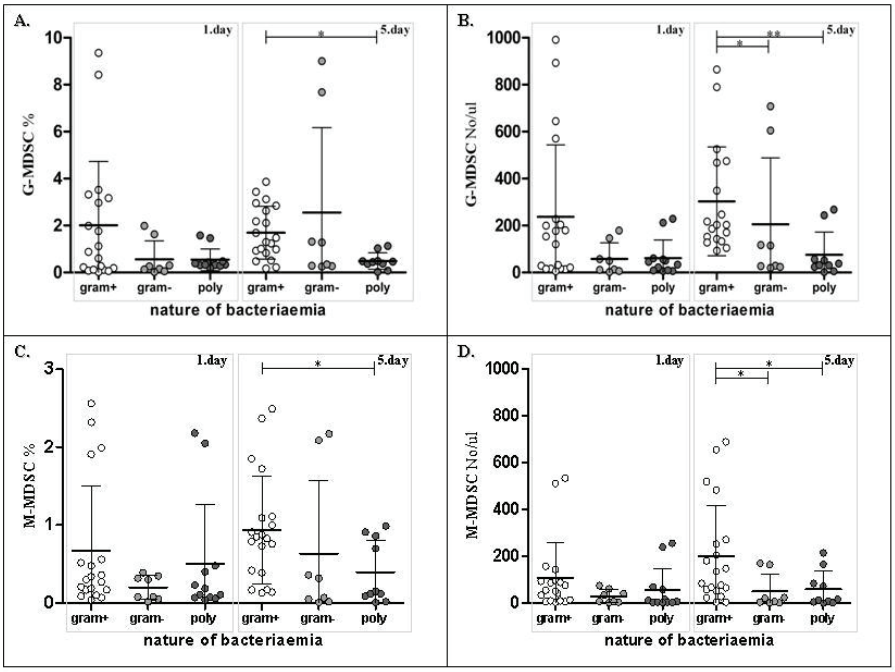

Fig. 1 – Comparison of MDSCs frequencies between groups of patients with different nature of bacteraemia (relative and absolute numbers are given as mean ± standard deviation; Mann Whitney test, *$p < 0.05$, **$p < 0.01$).
A. Relative number of G-MDSC (%); B. Absolute number of G-MDSC (N/μL); C. Relative number of M-MDSC (%); D. Absolute number of M-MDSC (N/μL).
MDSC – myeloid-derived suppressor cells; G – granulocytic; M – monocytic; poly – polymicrobial.

Initially, on the Day 1, patients with Gram-positive sepsis had more G-MDSCs and M-MDSCs (both relative and absolute number) comparing to other two groups, but without significant difference. Accumulation of G-MDSCs and M-MDSCs in patients with Gram-positive sepsis has become more intensive on the Day 5. This group had significantly more both G-MDSCs and M-MDSCs comparing to the Polymicrobial sepsis group ($p < 0.05$) (Figures 1 A,B,C,D). Also, patients with Gram-positive sepsis had significantly more G-MDSCs and M-MDSCs (absolute number) than patients with Gram-negative sepsis ($p < 0.05$) (Figures 1B and 1D).

Univariate logistic regression analyses were performed in order to determine whether associations of each individual variable with Gram-positive sepsis exist. 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 Gram-positive sepsis, without the effect of possible confounders. In Table 3 univariate ORs of variables for predicting Gram-positive sepsis in the patient population on the Day 1 and Day 5 are shown.

Table 3
Univariate odds ratio (ORs) of variables for predicting Gram-positive sepsis in the patient population on the Day 1 and Day 5

Variables	Standard β value	OR	95% CI		*p*
			lower bound	upper bound	
G-MDSCs					
frequencies					
Day 1	0.709	2.033	0.974	4.242	0.023
Day 5	0.084	1.087	0.767	1.542	0.638
absolute number					
Day 1	0.007	1.007	0.999	1.014	0.039
Day 5	0.003	1.003	1.000	1.007	0.043
M-MDSCs					
frequencies					
Day 1	0.580	1.786	0.689	4.624	0.232
Day 5	1.012	2.752	0.915	8.275	0.038
absolute number					
Day 1	0.005	1.006	0.998	1.013	0.166
Day 5	0.009	1.009	1.001	1.017	0.030

β – standardized regression coefficient; MDSCs – myeloid-derived suppressor cells; G-granulocytic; M – monocitic; OR – odds ratio; CI – confidence interval.

