THE CLINICAL UTILITY OF BRONCHOALVEOLAR LAVAGE CELLULAR ANALYSIS IN INTERSTITIAL LUNG DISEASE: AN ATS CLINICAL PRACTICE GUIDELINE

On-Line Supplement

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1. **BRIEF HISTORY OF BRONCHOALVEOLAR LAVAGE AND ITS APPLICATION TO ILD**

Bronchial irrigation with saline solution via a catheter passed through a rigid bronchoscope was first reported in 1927 (1), and the term "bronchial lavage" was coined by Stitt in 1932 (2) . Although initially used as a therapy for septic lung disease or pulmonary alveolar proteinosis (3, 4), lung lavage was subsequently employed to study lower respiratory tract immunity in various animal models in the 1960s (5, 6) and applied to the study of the human lung in the late 1960s and 1970s (7, 8). A seminal article reporting the application of BAL to the study of secretions obtained from the human lung was published in 1974 when saline lavage of a portion of the lung via a flexible bronchoscope was introduced as a research tool by Reynolds and Newball (9). Saline lavage of a defined area of the lung became known as bronchoalveolar lavage, and hundreds of articles on BAL appeared in the literature in the 1980s and 1990s as clinical use of the flexible fiberoptic bronchoscope expanded.

The technique rapidly gained acceptance, and a large number of centers began using the technique to obtain cells and proteins from the lower respiratory tract (10). However, many centers used their own technique for performing lavage, and concern arose that differences in technique could lead to significant differences in results obtained from BAL fluid analysis and the interpretation of such findings. Consequently, several groups established standardized methods for performing BAL. The European Respiratory Society has provided statements on the use of BAL for the clinical evaluation of patients with ILD including recommendations concerning the technical aspects of performing BAL (11-13) and on standardization of the BAL procedure (14), and a multi-center, NIH-sponsored investigation conducted under the auspices of the American Thoracic Society in the United States examined BAL cell profiles in patients with ILD and normal volunteer subjects (15). Specific recommendations regarding various aspects of performing and analyzing BAL were subsequently published. However, these statements were published prior to the use of high-resolution computed tomography (HRCT) of the chest as a routine clinical tool for the evaluation of patients with ILD and before the current classification of different forms of idiopathic interstitial pneumonia (IIP) had evolved (16) to become the system that is currently accepted. Despite the promotion of BAL standardization in Europe, the technique has never been standardized on a global basis, and considerable variation in technique and in analysis persists among bronchoscopists and analytical laboratories around the world.

Although BAL provides a means of retrieving secretions that coat the apical surfaces of bronchial and alveolar epithelium of normal individuals or patients with infectious or inflammatory lower respiratory tract disorders, these secretions are considerably diluted by the saline used to perform BAL. Many factors can affect the amount of fluid retrieved and/or the cellular and acellular profile of the retrieved bronchoalveolar epithelial surface fluid. Over the past quarter century that BAL has been applied to the clinical evaluation of patients with various forms of lung disease, its diagnostic potential for

ILD has been hampered by a relative lack of specificity. Although patterns in differential counts of BAL nucleated immune cells have correlated fairly well with certain forms of ILD such as sarcoidosis, their suboptimal specificity has limited its utility as a diagnostic tool in ILD.

Over the past decade, HRCT of the chest has greatly improved the clinician's ability to arrive at a likely diagnosis in the majority of cases of ILD. Nonetheless, tissue sampling is often performed via bronchoscopic transbronchial lung biopsy (TBLB) or surgical lung biopsy (SLB) to confirm or secure an accurate and confident ILD diagnosis. Transbronchial lung biopsy, while frequently diagnostic in certain forms of ILD such as granulomatous lung disease also has its limitations; tissue sampling may be inadequate and non-diagnostic, and the risk of a complication, while relatively low, is increased compared to bronchoscopy with BAL only (17, 18). Surgical lung biopsy allows sampling of lung tissue that is usually diagnostic, but the risk of complications, including death, is not negligible (19, 20).

Bronchoalveolar lavage (BAL) is a safe, easily performed and well-tolerated procedure. Importantly, BAL has rarely been reported to precipitate acute exacerbations or progression of ILD and can be safely performed by experienced bronchoscopists. BAL typically reveals variations in cellular (nucleated immune cells) and acellular components in patients with ILD that differ from normal control subjects, and many clinicians currently use BAL cellular analysis as a guide for narrowing the differential diagnosis of ILD. However, the role of BAL in routine clinical management has been a subject of ongoing debate and controversy. Although BAL should not be considered a stand-alone diagnostic test, when BAL cellular analysis is applied according to standardized protocols and considered in the appropriate clinical setting and context of other information obtained from ancillary diagnostic testing such as HRCT, it may allow the clinician to greatly narrow the broad differential diagnoses and avoid more invasive diagnostic procedures that entail some risk to the patient.

2. DEFINITIONS

Interstitial lung diseases (ILD) are defined as bilateral infiltrative diseases that are characterized by abnormalities in both lungs on chest radiographic imaging studies. These disorders typically manifest in immunocompetent adults with exertional dyspnea, abnormal pulmonary physiology and gas transfer without clinical suspicion of infection or malignancy and display an accumulation of inflammatory and immune effector cells and abnormal extracellular matrix in the distal airways and alveolar walls including the interstitium. The ILDs usually evolve over a period of months to years and include disorders of known cause such as the pneumoconioses and hypersensitivity pneumonitis (HP) or of unknown cause such as sarcoidosis, rheumatologic lung disease, and idiopathic interstitial pneumonia (IIP).

Idiopathic interstitial pneumonia (IIP) is a term that encompasses a heterogeneous group of ILD of unknown etiology that include idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia

(NSIP), desquamative interstitial pneumonia (DIP), respiratory bronchiolitis with interstitial lung disease (RBILD), acute interstitial pneumonia (AIP), cryptogenic organizing pneumonia (COP), and lymphoid interstitial pneumonia (LIP). In the appropriate clinical setting, IPF is a distinctive clinical entity characterized by the pattern of usual interstitial pneumonia (UIP) in HRCT and/or surgical lung biopsy (16). In the absence of surgical lung biopsy evidence of UIP, it has been suggested that a clinical diagnosis of IPF can be made by fulfilling all of the four major and three of the four minor criteria as set forth by an International Consensus Statement (21). Certain IIP pathologies may coexist in the same patient when multiple regions of the lung are biopsied; especially UIP and NSIP (22), and these patterns also occur in other ILDs and thus are not disease-specific.

Although most forms of ILD are chronic, some rare forms of ILD can present acutely. The committee defined acute ILD as a respiratory illness ≤4 weeks duration that is characterized by shortness of breath, hypoxemia, and diffuse infiltrates in a previously healthy patient who has no known lung disease and no obvious risk factors for ARDS such as sepsis, trauma, drowning, or overt aspiration. These include acute interstitial pneumonia (AIP), acute eosinophilic pneumonia (AEP), acute hypersensitivity pneumonitis (AHP), diffuse alveolar hemorrhage (DAH), cryptogenic organizing pneumonia (COP), drug reactions, and acute exacerbations of IPF or other forms of ILD. BAL can be performed safely under appropriate conditions and facilitate an expedient diagnosis of a few specific forms of acute ILD. Bronchoscopy with BAL has been safely performed in acutely ill patients with acute respiratory distress syndrome (ARDS) and is contraindicated (relative) if the patient has respiratory –cardio-pulmonary instability or a severe hemorrhagic diathesis**.**

3. TECHNICAL CONSIDERATIONS AND PROCEDURE DETAILS FOR BAL CELLULAR ANALYSIS

Reports in the literature that pertain to the performance of BAL document the considerable variability in technical aspects of performing BAL.This may in part be the reason of apparent reluctancy among pulmonologists worldwide to subject patients for BAL in the diagnostic evaluation of patients with ILD. This section reviews the features of obtaining and processing BAL that appear to affect the results of BAL cellular abnormalities and provides guidelines with the anticipation of standardizing the performance of the BAL procedure and analyses of BAL fluid (BALF) retrieved during BAL procedure plus subsequent reporting and interpretation of the results.

An underlying disease process or smoking may affect BAL bronchoscopic technique or analysis in several ways. Airway obstruction leads to changes in BAL fluid return. This is due to the mechanical problem of aspirating fluid through narrowed airways that collapse when negative pressure is applied to

facilitate fluid retrieval. In patients with chronic obstructive lung disease, the airway collapse is variable; the greater the negative pressure used to aspirate the fluid, significant airway collapse is more likely to occur and adversely influence the BALF return. In this regard, FEV-1/FVC ratio has been correlated with the yield of BALF return; the lower the ratio, the lower the proportion of BAL return (23, 24) Airway obstruction in asthmatics can be induced by the procedure itself (25, 26). A major concern in asthma is the risk of the procedure, and recommendations for performing BAL in asthmatics have been made (26, 27). Bronchoalveolar lavage can be performed in acute respiratory distress syndrome (ARDS) patients (28). However the procedure can lead to significant hypoxia (29), and modifications of the procedure, such as increasing inspired oxygen concentration, may be necessary to maintain oxygen saturation of >90% during the procedure (28). Nonetheless, BAL has been performed in various respiratory disorders; however, it is unknown if specific ILD and/or the severity of functional impairment of specific forms of ILD influence the BALF return during the BAL.

3.1. BAL Technique

3.1.1. Position of the patient:

Gravity may influence the lavage process. Because suction pressure affects airways, fluid flowing back toward the bronchoscope (when aliquot instillation is completed and aspiration is initiated) is relied upon. The position of the patient can affect the lavage, and gravity may impede lavage return from more gravity-dependent lung regions. There are no studies comparing the effects of upright, semi-recumbent, or supine positioning on BAL. Positioning should be determined according to the clinical situation affecting the patient and should be noted in the procedure report.

3.1.2. Area (bronchopulmonary segment) that is lavaged in the lung:

Another important consideration is the choice of the most appropriate area or areas of the lungs for lavage. When BAL was first introduced into clinical investigation in the 1970s, a main focus of research initially was to investigate immune and inflammatory mechanisms in IPF because of the especially poor prognosis and lack of effective therapies. Because infiltrates in IPF tend to be more prominent in the lung bases on thoracic imaging, some investigators have performed the BAL from the right lower lobe (30). Others, however, have preferred to sample the right middle lobe due to its easier access and higher fluid recoveries versus lower lobe lavages (9). Obtaining BAL from the lingula was generally avoided because biopsies from the tip of the lingula were often unsuitable for diagnostic purposes. A large number of reports in the 1970s and 1980s of BAL studies in the main groups of ILDs soon showed that differential BAL cell count findings were very similar from different centres using the

right middle lobe or the right lower lobe as their standard lavage site. There was discussion about whether an upper lobe site should be used for diseases with more prominent upper zone shadowing, but this approach was criticized at the time because of lack of a standardized procedure. This omission from earlier recommendations for performing BAL relates to the fact that such recommendations were made prior to the subsequent re-classification of the IIPs (16), and it is unknown if this necessitates a new approach to the choice of the site for lavage.

