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Review Article

Use of bronchoalveolar lavage in the diagnosis of pulmonary diseases and its potential clinical-laboratory applications: a review

Uso do lavado broncoalveolar no diagnóstico de doenças pulmonares e suas potenciais aplicações clínico-laboratoriais: uma revisão

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Abstract

Bronchoalveolar lavage (BAL) is a non-invasive bronchoscopic technique used to aspirate cells from the pulmonary interstitium, initially applied to detect neoplastic cells but later expanded for microbiological studies, leukocyte counts, molecular analyses, and immunophenotyping. This broader approach has linked BAL to the study of interstitial lung diseases (ILD). Despite its use since the 1970s, standardization of BAL cytopathological analysis is lacking, hindering its routine clinical application. The limited number of studies, particularly in Southern Hemisphere countries, reflects challenges in refining laboratory procedures and cytological findings. This article discusses the diagnostic potential of BAL when combined with clinical and radiological data. Integrating cytomorphologic findings with molecular techniques enhances its accuracy, particularly for cases with undefined etiopathogenesis, offering improved diagnostic and follow-up capabilities. The results make it clear that BALF cytology helps tell the difference between different types of ILDs. They also show that it is a useful tool for monitoring and diagnosing lung diseases.

Keywords: cytology, cell differential count, diagnosis, interstitial lung disease, bronchoalveolar lavage.

Resumo

A lavagem broncoalveolar (LBA) é uma técnica broncoscópica não invasiva usada para aspirar células do interstício pulmonar. Inicialmente aplicada para detectar células neoplásicas, sua utilização foi ampliada para estudos microbiológicos, contagem de leucócitos, análises moleculares e imunofenotipagem. Essa abordagem mais ampla vinculou o LBA ao estudo de doenças pulmonares intersticiais (DIP). Apesar de ser utilizada desde a década de 1970, a análise citopatológica do LBA carece de padronização, dificultando sua aplicação como rotina clínica. A escassez de estudos, especialmente em países do hemisfério sul, reflete os desafios na melhoria dos procedimentos laboratoriais e dos achados citológicos. O artigo discute o potencial diagnóstico do LBA quando combinado com dados clínicos e radiológicos e os reune aos achados citomorfológicos com técnicas moleculares, visando proporcionar melhores capacidades de diagnóstico e acompanhamento. Os achados enfatizam que a citologia oriunda do LBA contribui com o diagnóstico diferencial de diversos subtipos de DIPs, sugerindo um algoritmo celular de fácil uso clínico, além de realçar sua versatilidade como ferramenta de acompanhamento e diagnóstico na área pneumológica.

Palavras-chave: citologia, contagem celular diferencial, diagnóstico, doença pulmonar intersticial, lavado broncoalveolar.

1. Introduction

Pulmonary diseases are major causes of mortality worldwide, reaching 18% among neoplasms, excluding non-melanoma skin cancer (Bray et al., 2024). Among the non-neoplastic diseases of the pulmonary tissue, interstitial lung diseases (ILD) are highlighted in medical care due to the variety of clinical and radiological characteristics and, consequently, of diagnosis, providing subclassifications regarding their tissue alteration and their etiopathogenesis (Efared et al., 2017). ILD are infiltrative parenchymal alterations due to the accumulation of immune and inflammatory cells in the pulmonary interstitium and alveolar walls, and can be categorized as: acute or chronic;

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inflammatory or fibrotic; idiopathic or of known cause (Meyer et al., 2012).

To search for cells in the pulmonary interstitium and minimize the submission of pulmonary patients to biopsies, the bronchoalveolar lavage (BAL) technique was developed, which consists of recovering cells in the alveolar wall and associating them with the patient's clinical condition (Rice, 2022). Specifically, BAL has gained prominence in the field of scientific research due to its conclusive or indicative potential, knowledge of the cellular population, in addition to the identification of neoplastic cells and as a suggestive parameter of changes in the pulmonary parenchyma (Bergantini et al., 2021).

Thus, this review aims to address the cytomorphological criteria derived from BALF and how its versatility is fundamental for differentiating ILD, as well as for analyzing cancer cells, specifically in non-small cell lung cancer (NSCLC). In addition to contributing to the dissemination of information on basic bronchoscopy, technical procedures, microscopic analysis, as well as highlighting possible molecular methods using BAL fluid.