Univariate logistic regression analyses of investigated variables regarding Gram-positive sepsis on the Day 1 revealed that both G-MDSCs frequencies and absolute number had statistically significant power for predicting Gram-positive sepsis. Univariate logistic regression analyses of investigated variables regarding Gram-positive sepsis on the Day 5 revealed that G-MDSCs absolute number along with both M-MDSCs frequencies and absolute number had statistically significant power for predicting Gram-positive sepsis. Stepwise multivariate logistic regression analyses of the variables on the Day 5 determined that M-MDSCs absolute number was independent predictor of Gram-positive sepsis which is shown in Table 4.

The Spearman's rho test of correlation between frequencies and absolute numbers of G-MDSCs and M-MDSCs on one hand, and Gram-positive sepsis on the other hand, was performed to assess strength of association. On the Day, absolute numbers of G-MDSCs and M-MDSCs correlated significantly with Gram-positive sepsis. That positive correlation is shown in Table 5 and Figure 2.

On the Day 5, there were significantly positive correlations between all investigated variables and Gram-positive sepsis (Table 6).

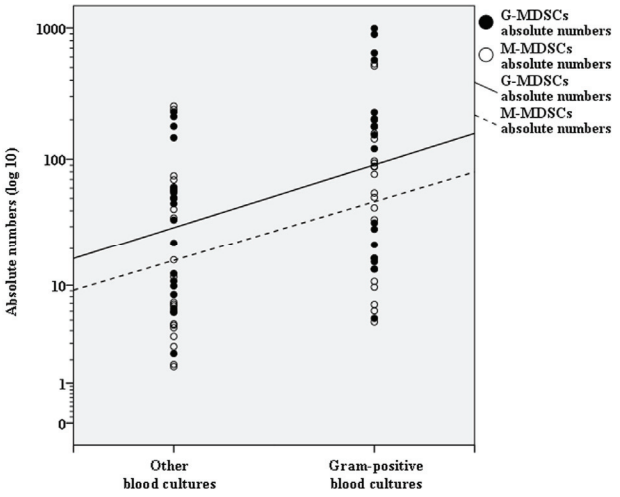

Fig. 2 – Scattergram on log_{10} scales of G-MDSCs and M-MDSCs absolute numbers vs. blood cultures in patients with secondary sepsis on the Day 1.

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

Table 4

Independent predictor of Gram-positive sepsis in the patient population by multivariate logistic regression analysis on the Day 5

Variables	Standard β value	OR	95% CI		*p*
			lower bound	upper bound	
M-MDSCs absolute number	0.012	1.012	0.999	1.026	0.035

β – standardized regression coefficient; MDSCs – myeloid-derived suppressor cells; M – monocitic; OR – odds ratio; CI – confidence interval.

Table 5

Spearman's rho correlations between variables and Gram-positive sepsis in the patient population on the Day 1

Variables	G-MDSCs frequencies	G-MDSCs absolute number	M-MDSCs frequencies	M-MDSCs absolute number
Gram positive blood culture	0.185; *p* = 0.261	0.328; *p* = 0.040	0.258; *p* = 0.113	0.378; *p* = 0.018
G-MDSCs frequencies		0.854; *p* = 0.000	-0.152; *p* = 0.356	-0.221; *p* = 0.177
G-MDSCs absolute number			0.043; *p* = 0.797	0.154; *p* = 0.350
M-MDSCs frequencies				0.866; *p* = 0.000

MDSC – myeloid-derived suppressor cells; G-granulocytic; M – monocitic.

