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Diagnosis of Diffuse Interstitial Lung Diseases: BAL Study - Its Utility for the Diagnosis

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Abstract

Introduction: Diffuse interstitial lung diseases are a hard-to-diagnose heterogeneous group of respiratory diseases. The study of bronchoalveolar lavage through flow cytometry may define typical cell patterns in different diseases and so help confirm the differential diagnosis. The purpose of this study was to retrospectively analyze the clinical utility of cell and lymphocyte subpopulations detected in the bronchoalveolar lavage by flow cytometry in order to define typical cell patterns that allow for making a differential diagnosis of granulomatous lung diseases.
Materials and methods: The retrospective study included 44 patients. The subjects were diagnosed with sarcoidosis or hypersensitivity pneumonitis during a period of 3 years. We performed the cellular analysis of bronchoalveolar lavage through flow cytometry and histological and imaging testing (HRCAT, High Resolution Computed Axial Tomography) as part of the diagnosis. The percentages of T cells, B cells, NK cells, CD4, CD8 and CD4/CD8 were analyzed by flow cytometry for the following markers: CD3 +, CD19 + CD4 +, CD8 +, CD3 + CD4-CD8- and CD3 + CD16-CD56-.
Results: We conclude that the most important parameters were lymphocytosis and especially the CD4/CD8 quotient. This quotient was high for diseases such as sarcoidosis and low for hypersensitivity pneumonitis, in comparison with the values found in the peripheral blood.
Conclusions: The BAL (Bronchoalveolar Lavage) study is useful for differentiating between granulomatous interstitial lung diseases and other DILDs (diffuse interstitial lung diseases).

Key words: Interstitial lung diseases; Flow cytometry; Bronchoalveolar lavage

Introduction

Diffuse interstitial lung diseases (DILDs) are a very heterogeneous group of entities that mainly affect the lung interstitium.

The diagnosis of such a heterogeneous group of diseases frequently poses a challenge to the clinical staff, for multiple reasons.

First, there are more than 150 causes of DILDs (but it is only possible to establish the etiologic diagnosis in 40-50% of the cases). Many factors are involved in the pathogenesis of the disease, both exogenous (metals, organic substances, wood, drugs, virus) and endogenous (autoimmune diseases, gastroesophageal reflux). Secondly, for many years there wasn't uniformity in the classification of these diseases. Finally, the clinical context in which these diseases are developed is frequently common and non-specific; on many occasions the symptoms can't be distinguished from other neoplastic or autoimmune diseases[1, 2].

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The most frequent clinical symptoms presented by patients with these diseases are dyspnea and cough that worsens after exercise.

Extrapulmonary symptoms are also common and sometimes help make a diagnosis. Clinical manifestations may be present, at the neurological, ocular, dermatologic, digestive or cardiac level.

Sarcoidosis and hypersensitivity pneumonitis (HN) are the most important of those diseases. Both diseases share their granulomatous nature (60% of HPs are manifested by granulomas) and the existence of common pulmonary and extrapulmonary manifestations, which extremely complicate the etiologic diagnosis.

The diagnostic process begins with a clinical evaluation (including the thorough knowledge of the medical record, physical examination, patient's biochemical and immunological analysis, etc.), chest x-ray, pulmonary functional tests and image-based testing such as HRCAT (High Resolution Computed Axial Tomography). If that weren't enough, the study could also be completed with other more invasive tests such as surgical lung biopsy or the study of bronchoalveolar lavage (BAL).

Bronchoalveolar lavage is a method that allows for the study of the lower airways through the analysis of cellular and biochemical components.

This technique consists in the instillation of a saline solution in boluses of about 20-50 ml (until getting 120-200 ml) at the lung segment level. After each instillation, the content is aspirated, obtaining a first aliquot (bronchial sample) that is representative of the cellularity of the airway.

It is a simple, safe (less than 3% complication rate), well-tolerated technique that provides a lot of clinical information for the study of lung diseases.

However, the usefulness of this technique is still controversial, since it appears to show high diagnostic value for certain lung diseases (alveolar proteinosis, eosinophilic pneumonia, histiocytosis X, alveolar infections or hemorrhage) but is just a guideline for others (pulmonary fibrosis, sarcoidosis, hypersensitivity pneumonitis, pneumoconiosis or drug toxicity)[3, 4].

Thus, we set forth the usefulness and diagnostic value of bronchoalveolar lavage cytological study, which seems to define certain cellular patterns typical of the disease that could be useful for the etiologic diagnosis of diffuse interstitial lung diseases.

The purpose of this study was to carry out a retrospective descriptive analysis of the cytological study and lymphocyte subpopulations in the BAL performed in patients with sarcoidosis and hypersensitivity pneumonitis in our hospital area.

We pretend to show that bronchoalveolar lavage can present cellular patterns typical of the disease, allowing us to differentiate the DILDs and greatly supporting the clinical diagnosis.