2. Bronchoscopy and Technical Processing

The bronchoscopic procedure uses a flexible bronchoscope, typically made of carbon fiber. A 0.9% saline solution is instilled serially, with a volume ranging from 100 to 300 milliliters (mL) at room temperature (Wahlstrom et al., 2009). The liquid is recovered by applying negative pressure. If laboratory processing exceeds 8 hours, 50% ethyl alcohol should be used as a preservative in a 1:1 ratio (volume/volume) immediately after aspiration of the instilled volume. The sample-alcohol mixture should be transported in a refrigerated environment at approximately 4 °C (Collins et al., 2014; Meyer et al., 2012). In our experience, processing should ideally be done within 24 hours to avoid cytomorphological changes and degenerative processes, which could compromise the cytological report. Samples to be processed within 8 hours do not require a preservative solution and can simply be transported at refrigerated temperature.

The lavage analysis begins with macroscopic evaluation, considering physical aspects, color, turbidity, and deposits due to specific pulmonary disease associations (Meyer et al., 2012; Miller et al., 2018). Samples are placed in sterile conical tubes and centrifuged at 600 rpm for 5 minutes. The sediment is analyzed for cellularity to guide cytocentrifugation: 100 rpm for 5 minutes in hypercellularity or 1500 rpm for 10 minutes when cells are scarce. Variations include 50×g for 3 minutes (Wahlstrom et al., 2009), 1640 rpm for 25 minutes (Meyer et al., 2012), or 600 rpm for 5 minutes (Bergantini et al., 2021). However, 1200 rpm for 5 minutes at room temperature is optimal, preserving cell integrity. Cytocentrifugation ensures slides without cell overlap and with homogeneous distribution, easing analysis. Sediment can also support microbiological and molecular studies (Subhagan et al., 2022).

It is recommended to perform at least two staining methods: Papanicolaou (wet fixation) and non-Papanicolaou (dry fixation) (Yang et al., 2001). While most laboratories opt for rapid staining, such as Diff-Quick, Papanicolaou staining is valuable for visualizing nuclear components (chromatin, nucleoli, and inclusions) in reactive, metaplastic, and atypical processes, as illustrated in Figure 1.

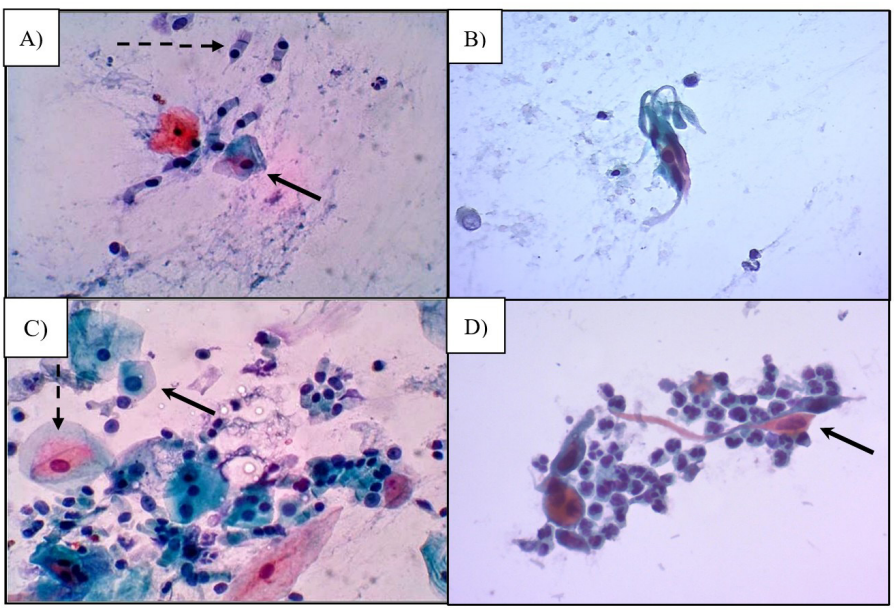

Figure 1. Epithelial cells on Papanicolaou stain. (A) Squamous epithelial cells (arrow) and ciliated columnar cells (dotted arrow) (magnification 400x); (B) Squamous cells in metaplasia (magnification 400x); (C) Squamous cells with reactive changes marked by increased nuclear volume (arrow) and cytoplasmic anophilia (dotted arrow) (magnification 400x); (D) Keratinized squamous cells in fusiform shape (arrow), atypical nuclei, compatible with Squamous Cell Carcinoma (magnification 400x).
Source: Cell microphotography in BAL. Designed by authors, 2025.

