Bronchoalveolar Lavage as a Diagnostic Tool

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ABSTRACT

Bronchoalveolar lavage (BAL) provides an important diagnostic tool that can facilitate the diagnosis of various diffuse lung diseases. BAL fluid can be analyzed to determine white blood cell (WBC) profiles and to detect respiratory pathogens. Although BAL is seldom useful as a "stand-alone" diagnostic test for the diagnosis of diffuse infiltrative lung disease, when combined with clinical data and high-resolution computed tomography of the chest, BAL WBC profiles can contribute significantly to the diagnosis of specific forms of interstitial lung disease (ILD). Additionally, BAL can play a very important role in the diagnosis of respiratory infection, and it is useful in monitoring the lung allograft and in evaluating pediatric lung disease. Examination of BAL cells or acellular components of BAL via gene microarray technology or proteomic analyses may allow BAL to assume a more prominent role in diagnosis and management of lung disease in the near future.

KEYWORDS: Bronchoalveolar lavage (BAL), interstitial lung disease (ILD), bronchoscopy

When bronchoalveolar lavage (BAL) was first introduced into clinical practice in the early 1980s, expectations for BAL as a diagnostic tool were high and outstripped its eventual utility as a diagnostic tool. Nonetheless, BAL has gained widespread acceptance as a clinical procedure that allows sampling of respiratory secretions with its leukocytes, other cellular components such as invading bacteria, and acellular components such as cytokines, viral particles, and microbial signatures (e.g., proteins and nucleic acids). Analysis of BAL fluid can lead to the diagnosis of pulmonary infection as well as provide WBC differential cell counts and other findings that can aid in the diagnosis and management of a variety of lung diseases (Table 1), but the results of BAL analysis must always be interpreted in the context of clinical presentation, radiographic imaging studies, and other pertinent testing. If BAL is used with a thorough understanding of its limitations, it may provide information that can establish a diagnosis. Even if it is not diagnostic, BAL can provide findings that are inconsistent with suspected diagnoses and help focus attention on pursuing alternative diagnoses. This article reviews the current status of BAL as a diagnostic tool in clinical pulmonary medicine.

TECHNICAL ASPECTS OF PERFORMING AND ANALYZING DIAGNOSTIC BRONCHOALVEOLAR LAVAGE

Recommendations for performing and analyzing BAL have been published by both the European Respiratory Society and the American Thoracic Society.[1–6] Some relatively simple technical considerations are key to retrieving an adequate specimen for analysis (Table 2).

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Table 1 Settings in Which Bronchoalveolar Lavage Can Play a Role in Diagnosis

PULMONARY INFECTION
Immunocompromised host with infiltrates
Ventilator-associated pneumonia
Evaluation of persistent infiltrates/inadequate clinical response
PULMONARY MALIGNANCY
Lymphangitic carcinomatosis
Bronchoalveolar carcinoma
Other malignancies
ACUTE RESPIRATORY FAILURE
DIFFUSE INFILTRATIVE LUNG DISEASE
Alveolar hemorrhage
Sarcoidosis
Pulmonary alveolar proteinosis
Eosinophilic pneumonia
Drug toxicity
Pulmonary Langerhans cell histiocytosis
Hypersensitivity pneumonitis
Idiopathic pulmonary fibrosis
OCCUPATIONAL LUNG DISEASE
Chronic beryllium disease
Asbestosis
POSTTRANSPLANT MONITORING OF THE LUNG ALLOGRAFT
PEDIATRIC LUNG DISEASE
Infection
Interstitial Lung Disease
Aspiration
Hemorrhage
Cystic fibrosis

Table 2 Key Technical Aspects of Diagnostic Bronchoalveolar Lavage

Position of the bronchoscope: the distal tip of the fiberoptic bronchoscope should be placed in a wedge position (preferably a segmental airway) when performing BAL
Volume of instilled normal saline: an adequate instilled volume (e.g., total of 100 to 240 mL divided into 3 to 5 aliquots) of normal saline should be used, and excessive total instilled volumes (e.g., those that exceed the retrieved volume by greater than 100 mL) should be avoided
Aspiration of BAL fluid: excessive negative suction pressures (>100 mm Hg) and visible airway collapse should be avoided (e.g., by using gently applied but adequate suction via a hand-held syringe)
Adequacy of airspace sampling: retrieved volume should be large enough to adequately sample distal bronchoalveolar airspace epithelial surface liquid (e.g., at least 5 to 10% of the total instilled volume, and preferably $\geq 30\%$ of total instilled volume)
Volume of BAL fluid used for cell analysis: an adequate amount of pooled BAL fluid aliquots should be provided for laboratory analysis of BAL cells (e.g., ≥ 2 mL as a minimum, but preferably 10 to 20 mL)

BAL, bronchoalveolar lavage.

The right middle lobe (RML) and lingula represent the areas of the lung that are more easily accessible and likely to allow good return of lavage fluid with patients placed in a supine position. These areas have traditionally been lavaged for evaluation of diffuse infiltrates, but using high-resolution computed tomographic (HRCT) images to target areas of more prominent change (especially areas with ground-glass attenuation) and lavaging these areas may increase the likelihood of retrieving diagnostic secretions. The distal end of the bronchoscope should be placed in a wedge position in a segmental or subsegmental bronchus to avoid contamination of distal airspace sampling with proximal large airway secretions. Multiple aliquots of normal saline should be sequentially instilled and withdrawn. Use of a hand-held syringe allows the bronchoscopist to adjust negative pressure such that airway collapse can be minimized as the instilled fluid is withdrawn. Warming of normal saline to 37°C just prior to instillation may reduce cough in patients with reactive airways. Protocols for performing BAL generally employ aliquots that range from 20 to 60 mL with up to six aliquots used for BAL in a single lavage site with a total instilled volume that ranges from 100 to 250 mL. Some investigators have considered the first aliquot as representing predominantly airway cells and secretions[7] and have kept this aliquot separate (used for microbiological analysis), whereas subsequent sequential aliquots (usually three to four in number) are considered more representative of distal airspaces ("alveolar sampling") and pooled for subsequent cellular analysis. Other centers pool all retrieved BAL aliquots prior to submitting BAL for laboratory analysis, and there are no data to support one approach over the other. If emphysema is present, airways may collapse with negative pressure and compromise fluid retrieval. If the percentage of fluid retrieved is less than 5 to 10% of the instilled total volume, the specimen undoubtedly represents inadequate sampling and does not represent secretions from distal bronchoalveolar airspaces,[8] which could especially be an issue if BAL is performed to evaluate interstitial lung disease (ILD). Ideally, the percentage of BAL fluid that is retrieved would be at least 30% for a patient with ILD. Postbronchoscopy fever (fever, chills, and extreme malaise) may occur within hours of the BAL procedure as a consequence of proinflammatory mediator release and is more likely to occur if larger volumes of fluid are used for BAL.[9]

