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ORIGINAL ARTICLE

The Role of CXC Chemokines in Pulmonary Fibrosis of Systemic Lupus Erythematosus Patients

Agnieszka Nielepkowicz-Goździńska[1] · Wojciech Fendler[2] · Ewa Robak[3] · Lilianna Kulczycka-Siennicka[3] · Paweł Górski[4] · Tadeusz Pietras[4] · Ewa Brzeziańska[5] · Małgorzata Pietrusińska[4] · Adam Antczak[1]

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Abstract The inflammatory process in systemic lupus erythematosus (SLE) affects many organs including the lungs. CXC chemokines are suggested to play an important role in the pathogenesis of SLE and pulmonary fibrosis. To estimate the concentrations of CXCL9, CXCL10, CXCL11 in bronchoalveolar lavage fluid (BALF) of patients with and without pulmonary involvements of SLE to evaluate CXC chemokines role in the pathogenesis of pulmonary fibrosis in SLE. Twenty-six SLE patients and 31 healthy controls were evaluated using high-resolution computed tomography (HRCT), pulmonary function tests, the SLE Disease Activity Index (SLEDAI), assessing CXCL9, CXCL11, CXCL10 level in BALF (an enzyme-immunosorbent assay kit). The mean CXCL9 and CXCL11 concentrations in BALF were higher in SLE patients compared to healthy controls (34.09 ± 102.34 vs 10.98 ± 14.65 pg/mL, $p < 0.001$; 72.65 ± 112.89 vs 16.12 ± 83.75 pg/mL, $p = 0.012$, respectively). The disease activity scored by SLEDAI and the concentration of CXCL10 in BALF were significantly higher in the SLE patients with pulmonary fibrosis when compared with patients with normal HRCT (8.23 ± 3.19 vs 5.01 ± 2.41; 73.45 ± 34.12 vs 40.76 ± 41.65, respectively, in both $p < 0.05$). In SLE patients positive correlations were found between SLEDAI and the percentage of lymphocytes in BALF ($r = 0.51$, $p < 0.05$); CXCL9 and CXCL10 concentrations in BALF ($r = 0.65$, $p < 0.001$); CXCL9 and CXCL11 concentrations in BALF ($r = 0.69$, $p < 0.001$). In lupus patients with pulmonary manifestations positive correlations were found between CXCL11 concentration in BALF and SLEDAI ($r = 0.55$, $p < 0.05$), CXCL11 concentration and the percentage of neutrophils in BALF ($r = 0.69$, $p < 0.05$), CXCL10 concentration and the percentage of neutrophils in BALF ($r = 0.57$, $p < 0.05$). Our observations indicate that CXCL9 and CXCL11 play an important role in the pathogenesis of SLE but it needs further studies. These results suggest that CXCL10 and CXCL11 are associated with neutrophils accumulation in the alveolar space of SLE patients with pulmonary fibrosis and should be considered as potential factor of interstitial fibrosis.

Keywords Bronchoalveolar lavage fluid · CXCL9 · CXCL10 · CXCL11 · Pulmonary fibrosis · Systemic lupus erythematosus

✉ Agnieszka Nielepkowicz-Goździńska
nielepkowicz@interia.pl

[1] Department of General and Oncological Pneumology, Medical University of Lodz, Kopcińskiego 22, 90-153 Lodz, Poland

[2] Department of Pediatrics, Oncology, Hematology and Diabetology, Medical University of Lodz, Lodz, Poland

[3] Department of Dermatology and Venereology, Medical University of Lodz, Lodz, Poland

[4] Department of Pneumonology and Allergy, Medical University of Lodz, Lodz, Poland

[5] Department of Molecular Bases of Medicine, Medical University of Lodz, Lodz, Poland

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the activation of T and polyclonal B lymphocytes, production of pathogenic autoantibodies and formation of immune complexes causing organ damage. The pathogenesis of SLE is still only partly understood.

Symptomatic pulmonary manifestations occur in half of SLE patients during the course of the disease (Kao et al. 2004). The pulmonary manifestation of lupus is an important indicator of overall prognosis (Abu-Shakra et al. 1995).

An imbalance of T helper (Th) 1 and Th2 cytokines, which is typical in SLE, appears to be an important factor responsible for the pathophysiology of organ damage in SLE (La Cava 2009). A number of chemokines have been investigated as possible factor playing a vital immunological role in SLE, but their role in pathogenesis of specific organ involvement is questionable (Vielhauer et al. 2007).