3. Interferences and Possible Errors in the Preparation of Bronchial Lavage

Despite being a technique with great diagnostic, prognostic and monitoring potential of ILD (Heron et al., 2012), the BAL can suffer several interferences, which include: scarce volume of the recovered lavage, limiting the relative count; cytological preparation and staining; delay in the delivery of the material to the laboratory; and, finally, the patient's clinical condition (Frye et al., 2020), since not every patient should undergo bronchoscopy. Attention should be paid to cardiopulmonary conditions, coagulation problems, hypersensitivity to sedatives and the diagnostic conclusion by high-resolution computed tomography (HRCT) (Meyer et al., 2012).

As mentioned, the patient's own condition can interfere with the cellular analysis. In cases of chronic obstructive pulmonary disease (COPD), emphysema, chronic bronchitis and pulmonary hypertension, there is difficulty in obtaining the lavage (He et al., 2017). Another factor is the production of mucus, which is a trivial factor, since it traps cells and can generate a false-negative result due to hypocellularity (Collins et al., 2014).

Variations in bronchoscopic procedures and BAL fluid analysis highlight the clinical-laboratory heterogeneity, compounded by local and regional references influencing cellular populations, making standardization difficult (Hetzel et al., 2021). The main challenge remains the lack of baseline studies and regional consensus. Climatic and genetic factors, particularly in multiethnic countries, add complexity to this issue, making it an ongoing subject of study (Cottin and Valenzuela, 2020). Most studies linking BAL cellular profiles with ILD come from Northern Hemisphere nations (Bergantini et al., 2021; Efared et al., 2017; Jara-Palomares et al., 2009; Meyer et al., 2012; Salonen et al., 2020) (Table 1), using the 2012 global/differential count parameters of the American Thoracic Society and European Respiratory Society, which do not reflect the reality of other countries.

4. Cytological Analysis of Bronchial-Alveolar Lavage and its Association with Pulmonary Interstitial Diseases

The cells present in the BAL are indicative of interstitial pathologies, having high informative value when added to the bronchoscopic findings, clinical tests and imaging tests, mainly CT. According to the consensus formed by the American Thoracic Society (ATS), the differential count of cells is totaled in a representative sum of 100% of that sample, as shown in Table 2 (Cottin and Valenzuela, 2020; Meyer et al., 2012). Some studies recommend a differential count of 200 (Frye et al., 2020), 300 (Rice, 2022), or even 500 cells (Wahlstrom et al., 2009). In addition to the differential

Table 1. Studies that associate BAL cytological patterns with ILD.

Author (place)	Objective	Results
Jara-Palomares et al. (2009) (Spain)	Associate cytological findings with ILD, specifying phenotypic of lymphocytes.	Lymphocyte predominance was found in sarcoidosis and extrinsic allergic alveolitis. CD4:CD8 immunophenotype pattern greater in sarcoidosis.
Meyer et al. (2012) (Unites States/Europe)	Guideline study, aiming to trace the quantitative cellular profile of the BAL and create guidelines for the preparation of the lavage sample	The cytology of the BAL has an indispensable factor in the multidisciplinary medical work, especially in cases where TCAR cannot conclude the pathology being investigated. In addition to creating initiatives for research on ILD regarding the pattern of lavages and reporting that quantitative patterns in the guideline do not exclude parenchymal diseases.
Efared et al. (2017) (Marroco)	To evaluate the diagnostic power of ILD through the cytology of BAL, comparing cytological findings of different types of parenchymal diseases.	With similar agreements to studies in the area, it was defined that BAL cytology is limited to differentiate types of ILD. However, it can be used in association with several methods to assist in diagnosis.
Salonen et al. (2020) (Finland)	Address the difference in BAL cell count of patients already diagnosed with ILD and compare them with patients with ILD with acute exacerbation.	A significant difference was reported between the studied population. An increase in the number of macrophages and neutrophils was observed, as well as the presence of basophils. Prognostic markers may also be defined. BAL can be used as a follow-up tool for patients with ILD
Bergantini et al. (2021) (Italy)	Insert a quantitative parameter called Bronchoalveolar Cytology Threshold (BCT)	The BCT index can be added to the BAL cell count, thus assisting in a more determined investigation for ILD.

