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Original Article

Bronchoalveolar Lavage Findings in Patients With Diffuse Interstitial Lung Disease: Prospective Study of a Cohort of 562 Patients

Luis Jara-Palomares,^{a,*} José Martín-Juan,^b Lourdes Gómez-Izquierdo,^c Aurelio Cayuela-Domínguez,^d Eulogio Rodríguez-Becerra,^b and Francisco Rodríguez-Panadero^b

^aNeumología, Hospital Sierra Norte, Constantina, Sevilla, Spain

^bUnidad Médico-Quirúrgica de Enfermedades Respiratorias (UMQER), Hospital Universitario Virgen del Rocío, Sevilla, Spain

^cServicio de Anatomía Patológica, Hospital Universitario Virgen del Rocío, Sevilla, Spain

^dUnidad de Apoyo a la Investigación, Hospital Universitario Virgen del Rocío, Sevilla, Spain

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ABSTRACT

Objective: Study of the bronchoalveolar lavage (BAL) fluid in some interstitial lung diseases can reveal patterns typical to each disease and that can support the diagnosis. The objective of this study was to perform a descriptive analysis of the cytologic study and of the lymphocyte subpopulations in BAL fluid from patients with interstitial lung disease.

Material and methods: In this prospective, observational study of 562 patients between January 1991 and January 2005, BAL fluid was analyzed to determine the distribution of cell populations and of lymphocyte subsets: CD3, CD4, CD8, CD3⁺CD4⁻CD8⁻, and CD56.

Results: The mean age was 53.4 years and 53.3% of the patients were women. The following diseases were studied: idiopathic pulmonary fibrosis (n=132), sarcoidosis (n=123), connective tissue diseases (n=133), cryptogenic organizing pneumonia (n=89), and extrinsic allergic alveolitis (n=85). Isolated lymphocytic alveolitis was common in sarcoidosis and extrinsic allergic alveolitis. Mixed alveolitis was the most common pattern in the other interstitial lung diseases. The CD4:CD8 ratio was the most useful parameter. It was high in sarcoidosis (median, 2.3); the ratio was low or inverted in the other interstitial lung diseases, with median values of 1.76 in idiopathic pulmonary fibrosis, 0.45 in extrinsic allergic alveolitis, 0.35 in cryptogenic organizing pneumonia, and 0.33 in the connective tissue diseases.

Conclusions: BAL parameters, in association with clinical and radiologic data, help to discriminate between interstitial lung diseases. BAL should therefore be considered a very useful tool in clinical management, particularly when pulmonary biopsy is not conclusive or is not possible.

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Hallazgos en el lavado broncoalveolar de pacientes con enfermedad pulmonar intersticial difusa. Estudio de una cohorte prospectiva de 562 pacientes

RESUMEN

Objetivo: En determinadas enfermedades pulmonares intersticiales difusas (EPID), el estudio del lavado broncoalveolar (LBA) define patrones típicos de cada enfermedad y tiene valor como apoyo al diagnóstico. El objetivo del estudio ha sido realizar un análisis descriptivo del estudio citológico y de las subpoblaciones linfocitarias en el LBA efectuado a pacientes con EPID.

Pacientes y métodos: Estudio prospectivo de 562 pacientes con EPID desde 1991 hasta 2005. Se realizó un estudio de la distribución celular y de la subpoblaciones linfocitarias en el LBA: CD3, CD4, CD8, CD3⁺CD4⁻CD8⁻ y CD56.

Palabras clave:

Enfermedad pulmonar intersticial difusa

Sarcoidosis

Lavado broncoalveolar

* Corresponding author.

E-mail address: luisoneumo@hotmail.com (L. Jara Palomares).

Resultados: La edad media de los pacientes era de 53,4 años y el 53,3% eran mujeres. Se estudiaron las siguientes enfermedades: fibrosis pulmonar idiopática (n = 132), sarcoidosis (n = 123), enfermedades del colágeno (n = 133), neumonía organizada criptogenética (n = 89) y alveolitis alérgica extrínseca (n = 85). Tanto en los casos de sarcoidosis como en los de alveolitis alérgica extrínseca fue frecuente la alveolitis linfocitaria aislada. En el resto de enfermedades la alveolitis mixta fue el patrón habitual. El índice CD4/CD8 fue el parámetro más útil, con un incremento en la sarcoidosis (mediana: 2,3). En el resto de las enfermedades el índice estaba invertido, con una mediana para la fibrosis pulmonar idiopática, la alveolitis alérgica extrínseca, la neumonía organizada criptogenética y la enfermedad del colágeno de 1,76; 0,45; 0,35, y 0,33, respectivamente.

