ORIGINAL ARTICLE

Myeloid-derived suppressor cells in bronchoalveolar lavage fluid in patients with chronic obstructive pulmonary disease

Beata Brajer-Luftmann[1], Agata Nowicka[1], Mariusz Kaczmarek[2], Marcin Grabicki[1], Barbara Kuźnar-Kamińska[1], Barbara Bromińska[3], Jan Sikora[2], Halina Batura-Gabryel[1]

1 Department of Pulmonology, Allergology and Pulmonary Oncology, Poznan University of Medical Sciences, Poznań, Poland
2 Department of Clinical Immunology, Poznan University of Medical Sciences, Poznań, Poland
3 Department of Endocrinology, Metabolism and Internal Diseases Poznan University of Medical Sciences, Poznań, Poland

KEY WORDS

bronchoalveolar lavage fluid, chronic obstructive pulmonary disease, myeloid-derived suppressor cells, pulmonary function tests

ABSTRACT

INTRODUCTION Myeloid-derived suppressor cells (MDSCs) have the potent ability to suppress T-cell function, and are important in the regulation of chronic inflammation and carcinogenesis. MDSCs may influence local and systemic inflammation and carcinogenesis in COPD; however, their presence in bronchoalveolar lavage fluid (BALF) and peripheral blood (PB) or their relationship with clinical parameters in COPD has not been studied yet.

OBJECTIVES The aim of the study was to assess MDSCs in BALF and PB and to analyze the relationship between MDSCs and clinical parameters in COPD.

PATIENTS AND METHODS The study included 64 patients with stable COPD. The clinical parameters of the patients were studied, and MDSCs were assessed using monoclonal antibodies directly conjugated with fluorochromes in flow cytometry.

RESULTS The percentage of MDSCs in BALF was lower than that in PB (0.63 ±0.90 vs 3.94 ±0.38). In BALF, MDSCs (% of mononuclear cells) correlated with forced expiratory volume in 1 second ($r_s = -0.30$, $P = 0.0185$), residual volume/total lung capacity ($r_s = 0.32$, $P = 0.0148$), PaO_2 ($r_s = -0.45$, $P = 0.0002$), arterial oxygen saturation (SaO_2; $r_s = -0.41$, $P = 0.0008$), and diffusion capacity of carbon dioxide ($r_s = -0.32$, $P = 0.0211$). There was a significant negative correlation between MDSCs (% of all leukocytes) and arterial oxygen pressure ($r_s = -0.42$, $P = 0.0006$) and SaO_2 ($r_s = -0.37$, $P = 0.0027$). No correlations were found in PB.

CONCLUSIONS MDSCs are present in human lung microenvironment and may be involved in local inflammation in COPD. Future studies should focus on a detailed assessment of MDSCs in local and systemic inflammation in COPD.

Correspondence to:
Beata Brajer-Luftmann, MD, PhD,
Katedra i Klinika Pulmonologii,
Alergologii i Onkologii Pulmonologicznej,
Uniwersytet Medyczny w Poznaniu,
ul. Szamarzewskiego 84,
60-569 Poznań, Poland,
phone: +48 61 841 70 61,
e-mail: bbrajer@ump.edu.pl
Received: November 29, 2016.
Revision accepted:
December 15, 2016.
Published online:
December 15, 2016.
Conflict of interest: none declared.
Pol Arch Med Wewn. 2016;
126 (12): 980-98888
doi:10.20452/pamw.3718

INTRODUCTION Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the world. The COPD burden is projected to increase in the next decades because of population aging and continued exposure to risk factors for COPD.[1] Inflammatory process and exposure to various environmental factors remain crucial in the pathogenesis of this disease.[2] COPD is characterized by a specific pattern of inflammation involving increased number of $CD8^+$ (cytotoxic) Tc1 lymphocytes. These cells with neutrophil and macrophages release inflammatory mediators and enzymes and interact with structural cells in the airways, lung parenchyma, and pulmonary vasculature. The wide variety of inflammatory mediators that have been shown to be increased in patients with COPD attract the inflammatory cells from the circulation (chemotactic factors) and amplify inflammatory process (proinflammatory cytokines).[1]

Myeloid-derived suppressor cells (MSDCs) are one of the major factors negatively regulating immune responses in cancer.[3] In mice these cells are defined as $Gr\text{-}1^+CD11b^+$ cells, comprising

TABLE 1 Characteristics of the study population

Parameter	n	Mean ± SEM	Min	Max
age, y	64	67.00 ±1.00	49	84
FEV_1, % pred.	64	47.69 ±2.30	19.2	93.2
FVC, % pred.	64	67.85 ±2.30	37.3	112.1
RV, % pred.	59	211.49 ±9.17	28.4	423.8
TLC, % pred.	59	121.65 ±2.93	67.2	188.5
RV/TLC, %	59	65.38 ±1.57	16.12	87.41
DL_{CO}, %	52	44.27 ±2.72	15.5	94.5
SaO_2, %	64	92.97 ±0.63	82.5	97.5
PaO_2, mmHg	64	66.53 ±1.22	49.2	87.5
BMI, kg/m^2	64	26.16 ±0.72	15.6	39.0
6MWT, m	59	317.63 ±16.77	60	545
BODE, 0–10	59	4.24 ±0.35	0	9
smoking, pack years	64	35.7 ±2.40	4	98
CRP, mg/l	64	5.12 ±0.91	0,5	12

Abbreviations: 6MWT, 6-minute walk test; BMI, body mass index; BODE index, B – BMI, O – obstruction, D – dyspnea, E – exercise; CRP, C-reactive protein; DL_{CO}, diffusion capacity of carbon dioxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; mMRC, modified British Medical Research Council; PaO_2, arterial oxygen pressure; RV, residual volume; SaO_2, arterial oxygen saturation; TLC, total lung capacity

TABLE 2 Specification of antibodies used in flow cytometry

Name	Format	Isotype	Clone
CD11b	APC	IgG1, κ	ICRF44
CD33	PE-Cy™7	IgG1, κ	P67.6
CD45	APC-Cy™7	IgG1, κ	2D1
HLA-DR	PerCP	IgG2, κ	L243
antihuman lineage cocktail 1 (Lin 1), (CD3, CD14, CD16, CD19, CD20, CD56)	FITC	IgG_1, κ IgG2b, κ	MφP9, NCAM16.2, 3G8, L27, SJ25C1, SK7