Significant differences have been reported among different lobes for both sarcoidosis (31) and idiopathic pulmonary fibrosis (32). The use of HRCT in recent years and the importance of HRCT patterns in the new classification of IIPs, raises the possibility that differences in HRCT patterns in the lungs of individual patients may prove useful to identify target areas for BAL that may more accurately reflect the inflammatory cell profile that is associated with a specific ILD. For example, areas of ground-glass opacity may reflect areas of more active inflammation and represent good target areas for lavage. Further research is needed to better clarify whether different HRCT patterns are associated with different BAL cell profiles in the same lung and whether this would lead to major differences in the diagnostic interpretation compared with the current approach to use a standardized lavage site. While this document is intended for management of non-infectious ILDs , there is evidence that lavage site variation is an important consideration in evaluation of respiratory infections; earlier reports have supported the BALF to be retrieved from the site of maximal involvement .In opportunistic lung infections (with diffuse pulmonary infiltrates), it is clear that when BAL was performed in patients with *P. carinii* pneumonia, lavage in the upper lobes had a higher yield of organisms than the traditional right middle lobe or lingual, and the yield was the same from the upper lobe and the more traditional right middle lobe or lingula (33).

While it is unclear whether BAL from selective sites (directed by HRCT abnormalities) increases the diagnostic yield of BAL cellular analyses in the evaluation of patients with ILD, the additional concern of lack of standardization for BAL from different bronchopulmonary segments warrants additional well designed studies to investigate this further. While BAL can be performed in any area of the lung in ILD, the area or areas of the lung lavaged should be specified in the BAL request form (see below).

3.1.3. Number of areas in the lung to be lavaged:

Generally, only one geographic area (lung segment) is lavaged when evaluating ILD. If multiple areas are lavaged, the specific lung regions that are lavaged should be noted. Some investigators have used multiple sites to evaluate ILD and pooled all the areas lavaged to get a higher number of cells and a more generalized representation of lung airspaces (32). However, occasionally a comparison of returns between lavage sites or correlation of lavage site and HRCT appearance is desired, and in this context the

returns will need to be kept separate. Pooling multiple lavages from different lavage sites also has the disadvantage of introducing greater variability into the procedure and compromising the efforts to improve BAL standardization, which is required for many research purposes. Using single aliquots to lavage different areas has the further disadvantage that such 'first aliquots' have been shown to contain a greater proportion of bronchial components than subsequent aliquots from the same site. If for any reason a non-standard site is used, it is recommended that the reason for this be stated and that the number of areas lavaged and whether lavage aspirates from different lung regions are pooled be noted and commented in the BAL request and report forms (see below).

3.1.4. Suction pressure during the procedure:

Airways collapse when the suction pressure is too high, and this should be apparent to the bronchoscopist if it occurs during the BAL procedure The majority of physicians in one survey used low pressure wall suction (<60 mm Hg). Some prefer negative pressure generated by a hand-held syringe, adjusting pressure based on visualization of the airways. Regardless of specific technique, visual examination of the airway allows the bronchoscopist to monitor for airway collapse during the aspiration process. It is suggested that negative suction pressure remain at least below 100 mm Hg and adjusted to avoid visible airway collapse.

3.1.5. Total volume of normal saline instilled /number of aliquots/ volume of saline instilled in each aliquot for BAL:

The volume of normal saline instilled during the BAL affects the BALF return and the results. This has been shown for both the cellular and protein contents of the BAL sample (34-36). The major changes seem to occur during the first 100 ml of the process. The fluid aspirated after 60 ml instilled is significantly different than obtained after 120 ml has been instilled. After two aliquots of 60 ml have been instilled and aspirated, the cells and protein returns appear to become consistent. In one study of ILD and control subjects, differences were not appreciated until at least 120 ml of fluid were instilled (37). This effect of volume on ILDs has been noted by others (38). Cellular analyses from BAL specimens collected from sequential BAL returns separately and analyzed separately from individual aliquots indicate that the BAL sample collected from the first aliquot tends to represent cells (epithelial cells) and protein (lactoferrin) from bronchiolar surfaces (23, 39, 40). It is suggested that at least a total volume of 100 ml of saline should be instilled to adequately sample cells and proteins on bronchoalveolar epithelial surfaces.

Although some experts have suggested a specific number of aliquots be used for BAL (11, 14), there is considerable variability in the number and volume of aliquots used by pulmonologists within and outside the United States. The choice of aliquot size can easily be standardized as it is apparent that this

is often determined merely by the size of the syringes available to the bronchoscopist in the local facility (usually either 20 or 60 ml). As noted above, the volume of the lavage is crucial for BAL sampling. In this regard 6 x 20 ml aliquots could provide similar returns to 2 x 60 ml lavages or other subdivisions of 120 ml totals. Larger volumes instilled into a broncho-alveolar units (spread over a larger 'alveolar' relative to 'bronchial' area than smaller instilled volumes) and use of larger volumes in humans has shown better alveolar sampling, with an increased proportion of cells and solutes found in the alveolar spaces (40). Larger total instilled volumes of a minimum of 100 ml and a recommended standard 240 ml using standard 4 x 60 ml aliquots have therefore been recommended by the European Respiratory Society (ERS) to improve standardization when more efficient alveolar sampling and accurate quantitative measurements are required (14, 40). This approach to standardization is important for investigators using the BAL samples for research as well as routine clinical purposes. However, when BAL is being performed in centers for routine clinical purposes as an aid to diagnosis of specific ILD, the BAL differential counts are not influenced by dilution and there is no evidence that they are influenced substantially by the variability in total introduction volumes or aliquot numbers and size used by different workers. A potential disadvantage of smaller instilled volumes (e.g.20 ml) is that they can recover a relatively higher proportion of 'bronchial components', thus this approach may be more useful for those studying airways diseases rather than ILDs.

An ERS Task Force has produced a comprehensive report on the many causes of variability in BAL, including variable dilution, and recommends better standardization of BAL procedure, including volume and number of aliquots instilled, to reduce this variability (14). Quantitative measurements of cell counts per ml and acellular components per ml are profoundly influenced by variable dilution, which may explain why their clinical utility remains poorly defined compared to differential cell counts that are not influenced by dilution. To facilitate future clinical developments, recommendations for standardization have been defined, which include the use of a standard total instilled volume of 200-240 ml introduced in 4 aliquots (14, 40).

3.1.6. Handling the first aliquot separately:

The aspirated fluid after the first 20 ml has been reported to be distinctly different from additional aliquots (39). Therefore, some investigators have proposed that the first aliquot be handled in a different manner (39) or even discarded if one is studying diffuse ILDs. Alternatively, the first aliquot could be sent to microbiology laboratory for stains and cultures of infectious pathogen/s. However, the numbers of cells in the later aliquots are much larger than those retrieved after the earlier volumes of lavage (34, 37). In one study, it was determined that combining aliquots of fluid retrieved after the first 60 ml aliquot did not significantly change the overall lavage results (34). The initial fluid can be handled separately,

especially if diseases that originate in the larger airways are being studied. However, this committee felt that it is acceptable to pool all the aspirated fluid for routine cellular analyses, but the technique that is used should be specified in the BAL request form (see below).

3.1.7. Variability of lavage return:

The percent of instilled saline that is retrieved is also variable. If less than 5% of the volume instilled is retrieved, alveolar sampling is probably inadequate. In one study of nonbronchoscopic lavage, it was shown that patients with a greater than 5% return of instilled fluid have a higher diagnostic yield (41). This committee recommends that at least 30% of the instilled volume be aspirated to be considered an optimal alveolar sample, although smaller volumes may still retrieve diagnostic material. Small volume returns (especially those <10% of the total instilled volume) should not be considered true BAL samples.

For safety reasons, the lavage should be stopped if the volume instilled and retained within the lung becomes too large (e.g. persistent recovery of <5% per aliquot). Because this decision will depend on the volume of the instilled aliquots, the bronchoscopist should be aware of the total instilled and aspirated volumes after each aliquot is instilled. If the difference between instilled and aspirated volumes becomes considerable (e.g. greater than 100 ml), instilling additional aliquots in the area of this lavage is not recommended, but a second lavage in a separate area could be undertaken if the patient is tolerating the procedure.

3.1.8. Safety aspects of BAL procedure:

Bronchoalveolar lavage procedure has a well-proven safety record in both human research and in clinical applications that range from evaluating patients with asthma and chronic obstruc tive pulmonary disease to patients with acute respiratory distress syndrome and ventilator-associated pneumonia. The two most common complications linked to BAL procedure are fever and hypoxemia (42, 43), but these BAL-associated adverse events are almost always self-limited and have been linked, to some degree, to the use of larger total lavage volumes (44) Nonetheless, the BAL procedure can precipitate acute phase responses with an increase in circulating pro-inflammatory cytokines, an increase in peripheral blood neutrophils, an influx and transient sequestration of neutrophils into the lung, and alterations in iron homeostasis (45-47). Despite the concern that BAL procedure can induce procedure associated acute inflammatory responses, fever, and hypoxemia, it has a well established safety record when used to evaluate patients with VAP (48), patients with ARDS (28, 49), lung transplant recipients (50), immunocompromised patients with acute infiltrates (51), patients with impaired coagulation (52) , or patients with evolving respiratory failure (53). Although pneumothorax and significant bleeding have

been reported (28, 52, 54, 55), such complications are exceedingly rare when bronchoscopy and BAL are performed without an accompanying biopsy. BAL has been implicated to precipitate an acute respiratory decline immediately following the procedure; two reports of acute exacerbation of IPF have been associated with to the BAL procedure in the clinical setting (56, 57).

Although BAL appears to be quite safe, adequate safety protocols should be utilized and be in place whenever bronchoscopy and BAL are performed to minimize the possibility of complications; the bronchoscopist should be prepared to identify and treat potential complications. A detailed history of complications associated with other procedures or trauma should be obtained, and the use of medications (e.g. clopidrogel, aspirin, coumadin, heparin) and/or medical conditions (e.g. uremia) that increase bleeding risk should be noted in keeping with good clinical practice. Coagulation studies (international normalized ratio, partial thromboplastin time, platelet count) may be performed to determine whether a significant coagulopathy is present that may increase the risk of bleeding complications. Intravenous access should be established in all patients who undergo conscious sedation, and personnel involved in the procedure should be adequately trained in conscious sedation protocols. Heart rate and oxyhemoglobin saturation should be monitored continuously throughout the procedure, and patients should not have an unstable medical condition such as recent myocardial infarction or unstable angina, Although adequate amounts of topical lidocaine (e.g. multiple 1-2 ml aliquots of 1% lidocaine) should be given to optimize patient comfort during the procedure, the total lidocaine dose should be kept to the minimum amount needed to achieve patient comfort and control cough, and contamination of BAL fluid with excessive lidocaine should be avoided to minimize the potentials of poor cell viability at the time of cellular analyses. Adherence to a stringent safety protocol has been shown to diminish and minimize the risk of complications in lung transplant recipients subjected to bronchoscopy (50). Similarly, adequate safety regimens should be in place and followed for every bronchoscopy procedure.

3.1.9. Summary of specific suggestions for the bronchoscopist performing the BAL procedure

►Routine clinical evaluation prior to performing BAL should include enquiry and appropriate testing for bleeding tendencies.

► For patients with suspected ILD in whom it has been decided that a BAL can be tolerated and will be performed, the BAL target site should be chosen on the basis of a HRCT performed prior to the procedure , rather than choosing a traditional BAL site.

►A safety protocol, standard for fiberoptic bronchoscopy procedure should be followed to minimize the likelihood of procedure-related complications.

►BAL should be performed with a fiberoptic bronchoscope in a wedge position in the selected bronchopulmonary segment.

►The total instilled volume should be no less than 100 ml and should not exceed 300 ml, and 3 to 5 aliquots should be used.

►Total unretrieved volume of instilled BAL fluid should not be excessive (e.g. greater than 100 ml from one segment).

►Negative suction pressure should not exceed 100 mm Hg and should be adjusted to avoid visible airway collapse.

