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BAL fluid cells and pulmonary function in different radiographic stages of newly diagnosed sarcoidosis

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ABSTRACT

Purpose: Sarcoidosis affected lungs detected in more than 90% of patients. The relationship between different stages of pulmonary sarcoidosis and pulmonary function tests (PFT) as well as bronchoalveolar lavage fluid (BALF) cells can be established. Geographic and ethnic factors are known to be linked to the specific characteristics of patients with sarcoidosis. The purpose of the study was to evaluate peculiarities of BALF cells pattern and pulmonary function tests at the time of the diagnosis of different radiographic types of sarcoidosis in a large group of Lithuanian sarcoid patients.

Material and methods: This is the prospective study of BALF cells and PFT of patients with newly diagnosed sarcoidosis. The study population consisted of 221 non-treated non-smoker patients. All patients underwent BAL and the majority of them underwent PFT.

Results: Comparing Stage I to Stage III groups, a slight increase in the macrophage and neutrophil count and a decrease of lymphocyte count was apparent. However, the leukocyte population difference was not statistically significant. We have observed significant increase of CD8 cell count, as well as a decrease of both the CD4 cell count and the CD4/CD8 ratio from Stage I to Stage III. We have determined statistically significant differences in all PFT parameters among the patient groups with different radiographic stages of sarcoidosis.The values of FVC, VC and TLC tended to decrease with an elevation of BALF neutrophils and/or eosinophils count. However BALF cells did not correlate well with PFT indices.

Conclusions: In newly diagnosed sarcoid patients, BALF cell and PFT markers depend on the sarcoidosis stage.

Key words: bronchoscopy, bronchoalveolar lavage, chest X-ray, pulmonary function, sarcoidosis

INTRODUCTION

Sarcoidosis can affect any organ system. In more than 90% of the patients involved [1], the lungs are the organs most likely to be affected. Approximately 50% of patients are asymptomatic at diagnosis, and some of them may never develop clinical symptoms. In these patients, the disease is usually discovered incidentally because of the chest radiograph findings. It should be noted that conventional chest roentgenography is an important diagnostic tool for both the initial evaluation of patients with sarcoidosis and patients monitored during the follow-up period [2]. The etiology of sarcoidosis remains unknown, and currently the course of sarcoidosis cannot be currently predicted at the onset of symptoms by any biological marker.

The current understanding in regard to the pathogenesis of the disease involves the exposure of a genetically susceptible individual to some environmental antigenic stimulus. Following the recognition of these still unknown antigens, the accumulation of immunocompetent cells in the lungs, i.e. alveolitis, occurs. The bronchoalveolar lavage (BAL) findings vary considerably, but a typical feature of pulmonary sarcoidosis is the increase in the percentage of lymphocytes, with predominance of T-helper cells, in the BAL fluid, but lymphocytosis (more than 10% lymphocytes) and/or a CD4/CD8 ratio of > 3.5 in the lavage fluid cell differential suggests

the diagnosis. The infiltration of activated CD4 positive T-cells represents the immunological hallmark of sarcoidosis [3]. Moreover, some findings suggest that a significant elevation of the CD4/CD8 ratio is associated with a favorable prognosis [4]. Conversely, it has been detected that an increased number of neutrophils can be related to more severe sarcoidosis [5]. However, many types of other immune cells, such as macrophages cells, involved in the inflammatory response and fibro genesis may also regulate the course of the disease [6].

Pulmonary function tests (PFT) are crucial to measuring initial lung impairment and provide the baseline for assessment of improvement or deterioration of the lung disease. The most common parameters indicating functional impairment are both diffusion capacity and vital capacity [2]. However, patients with pulmonary sarcoidosis may variously combine a decrease in the lung volume, a loss of diffusion capacity of the lungs for carbon monoxide, airway obstruction, airway hyperactivity, and gas exchange abnormalities during exercise [7-11].

