Radiographic Staging and Bronchoalveolar Lavage Cell Counts in Sarcoidosis

S. Vidal Serrano, J. Martín Juan, L. Gómez Izquierdo, I. Sánchez Rodríguez, E. Rodríguez Becerra, and F. Rodríguez Panadero

OBJECTIVE: Sarcoidosis is a multisystem granulomatous inflammatory disease of unknown etiology that mainly affects the lungs and lymph nodes. Bronchoalveolar lavage (BAL) is known to be useful in diagnosis of the disease but its value as a prognostic marker is unclear. The aim of this study was to assess whether there is a characteristic pattern in BAL cell counts according to radiographic stage and determine whether BAL offers information on disease course.

PATIENTS AND METHODS: The study included 34 patients with untreated sarcoidosis. Data were collected on the following variables: age, sex, smoking habit, treatment type, radiographic stage, respiratory function, serological parameters, and BAL cell counts. The patients were classified into 3 groups according to functional and radiographic change at 12-month follow-up.

RESULTS: No differences in age, sex, or smoking habit were found according to either radiographic stage or disease course. Although the proportion of lymphocytes in BAL fluid was higher in radiographic stage I than in stages II and III, the differences were not statistically significant. The differences in BAL cell counts between groups based on disease course were not statistically significant.

CONCLUSIONS: No differences were found in the characteristics of BAL fluid according to radiographic stage. The differential cell count in BAL fluid does not appear to predict the course of sarcoidosis in the first 12 months.

Key words: Sarcoidosis. Radiographic stages. Bronchoalveolar lavage.

Estadios radiológicos y lavado broncoalveolar en la sarcoidosis

OBJETIVO: La sarcoidosis es una enfermedad inflamatoria granulomatosa multisistémica de causa desconocida que afecta principalmente al pulmón y a los ganglios linfáticos. La utilidad del lavado broncoalveolar (LBA) en el diagnóstico es conocida, pero su valor como marcador pronóstico es controvertido. El objetivo de nuestro estudio es evaluar si existe un patrón característico en la celularidad del LBA según el estadio radiológico de presentación y determinar si el LBA aporta información sobre la evolución de la enfermedad.

PACIENTES Y MÉTODOS: Se incluyó en el estudio a 34 pacientes con sarcoidosis no tratados. Se recogieron las siguientes variables: edad, sexo, hábito tabáquico, tipo de tratamiento, estadio radiológico, exploración funcional respiratoria, parámetros serológicos y análisis celular del LBA. Se clasificó a los pacientes en 3 grupos según la evolución funcional y radiológica a los 12 meses.

RESULTADOS: No se encontraron diferencias entre la edad, el sexo y el hábito tabáquico ni entre los estadios radiológicos ni entre los grupos según evolución. En el estadio radiológico I el recuento porcentual de linfocitos del LBA fue mayor que en los estadios II y III, pero las diferencias no fueron estadísticamente significativas. Las diferencias en el LBA por grupos evolutivos no fueron estadísticamente significativas.

CONCLUSIONES: Al analizar las características del LBA según estadios radiológicos no se encontraron diferencias. El recuento diferencial de células en el LBA no parece predecir el curso de la sarcoidosis durante los primeros 12 meses.

Palabras clave: Sarcoidosis. Estadios radiológicos. Lavado broncoalveolar.

Introduction

Sarcoidosis is a multisystem granulomatous disease of unknown etiology that has a wide range of clinical manifestations. The histopathology of the disease is characterized by the presence of noncaseating granulomas that can appear in any organ but are most commonly located in the lungs and lymph nodes.

The normal course of the disease is highly variable and difficult to predict, and cases can range from being asymptomatic to displaying a progressive course that leads to pulmonary fibrosis. Despite advances in imaging technology and the analysis of immune and inflammatory processes that occur in sarcoidosis, prognostic factors that allow disease severity to be determined at diagnosis...
remain to be identified. The usefulness of Siltzbach’s²
geradiographic classification of sarcoidosis as an indicator
doctrine activity or prognosis has not been clearly
demonstrated.³ Furthermore, although bronchoalveolar
lavage (BAL) is used systematically for the diagnosis of
sarcoidosis, studies aimed at evaluating the usefulness of
BAL cell counts as predictors of disease course have
yielded inconclusive results.⁴⁻⁹

The aim of this study was to determine whether BAL
fluid exhibits a characteristic cell profile according to
radiographic stage in pulmonary sarcoidosis and to
determine whether cell analysis provides information
about disease course at 12-month follow-up.