Source: It is observed in this table that all articles are from the Northern Hemisphere, and although there is a certain equivalence of the studies, it is necessary to address them to a more inclusive sphere. The agreement is limited by the different methodologies adopted and by the population studied, so it is more relevant to consider qualitative rather than quantitative patterns. It is noted that the differential count of leukocytes, especially lymphocytes, usually does not have symmetry. Granulomatous and autoimmune diseases will usually present lymphocytosis (>15%), according to the ATS/ERS 2012 guideline (Meyer et al., 2012), however the amount does not follow a linear pattern. The survey of articles was based on studies that used relative cytology to differentiate the maximum number of ILD subtypes, regardless of clinical and radiological characteristics.

count, it is appropriate for the analyst to describe the morphological characteristics of the lavage cells (Rice, 2022).

Although BAL is a safe diagnostic technique with a wide spectrum to infer several pulmonary alterations (Bergantini et al., 2021), the lavage also presents difficulties in standardization, making the changes in the differential count indicate several pathological conditions; just like it can be just a variation of a non-pathological state. Even findings in the borderline pattern do not rule out pulmonary diseases (Meyer et al., 2012). The total count is also a pattern to be considered, since it has been reported that cigarette smoking increases the global amount of cells, mainly alveolar macrophages (Frye et al., 2020).

4.1. Cellular population of bronchoalveolar lavage

In a healthy individual, about 95% of the lavage is composed of macrophages and lymphocytes. Neutrophils and eosinophils can also appear, but in smaller quantities. Squamous and ciliated cylindrical epithelial cells should be present in less than 5% of the sample content, as a qualitative standard of non-contamination (Meyer et al., 2012). However, the cellular population is not limited to the cells mentioned above. Plasma cells, basophils, dendritic cells (DC), erythrocytes and type II pneumocytes can also be seen in BAL (Pesci et al., 2010; Speck et al., 2016).

Table 2. Differential count of BAL cells of an individual without pulmonary dysfunction.

Cellular type	Normal Count Cell (%)
Alveolar Macrophages	>85%
Lymphocytes	10-15%
Neutrophils	≤3%
Eosinophils	≤1%
Epithelial cells (squamous and collunar)	≤5%

Source: Epithelial cells should not exceed more than 5% of representation in the samples, according to the AST/ERS 2012 guideline (adapted from Meyer et al., 2012).

4.1.1. Macrophages

Macrophages (Figure 2) are the most abundant cells in lavage and can exhibit various morphological and phenotypic characteristics. Due to their prevalence, many studies associating cellular parameters with clinical conditions excluded them. However, other studies (Salonen et al., 2020) demonstrate their clinical and prognostic importance, especially when their phenotype is understood (Takiguchi et al., 2021). Pulmonary macrophages are classified into alveolar macrophages (AM), which are larger, and interstitial macrophages (IM), resembling monocytes with a high nucleus/cytoplasm ratio and vacuolated cytoplasm. Their physiological activity differs, and specific markers, like cluster of differentiation (CD) and HLA, define each subtype, influencing cytological findings (Liegeois et al., 2018).

Factors like smoking and drug use (Frye et al., 2020) increase macrophage numbers in BAL samples, making it nonspecific to associate their quantitative findings with clinical conditions. Macrophages play a key role in lung homeostasis and immunity, phagocytosing components, like nicotine and hemosiderin, that alter their morphology. They also differ in polarization, with distinct phenotypes, functions, enzymatic activation, and associations with various diseases (Forbes et al., 2014). In inflammatory conditions such as COVID-19 and COPD, monocyte recruitment and pro-inflammatory cytokine secretion worsen the disease, increasing neutrophil recruitment and the inflammatory score (Park et al., 2022).

4.1.2. Lymphocytes

Lymphocytes (Figure 3) are an important cellular component present in BAL, which is related to several interstitial lung diseases. Lymphocytosis is a very common condition in granulomatous pathologies, drug reactions and in autoimmune conditions, being one of the main

