

**BRONCHOALVEOLAR LAVAGE FOR THE EVALUATION OF INTERSTITIAL
LUNG DISEASE: IS IT CLINICALLY USEFUL?**

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Introduction

Bronchoalveolar lavage (BAL) has gained widespread acceptance as a procedure that can be performed safely to retrieve respiratory secretions for the examination of cellular and acellular components for both diagnostic and research purposes (1-6). When BAL was initially developed as a tool to sample respiratory secretions in animal models of lung disease and subsequently adapted as a clinical tool to study interstitial lung disease (ILD), it was perceived as holding considerable promise for the diagnosis and management of various forms of ILD such as sarcoidosis, idiopathic pulmonary fibrosis (IPF), and hypersensitivity pneumonitis (HP). Indeed, hundreds of manuscripts have been published in the medical literature over the ensuing decades as various centers around the world began to use BAL to identify agents of respiratory infections as well as examine correlates of changes in the composition of the airspace milieu associated with the presence of non-infectious parenchymal lung diseases. BAL is now routinely used as a diagnostic tool to diagnose respiratory infections, evaluate patients with acute respiratory failure or evidence of diffuse parenchymal lung diseases, or monitor the status of transplanted lung allografts (7). Despite the widespread use of BAL by pulmonologists, BAL cellular analysis, especially nucleated immune BAL cell differential counts, may be underutilized in ILD diagnosis. Information derived from BAL appearance and differential cell counts can provide useful diagnostic clues if recognized and interpreted appropriately with an adequate awareness of the potential diagnoses that are associated with specific BAL cellular patterns.

In the early years following its introduction into clinical practice, bronchoscopy with BAL was perceived to hold great potential for diagnosis and management of ILD. It eventually became clear, however, that although BAL nucleated immune cell patterns often had characteristics that were highly consistent with various forms of ILD such as sarcoidosis, BAL cell counts and differentials, lymphocyte subsets, or soluble components could not be relied upon to make a confident diagnosis for many specific forms of ILD if used as a “stand-alone” diagnostic test. As clinical pulmonary medicine evolved during the 1990s, high-resolution computed tomography (HRCT) of the chest came into widespread clinical use. As radiologists and clinicians became increasingly familiar with imaging patterns on HRCT that were consistent with specific forms of ILD, the likelihood of making a confident diagnosis of specific ILD such as IPF or sarcoidosis is high when characteristic patterns are present and the technical quality of the HRCT is good (8,9). Despite the increasing acceptance of obtaining a HRCT during the initial stages of evaluation of patients with ILD, many patients with new onset ILD may not have the characteristic patterns on HRCT that allow a diagnosis to be made with high level of confidence by HRCT imaging alone. However, when clinical information and HRCT findings are combined with BAL fluid analysis and/or transbronchial lung biopsy, confident diagnoses may emerge that obviate the need for surgical lung

biopsy. Although the BAL cell pattern can provide useful information that facilitates the establishment of a specific ILD diagnosis, this is only possible if the bronchoscopist uses appropriate technique to obtain the fluid and if the differential cell count is performed according to good clinical laboratory practice by personnel with adequate experience in BAL cytologic analysis and interpreted by an expert familiar with the diverse spectrum of specific forms of ILD. This article provides a perspective on the current and future use of BAL analysis in achieving a confident diagnosis of specific forms of ILD and the potential of BAL to assist in the management of various forms of ILD. Reaching a consensus on BAL technique and interpretive methods can bridge potential gaps that exist among pulmonologists worldwide in the application of BAL technique and analytic results to clinical practice.

Technical aspects of performing BAL

BAL technique is of paramount importance in obtaining a useful specimen (10,11). To retrieve a specimen that is likely to reflect the types of inflammatory cells that are present in distal airspaces, a sufficient amount of isotonic saline should be instilled to adequately sample bronchoalveolar secretions from distal airspaces. The bronchoscopist should avoid contamination of the sample with proximal large airway secretions by maintaining the distal end of the bronchoscope in a wedged position in a segmental or subsegmental bronchus throughout the period of time required for the instillation and retrieval of saline aliquots. Additionally, dwell time should be minimized (aliquots should be aspirated immediately once the entire aliquot volume has been instilled). Many different BAL protocols have been published and consist of multiple aliquots that usually range from five or six aliquots of 20 mL each to four aliquots of 60 mL each. Some investigators consider the first aliquot to represent predominantly airway cells and secretions (12) and have recommended that this aliquot be kept separate and used for microbiological analysis, while subsequent sequential aliquots, usually 3 to 4 in number, should be pooled and used for cellular analysis because these are more representative of distal airspaces (alveolar sampling). Others combine all aliquots and do not differentiate between “bronchial” and “alveolar” sampling. There are no data that support one approach over the other, and many centers pool all retrieved BAL aliquots prior to submitting BAL for laboratory analysis. Additionally, there is no consensus on the optimal magnitude of negative pressure to retrieve BAL fluid once an aliquot has been instilled.