Patients and Methods

Subjects and Study Protocol

The study group consisted of 34 patients diagnosed with
sarcoidosis that had not been treated with corticosteroids.
Diagnosis of the disease was confirmed by histology in patients
with compatible clinical and radiographic manifestations.¹⁰
Fiberoptic bronchoscopy with transbronchial biopsy and BAL
was performed in all patients. Other causes of granulomatous
inflammation were excluded by microbiological analysis of
BAL fluid for the presence of fungi and mycobacteria. In
patients with a negative transbronchial biopsy, pathologic
diagnosis was obtained by lung biopsy, mediastinoscopy,
lymph node biopsy, or biopsy of cutaneous lesions.

The following data were collected at diagnosis: age, sex,
smoking habit, radiographic stage, results of symptoms
questionnaire, respiratory function, serum concentration of
angiotensin-converting enzyme (ACE), calcium levels,
calcuria at 24 hours, and BAL cell counts.

Four radiographic stages were recognized at diagnosis
based on the classification described by Siltzbach:² stage 0
(no thoracic involvement), stage I (bilateral hilar
lymphadenopathy), stage II (bilateral hilar lymphadenopathy
and lung infiltrates), and stage III (lung infiltrates without
lymphadenopathy).

At 12-month follow-up, disease course was evaluated by
assessment of functional and radiographic change. The criteria
of Hunninghake et al¹¹ were used to assess deterioration of
respiratory function. A reduction of at least 15% compared
with initial values was considered significant for forced vital
capacity (FVC) and forced expiratory volume in the first
second (FEV₁), while a reduction of at least 10% of the initial
value was considered significant for total lung capacity (TLC)
and diffusion capacity for carbon monoxide corrected for
hemoglobin concentration and alveolar volume (DLCOc/VA).

Radiographs were obtained 2 months after treatment.
Eight patients (23.5%) had radiographic progression and 2
patients (5.8%) had radiographic improvement. The radiographic
findings were considered significant for forced vital
capacity (FVC) and diffusion capacity for carbon monoxide
corrected for carbon dioxide uptake corrected for
hemoglobin concentration and alveolar volume (DLCOc/VA).

Statistical Analysis

Since the variables did not obey a normal distribution,
results were expressed as the median and interquartile range.
Differences between radiographic stages and groups were
assessed using the χ² test for qualitative variables and the
Kruskal-Wallis test for quantitative variables. Correlations
were assessed using the Spearman correlation coefficient.
Statistical analyses were performed using the SPSS program
(SPSS version 12.0, Chicago, IL, USA) and statistical
significance was established a P≤.05.

Results

Noteworthy general characteristics were a
predominance of women (55.9%), a mean age of 44
years, and a percentage of smokers of 52.9% (Table 1).
Histologic confirmation of the diagnosis was obtained
for all patients: 24 through transbronchial biopsy, 2 by
mediastinoscopy, 6 by lung biopsy, 1 by lymph node
biopsy, and 1 by biopsy of a cutaneous lesion.
The most common symptoms were respiratory, particularly cough (60%) and dyspnea (48%). Four patients had cutaneous manifestations: 3 with erythema nodosum and 1 with lupus pernio. One patient displayed confusional syndrome as a result of hypercalcemia but was successfully treated. Over the course of the 12-month follow-up, no patients displayed other extrathoracic manifestations that could influence the course of the disease.

The median baseline values for FVC, FEV1, TLC, DLCOc/V A, and PaO2 were all within a normal range. The results of BAL cell analysis are shown in Table 1. These results were within the normal range in 3 patients. Nonsmokers had higher proportions of total lymphocytes and CD4 lymphocytes, and a higher CD4/CD8 cell ratio in BAL fluid than smokers; however, the differences did not achieve statistical significance. No other differences were found in BAL variables.

Corticosteroid treatment was indicated in 26 patients (76.4%), 2 patients received bronchodilator treatment for symptoms of bronchial hyperreactivity, and 6 patients received no treatment. The mean (SD) duration of corticosteroid treatment was 10.7 (2.8) months.