BAL fluid should be processed and analyzed to detect infectious agents and examine cellular components (Fig. 1), and attention to specific aspects of BAL processing and laboratory analysis can optimize the diagnostic potential of BAL (Table 3). Examination of at least 300 nucleated white blood cells (WBCs)

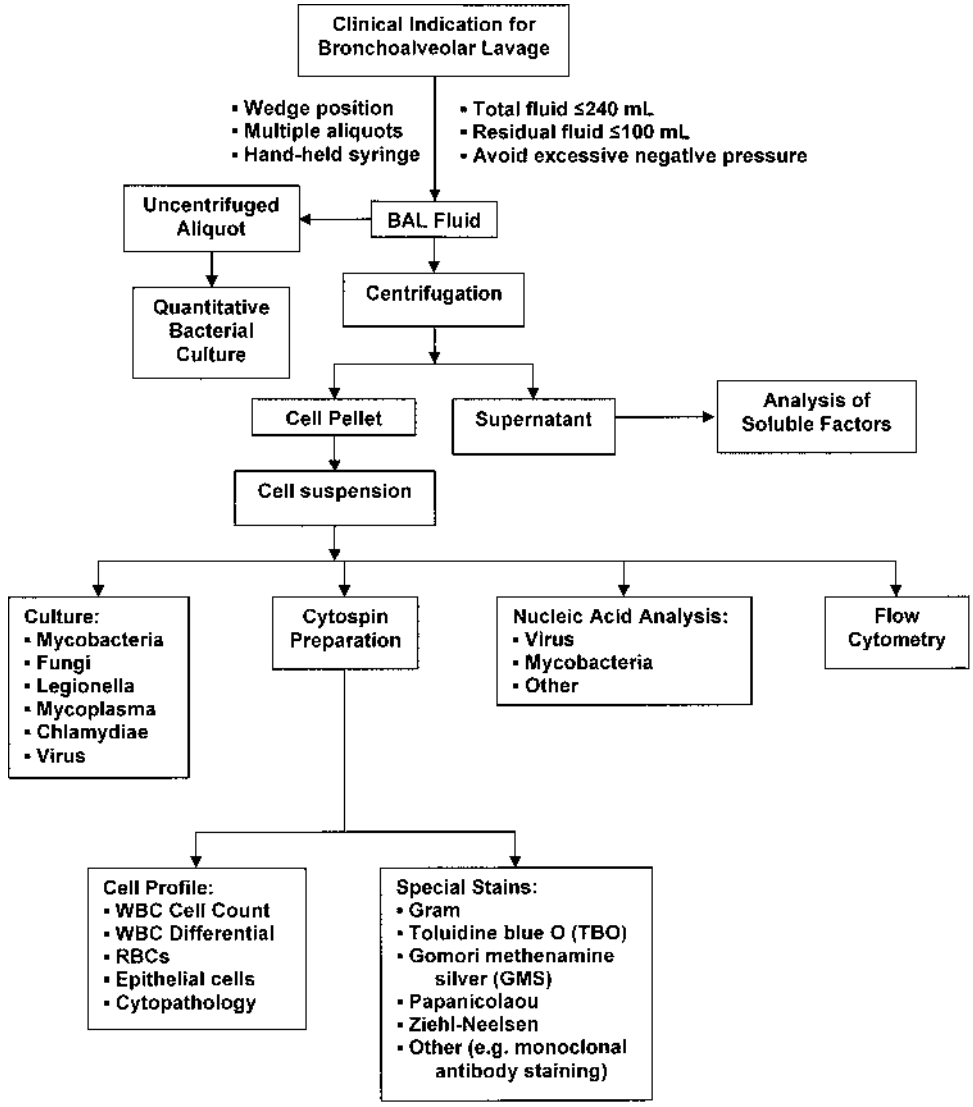


Figure 1 Processing and laboratory analysis of diagnostic bronchoalveolar lavage.

randomly on a single slide is recommended to provide an accurate and statistically meaningful enumeration of the BAL cell profile, and accuracy would be enhanced by analyzing 400 to 500 cells, especially if more than one slide is analyzed, with the differential counts from each slide averaged. BAL fluid obtained from healthy, never-smoking individuals should contain, on average, a majority of alveolar macrophages (80 to 90%), some lymphocytes (5 to 15%), and very few neutrophils ($\leq 3\%$) or eosinophils ($< 1\%$). Although many investigators have published BAL cell profiles for healthy volunteers, laboratories that perform BAL analysis should ideally study a sizable group of normal volunteers and establish their own normal values. The presence of squamous epithelial cells in BAL suggests that contamination by oropharyngeal secretions has occurred, which may be due to poor technique in performing the BAL or aspiration of upper airway secretions by the subject. BAL cell differentials and total cell counts from ex-smokers should be similar to those of never-smokers, but smokers generally have a significantly increased total BAL cell count as well as total macrophages and neutrophils per μL BAL fluid, although the BAL differential cell count for smokers does not appear to vary significantly from that for never-smokers or ex-smokers.[3,10] Elderly subjects appear to have increased percentages of lymphocytes and neutrophils in the differential cell counts, suggesting that advanced age may affect BAL cell differentials.[11,12] Additionally, the total volume of retrieved fluid declines with advanced age due to loss of elastic recoil and airway tethering that makes airways more likely to collapse with negative pressure.