The right middle lobe (RML) and lingula of the left upper lobe are the areas of the lung that are more easily accessible and likely to allow good return of BAL fluid from patients when lavaged while in a supine position (11). These areas have traditionally been used for lavage based on the perception that ILD is a ‘diffuse’ parenchymal lung disease, as demonstrated on routine chest radiographs. Because

patients with ILD are now routinely evaluated with HRCT images of the chest, HRCT images may be used to target areas of the lung that may yield cellular findings more reflective of the disease process, such as areas of ground glass attenuation, more prominent nodular profusion, or fine reticulation. Since HRCT images is sensitive in detecting patterns of otherwise occult segmental and/or patchy alveolar opacifications or ground glass attenuation in patients with ILD, the HRCT images should be used to guide to determine the site of BAL to include sampling from involved segments as indicated by the HRCT scan." However, no studies have been performed that demonstrate that the use of HRCT to target regions that show more prominent parenchymal change in certain geographic lung regions as compared to traditionally utilized sites such as the RML or lingula improve the diagnostic yield of BAL. Ideally, the percentage of BAL fluid that is retrieved would be in the range of 30% or greater for a patient with ILD. However, if ILD is superimposed on emphysema, airways may readily collapse with negative pressure and compromise fluid retrieval. Post-bronchoscopy fever (fever, chills, and extreme malaise) may occur within hours of the BAL procedure as a consequence of proinflammatory mediator release, and these symptoms are more likely to occur if larger volumes of fluid are used for BAL (13).

Processing of BAL fluid for cellular analyses

An accurate and statistically meaningful enumeration of the BAL nucleated immune cell profiles requires examining at least 300 of these BAL cells randomly on a single slide with accuracy further enhanced by analyzing 400 to 500 cells (especially if more than one slide is analyzed with the differential counts from each slide averaged). The relative numbers of bronchial and squamous epithelial cells should also be noted. Although many investigators have published BAL cell profiles for cohorts of healthy volunteers, laboratories that perform BAL analysis would ideally study a sizable group of normal volunteers and establish normal values in their own laboratories that correspond to their subject population and analytical methods. BAL slide preparations can be analyzed in clinical hematopathology laboratories, but it is of key importance that personnel who analyze the BAL slide preparations and determine differential counts should be adequately trained in the proper identification of BAL cells and in recognizing various atypia that may be present on such preparations (11). However, pulmonologists familiar with BAL cell patterns and ILD should interpret the BAL cell patterns and other findings.

BAL fluid obtained from healthy, never-smoking individuals should contain, on average, a majority of alveolar macrophages (80-90%), some lymphocytes (5-15%), and very few neutrophils ($\leq 3\%$) or eosinophils ($< 1\%$). While it is preferential to have laboratories establish normal values (including T lymphocyte subsets), normal values reported by others may be used, especially if the BAL procedure that is utilized conforms to published techniques and values obtained for normal subjects. The presence of squamous epithelial cells suggests that the BAL fluid has been contaminated by oropharyngeal secretions, which may be a consequence of poor technique in performing the BAL or because the subject aspirated upper airway secretions. BAL cell differentials and total cell counts for ex-smokers should be similar to that of never-smokers. Smokers, however, generally have a significantly increased total BAL cell count as well as total macrophages and neutrophils per μl BAL fluid, but the BAL differential cell count for smokers appears to vary little from that for never-smokers or ex-smokers except for a somewhat lower percentage of lymphocytes (5,14). Elderly subjects have been reported to have increases in lymphocytes and neutrophils in the differential cell counts, suggesting that advanced age may affect BAL cell differentials (15,16). Additionally, total volume of retrieved fluid declines with advanced age due to loss of elastic recoil and increased compliance of the aged lung. Some investigators have shown that total volumes of instilled saline that range from 100 ml to 250 ml appear to give similar cell differentials in individual patients with ILD (17,18).

Infection can cause the subacute onset of diffuse lung infiltrates or coexist with noninfectious ILD. Therefore, BAL should be examined and screened for mycobacterial or fungal infection if clinical suspicion for an infectious etiology warrants such testing. If bacterial infection is a possibility, an uncentrifuged BAL fluid specimen can be sent for quantitative bacterial culture. Uncentrifuged BAL fluid can also be sent for viral culture, although a centrifuged specimen may enhance virus detection via stains or viral nucleic acid probes. Centrifuged BAL specimens can also be used to culture *Legionella*, *Mycoplasma*, and *Chlamydiae* in addition to mycobacteria and fungi, and cytocentrifuged specimens can be stained to detect the presence of intracellular bacteria, *Pneumocystis jiroveci*, mycobacteria, or fungi (7).