Source: BD Biosciences, San Jose, California, United States

pathologically activated $CD11b^+Ly6CloLy6G^+$ immature granulocytes, $D11b^+Ly6ChiLy6G^-$ monocytes, and a small proportion of myeloid precursors. In humans predominantly, the immunophenotype of MDSCs is most commonly defined as $CD14^-CD11b^+$ or cells expressing a common myeloid cells marker, $CD33^+$, but not expressing the mature myeloid cell marker, the MHC class II molecules, HLA-DR, also markers of lymphoid cells (Lin-1: CD3, CD14, CD16, CD19, CD20, and CD56).[3] The main characteristic of these cells is their potent ability to suppress T-cell function. Inhibition of T-cell function by MDSCs may be mediated by the induction of regulatory T cells (T_{regs}) or by anti-inflammatory cytokines such as transforming growth factor β and interleukin 10. It may also involve the metabolism of L-arginine by arginase 1 or inducible nitric oxide synthase.[3-6] In addition, MDSCs promote tumor progression through a number of different immunological mechanisms.[6-8] Because of the ability of MDSCs to suppress both adaptive and innate immunities mainly through direct inhibition of cytotoxic functions of T cells and natural killer cells, they play pivotal role in cancer development. MDSCs facilitate cancer cell invasion and intravasation by secreting multiple proteolytic enzymes, including matrix metalloproteinases, which are necessary for extracellular matrix degradation and disruption of endothelial cadherins, adhesion proteins, or the basement membrane of vessels. Epithelial-mesenchymal transition (EMT) is one of the steps for dissemination of cancer cells. When cancer cells undergo EMT, they lose epithelial markers and gain mesenchymal phenotypes. Granulocyte-like MDSCs induce EMT in cancer cells using transforming growth factor β, epidermal growth factor, and hepatocyte growth factor. On the other hand, MDSCs also contribute to mesenchymal-epithelial transition of cancer cells by secreting versican. This function of MDSCs supports cancer cells in colonizing at metastatic niche.[9] In summary, MDSCs are implicated in human pathological conditions including some cancers, inflammatory diseases, and also lung diseases.[10-13]

It is known that smoking, the main risk factor for COPD, upregulates and activates circulating MDSCs in patients with COPD but not in smokers with normal lung function.[14] In patients with COPD, the activation of MDSCs is accompanied by downregulation of the T-cell receptor ζ chain expression.[14] In addition, MDSCs were shown to be elevated in the bone marrow, spleen, and lungs after a 4-month exposure to cigarette smoke, while this was paralleled by a decreased number of pulmonary dendritic cells.[15] However, these phenotypic MDSCs lacked immune suppressive activity, and thus were not bona fide MDSCs.[13,15] In a further study, blood MDSC levels were also increased in patients with COPD and correlated with elevated levels of T_{regs}, which is in agreement with studies that suggested reciprocal control of these 2 cell types.[16] In summary, these studies suggest that the accumulation of MDSCs in patients with COPD may underlie the blunted immune response observed in this disease.[13,16]

The occurrence of circulating MDSCs in inflammatory process in COPD has been relatively well documented, but its clinical meaning in COPD has not been determined yet. There have been a few reports on the prevalence of these cells in systemic inflammatory process in COPD,[2,14,16,17] and only one study on the prevalence of MDSCs in the lung microenviroment has been published.[18]

The aims of this study were to confirm the presence of MDSCs in peripheral blood (PB) and lung environment using bronchoalveolar lavage (BAL) and to determine their relationship with clinical parameters in patients with COPD.

PATIENTS AND METHODS

Study population

The study was conducted in the Department of Pulmonology, Allergology and Pulmonary Oncology, Poznan University of Medical Sciences, Poznań Poland. It included 64 patients (49 men, 15 women) diagnosed with COPD according to the 2010 criteria of the Global Initiative for Lung Disease