DIAGNOSIS OF PULMONARY INFECTION

BAL provides a very useful tool for diagnosing lower respiratory tract infection in both immunocompetent and immunocompromised patients. To detect and

Table 3 Recommendations for Processing and Laboratory Analysis of Diagnostic Bronchoalveolar Lavage

Process and analyze BAL promptly (e.g., cells in nutrient-poor media such as saline should be processed within 1 hour)
Avoid containers that promote cell adherence to container surfaces
Remove excessive mucus if present in amounts that are likely to interfere with specimen preparation (e.g., straining through loose gauze)
Use nutrient-supplemented media for prolonged storage (e.g., 12 to 24 hours) if necessary (discard specimens obtained more than 24 hours prior to processing and analysis)
Avoid centrifugation of cell suspensions at speeds that are likely to compromise cellular integrity and prevent uniform resuspension of retrieved BAL cells
Keep cell suspensions at 4°C if not analyzed immediately
Obtain nucleated cell counts via a hemocytometer and identify cell subpopulations via cytocentrifugation with staining (e.g., Wright-Giemsa or May-Grünwald-Giemsa) and enumeration of an adequate number of cells (e.g., ≥ 400 cells)
Perform analyses of BAL fluid and cells as needed to diagnose infection
Express concentrations of BAL cells or acellular components as per mL of fluid
Observe and report the following:
Volume and gross appearance (color and turbidity) of uncentrifuged BAL fluid
Absolute number of total nucleated cells and total number of red blood cells
White blood cell differential percentages
Percentage of epithelial cells that represent total nucleated cells
Other specific findings (e.g., plasma cells, mast cells, foamy alveolar macrophages, malignant cells, lipoprotein bodies, presence of foreign bodies or birefringent material)
BAL findings should be interpreted by an expert familiar with BAL analysis

BAL, bronchoalveolar lavage.

quantitate common bacterial respiratory pathogens, an uncentrifuged BAL fluid specimen can be sent for quantitative bacterial culture (Fig. 1). Centrifuged BAL specimens (resuspended cell pellets) can be used to culture viruses and atypical bacterial pathogens (*Legionella*, *Mycoplasma*, and *Chlamydia*) in addition to mycobacteria and fungi, and cytocentrifuged specimens (cytospin preparations) can be stained to detect the presence of bacteria, *Pneumocystis carinii*, mycobacteria, or fungi. Newer tools that utilize immunofluorescent staining with antibodies and/or nucleic acid analysis via polymerase chain reaction (PCR) methods are increasingly available for the detection of these pathogens and can facilitate more rapid diagnosis than culture techniques currently allow.[13–15] One useful application of BAL combined with PCR is in the diagnosis of pulmonary tuberculosis in smear-negative patients.[16]

BAL can be particularly helpful in the evaluation of pulmonary infiltrates in immunocompromised patients, who generally do not produce sputum that could facilitate a noninvasive diagnosis. BAL frequently identifies the offending pathogen in patients with human immunodeficiency virus (HIV) infection, solid organ transplants, bone marrow or stem cell transplants, and neutropenia associated with treatment of malignancy when lung infiltrates appear during the course of their disease. Furthermore, BAL can be performed safely and rapidly, even when significant renal failure or coagulopathies are present.[14,17–23] The diagnostic yield may be increased when BAL is combined with transbronchial lung biopsy (TBLB), but these patients are often at significant risk for hemorrhage when endoscopic lung biopsy is performed due to the presence of coagulopathy. Peikert et al[17] found that bronchoscopic findings led to management changes in 51% of a cohort of patients with febrile neutropenia accompanied by pulmonary infiltrates, and TBLB did not substantially increase the diagnostic yield when added to BAL and sputum analyses.

BAL has also been used to diagnose ventilator-associated pneumonia (VAP). Various sampling methods have been used to detect and quantify bacterial pathogens in patients suspected of having VAP, including endotracheal aspirate, blinded bronchial sampling, protected specimen brush, and BAL.[24–26] Blind BAL or specimen brushing can be performed via a catheter, and protected BAL has also been used on intubated patients. In contrast to blind techniques, bronchoscopy allows the clinician to reliably target areas that correspond to areas of infiltrate identified on radiological imaging as well as visualize and identify airways with purulent secretions. Although protected BAL may avoid contamination with upper airway secretions, cost is greater than conventional BAL performed without the use of protected catheters, and there is no evidence that protected BAL provides more reliable results than conventional BAL. One recently published, randomized clinical trial that compared BAL with quantitative culture to tracheal aspirates with qualitative culture found no advantage for BAL over blind tracheal aspiration, but patients known to be colonized with specific pathogens and immunocompromised patients were excluded from enrollment and randomization.[27]

DIAGNOSIS OF DIFFUSE INFILTRATIVE LUNG DISEASE

Bronchoalveolar lavage differential cell counts can provide important information that supports the diagnosis of specific ILD.[28–31] Differential cell counts obtained from slides prepared via cytocentrifugation usually show

variations in the WBC differential that differ from patterns found in normal subjects. These patterns tend to reflect inflammatory cell profiles in affected lung tissues,[32] 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 WBC.[33] Additionally, airway disorders (e.g., bronchiolitis, bronchitis, asthma, eosinophilic bronchitis, bronchiectasis, and allergic bronchopulmonary aspergillosis) can alter BAL WBC patterns. Nonetheless, certain cell patterns with significant changes in one or more WBC type can suggest the presence of specific types of ILD (Table 4), and BAL can play an important role in ILD differential diagnosis when BAL findings are combined with HRCT imaging and other clinical data (Fig. 2). A recently published study of the predictive value of BAL for ILD diagnosis for a large cohort of patients ($N = 3118$), however, suggested that BAL cell counts are most useful for the diagnosis of relatively common entities such as sarcoidosis, in contrast to relatively rare forms of ILD.[34]