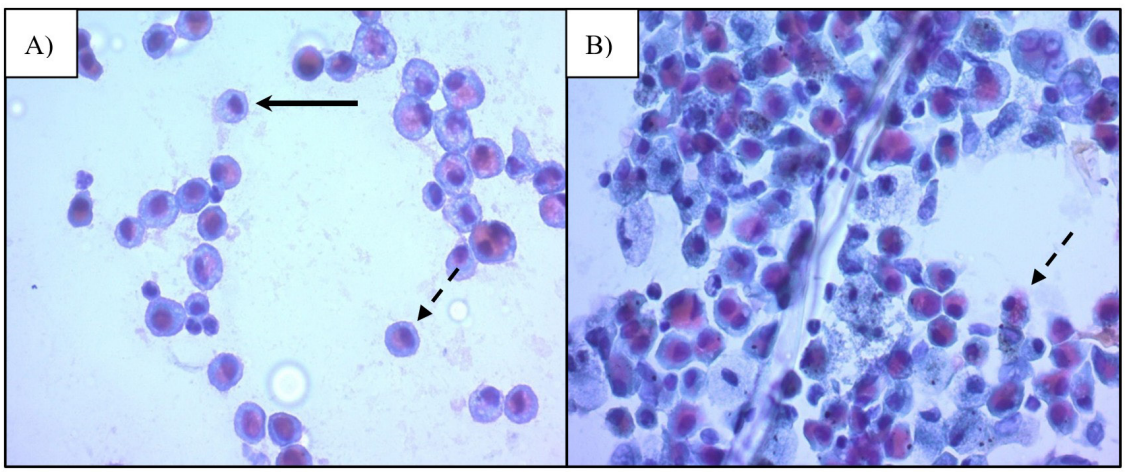

Figure 2. Macrophages from BAL samples. (A) Arrangement of foamy macrophages (arrow) and pigmented (hemosiderin-laden macrophage) (dotted arrow); (B) Typical and foamy macrophages and hemosiderin-laden macrophages (dotted arrow). Papanicolaou staining, 400x. **Source:** Cell microphotography in BAL. Designed by authors, 2025.

parameters to suspect interstitial diseases (Adderley et al., 2020; Tarasidis and Arce, 2020). Degrees of lymphocytosis are used as parameters to consider possible pulmonary diseases (Rice, 2022). There is a combination of cellular parameters found in the BAL that can form a logarithm for clinical assumption.

Many interstitial conditions cause or are caused by lymphocytosis, which can be present in several clinical conditions (Jara-Palomares et al., 2009). Some authors (Domagala-Kulawik, 2020; Gharsalli et al., 2018) recommend the evaluation of CD4 and CD8 for findings with >15% lymphocytes, since other lymphoproliferative diseases can also present similarly in the BAL.

4.1.3. Polymorphonuclears

Polymorphonuclears (Figure 4), especially neutrophils, have a determining significance in parenchymal samples, since they are the cause of fibrocystic reactions, have a high incidence, a greater propensity to bacterial load, and are of idiopathic origin (O'Dwyer et al., 2019) mainly in elderly patients (Patel et al., 2023). Despite not having statistical significance, the increase in polymorphonuclears may be associated with fibrosing interstitial pneumonia and Connective Tissue Disease associated with ILD (Efared et al., 2017).

Leukocytes, mainly lymphocytes and neutrophils, regardless of clinical suspicion, have a prognostic pattern, where neutrophilia associated with lymphopenia is indicative of an unfavorable prognosis (Kono et al., 2021). Other causes such as infections, idiopathic causes and metaplastic processes are characterized by an increase in polymorphonuclears in BAL samples (Patel et al., 2023; Pesci et al., 2010).

In cases of idiopathic pulmonary fibrosis (IPF), which has clinical and radiological characteristics similar to hypersensitivity pneumonitis (HP), it is common to find

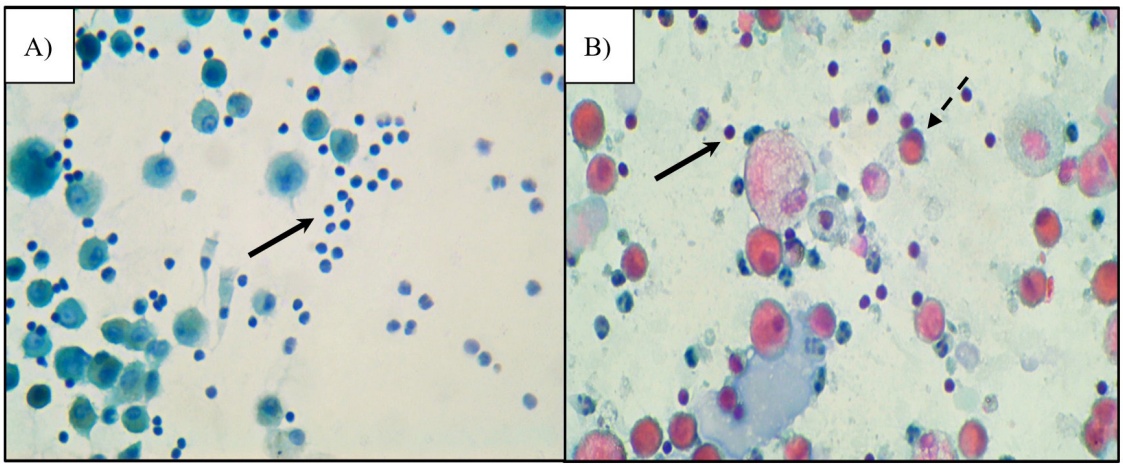

Figure 3. Lymphocytes from BAL samples. (A) Lymphocytes (arrow) next to collunar cells and macrophages. Lymphocyte predominance; (B) Lymphocytes (arrow) and hemosiderin-laden macrophage (dotted arrow). Papanicolaou staining, 400x.
Source: Cell microphotography in BAL. Designed by authors, 2025.