BAL characteristics and the diagnosis of specific types of ILD

When performed with a standardized technique, expertly examined, and combined with clinical and imaging data, BAL differential cell counts and other characteristics can provide important information that contributes significantly to the diagnosis of specific ILD (Table 1) (1-3,10,19-22). Additionally, cytopathologic examination may be useful to detect malignancies (e.g. lymphangitic

carcinoma, lymphomas) that can present as diffuse lung disease with similar appearance to non-malignant ILD on thoracic imaging (23). Other observations including the gross appearance of BAL fluid and identification of unusual cells (e.g. plasma cells, mast cells, foamy alveolar macrophages) or foreign material can yield important information that may lead to a specific diagnosis. In the appropriate clinical setting, certain gross and cellular findings in BAL are highly suggestive or even virtually diagnostic of specific ILD entities (Table 2), but these observations must be interpreted in the context of the patient's clinical presentation and radiologic findings. Additionally, clinicians should recognize that radiographic imaging, including HRCT, may not appear particularly abnormal when certain forms of ILD (e.g. non-IPF interstitial pneumonias or HP) are present. If BAL is performed on a symptomatic patient who has radiographic imaging that is not particularly suspicious for the presence of ILD, an abnormal cell profile consistent with the presence of an "alveolitis" can indicate the need for additional investigation including lung tissue biopsy.

When BAL fluid is obtained and analyzed from patients with ILD, differential cell counts obtained from slides prepared via cytocentrifugation usually show variations in the BAL cell differential that differ from patterns found in normal subjects. These patterns tend to reflect inflammatory cell profiles in affected lung tissues (1), but cell patterns may be obtained that are not typical for a specific ILD diagnosis, that show little or no change from normal profiles, or that show mixed patterns with significant changes in the relative percentage of more than one type of nucleated immune cell (2). Additionally, altered BAL cell profiles can be observed with various airway disorders (e.g. bronchiolitis, bronchitis, asthma, eosinophilic bronchitis, bronchiectasis, and allergic bronchopulmonary aspergillosis). Nonetheless, certain cell patterns with significant changes in one or more BAL cell type can be highly consistent with or strongly suggest the presence of specific types of ILD. A recently published study of the predictive value of BAL for ILD diagnosis for a large cohort of patients (N=3,118) suggested that BAL cell counts are most useful for relatively common ILD diagnoses such as sarcoidosis, in contrast to relatively rare forms of ILD where BAL differential cell counts tend to have less diagnostic utility (24).

A BAL lymphocyte differential count that exceeds 25% is quite likely to be caused by ILD associated with granuloma formation (e.g. sarcoidosis and hypersensitivity pneumonitis) or drug toxicity, if other possibilities, such as mycobacterial or fungal infection, are excluded. However, prominent BAL lymphocytosis can also be observed with other entities such as cryptogenic organizing pneumonia (COP), cellular non-specific interstitial pneumonia (NSIP), lymphoid interstitial pneumonia (LIP), or non-LIP lymphoproliferative disorders. Extreme increases in BAL neutrophils are likely caused by infection or relatively acute and diffuse lung injury. Eosinophil differential cell counts $\geq 25\%$ are highly likely to be

caused by eosinophilic lung disease, especially eosinophilic pneumonia (EP) if the presentation is acute (25,26). Increased numbers of mast cells have been associated with HP, drug reactions, sarcoidosis, ILD associated with collagen vascular disease (CVD), IPF, COP, eosinophilic pneumonia, or malignancy. Plasma cells have been observed in BAL in HP, drug reactions, EP, malignancy, or infection (27). Alveolar macrophages (AM) may also display certain morphologic changes such as a foamy appearance in HP, markedly vacuolated cytoplasm with positive staining of vacuoles for fat in chronic aspiration pneumonitis, cytoplasmic inclusions associated with viral infection (e.g. cytomegalovirus pneumonia), ingested RBCs and RBC fragments plus hemosiderin with diffuse alveolar hemorrhage, ingested asbestos bodies, or other dust particles.

