

## Role of Bronchoalveolar Lavage in Diagnosis of Interstitial Lung Diseases

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### ABSTRACT

**Background:** Individuals with interstitial lung disease (ILD) often have improved diagnostic outcomes when a bronchoalveolar lavage (BAL) is performed. Management of a wide range of lung illnesses, especially the extensive family of ILD, frequently includes cytological examination of BAL fluid.

**Objectives:** Using a comparison of cytological findings in BAL fluid to evaluate the usage of BAL in the diagnosis of common ILD. **Patients and methods:** This prospective, clinical study was performed between December 2017 and December 2020. We studied 60 adult patients of both sexes who presented to the Chest Department of Al-Azhar University Hospital (Assiut).

**Result:** There was a significant variance among the study population regarding comparison between spirometry results, spirometry in smokers and non-smokers, comparison between the two groups regarding BAL cells and CD4/CD8 ratio amongst the ILD cases. In terms of demographic information as well as BAL cells immunophenotyping, the studied cases were not significantly distinct from the control group.

**Conclusion:** BAL fluid is a crucial component in the challenging process of diagnosing ILD. We assembled a panel of biomarkers related to sarcoidosis. Both the CD4+/CD8+ ratio and the number of natural killer cells in BAL have been linked to sarcoidosis in decision tree analyses. These biomarkers may prove useful in identifying cases with ILD suspicions in clinical settings.

**Keyword:** BAL, ILD, Pneumonia.

### INTRODUCTION

In order to carry out the minimally invasive treatment known as BAL, the fiberoptic bronchoscope is maneuvered into a wedge position within the chosen bronchopulmonary region. This allows the treatment to be performed. When cells and solutes from the lower respiratory tract are analyzed, not only can a more precise diagnosis be made, but also novel insights into immunologic, inflammatory, and viral processes that occur at the alveolar level can be gained<sup>(1-3)</sup>. ILD are a large category of lung disorders, and the cytological evaluation of BAL fluid is frequently utilized in the management of these conditions<sup>(4-5)</sup>.

ILD was characterized as encompassing both acute and chronic bilateral parenchymal infiltrative lung illnesses with varying degrees of inflammation and fibrosis. These illnesses arise in immunocompetent hosts in the absence of an infection or tumor. ILD was given its name since it was thought to comprise both acute and chronic parenchymal infiltrative lung illnesses. Pneumoconioses, ILD related to central nervous system connective tissue disease (CTD-ILD), and hypersensitivity pneumonitis (HP) are all examples of ILD with a known cause. On the other hand, sarcoidosis and idiopathic interstitial pneumonias (IIP) are both examples of ILD with an unknown cause, as stated in a joint statement from the American Thoracic Society and the European Respiratory Society<sup>(3)</sup>.

The diagnosis of ILD is a difficult task, and the BAL fluid can add significantly to this process. Some authors were able to identify a panel of biomarkers that are connected to sarcoidosis. In accordance with the results of a decision tree analysis, the ratio of CD4+ to CD8+ cells and the percentage of natural killer cells in

BAL are both linked to sarcoidosis. These biomarkers can come in handy in clinical practice to discriminate among individuals who might have ILDs<sup>(3-5)</sup>.

The diagnostic utility of BAL cytological examination in the treatment of ILD is still a contentious topic of discussion and controversy<sup>(6-7)</sup>.

This study aimed at evaluating the usage of BAL in the diagnosis of common ILD by using a comparison of cytological findings in BAL fluid.

### PATIENTS AND METHODS

This prospective, clinical study was performed between December 2017 and December 2020. We studied 60 adult patients of both sexes with common interstitial lung diseases (IPF, extrinsic allergic alveolitis (EAA), sarcoidosis, and NSIP) and 20 adult apparently normal control cases who presented to the Chest Department of Al-Azhar University Hospital (Assiut), in whom airway sampling was indicated.

### Ethical considerations:

**The investigation was authorized by the Al-Azhar University (Assiut) Faculty of Medicine's Ethical Committee Board. Every individual provided written informed permission before enrollment. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.**

**Inclusion Criteria:** Sixty cases with interstitial lung disease (28 patients have been diagnosed as sarcoidosis, 16 patients have been diagnosed as usual interstitial pneumonia (UIP), 10 patients have been diagnosed as EAA, 6 patients have been diagnosed as NSIP). All

patients have been diagnosed in accordance with diagnostic algorithm of ILD (8); either by clinical evaluation, lung function tests, or HRCT pattern and distribution; alternatively, by clinical diagnosis and HRCT alone.

**Exclusion Criteria**

Patients with uncorrectable bleeding tendency disorder, Inability to lie in the supine position, Respiratory insufficiency requiring ventilatory support, FEV1 less than 1 liter, Serious cardiac arrhythmia, Hemodynamic instability, Patients with very poor general condition, Patients with respiratory distress and patients who refused to participate in the investigation. All patients were subjected to: Full medical history, Physical examination, Laboratory tests, Arterial blood gases analysis, pulmonary function tests, Coagulation studies, and high resolution computed tomographic scans (HRCT).