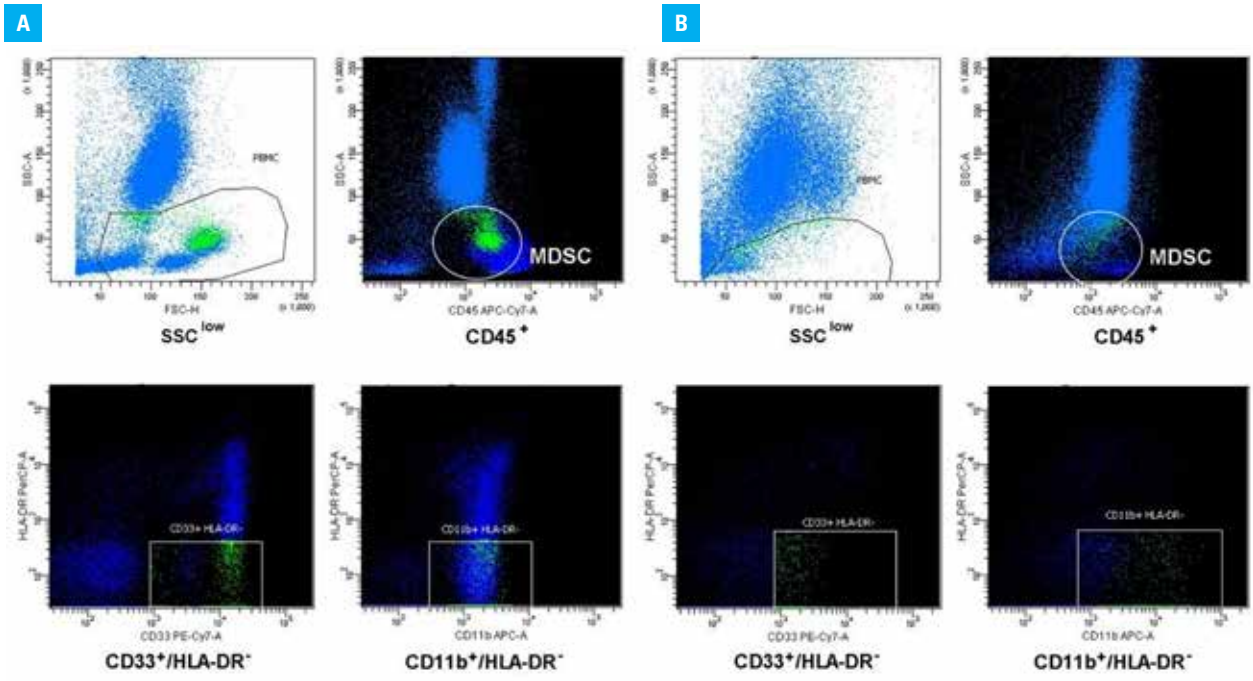


FIGURE 1 Assessment of myeloid-derived suppressor cell population in: **A** – myeloid-derived suppressor cell population in whole peripheral blood samples; **B** – bronchoalveolar lavage fluid

(GOLD),[1] who had a relevant history confirmed with a postbronchodilator ratio of forced expiratory volume in 1 second to forced vital capacity (FEV_1/FVC) of less than 0.7. All patients were older than 40 years and were current (23 patients) or former smokers (41 patients). Patients with other pulmonary disorders, such as asthma, tuberculosis, pulmonary thromboembolism, or interstitial pulmonary lesions, and with contraindications to fiberoptic bronchoscopy or pulmonary function tests, were excluded. A chest computed tomography was performed in all patients to identify individuals with coexisting lung cancer. No lesions suggesting malignancy were visualized, so only patients with COPD and without coexisting lung cancer were enrolled to the study.

All participants gave written informed consent to take part in the study, and approval from the Ethics Committee at the Poznan University of Medical Sciences was obtained. The characteristics of the study population are presented in TABLE 1.

Pulmonary function tests Spirometry and body plethysmography were performed and the diffusion capacity of carbon dioxide (DL_{CO}) was measured by an experienced technician using a Master Screen Body/Diffusion Jaeger device (Erich Jaeger GmbH, Hoechberg, Germany). Spirometry was performed 15 to 30 minutes after the inhalation of a short-acting bronchodilator. The results were shown as % predicted. The airflow limitation was defined as postbronchodilator FEV_1/FVC ratio of less than 0.70. The severity of airflow limitation was divided into 4 grades (1, mild; 2, moderate; 3, severe; and 4, very severe).[1] The BODE index (B, BMI; O, obstruction; D, dyspnea; and E, exercise) was calculated.[19] Dyspnea was assessed using the Modified British Medical Research Council (mMRC) Questionnaire. Two patients reported a score of 0; 13 patients, a score of 1; 18 patients, a score of 2; 26 patients, a score of 3; and 5 patients, a score of 4 in the mMRC scale. Exercise capacity was measured using the distance achieved in a 6-minute walk test (6MWT).[20] An arterial blood gas analysis was performed. Arterial blood samples (1 ml) were obtained from the patients' radial artery, after 5-minute rest in the sitting position.

Blood samples Peripheral blood samples were collected into EDTA tubes (9 ml) for cytometric immunophenotyping, while blood samples collected into tube without anticoagulant (9 ml) were centrifuged at 2500 rpm for 10 minutes at 4°C. The obtained serum samples were frozen immediately at –70°C for subsequent investigations.

Fiberoptic bronchoscopy and bronchoalveolar lavage Only the patients undergoing routine fiberoptic bronchoscopy for diagnostic purposes were enrolled to the study. BAL fluid (BALF) samples were collected according to international guidelines.[21,22] Topical lignocaine and intravenous fentanyl and propophol anesthesia were used. A special effort was made to use as low dose of lignocaine as possible.[23,24] The bronchoscope was wedged in the segmental or subsegmental bronchus of the middle lobe. The bronchus was lavaged with 50-ml aliquots of sterile saline solution at a temperature of 37°C, and then the fluid was aspirated. Two further 50-ml aliquots of saline solution were instilled and aspirated in the same way.[22,23,25-28]

Immunophenotypic assessment Fresh unfixed cells from BALF and PB were immunophenotyped using a flow cytometer. The evaluation of antigenic determinants characteristic for MDSC populations was performed using monoclonal antibodies directly conjugated with fluorochromes

TABLE 3 Percentage of myeloid-derived suppressor cells among all leukocytes and among mononuclear cells in bronchoalveolar lavage fluid and peripheral blood

Percentage	n	Mean ±SEM	Min	Max
BALF MDSCs, %	64	0.63 ±0.90	0.04	3.70
BALF MDSCs, % of MC	64	5.03 ±0.76	0.12	38.3
blood MDSCs, %	62	3.94 ±0.38	0.58	16.00
blood MDSCs, % of MC	62	11.94 ±0.90	2.27	36.40

Abbreviations: BALF, bronchoalveolar lavage fluid; MCs; mononuclear cells; MDSCs myeloid-derived suppressor cells

(TABLE 2). MDSCs were defined as cells with immunophenotype SSC^{low} /$CD45^{+}$/HLA-$DR^{-/low}$/$CD11^{+}$/$CD33^{++}$/Lin-1^{-} (FIGURE 1). The samples for analysis were prepared using flow cytometry in the following manner: briefly, initial preparation of BALF samples comprised sterile filtration to remove any mucus, blood clots, and tissue fragments, and concentration of the cells through centrifugation for 10 minutes at 1800 rpm. Resultant supernatants were removed, frozen, and allocated for the evaluation of the concentration of the soluble mediators in further analyses. Antibodies were added to the cell pellets from BALF as well as to PB samples: 5 µl of each antibody per $2 \times 10^5 - 1 \times 10^6$ of cells in a sample. Negative controls were samples without added antibodies. Cells were incubated with antibodies for 15 minutes in the dark. In the next step, erythrocyte residues were lysed using 2 ml of lysing solution (BD Biosciences, San Jose, California, United States). Lysis was stopped by adding phosphate buffer saline solution after 10 minutes. Finally, all lysed residues, morphotic particles, and soluble proteins were washed out by double centrifugation for 5 minutes at 1500 rpm. Appropriately stained cells were analyzed using a FACS Canto flow cytometer (BD Biosciences), and the results were processed using the FACS Diva software (BD Biosciences). Up to 50 000 events of each sample were collected. The percentage of positive cells was assessed.

