ORIGINAL PAPERS

Adv Clin Exp Med 2016, 25, 5, 871–878
DOI: 10.17219/acem/61953

© Copyright by Wrocław Medical University
ISSN 1899–5276

ELŻBIETA RADZIKOWSKA^{1, A–F}, ADRIANA ROŻY^{2, B–F}, PAULINA JAGUS^{2, B–F},
MAŁGORZATA POLUBIEC-KOWNACKA^{3, B, D–F}, ELŻBIETA WIATR^{1, C, E, F},
JOANNA CHOROSTOWSKA-WYNIMKO^{2, C, E, F}, KAZIMIERZ ROSZKOWSKI-ŚLIŻ^{1, C, E, F}

Clarithromycin Decreases IL-6 Concentration in Serum and BAL Fluid in Patients with Cryptogenic Organizing Pneumonia

¹ Third Department of Lung Disease, National Tuberculosis and Lung Disease Research Institute, Warszawa, Poland

² Laboratory of Molecular Diagnostics and Immunology, National Tuberculosis and Lung Disease Research Institute, Warszawa, Poland

³ Thoracic Surgery Department, National Tuberculosis and Lung Disease Research Institute, Warszawa, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

Abstract

Background. Inflammatory cytokines are involved in the development of cryptogenic organizing pneumonia (COP). It has been shown that macrolides inhibit cytokine production in the alveolar macrophages of COP patients. **Objectives.** The aim of the study was to assess the concentrations of interleukin 1 β (IL-1 β), IL-6, IL-8 and transforming growth factor β (TGF- β) in serum and in bronchoalveolar lavage fluid (BAL-f) in COP patients treated with clarithromycin (CAM).

Material and Methods. The study involved 26 patients (18 women and 8 men, mean age 56.46 $\pm$ 8.83 years) with biopsy-proven COP. After being treated with CAM, a complete recovery was achieved in 22 patients, while four patients did not respond to the treatment. The ELISA method was used to measure the serum and BAL-f concentrations of IL-1 β , IL-6, IL-8 and TGF- β .

Results. Before treatment, the serum IL-1 β , IL-6, IL-8 and TGF- β concentrations were similar in responders and non-responders. Significant decreases in serum concentrations of IL-6 (8.98 $\pm$ 13.26 pg/mL vs. 3.1 $\pm$ 6.95 pg/mL; p = 0.005), IL-8 (20.14 $\pm$ 25.72 pg/mL vs. 10.14 $\pm$ 6.8 pg/mL; p = 0.007) and TGF- β (37.89 $\pm$ 12.49 ng/mL vs. 26.49 $\pm$ 12.45 ng/mL; p = 0.001) were found after treatment, as well as a significant decrease in the BAL-f concentration of IL-6 (30.56 $\pm$ 56.78 pg/mL vs. 4.53 $\pm$ 5.84 pg/mL; p = 0.036). Clarithromycin treatment resulted in a significantly lower mean value of serum IL-6 responders than non-responders.

Conclusions. In COP patients, response to clarithromycin treatment was associated with decreases in serum concentrations of IL-6, IL-8 and TGF- β , and of ratios, and of the BAL-f concentration of IL-6 (*Adv Clin Exp Med* 2016, 25, 5, 871–878).

Key words: interleukin-1 β , transforming growth factor- β , organizing pneumonia, interleukin-8, bronchoalveolar lavage.

Cryptogenic organizing pneumonia (COP) is a rare disease that belongs to the larger group of idiopathic interstitial pneumonias [1, 2]. It is characterized by a specific histological pattern (intra-luminal exudates of fibromyxoid fluid involving the alveoli and small bronchioles, which form polyps with varying amounts of inflammatory infiltrates and fibrosis), non-specific clinical symptoms

(a flu-like syndrome, dyspnea, restrictive ventilatory impairment), and multiple, often migrated areas of opacities with air bronchograms, sometimes nodules or reticular lesions in radiological examinations. Histological examination of lung samples and exclusion of possible factors causing the secondary form of organizing pneumonia (such as infections, exposure to toxic substances, drugs, con-

nective tissue diseases, malignancies, autoimmune diseases, bone marrow or organ transplantation, and radiotherapy) are required for a firm diagnosis [3].

COP presents as an inflammatory disease. Usually serum inflammatory markers are elevated, and bronchoalveolar lavage fluid (BAL-f) shows an increased proportion of lymphocytes, neutrophils and eosinophils [4, 5].

