

## BRONCHOALVEOLAR LAVAGE IMPACT IN SARCOIDOSIS: Study of 40 cases

Dhouha Bacha, Aïda Ayadi-Kaddour, Olfa Ismail, Faouzi El Mezni

Department of Pathology  
Abderrahmen Mami Hospital, Ariana, Tunisia

D. Bacha, A. Ayadi-Kaddour, O. Ismail, F. El Mezni

IMPACT DU LAVAGE BRONCHO-ALVÉOLAIRE DANS LA SARCOÏDOSE : Etude de 40 cas

LA TUNISIE MEDICALE - 2009 ; Vol 87 (n°01) : 38 - 42

D. Bacha, A. Ayadi-Kaddour, O. Ismail, F. El Mezni

BRONCHOALVEOLAR LAVAGE IMPACT IN SARCOIDOSIS: Study of 40 cases

LA TUNISIE MEDICALE - 2009 ; Vol 87 (n°01) : 38 - 42

## R É S U M É

**Introduction :** Le profil du lavage bronchoalvéolaire (LBA) au cours de la sarcoïdose a longtemps été rapporté dans la littérature avec des résultats différents. Habituellement, ce profil montre une alvéolite lymphocytaire et un rapport CD4/CD8 élevé avec néanmoins une importante variabilité des valeurs rapportées.

**L'objectif** de cette étude est d'évaluer les différents paramètres du LBA dans la sarcoïdose avant et après corticothérapie et de déterminer l'apport du LBA dans l'évaluation du pronostic de la sarcoïdose.

**Méthodes :** L'étude a porté sur 40 patients atteints de sarcoïdose confirmée histologiquement dans tous les cas.

**Résultats :** Avant traitement, le LBA montre une augmentation de la cellularité globale par rapport aux valeurs normales et une hyperlymphocytose de phénotype T avec un rapport CD4/CD8 élevé. Après corticothérapie, on note une augmentation significative du taux des macrophages et une diminution significative de la lymphocytose alvéolaire et du rapport CD4/CD8.

Une augmentation significative de CD4/CD8 est notée d'une part chez les patients présentant une atteinte extrathoracique multiple par rapport à ceux n'ayant qu'une atteinte intrathoracique et d'autre part chez les patients ayant eu une évolution défavorable par rapport à ceux qui ont bien évolué.

**Conclusion :** Les résultats du LBA peuvent contribuer au diagnostic positif de la sarcoïdose lorsque le tableau radio-clinique est évocateur. Il ressort de notre étude qu'un rapport CD4/CD8 élevé est un facteur péjoratif de mauvais pronostic avec une atteinte extrathoracique multiple et une évolution clinique défavorable.

## S U M M A R Y

**Introduction :** Studies on bronchoalveolar lavage (BAL) fluid samples profile characteristics in sarcoidosis patients hitherto reported in literature give rise to conflicting data. The typical finding is lymphocytic alveolitis with a high CD4/CD8 ratio, although a broad range of values has been found.

**The aim** of the study was to evaluate different parameters of BAL fluid in patients with sarcoidosis before and after corticosteroid treatment and to determine the reliability of BAL in evaluating prognosis of sarcoidosis.

**Methods :** The study involved 40 patients with clinical and histological evidence of sarcoidosis.

**Results :** Before treatment, BAL fluid showed an increase in total cell count and a T lymphocytosis with an increase in the CD4/CD8 ratio. After treatment, we have noted a significantly increase in macrophage proportion, a significantly decrease in lymphocyte proportion and in CD4/CD8 ratio.

A significant higher CD4/CD8 ratio was noted in patients with multiple extrathoracic lesions compared with patients with only intrathoracic involvement and in patients with unfavourable evolution compared with patients with improved evolution.

**Conclusion :** When typical clinical and radiological findings definitely suggest sarcoidosis, BAL results may add a biological support. Our data suggests that a high CD4/CD8 ratio seems to be predictive of poor prognosis with a multiple extrathoracic organ involvement and an unfavourable outcome.