References in literature [12, 13] indicate that the relationship between different stages of pulmonary sarcoidosis and pulmonary function tests, as well as BAL fluid cells, can be found. Geographic and ethnic factors are also known to be linked to the specific characteristics of patients with sarcoidosis [14].

The present study is aimed at the investigation of the peculiarities of BALF cell pattern and pulmonary function tests, at the time of diagnosis, in different radiographic types of sarcoidosis in a large group of Lithuanian sarcoid patients (n = 221).

MATERIALS AND METHODS

This is the prospective study of BAL fluid cells and the pulmonary function of patients with newly diagnosed sarcoidosis. It is a part of the first large sarcoidosis study in Lithuania, launched in 1993. The patients underwent diagnostic methods only as part of routine clinical investigation. The study population consisted of 221 non-treated non-smoking patients who visited the Centre of Pulmonology and Allergology of Vilnius University Hospital Santariškių klinikos between the years 1995 and 2005. Sarcoidosis was diagnosed according to the WASOG guideline [2]. All study patients underwent BAL, and the majority of the participating subjects underwent PFT as well, although the number of them was smaller. Eighty-nine (89) (39%) patients were asymptomatic, 138 (61%) had sarcoidosis-related, mostly Löfgrens syndrome, symptoms. Chest radiography was performed in posterior/anterior and lateral projections. Radiographs were classified according to the chest radiographic stages of sarcoidosis (0-IV): Stage I, nodal enlargement only; Stage II, nodal enlargement and lung shadowing; Stage III, lung shadowing only; Stage IV, lung fibrosis. There were 6 patients with stage 0 sarcoidosis (clear chest radiograph), but due to the small number they were not included in the study. There was no Stage IV patient.

Table 1. **Demographic data of the study population according. To the chest radiographic stages**

Demographics	Stage I (n = 147)	Stage II (n = 46)	Stage III (n = 28)
Age, yr	36 ± 10	38 ± 12	36 ± 8
Sex, No			
Male	47	24	13
Female	100	22	15
Race, %			
White	100	100	100

None of the subjects had any relevant medical history or co-morbidity. No patient had a history of exposure to organic or mineral dust that is known to cause granulomatous lung disease. There was no difference in age distribution between the groups; the demographic data of the study population according to chest radiographic stages of sarcoidosis are summarized in *Tab. 1*. A signed information consent form was obtained from all participants. The study has been approved by the Committee on Biomedical Ethics of the Vilnius University Hospital Santariškių klinikos.

Pulmonary function testing included the measurement of the forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), a determination of total lung capacity (TLC), vital capacity (VC) with body plethysmography, and diffusing capacity of carbon monoxide (DL_{CO}) with standard single-breath technique (*WMAX*). Results were corrected for hemoglobin concentration. Respiratory function impairment was defined as DL_{CO} < 80%, FVC < 80%, VC < 80%, or FEV_1 < 80% [15]. All study related investigations for every single patient were performed within 1 week.

Oxygen tension ($P O_2$) and carbon dioxide tension ($P CO_2$) in arterialized capillary samples were measured at rest without supplementary oxygen. Furthermore, capillary blood gases are a sufficiently accurate substitution for arterial sampling in routine clinical practice for patients in the absence of hypotension [16, 17]; routinely, we do not obtain arterial blood samples for non-critically ill patients.

Fiberoptic bronchoscopy and BAL were performed as described elsewhere [18]. Subjects were premedicated with atropine, and lidocaine was topically delivered *via* an atomizer. The bronchoscope was inserted transnasally (in most cases) or orally and passed to segmental or subsegmental bronchus. BAL was performed in the right middle lobe, lingula or in the area of greatest radiological abnormality. Sterile isotonic saline at room temperature was instilled in two 50 ml aliquots. Each aliquot was retrieved with gentle manual aspiration. Only second aliquot was analyzed. Through all the study period we have used the same BAL as well as BALF analysis method.

