

Chemokine receptor CXCR3 ligands in bronchoalveolar lavage fluid: associations with radiological pattern, clinical course, and prognosis in sarcoidosis

Wojciech J. Piotrowski¹, Wojciech Młynarski², Wojciech Fendler²,
Krystyna Wyka², Jerzy Marczak¹, Paweł Górski¹, Adam Antczak³

¹ Department of Pneumology and Allergy, Medical University of Lodz, Łódź, Poland

² Department of Pediatric Oncology, Hematology and Diabetology, 1st Department of Pediatrics, Medical University of Lodz, Łódź, Poland

³ Department of General and Oncological Pulmonology, Medical University of Lodz, Łódź, Poland

KEY WORDS

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ABSTRACT

INTRODUCTION Sustained inflammation in sarcoidosis may lead to lung fibrosis. The activity of numerous chemokines responsible for proliferation and activity of T lymphocytes may play a crucial role in this process and may have predictive value. These include cytokines induced by interferon γ , such as CXCL9, 10, and 11—ligands of chemokine receptor CXCR3.

OBJECTIVES The aim of the study was to estimate the role of CXCR3 ligands in the pathogenesis of sarcoidosis and the predictive value of their concentrations in bronchoalveolar lavage (BAL) fluid.

PATIENTS AND METHODS CXCL9, 10, and 11 concentrations in BAL fluid were measured by an enzyme-linked immunosorbent assay in patients with sarcoidosis ($n = 59$) and controls ($n = 34$). A total of 46 patients were followed up for 24 months to compare the results between the subgroups with complete remission and with chronic disease.

RESULTS Protein-standardized CXCL11 concentrations in BAL fluid from patients with stage II sarcoidosis were higher than in those with stage I (median [interquartile range], 0.95 [0.26–2.39] vs. 0.32 [0.13–0.74] pg/ μ g protein, $P = 0.02$). CXCL10 levels in BAL fluid from patients without Löfgren syndrome were higher compared with those with the syndrome (0.69 [0.51–1.05] vs. 0.40 [0.27–0.70] pg/ μ g protein, $P = 0.05$). None of these markers predicted the chronic course of the disease. CXCL10 levels in BAL fluid correlated with serum angiotensin-converting enzyme, and CXCL11 levels with parenchymal lesions on high-resolution computed tomography. Only nonstandardized CXCL11 concentrations in BAL fluid were higher in sarcoidosis.

CONCLUSIONS Our results support the hypothesis that cytokines CXCL9, 10, and 11 may be involved in the pathogenesis of chronic sarcoidosis. However, the lack of notable differences between the sarcoidosis and control groups, as well as the lack of associations with the chronic course suggest that they should not be considered as potential prognostic markers.

INTRODUCTION Sarcoidosis is a multiorgan granulomatous inflammatory disease, which involves the lungs and intrathoracic lymph nodes in above 90% of the patients. Prognosis is usually good, especially in patients without lung parenchymal involvement and in those presenting with acute symptoms at onset (Löfgren syndrome

[LS]). However, from 5% to 10% of the patients may develop progressive lung fibrosis. At first, it may be difficult to estimate whether the disease will resolve without treatment or progress to irreversible fibrotic lung disease.^{1,2}

Noncaseating granulomas and lymphocytic infiltration with CD4⁺ predominance are

Correspondence to:

Wojciech J. Piotrowski, MD, PhD,
Klinika Pneumonologii i Alergologii,
Uniwersytet Medyczny w Łodzi,
ul. Kopcińskiego 22, 90-153 Łódź,
Poland, phone: +48-42-678-75-05,
fax: +48-42-678-21-29, e-mail:
wojciech.piotrowski@umed.lodz.pl
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the hallmarks of inflammation in sarcoidosis. Although the etiology is unknown, the persistent stimulation of the immune system with unidentified antigens seems to be critical for the chronic course of the disease. The mechanisms leading to the self-perpetuating activation of lymphocytes as well as the formation of granulomas have not been fully elucidated. The special role of interferon γ (IFN- γ) in granuloma formation can be illustrated by the fact that IFN- γ gene knockout mice are incapable of forming granulomas under experimental conditions.³ The activity of various low-molecular chemotactic cytokines (chemokines) seems to be equally important in the pathogenesis of this chronic inflammatory process. The subgroup of CXC chemokines is classified according to the presence or absence of the sequence: glutamic acid-leucine-arginine (ELR) near the NH₂-terminus. The ELR-CXC chemokines can be further divided into IFN- γ -inducible and non-IFN- γ -inducible chemokines. IFN- γ -inducible chemokines are represented by 3 cytokines: a monokine induced by IFN- γ , MIG (CXCL9); IFN- γ -inducible protein 10, IP10 (CXCL10); and IFN- γ -inducible T-cell α chemoattractant, ITAC (CXCL11). All of them act through the CXCR3 receptor; therefore, they are also known as CXCR3 ligands.⁴