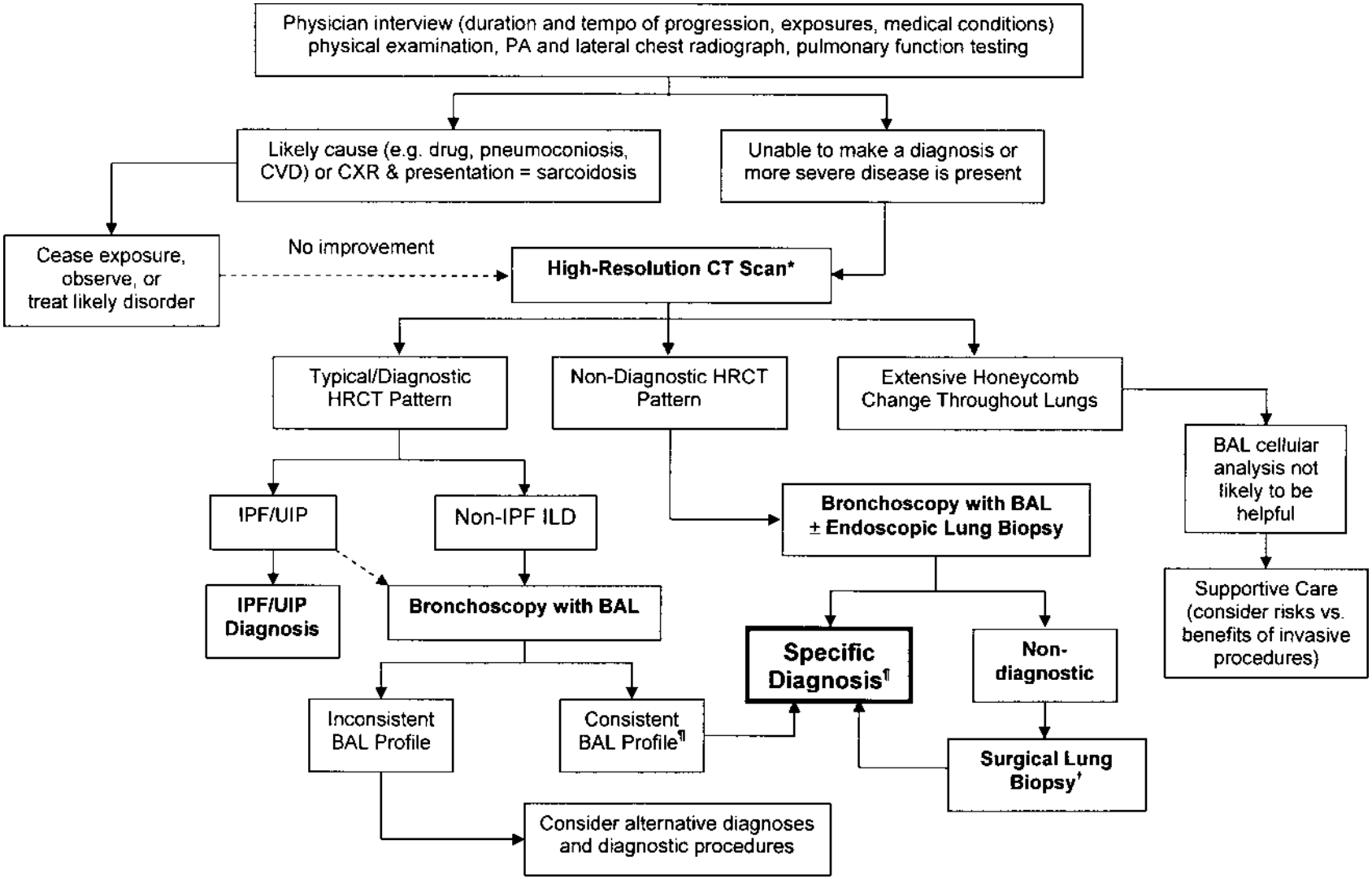
Flow cytometric analyses of BAL cells have been reported by numerous investigators,[33–35] and alterations in BAL lymphocyte subsets have been examined extensively, especially for sarcoidosis. However, whether BAL lymphocyte subset data contribute significantly to ILD diagnosis remains controversial. The BAL CD4 + T lymphocyte subset and CD4 + :CD8 + T lymphocyte ratio are usually increased in clinically active pulmonary sarcoidosis.[36] The CD4 + :CD8 + lymphocyte ratios in BAL from clinically healthy adults typically range from 1.5 to 2.0, and an elevated CD4 + :CD8 + ratio of ≥ 3.5 has been shown to be fairly specific for sarcoidosis.[37,38] However, the sensitivity of an increased CD4 + :CD8 + ratio is relatively low for sarcoidosis,[37,38] and many patients do not have an elevated ratio or may even have a low ratio.[39] A depressed CD4 + :CD8 + ratio has been reported for hypersensitivity pneumonitis (HP), drug-induced lung disease, cryptogenic organizing pneumonia (COP), eosinophilic pneumonia (EP), and idiopathic pulmonary fibrosis (IPF),[35] but the finding of a depressed CD4 + :CD8 + ratio has not been shown to be particularly helpful in the diagnosis of nonsarcoid ILD, and use of the CD4 + :CD8 + ratio as a routine component of BAL analysis increases the cost of the procedure. Flow cytometry may, however, be useful in certain situations. Staining for CD1a-positive cells can support a diagnosis of pulmonary Langerhans cell histiocytosis (PLCH) if clinical data and imaging suggest this entity. PLCH is associated with increased ($\geq 5\%$ of total cells) CD1a-positive cells in BAL, although this may no longer be the case in later stages of disease.[40] Additionally, if clinical data are consistent with the possibility of pulmonary lymphoma, the identification of monoclonal lymphocyte populations via flow cytometry can support the diagnosis of lymphoid malignancy.