Chemokines induce chemotaxis and activation of various leukocytes and are involved in tissue accumulation of a certain cell type during immune and inflammatory responses (Zlotnik and Yoshie 2000). In SLE, chemokines are involved in inflammatory cell recruitment to a great deal of tissues including the skin, joints, kidney and lungs. Chemokines are involved in regulation of tissue accumulation of specific leucocytes and organ inflammation, suggesting their important roles in the pathophysiology of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis and SLE (Galligan et al. 2004; Lee et al. 2009). The involvement of CXCL10 and CXCR3 has been observed in several types of tissue damages associated with SLE (Antonelli et al. 2014; Elia 2015).

Materials and Methods

The study comprised 26 never-smoking patients [fulfilling the revised American College of Rheumatology criteria for the diagnosis of SLE] (Hochberg 1997) recruited from outpatient clinics and 31 healthy controls (never-smoking members of the hospital staff and medical students with normal spirometry results) matched for sex and age. In all patients high-resolution computed tomography (HRCT), spirometry, body pletysmography, diffusion capacity for carbon monoxide corrected for hemoglobin concentration (DLCOc) measurement, and bronchoalveolar lavage fluid (BALF) sample collection were performed. In all healthy individuals spirometry was performed while BALF fluid collection was carried out in 20. Patients were divided into two groups, one with and one without pulmonary fibrosis in HRCT chest studies. The study was carried out with the approval of the Ethics Committee of the Medical University of Lodz (RNN/146/07/KE). Written informed consent was obtained from all patients.

Thirteen patients were taking corticosteroids (prednisone 5–20 mg per day), three of them were treated with a combination of corticosteroids and azathioprine (100–150 mg per day), and one patient was treated with mycophenolate mofetil (1000 mg per day). Ten patients were on a maintenance dose of immunosuppressive drugs (prednisone $\leq$10 mg per day, azathioprine $\leq$100 mg per day). Patients with a previous history of pulmonary disorders other than SLE (asthma, chronic obstructive pulmonary disease, lung cancer, pneumonia or allergies) and with current infection were excluded from the study. The disease activity was evaluated by the SLE Disease Activity Index (SLEDAI) (Bombardier et al. 1992) and the active disease was considered as SLEDAI $\geq$6 (Table 1).

HRCT studies were performed using a 64-Slice CT (GE Healthcare LightSpeed), with 1.25 mm single slices, collimation at 1-cm intervals through the lungs, 120 kV, 265 mA, and 0.6 s scan time. The images were obtained at a window level of −700 Hounsfield units (HU) and a window width of 1500 HU and examined by two independent experienced radiologists.

Spirometry was performed according to European Respiratory Society (ERS) standards (Miller et al. 2005). The equipment (MES LUNGTEST 1000) was calibrated once every day. FVC and FEV1 were measured and the Tiffenau index was calculated.

Body pletysmography was obtained with a plethysmograph (Jaeger) and performed according to ERS standards (Wanger et al. 2005). Residual volume and total lung capacity were measured.

DLCO diffusion capacity for carbon monoxide corrected for hemoglobin concentration was obtained with Lung Test 1000 Mes according to ATS/ERS guidelines (Macintyre et al. 2005).

BALF was collected with a flexible bronchoscope (Olympus B1-IT20) according to British Thoracic Society guidelines (British Thoracic Society Bronchoscopy Guidelines Committee 2001). The tip of the bronchoscope was wedged in the lingular segments and 5 aliquots of 50 ml of sterile 0.9 % NaCl solution at 37 °C were poured and recovered by gentle suction after each part. The fluid was collected, filtered through gauze and centrifuged. The pellet was suspended in phosphate buffer. Cytospins were prepared and the slides were stained with May-Grünwald Giemsa stain. The total cell count (TCC) was calculated ($n \times 10^6$) under a light microscope, and the numbers of macrophages, lymphocytes, neutrophils and eosinophils were presented as percentages of the TCC.

All samples of BALF were collected in the morning (8:00 and 10:00 a.m.) in order to avoid any influence of the circadian cycle and were immediately frozen at −80 °C until assayed.