Increased concentrations of the IFN- γ -inducible chemokines have been reported in serum^{5,6} and bronchoalveolar lavage (BAL)^{4,7-9} fluid from patients with sarcoidosis. However, associations between those increased levels and radiological, clinical, and laboratory markers as well as the chronic course of sarcoidosis have not been studied. Therefore, in the present study, we compared the levels of CXCR3 ligands in BAL fluid between patients with and without sarcoidosis. We also compared the levels of CXCR3 ligands in BAL fluid between patients with and without parenchymal involvement (radiological stage II vs. I) and between patients with and without symptoms of LS. Moreover, we evaluated the relations between chemokine levels in BAL fluid and selected clinical features characterizing the activity of sarcoidosis (BAL fluid cellularity and laboratory markers) and its severity (clinical, radiological, and functional). Finally, we compared the BAL fluid levels of CXCR3 ligands between patients with complete remission and those with a chronic course of sarcoidosis.

PATIENTS AND METHODS **Study group** The study included 59 patients with active sarcoidosis (44 men, 15 women; age 39 $\pm$ 11 years). The patients were recruited at the Department of Pneumology and Allergy of the Medical University of Lodz, Łódź, Poland. The diagnosis was based on international standards.¹ In the majority of the cases, the diagnosis was confirmed by biopsy, although it was postponed in cases with obvious clinical and radiological features and typical BAL results (symmetrical hilar enlargement

in patients with LS and signs of lymphocytic alveolitis with a ratio of CD4 to CD8 exceeding 3.5).

Patients were divided on the basis of chest X-ray results into subgroups with radiological stage I (hilar lymph node enlargement without signs of parenchymal involvement) and stage II disease (signs of parenchymal involvement in addition to hilar lymph node enlargement). We conducted an independent comparison between patients with acute onset (at least 2 of the following symptoms present: arthritis, erythema nodosum, elevated body temperature; LS subgroup) and those with an insidious onset of the disease (non-LS subgroup).

The results of the whole study group (n = 59) were analyzed to compare the sarcoidosis and control groups in terms of CXCR3 ligand levels in BAL fluid. For the purpose of comparisons within the group and for the calculation of correlations, the group size was limited to 44. The remaining cases were excluded owing to incomplete laboratory, functional, or high-resolution computed tomography (HCRT) data.

Forty-six patients were followed up every 6 months for a period of up to 24 months. Patients were classified according to a previously published scoring system.¹⁰ Briefly, complete remission was diagnosed when chest X-ray, lung function, and laboratory test results were normal, and there were no signs and symptoms of extrapulmonary sarcoidosis (n = 27). This group was compared with patients with chronic sarcoidosis (n = 19).

Control group The control group consisted of 34 nonsmokers referred for bronchoscopy because of chronic cough or undefined changes on chest X-ray. After a thorough examination, those patients were ultimately diagnosed either with idiopathic cough or were considered healthy (when radiological signs were defined as clinically insignificant changes or artifacts).

All patients signed written informed consent to participate in the study, and the study was approved by the Ethics Committee at the Medical University of Lodz (registration number: RNN/99/08/KE).

Bronchoscopy and bronchoalveolar lavage Bronchoscopy was performed with a flexible bronchoscope (Pentax, Japan) according to the Polish Respiratory Society Guidelines.¹¹ Patients optionally received midazolam and atropine before the examination; lidocaine (2%) was used as a topical anesthetic. BAL fluid was collected from the medial lobe (right bronchus [RB] 4 or RB5) by instillation and subsequent withdrawal of 4 $\times$ 50 ml of 0.9% NaCl. The fluid recovery rate was 52.1% $\pm$ 1.2%. Crude BAL fluid was filtered through gauze, centrifuged, and the pellet was suspended in a phosphate buffer. The total cell count was presented as n $\times$ 10⁶. Cytospin slides were prepared and stained using the May-Grünwald-Giemsa stain.