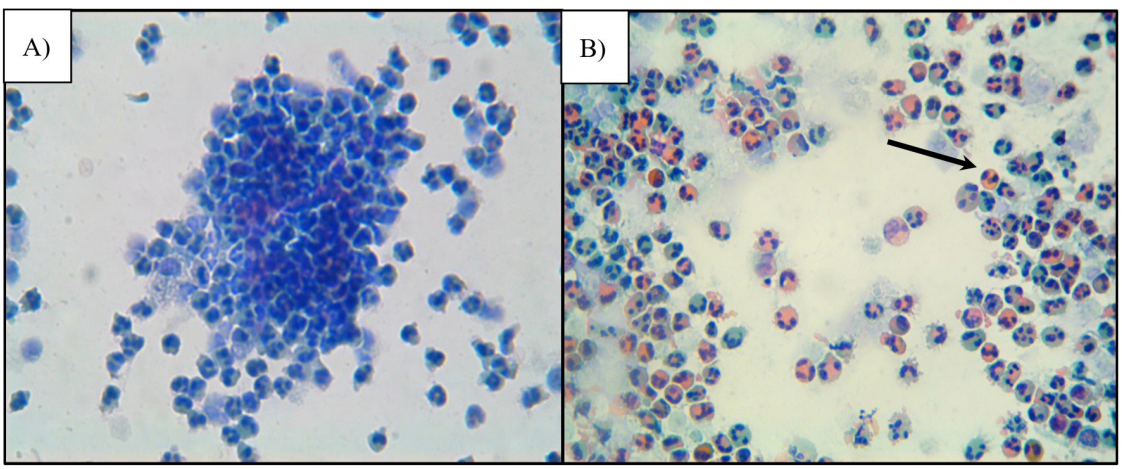

Figure 4. Polymorphonuclears from bronchoalveolar lavage samples. (A) Neutrophils arrangements. (B) Neutrophils and eosinophils (arrow). Papanicolaou staining, 400x.
Source: Cell microphotography in BAL. Designed by authors, 2025.

an increase in the number of lymphocytes (>15%) and neutrophils (>3%). However, Sobiecka et al. (2023) states that a higher cell percentile added to lymphocytosis can be used as a determining factor in HP; while Bergantini et al. (2021) proposes a cut-off point of >5.5% neutrophils for differentiating into IPF.

Cases of interstitial eosinophilia, mainly pneumonias, are more common in tropical regions (Mullerpattan et al., 2013), and can be classified etiologically as: secondary eosinophilic pneumonia, where there is a known cause (drug reactions and bacterial, fungal and parasitic infections); or primary eosinophilic pneumonia, encompassing idiopathic cases. Cases of increased eosinophils in the relative count can occur in transplants (Martinu et al., 2020) and in hypersensitivity (Pourakbari et al., 2016).

4.2. Interstitial diseases

It is difficult to classify interstitial diseases in a particular field, since they can be of idiopathic, occupational, exposure and autoimmune origin, and can cause acute and chronic systemic reactions. Its incidence is dependent on regional, ethnic, age and gender factors, however, due to its subdivisions, it is very complex to point out epidemiological data of these diseases (Wijsenbeek et al., 2022). These changes in the pulmonary parenchyma can be analyzed through BAL, however, the gold standard for identifying the complexity of ILD is the responsibility of multidisciplinary criteria (van der Staal et al., 2023), including cytology, which, when used alone, will most likely have low sensitivity to identify ILD.

In addition to idiopathic causes, approximately 15% of ILDs do not have classifications, making multidisciplinary choices more difficult, but fundamental (Collins and Luppi, 2021). Due to the previously described data, public health has difficulty in developing an adequate screening program, resulting in a high level of incidence and prevalence of ILD (Wijsenbeek et al., 2022).

