Do T-Lymphocyte Subtypes Profiles of Brochoalveolar Lavage Fluid Change According to Stages of Pulmonary Sarcoidosis?

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Summary

The aim of this study was to determine T lymphocyte subtypes in bronchoalveolar lavage (BAL) fluid of sarcoidosis patients in different stages of disease. Fourthy-eight patients mean age 45.9 ±1.7 years, (range 27 to 75 years.) with histologically verified sarcoidosis were studied. They were divided into three groups, based on their clinical presentation and radiologic findings. According to radiologic staging, 14 patients were Stage1, 29 were Stage 2, and 5 were Stage3. BAL was performed by the instillation of total of 100 ml in to the middle lobe or the lingula with immediate aspiration after each aliquot. After filtration through two layers of gauze, the recovered fluid was centrifuged and the cells were counted in a haemocytometer. Air dried smears were stained with May-Grunwald Giemsa stain for differantial cell counts. At least 600 cells were counted. Quantitative analysis of CD3+, CD4+, CD8+ and other T-lymphocyte subtypes was done by flow cytometry. There was not any significant difference in total cell count and lymphocyte percentages of patient with different stages of sarcoidosis . The mean CD4/CD8 ratio of the whole group was 4.1 ± 0.3, that was supporting the diagnosis of sarcoidosis .The CD4/CD8 ratio of patients in Stage 1 was higher than patients in Stage1 and Stage 3. The proportion of CD4+ cells in BAL fluid was elevated in patient with Stage 1 and Stage 2 sarcoidosis compared to Stage 3. There was not any significant difference in the CD3+, CD4+,CD8+, CD19+, CD56+, CD25+, lymphocytes subtypes among stages. There was a significant negative correlation between stages of the disease and CD4/CD8 ratio (r= -0.377, p<0.05). We came to the conclusion that the number and distributions of BAL T lymphocytes subsets may constitute a biological indicator for diagnostic orientation, but they do not distinguish sufficiently between the different groups of sarcoidosis to be of any prognostic value.
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Key Words: Sarcoidosis, BAL, lymphocytes subtypes

Özet

Akciğer Sarkoidozunun Evresine Göre BAL Sıvısı T-Lenfosit Alt Gruplarında Farklılık Olabilir mi?

Bu çalışmanın amacı sarkoidoz hastalarında, hastalığın farklı evrelerinde BAL sıvısında saptanan T lenfosit alt gruplarını belirlemekti. Araştırmaya histolojik olarak tanısı konmuş ortalama yaşı 45.9 ±1.7 yıl (27- 75 yaş) olan 48 hasta alındı. Hastalar klinik ve radyolojik bulgularına göre 3 gruba ayrıldı. Radyolojik evrelendirmeye göre 14 hasta evre 1, 29 hasta evre 2, 5 hasta evre 3 grubundaydı. BAL 100 ml serum fizyolojiğin orta lob veya lingulaya verilip hemen geri alınması ile yapıldı. Geri dönen BAL sıvısı iki kat gazlı bezden süzüldükten sonra santrifüj edildi ve hücre sayısı hemositometrede sayıldı. Preparatlar differensiyal sitolojik inceleme için May-Grunwald Giemsa boyası ile boyandı ve 600 hücre sayıldı. CD3+, CD4+, CD8+ ve diğer T-lenfosit alt gruplarının kantitatif analizi flow sitometre kullanılarak yapıldı. Sarkoidozun farklı evrelerindeki hastalarda BAL total hücre sayısı ve T lenfosit oranlarında farklılık saptanmadı. Sarkoidoz tanısını destekler şekilde bütün grubun ortalama CD4/CD8 oranı 4.1 ± 0.3 bulundu. Evre 1 deki hastaların CD4/CD8 oranı evre 2 ve evre 3 deki hastaların oranından daha yüksekti. BAL sıvısı CD4+ hücre oranı evre 1 ve evre 2 ' deki hastalarda evre 3'e göre daha yüksekti. Hastalar arasında BAL sıvısı CD3+, CD4+, CD8+, CD19+, CD56+, CD25+ lenfosit alt grupları oranları arasında fark yoktu. Hastalığın evreleri ile CD4/CD8 oranları arasında negatif korelasyon saptandı (r= -0.377, p<0.05). Araştırmanın sonucunda BAL sıvısı lenfosit sayılarının ve dağılımlarının tanısal olarak önemi olsa bile sarkoidozun farklı evrelerinin ayırımında yeterli olamadıkları için prognostik olamayacağı kanısına vardık.
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Anahtar Kelimeler: Sarkoidoz, BAL, T lenfosit alt grupları