Conclusiones: Los parámetros del LBA, junto a los datos clinicorradiológicos, ayudan a discriminar entre las EPID. Por lo tanto, debe considerarse una técnica de gran utilidad en el manejo clínico, sobre todo cuando la biopsia pulmonar no resulta diagnóstica o no es posible realizarla.

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Introduction

The diagnosis of interstitial lung disease (ILD) is based on a detailed medical history, physical examination, imaging studies (including high resolution computed tomography [HRCT]), blood tests, and lung function tests. Depending on the findings of HRCT, the following step is usually to perform bronchoalveolar lavage (BAL) and transbronchial biopsy, if there is no contraindication.

Transbronchial biopsy is very effective in the diagnosis of granulomatous disease, malignancy, and opportunistic infections,¹⁻⁴ but is rarely of value for the diagnosis of idiopathic interstitial pneumonias. If there are no relevant findings on transbronchial biopsy, the gold standard for diagnosis is open lung biopsy, most commonly performed by videothoracoscopy but also by thoracotomy. However, both open biopsy techniques have contraindications and are associated with a variable degree of morbidity and mortality, depending on the underlying disease and the patient's functional status and comorbid conditions. In Spain the frequency with which surgical biopsy techniques are used for confirmation of these diseases is very variable and does not usually reach 30%, as was demonstrated in 2 studies performed in this country^{5,6}; according to a study by Johnston et al⁷ in the United Kingdom with the participation of 200 patients with idiopathic pulmonary fibrosis (IPF), the rate may be even lower. The frequency of use of transbronchial biopsy has been reported as 33% and of open lung biopsy as 7.5%.⁷ Although there has been a slight increase in the use of videothoracoscopy⁸ as the technique of choice, diagnostic management has undergone no significant changes in recent years. There continues to be a certain pessimism about whether confirmatory lung biopsy will modify treatment plans and the diagnosis is therefore based on clinical and radiologic criteria, in particular HRCT, even though a third of cases of usual interstitial pneumonia cannot be diagnosed on HRCT findings alone.⁹

BAL is a technique initially conceived to investigate the immune and inflammatory mechanisms that are active in the lower respiratory tract; it is therefore essential in the study of the pathogenesis of diseases that affect the interstitium.²⁻⁴ In addition, it is very useful both for the isolation of opportunistic pathogens and for the specific diagnosis of certain diseases in which BAL findings have diagnostic value: alveolar proteinosis, diffuse alveolar hemorrhage, pulmonary eosinophilia, and bronchoalveolar carcinoma. However, in the most common diseases in our setting—IPF, sarcoidosis, extrinsic allergic alveolitis (EAA), cryptogenic organizing pneumonia (COP), and connective tissue disease-related interstitial lung disease (CTD-ILD)—patterns of a predominantly immune and cellular response have been described that are related to the alveolar-interstitial inflammatory process. Although the information from BAL will, in

the near future, be complemented with data on whether type 1 or type 2 T helper cell response predominates through determination of interleukin levels, the information of greatest clinical use is currently based on study of cell distribution and of the lymphocyte subpopulations. However, the true benefit of studying these parameters in BAL, in the absence of histological confirmation, is still a matter of debate.^{10,11}

The objective of this study was to carry out a descriptive analysis of the cytology findings and lymphocyte subpopulations in BAL performed on patients with the most common ILDs, in order to evaluate its diagnostic value.