The cytokine profile in the BAL-f of COP patients is characterized by elevated levels of cytokines mediated by the T-helper 1 response, such as interleukin-18 (IL-18), interleukin-12 (IL-12) and macrophage inflammatory protein [6]. However, it has been suggested that mainly alveolar macrophages are involved in the pathogenic process in COP [7]. They exhibit increase expression of interleukin-1 β (IL-1 β), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), fibronectin, tumor necrosis factor alpha (TNF α) and its soluble receptors sTNFR1/2, interferon gamma inducible protein 10, granulocyte-macrophage colony stimulating factor (GM-CSF) and chemokine (C-C motif) ligand 18 (CCL18) [8–13]. Additionally, factors involved in apoptosis and tissue remodeling play a major role in the pathogenesis of COP [14–15].

The aim of this study was to assess the concentration of selected cytokines in the serum and BAL-f of COP patients and to investigate any correlations between these parameters and the patients' response to treatment with clarithromycin (CAM).

The study was supported by National Tuberculosis and Lung Disease Research Institute grant No. 7.3. The local bioethics committee approved the study, and the participants signed informed consent.

Material and Methods

From 2004 to 2014, 26 patients with COP (18 women and 8 men, mean age 56.46 ± 8.83 years; range 39–71 years), were enrolled in this study. All the patients were evaluated regarding their concomitant diseases, use of medicines, symptoms and the duration of the symptoms. Routine blood and urine tests, along with microbiological, mycological and immunological examinations of bronchoalveolar lavage were performed.

The presence of connective tissue disease symptoms, antinuclear antibodies, antineutrophil cytoplasmic antibodies, rheumatoid factor were also tested for, and serological tests were carried out for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila*. In se-

lected cases tests for cytomegalovirus, influenza and parainfluenza virus, respiratory syncytial virus, hepatitis virus B and C were carried out. Chest X-rays and high resolution computed tomography (HRCT) scans, ultrasound examinations of the abdomen and thyroid gland and pulmonary function tests were also performed on all the patients.

The following criteria were required for a COP diagnosis: pulmonary infiltration suggesting organizing pneumonia in radiological examinations, characteristic changes in lung biopsy specimens, negative microbiological, mycological and cytological analyses of the BAL fluid and exclusion of other possible causes of organizing pneumonia.

Clarithromycin (CAM) was orally administered at a dose of 500 mg twice daily for three months. Patients who did not respond or who responded only partially to the clarithromycin treatment received prednisone orally at a dose of 0.5 mg/kg of weight for six weeks, with a continuous decrease in dosage over a period of six months.

The patients were assessed every month during the treatment and re-evaluated at the end of it. Subsequently, all the patients were examined in the 3rd and 6th months of the first year following treatment, and then every year for 3–5 years.

Complete or nearly complete regression of COP was defined as resolution of symptoms, decreases in serum inflammatory marker concentrations and complete or nearly complete resolution of pulmonary infiltrates on CT scans.

Bronchoalveolar Lavage

Bronchoalveolar Lavage (BAL) was performed during flexible fiberoptic bronchoscopy examinations under local anesthesia in the areas involved in the disease, according to established guidelines. The distal tip of the flexible fiberoptic bronchoscope was introduced into the small bronchus. Usually 100 or 200 mL of lukewarm, sterile, isotonic saline solution were administered in 20 mL portions and immediately recovered by means of gentle manual aspiration. The total recovery volume varied from 50 to 190 mL depending on the fluid recovery rate, the patient's condition and individual tolerance of the procedure.

Cytokine Assessments

Serum concentrations of IL-1 β (test sensitivity: minimum detectable level 1 pg/mL), IL-6 (test sensitivity: 1 pg/mL), IL-8 (test sensitivity: 1.5 pg/mL), and TGF- β 1 (test sensitivity: 1.7 pg/mL) were assessed by the ELISA method following the manufacturer's instructions (R & D Systems Inc., Minneapolis, USA). Serum samples were taken from

all the patients before and after the clarithromycin treatment, and BAL samples were collected before treatment, and 14 days after the therapy.

Five milliliters of blood and BAL-f samples were collected in serum separator tubes, allowed to clot for 30 min at 4°C, centrifuged at 4000 g for 10 min and stored in aliquots at -80°C. All measurements were done in duplicate, and the final result was the mean value of the two measurements.

Statistical Analysis

The statistical analysis was performed using STATISTICA 10 software (StatSoft Inc., Tulsa, OK, USA). Continuous unpaired variables were compared using the unpaired Student's t-test or the Mann-Whitney U-test, but for paired variables the paired Student's test and the Wilcoxon signed rank test were used. Fisher's exact test and the χ^2 test were used for proportion assessment. The Pearson r coefficient was used to measure the strength of associations between two variables. A p-value < 0.05 was considered statistically significant. All p-values are two-sided and unadjusted for multiple testing.