## M O T S - C L É S

Sarcoïdose, Lymphocyte, Lavage bronchoalvéolaire

## K E Y - W O R D S

Bronchoalveolar lavage, Lymphocyte, Sarcoidosis

## دور الغسيل السنخي القصبي أثناء مرض الغرناوية دراسة حول 40 حالة

الباحثون : باشا. ض - عيادي قدور. ع - إسماعيل. أ

الهدف من هذه الدراسة هو تقييم مختلف معالم الغسيل السنخي القصبي أثناء الغرناوية قبل وبعد العلاج القشري وتقييم دور هذا الغسيل في إنذار الغرناوية. إشتملت دراستنا على 40 مريضا مصابا بالغرناوية المؤكدة نسيجيا نستنتج أن نتائج الغسيل السنخي القصبي تساهم في التشخيص الإيجابي للغرناوية عندما توجي العلامات التصويرية السريرية بذلك ونلاحظ من خلال هذه الدراسة أنه عندما تكون نسبة CD4/CD8 مرتفعة يكون إنذار المرض سيئا.

الكلمات الأساسية : غسيل سنخي قصبي - الغرناوية.

Bronchoalveolar lavage (BAL) has become an important tool in the diagnostic and management of sarcoidosis. The typical finding is lymphocytic alveolitis with a high CD4/CD8 ratio, although a broad range of values has been found in case series [1,2,3,4]. The study involved 40 patients with clinical and histological evidence of sarcoidosis, who underwent BAL as part of their initial diagnostic evaluation.

The aim of this study is:

- To evaluate different parameters of BAL fluid in patients with sarcoidosis before and after corticosteroid treatment.
- To determine the reliability of BAL in evaluating prognosis of sarcoidosis.

## PATIENTS AND METHODS

### Patients

The study population included 40 patients with sarcoidosis confirmed by biopsy, at Abderrahmen Mami's Hospital of Ariana (Tunisia), over a three-year period (from May 2002 and May 2005). Demographic data, radiographic stage, pulmonary function tests and BAL cellular profile were collected. Follow-up study of 12 +/- 9 months was needed to investigate the value of BAL to evaluate disease improvement or progression and the possible influence of therapy on BAL profile.

### Methods

Bronchoalveolar lavage:

BAL was performed during fiberoptic bronchoscopy after premedication with atropine and local anesthesia of the larynx and bronchial tree. BAL was performed by washing of the right middle lobe with four 30-ml aliquots of sterile 0.9% saline solution at room temperature. Cytospin slides of BAL cells were stained with Papanicolaou, Haematoxylin-Eosin, MGG and Perls. Total and differential cell counts were determined. Lymphocyte subsets (CD4/CD8 ratio) were analysed when lymphocytosis is over 20% with an immunoperoxidase slide assay using a CD4 (helper/inducer) and CD8 (cytotoxic) monoclonal antibodies (Dako).

Forty-seven BALs were analysed as for 7 patients, 2 samples were collected for each one.

Normal values for BAL cellular analysis were determined from healthy no-smoking subjects [Table 1] [5].

**Table 1:** BAL cellular components in healthy no smoking subjects

Total cell count	150 000/ml-200 000/ml
Macrophages (%)	90-95
Lymphocytes (%)	<20
Neutrophils (%)	<5
Eosinophils (%)	<5
CD4/CD8 ratio	1,2

Repeated BAL studies after corticosteroid treatment were performed in 15 patients to allow measuring the changes in immune function that occurred with therapy.

### Statistical Analysis:

Mean values +/- Standard Deviation were calculated and groups were compared with the student test. A p value <0,05 was considered statistically significant. To investigate whether there were statistically significant differences between BAL before and after corticosteroid treatment, the Wilcoxon test was used.