Table 6

Spearman's rho correlations between variables and Gram-positive sepsis in the patient population on the Day 5

Variables	G-MDSCs frequencies	G-MDSCs absolute number	M-MDSCs frequencies	M-MDSCs absolute number
Gram positive blood culture	0.401; *p* = 0.013	0.428; *p* = 0.007	0.440; *p* = 0.006	0.466; *p* = 0.003
G-MDSCs frequencies		0.818; *p* = 0.000	0.484; *p* = 0.002	0.389; *p* = 0.016
G-MDSCs absolute number			0.663; *p* = 0.000	0.749; *p* = 0.000
M-MDSCs frequencies				0.899; *p* = 0.000

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

Positive correlations between G-MDSCs and M-MDSCs frequencies and Gram-positive sepsis are shown in Figure 3.

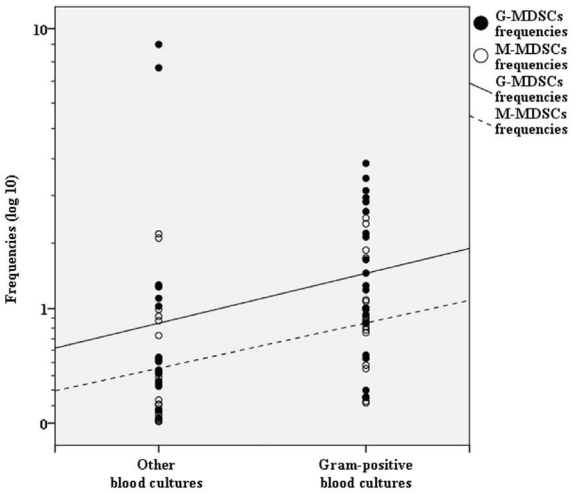

Fig. 3 – Scattergram on log_{10} scales of G-MDSCs and M-MDSCs frequencies vs. blood cultures in patients with secondary sepsis on the Day 5.

MDSC – myeloid-derived suppressor cells; G – granulocytic; M – monocitic.

Neutrophil (Ne) to G-MDSCs ratio and monocyte (Mo) to M-MDSCs ratio

Baseline characteristics of patient population regarding Ne/G-MDSCs and Mo/M-MDSCs according to nature of bacteremia on the Day 1 and Day 5 are shown in Table 7.

Ne/G-MDSCs and Mo/M-MDSCs ratios were lowest in critically ill patients with Gram-positive bacteremia both on the Day 1 and Day 5. On the first day, that difference did not reach statistical significance, but on the Day 5 both ratios were statistically significantly lower in patients with Gram-positive bacteremia compared to patients with Gram-negative or polymicrobial blood culture (Ne/G-MDSCs: χ^2 = 6.806, $p < 0.05$; Mo/M-MDSCs: $\chi^2 = 9.070$, $p < 0.01$).

Post hoc Mann-Whitney test revealed that on the Day 5 Mo/M-MDSCs ratio was significantly lower in patients with Gram-positive compared to Gram-negative blood culture (Z = -2.389; $p < 0.05$) Also, patients with Gram-positive blood culture had significantly lower both Ne/G-MDSCs and Mo/M-MDSCs ratios compared to patients with polymicrobial blood culture (Ne/G-MDSCs: Z = -2.781, $p < 0.01$; Mo/M-MDSCs: Z = -2.493, $p < 0.01$).

Also, levels of Ne/G-MDSCs were significantly lower in nonsurvivors, both on the Day 1 (Z = -1.921; $p < 0.05$) and the Day 5 (Z = -1.815; $p < 0.05$).