Introduction

Sarcoidosis is a systemic chronic granulomatous disease of unknown aetiology which primarily affects the lungs in more than 90% of patients (1-3). The disease characterized by an initial lymphocyte alveolitis and the development of granulomata within the lung interstitium. But these baseline knowledge of the immunopathogenesis of the disease has stemmed from analysis of bronchoalveolar lavage (BAL) (4,5).

A typical feature of pulmonary sarcoidosis is an increase

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in the percentage of BAL fluid (BALF) lymphocytes with an accumulation of T-helper cells in the lung, resulting in an increased BALF lymphocyte CD4/CD8 ratio (6). In early stages of the disease there is a mononuclear cell alveolitis dominated by activated CD4+ T cells and macrophages. These immunologically active cells release mediators, which appear to attract additional monocytes and induce formation of the characteristic non-caseating granulomas and, in a subgroup of patients, to fibrosis and permanently impaired lung function (3-5,7,8).

Several investigators have attempted, with conflicting results, to identify bronchoalveolar lavage indices that could give information on the inflammatory activity and progress of the disease in patients with pulmonary sarcoidosis (4) . The increase in lymphocyte numbers with a predominance of CD4+ T-lymphocytes in BAL fluid during the initial inflammatory process of the disease has been used as an adjunct to the clinical and histological assessment of the patient suspected to have sarcoidosis. But it is also reported that some patients with sarcoidosis had an alveolitis in which the CD8+ (suppressor/cytotoxic) T-lymphocyte was the predominant T-cell type (9,10,11). Although there are some studies investigating relationship between BAL fluid CD4/CD8 ratio and prognosis of sarcoidosis patients, they were not focused on changes in CD4/CD8 ratio according the staging of the diseases. In this study we aimed to investigate BAL findings and T lymphocyte subtypes of sarcoidosis patients in different stages of disease .

Method

Study population

Fourthy-eight patients (34 F, 14 M) mean age 45.9 ± 1.7 years, (range 27 to 75 years.) with histologically verified sarcoidosis were studied. They were divided into three groups, based on their clinical presentation and radiologic findings. According to radiologic staging, 14 patients were Stage1, 29 were Stage 2, and 5 were Stage 3.

Bronchoalveolar lavage

BAL was performed by the instillation of total of 100 ml of 0.9% saline solution in five 20 ml aliquots in to the middle lob or the lingula with immediate aspiration after each aliquot. After filtration through two layers of gauze, the recovered fluid was centrifuged and the cells were counted in a haemocytometer. Air dried smears were stained with May-Grunwald Giemsa stain for differantial cell counts. At least 600 cells were counted. Quantitative analysis of CD3+, CD4+, CD8+ and other T-lymphocyte subtypes was done by flow cytometry.

Statistical Analysis

Data were expressed as mean ± SD. Between the groups, the data were compared using the Kruskall-Wallis and the Mann Whitney U test. The Pearson test was used to examine the correlation between stage of disease and T lymphocyte subtypes. A p value of <0.05 was accepted as statistically significant.

Results

There was not any significant difference in total cell count and lymphocyte percentages of patient with different stages of sarcoidosis . (Table). The mean CD4/CD8 ratio of the whole group was 4.1 ± 0.3, that was supporting the diagnosis of sarcoidosis. The CD4/CD8 ratio of patients in Stage 1 was higher than patients in Stage2 and Stage 3 ($p>0.05$) (Figure 1).