Results

Out of the 26 participants in the study, 22 responded to the CAM treatment and were regarded as responders (R). Partial response was noted in two patients, and two patients did not respond to the treatment. These four patients were considered non-responders (NR). The responders were younger than the non-responders, but the difference did not reach statistical significance (mean age, 55.14 ± 8.53 years vs. 63.75 ± 7.84 years; $p = 0.071$).

There were no significant differences between responders and non-responders in terms of sex, smoking, the duration of symptoms, the observation period or concomitant diseases, with the exception of arterial hypertension, which was found among 32% of the responders but 100% of the non-responders ($p = 0.022$). The characteristics of the study participants are presented in Table 1.

During the observation period relapse was noticed in one responder and in one non-responder, 26 months and 6 months, respectively, after the end of the treatment. Both these women experienced multiple relapses.

Table 1. Characteristics of patients with organizing pneumonia

	Whole group number (%)	Responders number (%)	Non-responders number (%)	p-value
Sex				
all	26 (100)	22 (85)	4 (15)	0.786
women	18 (70)	15 (67)	3 (75)	
men	8 (30)	7 (33)	2 (25)	
Age mean (years)	56.46 ± 8.83	55.14 ± 8.53	63.75 ± 7.84	0.071
age range (years)	39–71	39–71	55–71	
Smoking				
non-smokers	20 (68)	17 (77)	3 (75)	0.723
ex-smokers	4 (16)	3 (14)	1 (25)	
smokers	2 (8)	2 (9)	0	
Additional diseases				
goiter	12 (46)	12 (55)	0	0.1
hypertension	11 (42)	7 (32)	4 (100)	0.022
diabetes mellitus	3 (12)	1 (5)	2 (50)	ns.
ischemic heart disease	2 (8)	2 (9)	0	ns.
stomach and duodenal ulcer, GERD	4 (15)	4 (18)	0	ns.
asthma	2 (8)	2 (9)	0	ns.
breast cancer	2 (8)	2 (9)	0	ns.
Biopsy				
open lung biopsy	10 (38)	7 (32)	3 (75)	0.264
transbronchial lung biopsy	16 (62)	15 (68)	1 (25)	
Observation period				
range (months)	5–96	5–96	56–86	0.055
mean $\pm$ SD months	53.08 ± 24.47	49.18 ± 24.07	74.5 ± 12.52	

COPD – chronic obstructive pulmonary disease; GERD – gastro-esophageal reflux disease; ns. – not significant.

Clinical Manifestations

The patients commonly presented weakness (92%), cough (85%), fever (80%), weight loss (62%), dyspnea (38%), sweats (50%), chest pain (31%) and sputum expectoration (27%) (Table 2). There were no significant differences between the responders and non-responders in terms of their clinical presentation, with exception of a higher frequency of sweats in non-responders (41% vs. 100%; $p = 0.048$).

Radiological Findings

Bilateral pulmonary lesions (96%), with air bronchograms (100%) and ground glass opacities (90%) were frequent radiological findings; nodules (50%), diffuse reticular lesions (23%), lymph node enlargement (12%) and pleural fluid (4%)

were also noted. Migratory lung lesions were observed in 77% of the patients. There were no significant differences between the responders and non-responders in terms of the radiological pattern of changes.

Laboratory Findings

All the patients had elevated erythrocyte sedimentation rates (mean: 76.82 ± 38.71 mm/h). The mean leukocyte count was 8420 ± 2710 /mL, and the mean number of eosinophils was 260 ± 330 mL. Antinuclear antibody titers, rheumatoid factor, antithyroid peroxidase and antithyroglobulin antibodies were elevated in three patients (12%), one patient (4%) and three patients (12%), respectively. The responders did not differ significantly from the non-responders in any of the laboratory parameters analyzed.