Table 7

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

Parameter	Gram-positive bacteremia (n = 20) mean ± SD, M (min-max)	Gram-negative bacteremia (n = 8) mean ± SD, M (min-max)	Polymicrobial bacteremia (n = 10) mean ± SD, M (min-max)
Neutrophils × 10^6/L			
Day 1	11,743.33 ± 8,567.86	11,701.25 ± 8,265.44	9,909.09 ± 4,823.16
	9,410 (1,390–28,600)	8,700 (1,960–22,800)	10,300 (1500–18300)
Day 5	12,893.33 ± 8,141.56	6,810.00 ± 3,868.92	9,585.00 ± 5,160.65
	14,500 (1,610–26,000)	6,265 (2,110–14,800)	7,960 (4,500–21,800)
Neutrophil to G-MDSC ratio			
Day 1	481.05 ± 1,039.91	863.99 ± 1,502.29	465.90 ± 818.14
	61.32 (5.55–4655.64)	375.70 (33.24–4,553.19)	229.67 (39.72–2,909.38)
Day 5	128.10 ± 250.63	143.12 ± 139.56	589.28 ± 890.21
	37.57 (9.60–1070.24)	91.52 (9.65–336.13)	217.10 (27.71–2625.67)
Monocytes × 10^9/L			
Day 1	718.09 ± 706.23	566.50 ± 341.08	533.63 ± 346.21
	600 (43–2,610)	510 (100–1090)	537 (50–1,120)
Day 5	801.00 ± 742.35	430.25 ± 189.21	699.80 ± 449.03
	697 (43–3,460)	411 (170–670)	525 (310–1,810)
Monocyte to M-MDSC ratio			
Day 1	16.02 ± 23.86	93.98 ± 128.28	47.59 ± 62.44
	8.38 (0,60–114.09)	12.72 (5.96–347.72)	16.09 (2.24–209.17)
Day 5	9.25 ± 10.64	134.07 ± 237.85	133.29 ± 222.34
	6.54 (0.65 – 36.47)	23.80 (2.32–671.14)	37.95 (4.03–702.78)

MDSCs – myeloid-derived suppressor cells; G-granulocytic; M – monocitic.
SD – standard deviation; M – median; min – minimum; max – maximum.

Clinical accuracy of Ne/G-MDSCs and Mo/M-MDSCs ratios in predicting nature of bacteremia and outcome

Clinical accuracy of Ne/G-MDSCs and Mo/M-MDSCs ratios in predicting nature of bacteremia and outcome was investigated. Discriminative power of both Ne/G-MDSCs and Mo/M-MDSCs ratios in predicting Gram-positive blood culture was statistically significant both on the Day 1 and Day 5. Results are shown in Table 8 and Figures 4 and 5.

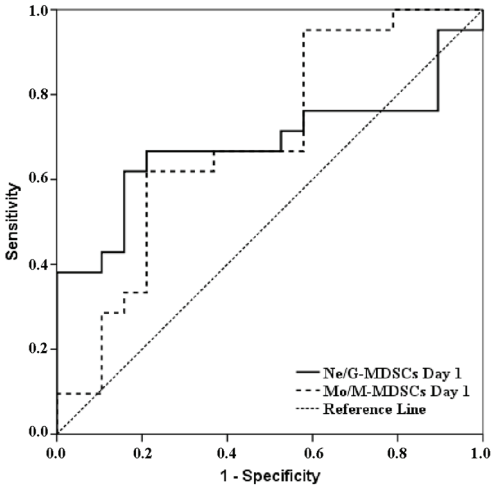

Fig. 4 – Receiver operating characteristic (ROC) curve for Ne/G-MDSCs and Mo/M-MDSCs on the Day 1 (Gram-positive blood culture).