## RESULTS

### Clinical and biological features

The study group was composed of 28 females and 12 males of median age 49 years (range 24-74 years). Main clinical characteristics of the patients at the time of diagnosis are shown in table 2. Data are presented as mean with proportion in parentheses.

**Table 2 :** Clinical features at time of diagnosis in 40 sarcoid patients

Features	Patients
<u>Smoking habits</u>	
No smokers	27 (67,5)
Smokers	13 (32,5)
<u>Respiratory manifestations</u>	
Cough	29 (72,5)
Dyspnea	24 (60)
Chest pain	18 (45)
<u>Extra respiratory manifestation</u>	
Systemic symptoms	17 (42,5)
Peripheral lymph nodes	7 (17,5)
Skin involvement	7 (17,5)
Arthralgia	6 (15)
Ocular manifestation	2 (5)
Löfgren's syndrome	1 (2,5)

All patients except two were symptomatic at the time of the initial evaluation. In asymptomatic patients, lung lesions were discovered during a routine chest radiograph. Respiratory manifestations were more frequent than extra respiratory manifestations. The most common presenting findings were cough, dyspnea, chest pain and constitutional symptoms (fever, fatigue). One patient presented with Löfgren's syndrome consisting in erythema nodosum, arthralgia, hilar lymph nodes and fever.

Blood lymphopenia was noted in 16 cases (40%). Inflammatory markers (erythrocyte sedimentation rate, C-reactive protein) were elevated in 22 patients (55%) and normal in 18 patients (45%). Angiotensin I-converting enzyme was performed in only 3 cases and it was elevated in all these cases.

### Pulmonary function test

Lung volumes were normal in 17 patients. A restrictive ventilatory defect was present in 14 cases. Obstructive and obstructive-restrictive ventilatory defects were detected in respectively 3 and 1 patients. Low pulmonary diffusing capacity was present in 11 of 18 patients.

### Radiographic findings

All sarcoid patients presented radiographic abnormalities. The majority of cases presented either stage 2 (bilateral hilar lymph nodes and pulmonary infiltrations) in 23 patients (57,5%) or stage 3 (pulmonary infiltrations) in 10 cases (25%). Stage 1 (bilateral hilar lymph nodes) and stage 4 (pulmonary fibrosis) were observed in respectively 4 (10%) and 3 cases (7,5%).

### Pathological findings

Histological confirmation of sarcoidosis was performed in all cases by the presence of non caseating granulomas. In most cases, histological features were observed in bronchial and lymph nodes biopsies [Table 3].

### Organ involvement

Respiratory tract involvement with or without intrathoracic lymph nodes were present in all cases. An associated extrathoracic disease was present in 21 patients (52,5%) mostly in peripheral lymph nodes and in skin [Table 3].

**Table 3 :** Initial biopsy sites and organ involvement in sarcoid patients

Features	Patients
<u>Initial diagnosis biopsy</u>	
Bronchial	18
Transbronchial	2
Lymph nodes	12
Lung	4
Labial	2
Skin	2
<u>Organ involvement</u>	
Lung and/or mediastinal lymph nodes	40
Peripheral lymph nodes	7
Skin	7
Musculoskeletal system	6
Spleen	3
Liver	2
Eyes	2
Nose	1
Heart	1
Nervous System	1
Cervix	1

### Treatment and clinical course

Thirteen patients had received no treatment with a favourable course in 4 cases, a deterioration of lung function in 3 patients and no significant change in 3 patients. Three patients were out of follow. Twenty-six patients had received corticosteroid

treatment (systemic treatment in 23 cases and inhaled corticosteroid in 3 cases). An improvement in pulmonary function was seen in 22 patients, an unfavourable course was seen in 3 patients and a stationary course was seen in 1 patient. Patient presented Löfgren's syndrome had received colchicine with a favourable evolution.