► For optimal sampling of distal airspaces the total volume (pooled aliquots) retrieved should be ≥30% of the total instilled volume. A total volume of retrieved fluid less than 30% may provide a misleading cell differential, especially if total retrieved volume is less than 10% of total instilled volume. If <5% of each instilled aliquot volume is recovered during the procedure due to retention of most of the fluid in the lavaged segment, the procedure should be aborted to avoid increased risk of tissue disruption and/or inflammatory mediator release due to overdistention of the lavaged segment..

► If <5% of each instilled aliquot volume is recovered during the procedure due to retention of most of the fluid in the lung, the procedure should be aborted to avoid increased risk to the patient.

►A minimal volume of 5 ml (optimal volume = 10-20 ml) of a pooled BAL sample is recommended for BAL cellular analysis (the rest of the sample should be used for microbiology, virology, and malignant cell cytology laboratory testing as clinically indicated).

► It is acceptable to pool all aliquots of the aspirated fluid for routine analyses (including the first retrieved aliquot), but the technique used should be specified in the BAL request form (see below).

3.2. Standardized Request Form for BAL cellular analyses

The committee recommends that a request form be completed and sent to the laboratory with the BAL fluid specimen for BAL cellular analysis (see Request form online appendix that should be utilized). This is useful for appropriate interpretation (see interpretation, below) and should include:

- Time and date of the procedure
- Smoking history
- Age and gender
- Pertinent clinical features (clinical suspicion of underlying diagnosis)
- Specific area(s) lavaged

- Total instilled volume
- Gross appearance of BAL fluid
- Patient position during the procedure
- Checkboxes for routine analysis and specific additional testing

3.3. Suggestions for specimen handling, transport to the laboratory and processing of the retrieved BAL fluid (BALF) specimen

3.3.1. Specific suggestions for handling and transport of BALF specimen to the laboratory

►BALF specimens should be collected in containers /aliquots that do not promote cell adherence to container surfaces (e.g. silicone-coated glass or polypropylene or other plastics that are designed for suspension tissue culture).

►BALF specimens can be transported 'fresh' at room temperature if the analytical laboratory is located within the same facility and there is minimal delay between BAL fluid retrieval and delivery to the laboratory to increase the likelihood of preservation of cell viability at the time of analyses trained and designated laboratory personnel.

► For anticipated delay in delivery to the laboratory beyond 30-60 minutes following retrieval of the fresh BALF, specimens should be transported at 4°C (e.g. on ice) and delivered within 1 hour.

►BALF specimens retrieved should not be frozen and/or not transported with dry ice for BAL cellular analyses.

►Transport periods greater than 1 hour in the original lavage saline are not recommended; the cells should be transferred to an appropriate tissue culture medium to preserve them for longer periods (e.g. MEM+25 mM HEPES or RPMI 1640+25mM HEPES).

►Rapid processing by designated laboratory technician trained in handling of BALF samples is ideal (i.e immediately after the specimen is delivered to the laboratory) and will provide optimal results; if significant delay in processing is unavoidable, viability of BAL cells are better preserved in nutrient-supplemented media (e.g. MEM+25mM HEPES or RPMI 1640+25mM HEPES) for up to 24 hrs. In selected facilities, such nutrient-supplemented media may be added to the container of the BALF specimen collected shortly after the procedure is completed; this will require appropriate coordination and elective arrangements between designated laboratory staff and clinical personnel handling the transport of the BALF specimen.

3.3.2. Specific suggestions for handling and processing the BALF specimen by laboratory technicians

3.3.2.a. Handling of aspirated BAL fluid:

The aspirated fluid can contain mucus material as well as the fluid itself. The handling of this fluid can affect the results of the BAL cellular analyses. Filtering BAL fluid though gauze prior to analysis has been used by some groups to remove the mucus. However, the cells in the BAL fluid may variably adhere to the gauze and adherent mucus. This may affect cell retrieval and the cellular profile of the BAL sample (30). The committee recommends that filtering BAL fluid with gauze should be avoided unless excessive amounts of mucus are present overtly. If filtration is performed, it should be specified in the report form (see below). Macrophages retrieved by BAL can be quite adherent to glass or similar surfaces, and macrophage adherence may be altered in various conditions, including cigarette smoking (58). Storage of cells at the time of the lavage should be in containers (e.g. polypropylene, other plastics designed for suspension tissue culture or silicone-coated glass) that do not promote cell adherence.

3.3.2.b. Centrifugation for cell concentration:

Techniques used to concentrate proteins and cells may lead to loss of cells (59-61). A centrifugation speed of 250-300g for 10 minutes is recommended and should yield a cell pellet that can be readily resuspended with gentle agitation. Cell counts can be made on unconcentrated samples. If concentration is performed prior to cell counting, the method should be specified.

3.3.2.c. Summary of specific suggestions for the laboratory technicians:

►BAL cellular analysis should be performed within one hour if in nutrient-poor media (e.g. saline).

►If BAL cellular analysis cannot be performed within 1 hour, cells can be transferred to tissue culture medium (e.g. MEM+25mM HEPES or RPMI 1640+25mM HEPES) for processing that should be performed within 2-3 hours for optimal results.

►BAL fluid and BAL-derived cell suspensions should be processed in labware that do not promote cell adherence to container surfaces (e.g. silicone-coated glass or polypropylene or other plastics - designed for suspension tissue culture).

►Mucus can be dissolved (e.g. dithiothreitol) if necessary.

►Specimens with gross mucus can be strained through 4x4 loose gauze if necessary.

►The BAL fluid should be centrifuged at an appropriate speed (e.g. 250-300g for 10 minutes) to maintain cellular integrity and allow uniform resuspension.

►Cell suspensions should be refrigerated at 4°C and resuspended in tissue culture medium (eg. MEM+25mM HEPES) if not analyzed immediately.

►Nucleated cell counts should be obtained via a hemocytometer.

►Cell viability should be determined (Trypan blue exclusion) and reported.

►Specimens obtained more than 24 hrs prior to analysis are not suitable for analysis.

►Differential cell counts should be performed via cytocentrifugation with staining (Wright-Giemsa or May-Grunwald-Giemsa) and enumeration of at least 400 cells.

►A Diff-Quick stain is not recommended, as mast cells will not be identified.

3.3.3.Specific suggestions for additional analysis based upon clinical setting or initial BAL cell analyses

► For patients with suspected ILD in whom BAL is performed, we suggest that lymphocyte subset analysis not be a routine component of BAL cellular analysis.

►Flow cytometry or immunocytochemistry for lymphocyte markers (B or T lymphocytes, Langerhans cells) should be performed as clinically indicated or when suspected from initial BAL cellular findings and the laboratory be alerted to the possibility of this additional analysis.

►Periodic Acid Schiff staining should be performed (alveolar proteinosis sediment) if Primary alveolar proteinosis is suspected clinically or based on initial BAL appearances.

►Oil red O staining should be performed if aspiration is suspected.

►Hemosiderin stain should be performed if hemorrhage is suspected and/or if initial BAL appearances raise suspicion of haemosiderin-laden macrophages.

►A separate report by a hematopathologist or cytopathologist is mandated for interpretation of isolated cells that are suspicious for malignancy.

►Inorganic dust bodies or particles within macrophages can be characterized by energy dispersive electron microprobe analysis if BAL cellular and microscopic appearances indicate their presence.

3.3.4. The storage of residual BAL fluid after cellular analyses:

Proper storage of BAL samples is crucial for the subsequent measurement of certain markers. Cells stored at 4° C can be analyzed up to 24 hours after the procedure without significant changes in the total cell count and differentials (62) However, neutrophil apoptosis is known to have commenced by 9 hours and such cells can be rapidly phagocytosed by macrophages(63, 64).

Certain proteins may be temperature sensitive and require that BAL aliquots be stored at -80° C until analysis is performed. The handling of many of the proteins measured in the lung lining fluid are

detailed elsewhere (14). The committee recommends that the conditions under which BAL samples are stored be specified in the BAL report form (see below).

4. BALF AND CELLULAR ANALYSIS FOR DIAGNOSTIC EVALUATION IN ILD

4.1. Gross analysis:

The appearance of BAL fluids can give important clues concerning the cause of ILD. The presence of blood with a progressive increase in the intensity of bloody discoloration in the retrieved BAL fluid with sequential aliquots during the BAL procedure strongly suggests a pulmonary/alveolar hemorrhage syndrome (65). If BAL fluid is grossly cloudy /'milky'appearance with a light brown/beige color appearance and contains whitish flocculent material that settles to the bottom of a container when left to stand, the diagnosis of pulmonary alveolar proteinosis is suggested (66). If cloudy material is present but low-speed centrifugation is required to clear the fluid, the diagnosis of microlithiasisis suggested (67). If oily material layers out on the top of the BAL fluid, the presence of lipid material and lipoid pneumonia is suggested. The presence of a black cell pellet after centrifugation suggests that the subject is a likely smoker or has been exposed to significant amounts of carbonaceous material in inhaled air.

4.2. Technical details for performing cell count and BAL cell differential counts in retrieved BALF:

Identification and enumeration of cellular elements is an important component of the evaluation of BAL fluid (11-13), although relatively few published studies regarding the technical aspects of specimen preparation exist. The single most critical prerequisite for successful cellular analysis is maintenance of cell viability following collection. Saline solutions, as are used for washing of the alveolar space when a BAL is performed, have low pH and are nutrient poor and incapable of sustaining cells for more than a limited time, i.e. less than one hour. If the sample will not be examined immediately, the collection and replacement of the saline cell-free supernatant with tissue culture medium such as MEM with 25 mM HEPES buffer or RPMI 1640 with HEPES buffer prior to transport provides much improved sample stability, which may not be provided by merely keeping the specimen at 4°C. Centrifugation of the sample, removal of the supernatant, and resuspension in medium is the preferred method of supplementation. Refrigeration of the sample will also retard cellular degradation and is advisable if the delay in specimen analysis is greater than 8 hours (e.g. overnight). Samples greater than 24 hours old are generally not suitable for analysis, even with supplementation. It is important that the fluid volume be recorded in the BAL request/report form prior to supplementation with any medium.

Following receipt in the laboratory, the cellular elements may need to be separated from the mucus contained in the fluid, if excessive mucus is overtly present on gross inspection of the fluid. Although the commonly used method to remove mucus is to strain the sample through a single layer of sterile gauze, this is not recommended by the committee because some loss of cell types may occur. For the occasional samples with very excessive mucus, treatment of the sedimented cell pellet with dithiothreitol after centrifugation to remove and collect the fluid supernatant is recommended as an alternative method to dissolve mucus without loss of other material. The cellular elements should then be washed with MEM+25mM HEPES of a balanced electrolyte solution such as Hank's Balanced Salt Solution (HBSS) or phosphate buffered saline (PBS) which lack calcium and magnesium followed by centrifugation at 250-300g for 5 minutes. Following careful aspiration of the supernatant, it is suggested that the cells can be resuspended in a known volume of tissue culture media by gentle agitation. Vigorous vortexing should be avoided.

The committee suggests that a minimum volume of 5 ml of BAL fluid should be used for cell analysis; a larger amount (10-20 ml), however, provides a more optimal specimen. A nucleated cell count should then be obtained manually in duplicate using a hemocytometer, either in the well-mixed original fluid or after the first wash with the caveat that cells are progressively lost with repeated washing. The presence of increased numbers of red blood cells should be noted, but need not be routinely enumerated. Automated cell counters are unlikely to provide accurate cell counts, as they are unable to correctly enumerate epithelial cells and other non-hematopoietic cellular elements. Stabilized cellular control materials are now commercially available for the quality control of body fluid cell counts and these should be used for the quality control of BAL cell counts. It is important to use pipettes and tubes for specimen handling that do not promote cell adherence (siliconized glass or polypropylene), as plain glass surfaces can cause macrophages to become activated and adherent, resulting in their underestimation. The viability of the nucleated cells may be estimated by using Trypan Blue in a counting fluid (i.e. Trypan blue exclusion), especially if specimens have not been freshly obtained and analyzed. The absolute number of nucleated cells, cellular concentration and percent viability in the original specimen can then be calculated, assuming the original and post-wash volumes are known. A viability of greater than 90% is considered acceptable, and less than 80% suggests the possibility of significant specimen compromise and thus be considered suboptimal for cellular analyses and may underestimate the true cellular components of the ILD within the alveolar walls.