Statistical analysis A statistical analysis was conducted using the Statistica 10.0 program (StatSoft, Inc., Tulsa, Oklahoma, United States). All parameters were checked for compatibility to the normal distribution using the Shapiro–Wilk test. A P value of less than 0.05 was considered significant. Results were shown as mean ± standard errors of the mean. To compare the percentage of MDSCs in PB and BALF, the Mann–Whitney test was used. To assess the presence of a relationship between the percentage of MDSCs in BALF and PB and the clinical parameters, the Pearson correlation coefficient was used.

RESULTS The study showed a significant difference between the percentages of MDSCs evaluated in PB and BALF. The percentage of MDSCs in BALF was lower than that in PB. The percentage of MDSCs among mononuclear cells (MCs) assessed in BALF was also lower than that in PB. The results are shown in TABLE 3 and Supplementary material online (*Figures S1* and *S2*).

In BALF, the percentage of MDSCs among MC correlated well with FEV_1 ($r_s = -0.30$, $P = 0.0185$), residual volume / total lung capacity (RV/TLC; $r_s = 0.32$, $P = 0.0148$) (FIGURE 2), GOLD stage ($r_s = 0.30$, $P = 0.0176$), arterial oxygen pressure (PaO_2; $r_s = -0.45$, $P = 0.0002$), arterial oxygen saturation (SaO_2; $r_s = -0.41$, $P = 0.0008$), and DL_{CO} ($r_s = -0.32$, $P = 0.0211$). Data are shown in FIGURE 3. No correlation was found between MDSCs (% of MCs) and age, distance achieved in 6MWT, mMRC, BODE index, smoking history, C-reactive protein levels, RV, or TLC. We revealed a significant negative correlation between the percentage of MDSCs among all leukocytes and PaO_2 ($r_s = -0.42$, $P = 0.0006$) and SaO_2 ($r_s = -0.37$, $P = 0.0027$) (FIGURE 4). The other parameters (FEV_1, TLC, RV, RV/TLC, GOLD stage, DL_{CO}, mMRC, age, BODE index, number of pack years, and 6MWT) did not correlate with the percentage of MDSCs in BALF.

In PB, no significant correlations were found between the percentage of MDSCs among all leukocytes or among MCs and all analyzed parameters.

The results are presented in TABLE 4.

DISCUSSION The presence of MDSCs was first described in animal models and patients with advanced stages of cancer, but was recently found also in patients with early cancer. By suppressing the protective immune response to malignant cells, they may promote the progression of the tumor and the development of metastasis.[29-32] However, there is strong evidence that these cells are also increased and play a regulatory role in the immune responses in bacterial and parasitic infections, acute and chronic inflammation, autoimmunity, traumatic stress, surgical sepsis, and transplantation.[33-35] MDSCs were shown to be an inherent part of chronic inflammation, so it was tempting to speculate that they may be directly involved in the chronic inflammatory process such as COPD.[13,35] There are only a few reports assessing the occurrence of circulating MDSCs in the blood of patients with COPD and only one in BALF.[18] For this reason, we attempted to assess the presence of MDSCs not only in blood but also in the bronchial tree, the site of local inflammatory process in COPD. It could be the reason why our study results were slightly different from the results of other researchers.

The most difficult task was to define the immunophenotype of the MDSCs. They are generated from a pool of myeloid progenitor cells that have failed the differentiation into mature cells, and for this reason do not express HLA-DR molecules. MDSCs express markers that are common for cells of myeloid origin. They are positive for CD11b and CD33, and negative for markers characteristic for lymphoid cells, which may be distinguished by use of antibodies Lin1 cocktail. More recently, particular subpopulations of MDSCs have been distinguished. The main division

FIGURE 2
Correlation between percentage of myeloid-derived suppressor cells (MDSCs) among mononuclear cells (MCs) in bronchoalveolar lavage fluid (BALF) and forced expiratory volume in 1 second (FEV_1) as well as residual volume to total lung capacity ratio (RV/TLC)