Radiographic Stages

The following distribution of radiographic stages was observed at diagnosis: 4 patients (11.7%) in stage I, 22 patients (64.7%) in stage II, and 8 patients (23.6%) in stage III. No statistically significant differences were found between different radiographic stages for age or smoking habit (Table 1), or for lung function (FVC, FEV1, FEV1/FVC, TLC, DLCOc/V A, or PaO2). Serum concentrations of ACE (Table 1) and calcium were higher in patients in radiographic stage II, while calciuria was higher in patients in stage III; these differences did not achieve statistical significance. Although BAL characteristics were not significantly different between radiographic stages, a higher percentage of lymphocytes was found in stage I than in other stages (Table 1).

Radiographic and Functional Evolution

At 12-month follow-up, 58.8% of patients were considered to be cured (group A) and 8.8% had deteriorated (group C). Remission or cure of the disease was observed in 75% of patients in stage I, 63.6% of patients in stage II, and 37.5% of patients in stage III (Table 2). No statistically significant differences were found between the three groups in terms of age, sex, or smoking habit; however, the percentage of smokers in group C was higher than in the remaining groups (Table 3). Lung function parameters were within a normal range except in group C, in which slight hypoxemia and a reduced FEV1/FVC ratio were observed; these differences were not statistically significant. Although no statistically significant differences in BAL cell

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (n=34)</th>
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<th>Stage II‡ (n=22)</th>
<th>Stage III§ (n=8)</th>
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</tr>
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<tr>
<td>Age, years</td>
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<td>33 (28-40)</td>
<td>44 (30-63)</td>
<td>44 (35-52)</td>
<td>.82*</td>
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<tr>
<td>Sex, % women</td>
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<td>50</td>
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<td>62.5</td>
<td>.89*</td>
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<tr>
<td>Smokers, %</td>
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<tr>
<td>ACE, U/mL</td>
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*Data are shown as medians (interquartile range). ACE indicates angiotensin-converting enzyme.
†Bilateral hilar lymphadenopathy.
‡Bilateral hilar lymphadenopathy and lung infiltrates.
§Lung infiltrates without lymphadenopathy.
||Kruskal-Wallis test.
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||Kruskal-Wallis test.
¶χ² test.

**TABLE 1**

General Characteristics and Analysis of Bronchoalveolar Lavage Fluid in the Total Population and According to Radiographic Stage

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<thead>
<tr>
<th>Variables</th>
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||Kruskal-Wallis test.
¶χ² test.

**TABLE 2**

Radiographic Stage at Diagnosis and Radiographic/Functional Evolution at 12-Month Follow-up

<table>
<thead>
<tr>
<th>Groups*</th>
<th>Stages†</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=20)</td>
<td></td>
<td>3</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>B (n=11)</td>
<td></td>
<td>1</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>C (n=3)</td>
<td></td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

*A indicates cured or improved; B, stable; C, deteriorated.
†I indicates bilateral hilar lymphadenopathy; II, bilateral hilar lymphadenopathy and lung infiltrates; III, lung infiltrates without lymphadenopathy.

The most common symptoms were respiratory, particularly cough (60%) and dyspnea (48%). Four patients had cutaneous manifestations: 3 with erythema nodosum and 1 with lupus pernio. One patient displayed confusional syndrome as a result of hypercalcemia but was successfully treated. Over the course of the 12-month follow-up, no patients displayed other extrathoracic manifestations that could influence the course of the disease.

The median baseline values for FVC, FEV1, TLC, DLCOc/V A, and PaO2 were all within a normal range. The results of BAL cell analysis are shown in Table 1. These results were within the normal range in 3 patients. Nonsmokers had higher proportions of total lymphocytes and CD4 lymphocytes, and a higher CD4/CD8 cell ratio in BAL fluid than smokers; however, the differences did not achieve statistical significance. No other differences were found in BAL variables.

The serum concentration of ACE was increased in 93% of patients. Comparison of ACE concentrations with BAL variables revealed a moderate correlation with the proportion of lymphocytes (r=-0.45; P=.03) and neutrophils (r=-0.42; P=.04).

Corticosteroid treatment was indicated in 26 patients (76.4%), 2 patients received bronchodilator treatment for symptoms of bronchial hyperreactivity, and 6 patients received no treatment. The mean (SD) duration of corticosteroid treatment was 10.7 (2.8) months.