Grossly bloody lavage fluid, which may range from pink to red with acute hemorrhage or to orange-brown if subacute, is characteristic of diffuse alveolar hemorrhage (DAH), especially if sequential retrieved BAL fluid aliquots do not show any decrease in the amount of bloody discoloration of the lavage fluid or (especially) if the discoloration increases (28). If DAH is present, RBCs should be identifiable on the cytospin, and AM will stain positively for hemosiderin when stained with an iron stain if the onset of hemorrhage has preceded the time of BAL by 24 to 48 hrs. Freshly retrieved BAL fluid that has a milky or light brown to whitish, cloudy appearance with flocculent debris that settles out of the fluid to the bottom of its container prior to centrifugation suggests PAP as the likely diagnosis (29). The diagnosis can be confirmed if the sediment, which is surfactant-derived lipoproteinaceous material, stains positive with periodic acid-Schiff (PAS) staining.

Flow cytometric analyses of BAL cells have been reported by numerous investigators (2,24,27), and alterations in BAL lymphocyte subsets have been examined extensively, especially for sarcoidosis (1,2,30-32). The presence of a high CD4⁺/CD8⁺ T lymphocyte ratio increases the likelihood of diagnosis of sarcoidosis when combined with BAL lymphocytosis, although the clinician must consider age as a factor in the elevated CD4⁺/CD8⁺ ratio if the patient is elderly (16). Nonetheless, it is debatable whether BAL lymphocyte subset data provides a clinical tool that contributes significantly to ILD diagnosis. The BAL CD4⁺ T lymphocyte subset and CD4⁺/CD8⁺ T lymphocyte ratio tend to be elevated in clinically active pulmonary sarcoidosis (31). CD4⁺/CD8⁺ lymphocyte ratios in BAL from clinically healthy, younger adults usually range from 1.0 to 3.5 with average value between 1.5 and 2.0. An elevated CD4⁺/CD8⁺ ratio of ≥ 3.5 is relatively specific for sarcoidosis (32,33). However, the sensitivity of an increased CD4⁺/CD8⁺ ratio is relatively low for sarcoidosis (32,33), and many patients do not have an elevated ratio or may even have a low ratio (34). A depressed CD4⁺/CD8⁺ ratio has been observed in HP, drug-induced lung disease, COP, EP, and IPF (27), but this ratio has not been shown to contribute

meaningfully to the diagnosis of non-sarcoid ILD. Use of the CD4⁺/CD8⁺ ratio as a routine component of BAL analysis is questionable and may increase the cost of the procedure considerably.

Flow cytometry may be useful to support a diagnosis of pulmonary Langerhans cell histiocytosis (PLCH) if clinical data and imaging suggest this entity. PLCH is associated with increased (>4-5% of total cells) CD1a-positive cells in BAL, although this may no longer be the case in later stages of disease (35,36). However, macrophage autofluorescence may present insurmountable difficulties in obtaining reliable data, and immunohistochemistry on prepared slides is more likely to provide reliable enumeration of the percentage of positively stained Langerhans cells (36). Flow cytometry and/or immunocytochemistry can be useful to identify monoclonal lymphocyte populations to support the diagnosis of a lymphoid malignancy and should be obtained if clinical data suggest the possibility of pulmonary lymphoma and the clinical laboratory can perform appropriate analyses. Although flow cytometric analysis of BAL cells may provide useful information in certain situations, the routine use of flow cytometry to analyze BAL cell subpopulations to assist in the diagnosis of specific forms of ILD is of unclear benefit. However, flow cytometry of BAL cells can be obtained if it is likely to provide important diagnostic information.

One application of BAL that evolved over the past decade was the suggested incorporation of the BAL cellular findings into the major and minor criteria for the clinical diagnosis of IPF when surgical lung biopsy is not performed (37). However, since the positive predictive value of the HRCT findings of usual interstitial pneumonia (UIP) pattern for a diagnosis of IPF/UIP is at least 85%, and a confident diagnosis of UIP by experienced radiologists can be made in 95% of cases with characteristic pattern of UIP on HRCT images (38-41), invasive procedures including bronchoscopy, BAL and transbronchial lung biopsy may not be required to make a confident diagnosis of IPF in the appropriate clinical setting (9). In fact, the features of the criteria for the UIP pattern has been precisely described in the new joint ATS-ERS-JRS-ALAT statement—evidence-based guideline for the diagnosis and management of IPF; the new guideline states that the presence of the described UIP pattern on HRCT imaging is sufficient to make the diagnosis of IPF in the appropriate clinical setting and eliminates the need for BAL cellular analyses (42,43). However, an increased lymphocyte BAL differential cell count in BAL from patients whose HRCT demonstrates a UIP pattern may raise the possibility of chronic HP or other diagnoses associated with BAL lymphocytosis. In a recent study, 8% of patients with a seemingly confident clinical and HRCT diagnosis of IPF had >30% lymphocytes in BAL, an atypical finding for the diagnosis of UIP/IPF that suggested HP or another disorder as the actual diagnosis (44). The new evidence-based guideline for diagnosis and management of IPF remarks on this point and defers the decision for the need of BAL cellular analyses to individual experts with established BAL laboratories (that provide reliable cell differential counts) who may opt to use BAL cellular analyses in patients undergoing evaluation for the diagnosis of IPF at their centers to ensure the lack of findings that suggest a non-IPF form of ILD as the diagnosis. (42). With the increasing utilization of HRCT to evaluate patients during early stages of ILD, it is likely for patients with ILD not to manifest the precise pattern of UIP on HRCT. In this setting many clinicians may routinely perform BAL for cellular analyses as a part of the initial evaluation of patients presenting with early and new onset ILD. Thus, the clinical utility for BAL cellular analyses as an useful intervention in narrowing the differential diagnoses of ILD in this setting is evident. It must