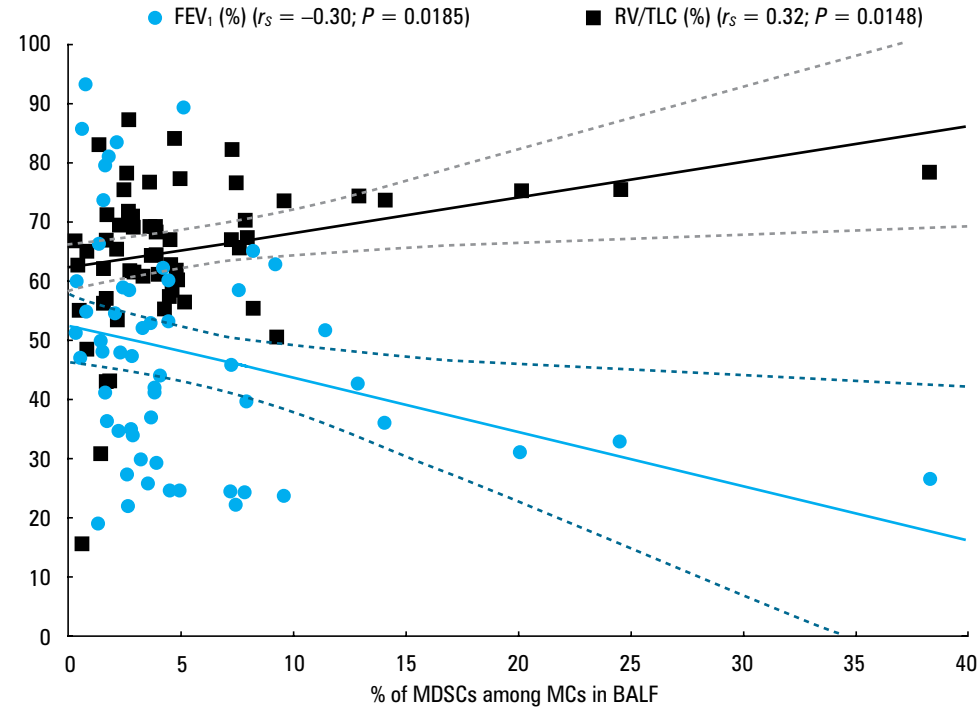
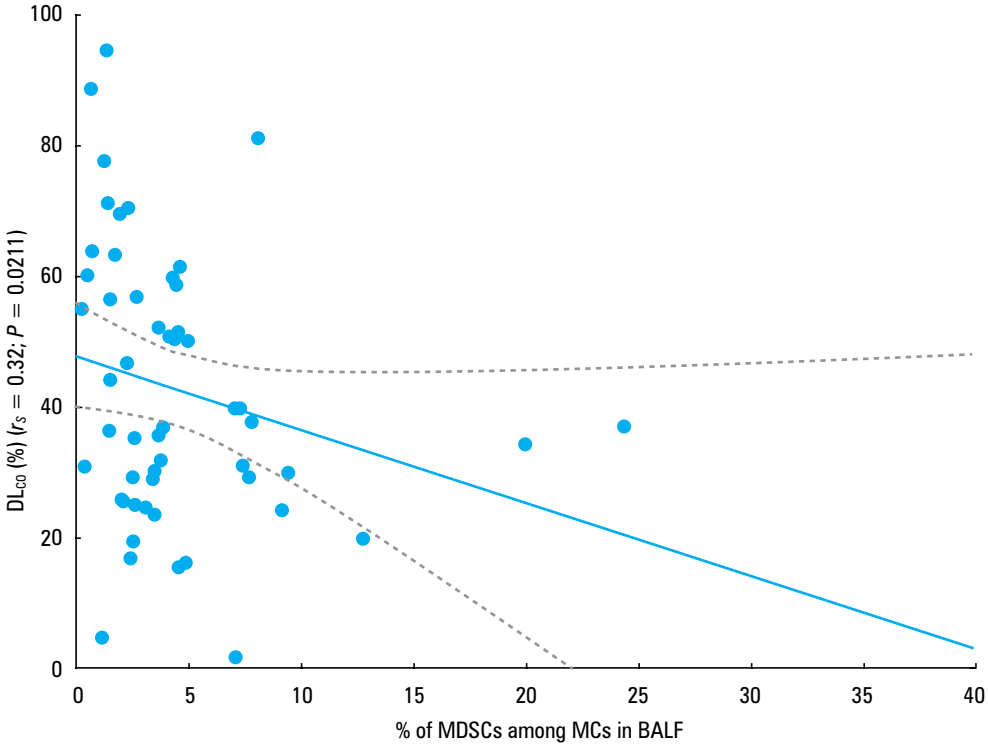


FIGURE 3
Correlation between percentage of myeloid-derived suppressor cells (MDSCs) among mononuclear cells (MCs) in bronchoalveolar lavage fluid (BALF) and diffusion capacity of carbon dioxide (DL_{CO})



indicates monocytic ($CD14^+/HLA\text{-}DR^-$) and granulocytic ($CD15^+/HLA\text{-}DR^-$) MDSCs. Such diversity generates a difficulty in clearly defining this cell population. To determine the full pool of this population, MDSCs were defined in the present study as $CD11b^+$ $CD33^+HLA\text{-}DR^-$ cells belonging to leukocytes ($CD45^+$) with low granularity (SSC^{low}).[36]

Scrimmini et al[14] assessed the proportion of circulating MDSCs in the blood of never-smokers, smokers with normal spirometry results, and patients with COPD. The proportion of circulating MDSCs in current smokers with normal lung function was significantly higher than that in former smokers, indicating that tobacco smoking is associated with an increased number of circulating MDSCs. Former smokers with COPD maintained similar proportions as current COPD smokers. The authors did not find any significant relationship between the proportion of circulating MDSCs and the severity of airflow limitation in patients with COPD. We also did not observe a significant correlation between the percentage of circulating MDSCs among all leukocytes and among MCs and the results of pulmonary function tests.

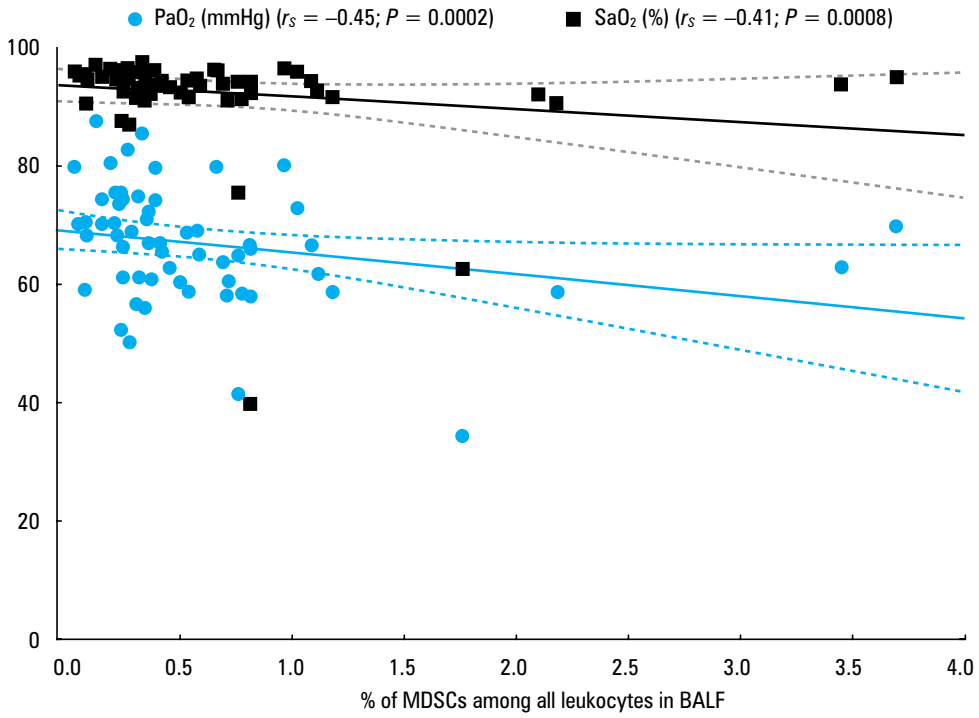


FIGURE 4
Correlation between percentage of myeloid--derived suppressor cells (MDSCs) among all leukocytes in bronchoalveolar lavage fluid (BALF) and arterial oxygen pressure (PaO_2) and arterial oxygen saturation (SaO_2)