BALF for cell analysis was filtered through a 70 mm pore filter to remove mucus, then sedimental cellular material was obtained by centrifugation (600g for 10 min. at 4°C). Differential counts of 600 BALF cells were performed on May-Grunwald-Giemsa stained preparations. The viability of BALF cells was determined by 0.4% Trypan blue dye

Table 2. **Characteristics of BALF Cell Counts*.**

Cells	Stage I (n = 147)	Stage II (n = 46)	Stage III (n = 28)
Cell (x 106/ml)	406 ± 299	447 ± 370	404 ± 487
Macrophages, %	49 ± 17	51 ± 16	52 ± 23
Lymphocytes, %	45 ± 17	42 ± 16	40 ± 21
Neutrophils, %	5 ± 3	6 ±4	7 ± 7
Eosinophils, %	1 ± 1	1 ± 1	1 ± 1

* Data are presented as mean ± SD. There was no significant difference among the groups.

Table 3. **BALF T lymphocyte subsets*.**

Cells	Stage I (n = 147)	Stage II (n = 46)	Stage III (n = 28)
CD4, %	81 ± 10	78 ± 10	68 ± 13
CD8, %	11 ± 6	15 ± 6	21 ± 11
CD4/CD8	10 ± 5	7 ± 4	5 ± 3

* Data are presented as mean ± SD. There was a significant difference in CD4, CD8, CD4/CD8 ratio among the groups (Kruskal-Wallis test, $p < 0.001$).

exclusion (this method is based on the principle that live cells do not take up certain dyes, whereas dead cells do). For lymphocyte subpopulation analysis, 100 µl of cell suspensions ($1x10^6$ cells) were incubated for 15 min at 4°C with 20 µl FITC or PE conjugated monoclonal antibody (Becton Dickinson). Following the incubation, 2 ml of cold PBS was added to the pellet and washed at 300g for 10 min at 4° C. Prepared BALF samples were examined by flow cytometry (FACSCalibur, Becton Dickinson).

Statistical methods: Group data were expressed as mean values ± SDs. The pulmonary function parameters were analyzed for variation with radiographic stage employing one-way ANOVA. Differential cell counts of BALF and lymphocyte subsets were compared with Kruskal-Wallis test. Spearman's rank correlation coefficient was used to study correlation between BALF cells and pulmonary function tests. In all tests a p-value of < 0.05 was considered to be statistically significant.

RESULTS

The volume of BAL fluid recovered was 65 ± 10 ml (range from 50 ml to 85 ml). The percentage the viability of the bronchoalveolar lavage fluid cells was 97 ± 3%.

Differential Cell Counts of BALF and Lymphocyte Subsets The BAL differential cell populations are shown in *Tab. 2*. Together with the well-known considerable increase of cellularity, e.g. lymphocytosis, a mild increase of the number of neutrophils can be noticed. When comparing groups of Stage I to Stage III, a slight increase in macrophage and the neutrophil count and diminishing in the lymphocyte count is apparent. However, the difference of leukocyte populations among the patient groups was not statistically significant. Spontaneous macrophage-lymphocytes rosettes (adherence of lymphocytes to alveolar macrophages) in BALF were found equally frequently in all groups – 69% of Stage I patients, 71% of Stage II and 75% of Stage III patients.

The increase of CD4/CD8 ratio in all groups is evident in *Tab. 3*. We have observed a significant increase of CD8 cell count, as well as the decrease of both the $CD4^+$ cell count and the CD4/CD8 ratio of Stage I to Stage III of sarcoidosis ($p < 0.001$).

Pulmonary function parameters. Only the minority of our patients had a significant reduction in the lung volume and/or airflow obstruction. The most frequently observed disorder of the lung function was impairment of the diffusing capacity of carbon monoxide. The percentage of the patients with normal lung function parameters is shown in *Fig. 1*. We determined statistically significant differences in all pulmonary function parameters among the patient groups with different radiographic stages of sarcoidosis. Results of PFT are summarized in Tab. 4.