Patients and Methods

This was a prospective, observational study that included all patients with a suspicion of ILD seen between January 1991 and January 2005. The majority of patients came from our units (Interstitial Lung Disease Unit and Internal Medicine Connective Tissue Diseases Unit). All patients entered a study protocol and were referred to the bronchoscopy unit (Medical-Surgical Unit for Respiratory Diseases, Hospital Universitario Virgen del Rocío, Seville, Spain). The following data were gathered from the clinical history: demographic (sex and age), past history (occupational and domestic exposures, smoking, underlying disease, previous treatments), clinical and radiologic data (symptoms, chest radiograph, computed tomography, and/or HRCT), laboratory data (complete blood count, angiotensin converting enzyme, complement, antinuclear antibodies, anti-DNA antibodies, anti-basement membrane antibodies), arterial blood gases, forced spirometry, cytology and microbiology of the BAL fluid, findings in the transbronchial biopsy or lung biopsy, and definitive diagnosis.

BAL was performed in all patients and transbronchial biopsy when there were no contraindications. BAL was carried out following a previously established protocol.^{12,13} In the samples that satisfied the criteria for tissue of the lower respiratory tract, the cell concentrations were determined and the differential cell counts were studied in cytocentrifuged samples stained with modified Giemsa stain (Panóptico Rápido, Química Clínica Aplicada SA, Amposta, Tarragona, Spain), with identification of alveolar macrophages, lymphocytes, neutrophils, eosinophils, and plasma cells. In the case of macrophages, a description was included of certain characteristics that were frequently associated with specific diseases (foamy cytoplasm, anthracosis, and fat or hemosiderin content). Any BAL fluid with differential cell counts within the following limits were considered normal: 80%-90% alveolar macrophages, 15% lymphocytes, 3% polymorphonuclear neutrophils, and 1% eosinophils; these are well-established normal ranges in

nonsmokers.¹³ In patients with lymphocytic alveolitis (defined as a BAL result with lymphocytes >15%), immunologic study was performed using immunocytochemical assay (immune peroxidase) on cryopreserved samples. The immunologic study included evaluation of the percentage expression of the T cell (CD3, CD4, CD8) and cytolytic T cell (CD56) associated antigens. In addition, the percentage of CD3⁺ T cells lacking CD4 and CD8 expression (CD3⁺CD4⁻CD8⁻ or double negative T cells) was evaluated. CD3 identifies the T-cell receptor; CD4 is related to the presence of the class II major histocompatibility complex receptor (helper T cells); CD8 identifies the class I major histocompatibility complex receptor (cytotoxic suppressor T cells), and CD56 identifies the neuronal cell adhesion molecule (cytolytic T cells).

Diagnosis Criteria

Clinical and/or histological diagnosis was available in all patients. All studies (imaging, biopsy, and BAL fluid) were performed by experienced specialists. Interpretation of the BAL findings was always based on the most relevant clinical data and the HRCT findings.

The diagnosis of sarcoidosis was confirmed histologically. In some patients without histological confirmation, a diagnosis of sarcoidosis was considered highly probable when the clinical and radiologic findings were compatible with the disease and the consensus criteria for BAL cellularity were satisfied.¹⁴⁻¹⁷

The diagnosis of IPF was made using lung biopsy, and in those cases in which the diagnosis was not reached, we applied the major and minor diagnostic criteria, also established by consensus, and which included the cell parameters of BAL.¹⁸

When the diagnosis of COP could not be confirmed by lung biopsy, it was established using the Poletti criteria,^{19,20} that is, more than 25% lymphocytes (with a CD4:CD8 ratio <0.9), combined with at least 2 of the following criteria: more than 20% foamy macrophages, more than 5% neutrophils, and more than 2% and less than 25% eosinophils (moderate neutrophilia and/or eosinophilia).

The diagnostic criteria of CTD-ILD were the same as those of the idiopathic ILDs not related to connective tissue disease¹⁶⁻²³: clinical and HRCT findings, lung function tests, and BAL fluid analysis.

The previously standardized major and minor criteria were required for the diagnosis of EAA.²⁴ The major criteria are the following: symptoms, evidence of exposure, CD3⁺CD8⁺ T cells in the BAL fluid, compatible histology, and a positive bronchial provocation test.

Follow-up lasted at least 16 months in all cases, this being the period necessary to assess the clinical course and to confirm the inclusion of each patient in each one of the groups.

The final diagnosis was established in 2 ways: *a*) with histological confirmation, or *b*) clinical diagnosis without histological confirmation, based on clinical and laboratory data, lung functional study, HRCT, cellular and immunologic parameters in the BAL, and the specific patient follow-up over time.