TABLE 4 Correlations between myeloid-derived suppressor cells in bronchoalveolar lavage fluid and peripheral blood and study parameters

Parameter	BALF MDSCs, %	BALF MDSCs, % of MCs	Blood MDSCs, %	Blood MDSCs, % of MCs
FEV_1, %	−0.15; 0.2496	−0.30; 0.0185	−0.07; 0.6153	−0.19; 0.1378
FVC, %	−0.04; 0.7761	−0.22; 0.0852	−0.05; 0.7190	−0.18; 0.1693
RV, %	0.08; 0.5590	0.20; 0.1247	−0.11; 0.4361	−0.05; 0.6893
TLC, %	0.09; 0.4862	0.16; 0.2256	−0.02; 0.8690	−0.04; 0.7591
RV/TLC, %	0.08; 0.5383	0.32; 0.0148	−0.05; 0.7274	0.12; 0.3895
DL_{CO}, %	−0.08; 0.5811	−0.32; 0.0211	0.09; 0.5247	−0.18; 0.2189
PaO_2, mmHg	−0.42; 0.0006	−0.45; 0.0002	−0.13; 0.3257	−0.19; 0.1452
$PaCO_2$, mmHg	0.05; 0.7081	0.00; 0.9825	0.08; 0.5299	0.07; 0.6063
SaO_2, %	−0.37; 0.0027	−0.41; 0.0008	−0.10; 0.4380	−0.17; 0.1770
age, y	0.08; 0.5331	0.12; 0.3253	0.11; 0.3879	0.17; 0.1878
BMI, kg/m^2	0.03; 0.7861	−0.05; 0.7144	0.07; 0.6256	−0.08; 0.5555
CRP, mg/l	0.25; 0.0546	0.2; 0.1329	0.14; 0.2912	0.26; 0.0509
mMRC	0.08; 0.5114	0.07; 0.5858	0.11; 0.4126	0.20; 0.1143
BODE, 0–10	0.04; 0.7667	0.18; 0.1795	0.01; 0.9220	0.20; 0.1454
6MWT, m	−0.09; 0,4965	−0.21; 0.0428	0.04; 0.7818	−0.21; 0.1204
GOLD stage, 1–4	0.12; 0.3527	0.30; 0.0176	0.05; 0.7032	0.22; 0.0876
smoking, pack years	−0.14; 0.3093	−0.05; 0.7283	0.25; 0.0634	0.15; 0.2673

Data are presented as r_s; P.

Abbreviations: see TABLES 1 and 3

However, our research showed a significant correlation between MDSCs assessed in BALF and the severity of airflow limitation. One of the important findings of our study is that we did not find any correlation between smoking history and MDSCs. It may indicate that airway MDSCs may play a crucial role in local inflammation in COPD independently of cigarette smoking and may aggravate inflammation with the severity of bronchial obstruction.

On the other hand, our results are in line with those of Tan et al.[17] They assessed proportions of MDSCs in PBMCs isolated from patient with stable COPD, smokers with no evidence of COPD, and healthy nonsmokers. Patients with COPD showed increased systemic immune activation but the proportions of MDSCs were similar to those in controls. We also observed no correlation between circulating and airway MDSCs and smoking history. In the available literature, we found only

one study in which BALF was used to identify and characterize human airway MDSCs in patients with COPD. Deshane et al[18] assessed BALF from 8 patients with moderate COPD, 10 healthy individuals, and 9 patients with mild asthma. The authors found that the proportions and numbers of MDSC subsets and their different functional profiles discriminate patients with mild asthma from those with COPD, and both disease groups from healthy individuals. This suggests a critical role for this myeloid lineage cells in the pathogenesis of asthma and COPD. The authors did not assess the correlation between airway MDSCs, pulmonary function tests, and other clinical parameters.[18] In our study, we confirmed the presence of airway MDSCs and also found the relationship between the number of these cells and the degree of airway obstruction.

It is known that hypoxia is a common feature of solid tumors.[37] Hypoxic zones in tumors attract immunosuppressive cells such as MDSCs. Noman et al[38] proved that the tumor microenvironment plays a role in the regulation of PD-L1 surface expression on MDSCs. As hypoxia is one of the major components of tumor microenvironment, they tested the effect of hypoxia on the expression of immune checkpoint receptors (PD-1 and CTLA-4) and their respective ligands (PD-L1, PD-L2, CD80, and CD86) on MDSCs. Hypoxia dramatically and significantly increased the percentage of PD-L1$^+$ MDSCs isolated from the spleen in B16-F10 and LLC tumor-bearing mice.[38] Similar results were reported by Corzo et al.[39] Hypoxia via HIF-1α dramatically alters the function of MDSCs in the tumor microenvironment and redirects their differentiation toward tumor-associated macrophages, hence providing a link between different MDSCs in the tumor microenvironment. We did not identify any study concerning the relationship of hypoxia and MDSCs in humans, especially in patients with COPD. The results of our study only partially correlate with the results of the above authors. The percentage of MDSCs among lung MCs negatively correlated with airflow limitation parameters. The significant negative correlation with FEV_1, RV/TLC, and DL_{CO} may indirectly prove that local inflammatory process is connected also with airway obstruction. It may promote worse oxygenation. Our study results indicated a significant correlation between lung MDSCs and PaO_2 and SaO_2, but further studies explaining this relationship are needed.