The proportion of CD4+ cells in BAL fluid was elevated in patient with Stage 1 and Stage 2 sarcoidosis compared to Stage 3, but not significant ($p>0.05$). There was not any significant difference in the CD3+, CD4+,CD8+ , CD19+, CD56+, CD11+, CD25+, CD45+ lymphocytes subtypes among stages ($p>0.05$).

Correlation analysis between different stages of sarcoidosis and BAL lymphocytes subtypes were done by using Pearson's correlation test. There was a significant negative correlation between stages of the disease and CD4/CD8 ratio ($r= -0.377$, $p<0.05$). There was not any other correlations among lymphocyte subtypes, and any stage of the disease .

Table I: BAL cytology in different stages of sarcoidosis patients.

	Stage 1	Stage 2	Stage 3	Total
Total Number of Cells x 10^6	21.9±10.5	13.4± 1.0	12.1± 0.4	14.2±1.4
Macrophages %	56.5±11.2	73.3±3.6	71.6±3.9	70.6±3.1
Lymphocytes %	42.5± 10.9	24.1±3.5	22.0±2.5	26.3±3
Neutrophils %	1.3±0.3	4.5±1.1	8.0±1.3	4.7±0.9
CD4 + % of Lymphocytes	62.2±8.5	59±4.1	40.3±6.7	57.5±3.5
CD8 + % of Lymphocytes	11.8±1.4	17±1.1	13.7±0.6	15.6±0.9
CD4 / CD8 ratio	5.2±0.5	3.8±0.3	3.4±0.3	4.1±0.3
CD19 + % of Lymphocytes	1.9±0.5	2.9±1.1	2.8±0.9	2.6 ± 0.7
CD25 + % of Lymphocytes	10.5±4.4	13.1±2.3	21.4±8.3	13.3±2.0
CD56 + % of Lymphocytes	2.1±0.3	6.6±1.3	2.2±0.8	5.7±2.1

Discussion

Sarcoidosis have a characteristic BAL finding of increased lymphocytes, especially CD4 type and high CD4/CD8 ratio. It has been suggested that increased number of lymphocytes with high proportion of CD4+ cells, without evidence of fungal or tuberculous infection, could be sufficient for the diagnosis of sarcoidosis (12). Therefore, BAL is one of the valuable techniques used to evaluate sarcoidosis patients (13-15). In this study, we found high CD4/CD8 ratio in all stages of the disease with lymphocytoses in BAL.

In diagnosing sarcoidosis, differential cytologic examination of BAL cell population is used with some limitations (16-18). Most of the patients with sarcoidosis have an increased percentage of lymphocytes in BAL, but this finding is not specific enough, since lymphocytic alveolitis might be detected in extrinsic allergic alveolitis, tuberculosis, drug induced lung diseases and various other disorders (19,20). Also, lymphocytosis in BAL is not a observed in all in sarcoidosis patients. Kantrow et all. was demonstrated that, one third of the patients with biopsy-proven sarcoidosis had lymphocytes lee than 16% in BAL (21).

Specificty of BAL findings in sarcoidosis could be enhanced by using the criteria of the CD4:CD8 ratio (22). The majority of patients with an elevated CD4/CD8 ratio have sarcoidosis, however, there were some interstitial lung diseases with high ratios such as tuberculosis, drug induced lung disease, collagen vascular diseases related lung fibrosis, and malignancy (15, 21, 23). Winterbauer et al. reported that among 55 patients with interstitial lung disease, and with lymphocytosis in BAL, CD4/CD8 ratio (determined only on subjects with >16% lymphocytes in BAL) greater than 4:1 had a positive predictive value of 94% for sarcoidosis. Katrow et al. demonstrated that over 40 % of the sarcoidosis patients had a CD4/ CD8 ratio greater than 3.5 (24) and 4 (21).