The number of macrophages, lymphocytes, neutrophils, and eosinophils were calculated using light microscopy and presented as the percentage of all nonepithelial cells.

CXCR3 ligand levels in bronchoalveolar lavage fluid

BAL fluid aliquots were stored in Eppendorf Tubes at a temperature of -70°C and thawed at room temperature. The frozen samples were used for measurements only once. Chemokines (CXCL9, CXCL10, and CXCL11) were assessed by standard methods using commercially available kits for enzyme-linked immunosorbent assays with mouse monoclonal antibodies specific for CXCL9, CXCL10, and CXCL11 (R&D Systems Inc., Minneapolis, Minnesota, United States). Samples were transferred into wells with chemokine-specific immobilized antibodies. After adding a secondary autoantibody conjugated with horseradish peroxidase and rinsing to remove excess of reagent, tetramethylbenzidine solution was added and the amount of substances was measured on the basis of color intensity at a wavelength of 450 nm using a microplate reader (BioRad, Hercules, California, United States). The protein concentration was measured using the Peterson's modification of the micro Lowry method, which utilizes sodium dodecyl sulfate facilitating the dissolution of relatively insoluble lipoproteins. A commercial kit was used (TP0300, Sigma-Aldrich, St. Louis, Missouri, United States). The reaction resulted in the reduction of the Folin & Ciocalteu's phenol reagent, which yielded a purple color. The protein content was measured based on the absorbance of the color solution at a wavelength of 500 to 800 nm in reference to a calibration curve. All measurements were performed in duplicate and the mean value was calculated.

Lung function tests Spirometry was performed according to the American Thoracic Society / European Respiratory Society standards¹² using a computer-based spirometer (Jaeger, Germany). Forced vital capacity (FVC) and forced expiratory volume in the first second (FEV_1) were measured, and the Tiffeneau index (FEV_1/FVC) was calculated. Diffusing capacity of the lung for carbon monoxide was measured only in patients with sarcoidosis using Lungtest 1000 SB (MES, Poland) with a single-breath method, according to the European Respiratory Society / American Thoracic Society standards.¹³ The values were corrected for hemoglobin. All data (except the Tiffeneau index) were presented as percent predicted.^{12,13}

Computed tomography Thin-section HRCT scans of the lungs were obtained in each subject. Sections of 1-mm collimation were acquired at 10-mm intervals from the apex to the dome of a diaphragm, at 120 kV, 200 mA, and a scan time of 0.6 s. All scans were obtained at full inspiration, and the images were obtained at a window level of -700 Hounsfield units and window width of 900 Hounsfield units. To estimate the extent of

parenchymal involvement and hilar lymph node enlargement, we used the classification by Drent et al.¹⁴ Briefly, the extent of bronchovascular bundle thickening, parenchymal nodules, septal/nonseptal lines, and parenchymal consolidation were estimated on a 4-grade scale and the sum of points described parenchymal involvement. Lymph node enlargement and pleural thickening were estimated separately, also on a 4-grade scale.

Laboratory activity markers A routine measurement of serum angiotensin-converting enzyme (ACE) and calcium concentrations, 24-hour urine calcium loss, and C-reactive protein (CRP) levels were measured in all patients.

Statistical analysis Chemokine concentrations were standardized to protein content within a given BAL fluid sample. In addition, a comparison between the control and study groups was performed for crude (nonstandardized) data. Continuous variables were presented as medians with interquartile ranges. Correlation analysis was performed using the Spearman's rank correlation. Comparisons between the 2 groups were performed using the Mann-Whitney test. A *P* value of less than 0.05 was considered statistically significant.

RESULTS Characteristics of the study group Demographic and laboratory data with relation to the radiological stage and the presence or absence of LS are presented in TABLES 1 and 2. We found higher concentrations of CRP in patients with LS compared with those without LS, while serum ACE levels and 24-hour urinary calcium loss were significantly higher in subjects without LS (TABLE 1).

CXCR3 ligand levels in bronchoalveolar lavage fluid from patients with sarcoidosis and controls

The crude CXCL11 concentration was significantly higher in the BALF of sarcoidosis patients (median, interquartile range: 77.6, 22.9–142.1 pg/ml) compared with controls (21.8, 7.2–105.8 pg/ml, $P = 0.006$). The crude concentrations of CXCL9 and CXCL10 tended to be higher in patients with sarcoidosis, but the difference was not significant. When standardized values were used, no significant differences were found between the sarcoidosis and control groups in any of the measured chemokines (TABLE 2).