MDSCs –myeloid-derived suppressor cells; G – granulocytic; M – monocytic; Ne – neutrophil; MO – monocyte;

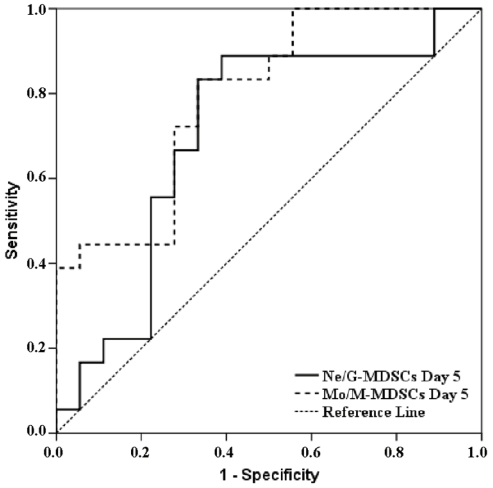

Fig. 5 – Receiver operating characteristic (ROC) curve for Ne/G-MDSCs and Mo/M-MDSCs on the Day 5 (Gram-positive blood culture).

MDSCs –myeloid-derived suppressor cells; G – granulocytic; M – monocytic; Ne – neutrophil; MO – monocyte.

Ne/G-MDSCs and Mo/M-MDSCs ratios lower than cut-off values were moderate predictors of Gram-positive blood culture both on the Day 1 and Day 5 in critically ill patients with secondary sepsis.

Clinical accuracy of Ne/G-MDSCs and Mo/M-MDSCs ratios in predicting polymicrobial blood culture was statistically significant on the Day 5 (Table 9 and Figure 6).

Table 8

Clinical accuracy of Ne/G-MDSCs and Mo/M-MDSCs ratios in predicting Gram-positive blood culture in patients with secondary sepsis on the Day 1 and Day 5

Parameter	AUC ROC	*p*	95% CI		Cut-off value	Sensitivity (%)	Specificity (%)	Youden index
			lower bound	upper bound				
Day1								
Ne/G-MDSCs	0.684	< 0.05	0.510	0.858	121.86	61.9	84.2	0.46
Mo/M-MDSCs	0.692	< 0.05	0.524	0.860	9.98	61.9	78.9	0.41
Day 5								
Ne/G-MDSCs	0.707	< 0.05	0.527	0.887	185.53	88.9	61.1	0.50
Mo/M-MDSCs	0.793	< 0.01	0.648	0.939	13.69	83.3	66.7	0.50

MDSCs – myeloid-derived suppressor cells; G – granulocytic; M – monocitic; Ne – neutrophil; Mo – monocyte; AUC – area under curve; ROC – reciever operating characteristic; CI – confidence interval.

Table 9

Clinical accuracy of Ne/G-MDSCs and Mo/M-MDSCs ratios in predicting polymicrobial blood culture in patients with secondary sepsis on the Day 5

Parameter	AUC ROC	*p*	95% CI		Cut-off value	Sensitivity (%)	Specificity (%)	Youden index
			lower bound	upper bound				
Ne/G-MDSCs Day 5	0.773	< 0.01	0.606	0.940	185.53	80.0	81.0	0.61
Mo/M-MDSCs Day 5	0.719	< 0.05	0.533	0.906	45.64	50.0	92.3	0.42

MDSCs – myeloid-derived suppressor cells; G – granulocytic; M – monocitic; Ne – neutrophil; Mo – monocyte; AUC – area under curve; ROC – reciever operating characteristic; CI – confidence interval.

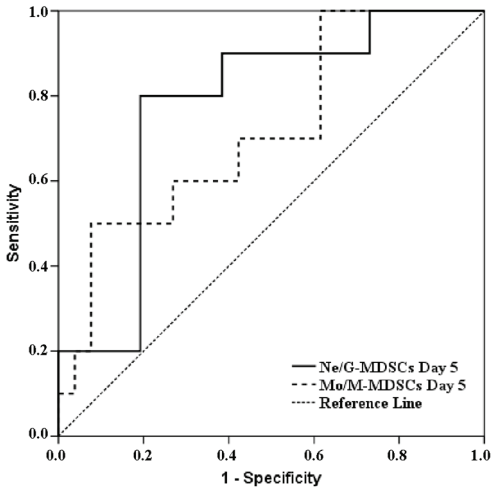

Fig. 6 – Receiver operating characteristic curve for Ne/G-MDSCs and Mo/M-MDSCs on the Day 5 (polymicrobial blood culture).