Table 4 Bronchoalveolar Lavage Cellular Changes Associated with Diffuse Lung Disease

↑ Lymphocytes ($\geq 15\%$)
Sarcoidosis
Hypersensitivity pneumonitis
CVD-associated ILD
Drug-induced pneumonitis
IIP (NSIP-cellular, COP)
Inflammatory bowel disease
Occupational disease (e.g., chronic beryllium disease)
Infection (mycobacteria, viral)
Radiation pneumonitis
↑ Neutrophils ($\geq 5\%$)
Infection
Lung injury
IIP (COP, DIP, IPF, NSIP)
CVD-associated ILD
Drug-induced pneumonitis
Hypersensitivity pneumonitis
Occupational lung disease
Aspiration pneumonia
Sarcoidosis
↑ Eosinophils ($\geq 3\%$)
Eosinophilic pneumonia
Drug-induced pneumonitis
Churg-Strauss syndrome
Hypereosinophilic syndrome
Parasitic infection
IPF, fibrotic NSIP
CVD-associated ILD
Pneumocystis pneumonia
↑ Plasma cells
Hypersensitivity pneumonitis
Drug-induced pneumonitis
Eosinophilic pneumonia
Malignancy
Infection (*Legionella*, *Pneumocystis*)
↑ Mast cells
Hypersensitivity pneumonitis
Drug-induced pneumonitis
IPF
CVD
COP
Eosinophilic pneumonia
Malignancy
Sarcoidosis

CVD, collagen vascular disease; COP, cryptogenic organizing pneumonia; DIP, desquamative interstitial pneumonia; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; IIP, idiopathic interstitial pneumonia; NSIP, nonspecific interstitial pneumonia.

Findings derived from BAL fluid analysis can be highly suggestive or even virtually diagnostic of specific ILD entities in the appropriate clinical setting (Table 5),



*HRCT may not be necessary if routine chest radiographic findings are typical/diagnostic of specific ILD (e.g. sarcoidosis) and fit with clinical data
¶ Infection, malignancy, and hemorrhage must be excluded as required by clinical features
†Surgical lung biopsy should sample at least two different lung regions and be guided by the HRCT image

Figure 2 Algorithm for the evaluation of suspected interstitial lung disease.

Table 5 Bronchoalveolar Lavage Findings That Strongly Support Specific Diagnoses

Abnormal appearance of lavage fluid
Bloody lavage with increasing intensity of bloody discoloration in sequential aliquots indicates diffuse alveolar hemorrhage unless coagulation disorders are present
Cloudy (milky or light brown color) unprocessed BAL fluid with flocculent material that settles by gravity to the container bottom within 15 to 20 minutes of fluid retrieval combined with identification of PAS-positive amorphous debris in the sediment is highly supportive of pulmonary alveolar proteinosis
Eosinophils: differential count $\geq 25\%$ is virtually diagnostic of eosinophilic lung disease
Neutrophils: differential count $\geq 50\%$ strongly supports acute lung injury or suppurative infection
Lymphocytes:
Lymphocyte differential count $\geq 25\%$ is strongly suggestive of granulomatous lung disease (sarcoidosis, HP, chronic beryllium disease), drug reaction, cellular NSIP, drug reaction, or viral infection
Lymphocyte differential count $> 50\%$ is strongly supportive of HP, drug reaction, or cellular NSIP
Assisting diagnosis of IPF: BAL is one criterion for establishing a diagnosis of IPF if other major and minor criteria for this diagnosis are met and BAL does not detect an alternative diagnosis

BAL, bronchoalveolar lavage; HP, hypersensitivity pneumonitis; IPF, idiopathic pulmonary fibrosis; NSIP, nonspecific interstitial pneumonia; PAS, periodic acid-Schiff.

but these observations must be interpreted in the context of the patient's clinical presentation and radiological findings. 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. Extreme lymphocytosis, especially with differential counts $\geq 50\%$, combined with a plausible exposure history to an antigen known to cause HP, is strongly suggestive of the diagnosis of HP. A high CD4+:CD8+ T lymphocyte ratio, if performed, increases the likelihood of sarcoidosis as the diagnosis 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.[12] 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 EP if the presentation is acute.[41,42] Increased numbers of mast cells have been associated with HP, drug reactions, sarcoidosis, ILD associated with collagen vascular disease (CVD), IPF, COP, EP, or malignancy. Plasma cells have been observed in BAL in HP, drug reactions, EP, malignancy, or infection.[35] Alveolar macrophages (AMs) may also display certain morphological 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 red blood cells (RBCs) and RBC fragments plus hemosiderin with diffuse alveolar hemorrhage, or ingested asbestos bodies. Because infection can cause the subacute onset of diffuse lung infiltrates or coexist with noninfectious ILD, BAL should be examined and screened for mycobacterial or fungal infection when performed to evaluate diffuse infiltrates. Other testing for the presence of infection or a malignancy should be considered and performed as clinically indicated.

The gross appearance of BAL fluid at the time of retrieval can provide diagnostic information. 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 sequentially retrieved BAL fluid aliquots do not show any decrease in the amount of bloody discoloration of the lavage fluid and the discoloration becomes more intense with retrieval of successive aliquots.[43] If DAH is present, RBCs should be identified on the cytospin, and AM will stain positively for hemosiderin when stained with an iron stain if hemorrhage has been occurring for 24 to 48 hours. 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 pulmonary alveolar proteinosis (PAP) as the likely diagnosis.[44] The diagnosis can be confirmed if the sediment, which is surfactant-derived lipoproteinaceous material, stains positive with a periodic acid-Schiff (PAS) stain.

BAL can be useful in the diagnosis of acute-onset ILD, defined as 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 acute respiratory distress syndrome (ARDS), such as sepsis or trauma. Diagnostic considerations in acute-onset ILD include infection, noninfectious ILD (acute interstitial pneumonia [AIP], 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 provide diagnostic information. If BAL analysis shows large numbers of eosinophils, this finding strongly supports a diagnosis of acute EP. Similarly, large numbers of lymphocytes suggest 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.