Materials and methods

Retrospective, observational study including all the patients diagnosed with sarcoidosis and hypersensitivity pneumonitis who underwent a bronchoalveolar lavage as part of the diagnosis between January 2015 and June 2018 at the H.U.V. Macarena de Sevilla.

All the patients underwent a bronchoalveolar lavage unless otherwise contraindicated, in order to identify and determine percentages and absolute lymphocyte counts as well as lymphocyte subpopulations. The same parameters were measured in peripheral blood, for the purpose of doing a comparative study as part of the diagnosis. Both samples, the BAL and peripheral blood were obtained and processed at the same time.

The patients: The study includes all the patients diagnosed with sarcoidosis and hypersensitivity pneumonitis (N = 44), from January 2015 to June 2018. Bronchoalveolar lavage was performed in all the patients as part of the diagnosis.

The final diagnosis was established in two ways: *a)* through histological confirmation, and *b)* clinical diagnosis without histological confirmation based on clinical and analytical data, functional study, HRCAT, cellular and immune parameters in the BAL and patient's follow-up over time.

The final diagnosis was established by the Pulmonology Service of our hospital center and included in the medical record of each patient (with the Diraya system of the Andalusian Health Service). Clini-

cal data for this study (clinical diagnosis of each patient) were taken from the Diraya, an electronic system of medical records, and were directly compared with the data provided by the personnel of the clinical biochemistry laboratory.

The study was approved by the Research Ethics Committee of our center.

Bronchoalveolar lavage: Bronchoalveolar lavage was performed through a fibrobronchoscope (Olympus). The instillation volume was 180 ml. At least 3 aliquots were recovered and sent to different laboratories (general biochemistry, microbiology and pathologic anatomy) to be studied fully.

Together with the collection of the BAL, a peripheral blood sample was taken from each patient through venipuncture.

Despite variability (smoking or age, mostly), it is an accepted fact that the cellularity present in the BAL fluid of normal subjects consists of: mainly macrophages (80-95%), lymphocytes (< 15%) and neutrophils (2-5%). Eosinophils, basophils and plasma cells represent the minority group (1-3%).

Processing of bronchoalveolar lavage: The antiserum used for identifying and determining percentages and absolute counts of lymphocytes was the Becton Dickinson BD Multitest 6-color TBNK reagent.

The immunological study, done by flow cytometry (BD FACSCanto II cytometer), included in both samples the identification and count of T, B and NK lymphocytes, monocytes and polymorphonuclear cells, as well as CD4+ and CD8+ lymphocyte T subpopulations and the existing relationship between them (CD4+/CD8+ quotient).

Results

The study includes 44 patients: 32 patients with sarcoidosis and 12 patients who showed hypersensitivity pneumonitis.

The mean age (± standard deviation) of the sample was 60.80 ± 15.46 years.

As regards gender, 62% were male (N = 27) and 38% were female (N = 17).

As for patients with sarcoidosis, there were 22 females (70%) and 10 males (30%); and the mean age of this population was 56.1 ± 11.07 years. Differences regarding age and gender were demonstrated in comparison with the rest of the diffuse respiratory diseases: in sarcoidosis, females predominated and the mean age was lower (56.1 ± 11.07).

In order to estimate the diagnostic utility of bronchoalveolar lavage, we will now describe the results obtained from each one of the respiratory diseases:

Firstly, in the study of patients with sarcoidosis, our results concluded that sarcoidosis is presented as lymphocyte alveolitis with a predominance of CD4+ lymphocytes: 60.94% (± 20.20) lymphocytes, 8.94% (±19.00) monocytes and 30.12% (± 16.19) polymorphonuclear cells. A clear increase in the lymphocyte population is observed compared to the rest of the cell populations of the BAL.

On the other hand, with respect to the quotient of CD4+/CD8+ lymphocyte subpopulations, we can say that there is a clear increase of such quotient in the bronchoalveolar lavage in comparison with its blood values. The mean CD4+/CD8+ quotient in blood was 1.09 (± 0.59), whereas in bronchoalveolar lavage it was 5.35 (± 3.75). Thus, we can see that most patients (68.8% of patients) show a CD4+/CD8+ quotient of more than 3.5.

Secondly, in patients with hypersensitivity pneumonitis, the BAL study showed an increase in lymphocytes, just like in the previous case: 63.33% (±10.40) lymphocytes, 5.00 (± 0.00) monocytes, 31.67 (± 10.40) polymorphonuclear cells.

But the BAL quotient in these patients had noticeably decreased in comparison with the values found in peripheral blood: the mean CD4+/CD8+ quotient in blood was 2.07 (± 1.47), whereas in the BAL it was 0.2 (± 0.119).

Discussion

The diagnostic utility of BAL has been the object of numerous studies. Most studies agree on the diagnostic value of BAL for certain diseases such as alveolar proteinosis, histiocytosis X or alveolar hemorrhage. But we should also add the fact that there are many studies still discussing its use for many other DILDs (sarcoidosis, pneumonias associated with drugs or connective tissue diseases), where it seems to have a mostly illustrative and not diagnostic value. For that reason, in most respiratory disorders the study of BAL doesn't seem enough for making the final diagnosis.