MDSCs –myeloid-derived suppressor cells; G – granulocytic; M – monocytic; Ne – neutrophil; MO – monocyte.

Ne/G-MDSCs and Mo/M-MDSCs ratios higher than respective cut-off values were predictors of polymicrobial blood culture on the Day 5 in critically ill patients with secondary sepsis. Ne/G-MDSCs ratio has very good discriminative power while Mo/M-MDSCs ratio has moderate one.

Clinical accuracy of both ratios in predicting lethal outcome was investigated. Ne/G-MDSCs ratio lower than cut-off value both on the Day 1 and Day 5 was moderate predictor of lethal outcome in this patient population. Discriminative power of Mo/M-MDSCs regarding outcome was not significant. Results are shown in Table 10 and Figure 7.

Underlying causes of secondary sepsis might not seem to influence the MDSCs accumulation

The underlying causes of secondary sepsis in examined patients were pancreatitis, peritonitis and trauma. The patients with different underlying causes of sepsis were perceived as separated groups and frequencies and absolute numbers of their G-MDSCs and M-MDSCs were compared. There were no statistically significant differences among these three subgroups neither on the Day 1 nor on the Day 5. So, MDSCs expansion was related to secondary infection regardless of nature of primary insult (pancreatitis, peritonitis, trauma).

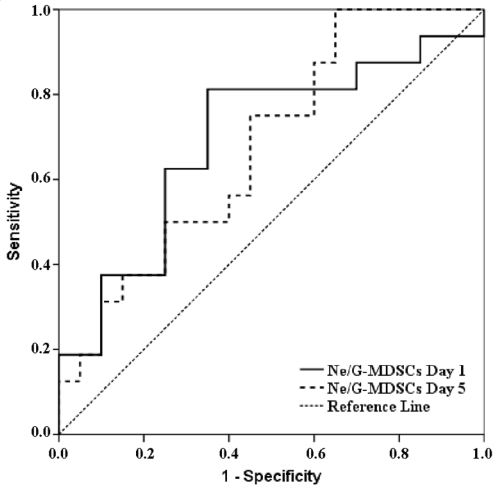

Fig. 7 – Receiver operating characteristic curve for Ne/G-MDSCs on the Day 1 and Day 5 and lethal outcome in patients with secondary sepsis

MDSCs –myeloid-derived suppressor cells; G – granulocytic; M – monocytic; Ne – neutrophil; MO – monocyte.

Discussion

Immune dysfunction is common in critically ill patients and it may modulate immune response and affect patient morbidity and mortality, particularly in severe trauma and/or sepsis. Immune cells' and mediators' role in immune response in critical illness is not yet fully elucidated [10, 11].

Expansion and activation of MDSCs, as part of immune response, are under the influence of several different factors, including infectious agents [12–14]. It seems that there is a difference between Gram-positive and Gram-negative sepsis regarding cytokine profile, for instance [1, 15]. It has also been shown that different types of microbes can induce specific subsets of MDSCs, with different impact on disease outcome [14].

A study by Janols et al. [16] showed predominant accumulation of $CD14^{low}$ polymorphonuclear MDSCs in patients with Gram-positive sepsis and septic shock. They also showed that the $CD14^{low}$ polymorphonuclear MDSCs accumulate in both, Gram-negative and Gram-positive sepsis, but are significantly more potent suppressors of T-cell proliferation when isolated from Gram-positive sepsis patients [16].

Table 10

Clinical accuracy of Ne/G-MDSCs ratio in predicting lethal outcome in patients with secondary sepsis on the Day 1 and Day 5

Parameter	AUC ROC	*p*	95% CI		Cut-off value	Sensitivity (%)	Specificity (%)	Youden index
			lower bound	upper bound				
Ne/G-MDSCs Day 1	0.694	< 0.05	0.513	0.875	241.53	81.3	65.0	0.46
Ne/G-MDSCs Day 5	0.678	< 0.05	0.504	0.853	262.90	100.0	35.0	0.35

MDSCs – myeloid-derived suppressor cells; G – granulocytic; M – monocitic; Ne – neutrophil; Mo – monocyte; AUC – area under curve; ROC – reciever operating characteristic; CI – confidence interval.