### Bronchoalveolar lavage

BAL results before treatment are shown in table 4

**Table 4 :** BAL results in sarcoidosis patients before treatment

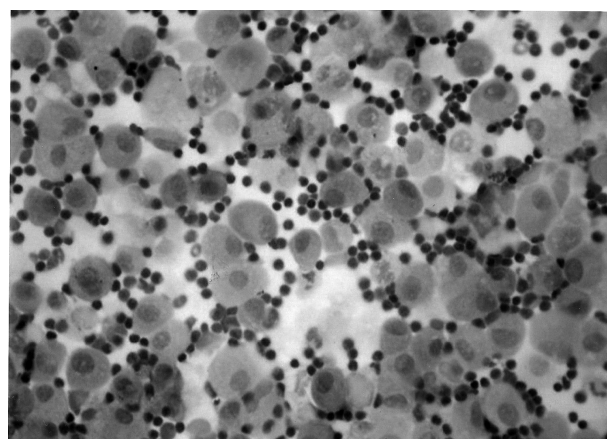
Total cell count	302 468+/-198 644/ml §
Macrophages (%)	52,9+/-18,5 §
Lymphocytes (%)	40+/-18 §
Neutrophils (%)	5,6+/-6,25
Eosinophils (%)	1,57+/-2,98
CD4/CD8 ratio	2,95+/-2,46 §

§:  $p < 0,01$ , Student test versus normal values in healthy non smoking subjects

In the sarcoid group, the mean value of total cell count before treatment was significantly increased. The total cell count was increased in 30 BAL, normal in 11 cases and decreased in 6 cases. Mean proportion of macrophages was significantly decreased. Macrophage proportions were decreased in 46 BAL and normal in 1 case.

Mean proportion of lymphocytes was significantly increased [Figure 1]. Lymphocyte proportion was increased in 40 BAL and normal in 7.

**Figure 1 :** Lymphocytic alveolitis (Haematoxylin-Eosin x 400)

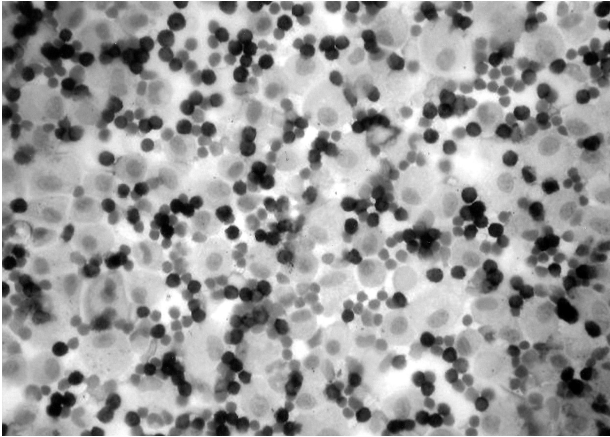


Mean proportion of neutrophils was over the normal value without significant difference.

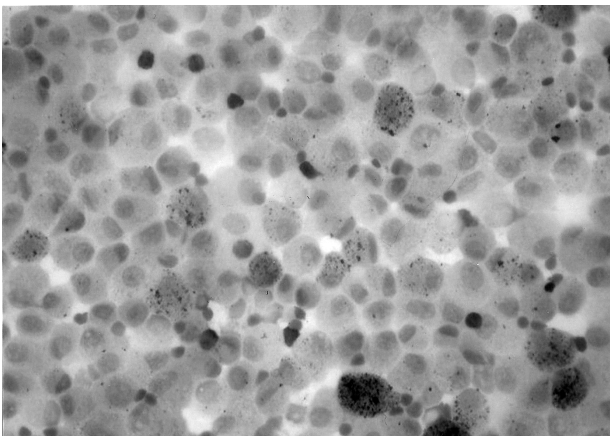
Mean BAL CD4/CD8 ratio was significantly increased 2,95+/-2,46 (range 0,15-12) [Figure 2]. The CD4/CD8 ratio was increased in 21 BAL, normal in 2 cases and decreased in 6 cases.