Assay of Chemokine Levels Using Specific Enzyme-Linked Immunosorbent Assay

Measurements of CXCL9, CXCL10, and CXCL11 were performed using a commercially available, specific enzyme-linked immunosorbent assays (ELISA, Quantikine; R&D Systems; USA). The detection limits for the

assays were as follows: 11.3 pg/mL for CXCL9, 4.46 pg/mL for CXCL10 and 13.7 pg/mL for CXCL11. The assay employs the quantitative sandwich immunoassay technique. Chemokines were quantitated according to the protocols provided by the manufacturer. As BALF procedure has a dilutive effect on the recovery of chemokines, the values of original chemokine levels were corrected by albumin concentrations in BALF.

Statistical Analysis

The normality of distribution of continuous variables was tested using *W* test. The Student's *t* test or Mann–Whitney's *U* test was used for intergroup comparisons, depending on the distribution of respective variables. For comparisons of more than two groups, nonparametric analysis of variance with post hoc tests was performed using a Bonferroni-corrected Mann–Whitney's *U* test for significant comparisons. Correlations were evaluated using the Pearson's test or Spearman's rank correlation test depending on normality of distribution. A p value of <0.05 was assumed to be statistically significant. The PQStat Software package was used for analysis.

Results

The active stage of SLE was found in 13 patients, including 8 persons treated with immunosuppressants (Table 1). Among 26 patients with SLE 13 patients were found to have pulmonary fibrosis in HRCT (Table 2). Demographic and clinical characteristics of SLE patients with and without pulmonary fibrosis are found in Table 1.

SLE patients had an increased percentage of neutrophils in BALF when compared with control group (1.23 ± 4.79 vs 0.00 ± 0.56, $p < 0.001$). The mean CXCL9 and

Table 1 Demographic and clinical characteristics of SLE patients and control group

	SLE with pulmonary fibrosis ($n = 13$)	SLE without pulmonary fibrosis ($n = 13$)	Control group ($n = 31$)
Age (mean ± SD)	37.2 ± 11.3	35.1 ± 13.5	31.2 ± 4.5
Smoker	0 (0 %)	0 (0 %)	0 (0 %)
Gender			
Female	12 (92.3 %)	12 (92.3 %)	27 (87.1 %)
Male	1 (7.7 %)	1 (7.7 %)	4 (12.9 %)
SLEDAI score median (range)	8.23 ± 3.19	5.01 ± 2.41	
Active	9 (69.2 %)	4 (30.8 %)	
Inactive	4 (30.8 %)	9 (69.2 %)	
ANA ≥1:160	13 (100 %)	13 (100 %)	
Positive anti-dsDNA	2 (15.4 %)	2 (15.4 %)	
Anti-RNP	0 (0 %)	0 (0 %)	
P/MP therapy	7 (53.8 %)	6 (46.1 %)	
NSAID therapy	5 (38.5 %)	4 (30.7 %)	
Immunosuppressive (MM, A) therapy	2 (15.4 %)	2 (15.4 %)	
Fever	1 (7.7 %)	2 (15.4 %)	
Skin and mucous membrane lesion	8 (61.5 %)	6 (46.1 %)	
Atritis	6 (46.1 %)	3 (23.0 %)	
Renal active disorders	2 (15.4 %)	1 (7.7 %)	
Cardiac disorders	1 (7.7 %)	0 (0 %)	
Neuropsychiatric disorders	5 (35.3 %)	4 (30.7 %)	
Gastrointerstitial disorders	4 (30.7 %)	2 (15.4 %)	
Lymphadenopathy	0 (0 %)	0 (0 %)	
Anemia (Hb <12 g/dl)	3 (23.0 %)	3 (23.0 %)	
Leucopenia (WBC <3.5 ccmm)	5 (35.3 %)	2 (15.4 %)	
Thrombocytopenia (<150,000 ccmm)	2 (15.4 %)	1 (7.7 %)	
ESR >25 mm/h	6 (46.1 %)	3 (23.0 %)	

A azathioprine, *ANA* antinuclear antibody, *anti-ds DNA* anti-double stranded DNA, *anti-RNP* antibody to ribonucleoprotein, *ESR* erythrocyte sedimentation rate, *MM* mycophenolate mofetil, *MP* methylprednisolone, *NSAID* non-steroidal anti-inflammatory drug, *P* prednisone, *SLEDAI* SLE Disease Activity Index

Table 2 HRCT positive radiological signs of pulmonary fibrosis in SLE patients

	SLE patients ($n = 26$)
HRCT changes	13 (50 %)
Reticular pattern	11 (42.3 %)
Areas of ground glass attenuation	2 (7.7 %)
Interlobular interstitial thickening	2 (7.7 %)
Air-space nodules	3 (11.5 %)
Honeycombing	2 (7.7 %)