CXCR3 ligand levels in bronchoalveolar lavage fluid from patients with stages I and II sarcoidosis

The CXCL11 concentration in patients with stage II sarcoidosis was significantly higher compared with those with stage I (median, interquartile range: 0.95, 0.26–2.39 pg/ μg protein 0.32 vs. 0.13–0.74 pg/ μg protein, $P = 0.02$, respectively; FIGURE 1). There were no significant differences between patients with stages I and II sarcoidosis in BAL fluid levels of CXCL10 and CXCL9 (0.49, 0.33–0.67 pg/ μg vs.

TABLE 1 Characteristics of the patients in relation to radiological staging of sarcoidosis and the presence or absence of acute symptoms

Parameters	LS	Non-LS	P value	Stage I	Stage II	P value
parenchymal lesions on HRCT	1.5 (1.0–3.0)	3.0 (2.0–4.0)	0.04	1.5 (0–3.0)	3.0 (2.0–4.0)	0.01
total CT score ^a	5.0 (4.0–6.0)	3.5 (2.0–5.0)	0.07	3.5 (2.0–5.0)	5.0 (4.0–6.0)	0.01
FVC, % predicted	99 (92–107)	98 (87–107)	0.5	99 (92–107)	94 (87–104)	0.5
FEV ₁ , % predicted	94 (83–100)	88 (80–96)	0.2	90 (83–100)	80 (78–98)	0.2
FEV ₁ /FVC, %	0.78 ± 0.08	0.77 ± 0.08	0.6	78 ± 8	77 ± 7	0.6
DL _{CO} , % predicted	0.96 ± 0.18	0.90 ± 0.13	0.3	95 ± 15	92 ± 21	0.6
TCC × 10 ⁴ /ml	18.6 (12.9–22.5)	19.6 (17.3–23.0)	0.5	19.1 (14.3–22.4)	18.7 (14.9–41.5)	0.4
macrophages, %	64.5 (46.0–77.0)	53.0 (45.0–63.0)	0.1	63.0 (47.5–70.5)	46.0 (44.0–80.0)	0.4
lymphocytes, %	30.5 (19.0–47.0)	35.0 (32.0–52.0)	0.2	32.0 (23.5–43.5)	40.0 (17.0–52.0)	0.4
neutrophils, %	1.1 (0–2.0)	2.0 (1.0–2.0)	0.4	1.3 (0.5–2.0)	2.0 (0–2.0)	0.9
eosinophils, %	0.6 (0–1.0)	1.0 (0–2.0)	0.2	0.9 (0–1.5)	2.0 (0–2.2)	0.2
ACE, IU/ml	45.8 (27.9–66.6)	76.0 (55.4–98.2)	0.004	54.3 (38.0–72.1)	78.7 (29.6–120.3)	0.1
CRP, mg/l	25.9 (9.7–66.1)	4.0 (2.4–8.3)	0.001	16.0 (2.4–38.8)	8.3 (4.7–20.5)	0.9
serum Ca ²⁺ , mmol/ml	2.49 (2.41–2.56)	2.51 (2.47–2.54)	0.7	2.49 (2.45–2.55)	2.51 (2.47–2.58)	0.3
urine Ca ²⁺ , mmol/24 h	4.38 (2.8–6.4)	7.17 (4.15–8.19)	0.02	4.39 (2.86–7.20)	6.4 (4.15–8.16)	0.2

Data are presented as median (interquartile range) for nonnormal distribution or mean ± standard deviation for normal distribution.

a a sum of the parenchymal score (4 grades) and lymph node enlargement and pleural thickening score (4 grades)

Abbreviations: ACE – angiotensin-converting enzyme, BAL – bronchoalveolar lavage, CRP – C-reactive protein, CT – computed tomography, DL_{CO} – diffusing capacity of the lung for carbon monoxide corrected for hemoglobin, FEV₁ – forced expiratory volume in the first second, FVC – forced vital capacity, HRCT – high-resolution computed tomography, LS – Löfgren syndrome, TCC – total cell count

TABLE 2 Chemokine concentrations in the control and sarcoidosis groups

Chemokine		Control group	Sarcoidosis group	<i>P</i> value
CXCL9	pg/ml	15.9 (9.6–32.5)	27.8 (16.9–38.9)	NS
	pg/μg protein	0.16 (0.09–0.52)	0.18 (0.12–0.32)	NS
CXCL10	pg/ml	47.0 (21.6–101.4)	60.5 (30.8–138.2)	NS
	pg/μg protein	0.83 (0.30–1.09)	0.61 (0.27–0.90)	NS
CXCL11	pg/ml	21.8 (7.2–105.8)	77.6 (22.9–142.1)	0.006
	pg/μg protein	0.47 (0.24–2.82)	0.40 (0.19–0.95)	NS

Data are presented as median (interquartile range).