No significant correlation was found between these different variables (gender, smoking, respiratory and general symptoms, skin, ocular and musculoskeletal involvements, lymphocytopenia and low pulmonary diffusing capacity) and BAL parameters.

**Figure 2 :** 2a. Lymphocytes show strong immunoreactivity for CD4 (CD4 x 400)



**Figure 2 :** 2b. Few lymphocytes stain positively for CD8 (CD8 x 400)



LBA profile in patients with multiple extrathoracic lesions showed a significantly increased CD4/CD8 ratio compared with patients with only intrathoracic involvement ( $5.95 \pm 0.76$  vs  $3 \pm 2.24$ ) ( $p=0.01$ ). BAL profile in patients with unfavourable evolution (6 patients) showed a significant higher CD4/CD8 ratio compared with patients with improved evolution (27 patients) ( $6.45 \pm 3.84$  vs  $2.39 \pm 1.79$ ).

BAL results after corticosteroid treatment analysed in 15 patients are defined in table 5.

We have noted a significantly increase in macrophage proportion ( $p=0.036$ ) and a significantly decrease in lymphocyte proportion ( $p=0.043$ ) and in CD4/CD8 ratio ( $p=0.054$ ). A modest change in total cell count and in proportion of neutrophils and eosinophils, was noted.

**Table 5 :** BAL results in sarcoidosis patients before and after treatment (15 patients)

	Before treatment	After treatment	p
Total cell count	329 875 $\pm$ 111 165/ml	328 028 $\pm$ 193 515/ml	NS
Macrophages (%)	41,87 $\pm$ 17,5	51,9 $\pm$ 18,2	S
Lymphocytes (%)	50,67 $\pm$ 19,2	40,75 $\pm$ 17,45	S
Neutrophils (%)	4,93 $\pm$ 5,68	4,81 $\pm$ 4,46	NS
Eosinophils (%)	2,51 $\pm$ 4,5	2,5 $\pm$ 3,26	NS
CD4/CD8 ratio	3,81 $\pm$ 2,98	1,98 $\pm$ 1,09	S

S: Significant difference, NS: No significant difference

## DISCUSSION

BAL is regarded as an important diagnostic method in sarcoidosis. The cellular profile in BAL fluid samples reflects the presence of alveolitis as a local expression of disseminated immunologic disorder. Patients with pulmonary sarcoidosis frequently have increased numbers of lymphocytes (usually range between 20 and 40% of total cell count but may reach 70% in some cases) and a high CD4/CD8 ratio [3,6]. In agreement with literature, BAL fluid showed in our study an increase in total cell count and a T lymphocytosis with an increase in the CD4/CD8 ratio. BAL lymphocytosis is not a universal finding. Lymphocytosis was lower than 16% of total cell count in 30% of the sarcoid patients in the study of Kantrow [6] and in 23% of cases in the study of Winterbauer [3]. In our study, lymphocytosis was lower than 20% in 7 patients (17,5%). An elevated CD4/CD8 ratio in patients with a clinical picture typical of sarcoidosis, may prove this diagnosis and obviate the need for confirmation by additional biopsy. Therefore, BAL CD4/CD8 ratio measured during the initial diagnostic evaluation of patients with biopsy-proven sarcoidosis is highly variable [6]. It ranged in our study from 0,15 to 12. Furthermore, a low CD4/CD8 ratio is not a new finding in sarcoidosis, 6 patients in our study has a ratio lower than the normal value.

Cigarette smoking modifies the immunologic bronchoalveolar fluid sample profile, since alveolitis was less pronounced in smokers with a tendency to high numbers of mast cells [7]. In our study, no significant differences were demonstrated in BAL between smokers and non smokers patients.