Radiographic Stages

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Radiographic and Functional Evolution

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counts were observed between the groups, the percentage of lymphocytes was higher in group A. Lymphocyte subpopulations were analyzed in 22 patients (15 from group A, 6 from group B, and 1 from group C). No statistically significant differences were observed between groups A and B (Table 3). Although the serum concentration of ACE tended to be higher in group A \( (P = .07) \) and the concentrations of calcium in serum and urine were slightly higher in group B, these differences were not statistically significant.

Corticosteroid treatment was administered in 14 patients (70%) from group A, 9 (81.8%) from group B, and 3 (100%) from group C. No statistically significant differences in disease course were observed between patients who received corticosteroid treatment and those who did not \( (P = .32) \). A similar result was obtained when the results were analyzed according to radiographic stage at presentation (stage I, \( P = .24 \); stage II, \( P = .75 \); stage III, \( P = .80 \)).

**Discussion**

In the present study, the differential cell count and analysis of lymphocyte subpopulations in BAL fluid did not distinguish radiographic stages at diagnosis of sarcoidosis. Nevertheless, it is noteworthy that the percentage of lymphocytes observed in radiographic stage I was higher than in the other stages. This finding suggests the presence of damage to the lung parenchyma that is revealed by BAL at stages in which radiography does not detect interstitial alterations. The classification of sarcoidosis based on radiographic observations does not appear to be related to the results obtained by cell analysis of BAL fluid. Thus, radiography alone is of limited use in the evaluation of disease activity or as a basis for therapeutic decisions.

The results of BAL cell analysis were normal in 3 patients: 2 patients in stage II and 1 patient in stage III. This indicates that such normal results from BAL cell analysis in a patient with a clinical and radiographic profile highly indicative of sarcoidosis do not exclude the possibility that the disease is present. A possible explanation for such cases is a predominance of fibrosis with a less apparent inflammatory phase and, consequently, the absence or severe reduction of indicators in BAL fluid.

Given the effect of smoking on cell populations in BAL fluid, the influence of smoking habit on the differential cell count and cell subpopulations was analyzed. Our results did not differ from those obtained in a multicenter study based on a large population of healthy subjects.\(^{16}\)

Although at 12-month follow-up a large percentage of patients (91.2%) were either cured or showed improvement, 8.8% displayed disease progression. These findings are consistent with results obtained in other studies and demonstrate that, although sarcoidosis generally has a good prognosis, in 10% to 30% of patients the disease follows a progressive and irreversible course that leads to pulmonary fibrosis.\(^{17}\)

Lung function testing revealed a reduction in the FEV\(_1\)/FVC ratio and slight hypoxemia in group C that could be related to the presence of a higher percentage of smokers in this group. Although BAL cell analysis showed a slightly higher percentage of lymphocytes in group A, the differences observed were not statistically significant.