however be recognized that for an accurate diagnosis, biopsy of the tissue with adequate sampling may still be required. The performance of transbronchial biopsy in the peripheral lesions of UIP, NSIP, DIP is not advised.

Bronchoalveolar lavage can also assist in the diagnosis of acute onset ILD (defined as a combination of illness ≤ 4 weeks duration, shortness of breath, hypoxemia, and diffuse radiographic infiltrates in a patient with no history of prior lung disease and no obvious risk factors for ARDS such as sepsis or trauma). Diagnostic considerations in acute onset ILD include infection, non-infectious ILD (acute interstitial pneumonia, acute EP, DAH, acute HP, acute COP, drug toxicity, or acute exacerbation of previously undiagnosed IPF). Examination of BAL fluid can detect infection or hemorrhage, and the BAL cell profile may show large numbers of eosinophils, which is strongly supportive of a diagnosis of acute EP. Additionally, large numbers of lymphocytes would suggest entities such as acute HP or drug toxicity, especially if accompanied by plasma cells and an appropriate exposure history. Bronchoscopy with BAL at the time of acute presentation may facilitate diagnosis and minimize procedural risk if a diagnosis can be made and obviate the need to progress to a surgical lung biopsy.

BAL and the Management of ILD

A role for BAL cellular analyses in the treatment of ILD (e.g. to initiate, direct and/or monitor pharmacologic therapies) has not been established. One application of BAL to disease management, however, is its use to evaluate acute changes in symptoms and lung function. Several adverse events may occur in the course of interstitial diseases including respiratory infection, drug reactions, hemorrhage, or an acute exacerbation of the disease process, and BAL may help to identify the cause of clinical deterioration and facilitate timely intervention.

Many investigators have examined the role of BAL cellular profiles for monitoring disease activity and response to therapies in specific ILD, and BAL cellular findings at the time of diagnosis have been reported by some investigators to reflect disease severity and predict the likelihood of disease progression. The degree of increase in BAL neutrophils has been correlated with disease severity and prognosis for both HP (45,46) and IPF (37,47-49), and BAL eosinophilia has been linked to more severe disease and worse prognosis in IPF (51,51). Similarly, the presence of increased neutrophils in BAL from patients with sarcoidosis has been associated with more progressive disease that is less likely to respond

to immunosuppressive therapy (52). BAL lymphocytosis has been linked to prognosis and response to therapy for some forms of IIP. BAL findings from patients diagnosed with IPF when this diagnostic term did not necessarily exclude other IIP such as NSIP suggested that higher BAL lymphocyte differential cell counts correlated with better prognosis and/or response to therapy (37,48-50,53). A more recent study, although retrospective, demonstrated that BAL lymphocytosis in patients who appear to have fibrotic IIP suggests that the diagnosis is not IPF but rather NSIP, and BAL lymphocytosis was found to correlate with a better prognosis (54).

Lung lavage was initially used as a therapy for septic lung disease and PAP (55,56). Although its therapeutic application is quite limited at this time, whole lung lavage (WLL) is considered standard of care for PAP (57). However, standardization of technique and controlled clinical trials to determine the efficacy and durability of WLL as a therapy for PAP are lacking (57).

Future directions and research questions

BAL holds the potential to play an increasingly useful role in both the diagnosis and monitoring of patients with ILD, but many important issues have not been adequately resolved. These unresolved questions include:

- What is the best technique for performing BAL?
 - Aliquot volume and number of aliquots
 - Type and magnitude of negative pressure to retrieve instilled fluid
 - Pooling of aliquots for subsequent analysis
- When and how should HRCT be used as a guide to determine the site of BAL in patients demonstrating diffusely distributed lesions in routine chest radiographs ?
- Should microbiologic testing be performed whenever BAL is performed for cytologic analysis to determine BAL cell total and differential cell count?
- Should cytologic analysis to detect malignant cells be performed whenever BAL is performed to evaluate diffuse lung disease?
- Who should interpret BAL cellular preparations?
- Can BAL combined with transbronchial lung biopsy synergistically increase diagnostic power and diminish need for surgical lung biopsy?