To evaluate the diagnostic value of BAL, a second analysis was performed after selecting patients with sarcoidosis and COP with histological confirmation; the sensitivity, specificity, and positive and negative predictive values were then calculated for the consensus diagnostic criteria for BAL.^{1,16,17,19,20}

Statistical Analysis

In the descriptive analysis, qualitative variables are expressed as absolute and relative frequencies and quantitative variables as means and standard deviations, except for calculation of the measure of central tendency in the study of CD3, CD4, CD8, and CD56 antigen expression and the CD4:CD8 ratio, in which the median and interquartile range are used, as the sample had a nonnormal distribution. The Kruskal-Wallis and Mann-Whitney U tests were used for comparisons between groups. A *P* value less than .05 was considered significant in all cases.

Results

Study Population

The study population was formed of a total of 562 patients with the following diseases: IPF (n=132), sarcoidosis (n=123), COP (n=89), EAA (n=85), and connective tissue diseases (n=133). The most common connective tissue diseases in the study were lupus (n=32), rheumatoid arthritis (n=25), progressive systemic sclerosis (n=24), mixed connective tissue disease (n=20), polymyositis/dermatomyositis (n=10), and primary Sjögren syndrome (n=7). The mean (SD) age of the patients in the sample analyzed was 53.4 (16.2) years. Sex and age differences were detected between the different diseases (Table 1): the mean age of presentation was lower in sarcoidosis and higher in IPF, and there was a female predominance in the connective tissue diseases, COP, and sarcoidosis, whereas IPF was more common in men. In all the diseases except the connective tissue diseases, there was a higher frequency of nonsmokers, although this trend was only significant for IPF and COP.

Cellular and Immunologic Parameters in the Bronchoalveolar Lavage Fluid

In the study of differential cell counts (Table 2), the pattern of isolated lymphocytic alveolitis was observed almost exclusively in

Table 1
Epidemiologic Data of the Study Population

Disease	Patients	Age, y, mean (SD)	Sex ^a		Smoking ^b		Diagnosis	
			Women	Men	Yes	No	DHC	DNHC
Sarcoidosis	123 (21.9%)	43.2 (15.5)	73 (59.3%)	50 (40.7%)	52 (42.2%)	71 (57.8%)	30 (24.3%)	93 (75.6%)
IPF	132 (23.5%)	62.0 (12.3)	36 (27.2%)	96 (72.8%)	33 (25%)	99 (75%)	10 (7.6%)	122 (7.6%)
EAA	85 (7.6%)	56.0 (15.3)	44 (7.6%)	41 (7.6%)	35 (7.6%)	50 (7.6%)	13 (7.6%)	72 (7.6%)
COP	89 (7.6%)	56.0 (16.2)	57 (7.6%)	32 (7.6%)	19 (7.6%)	70 (7.6%)	8 (7.6%)	81 (7.6%)
CTD	133 (7.6%)	51.7 (15.3)	90 (7.6%)	43 (7.6%)	77 (7.6%)	56 (7.6%)	-	133 (7.6%)
Total	562 (7.6%)	53.6 (16.2)	300 (7.6%)	262 (7.6%)	216 (7.6%)	346 (7.6%)	61 (7.6%)	501 (7.6%)

^a Kruskal-Wallis test statistically significant (*P*<.001) (comparative study between groups).

^b Kruskal-Wallis test not significant (*P*=.204) (comparative study between groups).

Abbreviations: COP, cryptogenic organizing pneumonia; CTD, connective tissue diseases; DHC, diagnosis with histological confirmation; DNHC, diagnosis with no histological confirmation; EAA, extrinsic allergic alveolitis; IPF, idiopathic pulmonary fibrosis.

Table 2
Study of Cell Differential Percentages in the Bronchoalveolar Lavage Fluid^a

Cell Differential	Disease				
	Sarcoidosis (n=88)	IPF (n=76)	EAA (n=64)	COP (n=39)	CTD (n=104)
Alveolar macrophages, %	58.3 (21.7)	62.3 (22.3)	39.1 (20.6)	47.3 (20.5)	58.7 (23.4)
Lymphocytes, %	37.9 (21.8)	17.3 (15.4)	47.3 (23.5)	28.9 (17.2)	28.1 (21.9)
Neutrophils, %	3.2 (4.7)	15.8 (16.3)	9.5 (11.7)	18.3 (18.7)	7.6 (11.3)
Eosinophils, %	1.4 (1.9)	4.5 (4.7)	3.4 (6.1)	6.0 (11.1)	2.5 (3.4)
Plasma cells, %	1.0 (0.7)	2.1 (4.0)	2.9 (4.3)	2.9 (4.9)	4.0 (7.7)

^aData are given as mean (SD).