Table 2. Pulmonary function tests in patients with organizing pneumonia

Pulmonary function test	All		Responders		Non-responders		p-value
	examined patients	number (%)	examined patients	number (%)	examined patients	number (%)	
VC mean % pred.	96.38 ± 23.11	26 (100)	101.85 ± 18.58	22 (100)	69.0 ± 23.05	4 (100)	0.006
VC < 80% pred.		4 (15)		2 (9)		2 (50)	0.037
FEV1 mean % pred.	93.42 ± 16.45	26 (100)	97.35 ± 13.91	22 (100)	73.75 ± 13.75	4 (100)	0.006
FEV1 < 80% pred.		1 (4)		0		1 (25)	0.154
FEV1% mean % pred.	92.42 ± 9.45	26	91.4 ± 9.88	30	97.5 ± 4.33	4	0.246
FEV1% < 70% pred.		1 (4)		0		1 (25)	ns.
TLC mean % pred.	99.95 ± 19.25	23 (89)	105.35 ± 19.25	19	77.0 ± 20.79	4	0.2
TLC < 80% pred.		4 (17)		2 (9)		1 (25)	0.125
TLC > 120% pred.		3 (13)		3 (14)		0	ns.
RV mean % pred.	107.18 ± 24.25	23 (89)	111.11 ± 24.45	19 (86)	89.5 ± 15.26	4 (100)	0.112
RV < 80% pred.		3 (13)		1 (5)		2 (50)	0.067
RV > 120% pred.		8 (35)		8 (36)		0	ns.
DLCO mean % pred.	75.95 ± 18.22	23 (89)	79.67 ± 16.97	19 (86)	59.25 ± 13.37	4	0.039
DLCO < 70% pred.		9 (39)		6 (27)		3 (75)	0.065
PaO ₂ mean – mm Hg	74.52 ± 9.89	26 (100)	76.76 ± 8.99	22 (100)	62.75 ± 4.2	4	0.006
PaO ₂ < 60 mm Hg		5 (19)		6 (27)		3 (75)	0.114
PaCO ₂ mean – mm Hg	36.0 ± 3.79	26 (100)	35.86 ± 3.93	22 (100)	36.75 ± 2.86	4 (100)	0.679
6MWT – distance < pred.		2 (8)		0		2 (50)	0.218
6MWT desaturation		6 (24)		2 (9)		4 (100)	0.018

FVC – forced vital capacity; FEV1 – forced expiratory volume in 1 s; TLC – total lung capacity; RV – residual volume; DLCO – diffusion lung capacity for carbon monoxide; 6MWT – 6-min walk test; PaO₂ – partial pressure of oxygen in the arterialized blood; PaCO₂ – partial pressure of carbon dioxide in the arterialized blood; HRCT – high resolution computed tomography; ns. – not significant.

Lung Function Parameters

Spirometry data were available for all the patients, but body plethysmography and the diffusing capacity of the lungs for carbon monoxide for only 23 (89%) (Table 2). The patients who did not respond to CAM had significantly decreased pulmonary function parameters in comparison to the responders: The mean FVC % of predicted was 69.0 ± 23.05 in non-responders as opposed to 101.85 ± 18.58 in responders ($p = 0.006$), and the FEV1 % of predicted was 73.75 ± 13.75 in non-responders, compared to 97.35 ± 13.91 ($p = 0.006$). The non-responders displayed obturation significantly more frequently than the responders (50% vs. 9%; $p = 0.037$); likewise impaired DL, CO (mean DL, CO% of predicted 59.25 ± 13.37 vs. 79.67 ± 16.97 ; $p = 0.039$).

The patients who responded to CAM had significantly higher values of oxygen tension in arterialized capillary blood (76.76 ± 8.99 mm Hg vs. 62.75 ± 4.2 mm Hg in the nonresponders; $p = 0.006$). Desaturation in 6MWT was observed significantly more frequently in non-responders than responders (100% vs. 9%; $p = 0.018$).

Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) was performed on all 26 patients. The mean percentages of macrophages, lymphocytes, eosinophils and neutrophils were $64.51\% \pm 24.28\%$, $26.3\% \pm 18.06\%$, $4.05\% \pm 5.75\%$ and $6.48\% \pm 9.83\%$, respectively.

Data regarding subpopulations of lymphocytes in BAL-f were available for 16 patients. The mean ratio of CD4⁺/CD8⁺ cells was 3.71 ± 2.66 ; 6 patients showed a ratio of < 1.0 , and in 4 patients the ratio was over 2. BAL-f eosinophil levels between 20% and 25% were present in 2 patients, and neutrophil levels of $> 10\%$ were present in 3 patients.

Examination of the cell populations in the BAL fluid showed no significant differences in the distribution of macrophages, lymphocytes, or subpopulations of lymphocytes between the groups, but individual patients showed a wide variety of results. There were no significant differences between the responders' and non-responders' BAL profiles.