BAL may prove particularly helpful as treatments become available that may alter the profiles of BAL cells and/or non-cellular components of lower respiratory tract bronchoalveolar surface liquid if patients have a favorable response to such therapies, and detection of these changes may support continued, ongoing treatment with specific agents. Additionally, multi-center studies that prospectively compare BAL with HRCT and histological patterns in large groups of patients will be facilitated by improvements in BAL standardization (11). Such studies have the potential to better define the underlying inflammatory and histological events associated with different HRCT appearances, which may reduce the need for biopsies plus provide information that can guide effective therapies.

New tools that utilize genomic and proteomic characterization of BAL cells and soluble components may eventually prove extremely useful in making an accurate ILD diagnosis, choosing and implementing potentially effective therapies, monitoring disease activity, and assessing the effect of therapeutic pharmacologic interventions. The recent development of DNA microarray and other technologies that identify and monitor expression patterns of vast numbers of genes promise to provide important information about the pathobiology of various types of ILD (58,59). Similarly, recent advances in proteomics that allow mapping and identification of multiple protein expression patterns by 2D gel electrophoresis, image analysis, protein spot transfer and mass spectrometry now raise the real hope that the products of genes linked to disease pathogenesis can be identified and quantitated (60).

Determining gene and protein expression patterns over time as reflected in BAL can potentially identify the key molecules involved in the initiation and progression of the different ILDs, provide an accurate clinical diagnosis of specific ILD without resorting to lung biopsy, and indicate targets for new and effective therapies. A global analysis approach has identified many genes that display increased expression in IPF (58). Additionally, certain gene products that may play an important role in IPF pathogenesis including pigment epithelium-derived factor (61), matrilysin (62), and osteopontin (63) have been identified via these methods and may serve as disease biomarkers. Thonhofer et al (64) demonstrated that stimulated BAL cells from patients with sarcoidosis displayed up- or down-regulation of over a thousand genes, including selective up-regulation of *B-MYB*, a potent growth factor for lymphocytes and regulator of apoptosis, and *FABP4*, a regulator of lipid metabolism and arachidonic acid uptake by macrophages. Not only can microarray genetic analysis provide characteristic gene expression patterns for specific ILD, but distinct expression patterns may differentiate one ILD from another. Selman et al. (65) recently validated the concept that gene expression patterns can be characteristic of

specific ILD by demonstrating that distinct gene expression patterns in lung tissue differed for patients with IPF versus those with HP.

A proteomics approach may also revolutionize the utility of BAL analysis for ILD diagnosis and management (60). Characterizing and quantitating proteins in biological specimens allow one to circumvent modifications of RNA that affect protein production and expression, and proteomic analytic techniques can be applied to acellular compartments such as epithelial surface liquid retrieved via BAL or peripheral blood plasma. Early investigations that utilized electrophoretic techniques to examine protein profiles in BAL were able to show some differences between IPF and sarcoidosis (66,67), and later studies using two-dimensional electrophoretic techniques allowed enhanced fingerprinting of digested proteins from BAL supernatant fluids and demonstrated different profiles for IPF, sarcoidosis, and HP (68,69). More recently Rottoli et al. (70) used 2-dimensional electrophoresis to construct protein maps from BAL fluid that were able to demonstrate differences among IPF, systemic sclerosis, and sarcoidosis. The development of techniques that employ (1) mass spectrometry combined with ionization of peptides via matrix-assisted laser-desorption ionization (MALDI) or electrospray ionization (ESI) or (2) mass spectrometry combined with the use of surface chromatography to capture proteins on a chip surface (SELDI) coupled with greatly enhanced computational abilities and proteomics databases hold considerable promise for the study of ILD via this emerging technology, and these techniques have recently been applied to the study of markers in BAL fluid in patients with lung inflammation (71).

Summary and Conclusions.

Although investigators have recognized the importance of the BAL procedure in clinical pulmonary medicine, unresolved questions concerning the BAL procedure and the clinical utility of BAL cellular analyses for the evaluation and management of ILD in the present era need to be addressed by consensus reached among international experts. The technical aspects of performing BAL and analyzing cellular features of BAL fluid need standardization, and the status of the clinical utilization of BAL findings in day-to-day clinical practice needs to be clarified so that principles of performing, analyzing, and interpreting BAL can be applied globally and avoid wide variations in BAL technique and specimen analysis. BAL cellular patterns and other BAL findings can play an important role in the diagnosis of many forms of ILD, especially when these findings are obtained via a properly performed BAL procedure and analysis and then combined with clinical data and HRCT imaging. In many instances the combination of clinical information (history, examination,

and pulmonary function testing), HRCT imaging, and BAL analysis can provide enough information to allow the clinician to be assured of a confident diagnosis and avoid more invasive testing to obtain lung tissue for histopathologic diagnostic examination. BAL may be particularly beneficial for the fragile patient or patients at high risk for significant complications if subjected to surgical lung biopsy.

