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

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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.
pathologically activated CD11b-Ly6CloLy6G-immature granulocytes, D11b-Ly6ChiLy6G-mono-
cytes, and a small proportion of myeloid precursors. In humans predominantly, the immuno-
phenotype 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). 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
(Tregs) or by anti-inflammatory cytokines such as transforming growth factor β, and interleu-
kin 10. It may also involve the metabolism of L-arginine by arginase 1 or inducible nitric oxide
synthase. In addition, MDSCs promote tumor progression through a number of different immu-
noregulatory mechanisms. Because of the ability of MDSCs to suppress both adaptive and in-
nate immune responses mainly through direct inhibition of cytotoxic functions of T cells and natural
killer cells, they play pivotal role in cancer develop-
ment. MDSCs facilitate cancer cell invasion and intravasation by secreting multiple proteo-
lytic enzymes, including matrix metalloprotein-
ases, which are necessary for extracellular matrix
degradation and disruption of endothelial cad-
erins, adhesion proteins, or the basement mem-
brane of vessels. Epithelial-mesenchymal transi-
tion (EMT) is one of the steps for dissemination of
cancer cells. When cancer cells undergo EMT,
they lose epithelial markers and gain mesenchy-
mal phenotypes. Granulocyte-like MDSCs induce
EMT in cancer cells using transforming growth factor β, epidermal growth factor, and hepato-
cyte growth factor. On the other hand, MDSCs also contribute to mesenchymal-epithelial tran-
sition of cancer cells by secreting versican. This
function of MDSCs supports cancer cells in col-
onizing at metastatic niche. In summary, MDSCs are implicated in human pathological condi-
tions including some cancers, inflammatory dis-
eases, and also lung diseases.

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

The occurrence of circulating MDSCs in inflam-
atory 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 sys-
temic inflammatory process in COPD, and only one study on the prevalence of MDSCs in
the lung microenvironment has been published.

The aims of this study were to confirm the pres-
ence 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 Oncolo-
ogy, Poznan University of Medical Sciences, Poznań
Poland. It included 64 patients (49 men, 15 wom-
en) diagnosed with COPD according to the 2010
criteria of the Global Initiative for Lung Disease

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**Original Article** MDSCs in BALF in patients with COPD

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Exercise capacity was measured using the distance achieved in a 6-minute walk test (6MWT). 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.

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. Topical lignocaine and intravenous fentanyl and propofol anesthesia were used. A special effort was made to use as low dose of lignocaine as possible. 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.

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.
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. 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. 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. There are only a few reports assessing the occurrence of circulating MDSCs in the blood of patients with COPD and only one in BALF. 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 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 FEV1 (r = −0.30, P = 0.0185), residual volume / total lung capacity (RV/TLC; r = 0.32, P = 0.0148) (Figure 2). GOLD stage (r = 0.30, P = 0.0176), arterial oxygen pressure (PaO2; r = −0.45, P = 0.0002), arterial oxygen saturation (SaO2; r = −0.41, P = 0.0008), and DLco (r = −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 PaO2 (r = −0.42, P = 0.0006) and SaO2 (r = −0.37, P = 0.0027) (Figure 4). The other parameters (FEV1, TLC, RV, RV/TLC, GOLD stage, DLco, mMRC, age, BODE index, number of pack years, and 6MWD) 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**

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**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. 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.

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. There are only a few reports assessing the occurrence of circulating MDSCs in the blood of patients with COPD and only one in BALF. 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...
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.

indicates monocytic (CD14+/HLA-DR-) and granulocytic (CD15+/HLA-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-DR- cells belonging to leukocytes (CD45+) with low granularity (SSC). Scrimmini et al. 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.
On the other hand, our results are in line with those of Tan et al. They assessed proportions of MDSCs in PBMCs isolated from patients 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 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.

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one study in which BALF was used to identify and characterize human airway MDSCs in patients with COPD. Deshane et al18 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 al39 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 FEV1, RV/TLC, and DLCO may indirectly prove that local inflammatory process is connected also with airway obstruction. It may promote worse oxygenation. Our study results indicated a significant negative correlation between lung MDSCs and PaO2 and SaO2, 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.

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

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ARTYKUŁ ORYGINALNY

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

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

STRESZCZENIE
Komórki supresorowe pochodzenia szpikowego (myeloid-derived suppressor cells – MDSC) charakteryzują się silną zdolnością hamowania funkcji komórek T oraz stanowią ważny element w regulacji przewlektka (ogólnego i miejscowego) zapalenia i nowotworzenia. MDSC mogą mieć wpływ na lokalny i systemowy stan zapalny oraz nowotworzenie w przewlekiej 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 DLCO (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 SaO_2 (r_s = −0,37; p = 0,0027). W PB nie wykazano żadnych korelacji.

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