Table 3 Concentrations of cytokines and percentage of BALF cells in SLE patients and control group

Parameter	SLE patients ($n = 26$)	Control group ($n = 31$)	p
TCC BALF $\times$ 10^6 cells	34.3 ± 11.5	29.2 ± 9.3	ns
BALF lymphocytes (%)	17.11 ± 10.13	11.00 ± 7.43	ns ($p = 0.08$)
BALF macrophages (%)	79.42 ± 12.41	82.5 ± 9.83	ns
BALF eosinophils (%)	1.11 ± 1.54	0.0 ± 0.5	ns
BALF neutrophils (%)	1.23 ± 4.79	0.00 ± 0.56	<0.001
CXCL9 BALF	34.09 ± 102.34	10.98 ± 14.65	<0.001
CXCL10 BALF	57.03 ± 91.89	62.73 ± 51.35	ns
CXCL11 BALF	72.65 ± 112.89	16.12 ± 83.75	0.012

Values are given as the mean ± SEM (median) (25th percentile; 75th percentile)

ns not significant

CXCL11 concentrations in the BALF were higher in SLE patients compared to healthy controls (34.09 ± 102.34 vs 10.98 ± 14.65 pg/mL, $p < 0.001$; 72.65 ± 112.89 vs 16.12 ± 83.75 pg/mL, $p = 0.012$, respectively; Table 3). The mean CXCL9, CXCL10 and CXCL11 concentrations in BALF did not correlate with SLE activity. In SLE patients positive correlations were found between SLEDAI and the percentage of lymphocytes in BALF ($r = 0.51$, $p < 0.05$); between CXCL9 and CXCL11 concentrations in BALF ($r = 0.69$, $p < 0.001$); between CXCL9 and CXCL10 concentrations in BALF ($r = 0.65$, $p < 0.001$) (Table 4; Fig. 1). The disease activity scored by SLEDAI and the concentration of CXCL10 in BALF were significantly higher in the SLE patients with pulmonary fibrosis when compared with patients with normal HRCT (8.23 ± 3.19 vs 5.01 ± 2.41; 73.45 ± 34.12 vs 40.76 ± 41.65, respectively, in both $p < 0.05$; Fig. 2; Table 5). In patients with pulmonary manifestation positive correlations were found between CXCL11 concentration in BALF and SLEDAI ($r = 0.55$, $p < 0.05$), CXCL11 and the percentage of neutrophils in BALF ($r = 0.69$, $p < 0.05$), CXCL10 and the percentage of neutrophils in BALF ($r = 0.57$, $p < 0.05$); CXCL9 and CXCL11 concentrations in BALF ($r = 0.61$, $p < 0.05$); CXCL9 and CXCL10 concentrations in BALF ($r = 0.81$, $p < 0.001$) (Table 6; Fig. 3). Albumin concentrations in BALF had no dilutive effect on the concentrations of chemokines in BALF.

Table 4 Correlations in SLE patients

	r	p
CXCL9 vs CXCL10	0.65	<0.001
CXCL9 vs CXCL11	0.69	<0.001
CXCL10 vs CXCL11	0.24	ns
CXCL9 vs SLEDAI	0.00	ns
CXCL9 vs BALF lymphocytes (%)	0.18	ns
CXCL9 vs BALF eosinophils (%)	−0.11	ns
CXCL9 vs BALF neutrophils (%)	0.11	ns
CXCL10 vs SLEDAI	0.23	ns
CXCL10 vs BALF lymphocytes (%)	0.25	ns
CXCL10 vs BALF eosinophils (%)	−0.09	ns
CXCL10 vs BALF neutrophils (%)	0.3	ns
CXCL11 vs SLEDAI	0.32	ns
CXCL11 vs BALF lymphocytes (%)	0.14	ns
CXCL11 vs BALF eosinophils (%)	0.09	ns
CXCL11 vs BALF neutrophils (%)	0.09	ns
SLEDAI vs BALF lymphocytes (%)	0.51	<0.05
SLEDAI vs BALF neutrophils (%)	−0.01	ns
SLEDAI vs BALF macrophages (%)	−0.42	ns
SLEDAI vs BALF eosinophils (%)	−0.4	ns

ns not significant

The concentration of CXCL9, CXCL11 in BALF, the spirometric results (FEV_1/FVC, FEV_1, FVC, DLCO) and the numbers of macrophages, lymphocytes, neutrophils and eosinophils in BALF were not significantly different in the patients with pulmonary fibrosis when compared with patients without pulmonary fibrosis (Table 5).