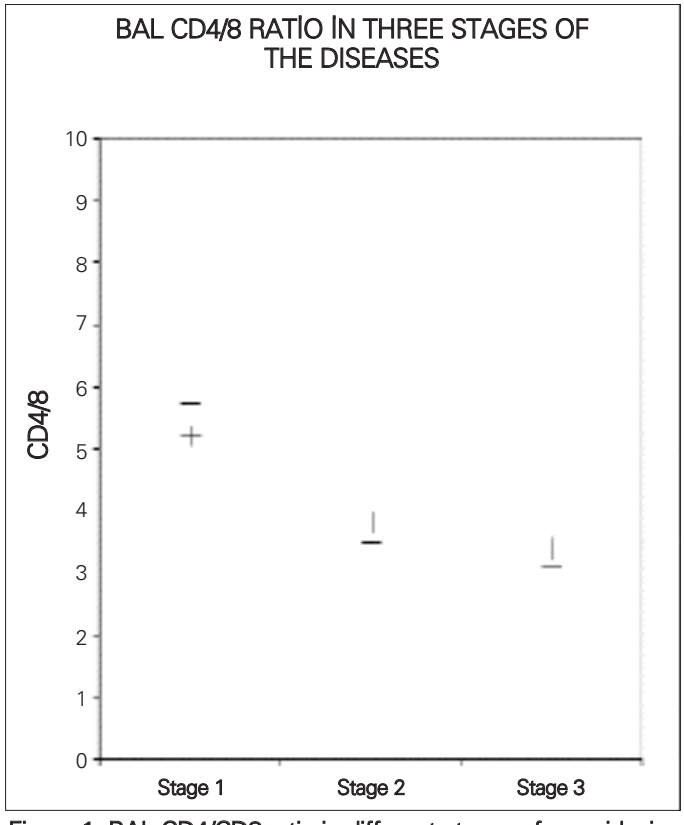

Figure 1: BAL CD4/CD8 ratio in different stages of sarcoidosis.

Although a high CD4:CD8 ratio supports the diagnose of the disease, a normal or low ratio does not exclude the sarcoidosis. The CD8+ lymphocytes predominance is an unexplained finding in patients low or normal CD8 ratio. BAL and lung biopsy findings from patients with sarcoidosis suggested that the influx of CD4+ lymphocytes typically seen early in the alveolitis may be replaced by CD8+ lymphocytes as the disease stabilizes or becomes inactive (25, 26).

Finding of BAL fluid CD4:CD8 ratio within the normal limits (19) might indicate resolution of the disease or response to therapy (27,28). On the other hand, in some studies it was demonstrated that a small number of sarcoidosis patients had a low CD4:CD8 ratio and a poor prognosis (11). Subsequent reports have not demonstrated an association of the CD4/CD8 ratio with duration of symptoms or radiographic findings (29). In predicting the course and prognosis of sarcoidosis, usefulness of BAL fluid cellular analysis is still controversial. Most researchers have focused upon the intensity of lymphocytic alveolitis and the CD4/CD8 ratio with various results. Some investigators have observed a strong correlation between the clinical presentation of sarcoidosis and an elevated CD4/ CD8 ratio (25,26,30) .In patients with stage 1 disease and particular type of clinical presentation (bilateral hilar adenopathy with erythema nodosum or uveitis), the CD4/CD8 ratios tend to be substantially higher than asymptomatic patients with sarcoidosis (30). Whereas others demonstrated that a high lymphocyte count and CD4/CD8 ratio might be a sign of resulition in the disease (25,31).

Some authors found that a high percentage of BALF lymphocytes predicts functional deterioration (32). In other studies, neither the percentage of BALF lymphocytes nor the CD4/CD8 ratio was of predictive value (33,34), such as Ziegenhagen et al demonstrated that the percentage of lymphocytes and CD4/CD8 ratio can not be a reflection of severity disease, and also can not indicate higher risk of necessity of steroid therapy (6).

Finally, in this study we evaluated if the T lymphocytes subtypes of BAL fluid reflect stage of the sarcoidosis. Although previously it was well established that BAL fluid lymphocytes with high CD4/CD8 ratio were helpful in diagnosis of sarcoidosis, we found that the percentage of BAL lymphocytes and the BAL lymphocyte CD4/CD8 ratio did not distinguish sufficiently the different stage of sarcoidosis, and any T cell subtypes is not corraletes with the stage of the disease.

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