Abbreviations: NS – nonsignificant

0.73, 0.21–1.80, respectively, for CXCL10 and 0.19, 0.15–0.30 pg/μg vs. 0.15, 0.05–0.76 pg/μg respectively, for CXCL9).

CXCR3 ligand levels in bronchoalveolar lavage fluid from patients with and without Löfgren syndrome

The BAL fluid levels of CXCL10 in patients without LS were higher compared with those in patients with LS (0.69, 0.51–1.05 pg/μg vs. 0.40, 0.27–0.70 pg/μg, $P = 0.05$, **FIGURE 2**). No significant differences were found between the LS and non-LS subgroups in the BAL fluid levels of CXCL11 (0.27, 0.19–0.95 pg/μg vs. 0.41, 0.18–0.88 pg/μg) and CXCL9 (0.19, 0.12–0.34 pg/μg vs. 0.16, 0.13–0.32 pg/μg).

Clinical and radiological characteristics during 24-month follow-up

Of 46 patients, 27 were not diagnosed with sarcoidosis (complete remission, 59%). Of 27 patients with complete remission, 24 (89%) were initially diagnosed with stage I sarcoidosis. In 21 patients (78%),

the disease started with acute symptoms (LS). There were 4 cases (15%) of extrapulmonary sarcoidosis (1 with facial nerve palsy, 1 with skin lesions, and 2 with ocular manifestations). Six patients were treated with glucocorticosteroids (owing to extrapulmonary sarcoidosis, prolonged and extensive acute symptoms). In the subgroup with chronic sarcoidosis ($n = 19$), 13 patients (68%) were initially diagnosed with stage I disease; 9 patients (48%) presented the signs of LS; there were 5 patients (26%) with extrapulmonary locations (2 with ocular manifestations, 2 with skin lesions, and 1 with bone marrow and spleen involvement); and 2 patients had recurrent sarcoidosis. Eight patients (42%) received treatment because of recurrent symptoms, radiological progression, or extrapulmonary disease.

CXCR3 ligand levels in bronchoalveolar lavage fluid in patients with complete remission vs. patients with chronic sarcoidosis

There were no differences between patients with complete remission and those with chronic sarcoidosis in the BAL fluid concentrations of CXCL9 (0.17; 0.11–0.34 pg/μg protein vs. 0.16; 0.02–0.29 pg/μg protein), CXCL10 (0.50; 0.27–0.80 pg/μg protein vs. 0.55; 0.24–0.91 pg/μg protein), or CXCL11 (0.45; 0.24–1.15 pg/μg protein vs. 0.38; 0.19–0.72 pg/μg protein). The differences were not significant also for nonstandardized values.

Correlations between CXCR3 ligands and laboratory and radiological markers

CXCL10 concentrations in BAL fluid correlated positively with serum ACE concentrations ($r = 0.38$; $P = 0.013$) and 24-hour urinary calcium loss ($r = 0.37$;