Endobronchial involvement. Endobronchial abnormality (erythema, nodules, etc.) has been found in 6% of our patients. Interestingly, these changes were more common in advanced radiographic stages: 2% of Stage I patients, 11% of Stage II and 21% of Stage III patients had endobronchial abnormalities.

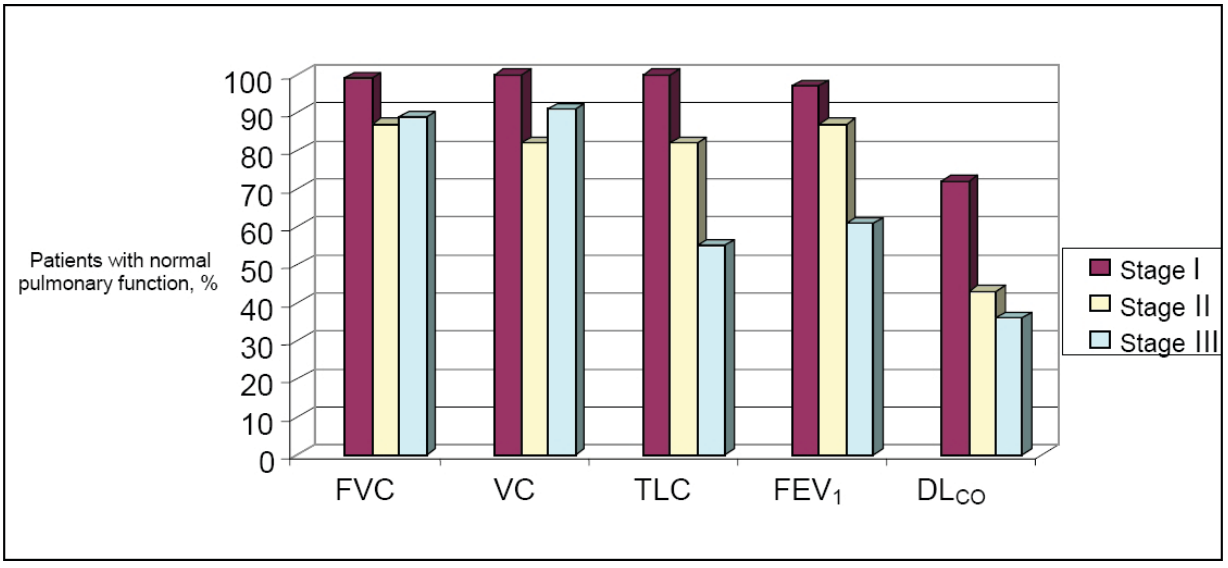
Correlation of BALF cell count with the results of pulmonary function tests. The values of FVC, VC and TLC tended to decrease with the elevation of the BALF the

Table 4. **Parameters of pulmonary function*.**

Parameter	Stage I (No of pts)	Stage II (No of pts)	Stage III (No of pts)	p
FVC, % pred	106 ± 14 (102)	99 ± 18 (31)	87 ± 15 (18)	< 0.001
FEV1, % pred	102 ± 15 (102)	95 ±1 6 (31)	86 ± 17 (18)	< 0.001
VC, % pred	109 ± 13 (49)	104 ± 19 (17)	90 ± 15 (11)	< 0.01
TLC, % pred	100 ± 11 (47)	95 ± 14 (17)	84 ± 15 (11)	< 0.01
DLCO, % pred	90 ± 14 (47)	79 ± 15 (14)	75 ± 12 (11)	< 0.01
P O2, mm Hg	70 ± 6 (93)	66 ± 5 (27)	64 ± 5 (20)	< 0.001
P CO2, mm Hg	39±3 (93)	41 ± 4 (27)	42 ± 4 (20)	< 0.05

* Data are presented as mean ± SD. FVC – forced vital capacity, VC – vital capacity, TLC – total lung capacity, FEV1 – forced expiratory volume in one second, DLCO – diffusing capacity of carbon monoxide, P O2 – oxygen tension, P CO2 – dioxide tension. Pred –predicted. There was a significant difference in all parameters among the groups (ANOVA).