5. Bronchoalveolar Lavash in the Clinical Context

Developed to avoid unnecessary surgical biopsies for all patients with pulmonary symptoms, BAL gained prestige for investigating parenchyma pulmonary infiltration, including both neoplastic and non-neoplastic conditions, particularly opportunistic infections (Pesci et al., 2010). Clinical algorithms, based on ATS/ERS guidelines (Meyer et al., 2012; Valentini et al., 2019), suggest BAL can be disregarded if HRCT is conclusive or bronchoscopy isn't feasible. When performed, clinical suspicion and radiological findings form a diagnostic triad. BAL samples can also undergo microbiological testing, including Polymerase Chain Reaction (PCR) for viruses or intracellular microorganisms (Recio et al., 2021). In the cellular count, the sum of medical data, lymphocytosis, neutrophilia and eosinophilia, or their combinations, provide different prognoses (Gharsalli et al., 2018), which may reflect in the medical conduct.

For the diagnosis of ILD, where imaging tests are usually inconclusive, the use of BAL is essential for clinical research. However, the use of BAL is not limited to the study of interstitial disease, but also in research on secondary immunological pathologies, lung transplantation and determining prognosis (Bezel et al., 2021; Martinu et al., 2020).

5.1. Sensitivity and specificity of BAL cytology

The implementation of new techniques and systems that aid in diagnosis, being of a qualitative-quantitative nature, can become a reality in the routine of the clinical pathology laboratory (Domagala-Kulawik, 2020), with the aim of providing more reliable results in the detection at the cellular level of the ILD. Even with limitations to close diagnosis (Efared et al., 2017), cytology cannot be ruled out in an initial investigation, as it's non-invasive nature is more accessible than biopsies, avoiding unnecessary tissue damage to patients. Parameters that measure and seek to increase the specificity and sensitivity of BAL cytology should be considered in order to elaborate standards that accommodate as many people as possible.

The study by Jara-Palomares et al. (2009) had sensitivity calculated from 5 ILD diagnosed without histological confirmation. Sensitivity and specificity were calculated from the findings of association of cytology with the immunological characteristics of Sarcoidosis (N=30; Sensitivity = 40%; Specificity = 96.8%) and COP (N=8; Sensitivity = 87.5%; Specificity = 94.3%). In studies to determine the predictive values of BAL in neoplastic lesions, Bezel et al. (2016) and Tomar et al. (2016) generated similar results, where specificity was greater (43% and 75%) than sensitivity (29% and 47.6%). While the study by Girard et al. (2017) showed low sensitivity (15%) for the diagnosis of tumor lesions. Factors such as lesion size and anatomical position are determinants of diagnostic yield (Boonsarngsuk et al., 2015).

The studies that involve BAL as an associative tool for ILD (Bergantini et al., 2021; Bezel et al., 2016; Frye et al., 2020; Kono et al., 2021), used Relative Operating Characteristic curve (ROC curve) to obtain the results of sensitivity and specificity. The importance of the population studied, the sample size, exogenous factors and methodologies adopted for cell processing and analysis can limit the results (Bollmann et al., 2017), which cannot exclude the authenticity and legitimacy of the research. However, it is known that there are problems outside the technical-laboratory sphere and that new technological concepts may emerge to recalculate these conclusions, such as the method of sample acquisition, technical processing and consensus analyses of the washing for a given population.

The diagnosis of lung cancer is made late (Sung et al., 2020), making it essential to include cytopathological research. The predictive values for the cytological diagnosis of lung lesions are variable, including samples from fine needle aspiration (FNA) and bronchoscopic techniques. Thus, a prior detection is necessary, since the search for malignant lesions by cytology has positive predictive values ranging from 48.15 to 72.69%. In addition to being safe, fast and more cost-effective than biopsies. It is worth noting that the sensitivity of BAL cytology has a direct implication with the location of the lesion (Bezel et al., 2016; Sareen and Pandey, 2016).

5.2. Autoimmune causes and transplant recipients

As previously mentioned, immunosuppressive conditions are moderators of microbiological infection that can cause ILD in about 22% of cases (Hurst et al., 2017). In the context of autoimmune diseases, changes in leukocyte counts are almost unanimous. Systemic sclerosis can manifest itself in ILD by IPF or Pulmonary arterial hypertension (PAH). In rheumatoid arthritis, which affects 1% of the world's population (Mikuls et al., 2021), it is related to the development of ILDs in 10% of fibrotic cases, its clinical presentations are worrying due to the high levels of morbidity and mortality. Rheumatoid arthritis associated with ILD (AR-ILD) can manifest itself in various ways, as well as alarming conditions when associated with smoking, genetic susceptibility and recurrent inflammatory episodes, thus having an unfavorable prognosis (Akiyama and Kaneko, 2022). This singularity qualifies it as an idiopathic multifactorial disease, and multidisciplinary action is essential for a clinical-pathological conclusion (Cottin and Valenzuela, 2020).