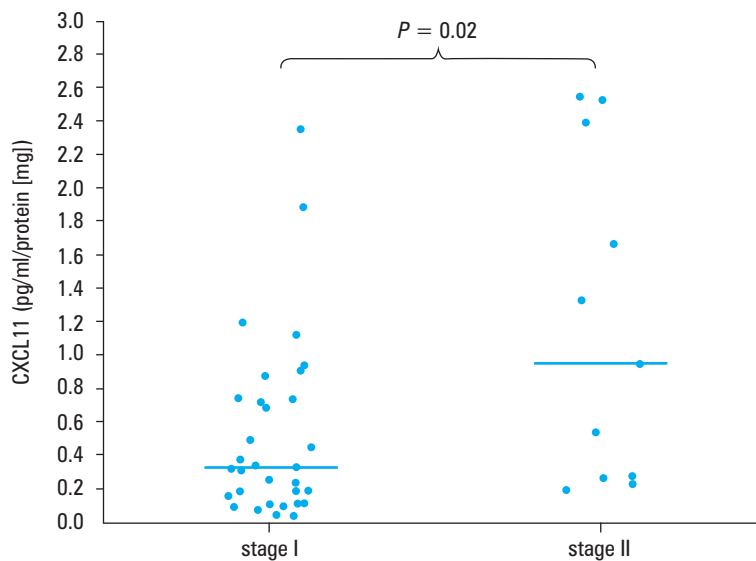


FIGURE 1 CXCL11 (ITAC) concentrations in bronchoalveolar lavage fluid from patients with stages I and II sarcoidosis

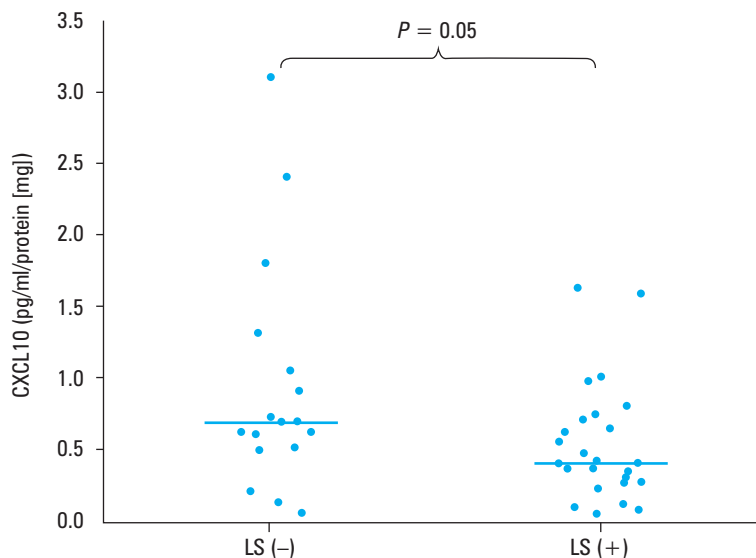


FIGURE 2 CXCL10 (IP10) concentrations in bronchoalveolar lavage fluid from patients with sarcoidosis with and without Löfgren syndrome (LS)

$P = 0.016$). CXCL11 concentrations in BAL fluid correlated positively with parenchymal lesions on HRCT ($r = 0.33$; $P = 0.028$).

Other correlations Parenchymal lesions on HRCT correlated with the percentage of lymphocytes in BAL fluid ($r = 0.30$; $P = 0.049$).

DISCUSSION We found that only crude CXCL11 concentrations in BAL fluid were higher in patients with sarcoidosis compared with those without. The within-group analysis revealed elevated standardized concentrations of CXCL11 in BAL fluid in more advanced lung disease (patients with lung parenchymal involvement) compared with those with sole hilar lymph node enlargement. We also found increased CXCL10 levels in patients with an insidious compared with those

with acute onset of sarcoidosis (the latter have an excellent long-term prognosis). CXCL11 levels correlated with HRCT scores describing the extent of lung parenchymal changes, and CXCL10 levels correlated with laboratory activity markers known to be linked to a chronic course and worse prognosis. Moreover, we did not find any differences between patients with remission and those with a chronic course. Therefore, our findings are only partly consistent with the results of other authors who suggested that IFN- γ -inducible chemokines were involved in the inflammatory process in the course of sarcoidosis.^{4,7-9}

CXCR3 and its ligands are mainly expressed in the cells of monocyte lineage, especially epithelioid and multinucleated giant cells, the main components of sarcoid granulomas. Other sources of these chemokines include lymphocytes, eosinophils, neutrophils, fibroblasts, and endothelial cells.^{4,7,15} Many investigators confirmed the importance of the CXCR3–CXCR3 ligand axis in the mobilization of Th1 lymphocytes to the loci of inflammation.¹⁶ The most extensively studied molecule is CXCL10. Agostini et al.⁷ showed that cells bearing CXCL10 were mainly epithelioid cells and CD68⁺ macrophages located inside the granulomatous areas. However, dominant CD4⁺ T cells in BAL fluid, which express Th1 cytokines (IFN- γ), expressed also high levels of CXCR3. The increased concentrations of CXCR3 ligands in BAL fluid from patients with sarcoidosis have been reported by a number of studies^{4,7-9}; however, their results were inconsistent. For instance, Busuttill et al.⁴ reported elevated CXCL9 levels but did not confirm elevated CXCL10 levels in BAL fluid in this patient group, while Arakelyan et al.¹⁷ did not find significant differences in the BAL fluid levels of CXCL9 and CXCL10 between the sarcoidosis and control groups, which is in line with our study. These discrepancies may be explained by the polymorphic and highly variable clinical course of the disease and different patient selection criteria.