One application for BAL that has been adapted recently is its incorporation into the criteria for the clinical diagnosis of IPF when surgical lung biopsy is not performed.[45] All four of the major criteria (exclusion of other known causes of ILD, demonstrating the presence of a restrictive ventilatory defect and impaired gas exchange with pulmonary function testing, bibasilar reticular abnormalities with little or no ground-glass opacities on HRCT, and transbronchial biopsy *or* BAL showing no features to support an alternative diagnosis) and at least three of the four minor criteria (age >50 years, the insidious onset of otherwise unexplained dyspnea on exertion, illness duration ≥ 3 months, and chest auscultation showing bibasilar inspiratory crackles) must be present to make a confident diagnosis of IPF without resorting to a surgical lung biopsy. Some experts would suggest, however, that when lower lung zone honeycomb change and upper lung zone reticular lines are present, the positive predictive value of these HRCT findings for a diagnosis of IPF/usual interstitial pneumonia (UIP) is at least 85%, and a confident diagnosis of UIP can be made when these findings are present.[46,47]

Although BAL can be virtually diagnostic of specific types of ILD when performed with a standardized technique, expertly examined, and combined with clinical and imaging data, it cannot be used as a stand-alone diagnostic test. Furthermore, 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.

BRONCHOALVEOLAR LAVAGE AND INTERSTITIAL LUNG DISEASE MANAGEMENT

With the exception of whole lung lavage as a treatment for pulmonary alveolar proteinosis,[48] a role for BAL in the management of ILD (e.g., to direct or monitor pharmacological therapies) has not been established. One application of BAL to disease management is its use to evaluate acute changes in symptoms and lung function. Several adverse events may occur in the course of the ILD, including respiratory infection, drug reactions, hemorrhage, or an acute exacerbation of the disease process, and BAL may play an important role in identifying the cause of clinical deterioration.

Many investigators have examined the role of BAL for monitoring disease activity and response to therapies in specific ILDs, and BAL 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[49,50] and IPF,[45,51–53] and increased eosinophils have been linked to more severe disease and worse prognosis in IPF.[54,55] Similarly, increased neutrophils in BAL from patients with sarcoidosis have been associated with more progressive disease that is less likely to respond to immunosuppressive therapy.[56]

BAL lymphocytosis has been linked to prognosis and response to therapy for some forms of idiopathic interstitial pneumonia (IIP). BAL findings from patients diagnosed with IPF when this diagnostic term did not necessarily exclude other IIP such as nonspecific interstitial pneumonia (NSIP) suggested that higher BAL lymphocyte differential cell counts correlated with either or both better prognosis and response to therapy.[45,52–54,57] A more recent study, although retrospective, demonstrated that BAL lymphocytosis in fibrotic IIP suggests that the diagnosis is NSIP and not UIP, and BAL lymphocytosis was found to correlate with a better prognosis.[58]

EVALUATION OF OCCUPATIONAL LUNG DISEASE

BAL has been used to evaluate various occupational ILDs, including asbestosis, coal workers' pneumoconiosis, silicosis, hard metal lung disease, and chronic beryllium disease. Although silica particles or asbestos fibers can be detected in BAL,[59–63] differentiating exposure from dust-induced pneumoconiosis remains problematic and cannot be accomplished with BAL alone. Similarly, although giant cells have been associated with hard metal lung disease,[64] limited evidence exists to support their diagnostic use in clinical settings. However, BAL can prove useful in diagnosing chronic beryllium disease by allowing detection of a lymphocytic alveolitis and beryllium-sensitized BAL lymphocytes.[65] A positive beryllium lymphocyte proliferation test can be diagnostic of chronic beryllium disease and may be present when peripheral blood mononuclear cell proliferation in response to beryllium is absent.

BRONCHOALVEOLAR LAVAGE AND PULMONARY MALIGNANCY

BAL can retrieve diagnostic malignant cells in patients with primary lung cancer. Lam et al[66] obtained diagnostic specimens via BAL in 69% of cases of bronchial carcinoma, and the addition of endobronchial brushings,

endobronchial biopsies, and postbronchoscopic sputum analysis did not significantly increase the diagnostic yield. Wongsurakiat et al[67] retrieved diagnostic malignant cells in 47% of patients with peripheral lesions, whereas transbronchial biopsy was diagnostic in only 17%). Similarly, Tang et al[68] retrieved malignant cells via BAL in 49% of patients with peripheral lesions, and the diagnostic yield of BAL combined with TBLB increased to 78%.

Bronchoalveolar carcinoma, lymphangitic carcinoma, or infiltration of the lung with bone-marrow-derived malignant cells can all give interstitial patterns on chest radiographic imaging and masquerade as ILD. Bronchoalveolar carcinoma can be diagnosed via sputum cytology, examination of BAL fluid, or transbronchial lung biopsy, and surgical lung biopsy is usually unnecessary.[69,70] Similarly, bronchial brushings, BAL analysis, or bronchoscopic lung biopsies usually show either or both positive cytology and histopathology when lymphangitic carcinomatosis is present.[69] Additionally, examination of BAL cells via surface marker analysis or polymerase chain reaction (PCR) can be used to identify B or T cell lymphomas.[71,72]

BRONCHOALVEOLAR LAVAGE AND EVALUATION OF THE LUNG ALLOGRAFT

Bronchoscopy is the principal invasive diagnostic modality for evaluating the lung allograft. When bronchoscopy is performed on lung transplant recipients to diagnose acute deterioration in graft function or for surveillance purposes to rule out infection or rejection, BAL and TBLB are usually performed together. BAL is an extremely useful tool for the detection of infection via culture in combination with cytological methods that employ special staining, and PCR is now used by most centers to detect cytomegalovirus (CMV) DNA and to quantitate CMV viral load in peripheral blood as well as to detect other viral pathogens.