Abbreviations: COP, cryptogenic organizing pneumonia; CTD, connective tissue diseases; EAA, extrinsic allergic alveolitis; IPF, idiopathic pulmonary fibrosis.

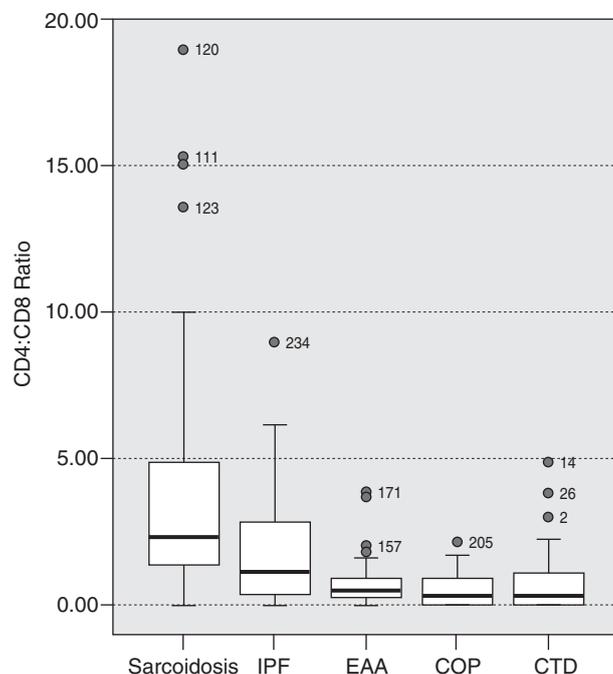


Figure. Plot of the CD4:CD8 ratio. The horizontal black lines indicate the median, and the 2 limits of the box are the 25th and 75th percentiles. The vertical bars represent the sample distribution. The circles indicate extreme values. COP indicates cryptogenic organizing pneumonia; CTD, connective tissue diseases; EAA, extrinsic allergic alveolitis; IPF, idiopathic pulmonary fibrosis.

sarcoidosis and EAA. Mixed alveolitis occurred in diseases such as IPF, COP, and the connective tissue diseases. Plasma cells were observed more frequently in the connective tissue diseases (4%), followed by EAA and COP (2.9%). Evaluation of the immunologic study in the BAL fluid showed the CD4:CD8 ratio to be the most useful parameter, as it clearly increased discriminatory diagnostic value (Figure). Sarcoidosis presented a higher ratio (median, 2.3) than the other diseases, in which the ratio was clearly low, with medians for IPF, EAA, COP, and CTD-ILD of 1.76, 0.455, 0.35, and 0.33, respectively. Inversion of the CD4:CD8 ratio did not discriminate between these diseases, as there was a degree of overlap. Only the presence of CD3⁺CD4⁻CD8⁻ cells (median, 20) was a discriminatory finding for the connective tissue diseases versus other diseases. Study of T-cell expression of CD3, CD4, CD8, and CD56 antigens (Table 3) revealed statistically significant differences between all the groups studied with respect to the percentage of cells with CD4⁺, CD8⁺, and CD3⁺CD4⁻CD8⁻ expression, and the CD4:CD8 ratio ($P < .001$). The CD56⁺ cell count (cytolytic cell marker) was only increased in a proportion of patients with EAA.

Comparison of the distinct diseases showed statistically significant differences in the various lymphocyte subpopulations between connective tissue diseases and sarcoidosis ($P < .05$, Mann-Whitney U test); these parameters presented extreme values in those diseases.

Diagnostic Value of the Bronchoalveolar Lavage Criteria in Patients With Histological Confirmation

In cases with histological confirmation, the sensitivity, specificity, and positive and negative predictive values were calculated for the diagnostic criteria of COP and sarcoidosis; these diseases can be diagnosed on clinical, radiologic, and BAL criteria when histological confirmation is not available.