6. Perspectives

With the necessary investments, BAL can bring a greater screening power for pathological conditions that still do not have precise diagnoses or well-defined etiological characteristics. Currently, there is an investment in the study of macrophages, focusing on their genesis, morphological differentiation, association with the patient's clinical condition (Byrne et al., 2016; Park et al., 2022) and the secretion of cytokines and microvesicles (Zhang et al., 2022), being considered a branch of biotechnology omics studies.

In the academic field, BAL gains multitasking with potential indications of lung lesions. Its use to reduce the performance of biopsies is already a reality, but using its cytopathological findings, secretion of cytokines and proteins, submission to microbiological culture or molecular research makes it a very comprehensive research material, with considerable specificity power, however not self-sufficient (Zaidi et al., 2020). In addition, the clinical hypothesis added to the clinical-social data helps the cytopathologist's research, making him produce a high-quality investigation.

6.1. Immunophenotyping

The cytometry can assist in medical management, prognosis and scientific/academic knowledge of the other cells of the bronchoalveolar population, in addition to the secretion of cytokines by these cells (De Luca et al., 2012). The great functional demand of alveolar macrophages, thus reflecting in its wide morphological spectrum, can provide specific data on infiltrative diseases, its differentiation of activated macrophages and interstitial macrophages, in addition to its embryogenesis in the organism (Byrne et al., 2016). Cytometry can also identify neoplastic cells, mainly of hematologic origin, when present in BAL samples (Cesana et al., 2010).

Phenotypical studies in BAL cells are essential for therapeutic research, such as immunotherapy. By differentiating macrophages and lymphocytes, it is possible to obtain a deeper knowledge of the microenvironment and create panels to characterize pulmonary diseases, whether neoplastic or non-neoplastic (Kwiecień et al., 2023). In addition, in cases of ILD that present similar cytological characteristics, flow cytometry is crucial for differential diagnosis and, consequently, for the choice of the most appropriate therapeutic approach.

6.2. Molecular techniques

Molecular diagnosis is very present in the scientific laboratory routine, especially PCR. Its use as a research tool in BAL samples is very relevant, especially for suspected viral and fungal infections (Hardak et al., 2019; Ishiguro et al., 2019). The introduction of PCR as a research tool in BAL was very relevant because it allows the identification of multiple infections and has greater sensitivity than conventional microbiological cultures, as observed in the study by Subhagan et al. (2022).

Other molecular techniques such as immunohistochemistry (IHC) and immunocytochemistry (ICC) are also present in BAL research, and are generally associated with the search for specific biomarkers of neoplastic lesions, whose association is made with cytopathology (Sung et al., 2020). ICC used in pulmonary cytology can also be used for diagnostic and prognostic predictive markers, mainly to detail neoplastic lesions, and can mark cytokines and associate them with some ILD (Metovic et al., 2020).

7. Conclusion

Currently, BAL is used as a complementary research tool when imaging tests do not provide ideal specificity and sensitivity. Its results, when not conclusive, can overlap with pulmonary diseases, whether of a neoplastic, interstitial, immunosuppressive or infiltrative nature, as found in the study by Choi et al. (2020), whose main use of BAL stands out in the primary research of pulmonary infiltrate, mainly for immunocompromised patients.

Being one of the main criteria for performing BAL, the relative cell count, when used as the only parameter for conclusion, is limited. Multidisciplinarity is essential for confident conclusion, with morphological characteristics being very important.

Simplifying the classifications of ILD can also be a facilitator for diagnosis and epidemiological data, since studies have categories according to regional incident cases based on local demographic consensus. The need for systematization in this area is fundamental, the guideline made in 1999 by ERS, whose update occurred in 2012 together with AST, has the potential to include organizations from other continents and hemispheres, resulting in a multicenter study of high reliable power, resulting, in such a way, that the BAL fluid enjoys cytomorphological criteria suitable for diagnosing pulmonary diseases.

Data Availability Statement

The research data analyzed in this study are not publicly available by any means, as they were obtained from

secondary sources indexed in scientific databases such as PubMed, Scopus, Web of Science, and ScienceDirect. All articles used for data extraction are properly cited throughout the manuscript. No original datasets were generated during the current study.

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