Also there were no differences in the concentration of CXCL9, CXCL11, CXCL10 in BALF, the lung function tests or SLEDAI in SLE patients associated with immunosuppressive treatment (data not shown).

Discussion

Approximately 50 % of lupus patients will experience lung involvements of SLE during the course of their disease (D'Cruz et al. 2007). Recent studies have shown that several chemokines have been implicated both in the pathogenesis of pulmonary fibrosis and SLE.

Moreover, the CXC chemokine family is involved in regulating angiogenesis and vascular remodeling, and in promoting the mobilization and trafficking of mesenchymal progenitor cells such as fibrocytes (Strieter et al. 2007).

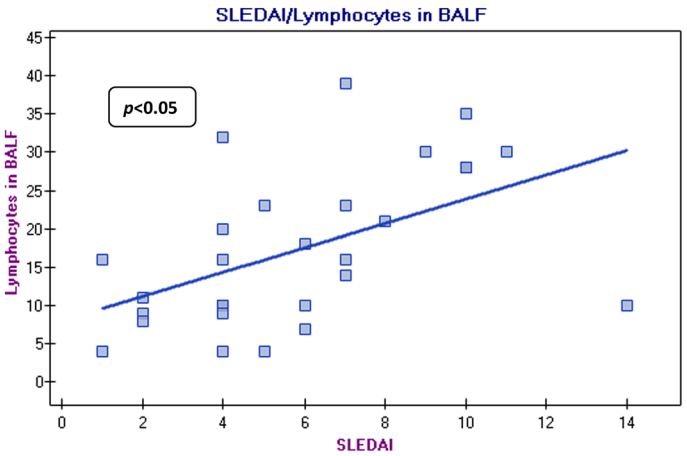

Fig. 1 Correlation of SLEDAI and percentage of lymphocytes in BALF of SLE patients

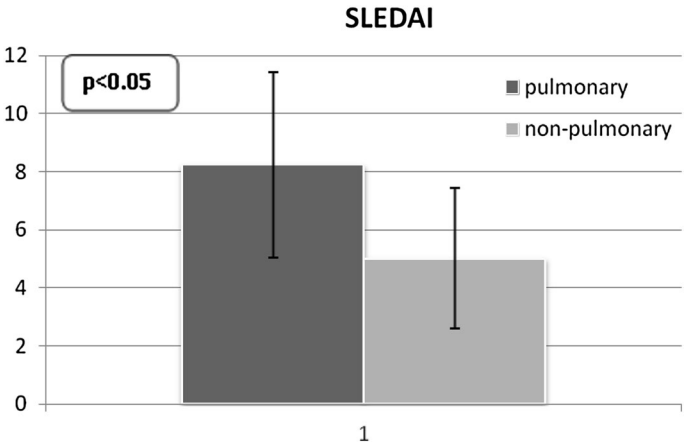

Fig. 2 The comparison of SLE activity in lupus patients with and without pulmonary fibrosis

Table 5 Results of measurements in SLE patients in relation to pulmonary manifestation of disease

Parameter	Pulmonary ($n = 13$)	Non-pulmonary ($n = 13$)	p
SLEDAI	8.23 ± 3.19	5.01 ± 2.41	<0.05
BALF lymphocytes (%)	21.75 ± 11.17	14.70 ± 8.01	ns
BALF eosinophils (%)	0.00 ± 1.57	0.97 ± 1.13	ns
BALF neutrophils (%)	1.87 ± 7.35	0.87 ± 1.72	ns
BALF macrophages (%)	88.22 ± 13.34	79.01 ± 8.00	ns
FEV1/FVC (%)	80.14 ± 7.94	83.5 ± 4.74	ns
FEV1 (% pred)	90.1 ± 20.01	101 ± 16.01	ns
FVC (% pred)	91.98 ± 21.64	104 ± 15.00	ns
TLC (% pred)	87.71 ± 27.21	111.82 ± 20.02	0.01
CXCL9 BALF	33.41 ± 23.10	26.52 ± 33.05	ns
CXCL10 BALF	73.45 ± 34.12	40.76 ± 41.65	<0.05
CXCL11 BALF	83.20 ± 96.99	54.34 ± 111.98	ns

Values are given as the mean ± SEM (median) (25th percentile; 75th percentile)

ns not significant, *BALF* bronchoalveolar lavage fluid, *EBC* exhaled breath condensate, *FEV1* forced expiratory volume in one second, *FVC* forced vital capacity, *SLEDAI* SLE Disease Activity Index, *TLC* total lung capacity

These functions of CXC chemokines are important in the pathogenesis of fibroproliferative disorders like pulmonary fibrosis (Strieter et al. 2007).