When we analyzed the cases of COP (n=8), the sensitivity and specificity were 87.5% and 94.3%, respectively, and the positive and negative predictive values were 70% and 98%, respectively. In the case of sarcoidosis (n=30), the sensitivity and specificity were 40% and 96.8%, respectively, and the positive and negative predictive values were 92.3% and 62.5%, respectively (Table 4).

Discussion

The diagnosis of ILD continues to be a clinical problem. Evaluation of the clinical presentation, previous medications, systemic alterations, and identification of radiologic patterns, particularly in HRCT, provide essential information that enables us to establish a suspected diagnosis in the majority of cases. The diagnostic value of transbronchial biopsy is only truly evident in granulomatous disease.^{12,15} With respect to BAL, its capacity for orienting diagnosis in certain patients with ILD has been evaluated in numerous studies.^{19,24-30}

In our study, histological confirmation was achieved in only 11% of cases (14% if we exclude patients with connective tissue diseases, as lung biopsy is not usually performed in those patients). In other series, and depending on the characteristics of the center, histological confirmation has varied between 34% in the study by Welker et al²⁷ and 7.5% in the one by Johnston et al.⁷ There are numerous conditioning factors that determine this diagnostic behavior: comorbid illnesses, the morbidity and mortality associated with the diagnostic procedure itself, and the resources available in each center. Furthermore, in some patients it is possible that confirmatory lung biopsy will not modify the treatment plan.

In a large proportion of cases, the diagnosis cannot be established despite performing transbronchial biopsy, and surgical biopsy is not possible or is refused by the patient. In these cases, the clinical, radiologic, and functional data and the parameters of the BAL performed during fiberoptic bronchoscopy are the only data available on which to base the diagnostic suspicion.

Table 3
Distribution of the T cell Subpopulations in the Bronchoalveolar Lavage Fluid in Each Disease^a

Subpopulations (Immunoperoxidase)	Disease					
	Sarcoidosis	IPF	EAA	COP	CTD	P
CD3 ⁺ T cells	95.0 (90-95)	90.0 (90-95)	95.0 (85-95)	90.0 (88.2-95)	95.0 (90-95)	.110
CD4 ⁺ T cells ^b	63.0 (40-80) ^c	50.0 (25-58)	26.0 (17.5-34.2)	20.5 (5-48.2)	18.5 (2.5-33) ^c	<.001
CD8 ⁺ T cells ^b	24.0 (15-36) ^c	30.0 (21-60)	60.5 (37-70)	44.0 (32-74.7)	43.0 (27.5-43) ^c	<.001
CD56 ⁺ T cells ^b	0.9 (0.9-0.9)	0.9 (9-0.9)	0.9 (0.9-9.2)	0.9 (0.9-2.5)	0.9 (0.9-0.9)	.025
CD3 ⁺ CD4 ⁻ CD8 ⁻ T cells ^b	0.9 ^c (0.9-6)	5.0 (0.9-17)	6.0 (0.9-15)	11.0 (0.9-32.5)	20.0 ^c (3-41)	<.001
CD4:CD8 ratio ^b	2.3 (1.3-4.9) ^c	1.8 (0.4-2.9)	0.4 (0.2-0.9)	0.3 (0.1-0.9)	0.3 (0.1-1) ^c	<.001

^a Values expressed as median (interquartile range, 25-75).

^b Parameters in which the Kruskal-Wallis test was statistically significant ($P<.05$) (comparative study between groups).

^c Parameters in which the Mann-Whitney U test was statistically significant.

Abbreviations: COP, cryptogenic organizing pneumonia; CTD, connective tissue diseases; EAA, extrinsic allergic alveolitis; IPF, idiopathic pulmonary fibrosis.

Table 4
Evaluation of the Diagnostic Utility of the Criteria Based Exclusively on Cellular and Immunological Parameters of the Bronchoalveolar Lavage in Patients With Histologically Confirmed Cryptogenic Organizing Pneumonia or Sarcoidosis

	COP	Sarcoidosis
Sensitivity, %	87.5	40
Specificity, %	94.3	96.8
Positive predictive value, %	70	92.3
Negative predictive value	98	62.5

Abbreviations: COP, cryptogenic organizing pneumonia.