For the identification and enumeration of cellular subpopulations, the committee suggests that six cytocentrifuge preparations should be made. Although, it is recognized that the cytocentrifuge technique may underestimate lymphocytes, particularly if their numbers are high (61, 68), cytocentrifugation is a technique commonly available in clinical laboratories, is rapid, and provides improved cellullar detail

compared with other methods. Two of the air-dried preparations should be stained with either Wright-Giemsa or May-Grunwald-Giemsa and subsequently protected by a coverslip. A Diff-Quick stain is suboptimal, as mast cells are poorly identified. Under low power magnification (200x), the presence of the morphologic abnormalities may be identified, e.g. dust particles, microorganisms, clusters of malignant cells, or acellular aggregates suggestive of alveolar proteinosis. Under high power magnification (500x), a differential count of the nucleated BAL immune cells should be performed, to include: macrophages, lymphocytes, neutrophils, and eosinophils. To provide a statistically meaningful estimate for populations representing less than 10% of the total, 400 cells should be evaluated, as is currently recommended for differential WBC counts in peripheral blood (69). The cells evaluated should be equally divided between the two slides and a random search method used to minimize processing and distributional artifacts. For cytocentrifuge preparations, the speed of the centrifuge, the area of the slide, and the number of cells counted have all been shown to lead to differences in counts (70). The presence of bacteria or other organisms as well as malignant cells should also be noted. The other remaining unstained slides should be retained for potential use for more specialized stains as indicated by clinical history or morphologic findings (e.g. iron stain for hemosiderin-laden macrophages, PAS for alveolar proteinosis, Oil Red O for aspiration, silver stain for fungal infection, etc). Specimens that are considered to accurately reflect distal airspace inflammatory cell patterns should not contain more than 5% epithelial cells.

Specific cutoffs that define abnormal increases in BAL cell differential counts are not supported by strong evidence, but differential counts in non-smoking, clinically normal individuals have been stated to show lymphocytes ≤ 15%, neutrophils ≤3%, eosinophils ≤ 0.5%, mast cells ≤0.5%, and macrophages > 80%(11) . Other studies in which BAL was performed in large cohorts of patients have used slightly different cutoffs to define BAL eosinophilia (eosinophils > 2%, BAL neutrophilia (neutrophils > 4%), or BAL lymphocytosis (BAL lymphocytes >14%) (71). Additionally, percentages of neutrophils and lymphocytes tend to be somewhat higher for clinically normal, elderly individuals compared to younger adults (72, 73).

Our analysis of the data presented in various published reports of BAL in normal individuals with or without smoking as an additional variable and in reports of BAL performed in patients with lung disease suggest that reasonable thresholds for increases in BAL cell differential counts are >15% for lymphocytes, >3% for neutrophils, and >1% for eosinophils. Healthy smokers can have up to a 10 fold increase in the number of macrophages per ml of BAL fluid but no increase in lymphocytes compared with healthy non-smokers. This results in their having slightly, but significantly, higher differential percentage counts of macrophages (≥85%) and slightly lower percentages of lymphocytes compared with healthy non-smokers.

4.3. Infection screening:

Pulmonary infection may present as a diffuse infiltrative lung disease, or infection may complicate the course of ILD. Because fungal or mycobacterial infections can masquerade or coexist with various forms of ILD, BAL fluid should be examined for infectious agents as clinically indicated when used in the evaluation of diffuse infiltrative lung disease. For patients with possible granulomatous diseases such as sarcoidosis, the committee suggests that minimal screening of BAL should include testing to identify mycobacterial and fungal infection in addition to BAL cellular component analysis. Other testing should also be performed as clinically indicated.

4.4. Flow cytometry:

In some instances, it may be desirable to enumerate the percentage of T cells, T cell subsets, or expression of other antigens on specific cellular subsets. In particular, assessment of the CD4/CD8 ratio could be considered in the presence of ≥15% lymphocytes. However, the determination of T lymphocyte subsets on specimens that contain less than 15% lymphocytes is unlikely to yield useful information. These studies can be easily accomplished by standard flow cytometric techniques using the same cell preparation described above. In patients having known hematopoietic neoplasms, flow cytometry can provide evidence of pulmonary involvement by the identification and quantitation of abnormal blast or neoplastic lymphocyte populations with a high degree of sensitivity and specificity. For Langerhans cell histiocytosis, immunophenotying by either immunocytochemical or flow cytometric techniques can be important to support the diagnosis. Although flow cytometry may provide useful diagnostic clues in certain situations, the committee does not recommend analyses for T cell subsets by flow cytometry as a routine component for the cellular analysis of all BAL specimens.

4.5. Reporting of BAL Cellular Analysis

4.5.1. The effect of underlying disease processes or tobacco smoking on BAL cellular analysis:

Cigarette smoking can also have profound effects on the cell populations found in the BAL fluid (11). The greatest effect is an increase in the number of macrophages, which are often 10-fold greater in numbers than in nonsmokers. Neutrophils also appear in increased numbers. Because of this, it is essential to establish control ranges not only for healthy non-smokers but also for ex-smokers and current smokers for routine and research purposes. If a patient is a current cigarette smoker, changes in the BAL cellular profile that are attributed to a disease process may actually be due to inflammatory changes caused by the smoking habits of the patient. Because of the potential effects of cigarette smoking and

underlying lung disease on technical aspects of BAL and WBC total cell counts and profiles, the underlying disease of the patient and cigarette smoking history should be included in the BAL request form for appropriate interpretation of the BAL cellular analyses.

4.5.2. The reporting of cellular components of the BAL fluid:

The committee suggests that the results of the BAL cellular analysis be reported in a standardized manner; this report should include: patient demographic information, clinical information supporting the indication for performing the BAL, the initial specimen volume, color and turbidity, % viability for the nucleated cells, absolute total nucleated cell numbers, and immune cell differential percentages. The columnar ciliated epithelial cells should be reported as a percentage of the total nucleated cells, with less than 5% ciliated columnar epithelial cells (generally simply stated as 'epithelial cells') characteristic of adequate alveolar sampling. The presence of greater than 5% ciliated columnar epithelial cells suggests increased proximal airway sampling. Such samples should be considered suboptimal, and the interpretation qualified as potentially not being representative of the alveolar space. The differential cell counts should be reported as a percentage of total viable immune/inflammatory cells, excluding epithelial cells, and reference ranges should be included (15). The presence of other specific morphologic findings (e.g. malignant cells, lipoprotein bodies, etc.) should also be reported, and the results of any special stains should be described. If T cell subset analysis or other immunophenotypic studies are performed, the CD4/CD8 ratio or other findings should be reported. An interpretation of the quality of the study and significance of the laboratory findings in the patient's clinical context should also be provided, but requires that the appropriate clinical information be supplied in the request form for BAL cellular analyses (see below). The committee suggests that the report of the BAL cellular pattern should be accompanied by a formal interpretation by an expert in ILD or pathologist familiar with ILD.

4.5.3. The reporting of acellular components:

While the clinical significance of the acellular component of the BAL fluid is unknown, this has been the topic of a specific ERS report (14). One aspect of that report was an analysis of the limitations of the currently available markers of BAL dilution (40). Instilled fluid is mixed with the endogenous fluid in the alveoli (epithelial lining fluid) during the lavage process. The aspirated fluid contains a mixture of the instilled and epithelial lining fluid. Determining the percentage of epithelial lining fluid, and hence the concentration of these constituents in the lung lining fluid, has been estimated by using a dilution marker.

The most common endogenous marker has been urea (74). Measurements of urea in the peripheral blood and epithelial lining fluid are assumed to be the same. Knowing the concentration of urea in the blood and in the aspirated BAL fluid, one can calculate the dilution. This does provide a

relatively good marker of dilution in many conditions. However, the amount of urea in the lung rises during theBAL procedure and the dwell time during the bronchoscopy itself affects the measurements (75). Conditions, which increase epithelial permeability, such as ARDS and pneumonia, will also affect the results using this internal marker. Water has been shown to rapidly pass into the lung during the BAL procedure (76). Thus, it is unclear whether any internal marker can be reliably used to measure all the changes that occur during BAL (77).

The use of an external marker has also several limitations. While methylene blue was reported to be a relatively useful marker for some diseases (78), methylene blue is also taken up by the cells in the airway, reducing the concentration in the aspirated BAL fluid. To date, all external markers have been taken up to a varying degree by the biologically active cells, such as alveolar macrophages. One suggested method is to report the cells per ml of aspirated fluid (40). Using this correction method has allowed clinicians to quantitate the number of bacteria in the alveolar space and to therefore diagnose bacterial pneumonia (79, 80). Although this technique may fail to detect mild differences between groups, it should be hypothesized to detect changes that are one hundred fold or greater. Because all methods used to estimate the initial volume (prior to dilution with instilled normal saline and retrieval of the diluted specimen) of bronchoalveolar surface ("alveolar lining fluid") are problematic and may introduce considerable error, the committee recommends that total nucleated cell count can be reported as per ml of fluid aspirated. To improve the reliability of quantitative measurements of BAL acellular components, the committee acknowledges the ERS recommendations be followed using a standard total instilled volume of 200-240ml and a standard number of 4 aliquots (40)

The committee did not identify any benefit for analyzing and reporting acellular components of the BAL for routine clinical management of patients with ILD.

4.5.4. A formal standardized report form for BAL cellular analysis:

The committee suggests that the interpretation of the cellular analyses be reported in a formal report form.The report should include/note the following:

- Clinical suspicion of the specific differential diagnosis of the ILD in question
- Cigarette smoking history
- Radiographic HRCT pattern
- Drug use (especially corticosteroids and other immunosuppressants)

The above should be sought and provided in the form requesting cellular analyses

- Volume and gross appearance (color and turbidity) of uncentrifuged BAL fluid
- Percent viability of nucleated cells (if performed)

- Absolute total nucleated cell number per ml of retrieved, pooled BAL
- BAL immune cell differential percentages
- Percentage of epithelial cells that comprise total nucleated cells
- Other specific morphologic findings (e.g. plasma cells, malignant cells, lipoprotein bodies, unusual appearance such as foamy AM, presence of foreign bodies, birefringent material)
- Quantitative measurements of BAL cells or acellular components should be expressed as per ml of aspirated fluid

The committee suggests that all of the above essential clinical and cellular details should be integrated in a formal report (see on line appendix) interpretated by an expert in ILD and/or specialist with adequate familiarity with hematopoietic cellular morphology and ILD as well as technical aspects of cellular analyses of the BAL fluid that has been properly retrieved from the lung.