Serum Cytokines

CAM treatment was associated with significant decreases in serum concentrations of IL-6 (8.98 ± 13.26 pg/mL before treatment vs. 3.1 ± 6.95 pg/mL after treatment; $p = 0.005$), IL-8 (20.14 ± 25.72 pg/mL before treatment vs. 10.14 ± 6.8 pg/mL after treatment; $p = 0.007$) and TGF- β 1 (37.89 ± 12.49 ng/mL before treatment vs. 26.49 ± 12.45 ng/mL after treatment; $p = 0.001$) (Table 3). There was no difference between IL-1 β serum concentrations before and after the treatment. Initially, the patients who responded to CAM displayed similar serum concentrations of these cytokines as the non-responders. In the responders, the median values of IL-6, IL-8 and TGF- β 1 were significantly decreased after the treatment: The median for IL-6 was 8.29 ± 13.7 pg/mL before

Table 3. Serum concentration of cytokines before and after the treatment

	All mean $\pm$ SD	Responders mean $\pm$ SD	Non-responders mean $\pm$ SD	p-value
IL1 pg/mL	2.53 ± 4.52	2.54 ± 4.34	2.51 ± 4.9	0.992
IL1 pg/mL – post	2.1 ± 2.74	2.51 ± 2.84	2.49 ± 1.59	0.765
p-value	0.968	0.528	ns.	
IL 6 pg/mL	8.98 ± 13.26	8.29 ± 13.7	12.75 ± 11.32	0.547
IL 6 pg/mL – post	3.1 ± 6.95	1.84 ± 3.08	9.97 ± 15.69	0.029
p-value	0.005	0.003	ns.	
IL 8 pg/mL	20.14 ± 25.72	19.86 ± 27.5	22.18 ± 14.69	0.872
IL 8 pg/mL – post	10.14 ± 6.8	9.81 ± 6.98	11.83 ± 4.88	0.598
p-value	0.007	0.036	ns.	
TGF β ng/mL	37.89 ± 12.49	39.14 ± 12.49	31.02 ± 23.52	0.307
TGF β ng/mL – post	26.49 ± 12.45	26.12 ± 10.56	28.45 ± 21.29	0.738
p-value	0.001	0.001	ns.	

ns. – not significant.

treatment vs. 1.84 ± 3.08 pg/mL after ($p = 0.003$); for IL-8 it was 19.86 ± 27.5 pg/mL before and 9.81 ± 6.98 pg/mL after ($p = 0.036$); and for TGF- β 1 it was 39.14 ± 12.49 ng/mL before treatment vs. 26.12 ± 10.56 ng/mL after ($p = 0.001$). In the non-responding patients the differences were not analyzed because of small number of cases.

There were no significant differences between the responders and non-responders in the median values of IL-1 β , IL-8 and TGF- β 1 serum concentration before and after the CAM treatment, but the responders displayed significant decrease of IL-6 after the CAM treatment in comparison with the non-responders (1.84 ± 3.08 pg/mL in the responders vs. 9.97 ± 15.69 pg/mL in the non-responders; $p = 0.029$).

BAL-f Cytokines

CAM treatment resulted in a significant decrease in IL-6 concentration in the BAL fluid (30.56 ± 56.78 pg/mL before treatment vs. 4.53 ± 5.84 pg/mL after treatment; $p = 0.036$). There was no difference between IL-1 β , IL-8, TGF- β 1 concentrations in the BAL-f before and after the treatment. The patients who responded to CAM displayed similar BAL-f concentrations of the cytokines examined as those who did not respond to CAM. The responders showed a significant decrease in the mean values of IL-6 in the BAL-f after the treatment (32.34 ± 60.65 pg/mL before and 4.13 ± 6.37 pg/mL after treatment;

$p = 0.018$). In the non-responding patients the differences were not analyzed because of small number of cases (Table 4).

Discussion

Beyond specific antibacterial activity, macrolides have unspecific anti-inflammatory activity, which has been used in diffuse panbronchiolitis, atopic dermatitis, asthma, bronchiectases, cystic fibrosis, bronchiolitis obliterans and inflammatory bowel diseases [16–19]. The efficacy of these antibiotics in COP has been demonstrated in few studies [20–21] and in previous research by the present authors [22]. Macrolides influence the production and release of proinflammatory cytokines [23]. It has been shown that these antibiotics inhibit the production of IL-1 and TNF- α in monocytes; IL-1, TNF- α and GM-CSF in mast cells; and IL-8, macrophage inflammatory protein-1 (MIP-1) in macrophages and lymphocytes [7, 24]. The suppressor activity of macrolides on fibroblast-like cells lowers IL-6 and IL-1 concentrations [25]. By inhibiting nuclear factor kappa B (NF- κ b), macrolides decrease the expression of genes involved in the immune response (e.g. i-NOS, COX-2, TNF- α , IL-1 and IL-6) [7, 26]. Macrolides downregulate the activation and migration of neutrophils and IL-8 production, elastase activity and the neutrophil oxidative burst [23–26].