Welker et al²⁷ performed a prospective, observational study with 1748 patients in order to measure the predictive value of BAL in ILD. Using Bayesian methods, they calculated the diagnostic probability of a given ILD before and after BAL. In this way they demonstrated that BAL provides substantial diagnostic information in certain diseases (sarcoidosis, IPF, and EAA).

In our series, 92.5% of cases of IPF were not confirmed histologically and the diagnosis was reached using consensus criteria.¹⁸ In the BALs analyzed, there was a high proportion of mixed alveolitis, with a predominance of lymphocytes, neutrophils, and eosinophils, a similar pattern to that of other fibrosing conditions (EAA, COP, connective tissue disease), although, in contrast to those conditions, the CD4:CD8 ratio was closer to normal. At the present time, the value of BAL in IPF resides in reaching a diagnosis by the exclusion of other disorders, as stated in the various international consensus statements.^{1,18} In our study, the diagnosis was supported both by compliance with these criteria and by the long-term follow-up.

The value of transbronchial biopsy in the diagnosis of sarcoidosis is variable, depending on the radiologic stage; the value may be lower in cases with predominantly lymph node disease compared with the interstitial forms.^{1,15,31,32} In these cases, the CD4:CD8 ratio is useful in the diagnosis, together with other techniques (transbronchial biopsy or fine-needle biopsy of mediastinal lymph nodes), as it avoids having to perform more invasive tests such as mediastinoscopy. On this subject, attention is drawn to the results of 4 studies that concluded that, in patients with a clinical picture compatible with the disease, a raised CD4:CD8 ratio (>3.5) is diagnostic of sarcoidosis, and surgical biopsy is unnecessary.^{16,17,26,27,29}

Of the 123 cases in our series diagnosed with sarcoidosis, histological confirmation was only available in 30 and the diagnosis was made on clinical grounds and HRCT and BAL findings in 93. In the subgroup with histological confirmation, a CD4:CD8 ratio equal to or greater than 3.5 had a sensitivity and specificity of 40% and 96.8%, respectively, and positive and negative predictive values of 92.3% and 62.5%, respectively. These values are similar to those

reported by other authors such as Costabel¹⁶ and Winterbauer et al,¹⁷ who were also seeking a highly specific variable to help in the diagnosis. However, it should not be forgotten that the cellularity in this disease varies depending on the radiographic stages, as reported by our group in a recent study²⁸ that confirmed the findings of other authors. In early stages, with a predominance of hilar or mediastinal lymph nodes and with no interstitial disease visible on HRCT, BAL can reveal an intense lymphocytic alveolitis and a very significant increase in the CD4:CD8 ratio in comparison with more advanced stages, in which the ratio can even normalize or be low.

The study by Costabel¹⁶ of 117 consecutive patients with histological confirmation showed a sensitivity of 52% and specificity of 94% for the same cutoff point of the CD4:CD8 ratio. Winterbauer et al,¹⁷ in a study with 27 patients, reported a sensitivity of 59% and a specificity of 96% for a CD4:CD8 ratio equal to or greater than 4.0, and Thomeer²⁹ found a sensitivity of 55% and a specificity of 94% with a CD4:CD8 ratio equal to or greater than 4.0 in a group of 42 patients with histological confirmation. From a clinical point of view, it is more useful for diagnostic purposes if this cutoff point has a high specificity. As has been mentioned, the specificity in our study is high for a cutoff point of 3.5, with a slightly higher value than has been published previously.

The clinical presentation in some forms of sarcoidosis is similar to that of the connective tissue diseases. We believe it would be useful to establish a distinctive trait to differentiate between these 2 conditions, as both usually have an insidious onset and are associated with systemic manifestations. The systematic study of the BAL in these 2 diseases shows clearly significant differences: in the connective tissue diseases there is an evident inversion of the CD4:CD8 ratio and change in the expression of the 2 antigens, with a high percentage of CD3⁺CD4⁻CD8⁻ cells. This immunophenotype is frequently found in the most common connective tissue diseases, such as systemic lupus erythematosus, scleroderma, and Sjögren syndrome, when they present with radiologic evidence of interstitial disease, and this is also of great interest for identifying early involvement of the pulmonary interstitium, even though significant findings are absent from HRCT and lung function tests. The more advanced phases of these diseases, and progressive systemic sclerosis in particular, have clinical, radiographic, and histological findings, and a functional decline that are very similar to those of IPF. However, BAL offers distinctive traits by demonstrating a clearly reduced ratio,^{23,33} with a greater fall in CD4⁺ T cells than in the CD8⁺ population. In this disease, the role of the CD8⁺ T cell population (with cytotoxic suppressor function) as producers of type 2 helper T cell cytokines has been studied in recent years.^{25,30,33}