Vascular remodeling and angiogenesis in the lung has a major role in contributing to pulmonary fibrosis (Keane et al. 2001). Renzoni et al. (2003) have observed vascular

Table 6 Correlations in SLE patients with pulmonary fibrosis

	r	*p*
CXCL9 vs CXCL10	0.81	<0.001
CXCL9 vs CXCL11	0.61	<0.05
CXCL10 vs CXCL11	0.28	ns
CXCL9 vs SLEDAI	0.12	ns
CXCL9 vs BALF lymphocytes (%)	0.3	ns
CXCL9 vs BALF eosinophils (%)	−0.11	ns (0.054)
CXCL9 vs BALF neutrophils (%)	−0.01	ns
CXCL10 vs SLEDAI	0.31	ns
CXCL10 vs BALF lymphocytes (%)	0.23	ns
CXCL10 vs BALF eosinophils (%)	−0.16	ns
CXCL10 vs BALF neutrophils (%)	0.57	<0.05
CXCL11 vs SLEDAI	0.55	<0.05
CXCL11 vs BALF lymphocytes (%)	0.39	ns
CXCL11 vs BALF eosinophils (%)	0.18	ns
CXCL11 vs BALF neutrophils (%)	0.69	<0.05
SLEDAI vs BALF lymphocytes (%)	0.26	ns
SLEDAI vs BALF neutrophils (%)	0.4	ns
SLEDAI vs BALF macrophages (%)	−0.28	ns
SLEDAI vs BALF eosinophils (%)	−0.47	ns

ns not significant

remodeling in idiopathic pulmonary fibrosis (IPF) and the fibrosing alveolitis associated with systemic sclerosis. Moreover several studies have supported the hypothesis that expression of angiogenic cytokines [interleukin (IL)-8/CXC ligand (CXCL)-8, epithelial neutrophil-activating protein-78/CXCL-5, and CXCL-1, -2, -3] and angiostatic cytokines (CXCL9, CXCL10 and CXCL11) is associated with the development of pulmonary fibrosis (Belperio et al. 2000; Keane et al. 1997, 1999; Nor et al. 2001). CXCL9, CXCL10 and CXCL11 cascade connect the Th1 cytokine profile with angiostasis and Pan et al. (2006) create the concept of immunoangiostasis. There is a growing body of evidence that several chemokines have been implicated in the pathogenesis of diffuse lung disease (IPF and sarcoidosis) (Antoniou et al. 2006; Strieter et al. 2002; Ziegenhagen et al. 1998).

In an animal model of pulmonary fibrosis, CXCL11 inhibited vascular remodeling. Mice that received bleomycin and were treated with CXCL11 had decreased fibrosis and decreased intrapulmonary angiogenesis. Interestingly, these decreased fibrosis and intrapulmonary angiogenesis could be blocked using an antibody specific to CXCR3 (Burdick et al. 2005). CXC chemokine receptor 3 (CXCR3) is the receptor for the interferon (IFN)-γ-inducible chemokines (CXCL9, CXCL10 and CXCL11) and is expressed on activated immune cells and proliferating endothelial cells. CXCR3 is suggested to play role in limiting tissue fibroproliferation. This effect may be mediated by production of IFN-γ in the process of lung injury (Jiang et al. 2004).

Tager et al. (2004) findings suggest that CXCL10 inhibits pulmonary fibrosis by inhibition of fibroblast responses to the chemotactic and interruption of fibroblast recruitment. Inhibitory activity of CXCL10 on fibroblast accumulation in the lung may represent a novel treatment for pulmonary fibrosis. CXCL10 in SLE is also suggested to play role in the perpetuation of inflammation and concurrent in tissue damage (Lee et al. 2009).