5. CLINICAL APPLICATION OF BAL CELLULAR FINDINGS TO SPECIFIC TYPES OF ILD

5.1 Idiopathic Interstitial Pneumonia

5.1.1. Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF), the most common of the idiopathic interstitial pneumonias (IIP) and the classic progressive fibrosing interstitial lung disease, has been studied extensively with BAL. Knowledge of IPF has increased significantly over the last decade. Various definitions pertaining to ILD have evolved, and the clinical manifestations of different forms of ILD have been recognized. Initially the term IPF (or cryptogenic fibrosing alveolitis [CFA]) was liberally used to describe essentially all of the progressive, fibrosing non-granulomatous lung diseases of unknown cause. As the definition evolved, it was used to describe the disorders we now call the IIP. We now know that clinicopathologically and prognostically distinct disorders exist within the group of IIP (16), and that they can be separated by the pathologic patterns seen on surgical lung biopsy. IPF is now recognized as a distinct clinical entity manifested in adults and charecterized by the presence of the usual interstitial pneumonia (UIP) pattern, on surgical lung biopsy and /or HRCT scan of chest (16). Because of this diagnostic evolution, and the evolved knowledge of IIP over the last decade, many studies performed before 2000 are difficult to interpret. Therefore this section focuses on those studies where subjects met the ATS/ERS criteria published in 2000 (21) for diagnosis by surgical lung biopsy or clinical, radiographic, physiologic, and bronchoscopic data. All responses to PubMed searches using BAL and IPF or CFA were reviewed to assure the use of a surgical lung biopsy of UIP or a confident clinical diagnosis utilizing current criteria.

The typical BAL cellular profile in IPF consists predominantly of macrophages together with moderately increased percentages of neutrophils and eosinophils while lymphocyte percentages are generally normal (15, 81). However, these findings are regularly seen in a wide variety of fibrosing lung diseases other than IPF and therefore nonspecific (82). Neutrophil counts greater than 5% and sometimes up to 30% are seen in up to 90% of patients. The numbers of neutrophils may be directly proportional to the extent of disease seen on HRCT (83). Eosinophil counts greater than 5% are seen in up to 60% of patients. Atypical BAL cellular profiles include eosinophil counts greater than 20% or lymphocyte counts greater than 15%. With atypical findings like these, differential diagnoses such as eosinophilic lung disease (84), NSIP, COP, infection, hypersensitivity pneumonitis, and sarcoidosis (16) should be considered.

The value of BAL cell counts and patterns as a prognostic tool for an individual patient is limited. While increases in BAL neutrophils or eosinophils have been associated with shortened survival, not all studies have confirmed this (85). The presence of increased numbers of lymphocytes has been associated

with greater responsiveness to corticosteroid therapy and an improved survival; however, most of these studies were performed prior to the exclusion of NSIP from the diagnosis of IPF. Nevertheless, a recent paper on IPF (defined according to currently accepted diagnostic criteria) demonstrated that an increased baseline BAL neutrophil percentage was an independent predictor of early mortality, whereas lymphocyte and eosinophil percentages were not (86). This study supported the results of earlier studies that used older criteria for the diagnosis of IPF. A recent study of a large cohort of patients who met consensus criteria for the diagnosis of IPF revealed significant BAL lymphocytosis (>30%) in 6 of 74 patients (87), and the diagnosis for these 6 patients was ultimately determined to be NSIP or HP rather than IPF.

Overall, the data published in the literature to date have significant limitations and the BAL techniques utilized in the studies are highly variable to make a recommendation for the use of BAL cellular analysis to provide prognostically useful information for an individual patient at the time of diagnosis. Additionally, there is assuredly no role for serial BAL for the sole purpose of assessing response to therapy or prognosis.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for IPF:

AS AN AID FOR THE DIAGNOSIS:

- The criteria for the diagnosis of IPF should be in accordance with current published guidelines for the clinical management of IPF
- BAL cellular profile alone cannot establish a definitive diagnosis of IPF
- Typical BAL cellular findings that do not suggest an alternative diagnosis may provide a more confident diagnosis of IPF when combined with a HRCT pattern that is suggestive of UIP (possible UIP pattern) plus supporting clinical criteria as described in current published guidelines for the clinical management of IPF
- Significant BAL lymphocytosis (> 30 %) suggests a diagnosis other than IPF

FOR PROGNOSIS

- Literature to date do not convincingly support the use of BAL cell profiles as prognostic indicators

5.1.2. Nonspecific Interstitial Pneumonia (NSIP)

In 1994, Katzenstein and Fiorelli reported that NSIP could be distinguished histopathologically from other forms of IIP (88). Several subsequent reports and reviews have confirmed the characterization of NSIP, and the clinico-radiological-pathological features of idiopathic NSIP (patients who have no rheumatologic disease such as scleroderma) have helped to discriminate IPF/UIP from other forms of IIP

(21, 89-93). NSIP is now recognized as an IIP with a relatively favorable prognosis compared to IPF. Recent studies have shown that a histological pattern of NSIP is rather common (93), and it can exhibit both interlobar and intralobar variability (22). Its histopathology is variable (cellular, mixed, or fibrotic), and clinical presentation ranges from subacute to chronic modes of onset. Although two studies have described an unfavorable prognosis in some NSIP patients (16, 93), IPF/UIP patients have poorer survival at 5 years after diagnosis compared to patients with NSIP, though this difference is considerably reduced at 10 years after detection (16, 93). Fibrotic changes in NSIP are temporally homogeneous (92), and fibroblastic foci with dense fibrosis are not prominently found on specimens obtained from surgical lung biopsy.

The BAL fluid cell profile in patients with cellular NSIP typically has an increased lymphocyte percentage with a reduced CD4/CD8 ratio. These characteristics are of limited diagnostic use in the absence of other findings, as both are common to a variety of disease processes such as infection, collagen vascular diseases, hypersensitivity pneumonitis, drug-induced reaction, and malignancy. When observed in association with clinico-radiological features compatible with NSIP, however, they may strongly suggest the presence of NSIP. Although BALF from patients with cellular NSIP usually exhibit an increase in the lymphocyte percentage with a reduced CD4/CD8 ratio (94), patients with fibrotic NSIP rarely show these features. This discrepancy limits the utility of the BAL fluid cell profile as a sole diagnostic criterion for differentiating NSIP from other forms of IIP. Among patients with fibrotic NSIP (Katzenstein's group 2 and 3), some show BALF CD8 T lymphocytosis, while others exhibit BALF fluid cell profiles similar to those observed in IPF/UIP patients (low lymphocyte percentage but frequent increases in neutrophils or eosinophils (90, 94) . This BAL cell profile is similar to that observed for NSIP associated with collagen vascular disease (90, 92, 94-96). Anecdotal findings have shown resolution of BAL fluid lymphocytosis with clinical improvement.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for NSIP:

AS AN AID FOR DIAGNOSIS:

- BAL cell profiles usually show an increase in lymphocyte percentage with cellular NSIP, and the CD4/CD8 ratio tends to be decreased if BAL lymphocyte subsets are characterized
- BAL lymphocytosis is usually not observed with fibrotic NSIP
- The finding of BAL lymphocytosis combined with diffuse ground glass opacities on HRCT supports a diagnosis of cellular NSIP, but other entities such as HP, COP, drug toxicity, or sarcoidosis are also associated with BAL lymphocytosis.

FOR PROGNOSIS: Unknown; no convincing reports

5.1.3. Cryptogenic Organizing Pneumonia (COP)

Cryptogenic organizing pneumonia is an idiopathic interstitial pneumonia of unknown cause (16) that has also been studied with BAL. Previously called idiopathic bronchiolitis obliterans with organizing pneumonia (BOOP), the disease is differentiated from organizing pneumonias that occur with inhalation injuries, radiation, connective tissue diseases, transplant rejection, various drugs and viral pneumonias (97); (98); (99).

Because the radiograph and clinical course are similar to many pulmonary diseases, diagnostic bronchoscopy is usually performed using BAL and biopsy to exclude fungal, mycobacterial, and malignant diseases. The BAL cellular profile in COP is mixed, consisting of lymphocytes, neutrophils, eosinophils, plasma cells, mast cells, and foamy macrophages in variable degrees. Although some studies have attempted to correlate the radiographic presentation with expected BAL profile (100), there is no consensus that any cell profile is diagnostic for COP. Some estimation of disease chronicity helps define whether the BAL is compatible with COP. Lymphocytes typically predominate in chronic cases. Fulminant COP typically has higher BAL neutrophil percentages (101). Eosinophil percentages are usually between 2% and 25% of cells. Higher percentages should prompt consideration of eosinophilic pneumonia. Because COP has a high proclivity for relapse after corticosteroids are stopped, BAL profiles have been compared between patients who relapse and those who continue to improve after corticosteroids; no differences have been found (102).

When compared to other interstitial lung diseases, BAL from COP has increased numbers of lymphocytes (103, 104). A mouse model of COP has been developed in CBA/J mice infected with 1 x 106 plaque-forming units of reovirus 1/L (105). These mice develop corticosteroid responsive follicular bronchiolitis and intraalveolar fibrosis similar to human COP. Depletion of either CD4 (+) or CD8(+) lymphocytes has been shown to limit fibrosis and development of the COP lesion (105). Although HP and sarcoidosis may also have an increased lymphocyte percentage, the clinical presentations and HRCT are usually different from COP, and an increase in BAL eosinophils is usually absent in sarcoidosis.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for COP:

AS AN AID FOR THE DIAGNOSIS:

- The cellular profile of excess BAL lymphocytes in the setting of peripheral alveolar infiltrates in which infectious and malignant disease has been excluded by bronchoscopy strongly suggests COP

FOR PROGNOSIS

▪ BAL cellular analysis is not helpful in determining the response to therapy, predicting relapse, or monitoring the course of disease

5.1.4. Eosinophilic Interstitial Lung Diseases

Eosinophilic lung diseases (ELD) are a heterogeneous group of disorders defined by prominent accumulations of eosinophils. These include drug-induced pulmonary eosinophilia, acute or chronic eosinophilic pneumonia, parasitic infections, hypereosinophilic syndrome, allergic angiitis and granulomatosis (Churg-Strauss syndrome), and allergic bronchopulmonary aspergillosis. Additionally, prominent eosinophil infiltration may occur with malignancy, pulmonary Langerhans cell histiocytosis (PLCH), other systemic vasculitides, or sarcoidosis. Peripheral blood and tissue eosinophilia have been noted in various inflammatory disorders: skin conditions (eczema, dermatitis, and generalized drug reactions), malignancies (Hodgkin's disease and lung cancer), chronic granulomatous disorders (tuberculosis, sarcoidosis), fungal diseases (coccidioidomycosis, aspergillosis), drug and chemical-related conditions, and idiopathic pulmonary infiltrates with eosinophilia syndromes. In adults, drug-induced eosinophilic pneumonia (drug-EP) constitutes a frequent cause of pulmonary infiltrate with or without eosinophilia and/or alveolar eosinophils.

The introduction of BAL in the diagnostic evaluation of ILD has expanded the list of ELD substantially by identifying disorders characterized by an increase in the number of eosinophils in BAL fluid but not necessarily in blood eosinophils. The diagnostic value of BAL eosinophilia may be particularly helpful if the level of blood eosinophils is normal. The diagnosis of pulmonary eosinophilia can be made if BALF shows 25% or more eosinophils on the cell differential count (106). Because ELD may be mistaken for other diseases, especially severe community-acquired pneumonia, the diagnosis may be missed or delayed (107). Because of the diversity of clinical conditions that cause pulmonary eosinophilia, the differential diagnostic process is often tedious and difficult. A working diagnosis can usually be reached by obtaining a complete clinical history (including occupations, hobbies, drug exposures, and recreational activities), a thorough physical examination, a chest radiograph, skin testing, and serologic studies for fungi and paragonimiasis. An increased number of eosinophils in BAL fluid can be seen in a variety of inflammatory disorders, including chronic granulomatous disord ers (e.g. tuberculosis or sarcoidosis) (108), fungal diseases (e.g. coccidiodomycosis or aspergillosis), extrinsic allergic alveolitis (EAA), hypersensitivity pneumonitis (HP) (109, 110), drug- and chemical-related conditions (111), idiopathic pulmonary fibrosis (IPF) (112, 113), lung involvement with collagen vascular disease (15, 106, 112, 114), and *P. carinii* pneumonia (115, 116). However, these conditions rarely include more than 10% eosinophils. Patients with a higher percentage of eosinophils most likely have

ELD. The clinical presentation of a chronic eosinophilic pneumonia (CEP) may mimic IPF, but CEP has a more favourable outcome.