Figure 1. **Percentage of patients with normal value of pulmonary function parameters*.**



* FVC – forced vital capacity, VC – vital capacity, TLC – total lung capacity, FEV1 –forced expiratory volume in one second, DLCO – diffusing capacity of carbon monoxide.

neutrophils and/or the eosinophils count. However, BALF cells did not correlate well with PFT indices. We observed the deterioration of FVC and PO_2 parameters with the decrease in CD4 and the increase in CD8 cells counts (data not shown).

DISCUSSION

This study evaluated the BALF cells and pulmonary function tests in different radiographic types of sarcoid patients with newly diagnosed disease. The key findings in our study are as follows: (1)There is a statistically significant decrease of the CD4 T-cells count and the CD4/CD8 ratio as well as a heightened CD8 cell count with an increasing radiographic stage of sarcoidosis; and, (2) there is a statistically significant decrease of the parameters of pulmonary function in advanced stages of sarcoidosis; and, (3) there is an absence of good correlation between BAL fluid cells and PFT indices. An increase in the endobronchial abnormality rate together with an increase in the stage of sarcoidosis has also been found.

Our finding is that the decrease of both the CD4 T-cell count and the CD4/CD8 ratio with an increase in the stage of sarcoidosis is in agreement with Capelli and co-workers [13]. Previous studies [4,6] demonstrated that a high lymphocyte count, a high CD4 cell percentage, and a high CD4/CD8 ratio in BALF were associated with good prognosis. Ziora et al. [19] evaluated the homogeneity of alveolitis by estimating lymphocyte subsets in BALF and performing high-resolution computed tomography among patients with sarcoidosis. Interestingly, they demonstrated that, in the extensively involved lung sites of the patients with non-homogenous involvement of disease, there were substantially more lymphocytes and higher CD4 to CD8 ratios compared to the less involved sites.

The percentage of BALF neutrophils tended to increase with radiographic stage, although it was not statistically significant. Capelli and co-workers [13] observed augmented neutrophil and eosinophil counts with the more advanced sarcoidosis stage. Increased BALF neutrophil and/or eosinophil counts reflect an ongoing inflammatory process in the lungs, and it is associated with a more advanced, chronic disease course, functional pulmonary impairment, poor response to corticosteroid treatment and persisting abnormal chest radiographs [6, 20]. The time point of the onset of the disease for the individual patient, with the exception of patients with Löfgren‘s syndrome, is not commonly known. We have assumed that the investigation of our patients took place shortly after the onset of the disease, while none of our patients had the final stage of lung fibrosis (IV stage of sarcoidosis). The explanation for this phenomenon could be simple; most of the population of Lithuania underwent chest X-ray yearly or once in two years.

Spontaneous macrophage-lymphocytes rosettes in BALF from active sarcoid patients [21] have been found, probably because of active antigen presentation at the focus of inflammation. It is known that alveolar macrophages (AM) of sarcoid patients express larger surface amounts of class II MHC molecules and different adhesion molecules necessary for interactions with T cells, and they have been shown to be a more potent antigen presenting cells than normal AM [22]. The increase in macrophage-lymphocytes clusters in BAL cells is not specific for granulomatous disease and is associated with the increase in BAL lymphocytes count [23]. We can speculate that the finding of macrophage-lymphocytes rosettes in nearly all the examined samples is the biomarker of the active persistent immune inflammation in the lung of our patient groups.