Unlike other authors,^{4,9} we found the values standardized to the protein concentration to be more reliable than crude data. Although we did not find any differences between subjects with and without sarcoidosis, we showed several correlations within the sarcoidosis group. The only difference between the sarcoidosis and control groups was observed in the crude CXCL11 concentration, but it was not significant when standardized values were compared. We believe that protein-standardized values are more accurate and relevant for the estimation of inflammatory processes in BAL fluid, which is in line with the current knowledge and published guidelines.^{18,19}

Our study showed the elevated concentrations of CXCR3 ligands in more advanced lung disease, which is in agreement with the studies by other authors. The prognostic role of radiological classification is well established, and all the available data show that stage I sarcoidosis has a more favorable outcome than stages

II and III.^{1,2} Arakelyan et al.¹⁷ reported elevated concentrations of CXCL9 and CXCL10 in patients with parenchymal disease (stage III) compared with patients with LS. In the study by Nishioka et al.,⁹ CXCL9 and CXCL10 concentrations in BAL fluid were elevated only in stage II disease compared with healthy controls (but not in the whole sarcoidosis group), and serum CXCL11 levels were elevated only in patients with stage II sarcoidosis.⁹

Our study has been the first to show the elevated concentrations of CXCL11 in BAL fluid from patients with parenchymal lung disease compared with those with the disease limited to intrathoracic lymph nodes. Moreover, a notable correlation was found between CXCL11 concentrations in BAL fluid and parenchymal lesions on HRCT. Of note, also a trend towards higher CXCL10 concentrations in BAL fluid was noted in stage II compared with stage I sarcoidosis; however the difference was not significant. Busuttill et al.⁴ did not confirm such correlations between CXCL11 concentrations and radiological stages of the disease.

Patients with LS have a much higher chance of early and spontaneous recovery; consequently, the risk of relapsing, chronic, or progressive disease in these patients is very low. Therefore, the acute onset has a well-established positive prognostic value.^{1,2} Other authors published similar results showing the lower concentrations of CXCL10 in BAL fluid of patients with LS.¹⁷ It is particularly interesting that CXCL10 concentrations in BAL fluid in our study correlated positively with serum ACE concentrations and 24-hour urinary calcium loss. ACE is produced by granuloma cells as well as active form of vitamin D₃. Therefore, both markers are elevated in a subset of patients with sarcoidosis and has been used in everyday practice as activity markers. Although they are not ideal biomarkers, mostly due to low sensitivity and specificity,²⁰ the elevated serum concentrations of ACE and impaired calcium metabolism are characteristic of a serious and chronic disease rather than of self-limiting and nonextensive one.^{1,2} Takeuchi et al.⁵ found a positive correlation between serum CXCL9 and CXCL10 concentrations and ACE. Although the monitoring of calcium metabolism is mandatory in sarcoidosis, the role of ACE is rapidly decreasing. Several novel markers have been reported to be more relevant for estimating the activity of sarcoidosis (such as soluble human interleukin 2 receptor or neopterin),²¹ while others (such as transforming growth factor β) may be used for progressive disease.²² Cytokines characterizing the Th1/Th17 lymphocyte subsets may also be useful in determining disease activity and prognosis.²³ Current data indicate that there is no single ideal biomarker to assess disease activity and prognosis with reliable sensitivity and specificity.²⁴

A correlation between CXCR3 ligand concentrations in BAL fluid and the intensity of lymphocytic alveolitis would be expected. A number

of investigators reported positive correlations between the number of CD4⁺ lymphocytes and all CXCR3 ligands in BAL fluid.⁹ Similarly, other authors found positive correlations between CXCL9 and CXCL10 levels and the percentage and number of lymphocytes in BAL fluid. On the other hand, in the study by Busuttill et al.,⁴ the presence of lymphocytic alveolitis had no impact on the BAL fluid levels of CXCL9, CXCL10, and CXCL11. In our study, we did not observe any correlations between the levels of CXCR3 ligands and the percentage and number of lymphocytes in BAL fluid. We only found a correlation between the percentage of lymphocytes in BAL fluid and parenchymal lesions on HRCT.