**Statistical analysis**

All statistical analyses were conducted utilizing SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Quantitative variables were reported as means±SD and qualitative variables as absolute and relative frequencies in the descriptive analysis. One-way ANOVA test was used to compare the subsets of macrophages, lymphocytes, and polymorphonuclear cells in BAL from each group.

To analyze how the incidence of ILD disorders shifted in response to differences in patient cell counts, we used the Pearson's Chi-square test. Arbitrary intervals based on normal values and previous research have been set for the range of inflammatory cell subpopulation variation(3,8,9). P<0.05 was considered significant.

**RESULTS**

There was not a significant distinction in age, gender, smoking, and BMI among the cases and controls (Table 1).

**Table (1):** Demographic data of the cases

Variables	Cases n=60	Controls n= 20	p value
<b>Age (years)</b>			
Mean± SD	42.7± 12.1	40.2± 14.3	0.219
<b>Gender</b>			
Male n (%)	34 (56.7)	12 (60)	0.794
Female n (%)	26 (43.3)	8 (40)	
<b>Smoking</b>			
Smokers n (%)	22 (36.7)	8 (40)	0.790
Non-smokers n (%)	38 (63.3)	12 (60)	
<b>BMI (Kg/m<sup>2</sup>)</b>	24.5± 2.6	23.7± 3.1	0.260

There was a significant variation amongst cases and controls regarding spirometry results; FEV<sub>1</sub>, forced expiratory volume in first second, and FEV<sub>1</sub>/ FVC (%) (Table 2).

**Table (2):** Comparison amongst spirometry results among cases and controls

Variables	Cases Mean± SD	Controls Mean± SD	P value
<b>FVC (% pred)</b>	80.7± 15.2	85.6± 12.5	0.197
<b>FEV<sub>1</sub> (% pred)</b>	81.3± 16.4	88.1± 15.5	0.108
<b>FEV<sub>1</sub>/FVC (%)</b>	82.3± 7.2	90.3± 11.4	<0.001*

\*: Significant

The results of spirometry tests showed a significant distinction among smokers and non-smokers (Table 3).

**Table (3):** Comparison among smokers and non-smokers regarding spirometry

Variables	Smokers Mean± SD	Non- smokers Mean± SD	P value
<b>FVC (% pred)</b>	79.6± 13.1	86.4± 14.5	<0.039*
<b>FEV<sub>1</sub> (% pred)</b>	76.2± 15.3	89.1± 13.9	<0.001*
<b>FEV<sub>1</sub>/FVC (%)</b>	83.4± 6.7	89.2± 12.6	0.023*

\*: Significant

There was statistically significant distinction among the 2 studied groups regarding BAL cells (Table 4).

**Table (4)** Comparison among the 2 groups as regard BAL cells

Cells	Cases	Controls	P value
<b>Macrophage (%)</b>	56.1± 25.9	88.3± 13.6	0.045
<b>Lymphocytes (%)</b>	31.3± 22.5	11.7± 7.3	0.011*
<b>Neutrophils (%)</b>	13.7± 12.9	1.3± 0.2	0.042*
<b>Eosinophils (%)</b>	1.6± 3.6	0.3± 0.2	0.041*

\*: Significant

There was significant difference between groups as regard CD<sub>4</sub> /CD<sub>8</sub> ratio and it was higher among the participants who had sarcoidosis (Table 5).

**sTable (5):** CD<sub>4</sub> /CD<sub>8</sub> ratio amongst the ILD cases

Disease	Mean± SD	<3.5 n (%)	≥3.5 n (%)	P value
<b>Sarcoidosis (n=28)</b>	2.6± 1.1	18	10	<b>0.001*</b>
<b>Usual interstitial pneumonia (n=16)</b>	1.9± 0.5	10	6	
<b>Extrinsic allergic alveolitis (n=10)</b>	2.1± 0.8	6	4	
<b>Non-specific interstitial pneumonia (n=6)</b>	1.3± 0.6	6	0	

\*: Significant

In terms of the immunophenotyping of BAL cells, there was not a noticeable distinction among these individuals and the controls (Table 6).

**Table (6):** A comparison of the immunophenotyping of BAL cells based on the two groups that were examined.

Cells	Cases	Controls	P value
<b>CD<sub>3</sub></b>	89.4± 5.7	95.3± 3.9	<b>&lt;0.001*</b>
<b>CD<sub>4</sub></b>	53.7± 15.4	53.6± 10.2	0.232
<b>CD<sub>8</sub></b>	32.9± 16.4	35.6± 12.4	0.056
<b>CD<sub>4</sub>/CD<sub>8</sub></b>	2.0± 0.3	1.5± 0.2	<b>&lt;0.001*</b>
<b>CD<sub>19</sub></b>	1.4± 0.4	2.1± 1.1	0.078

**DISCUSSION**

BAL is frequently employed as a diagnostic instrument in the treatment of ILD. Nevertheless, its diagnostic value in distinguishing among entities comprising the extremely heterogeneous group of ILD remains a contentious topic. Our study's objective is to determine the diagnostic value of BAL in the management of ILD by contrasting the cytological findings in BAL fluid amongst the various diseases in this cohort<sup>(10)</sup>.