Lung function tests provide information about the presence of respiratory functional impairment, one of the indicators of disease severity [2]. The study data show that PFT determination during the spirometry is not sufficient for the full evaluation of the respiratory function. These determinants were normal for most of I and II Stage patients; nevertheless, dysfunction has been found while performing the gas diffusion test (*Fig. 1.*).

In sarcoidosis, alteration of DL_{CO} may reflect changes in the gas exchange area, barrier thickness, and ventilation-perfusion-diffusion mismatching of the lung. Alveolar capillary membrane diffusing capacity is the major determinant of impaired DL_{CO} [11].

Recently Handa et al. [24] demonstrated that the frequency of airflow limitation in Japanese sarcoid patients was 8.8%. Similar to our findings, they had shown that patients with airflow limitations predominantly had the advanced chest radiographic stage. Granulomatous sarcoid lesion, bronchial wall compression by enlarged lymph nodes, airway fibrotic scarring or fibrosis of the respiratory tract can stipulate bronchial obstruction. Endobronchial involvement significantly increases the risk for airway hyperactivity [10], causes obstructive ventilatory defect, and occasionally bronchial stenosis [25]. Previous studies [26], which demonstrated racial differences in endobronchial involvement, established both visually as well as endobronchial biopsy results.

We had found significant differences in all PFT parameters among the groups with different radiographic stages of sarcoidosis *(Tab. 4)*: PFT parameters significantly decreased from sarcoidosis Stage I to Stage III. These findings are similar to those described by Baydur et al. [8] and Bergin et al. [12]. These authors had determined the trend that the PTF markers decrease with the increase of the radiological sarcoidosis stage. Our suggestion is that our results are statistically significant due to a much larger group of patients in our study (There were 221 patients in our group versus 36 patients [8] and 27 patients [12]).

Computer tomography (CT) is more sensitive in the evaluation of the lung parenchyma, and in assessing response to therapy or the progression to fibrosis [27,28]. Moreover, patterns of parenchymal sarcoidosis seen at CT correlate with the pulmonary function tests [12,28]. Unfortunately, the interpretation of functional-morphologic correlation in sarcoidosis is complicated by the variable CT features and the heterogeneity of functional impairment, which may be restrictive, obstructive, or a complex combination of both types; therefore, CT may not be superior in enabling predictions of functional impairment in comparison to chest radiography [29]. Consequently, conventional chest X-ray examination is currently recommended for sarcoidosis classification and patients' follow-up in clinical practice [2].

Nevertheless, we have to notice that there are several limitations to our study. A comprehensive PTF study was not available for all of the patients at the time of diagnosis, but the majority of the patients were available for the follow up (up to 10 years). They periodically underwent chest radiography and PFT examination. (The data is being prepared for publication.) We measured oxygen tension ($P O_2$) and carbon dioxide tension ($P CO_2$) in arterialized capillary instead of in arterial blood samples. It is important to mention that several authors [16,17] concluded that capillary blood gases are sufficiently accurate as a substitution for arterial sampling in routine clinical practice in the absence of hypotension. Although our aim was not only to investigate particular blood gas markers but to evaluate the change during the course of the disease, we had chosen capillary blood gases investigation, which is much easier both for the investigator and the patient. The BAL method currently in use (two 50 ml boluses) is also slightly different from used by other authors. However, the greatest technical variation in carrying out bronchoalveolar lavage is related to the volume of fluid used [18]. Therefore, our investigation shows a difference in the BALF cell pattern in newly diagnosed sarcoid patients with each different stage. In the more advanced stage of the sarcoidosis, the cell count associated with early alveolitis decreases, while the cell count associated with pulmonary fibrosis is increases. Most of the newly diagnosed patients do not have (or have slightly) abnormal PFT findings. In more advanced stages of the disease, greater PFT lesion and endobronchial abnormality is found.

We conclude that, in newly diagnosed sarcoid patients, the BAL fluid cell and PFT markers depend on the stage of sarcoidosis.

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