Acute eosinophilic pneumonia (EP) is an acute febrile illness that can result in life-threatening respiratory failure, and the presence of eosinophils in BAL is often striking and can be of diagnostic value. The diagnosis of lung eosinophilia can usually be made quickly and safely with examination of BAL fluid (BAL fluid differential with ≥ 25% eosinophils) together with clinical information in most cases even when the patient is already critically ill. Moreover, lung biopsy is often not an option in patients who are acutely ill due to acute EP. Furthermore, the presence of BAL fluid findings consistent with diffuse alveolar damage (DAD) may strengthen the suspicion of acute EP (117). Intervention with corticosteroids results in rapid complete recovery without relapse. Chronic eosinophilic pneumonia (CEP) is an illness of unknown aetiology occurring predominantly in women (106, 118). BAL fluid analysis demonstrates high percentages of eosinophils in the acute stage of CEP with normalization of the BALF cell profile after treatment with corticosteroids. A typical clinical presentation, consisting of an increase of the percentage eosinophils in BAL fluid and a prompt response to corticosteroids, can be diagnostic of CEP in most cases and obviate the need for lung biopsy.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for ELD:

AS AN AID FOR THE DIAGNOSIS:

- ELD is usually associated with a prominent increase in BAL eosinophils
- The finding of a large increase in BAL eosinophil differential percentage (≥25%) combined with peripheral radiographic opacities or consolidation on CXR or HRCT strongly supports a diagnosis of AEP, CEP, or drug-induced ELD
- When combined with consistent clinical data, the finding of BAL eosinophilia provides strong support for a confident diagnosis of an ELD syndrome

FOR PROGNOSIS: unknown

5.1.5. Desquamative Interstitial Pneumonia and Respiratory Bronchiolitis with Interstitial Lung Disease

Respiratory bronchiolitis (RB), respiratory bronchiolitis-associated interstitial lung disease (RB-ILD), and desquamative interstitial pneumonia (DIP) have overlapping features and are all highly associated with smoking. RB-ILD has a histopathologic pattern indistinguishable from smoking-related RB, which is a relatively common finding on histopathologic examination of lungs from heavy smokers. RB-ILD is characterized by accumulation of pigmented macrophages and mild inflammatory changes

centering on respiratory bronchioles and neighbouring alveoli, mild alveolar septal thickening, and fibrosis (119-122). The diagnosis of RB-ILD is based upon clinical features (symptoms, clinical signs, pulmonary function changes and radiological or preferably HRCT abnormalities). Variability in the classification of patients in some previous studies as having RB versus RB-ILD poses problems in interpreting studies of RB-ILD. RB is generally differentiated from RB-ILD by a relative lack of symptoms and imaging studies (HRCT) that do not show diffuse parenchymal changes that are seen in RB-ILD. The histological features of DIP are similar to RB-ILD. Lung tissue is characterized by greatly increased numbers of tan-brown macrophages in alveolar spaces with mild alveolar septal thickening, with or without mild fibrosis, but bronchiolar inflammation is not prominent as in RB-ILD(123). In some studies DIP and RB-ILD have been considered distinct pathological conditions, but in others these entities are described as extremes of a spectrum of the same smoking-related process (81, 119-121, 123, 124).

There are relatively few BAL data in the literature for RB-ILD and DIP, probably due to the rarity of these diseases combined with the difficulty of studying patients with well-defined clinical features. In the ATS/ERS consensus on IIP (16) an increase in alveolar macrophages containing brown golden or black pigmented inclusions, indistinguishable from those found in normal smokers, is reported in RB-ILD. The absence of this finding should prompt the consideration of other diagnoses. A slight increase in neutrophils may also be observed. The BAL cellular pattern typically shows an increase in pigmented macrophages in DIP, but it has been associated with increases in neutrophils, eosinophils and lymphocytes as well. However, these descriptions of BAL findings have been based upon relatively little data for these forms of IIP (125-128). Since the ATS statement on IIP was published, comprehensive reviews on RB-ILD and DIP (119-122, 129, 130) and on the role of BAL in ILD (131-135) have appeared in the literature. A role for BAL in the diagnosis of RB-ILD is suggested with characteristic BAL findings consisting of a considerable increase in brown pigmented macrophages accompanied by a modest increase in neutrophils, but not eosinophils or lymphocytes (130). Clearly, there is an overlap between RB-ILD and DIP not only for BAL but also for histological, HRCT and clinical features (136). BAL cannot be considered diagnostic for DIP or RB-ILD, but BAL cellular analysis may aid in the differential diagnosis. The recent classification of RB-ILD and DIP opens new perspectives for future BAL data, improving characterization of patients with more accurate and precise criteria (16, 137).

The committee concludes the following regarding the clinical utility of BAL cellular analyses for DIP and RB-ILD

AS AN AID FOR THE DIAGNOSIS:

- Large numbers of pigmented macrophages in BAL fluid are consistent with a diagnosis of DIP or RB-ILD and may be accompanied by increased numbers of neutrophils and/or eosinophils

FOR PROGNOSIS: unknown

5.1.6. Acute Interstitial Pneumonia (AIP)

The currently recognized entity of AIP (16) corresponds to the majority of the cases of rapidly progressive, fibrotic pneumonitis that were first described by Hamman and Rich in 1935 (138). The initial reports by Hamman and Rich drew attention to ILD and pulmonary fibrosis, and the eponym, Hamman-Rich syndrome, was used to describe progressive pulmonary fibrosis for which no cause could be identified. Although the term Hamman-Rich syndrome was at one time used synonymously with IPF and thought to be a variant of UIP in Liebow's classification system as described in 1975 (139), the Hamman-Rich syndrome was eventually linked to the histopathologic finding of DAD (140, 141), and the clinical descriptor, AIP, was chosen as the name for the clinical entity. AIP is now recognized as a form of IIP that is distinct from IPF/UIP and other forms of IIP (16). The cardinal features of AIP include the rapid onset of symptoms, the absence of an identifiable cause, and the presence of DAD on examination of lung tissue (16).

Bronchoalveolar lavage cellular findings may vary according to the time that it is performed during the natural course of AIP, but generally an increase in total cells and neutrophils, which may be accompanied by evidence of hemorrhage (presence of RBCs and/or hemosiderin), has been reported (127, 142). However, very little data from a cohort that is larger than a case series or case reports exists in the literature, in contrast to BAL data for ARDS or other ILD entities. Although BAL cellular findings in AIP are non-specific, the cellular pattern can provide supportive data and assist in the differential diagnosis by identifying or excluding other diagnoses such as acute EP, acute HP, infection, or DAH. Nonetheless, performance of a surgical lung biopsy is advocated if BAL does not provide a definitive diagnosis and the clinical presentation does not fit with a diagnosis of ARDS.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for AIP :

AS AN AID FOR THE DIAGNOSIS :

- BAL cellular findings are non-specific and not diagnostic of AIP
- A prominent increase in neutrophils in BALF that cannot be explained by pulmonary infection is consistent with a diagnosis of AIP in the appropriate clinical setting
- A surgical lung biopsy may be required to detect histopathologic changes that characterize this entity and make a confident clinical diagnosis

FOR PROGNOSIS : unknown

5.1.7. Lymphoid Interstitial Pneumonia (LIP)

The term LIP has persisted as a clinicopathologic entity from the time that it was initially coined by Liebow and Carrington in 1969 (143). However, our understanding of this disorder has changed since its initial description and classification, and many experts would classify LIP as a pulmonary lymphoproliferative disorder and possibly a preneoplastic lesion. The development of immunohistochemical methods, however, has allowed the differentiation of LIP from truly neoplastic lesions including mucosa-associated lymphoid tissue (MALT) lesions, which are low-grade B cell lymphomas, and has shown that malignant transformation is unusual in LIP (144-146). The ATS consensus statement on IIP (16) suggested that LIP should be "regarded as a histologic variant of diffuse pulmonary lymphoid hyperplasia with predominantly interstitial changes" that is related to follicular bronchiolitis, a peribronchiolar lymphocytic infiltrate that has germinal centers (147). Whatever its etiology, its histologic pattern is that of an IIP, although its incidence is very low and some cases have been reexamined and reclassified as cellular NSIP (16).

Due to the rarity of LIP, especially idiopathic LIP that is not associated with infection or autoimmunity, there is relatively little literature concerning BAL in LIP. Many publications are only case reports or pertain exclusively to children. Taking into consideration the paucity of literature reports on BAL in LIP, some investigators have reported a significant increase in both total lymphocytes and the differential percentage of lymphocytes in BAL fluid from patients with LIP (148-150). The largest case series of patients with LIP that was not associated with infection consisted of 15 subjects of whom three had idiopathic LIP. BAL was performed in 6 patients, and the mean percentage of lymphocytes on differential cell count was 31% (148). Examination of immunoglobulin gene expression by BAL lymphocytes can assist in diagnosis. The detection of monoclonal immunoglobulin gene rearrangement of BAL lymphocytes suggests the presence of pulmonary lymphoma rather than LIP (149-151).

The committee concludes the following regarding the clinical utility of BAL cellular analyses for LIP:

AS AN AID FOR THE DIAGNOSIS:

- BAL cellular analysis by itself cannot establish a diagnosis of LIP
- BAL lymphocytosis in combination with clinical features and HRCT findings that are suggestive of LIP can be supportive of this diagnosis

FOR PROGNOSIS: unknown

5.2. Sarcoidosis

Sarcoidosis is a multisystem disease of unknown etiology, histopathologically defined by the presence of epithelioid cell granulomata in multiple organs. These granulomas are characterized by a

surrounding rim of lymphocytes. Bronchoalveolar lavage allows recovery of airspace lymphocytes which have been shown to be predominantly activated T-cells of the Th-1-phenotype that typically accumulate in all involved organs. Phenotypically, these cells are characterized by expression of CD4, HLA-DR and other activation markers. The increased CD4/CD8 ratio at sites of involvement has been included in the definition of sarcoidosis in 1991(152), whereas the more recent update of the definition in the ATS/ERS Statement of 1999 replaced the CD4/CD8 ratio with "heightened Th-1 immune response" (153). The reduction in lymphocyte numbers that is frequently observed in peripheral blood of patients with sarcoidosis has been attributed to the localized T-cell accumulation in tissues affected by granulomatous inflammation.

BAL has added immensely to our understanding of the immunopathogenesis of sarcoidosis (12, 132, 154-173), and a number of studies have shown a good correlation between the type and number of inflammatory cells obtained by BAL and those observed in histological sections of lung biopsy specimens as well as cells derived from mechanically disaggregated lung tissue (174-176). In sarcoidosis, BAL shows an excess of lymphocytes in 90% of patients at the time of diagnosis. The total cell count in BAL is usually only mildly elevated in pulmonary sarcoidosis, in contrast to the marked elevation in hypersensitivity pneumonitis, (157-159). The relative proportion of lymphocytes is higher in clinically active disease (range 20 – 80, mean around 40 %), but clinically inactive sarcoidosis patients have a lower percentage (mean of 30 %). There is considerable overlap in lymphocytes between active and inactive disease, and BAL cell counts may be normal in 10 – 15 % of patients (176). In late or advanced sarcoidosis, neutrophils may also be increased, as well as the number of mast cells (177).