In conclusion, there are still more questions than answers about MDSCs and their role in inflammatory process in COPD. The limitation of the current study is the absence of healthy control group. This study had a cross-sectional design. We concentrated on BALF collection and clinical parameters assessment only in patients with COPD. The results confirm that MDSCs occur in the lung microenvironment and PB in patients with COPD. The number of these cells may not be influenced by smoking history. It may suggest that these cells are involved in local inflammatory process in COPD, independently from smoking. Future studies are necessary and should focus on explaining the role of MDSCs in inflammatory process in COPD.

Contribution statement HG-B, AN, BB-L, MK, and JS designed the study. BB-L, AN, MK, and BB performed the research and collected data. BB-L, AN, MK, HG-B, JS, MG, and BK-K analyzed and interpreted the results. AN, BB-L, HG-B, and MK wrote the paper.

Acknowledgments The project received financial support from the National Science Centre, Poland (N N402 598 040; to HB-G). We would like to thank the participants for taking part in the study and Magdalena Lewandowska, MSc, for statistical analysis.

Supplementary material online Supplementary material is available with the online version of the article at www.pamw.pl.

REFERENCES

1 Global Initiative for Obstructive Lung Disease. Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease. 2010 update. www.goldcopd.org. 2010. Accessed January 18, 2011.

2 Vaguliene N, Zemaitis M, Lavinskiene S, et al. Local and systemic neutrophilic inflammation in patients with lung cancer and chronic obstructive pulmonary disease. BMC Immunol. 2013; 14-36.

3 Peranzoni E, Zilio S, Marigo I, et al. Myeloid-derived suppressor cell heterogeneity and subset definition. Curr Opin Immunol. 2010; 22: 238-244.

4 Srivastava MK, Zhu L, Harris-White M, et al. Myeloid suppressor cell depletion augments antitumor activity in lung cancer. PLoS One. 2012; 7: 40677.

5 Domagala-Kulawik J, Osinska I, Hoser G. Mechanisms of immune response regulation in lung cancer. Transl Lung Cancer Res. 2014; 3: 15-22.

6 Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. Nat Rev Cancer. 2013; 13: 739-752.

7 Lindau D, Gielen P, Kroesen M, et al. The immunosuppressive tumour network: Myeloid-derived suppressor cells, regulatory T cells and natural killer T cells. Immunology. 2013; 138: 105-115.

8 Keskinov A, Shurin MR. Myeloid regulatory cells in tumor spreading and metastasis. Immunobiology. 2015; 220: 236-242.

9 Katoh H, Watanabe M. Myeloid-derived suppressor cells and therapeutic strategies in cancer. Mediators Inflamm. 2015; 159269.

10 Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. J Immunol. 2009; 182: 4499-506.

11 Greten TF, Manns MP, Korangy F. Myeloid derived suppressor cells in human diseases. Int Immunopharmacol. 2011; 11: 802-807.

12 Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009; 9: 162-174.

13 Kolahian S, Öz HH, Zhou B, et al. The emerging role of myeloid-derived suppressor cells in lung diseases. Eur Respir J. 2016; 47: 967-977.

14 Scrimini S, Pons J, Agustí A, et al. Differential effects of smoking and COPD upon circulating myeloid derived suppressor cells. Respir Med. 2013; 107: 1895-1903.

15 Ortiz ML, Lu L, Ramachandran I, et al. Myeloid-derived suppressor cells in the development of lung cancer. Cancer Immunol Res. 2014; 2: 50-58.

16 Kalathil SG, Lugade AA, Pradhan V, et al. T-Regulatory Cells and Programmed Death 1 + T Cells Contribute to Effector T-Cell Dysfunction in Patients with Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med. 2014; 190: 40-50.

17 Tan DB, Fernandez S, Price P, et al. The proportion and function of peripheral myeloid-derived suppressor cells do not correlate with systemic inflammation in chronic obstructive pulmonary disease. Hum Immunol. 2014; 75: 5-9.

18 Deshane JS, Redden DT, Zeng M, et al. Subsets of airway myeloid--derived regulatory cells distinguish mild asthma from chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2015; 135: 413-424.e15.

19 Cote CG, Celli BR. Pulmonary rehabilitation and the BODE index in COPD. Eur Respir J. 2005; 26: 630-636.

20 Pinto-Plata VM, Cote C, Cabral H, et al. The 6-min walk distance: Change over time and value as a predictor of survival in severe COPD. Eur Respir J. 2004; 23: 28-33.

21 Klech H. PW. Technical recomendations and guidelines for bronchoalveolar lavage (BAL). Report of the European Society of Pneumonology Task Group on BAL. Eur Respr J. 1989; 2: 561-585.

22 Meyer KC, Raghu G, Baughman RP, et al. An official American Thoracic Society clinical practice guideline: The clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. Am J Respir Crit Care Med. 2012; 185: 1004-1014.

23 Kelsen SG, Mardini IA, Zhou S, et al. A technique to harvest viable tracheobronchial epithelial cells from living human donors. Am J Respir Cell Mol Biol. 1992; 7: 66-72.

24 Hodge SJ, Hodge GL, Holmes M, et al. Flow cytometric characterization of cell populations in bronchoalveolar lavage and bronchial brushings from patients with chronic obstructive pulmonary disease. Cytometry B Clin Cytom. 2004; 61: 27-34.

25 Chmielowski A, Chrostowska-Wynimko J, Fil A, et al. [Recommendation of the Polish Respiratory Society for bronchoalveolar lavage (BAL) sampling, processing and analysis methods]. Pneumonol Alergol Pol. 2011; 79: 75-89. Polish.

26 Osińska I, Wołosz D, Domagała-Kulawik J. Association between M1 and M2 macrophages in bronchoalveolar lavage fluid and tobacco smoking in patients with sarcoidosis. Pol Arch Med Wewn. 2014;124: 359-364.

27 Piotrowski WJ, Młynarski W, Fendler W, et al. Chemokine receptor CXCR3 ligands in bronchoalveolar lavage fluid: associations with radiological pattern, clinical course, and prognosis in sarcoidosis. Pol Arch Med Wewn. 2014; 124: 395-402.