The findings of Janols et al. [16] suggest that different types of bacteria can influence myeloid response of the septic host, and accordingly, generation of specific MDSCs subset with possible distinct functions. In our study, we found significantly higher frequencies of both detected MDSCs subpopulations, G-MDSCs and M-MDSCs, in patients with Gram-positive sepsis when compared with Polymicrobial sepsis patients on the Day 5. Also, patients with Gram-positive sepsis had significantly more both G-MDSCs and M-MDSCs (absolute number) than patients with Gram-negative sepsis ($p < 0.05$). Stepwise multivariate logistic regression analyses of variables on the Day 5 determined that M-MDSCs absolute number was independent predictor of Gram-positive sepsis. Positive correlations between G-MDSCs and M-MDSCs frequencies and Gram-positive sepsis are confirmed by the Spearman's rho test. Possible explanation of these differences may lie in the basic understanding of MDSCs expansion seen in malignant diseases and protracted infections [12, 17]. Prompt reaction of the bone marrow in response to Gram-negative, and possible to polymicrobial causative infectious agents, may leave no time for different proinflammatory factors to act on myeloid precursors in different stages of maturation and to activate/convert them into immunosuppressive cells. On the contrary, more indolent, in terms of an acute inflammatory response, Gram-positive infectious agents could lead to prolonged bone marrow exposure, creating the environment conducive for MDSCs accumulation [18]. In addition, our finding that there were no significant differences in MDSCs accumulation between patients with different underlying causes of secondary sepsis (pancreatitis, peritonitis or trauma injury as primary insults) also speaks in favor of the causative infectious agent being more important for MDSCs generation than the type of primary insult leading to secondary sepsis.

Uhel et al. [19] performed peripheral blood transcriptomic analysis on 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. In sepsis patients they demonstrated up-regulation of gene profile associated with MDSCs regrutation and immunosuppression (MMP8, MMP9, ARG1, S100A8, S100A9, S100A12, PD-L1, IL-4R, and IL-10), but down-regulation of gene profile associated with inflammatory response (CD4, CD20, CD8, CD3, IL-8 and IL-6). They concluded that $CD14^{+}HLA\text{-}DR^{low/-}$ M-MDSCs and $CD15^{+}$ G-MDSCs strongly contributed to T-cell dysfunction in patients with sepsis. Contrary to our results, they found no association with Gram-staining of the causative organism. Interestingly, they also demonstrated that expression of two, among key MDSCs parameters, ARG1 and S100A9, significantly directly correlated to granulocyte count and inversely correlated to number of lymphocytes. Furthermore, Uhel et al. [19] showed that beside MDSCs, $CD14^{+}$ monocytes and $CD15^{+}$ low density granulocytes from sepsis patients were suppressive *in vitro*, similarly to MDSCs. They also showed that population of low density granulocytes is very heterogenous, being composed of immature and mature granulocytes both expressing degranulation markers.