WBC counts and differentials provide potentially useful information but must be interpreted carefully. During the immediate posttransplant period, cell counts are quite elevated, with cell differential counts showing a predominance of neutrophils, which is likely the consequence of reperfusion injury.[73–75] This neutrophil influx gradually subsides during the first 2 to 4 weeks posttransplant, and BAL differential counts tend to approach those of normal individuals by 3 months posttransplant if recipients have well-functioning grafts without evidence of rejection or infection, although total cell counts tend to remain somewhat increased in comparison with normal controls.[75] BAL lymphocytosis may occur with acute rejection (AR) or viral infection and can also be seen with obliterative bronchiolitis, whereas bacterial infection is typically accompanied by increased neutrophils. The CD4 lymphocyte subset declines in respiratory secretions posttransplant, and the BAL CD4:CD8 lymphocyte ratio is typically depressed.

Unfortunately, considerable interindividual variation in BAL WBC profiles precludes the use of BAL WBC patterns to provide a definitive diagnosis of posttransplant pulmonary complications. However, a significant increase in lymphocytes or neutrophils in BAL that occurs outside of the perioperative window should increase suspicion of allograft pathology. Although the diagnosis of chronic rejection currently depends upon spirometry as a surrogate marker to detect and grade it,[76] techniques to diagnose chronic rejection via microarray analysis of BAL cell gene expression[77] or BAL fluid protein analysis via matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) profiles[78] have been reported and may provide a sensitive and accurate means of detecting the onset of obliterative bronchiolitis. Similar techniques are being investigated to detect AR.[79] Although the use of bronchoscopy with BAL and TBLB for surveillance purposes to evaluate clinically stable patients following transplant is somewhat controversial,[80] surveillance BAL may prove quite useful if it can be shown to have adequate sensitivity and specificity for the diagnosis of acute or chronic rejection when newer techniques such as profiling gene expression are applied to BAL analysis. Additionally, gastroesophageal reflux has been recognized as a significant risk for posttransplant allograft dysfunction.[81] Measurement of pepsin in BAL fluid[82] has the potential to facilitate the identification of patients with persistent reflux but remains to be clinically evaluated.

PEDIATRIC LUNG DISEASE AND CYSTIC FIBROSIS

BAL has been used as a diagnostic modality for evaluating possible infection or ILD in children, and it has been shown to be very useful in the diagnosis of infection in both immunocompetent and immunocompromised children with unexplained infiltrates. The constituents of BAL fluid for normal children have been shown to be very similar to those in adults,[83–85] and total instilled BAL volume can be modified for variation in age by adjusting for body weight.[86] ILD is relatively rare in children, but various forms of ILD such as lymphocytic interstitial pneumonitis, sarcoidosis, and hypersensitivity pneumonitis can occur in both infants and older children. Although BAL WBC patterns may be abnormal in children with ILD, no reliable diagnostic characteristics have been identified. However, BAL cell patterns may occasionally be diagnostic or at least narrow the differential diagnostic possibilities.[87] BAL may also be useful in detecting aspiration or alveolar hemorrhage.[87] Detection of lipid-laden macrophages is suggestive of aspiration, but its specificity is problematic.[87–91] Measurement of pepsin levels in BAL, however, may provide a

measurement that has specificity for gastroesophageal reflux that the lipid-laden macrophage index appears to lack.[82]

BAL has also been used to study cystic fibrosis (CF) lung disease in both children and adults.[92–95] Studies have demonstrated that BAL can detect the presence of specific pathogens and inflammatory cell infiltrates in infants and young children, even when they do not expectorate sputum.[95,96] Additionally, oropharyngeal sampling has a poor predictive value for pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and cultures of sputum and BAL may match poorly with oropharyngeal specimens.[97,98] BAL in children with CF at the time of diagnosis can detect lower respiratory tract infection in children without respiratory symptoms,[99] and a multicenter, randomized clinical trial is under way in Australia to assess BAL-driven therapy for the management of children with CF.[100]

Table 6 Future Applications of Bronchoalveolar Lavage to Diagnosis and Management of Lung Diseases

Characterization of BAL cell gene expression patterns (nucleic acid microarray analysis)
Determination of BAL acellular glycoprotein ''fingerprints'' (proteome analysis)
Correlation of gene analysis profiles and proteomic analysis with specific diagnoses (e.g., specific types of ILD, lung allograft rejection)
Detection of specific infection via microbial oligonucleotide analysis (GreeneChip)
Construction of diagnostic algorithms that combine clinical data, HRCT imaging, and BAL gene/protein signatures to make specific ILD diagnoses
Use of BAL gene/protein analyses to determine disease activity and guide management
Use of BAL gene/protein profiling to identify useful peripheral blood surrogate markers that may facilitate diagnosis and monitoring of ILD or lung allograft status

BAL, bronchoalveolar lavage; HRCT, high-resolution computed tomography; ILD, interstitial lung disease.

NEW APPLICATIONS OF BRONCHOALVEOLAR LAVAGE

BAL is clearly useful in diagnosing infection, and when BAL is performed and analyzed in a standardized fashion and interpreted within the context of a patient's clinical presentation and HRCT imaging, it can provide compelling supportive information that can lead to an accurate and reasonably confident ILD diagnosis in many instances.[29,32] As our understanding of the role and interplay of inflammatory cells, nonmotile cells, cytokines, chemokines, reactive oxygen species, and proteases in the pathogenesis of ILD evolves, BAL may play an increasingly useful role in both the diagnosis and monitoring of patients with ILD. It may prove particularly helpful as treatments become available that may alter the profiles of cells or noncellular components of lower respiratory tract bronchoalveolar surface liquid when patients have a favorable response to such therapies, and detection of these changes may support continued, ongoing treatment with specific agents. Multicenter studies that prospectively compare BAL with HRCT and histological patterns in large groups of patients will be facilitated by improvements in BAL standardization. Such studies have the potential to better define the underlying inflammatory and histopathological events associated with different HRCT appearances, which may reduce the need for biopsies plus provide information that can guide effective therapies.