28 Przybylski G, Chorostowska-Wynimko J, Dyczek A, et al. Studies of hepatocyte growth factor in bronchoalveolar lavage fluid in chronic interstitial lung diseases. Pol Arch Med Wewn. 2015; 125: 260-271.

29 Dolcetti L, Marigo I, Mantelli B, et al. Myeloid-derived suppressor cell role in tumor-related inflammation. Cancer Lett. 2008; 267: 216-225.

30 Diaz-Montero M, Labib SM, Nishimura M, et al. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin–cyclophosphamide chemotherapy. Cancer Immunol Immunother. 2009; 58: 49-59.

31 Clark CE, Hingorani SR, Mick R, et al. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. Cancer Res. 2007; 67: 9518-9527.

32 Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. J Immunol. 2009; 182: 4499-4506.

33 Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009; 9: 162-174.

34 Bunt SK, Clements VK, Hanson EM, et al. Inflammation enhances myeloid-derived suppressor cell crosstalk by signaling through Toll-like receptor. J Leukoc Biol. 2009; 85: 996-1004.

35 Cosio MG, Saetta M. Evasion of COPD in smokers: at what price? Eur Respir J. 2012; 39: 1298-1303.

36 Ohki S, Shibata M, Gonda K, et al. Circulating myeloid-derived suppressor cells are increased and correlate to immune suppression, inflammation and hypoproteinemia in patients with cancer. Oncol Rep. 2012; 28: 453-458.

37 Semenza GL. Oxygen sensing, homeostasis, and disease. N Engl J Med. 2011; 365: 537-547.

38 Noman MZ, Desantis G, Janji B, et al. PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. J Exp Med. 2014; 211: 781-790.

39 Corzo CA, Condamine T, Lu L, et al. HIF-1α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. J Exp Med. 2010; 207: 2439-2453.

ARTYKUŁ ORYGINALNY

Mieloidalne komórki supresorowe w płynie z płukania oskrzelowo-pęcherzykowego u chorych na przewlekłą obturacyjną chorobę płuc

Beata Brajer-Luftmann[1], Agata Nowicka[1], Mariusz Kaczmarek[2], Marcin Grabicki[1], Barbara Kuźnar-Kamińska[1], Barbara Bromińska[3], Jan Sikora[2], Halina Batura-Gabryel[1]

1 Katedra i Klinika Pulmonologii, Alergologii i Onkologii Pulmonologicznej, Uniwersytet Medyczny im. Karola Marcinkowskiego w Poznaniu, Poznań
2 Katedra i Zakład Immunologii Klinicznej, Uniwersytet Medyczny im. Karola Marcinkowskiego w Poznaniu, Poznań
3 Klinika Endokrynologii, Przemiany Materii i Chorób Wewnętrznych, Uniwersytet Medyczny im. Karola Marcinkowskiego w Poznaniu, Poznań

SŁOWA KLUCZOWE

badanie czynnościowe układu oddechowego, komórki supresorowe pochodzenia szpikowego, popłuczyny oskrzelowo-pęcherzykowe, przewlekła obturacyjna choroba płuc

STRESZCZENIE

WPROWADZENIE Komórki supresorowe pochodzenia szpikowego (*myeloid-derived suppressor cells* – MDSC) charakteryzują się silną zdolnością hamowania funkcji komórek T oraz stanowią ważny element w regulacji przewlekłego (ogólnego i miejscowego) zapalenia i nowotworzenia. MDSC mogą mieć wpływ na lokalny i systemowy stan zapalny oraz nowotworzenie w przewlekłej obturacyjnej chorobie płuc (POChP), ale ich obecność w popłuczynach oskrzelowo-pęcherzykowych (*bronchoalveolar lavage fluid* – BALF) i krwi obwodowej (*peripheral blood* – PB) oraz ich zależność od parametrów klinicznych w POChP nie zostały dotychczas zbadane.

CELE Celem badania była ocena MDSC w BALF i PB oraz analiza związku między MDSCs a parametrami klinicznymi w POChP.

PACJENCI I METODY Do badania włączono 64 chorych na POChP w stabilnym okresie choroby. U pacjentów oznaczono parametry kliniczne, a MDSC oceniono za pomocą przeciwciał monoklonalnych bezpośrednio srzężonych z fluorochromami metodą cytometrii przepływowej.

WYNIKI Odsetek MDSC w BALF był niższy niż w PB (0,63 ±0,90 *vs* 3,94 ±0,38). W BALF MDSC (odsetek komórek jednojądrzastych) korelowały z natężoną objętością wydechową pierwszosekundową ($r_s = -0,30$; $p = 0,0185$), wskaźnikiem rozdęcia płuc ($r_s = 0,32$; $p = 0,0148$), PaO_2 ($r_s = -0,45$; $p = 0,0002$), SaO_2 ($r_s = -0,41$; $p = 0,0008$) i DL_{CO} ($r_s = -0,32$; $p = 0,0211$). Wykazano istotną negatywną korelację między MDSC (odsetek wszystkich leukocytów) a PaO_2 ($r_s = -0,42$; $p = 0,0006$) i SaO2 ($r_s = -0,37$; $p = 0,0027$). W PB nie wykazano żadnych korealcji.

WNIOSKI MDSC są obecne w mikrośrodowisku dróg oddechowych człowieka i mogą brać udział w lokalnym procesie zapalnym w POChP. Dalsze badania powinny skupić sie na szczegółowym wyjaśnieniu ich roli w kontekście lokalnego i systemowego zapalenia w POChP.

Adres do korespondencji:
dr hab. n. med. Agata Nowicka, Katedra i Klinika Pulmonologii, Alergologii i Onkologii Pulmonologicznej, Uniwersytet Medyczny im. Karola Marcinkowskiego w Poznaniu, ul. Szamarzewskiego 84, 60-569 Poznań, tel.: 61 841 70 61, e-mail: agnowicka@ump.edu.pl
Praca wpłynęła: 29.11.2016.
Przyjęta do druku: 15.12.2016.
Publikacja *online*: 15.12.2016.
Nie zgłoszono sprzeczności interesów.
Pol Arch Med Wewn. 2016; 126 (12): 980-988
doi:10.20452/pamw.3718