Regarding the demographic data of the participants, we found that the participants age ranged from 30 to 56 and mean age was 42.7± 12.1. Also, 56.7% of the participants were males. The mean BMI was 24.5± 2.6. The former smokers represented 6.3% of the participants.

The study by **d'Alessandro et al.**<sup>(11)</sup> employed a panel of BAL indicators to make a differential diagnosis of ILD on 100 Caucasian cases who had BAL as part of their diagnostic workup for suspected ILD. The median age (IQR) of these individuals was 65 years, and 50% of them were men. Smokers made up 54% of the patient population. While **Efared et al.**<sup>(10)</sup> studied the

diagnostic value of the bronchoalveolar lavage in ILD, they included 151 patients, 74.83% were women. The average age was 52.78 years (age was varying from fifteen to eighty).

Results of this study indicated that there was no significant difference among groups of ILD (sarcoidosis, UIP, EAA and NSIP) regarding age, gender, smoking and BMI.

While the study by **d'Alessandro et al.**<sup>(11)</sup> revealed that sarcoidosis cases tended to be younger ( $p = 7.4E^{-03}$ ) than other individuals. There were no statistically significant variations in smoking history or RX scadding phases across the ILD patient groups.

Regarding the spirometry values of the participants, we found that there was statistically significant variation among interstitial lung diseases groups as regard spirometry values using Kruskal Wallis test and p was significant at <0.05.

The study by **Domagala-Kulawik et al.**<sup>(12)</sup> reported that mild and moderate impairments of pulmonary function (both obstructive and restrictive) were observed. The mean value of lung diffusion capacity for CO was 58 ±14% predicted. Analysis of blood gases revealed slight hypoxemia. They found no patients with respiratory failure.

Regarding the BAL cells among the participants, we found that the neutrophils percentages were statistically significantly lower in sarcoidosis than other ILD. But macrophages, lymphocytes and eosinophils had no significant difference.

Differentiating ILDs is often aided by the information provided by BAL cell patterns and lymphocyte phenotyping, particularly when it comes to validating the diagnosis of granulomatous lung disorders. The profile of cells collected by BAL has been extensively studied, and has shed light on a number of diseases, involving HP and sarcoidosis<sup>(3)</sup>.

Also, statistically significant variations were found in the present investigation among the groups with respect to CD<sub>4</sub> /CD<sub>8</sub> ratio and it was higher among the participants who had sarcoidosis. The mean of the ratio was 2.6±1.1 among participants who had sarcoidosis. As well we found that natural killer cells were statistically significantly higher in sarcoidosis. But there was no statistically significant difference regarding, CD<sub>4</sub>, CD<sub>8</sub>, and NK-like cells.

A diagnosis of sarcoidosis is supported by the accumulation of CD<sub>4</sub>+T-helper cells in afflicted tissues and an increased CD<sub>4</sub>+ /CD<sub>8</sub>+ ratio in BAL, although histological inspection is still required<sup>(13)</sup>. Previous research has also shown that people with sarcoidosis have a reduced number of NK cells and CD<sub>103</sub>+CD<sub>4</sub>+ cells in their BAL fluid, and a higher CD<sub>4</sub>+ /CD<sub>8</sub>+ ratio<sup>(8, 14)</sup>.

While the study by **Efared et al.**<sup>(10)</sup> reported that the mean CD<sub>4</sub>/CD<sub>8</sub> ratio was 2.18 overall. Sarcoidosis had the highest mean CD<sub>4</sub>/CD<sub>8</sub> ratio in comparison with other diseases, at 2.56. Lymphocyte subsets in the BAL fluid, namely HLA-DR+ CD<sub>8</sub>+ T-cells and

natural killer T-cells, have also been examined as potential diagnostic markers for distinguishing sarcoidosis from HP. Activated lymphocytes in the BAL fluid from individuals with either sarcoidosis or HP express the human leucocyte antigen (HLA)-DR<sup>(15)</sup>. Sarcoidosis cases appear to have lower levels of HLA-DR expression on CD8+ lymphocytes in contrast to HP individuals<sup>(8)</sup>. Participants with sarcoidosis have less natural killer-T (NKT) cells in their BAL than those with HP, which express CD56 and/or CD16 as well as T cell receptors<sup>(16)</sup>.

## CONCLUSION

The diagnosis of ILD is a difficult task, and the BAL fluid can aid significantly in this process. We were able to identify a panel of biomarkers that are connected to sarcoidosis. In accordance with the results of a decision tree analysis, the ratio of CD4+ to CD8+ cells and the percentage of natural killer cells in BAL are both linked to sarcoidosis. These biomarkers can come in handy in clinical practice to discriminate among individuals who might have ILDs.

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