New diagnostic tools can also allow genomic and proteomic characterization of BAL cells and soluble components (Table 6). Rapidly expanding computational capabilities combined with evolving high-throughput gene expression technologies are now being exploited to establish gene expression profiles for various specific disease states, including ILD and lower respiratory tract infection. These cutting edge methods may eventually prove extremely useful in making an accurate diagnosis, choosing and implementing effective therapies, monitoring disease activity, and assessing the effect of therapeutic interventions. Acquisition of new knowledge of the pathobiology of various lung diseases is now being aided by the recent development of DNA microarray and other technologies that identify and monitor expression patterns of vast numbers of genes.[101,102] In addition, recent advances in proteomics that allow mapping and identification of multiple protein expression patterns by two-dimensional 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.[103] 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 ILDs without resorting to lung biopsy, and indicate targets for new and effective therapies. Additionally, the diagnosis of infectious agents via nucleic acid amplification techniques performed on BAL can provide diagnostic information that surpasses the capabilities of conventional analytic methods.[13] Furthermore, a new diagnostic tool (GreeneChipPm, Agilent Technologies, Santa Clara, CA) that can rapidly detect a broad spectrum of pathogen oligonucleotides (viruses, bacteria, fungi, and parasites) in body fluids has recently been used for pathogen detection[15] and holds the potential to greatly improve the differential diagnosis of lower respiratory tract infection when applied to BAL analysis.

A potential advantage of characterizing and quantitating proteins in biological specimens is that it circumvents modifications of RNA that affect protein production and expression, and it can be applied to acellular compartments such as epithelial surface liquid retrieved via BAL or peripheral blood plasma. Some early investigations that utilized electrophoretic techniques to examine protein profiles in BAL from patients with ILD were able to show some differences between IPF and sarcoidosis,[104,105] 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.[106,107] Rottoli et al[108] were able to use protein mapping via two-dimensional electrophoresis to construct protein maps from BAL fluid that were able to profile differences among IPF, systemic sclerosis, and sarcoidosis, and Sabounchi-Schütt et al[109] have used similar techniques to identify inflammatory markers from the serum of patients with sarcoidosis and speculated that proteins in BAL fluid and peripheral blood may eventually provide disease-specific markers. The development of techniques that employ (1) mass spectrometry combined with ionization of peptides via (MALDI) or electrospray ionization (ESI) or (2) mass spectrometry combined with the use of surface chromatography to capture proteins on a chip surface (SELDI [surface-enhanced laser desorption ionization]) coupled with greatly enhanced computational abilities and proteomics databases hold considerable promise for the study of ILD via this emerging technology. Although these newer techniques for proteome analysis are still being developed and have yet to be widely applied to the study of ILD, they have already been applied to the study of markers in BAL fluid in patients with lung inflammation.[110]

A global analysis approach has identified many genes with increased expression in IPF,[102] and certain gene products that may play an important role in IPF pathogenesis or that serve as disease biomarkers, such as pigment epithelium-derived factor,[111] matrilysin,[112] and osteopontin,[113] have been identified via these methods. Thonhofer et al[114] examined gene expression by stimulated BAL cells from patients with sarcoidosis and showed that over a thousand genes were up- or downregulated, including selective upregulation 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 ILDs, but distinct expression patterns may differentiate one ILD from another. Validation of this concept has been provided by Selman et al,[115] who demonstrated distinct gene expression patterns in lung tissue for patients with IPF versus HP, and an exciting application of microarray-derived gene expression patterns to BAL cells has now been used to differentiate patients with chronic rejection versus those without via sequential profiling of BAL cells based on extracted RNA as the indicator of gene activity.[77]

In addition to profiling of lung tissue and BAL cells, comparisons of BAL cell gene expression with peripheral blood may allow the identification of signatures that may give diagnostic information, provide an index of disease activity, or assist in the selection of specific pharmacological interventions for effective treatment of the disorder. Microarray investigations of peripheral blood mononuclear cells from patients with acute sarcoidosis have demonstrated that expression of the *Bcl-2* family of genes shows a prosurvival profile, whereas nuclear factor kappa B (*NF-KB*) is upregulated in sarcoidosis patents with progressive disease.[116] Additionally, Rutherford et al[117] have demonstrated specific peripheral blood gene expression patterns that correlate with self-limited disease. Such studies, especially when combined with examination of biopsy specimens or BAL cells or both, may allow the identification of circulating biomarkers, such as osteopontin in peripheral blood,[118] that may eventually prove useful in clinical practice.

The fact that noninvasive or minimally invasive methods which can accurately and reliably identify specific ILDs, assess disease activity, stage various diseases and render a prognosis, aid the selection of optimal approaches to treatment, or monitor response to therapeutic interventions are lacking constitutes a major problem that needs to be resolved through additional clinical investigation. Universal adoption of a reasonably standardized BAL procedure will undoubtedly improve the chances of identifying clinically relevant differences between disorders and stages of disease. It will also enable centers to pool information to achieve the large patient groups necessary to more adequately investigate the value of BAL markers to identify a specific disease entity, indicate clinical disease activity, detect disease progression, provide a prognosis, and guide treatment strategies. Identifying specific and sensitive markers that can address these problems remains an elusive but vitally important target, but new developments in genomics and proteomics may allow us to attain these goals.

SUMMARY AND CONCLUSIONS

BAL can play an important role in the evaluation of various lung diseases and may provide or strongly support specific diagnoses. It is particularly useful for identifying pulmonary infection, and WBC differential cell patterns can prove useful in evaluating patients with diffuse lung infiltrates. Newer techniques that utilize advanced computational and analytical techniques such as the detection of specific patterns of gene expression via microarrays, the characterization of protein components

that reflect specific gene expression, or the detection of microbial oligonucleotide fingerprints may revolutionize the ability of BAL to diagnose specific disorders as well as allow BAL to assume a role in disease management by using gene expression patterns to gauge responses to therapeutic interventions.

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