Initially, it had been hoped that BAL parameters, particularly abnormalities in the cell populations would prove more useful in the assessment of activity and prognosis of sarcoidosis than peripheral blood tests. However, conflicting results have been reported in studies evaluating the utility of BAL in assessing the prognosis of the disease [8]. In early 1980s, a high lymphocyte count in BAL was considered a key sign of the unfavourable sarcoidosis outcome accompanied by gradual lung deterioration. However, subsequent studies do not confirm this hypothesis [9]. Previous studies have shown that there is an association between low CD4/CD8 ratio and lower remission

rates in sarcoidosis patients. At present, the consensus reached is that the intensity of the alveolitis as assessed by either BAL lymphocyte counts or CD4/CD8 ratio, does not predict outcome in criterion for treatment decisions [10]. In our study, we have noted a significant increase in CD4/CD8 ratio in patients with multiple extrathoracic lesions compared to patients with only intrathoracic involvement and in patients with unfavourable evolution compared with patients with improved evolution. Therefore, in contrast with some series, a high CD4/CD8 ratio seems to be predictive of poor prognosis in our study. The most satisfying therapy for the patient and physician in sarcoidosis is no treatment at all. Systemic therapy varies between treatment centres, with some groups treating only a third of their patients and others more than two-thirds [7]. The evidence lends support to the use of corticosteroids for patients with neurological, cardiac, ocular, serious respiratory

involvement and hypercalcaemia. Corticosteroids suppress disease activity in pulmonary sarcoidosis and their use produces symptomatic radiological and functional improvement. They are known to affect T lymphocyte function [11]. In our study, we have noted a significantly decrease in lymphocyte proportion and a significantly decrease in CD4/CD8 ratio after treatment.

## CONCLUSION

This study confirms characterized features in BAL fluid in patients with sarcoidosis. Then, when typical clinical and radiological findings definitely suggest sarcoidosis, BAL results may add a biological support.

Our data suggests that a high CD4/CD8 ratio seems to be predictive of poor prognosis with a multiple extrathoracic organ involvement and an unfavourable outcome.

## RÉFÉRENCES

1. Baughman RP, Drent M. Role of bronchoalveolar lavage in interstitial lung disease. *Clin Chest Med* 2001; 22: 331-41.
2. Klech H, Hutter C, Costabel U. Clinical guidelines and indications for bronchoalveolar lavage (BAL). *Eur Respir Rev* 1992; 2: 47-127
3. Winterbauer R, Lammert J, Selland M, Wu R, Corley D, Springmeyer S. Bronchoalveolar lavage cell populations in the diagnosis of sarcoidosis. *Chest* 1993; 104: 352-61.
4. Shim S. Diagnosis and treatment of sarcoidosis. *JAAPA* 2006; 19: 30-4.
5. Capron F. Lavage broncho-alvéolaire. *Arch Anat Cytol Path* 1997; 45: 255-260.
6. Kantrow S, Meyer K, Kidd P, Raghu G. The CD4/CD8 ratio in BAL fluid is highly variable in sarcoidosis. *Eur Respir J* 1997; 10: 2716-21.
7. Judson MA. The management of sarcoidosis by the primary care physician. *Am J Med* 2007; 120: 403-7.
8. Drent M, Jacobs J, Vries J, Lamers R, Liem I, Wouters E. Does the cellular bronchoalveolar lavage fluid profile reflect the severity of sarcoidosis? *Eur Respir J* 1999; 13: 1338-44.
9. Judson MA. Sarcoidosis: Clinical presentation, diagnosis, and approach to treatment. *Am J Med Sci* 2008; 335: 26-33.
10. Chorostowska J, Lesniewska D, Krychniak A, Remiszewski P, Sopinski J. Cellular components of the bronchoalveolar lavage correlate with lung function impairment and extrapulmonary involvement markers in active sarcoidosis. *Journal of Physiology and pharmacology* 2004; 55: 41-7.
11. Baughman R, Lower E, Roland M. Sarcoidosis. *The Lancet* 2003; 361:1111-8.