COP is an ILD that, from a pathologic point of view, is characterized by the presence of organizing pneumonia, with a subacute course of

cough and progressive dyspnea, and radiologically presents unilateral or bilateral condensations, that can be migratory and recurrent. Although histological diagnosis would be ideal, it is sometimes not possible and, given the natural history of the disease, confirmatory lung biopsy is often not performed. For this reason, BAL is a technique that gives diagnostic support and that is useful in the evaluation of these patients; analysis of the BAL reveals marked lymphocytosis with a low CD4:CD8 T cell ratio. Using a series of arbitrary criteria based on the cytologic and immunologic parameters of the BAL, Poletti et al¹⁹ analyzed 35 patients with COP and assessed the utility of BAL and of transbronchial biopsy in those with clinical and radiologic findings compatible with the disease. In their study, the probability of correct diagnosis using the results of both BAL and transbronchial biopsy was 62% and the positive predictive value of transbronchial biopsy was 94%. For those authors, the presence of mixed cellularity with lymphocyte predominance, inversion of the CD4:CD8 ratio, and macrophages with foamy cytoplasm, enabled the diagnosis of COP to be made with a high degree of certainty and without the need for confirmatory surgical biopsy. On studying the diagnostic utility of the cellular parameters of BAL, they demonstrated a sensitivity and specificity of 63% and 57%, respectively, and positive and negative predictive values of 85% and 29%, respectively.

In our study, in the subgroup of patients with histologically confirmed COP, the criteria described by Poletti et al¹⁹ had a sensitivity of 87.5%, a specificity of 94.3%, and positive and negative predictive values of 70% and 98%, respectively. From the results obtained, we can conclude that application of the Poletti criteria in our study gave rise to an adequate sensitivity, specificity, and negative predictive value, and, in contrast to what was reported in that study, we found the criteria to be more specific and to have a higher negative predictive value. When these criteria are satisfied and are associated with clinical data and HRCT findings, a diagnosis of a high suspicion of COP can be made. In view of the frequency of this disease, probably underestimated, further studies on this subject should be performed in order to confirm these results.

Finally, in the case of EAA, diagnosis is based mainly on criteria clinical, and BAL is useful when these criteria are not fully satisfied (particularly when there is no clinical response after elimination of the antigen) or the HRCT suggests other disorders that must be excluded. Briefly, BAL can detect abnormalities in all EAA patients, and a normal BAL practically rules out the disease (it has a high negative predictive value). Although the cellularity is variable depending on the presentation and course of the disease, BAL typically presents a pattern of mixed cellularity with a clear lymphocyte predominance and inversion of the CD4:CD8 ratio.¹⁹

In summary, as in other studies, we found a low frequency of histologically confirmed diagnoses. In this situation, the BAL parameters, if they are correctly interpreted, based on clinical and HRCT data, will help to establish diagnostic suspicion. Although the differential cell count in BAL fluid has shown a degree of overlap with other conditions, immunologic study can narrow the differential diagnosis. In sarcoidosis, the CD4:CD8 ratio is a useful parameter that provides clear diagnostic support. In COP and EAA, the cellular and immunologic study of BAL is very useful to demonstrate inversion of the CD4:CD8 ratio with an increase in CD56⁺ T cells. In IPF and CTD-ILD, 2 diseases with similar clinical and radiologic presentations, BAL shows distinctive traits in differential cell counts and in the immunologic pattern, which presents a marked inversion of the CD4:CD8 ratio and the presence of CD3⁺CD4⁻CD8⁻ T cells.

Further studies of BAL fluid in ILD are necessary to confirm these findings and to define other cytologic markers and markers of immune expression, or to measure cytokines or more specific soluble

components that contribute to improving its potential diagnostic role as a complementary technique together with biopsies.

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