Certain BAL parameters other than lymphocytosis can also be helpful in differentiating sarcoidosis from other entities. A normal or only mildly elevated total cell count with a predominance of lymphocytes, a normal percentage of eosinophils and neutrophils, and the lack of plasma cells or foamy alveolar macrophages is characteristic for sarcoidosis. Hypersensitivity pneumonitis, in contrast, usually shows an additional mild increase in BAL neutrophils and eosinophils plus the presence of plasma cells (178-180). In IPF, the lymphocyte count is normal or only mildly elevated. Recently, Drent and colleagues were able to differentiate between the three most prevalent forms of ILD (sarcoidosis, IPF, and hypersensitivity pneumonitis) via computer program for analyzing BAL data, using a discriminate analysis of logistic regression (181).

The BAL CD4/CD8 ratio is increased in about 50 – 60 % of patients with sarcoidosis. The diagnostic value of this ratio has been debated recently because of the high variability in sarcoidosis (181, 182). The ratio may even be decreased in 15 % of patients. Nevertheless, three independent groups found almost identical values for the sensitivity and specificity of an elevated ratio for diagnosing sarcoidosis (183-185). The sensitivity was rather low, reaching 55 %. The specificity was high at 95 %. The

specificity of the CD4/CD8 ratio was higher than the specificity of transbronchial biopsy in one study (184), and an elevated CD4/CD8 T cell ratio may have a diagnostic specificity that is similar to the histopathological demonstration of granulomata in patients with a compatible clinical and radiological profile that fits with the diagnosis of sarcoidosis. A recent study sought to quantify how the likelihood for a given diagnosis changes with the knowledge of BAL cell differentials and the CD4/CD8 ratio. Welker et al, found that, when lymphocytes were combined with the CD4/CD8, the probability of sarcoidosis was doubled if the CD4/CD8 ratio was high (186). They were able to demonstrate an added informative value of the CD4/CD8 ratio, especially in sarcoidosis and hypersensitivity pneumonitis.

The presence of abnormal BAL cell counts (i.e increased) has been considered as an 'alveolitis' in early reports of BAL cellular findings. Because the BAL procedure collects cells from airways and alveoli, the term "alveolitis" has remained controversial, and the use of this term is generally discouraged. It had been suggested that patients with a 'high intensity alveolitis' showed clinical deterioration, but patients with a 'low intensity alveolitis' remained stable (187). However, the prognostic value of BAL lymphocytes and its value as an indicator for corticosteroid therapy was subsequently shown to be questionable. Many subsequent studies have shown that the degree of lymphocytosis at time of diagnosis is of no prognostic significance (177, 188-191). Additionally, patients with a good prognosis and high likelihood of spontaneous remission, such as those with acute sarcoidosis (Löfgren's syndrome), can have very high CD4/CD8 ratios (192). Therefore, the quantification and also the subtyping of lymphocytes in BAL fluid from patient with sarcoidosis have not fulfilled the early promise of a useful marker of disease activity in sarcoidosis. However, the finding of an asociated increased BAL neutrophils may have prognostic value. Two independent groups have shown that increased neutrophils in BAL correlated with clinical deterioration during follow-up (172, 173), but additional prospective studies are needed to confirm this finding.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for sarcoidosis:

AS AN AID FOR THE DIAGNOSIS:

- BAL fluid analysis usually reveals a prominent increase in lymphocytes on the differential cell count, although a BAL lymphocytosis is not always present
- The finding of an increase in the CD4/CD8 ratio combined with a BAL lymphocytosis strongly supports a diagnosis of sarcoidosis, particularly when combined with consistent clinical and radiographic findings

FOR PROGNOSIS:

▪ BAL cellular findings such as a very high proportion of lymphocytes (‘intense lymphocytic alveolitis’) or CD4/CD8 lymphocyte ratio do not have prognostic value

5.3. Hypersensitivity Pneumonitis

Hypersensitivity pneumonitis(HP), also known as extrinsic allergic alveolitis, is an immunologically mediated lung disease that results from repeated inhalation of a variety of finely dispersed antigens such as organic dusts and low molecular weight chemical compounds (193). The disease is characterized by a diffuse lymphocytic inflammation affecting the small airways and pulmonary parenchyma. HP has been classified into acute, subacute and chronic on the basis of its clinical presentation. Long-term outcome of the subacute/chronic forms is variable, but the disease may evolve to fibrosis or emphysematous changes and lead to irreversible lung damage (193). The clinical presentation seems to depend on several factors, including the amount and duration of exposure to the antigen, the nature of the inhaled dust, and the host response. HP occurs more frequently in nonsmokers than in smokers, despite the same exposure risk (194-197). The reasons for the apparent protection provided by smoking is unknown, but it is possibly related to the local immunosuppressive effect of cigarette smoking, which may decrease the immune reaction against the antigen, thus preventing the exaggerated response necessary to develop HP (198, 199). Although cigarette smoking has an apparent suppressive effect on the development of HP, smoking has no suppressive effect on disease progression once the disease is established (196). Moreover, when the disease occurs in smokers, the clinical course seems to be more insidious and chronic, suggesting that cigarette smoking worsens the clinical behavior of the disease (200, 201). It is important to emphasize that the characteristics of the inhaled antigen (i.e. fungi versus proteins), the clinical form of presentation (acute versus subacute/chronic) and the smoking status of patients can affect the BAL findings, and they should be considered when BAL cells and non-cellular BAL constituents are evaluated.

The BAL cell profile in HP is characterized by a significant increase in the total cell count, especially a remarkable elevation in the percentage of lymphocytes, which often exceeds 50%. This marked BAL lymphocytosis is the most consistent finding in this disease, and it has been reported in all the clinical presentations (202-205). In pigeon breeder´s disease, BAL lymphocytes in subacute (< 3 months of symptoms before diagnosis) and chronic (> 12 months) cases are usually over 50%, although levels are significantly higher in subacute compared with chronic HP (206). In general, BAL lymphocyte percentage of less than 30% makes the diagnosis of HP uncertain, and it is important to recognize that BAL lymphocytosis may be also found in a number of asymptomatic individuals who are exposed to the antigen but do not develop the disease (207). It is unknown whether the latter finding represents an

appropriate, normal inflammatory response or whether the apparent "normal" individuals exposed to antigens that can induce HP have a subclinical, low-intensity lower airway inflammation.

Drent and coworkers evaluated BAL inflammatory cell profiles from 59 nonsmoking HP patients at various time-points after termination of antigen inhalation (109). When patients were lavaged soon after exposure (less than 24 hours), BAL contained increased numbers of neutrophils, lymphocytes, eosinophils and mast cells, with a concomitant reduction of alveolar macrophages. At 2-7 days after antigen exposure, BAL was primarily characterized by an increase in lymphocytes, plasma cells and mast cells. Although higher than controls, a significant drop in BAL neutrophils was observed compared with the group of patients studied in the first 24 hours. In BAL obtained more than one week after exposure, the distribution of cell types displayed a tendency to return to normal values, with the exception of the lymphocytes.

The majority of the BAL lymphocytes are T cells that express a variety of activation markers such as the p75 chain of interleukin (IL)-2 receptor, "very late activation antigen" (VLA-1), and class II antigens of the major histocompatibility complex (HLA)-DR (208, 209). Flow cytometry and polymerase chain reaction analyses of T cell receptor β chain variable regions have demonstrated an overexpression of cells bearing Vβ2, Vβ3, Vβ5, Vβ6, and Vβ8 gene segments (210). Evaluation of CD4+/CD8+ T lymphocyte subpopulations has given contradictory results. It is the general belief that HP is characterized by an exaggerated accumulation of $CD8^+$ T cells with a decrease in the $CD4^+/CD8^+$ ratio, and a number of studies have supported this notion (209-213). Nevertheless, some authors have found that both subsets are increased without changes in the $CD4^+/CD8^+$ ratio, while others have reported an opposite finding with a predominance of $CD4^+$ on the surface phenotypes of BAL T cells and an increased $CD4^+/CD8^+$ ratio (194, 204, 205). This apparent discrepancy may, in part, be related to the antigen that causes the HP. Ando et al.(214) found significant differences between summer-type HP ($CD4^+/CD8^+$ ratio: 0.6 ± 0.1) versus farmer´s lung (4.4 ± 0.7) and also reported intermediate values in bird fancier´s lung and ventilation pneumonitis (~2.0). An additional finding was that, in general, smokers with HP exhibited higher levels of CD4 with an increased $CD4^+/CD8^+$ ratio, suggesting that exposure to tobacco smoke may elevate this ratio in patients with HP (214). An increase in the CD4+ subpopulation has been also reported in chronic cases that may evolve to fibrosis (215).

Thus, BAL $CD4^+/CD8^+$ ratio in HP is quite variable and is probably affected by the clinical form (acute versus chronic), exposure to tobacco smoke, the type and dose of inhaled antigen, and, possibly, the time elapsed since antigen exposure. The influence of other cofactors such as viral infection, nonspecific irritants, immunological conditions, and genetic background is unknown. A predominant increase in $CD8^+$ appears to be observed primarily in nonsmokers with acute HP, while a prevalence of $CD4^+$ T cells is frequently found in smokers or those with chronic/fibrotic forms of the disease (205).

Additionally, increased natural killer cells, nonmajor histocompatibility complex (MHC)-restricted cytotoxic lymphocytes, and lymphokine-activated killer cells are usually detected in BAL from patients with HP (208).

Other changes in BAL cellular composition in addition to a T cell lymphocytosis have been reported. High titres of BAL specific antibodies and a lung tissue B cell expansion are also found in HP (216), and a number of studies have documented a modest but significant increase of plasma cells (217). In a study in which 1,260 BAL fluids from patients with different interstitial lung diseases were analyzed, plasma cells were found more often in patients with HP and drug-induced pneumonitis (218). Interestingly, for HP patients with plasma cells in BAL, the plasma cells are higher at 2-7 days after the last antigen exposure and then progressively decrease over time, and the plasma cells are accompanied by increased numbers of lymphocytes, eosinophils and mast cells and a lower $CD4^+/CD8^+$ ratio as compared with HP patients without BAL plasma cells (217).

Although the percentage of BAL macrophages in HP patients is very low, the absolute number of macrophages is comparable to those in controls because of the increase in total BAL cells. Alveolar macrophages are in a high state of activation and display a higher intensity expression of HLA-DQ and HLA-DP than those from patients with IPF or from healthy subjects (219). Likewise, alveolar macrophages from HP patients overexpress intercellular adhesion molecule (ICAM)-1, an important cell surface glycoprotein that functions as an adhesion molecule (220). In addition, an increase in the percentage of AM containing surfactant protein A (SP-A) has been found in HP patients (221).

A moderate, but significant increase of the percentage of neutrophils has been found after inhalation challenge (acute transient increase in neutrophils), and BAL neutrophilia is found in about half of the patients with chronic bird breeder´s disease (109, 180, 222, 223). Acute episodes of HP are usually associated with an increase in the number of circulating neutrophils with the subsequent migration of these cells into the lungs (223-225). Because most patients are lavaged days and even weeks after the last exposure, the early increase of neutrophils is only revealed if BAL is performed soon after antigen challenge, although BAL neutrophils can remain higher than control subjects even one month after antigen challenge (109). In a study of chronic HP, Haslam and coworkers (180) evaluated 15 patients with chronic changes, most of whom were exposed to the antigen up to the time of BAL. A significant increase of neutrophils was observed in half of them. A more recent study has shown an increase in BAL neutrophils in one third of patients with chronic pigeon breeder´s disease (226). Currently, it is not clear if this increase in BAL neutrophils is related to a recent exposure or is part of the chronic response.