Universal adoption of a standardized BAL procedure will undoubtedly improve the chance of identifying clinically relevant differences between specific disorders and provide results that can be compared from center to center. A standardized procedure will enable centers to pool information to achieve the large patient groups necessary to more adequately investigate the value of BAL markers for the identification of specific disease entities, determining clinical disease activity, detecting disease progression, providing a prognosis, and guiding treatment strategies. Identifying specific and sensitive markers that can address these problems remains an elusive but vitally important goal. Future application of BAL to the evaluation of ILD in concert with an evolving, improved understanding of the pathogenesis of specific ILD and the development of novel testing that utilizes genomic and proteomic characterization of BAL fluids holds the potential to provide accurate diagnostic and prognostic information for patients with ILD and potentially play a role in choosing novel therapies for specific forms of ILD. Despite this significant diagnostic potential, very few patients have been investigated so far using gene and protein mapping approaches, and applications of the powerful techniques of gene and protein mapping to the study of ILD are still at a very early stage but have the potential to evolve rapidly in the coming years. Access to large collections of well-characterized clinical samples (BAL, lung biopsies, and peripheral blood) to which these techniques can be applied combined with HRCT or newer forms of thoracic imaging will greatly facilitate our understanding of basic and clinical aspects of ILD pathobiology and facilitate the identification of clinically useful biomarkers.

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Table 1. Bronchoalveolar lavage findings that are useful in ILD diagnosis.

BAL Finding	Consistent Interpretation/Suggested Diagnosis
Eosinophils $\geq 25\%$	Eosinophilic pneumonia
Lymphocytes $\geq 25\%$	Sarcoidosis, HP, cellular NSIP, drug reaction, CBD, LIP, lymphoproliferative disorder
Neutrophils $\geq 50\%$	AIP, DAD, AEIPF, pulmonary infection
Bloody fluid	Pulmonary hemorrhage, DAH
High hemosiderin score	DAH, DAD
Cd1a+ cells $>4\%$	Langerhans cell histiocytosis
Milky BAL fluid with PAS-positive amorphous debris	Pulmonary alveolar proteinosis
Monotypic lymphocytes	Pulmonary lymphomatous malignancy
Malignant cells	Pulmonary malignancy
Squamous epithelial cells $>5\%$	Unsuitable sample due to upper airway secretion contamination
Bronchial epithelial cells $>5\%$	BAL sample may be unsuitable for cell analysis

Table 2. Bronchoalveolar lavage findings: diagnostic value and disease correlations.

ILD Type Suspected	BAL Helpful?	Useful BAL Findings	Diagnostic Value of BAL*
Sarcoidosis	Yes	↑↑Lymph ↑↑CD4/CD8 T-cell ratio**	Highly consistent if CXR and/or HRCT shows typical changes of BHL, nodules along bronchovascular structures*
Hypersensitivity Pneumonitis	Yes	↑↑Lymph Plasma cells Foamy macrophages	Highly consistent if significant exposure to known antigen combined with typical HRCT pattern (ground glass nodules in mid/upper lung regions) *
Cellular NSIP	Yes	↑↑Lymph	Highly consistent if mid/lower lung region diffuse GGO present*
Fibrotic NSIP	+/-	Variable ↑Lymph, ↑Eos, ↑Neut	Rule out infection/malignancy; absence of inconsistent BAL findings plus typical/highly consistent HRCT imaging is supportive of diagnosis
Drug Reaction	Yes	↑Lymph, ↑Eos	Highly consistent if patchy/diffuse infiltrates plus drug ingestion known to be pneumotoxic and cause interstitial change*
Acute Interstitial Pneumonia	Yes	↑/↑↑Neut Exclude infection	Rule out infection/malignancy
Diffuse Alveolar Damage	Yes	↑Neut Reactive/desquamated Type II pneumocytes	Type II pneumocyte changes are highly suggestive in appropriate clinical setting with patchy/diffuse infiltrates
Lymphangitic Carcinoma	Yes	Malignant cells	Diagnostic
Lymphoid Malignancies	Yes	Monoclonal Lymph	Diagnostic
Langerhans Cell Histiocytosis	Yes	>4% CD1a+ cells	Highly consistent/diagnostic if typical HRCT changes present
Lymphangiomyomatosis	+/-	Exclude infection	Rule out infection/malignancy; absence of inconsistent BAL findings plus typical/highly consistent HRCT imaging is supportive of diagnosis
Alveolar Proteinosis	Yes	Milky fluid White/tan sediment PAS+ sediment	Diagnostic if typical/consistent HRCT changes present
Radiation Pneumonitis	+/-	↑Lymph	Highly consistent if thoracic irradiation occurred within window*
Eosinophilic Pneumonia	Yes	↑↑Eos	Eos >25% are diagnostic if correlated with typical/highly consistent HRCT imaging (or typical/classic CXR pattern)
Cryptogenic Organizing Pneumonia	+/-	Variable alveolitis ↑Lymph, ↑Eos, ↑Neut	Rule out infection/malignancy; evidence of inflammation with typical/highly consistent HRCT imaging is supportive of diagnosis
Scleroderma	+/-	Variable ↑Lymph, ↑Eos, ↑Neut	Rule out infection/malignancy; evidence of inflammation with typical/highly consistent HRCT imaging plus typical clinical findings plus positive serologic testing is supportive of diagnosis
Other CTD	+/-	Variable alveolitis ↑Lymph, ↑Eos, ↑Neut	Rule out infection/malignancy; evidence of inflammation with typical/highly consistent HRCT imaging plus typical clinical findings plus positive serologic testing is supportive of diagnosis
Chronic Beryllium Disease	Yes	↑Lymph	Rule out infection/malignancy; BAL Be-LPT plus typical/highly consistent HRCT imaging