Cai et al. showed that alveolar macrophages increased spontaneous and lipopolysaccharide-stim-

Table 4. Concentration of cytokines in BAL-f in COP patients, before and after the treatment with clarithromycin

	All		Responders		Non-responders		p-value
	number	mean $\pm$ SD	number	mean $\pm$ SD	number	mean $\pm$ SD	
IL1 pg/mL	26	9.29 ± 19.55	22	10.25 ± 20.85	4	3 ± 1.88	0.501
IL1 pg/mL – post	12	5.63 ± 7.37	10	6.23 ± 7.92	2	2.65 ± 3.45	0.556
p-value		0.537		0.2		NA	
IL 6 pg/mL	26	30.56 ± 56.78	22	32.34 ± 60.65	4	14.6 ± 2.87	0.574
IL 6 pg/mL – post	12	4.53 ± 5.84	10	4.13 ± 6.37	2	6.53 ± 0.81	0.62
p-value		0.036		0.018		ns.	
IL 8 pg/mL	26	37.71 ± 34.26	22	38.45 ± 36.53	4	32.54 ± 11.26	0.78
IL 8 pg/mL – post	12	29.91 ± 37.58	10	31.13 ± 40.45	2	17.84 ± 22.85	0.909
p-value		0.756		0.469		ns.	
TGF β pg/mL	26	395.19 ± 274.89	22	420.28 ± 275.31	4	456.75 ± 389.67	0.836
TGF β pg/mL – post	12	356.9 ± 211.21	10	340 ± 220.59	2	424 ± 27.58	0.641
p-value		0.502		0.502		ns.	

ns. – not significant.

ulated production of several cytokines in patients with COP, such as TNF- α , sTNFR1, sTNFR2, IL-1 β , IL-6, IL-8, IL-10 IP-10 and CCL18 [7]. Partial inhibition (30–50%) of this reaction was observed when CAM or azithromycin were added. It was a dose-dependent process comparable to changes induced by dexamethasone. Additionally, a stronger inhibitory effect of CAM than azithromycin on cytokine production was revealed.

Similarly, but in a clinical situation, the present study showed a decrease of serum concentrations of inflammatory cytokines such as IL-6, IL-8, and TGF- β in the COP patients successfully treated with CAM. The patients who did not respond to treatment displayed higher levels of IL-6 in serum after the treatment than the responders. Also, successful therapy with CAM was correlated with a decrease in IL-6 concentration in BAL-f. Interleukin-6 is a pleiotropic cytokine exerting multidirectional effects on cells, and is a key regulator of defense mechanisms. Moreover, it is a stimulator of acute phase protein synthesis in the liver [28]. Overproduction of IL-6 results in a shift from acute to chronic inflammation, neutrophil recruitment, inhibition of neutrophil apoptosis and promotion of monocyte differentiation [29]. Inhibition of IL-6 production is probably one of the main mechanisms of clarithromycin's effects in COP.

Despite theoretical assumptions that IL-1 gene expression is inhibited by macrolides, the patients in the present study did not show any changes in serum or BAL-f concentrations of IL-1 β in connection with the treatment [7, 10, 24].

It has previously been reported that not all patients respond to CAM treatment [20–22]. In the present study, 15% of the patients responded on-

ly partially or did not respond to CAM treatment. The lack of CAM effect was frequently observed in more severely ill patients (those with severe respiratory impairment and lower values of blood oxygenation). In addition, the non-responders did not display a decrease of IL-6 in serum, as was noticed in the patients who responded to treatment and were assessed as healthy. There are no data regarding the correlation between response to CAM treatment and clinical status and/or pulmonary function parameters. However, studies with prednisone in COP revealed that delayed treatment, elevated levels of cholestasis enzymes and hypoxemia were correlated with relapses [30]. In the present study, two relapses were noticed during the observation period (53.08 ± 24.47 months), one in a responder, and one in a non-responder). Because of the small number of patients who relapsed, the specific factors that could have led to reactivation of the disease were not analyzed.

The present study has some limitations. It analyzed only the four cytokines thought to be the most important in the pathogenesis of COP. They represent only a small part of all the possible inflammatory mediators involved in the development of the disease. The most important limitation, however, is the small number of patients, particularly those who did not respond to treatment and in whom BAL was performed. Additionally, the levels of cytokines varied widely. These parameters influenced the power of the statistical analysis.