With respect to our work, we agree with the literature that was consulted about the diagnostic value of BAL for certain DILDs, including sarcoidosis and hypersensitivity pneumonitis, where BAL shows a high value[5-7].

Sarcoidosis is a multisystemic disease of unknown origin, characterized by the presence of non-caseating granulomas. Due to this multisystemic characteristic, the diagnostic approach may be complex (there isn't an individual diagnostic test for sarcoidosis).

Despite the fact that sarcoidosis may affect multiple organs, the lung is involved in most cases (80-95% of cases). Lung symptoms include dyspnea, cough, distress and wheezing. Other frequently affected organs include: eyes, skin and lymph nodes[8].

Sarcoidosis predominantly affects young adults. Most of them are 20-40 years old and it is clearly most common in women than in men (ratio 3:1). This fact was confirmed in our patients, and we concluded there is a higher incidence in young women.

Even though its etiology is unknown, we suggest a cause of genetic origin together with certain environmental factors. As regards the genetic factors, the individuals more susceptible to the disease are those with HLA DR11, 12, 14,15 and 17; on the other hand, the studies seem to conclude that having HLA DR1 and DR2 provides a protection capacity. As for the exogenous factors, it is important to mention: microbial agents (mycobacteria), organic agents (pine pollen), inorganic agents (beryllium, aluminum) or drugs (methotrexate).

So, the characteristic granulomas that are formed are caused by a persistent immune response to antigens acting in a continuous way, capable of inducing an exaggerated response in genetically predisposed individuals.

As a consequence of this immune response, patients diagnosed with sarcoidosis showed lymphocyte alveolitis with noticeable increase in the BAL CD4+/CD8+ quotient. Thus, 68.8% of patients showed a CD4+/CD8+ quotient of more than 3.5. An increase in said quotient is very specific of sarcoidosis (whereas an increase in lymphocytes is more sensitive). So, according to the consulted literature, those patients with a CD4+/CD8+ quotient > 4 have a high probability of developing sarcoidosis (predictive value of 94%), whereas CD4+/CD8+ quotients < 1 allow for the exclusion of the disease[9-11].

This fact seems to be related to the nature of the disease itself, characterized by the presence of non-caseating granulomas which consist mainly of macrophages and CD4+ lymphocytes that appear as a consequence of an exaggerated immune response.

Also, hypersensitivity pneumonitis is a diffuse interstitial disease normally characterized by the presence of granulomas (60% of the cases) caused by the chronic inhalation of a wide variety of organic products. In this group we include a lot of substances: soy, hay, wood, coffee grains, sugar cane, cured meats. In almost every case its origin is occupational. The most frequent of these diseases are: the "farmer's lung disease" (moldy hay, Ag. T. vulgaris) and the "bird fancier' s lung disease" (serum, bird proteins and droppings, Ag. T. vulgaris).

The pathogenesis is based on an immune response to these inhaled particles giving place to type III hypersensitivity reactions (mediated by immune complexes and CD8+ lymphocytes) and type IV hypersensitivity reactions (mostly mediated by CD8+ lymphocytes responsible for the formation of granulomas)[12].

In patients with hypersensitivity pneumonitis, the immune study through BAL cytometry yielded a high value. HP has a complex diagnosis, due to its granulomatous nature and extrapulmonary clinical

manifestation (cough, dyspnea or weight loss); it can be clinically similar to other diseases, such as sarcoidosis.

All the patients with HP show alterations in the BAL in such a way that an unaltered BAL excludes the HP diagnosis. It is the most sensitive method. Lymphocytosis is predominant, with CD8+ lymphocytes that appear as a consequence of type III and type IV hypersensitivity reactions to inhaled particles, thus, the quotients in these patients are lower than one**.** An increase in CD4+ lymphocytes suggest a bad prognosis, given that an increase in CD4+ (as well as neutrophils) has been related to a higher possibility of suffering from fibrosis[13].

As can be observed, these diseases can show common clinical symptoms that greatly complicate their diagnosis; however, there are clear differences regarding the cellular populations present in the bronchoalveolar lavage. Thus, under certain circumstances where it is difficult to distinguish between HP and sarcoidosis, the cytological study of BAL has allowed us to guide the diagnosis: CD4/CD8 quotients are reduced in BAL for HP, and those quotients increase considerably for sarcoidosis.

Conclusions

Diffuse interstitial lung diseases (DILDs) entail in most cases a diagnostic challenge for the clinician.

But, in the adequate clinical and diagnostic context, the analysis of bronchoalveolar lavage by flow cytometry is useful to study lung diseases, since it shows high diagnostic value for certain diseases such as sarcoidosis and hypersensitivity pneumonitis.

Likewise, after the review that was conducted, we believe more studies about bronchoalveolar lavage are necessary to discover new BAL markers of any nature (immune, biochemical, cellular), more specific, that contribute to a better understanding of diffuse interstitial lung diseases.

Conflict of interests: The authors declare that there is no conflict of interests.

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