			is highly consistent with diagnosis
Asbestosis	+/-	Variable alveolitis Asbestos fibers	Rule out infection/malignancy; absence of inconsistent BAL findings plus typical/highly consistent HRCT imaging is supportive of diagnosis
Silicosis	+/-	↑AM with ↑silica particles	Rule out infection/malignancy; absence of inconsistent BAL findings plus typical/highly consistent HRCT imaging is supportive of diagnosis
Chronic Aspiration of Gastric Secretions	Yes	Lipid-laden AM Pepsin-like activity	Rule out infection/malignancy; consistent BAL findings plus typical/highly consistent HRCT imaging plus consistent clinical history is supportive of diagnosis
IPF	+/-	↑Neut, ↑Eos	Rule out infection/malignancy; absence of inconsistent BAL findings plus typical/highly consistent HRCT imaging is supportive of diagnosis; lymphocytosis suggests alternative diagnosis
DIP/RBILD	+/-	Pigmented AM ±↑Neut ±↑Eos	Rule out infection/malignancy; presence of elevated total “smoker” macrophage numbers plus absence of inconsistent BAL findings plus typical/highly consistent HRCT imaging is supportive of diagnosis
Lymphoid Interstitial Pneumonia	+/-	↑Lymph	Rule out infection/malignancy; lymphocytosis plus absence of inconsistent BAL findings plus typical/highly consistent HRCT imaging is supportive of diagnosis
Lymphangioliomyomatosis	+/-	Infection excluded	Rule out infection/malignancy; absence of inconsistent BAL findings plus typical/highly consistent HRCT imaging is supportive of diagnosis
Diffuse Alveolar Hemorrhage	Yes	Bloody lavage High Golde score	Progressively bloody BAL fluid with sequential lavage aliquots plus BAL macrophages with significant amounts of hemosiderin are highly diagnostic but do not establish specific cause
Acute onset ILD	Yes	↑Lymph, ↑Eos, ↑Neut Infection/malignancy excluded	Marked lymphocytosis suggests HP or drug toxicity; marked eosinophilia suggests acute EP as diagnosis; reactive/desquamated Type II pneumocytes suggests DAD
AEIPF	+/-	Infection excluded	Rule out infection/malignancy; absence of inconsistent BAL findings plus typical/highly consistent HRCT imaging is supportive of diagnosis

*BAL cytologic analysis and culture is useful to rule out infection and/or malignancy

**BAL lymphocyte subset determination is optional but is particularly supportive if CD4/CD8 ratio ≥ 3.5

Abbreviations: AM=alveolar macrophage; Eos=eosinophil; Lymph=lymphocyte; Neut=neutrophil; CXR=routine chest x-ray; HRCT=high-resolution computed tomography of thorax; BHL=bilateral hilar lymphadenopathy; GGO=ground-glass opacity; Be-LPT=beryllium-induced lymphocyte proliferation test; DIP/RBILD=desquamative interstitial pneumonia/respiratory bronchiolitis with interstitial lung disease; AEIPF=acute exacerbation of IPF; CTD=connective tissue disease; PAS=periodic acid Schiff stain