In summary, the elevated concentrations of CXCL11 in BAL fluid from patients with stage II sarcoidosis, unlike in that from patients with stage I, and of CXCL10 in patients with an insidious onset, unlike in those with an acute one, support the hypothesis that these molecules are involved in sustaining inflammation in sarcoidosis and may be responsible for the chronic course of the disease.

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Ligandy receptora cytokinowego CXCR3 w popłuczynach oskrzelowo-pęcherzykowych – związek z obrazem radiologicznym, przebiegiem klinicznym i rokowaniem w sarkoidozie

Wojciech J. Piotrowski¹, Wojciech Młynarski², Wojciech Fendler²,
Krystyna Wyka², Jerzy Marczak¹, Paweł Górski¹, Adam Antczak³

1 Klinika Pneumonologii i Alergologii, Uniwersytet Medyczny w Łodzi, Łódź

2 Klinika Pediatrii, Onkologii, Hematologii i Diabetologii, I Katedra Pediatrii, Uniwersytet Medyczny w Łodzi, Łódź

3 Klinika Pulmonologii Ogólnej i Onkologicznej, Uniwersytet Medyczny w Łodzi, Łódź

SŁOWA KLUCZOWE

biomarkery,
popłuczyny
oskrzelowo-
-pęcherzykowe,
rokowanie, sarkoidoza

STRESZCZENIE

WPROWADZENIE Przewlekające się zapalenie w przebiegu sarkoidozy może prowadzić do włóknienia płuc. Aktywność wielu chemokin odpowiedzialnych za proliferację i regulujących aktywność limfocytów T może odgrywać kluczową rolę w tym procesie i może mieć znaczenie rokownicze. Należą do nich cytokiny indukowane przez interferon- γ , takie jak CXCL9, 10 i 11 – ligandy receptora CXCR3.

CELE Celem badania była ocena roli ligandów receptora chemokinowego CXCR3 w patogenezie sarkoidozy i znaczenia rokowniczego stężeń tych chemokin w popłuczynach oskrzelowo-pęcherzykowych (*bronchoalveolar lavage fluid* – BALF).

PACJENCI I METODY Standaryzowane stężenia CXCL9, 10 i 11 w BALF mierzono za pomocą testu ELISA u chorych na sarkoidozę ($n = 59$) i w grupie kontrolnej ($n = 34$). 46 pacjentów obserwowano przez 24 miesiące w celu porównania uzyskanych wyników w podgrupach z pełną remisją choroby i z przewlekłym przebiegiem.

WYNIKI Stężenia CXCL11 w BALF u chorych ze stopniem II sarkoidozy były większe niż u chorych ze stopniem I (mediany [przedział międzykwartylowy] 0,95 [0,26–2,39] vs 0,32 [0,13–0,74] pg/ μ g białka; $p = 0,02$). Stężenia CXCL10 w BALF chorych bez zespołu Löfgrena były większe niż u chorych z zespołem (0,69 [0,51–1,05] vs 0,40 [0,27–0,70] pg/ μ g białka; $p = 0,05$). Żaden z tych markerów nie był związany z przewlekłym przebiegiem choroby. Stężenia CXCL10 w BALF korelowały ze stężeniem enzymu konwertującego angiotensynę w surowicy, a CXCL11 z zaawansowaniem zmian śródmiąższowych w badaniu HRCT. Tylko niestandaryzowane stężenia CXCL11 w BALF były większe w sarkoidozie.

WNIOSKI Uzyskane wyniki przemawiają za hipotezą, że cytokiny CXCL9, 10 i 11 mogą odgrywać pewną rolę w patogenezie przewlekłej sarkoidozy. Jednak brak wyraźnych różnic między grupą chorych na sarkoidozę a grupą kontrolną, a także brak związku z przewlekłym przebiegiem przemawiają przeciwko ich potencjalnej roli jako markerów prognostycznych.

Adres do korespondencji:
dr hab. med. Wojciech J. Piotrowski,
Klinika Pneumonologii i Alergologii,
Uniwersytet Medyczny w Łodzi,
ul. Kopcińskiego 22, 90-153 Łódź,
tel.: 42-678-75-05,
faks: 42-678-21-29, e-mail:
wojciech.piotrowski@umed.lodz.pl
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