In other words, beside MDSCs, mature monocytes and granulocytes of investigated patients demonstrated function and phenotype alterations. These findings are hard to explain from the aspect where MDSCs increment is a consequence of emergency myelopoesis followed by export of immature myeloid cells from bone marrow into blood stream. But, several recent articles pointed out that MDSCs increase could be achieved by reprogramming of existing monocytes, arguing that monocyte to M-MDSCs relation is very dynamic and plastic [4, 20]. Of course, both mechanisms could be operative at the same time, they are not mutually exclusive. According to this, we have analyzed ratio of monocytes to M-MDSCs and neutrophils to G-MDSCs, in every individual patient and in both time points. All sepsis patients from our study demonstrated decrement of Ne/G-MDSCs ratio and increment of Mo/M-MDSCs ratio from the 1th to 5th day. But, stratification of patients according to the type of microbial culture demonstrated significant differences. Patients with Gram-positive sepsis demonstrated significant decrement of Ne/G-MDSCs ratio and less prominent decrement of Mo/M-MDSCs ratio from the 1th to 5th day. Although Gram-negative sepsis patients also demonstrated significant Ne/G-MDSCs ratio decrement, the number of their monocytes increased comparing to detected number of M-MDSCs. Contrary to both previous groups, sepsis patients with polymicrobial cultures on the Day 5 demonstrated increase of both Ne/G-MDSCs and Mo/M-MDSCs ratios. All these indicate that type of microbial infection in sepsis is significantly associated with particular profile of MDSCs, dynamic of their change and their relation to mature – like counterpart cells. Finally, in our investigation, decrease of Ne/G-MDSCs ratio was associated with worse outcome, being significantly lower in nonsurvivors comparing to survivors.

Bergenfelz et al. [21] demonstrated that systemic M-MDSCs are generated from monocytes and that their number correlates with disease progression in breast cancer patients. Additionaly, they observed significant increase of monocytes with altered phenotype both in breast cancer group as well as in control sepsis group. These monocytes exhibited $CD14^{+}HLA^{-}DR^{low/-}$ phenotype, which is specific for M-MDSCs, and were already documented in few earlier studies in sepsis patients with compensatory antiinflammatory response syndrome [20, 22–25]. Gene profiling further delineated that these populations of monocytes/M-MDSC were similarly immunosuppressive in both breast cancer and sepsis patients, but not other infective diseases and healthy controls [21]. Monocytes from early breast cancer group produced comparable levels of IL-1β, IL-6, IL-8 and TNF as monocytes from metastatic group, indicating change of monocyte function early in the disease. Furthermore, sepsis patients had significantly more total $CD14^{+}$ cells, $CD14^{+}CD16^{-}$ cells, $CD14^{++}CD16^{+}$ intermediate monocytes and $CD14^{++}CD16^{++}$ nonclassical monocytes comparing to both early and metastatic breast cancer patients and healthy controls, with in-

creased $CD16^{+}/CD16^{-}$ monocyte ratio. Authors concluded that these Mo/M-MDSCs were induced early during the tumor growth and progression and that monocytes are affected by the tumor much before their extravasation into the tumor tissue. Based on observation of similar phenotypic and molecular findings in breast cancer and sepsis patients, we could assume that sepsis progression could reprogramme monocytes and granulocytes in the same way.

Reprogramming process is not a rare event and could have physiological implications. Zhao et al.[26] demonstrated that human trophoblast cells efficiently change differentiation programme in monocytes, inducing their maturation toward dendritic cells. Those trophoblast cells induced monocyte derived dendritic cells display altered, hypostimulatory capacity to T lymphocytes and induce generation of inhibitory regulatory T lymphocytes. Sepsis itself induces numerous changes in monocyte functions. Shalova et al.[27] demonstrated that sepsis patients' monocytes exert significant upregulation of genes associated with inflammation (IL-1b, IL-6, CCL3, CCL5), but also with tissue remodeling genes (VEGF, MMPs). Authors found that hypoxia inducible factor-1 (HIF-1a) was specifically upregulated in sepsis patients' monocytes but not in the control ones. HIF-1a negatively regulated Toll-like receptors (TLR) monocyte activation, resulting in diminished proinflammatory response to endotoxin challenge, so called endotoxin tolerance. Although that study did not investigate MDSCs, authors concluded that HIF-1a is important regulator of monocyte reprogramming toward immunosuppressive functions in sepsis patients.

The 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.

Conclusion

Gram-positive infectious agents were powerful inducers of MDSCs generation in sepsis. Also, underlying causes of secondary sepsis might not seem to influence the MDSCs accumulation. Larger trial is essential for possible confirmation of our findings.

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