The authors concluded that IL-6, IL-8 and TGF- β 1 play a significant role in the pathogenesis of COP, and decreases in their concentrations are connected with the resolution of the disease.

References

- [1] Cotin V, Cordier JF: Cryptogenic organizing pneumonia. *Semin Respir Crit Care Med* 2012, 33, 462–475.
- [2] Travis WD, Costabel U, Hansell DM, Lynch DA, Nicholson AG, Ryerson ChJ, Ryu JH, Selman M, Wells AU, Behr J, Bouros D, Brown KK, Colby TV, Collard HR, Cordeiro CR, Cottin V, Crestani B, Drent M, Dudden RF, Egan J, Flaherty K, Hogaboam C, Inoue Y, Johkoh T, Soon Kim D, Kitaichi M, Loyd J, Martinez FJ, Myers J, Protzko S, Raghu G, Richeldi L, Sverzellati N, Swigris J, Valeyre D, ATS/ERS Committee on Idiopathic Interstitial Pneumonias: An official American Thoracic Society/European Respiratory Society statement: Update of international multidisciplinary classification of idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2013, 188, 733–748.
- [3] Drakopanagiotakis F, Paschalaki K, Abu-Hijleh M: Cryptogenic organizing pneumonia. Clinical presentation, radiographic findings, treatment response and prognosis. *Chest* 2011, 139, 893–900.
- [4] Poletti V, Cazzato S, Minicuci N, Zompatori M, Burzi M, Schiattone ML: The diagnostic value of bronchoalveolar lavage and transbronchial biopsy in cryptogenic organizing pneumonia. *Eur Respir J* 1996, 9, 2513–2525.
- [5] Florani S, Ratta L, Bulgheroni A, Cascina A, Paschetto E, Cervio G, Luinetti O, Fietta AM, Meloni F: Cytokine profile of bronchoalveolar lavage in BOOP and UIP. *Sarcoidosis Vasc Diffuse Lung Dis* 2002, 19, 47–53.
- [6] Cai M, Bonella F, He X, Sixt SU, Sarria R, Guzman J, Costabel U: CCL18 in serum, BAL fluid and alveolar macrophage culture supernatant in interstitial lung diseases. *Respir Med* 2013, 107, 1444–1452.
- [7] Cai M, Bonella F, Dai H, Sarria R, Guzman J, Costabel U: Macrolides inhibit cytokine production by alveolar macrophages in bronchiolitis obliterans organizing pneumonia. *Immunobiology* 2013, 218, 930–937.
- [8] Carre C, King TE, Mortensen R, Riches DWH: Cryptogenic organizing pneumonia: increased expression of interleukin 8 and fibronectin genes by alveolar macrophages. *Am Rev Respir Cell Mol Biol* 1994, 10, 100–105.