CXCL9, CXCL10 and CXCL11 induce chemotaxis of different leukocyte subtypes and interact with one single chemokine receptor, CXCR3 that is expressed in some subgroups of Th and T killer cells (Tensen et al. 1999). In inflammatory diseases they recruit leukocytes to settle of injury. Sustained neutrophil accumulation in the alveolar space (neutrophilic alveolitis) causes lung injury and leads to interstitial fibrosis (Lynch et al. 1992; Pantelidis et al. 1997). Ziegenhagen et al. (2003) showed that increased percentages of neutrophils in BALF from patients with newly diagnosed pulmonary sarcoidosis were associated with progressive disease and need for treatment.

In the presented study, the percentage of neutrophils in BALF was found to be higher in SLE patients when compared with healthy individuals and the findings are in accordance with previous researches (Groen et al. 1993; Witt et al. 1996). Wallaert et al. (1990) proposed that subclinical alveolitis is present in SLE, with an accumulation of inflammatory and immune cells in the BAL fluid in patients without clinical and radiological respiratory abnormalities.

CXCL8 leads to neutrophil recruitment in the pulmonary interstitium and airspace (Knall et al. 1996). Our previous findings are similar and support the concept that CXCL chemokines are involved in pulmonary fibrosis in SLE patients. We have previously reported increased IL-8 concentration in BALF and exhaled breath condensate in SLE patients compared to healthy controls and correlation of IL-8 concentration in BALF with SLE activity in the group of SLE patients with fibrotic changes of the lungs (Nielepkowicz-Goździńska et al. 2014).

Lit et al. (2006) have shown the correlation of increased plasma concentration of CXCL9 and CXCL10 with SLE activity, and their association with IL-18, supports the significance of chemotaxis of Th1/Th2 lymphocytes and neutrophils in pathogenesis of SLE. We observed positive correlations of CXCL10 and CXCL11 levels with number of neutrophils in BALF of SLE patients with pulmonary fibrosis. But there was no correlation of CXCL9 with number of neutrophils in BALF of SLE patients with pulmonary fibrosis. We also observed positive correlation of CXCL11 level in BALF with the disease activity in SLE patients with pulmonary fibrosis. The correlations of the