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**Table 3**

**General Characteristics, Lung Function, and Bronchoalveolar Lavage According to Disease Course**

|                  | Group A (n=20) | Group B (n=11) | Group C (n=3) | \( P \)  \\
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>39 (31-51)</td>
<td>44 (31-52)</td>
<td>53 (50-61)</td>
<td>.15(^a)</td>
</tr>
<tr>
<td>Sex, % women</td>
<td>40</td>
<td>72.7</td>
<td>100</td>
<td>.07(^b)</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>60</td>
<td>36.4</td>
<td>66.7</td>
<td>.39(^c)</td>
</tr>
<tr>
<td>Lung function, % of predicted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td>86 (71-97)</td>
<td>86 (71-106)</td>
<td>90 (68-96)</td>
<td>.90(^d)</td>
</tr>
<tr>
<td>FEV(_1)</td>
<td>80 (63-93)</td>
<td>86 (64-106)</td>
<td>88 (65-91)</td>
<td>.79(^e)</td>
</tr>
<tr>
<td>FEV(_1)/FVC</td>
<td>78 (71-87)</td>
<td>86 (77-87)</td>
<td>75 (68-81)</td>
<td>.63(^f)</td>
</tr>
<tr>
<td>TLC</td>
<td>83 (72-94)</td>
<td>95 (80-108)</td>
<td>87 (84-97)</td>
<td>.34(^g)</td>
</tr>
<tr>
<td>DLCOc/VA</td>
<td>100 (91-124)</td>
<td>99 (90-123)</td>
<td>95 (78-103)</td>
<td>.87(^h)</td>
</tr>
<tr>
<td>PaO(_2), mm Hg</td>
<td>93 (83-98)</td>
<td>89 (82-97)</td>
<td>77 (76-77)</td>
<td>.11(^i)</td>
</tr>
<tr>
<td>ACE, U/mL</td>
<td>78 (44-96)</td>
<td>71 (31-104)</td>
<td>17 (14-20)</td>
<td>.07(^j)</td>
</tr>
<tr>
<td>Bronchoalveolar lavage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages, %</td>
<td>57 (44-72)</td>
<td>55 (40-85)</td>
<td>50 (44-70)</td>
<td>.95(^k)</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>39 (26-49)</td>
<td>34 (15-57)</td>
<td>35 (29-44)</td>
<td>.93(^l)</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>1 (0-3)</td>
<td>3 (0-8)</td>
<td>2 (1-8)</td>
<td>.46(^m)</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>0 (0-2)</td>
<td>0 (0-1)</td>
<td>1 (0-1)</td>
<td>.59(^n)</td>
</tr>
<tr>
<td>CD4, % of total lymphocytes</td>
<td>60 (45-82)</td>
<td>68 (54-85)</td>
<td>61</td>
<td>.77(^o)</td>
</tr>
<tr>
<td>CD8, % of total lymphocytes</td>
<td>23 (15-34)</td>
<td>28 (6-43)</td>
<td>31</td>
<td>.75(^p)</td>
</tr>
<tr>
<td>CD4/CD8 cell ratio</td>
<td>2 (1-6)</td>
<td>2 (1-14)</td>
<td>2</td>
<td>.89(^q)</td>
</tr>
</tbody>
</table>

\(^a\)Data are shown as medians (interquartile range). FVC indicates forced vital capacity; FEV\(_1\), forced expiratory volume in the first second; TLC, total lung capacity; DLCOc/VA, diffusion capacity for carbon monoxide corrected for hemoglobin concentration in blood and alveolar volume; ACE, angiotensin-converting enzyme.

\(^b\)Cured or improved.

\(^c\)Stable.

\(^d\)Deteriorated.

\(^e\)Kruskal-Wallis test.

\(^f\)\( \chi^2 \) test.

\(^g\)\( \chi^2 \) test.
Published data regarding the prognostic value of BAL cell analysis is highly variable. Some authors have found that an increased proportion of lymphocytes (>28%) in untreated patients correlates with deterioration of lung function in the first 6 months. In contrast, Foley et al. found that initial lymphocyte levels of more than 28% were accompanied by an improvement in lung function at 2-year follow-up. A recent study found that patients showing clinical improvement had higher numbers of lymphocytes. Finally, other authors have found that the lymphocyte count at diagnosis does not predict either deterioration of respiratory function or treatment response.

The prognostic value of lymphocyte subpopulations has also been evaluated, again with variable results. Some authors have reported that patients with an increased CD4/CD8 lymphocyte ratio show greater deterioration than those whose ratio is normal, while others have found no link between either lymphocyte count or CD4/CD8 cell ratio and disease prognosis.

A recent study concluded that an increase in the percentage of neutrophils (>3%) and eosinophils (>1%) is associated with a greater likelihood of needing corticosteroid treatment. That study is consistent with the findings of Drent et al. in suggesting that the neutrophil count allows patients in whom the disease is in remission to be differentiated from those with severe progressive disease. These conclusions are also consistent with those of Lin et al., who, some years ago, found a link between an increased neutrophil count and worse prognosis.

The discrepancies in reported results appear to be related to methodological differences between studies and the heterogeneity of study populations.

The small sample size used in the present study does not allow us to draw conclusions about the prognostic value of BAL cell analysis. Nevertheless, it is possible to make the following observations: a) radiographic classification of sarcoidosis has limited practical use when considered in isolation, since it does not provide information on disease activity; b) although BAL cell counts aid diagnosis and allow the intensity of the pulmonary inflammatory response to be determined, they do not appear to predict disease course over the first 12 months in our patient group. It will be necessary to increase the number of patients in each group to better assess the prognostic value of lymphocyte subpopulation (CD4 and CD8) counts.

In the future, increased understanding of the pathogenesis of pulmonary sarcoidosis may facilitate the identification of new soluble components of BAL fluid that may be used as prognostic markers to aid disease management and the development of new therapeutic strategies.

REFERENCES