- [9] Martinez JAB, King TE, Brown K, Jennings CA, Borish L, Mortenson RL, Khan TZ, Bost TW, Riches DW: Increased expression of the interleukin-10 gene by alveolar macrophages in interstitial lung disease. *Am J Physiol* 1997, 273, 676–683.
- [10] Matsuno O, Okubo F, Masutomo K, Yoshida F, Okubo T, Miyazaki E, Kumamoto T: Elevated concentrations of soluble IL-2 receptor in both bronchoalveolar lavage fluid and serum in patients with BOOP. *Tohoku J Exp Med* 2003, 201, 61–65.
- [11] Ye Q, Dai H, Sarria R, Guzman J, Costabel U: Increased expression of tumor necrosis factor receptors in cryptogenic organizing pneumonia. *Respir Med* 2011, 105, 292–297.
- [12] Choi KH, Lee HB, Jeong MY, Jeong MY, Rhee YK, Chung MJ, Kwak YG, Lee YC: The role of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in cryptogenic organizing pneumonia. *Chest* 2002, 121, 1478–1485.
- [13] El-Zammar O, Rosenbamm P, Katzenstein AL: Proliferate activity in fibrosing lung diseases: A comparative study of Ki-67 immunoreactivity in diffuse alveolar damage, bronchiolitis organizing pneumonia, and usual interstitial pneumonia. *Human Pathol* 2009, 40, 1182–1188.
- [14] Lappi-Blanco E, Soni Y, Paako P: Apoptotic activity is increased in the newly formed fibromyxoid connective tissue in bronchiolitis obliterans organizing pneumonia. *Lung* 1999, 177, 367–376.
- [15] Majeski E, Harley RA, Bellum SC, London SD, London L: Differential role for T cells in the development of fibrotic lesions associated with reovirus 1/l-induced bronchiolitis obliterans organizing pneumonia versus acute respiratory distress syndrome. *Am J Respir Cell Mol Biol* 2003, 28, 208–217.
- [16] Amayasu H, Yoshida S, Ebana S, Yamamoto Y, Nishikawa T, Shoji T, Nakagawa H, Hasegawa H, Nakabayashi M, Ishizaki Y: Clarithromycin suppresses bronchial hyperresponsiveness associated with eosinophilic inflammation in patients with asthma. *Ann Allergy Asthma Immunol* 2000, 84, 594–598.
- [17] Corris PA, Ryan VA, Small T, Lordan J, Fisher AJ, Meachery G, Johnson G, Ward C: A randomised controlled trial of azithromycin therapy in bronchiolitis obliterans syndrome (BOS) post lung transplantation. *Thorax* 2015, 70, 442–450.
- [18] Haghi M, Saadat A, Zhu B, Colombo G, King G, Young PM, Traini D: Immunomodulatory effects of a low-dose clarithromycin-based macrolide solution pressurised metered dose inhaler. *Pharm Res* 2015, 32, 2144–2153.
- [19] Southern KW, Barker PM, Solis-Moya A, Patel L: Macrolide antibiotics for cystic fibrosis. *Cochrane Database Syst Rev* 2012, 11, CD002203.
- [20] Ding QL, Lv D, Wang BJ, Zhang QL, Yu YM, Sun SF, Chen ZB, Ma HY, Deng ZC: Macrolide therapy in cryptogenic organizing pneumonia: A case report and literature review. *Exp Ther Med* 2015, 9, 829–834.
- [21] Pathak V, Kuhn JM, Durham C, Funkhouser WK, Henke DC: Macrolide use leads to clinical and radiological improvement in patients with cryptogenic organizing pneumonia. *Ann Am Thorac Soc* 2014, 11, 87–91.
- [22] Radzikowska E, Wiatr E, Gawryluk D, Langfort R, Bestry I, Chabowski M, Roszkowski K: Organizing pneumonia – clarithromycin treatment. *Pneumonol Alergol Pol* 2008, 76, 334–339.
- [23] Kanoh S, Rubin BK: Mechanisms of action and clinical application of macrolides as immunomodulatory medications. *Clin Microbiol Rev* 2010, 23, 590–615.
- [24] Asano T, Ogushi F, Tani K, Tamiya H, Nishioka Y, Sone S: Increased macrophage inflammatory protein-1 alpha and -1beta in BAL fluid of bronchiolitis obliterans organizing pneumonia. *Respirology* 2003, 8, 461–466.
- [25] Shinkai M, Henke MO, Rubin BK: Macrolide antibiotics as immunomodulatory medications: Proposed mechanism of action. *Pharmacol Ther* 2008, 117, 393–405.
- [26] Ichiyama T, Nishikawa M, Yoshitomi T, Hasegawa S, Matsubara T, Hayashi T, Furukawa S: Clarithromycin inhibits NF-kappa B activation in human peripheral blood mononuclear cells and pulmonary epithelial cells. *Antimicrob Agents Chemother* 2001, 45, 44–47.
- [27] Ribeiro CM, Hurd H, Wu Y, Martino ME, Jones L, Brighton B, Boucher RC, O'Neal WK: Azithromycin treatment alters gene expression in inflammatory, lipid metabolism, and cell cycle pathways in well-differentiated human airway epithelia. *PLoS One* 2009, 4, e5806.
- [28] Streetz KL, Wüdstefeld T, Klein C, Manns MP, Trautwein C: Mediators of inflammation and acute phase response in the liver. *Cell Mol Biol (Noisy-le-grand)* 2001, 47, 661–673.
- [29] Rubin A: Interleukin-6 and lung inflammation: Evidence for a causative role in inducing respiratory system resistance increments. *Inflamm Allergy Drug Targets* 2013, 12, 315–321.
- [30] Lazor R, Vandevenne A, Pelletier A, Leclerc P, Court-Fortune I, Cordier JF: Cryptogenic organizing pneumonia. Characteristics of relapses in series of 48 patients. *Am J Respir Crit Care Med* 2000, 162, 571–577.

Address for correspondence:

Elżbieta Radzikowska
Third Department of Lung Diseases
National Tuberculosis and Lung Disease Research Institute
ul. Płocka 26
01-138 Warszawa
Poland
E-mail: e.radzikowska@wp.pl

Conflict of interest: None declared

Received: 22.11.2015

Revised: 1.01.2016

Accepted: 25.02.2016