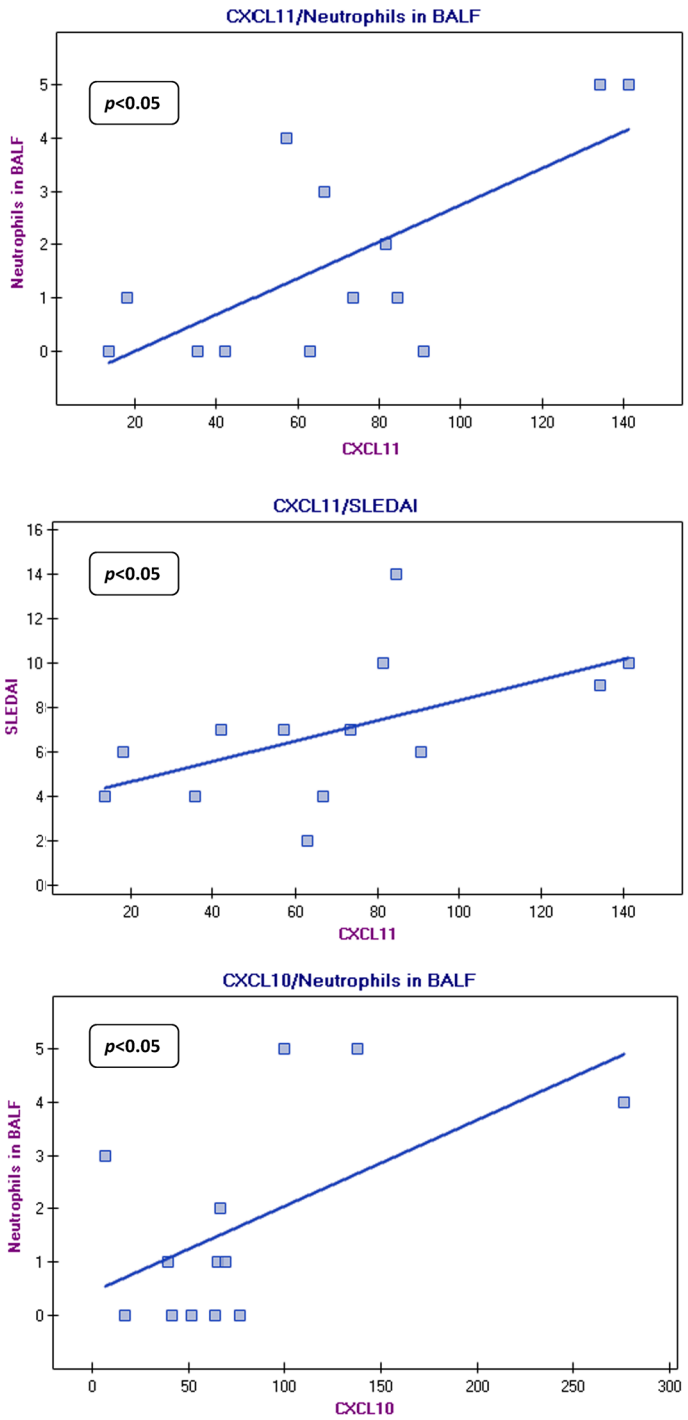

Fig. 3 Correlations in SLE patients with pulmonary fibrosis

CXCL chemokines concentration in BALF with SLE activity in lupus patients with pulmonary fibrosis could make CXCLs as biomarkers for pulmonary fibrosis.

Growing number of study results suggested that CXCL10 may be valuable indicator to assess SLE activity and prediction of disease exacerbations. Antonelli et al. (2014) emphasized the leading role of CXCL10 in autoimmune diseases (such as SLE, rheumatoid arthritis, psoriatic arthritis). High level of CXCL10 in peripheral fluids is a marker of host immune response, especially Th1 orientated T cells. Shah et al. (2011) have showed that CXCL10 was higher in sera of SLE patients and plasma CXCL10 concentration showed strong positive correlation with the disease activity. Also Lit et al. (2006) and Bauer et al. (2009) have showed CXCL10 was higher in SLE patients. Kong et al. (2009) have shown in studies

conducted at cellular level that enhanced expression of CXCL10 correlates with disease activity and clinical manifestations in SLE. Furthermore concentration of CXCL10 correlated positively with anti-DNA autoantibody levels and negatively with serum complements concentrations (Eriksson et al. 2003; Narumi et al. 2000). Abujam et al. (2013) reported that urinary and serum CXCL10 is potentially useful marker of lupus activity. CXCL10 concentration in the cerebrospinal fluid was correlated with central nervous system involvement in SLE (Okamoto et al. 2004).

In the presented study we demonstrated that the concentration of CXCL9, CXCL11 in BALF were not significantly different, while CXCL10 in BALF were significantly higher, in the patients with pulmonary fibrosis when compared with patients without pulmonary fibrosis. Nevertheless CXCL10 level in patients without pulmonary manifestations was lower than in controls and we observed a positive correlations between CXCL9 and CXCL10, CXCL9 and CXCL11 concentrations in BALF in SLE patients. The possible reason for this observation could be the immunosuppressive therapy influence. This observation could confirm those made by Capper et al. (2004) regarding the coexistence of SLE patients with different pattern of cytokine activity. They found a group of patients with very different cytokine profile to that of other SLE patients; the differences may result from different aetiologies of SLE.

An antibody to CXCL12 administered to NZB/W lupus mice inhibited disease progression, production of autoantibodies and proteinuria (Balabanian et al. 2003). Antichemokine specific immunomodulatory therapies could be beneficial to patients with inflammatory rheumatic diseases including SLE patients (Szekanecz et al. 2006).

These findings altogether support the notions that CXC chemokines are involved in promoting angiogenesis and fibrosis in the lung. In presented study the immunosuppressive therapy altered the results and is a limiting factor for the study. The inhibitory effects of steroids on CXCLs secretion in SLE needs further studies. Additionally the number of assessing patients is not high and could be a subsequent limiting factor for the study accuracy.

In conclusion, to the best of our knowledge, this is the first study investigating the local expression of CXCL9, CXCL10 and CXCL11 in SLE patients with and without pulmonary fibrosis. Our observations indicate that CXCL9 and CXCL11 in BALF distinguish SLE patients from healthy subjects and could play an important role in the pathogenesis of SLE but it needs further studies.

These results suggest that CXCL10 and CXCL11 are associated with neutrophils accumulation in the alveolar space of SLE patients with pulmonary fibrosis and should be considered as potential factor of interstitial fibrosis. The fact that CXCLs are chemotactic for the inflammatory cells to a particular location might cause the chemokines to be perceived as parameters of localized inflammation. Potential usefulness of these chemokines as prognostic and monitoring marker of pulmonary fibrosis merit further studies.

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Compliance with Ethical Standards

Conflict of interest None declared.

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