Mast cells play an important role in inflammation, fibrosis and remodeling. Several studies have documented a small but significant increase in HP (180, 227, 228), and electron microscopy suggests that these cells resemble bronchial subepithelial tissue mast cells rather than those from dermal connective

tissue and alveolar interstitial tissue (180). Additionally, several ultrastructural changes suggestive of activation and degranulation have been describe (180, 228), and histamine is present in BAL supernatants (227). A recent study including patients with sarcoidosis, IPF, COP, rheumatoid lung, RB-ILD and HP, showed that mast cells are increased in COP and HP (229), but no correlations were found with smoking history or the lymphocyte surface markers CD3, CD4, and CD8. Interestingly, increased mast cells have not only been reported in acute farmer´s lung, but also in a number of ex-farmer´s lung patients who had quit farming and even in some normal farmers (230).

The committee concludes the following regarding the clinical utility of BAL cellular analyses for hypersensitivity pneumonitis:

AS AN AID FOR THE DIAGNOSIS:

- BAL lymphocytosis supports the diagnosis.
- The diagnosis of HP can be confidently made in a patient with an interstitial lung disease that is accompanied by: a) an antecedent exposure and positive serum antibodies against the putative antigen; b) the presence of small poorly defined nodules, ground glass and a mosaic attenuation pattern on HRCT; and c) *greater than 50% lymphocytes in the BAL differential cell count.*
- Alternative diagnoses should be suspected if BAL lymphocytosis is absent.
- Non-smoking patients with an acute or subacute presentation of HP may display an increase of CD8+ T-cells with a decrease in the CD4/CD8 T lymphocyte ratio, whereas patients who are active smokers or have a chronic presentation usually have an increased CD4/CD8 ratio.

FOR PROGNOSIS: Uncertain

5.4. Connective Tissue Disease (CTD) /Collagen Vascular Disease (CVD)

Our understanding of the rheumatological diseases, particularly the different patterns of diffuse lung disease that occur in these diseases, has evolved significantly over recent years. Much of this has been stimulated by the discussion around, and publication of, the document on classification of IIP (16). Although the statement on idiopathic disease cannot be applied directly to rheumatological diseases, it is very clear that a number of common patterns exist. Importantly, in many of the rheumatological diseases, different patterns of diffuse lung disease may occur in different patients and, of relevance, a combination of airway or other intrathoracic disease may co-exist with diffuse lung disease in the same patient, and subclinical disease can be identified in individuals with no pulmonary symptoms when their lungs are investigated as part of routine evaluation. The pathological patterns of UIP, NSIP, LIP, DAD, COP and DIP can all be found in patients with rheumatological disease, and these pathological patterns of diffuse lung disease can coexist with airway diseases including bronchiolitis obliterans and bronchiectasis.

Historic data on BAL cell patterns do not take into account more recent detailed knowledge of the different patterns of diffuse lung disease that occur in the rheumatological diseases. Additionally, the absence of prospective studies of treatment (with the exception of systemic sclerosis) does not allow any statement of the prognostic significance of BAL cell findings to be made in most circumstances, and correlations of lung histopathology with BAL cell profiles have been fairly limited, although some data are available for systemic sclerosis (SSc). There are little data concerning the clinical significance of BAL cell patterns and CTD-associated ILD in the context of currently available HRCT imaging, although this has been studied to some degree in SSc. Although there are a number of studies that have explored BAL cellular findings longitudinally, these findings have not been correlated with treatment response, prognosis, or the HRCT pattern. In historic series, the presence of excess neutrophils, eosinophils and lymphocytes is reported in a small number of patients with "collagen vascular disease" (231).

In the Clinical Guidelines and Indications for Bronchoalveolar Lavage that was published by the task force on BAL in 1992 (13), CTD cases were subdivided into those with and without "ILD." The predominant abnormality reported was an excess of BAL neutrophils, with eosinophils reported as increased in SSc only while neutrophils were elevated in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Sjögren's syndrome. This document stated that there was little diagnostic value of BAL in CTD except to differentiate associated lung disorders such as infection, pulmonary hemorrhage, alveolar proteinosis or a superimposed drug-induced disorder. Similarly, statements about prognosis need to be somewhat more guarded now than those reported in previous studies when an increase in lymphocytes was thought to be predictive of a relatively good prognosis but predominant neutrophils and eosinophils indicative of a poorer prognosis, because of our currently more refined appreciation of the varied patterns of diffuse lung disease and combinations with airway disease that can be observed in patients with CVD. In the American Review of Respiratory Disease Supplement on BAL (15) BAL cell counts were reported in 14 patients with "connective tissue disease," and the major differences were between the numbers of neutrophils and eosinophils in comparison to controls. More recent studies have focused on individual rheumatological diseases and some clearer messages are emerging.

5.4.1. Systemic sclerosis

There have been a number of studies of BAL in SSc. Frigieri (232) performed bilateral BAL in the right middle and left upper lobe and found a combination of lymphocytes, eosinophils and neutrophils in the BAL cell differential. They concluded that a single lavage performed in the right middle lobe was able to accurately identify lung inflammation associated with SSc. In the largest study of BAL cell findings in SSc, Bouros et al (95) reported BAL findings in 73 patients, and increases in numbers of neutrophils

and/or eosinophils and/or lymphocytes were observed. More eosinophils were present in those individuals with an NSIP pattern of diffuse lung disease in contrast to those with a UIP/end stage lung pattern of disease. Neutrophil and lymphocyte numbers did not differ between these two subgroups. More lymphocytes were present in individuals whose biopsies showed cellular NSIP versus those with a fibrotic NSIP pattern. In separate studies from the same group it has been shown that increased BAL neutrophils are associated with the finding of extensive changes on HRCT, especially reticular abnormalities (83). Lymphocytes were present irrespective of whether there was any disease observed on HRCT. These findings are consistent with a number of other studies of BAL cellular analysis in patients with SSc.

BAL differential cell counts in SSc are of questionable prognostic value. The presence of excess granulocytes was associated with greater impairment of lung function in one study (233), and Schnabel et al (234) confirmed the studies of Silver et al (233, 235) in reporting reduction in BAL neutrophils following cyclophosphamide treatment, which coincided with improvement in lung function. However, in another study of cyclophosphamide therapy given intravenously, this lavage cell response was not seen even though some of the patients were found to have improvement in lung function. Excess numbers of neutrophils has been described by some authors to predict more progressive disease (233, 236, 237). However, it has been shown that an increase in neutrophils reflects an increase in the extent of disease on HRCT, particularly the reticular pattern, and this increase is, therefore, likely a marker of more extensive disease rather than an independent index of progressive disease (238). In one retrospective, uncontrolled study, a decreased likelihood of response to treatment was associated with the presence of neutrophils (239). In another study (240) BAL cellular analysis showing excess neutrophils or eosinophils was used as the index to initiate treatment with immunosuppressive therapy, and these patients were allocated to receive treatment or no treatment. Patients with increased inflammatory cells in their BAL had a better outcome with treatment than those without treatment, but a significant proportion of patients with normal BAL cell profiles deteriorated. Furthermore, increased inflammatory cells in BAL may be present in individuals with normal chest radiography and pulmonary function tests (232). Because many patients with apparently normal BAL cellular profiles may exhibit progressive disease, the presence of an abnormal BAL cell profile as the only index for treatment with immunosuppressive therapy is not supported. Additionally, serial BAL as a monitor of disease activity cannot be recommended as a clinical tool and and should be reserved for research purposes, although one study has shown that a abnormal BAL cell profile was associated with lung function deterioration (233). Lastly, two recent, large clinical trials that included BAL cellular analysis did not find useful correlation of BAL cell profiles with disease progression or response to treatment (71, 241).

The committee concludes the following regarding the clinical utility of BAL cellular analyses for SSc:

AS AN AID FOR THE DIAGNOSIS:

▪ The predominant lavage pattern in SSc consists of neutrophilia with or without increased eosinophils.

▪ Some patients have a lymphocytosis, especially those with a cellular NSIP pattern.

FOR PROGNOSIS:

▪ BAL cell profiles should not be used to determine whether treatment should be started or to monitor disease activity.

▪ BAL cell profiles do not predict response to therapy or prognosis.

5.4.2. Rheumatoid arthritis:

Twenty-four patients were evaluated in one of the first studies of rheumatoid arthritis (242). Nine patients with clinical and chest imaging evidence of diffuse lung had an increased percentage of neutrophils in BAL. Five patients who lacked clinical evidence of lung disease but had imaging or lung function changes showed an increase in BAL lymphocytes in comparison to a third group of patients that had no evidence of lung disease and lacked BAL cellular changes. The findings were interpreted as showing that subclinical lung disease was characterized by BAL lymphocytosis, whereas the presence of diffuse lung disease was associated with an increase in BAL neutrophils. However, the co-existence of airway and diffuse lung disease was not considered. In a second study in 1990 of 39 patients (243), 25 individuals had an excess of lymphocytes and 8 an excess of granulocytes. The abnormal BAL cell profile was independent of the presence or absence of rheumatoid factor. Another study from Japan (244) showed increased BAL neutrophils 55% of the cohort but an increase in lymphocytes in only 25%. Gabbay et al. reported BAL findings in 36 patients who underwent intensive imaging, lung function testing, and BAL. Evidence of lung inflammation (defined as elevations in neutrophils, eosinophils and/or lymphocytes) were reported for 52% of the patients, and more than one cell type was increased in 7 patients. An abnormal BAL cell profile was present in all 5 individuals who had symptoms combined with chest imaging and physiological abnormalities as compared to 10 of 13 asymptomatic patients with radiological and physiological abnormality and 0 of 11 who were asymptomatic and had no abnormalities on chest imaging or pulmonary function testing.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for RA :

AS AN AID FOR THE DIAGNOSIS:

▪ Lymphocytes are a more prominent feature in RA than in SSc.

▪ Good studies to correlate BAL with HRCT patterns of disease are lacking

FOR PROGNOSIS: unknown

5.4.3. Primary Sjögren's syndrome.

There have been few studies of this condition using BAL. Delavanga, et al. (245) performed BAL on 23 patients and found that the major abnormality was an increase in lymphocytes. Patients with higher numbers of lymphocytes had more frequent respiratory symptoms and chest radiographic evidence of lung disease. Deheinzeiln et al. (246) demonstrated an excess of lymphocytes and/or polymorphonuclear leukocytes in 13 of 20 patients who had BAL. Lung biopsies that were performed in 12 of these patients showed a wide range of abnormalities that likely accounted for the variability in the BAL cell profile. A longitudinal study (247) of pulmonary involvement in primary Sjögren's syndrome examined 18 individuals who had a previous documentation of an abnormal BAL cell profile consistent with inflammation (14, lymphocytes alone; 4, lymphocytes together with neutrophils) and were lavaged a second time after a two-year interval. Six of the 14 patients with lymphocyte excess when first lavaged had normal differential cell counts, and only one of the remaining 8 had developed an increase in neutrophils. The pattern of mixed BAL inflammatory cell patterns did not change in 4 patients with a mixed BAL cell pattern when initially lavaged, and all five patients with an abnormal HRCT (subpleural lines in 3 patients, ground glass opacities in one and ground glass opacities with subpleural lines in the fifth patient), showed an excess of neutrophils and lymphocytes in BAL. The presence of alveolar neutrophils was associated with a greater rate of decline in gas transfer.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for Primary Sjögren's syndrome:

AS AN AID FOR THE DIAGNOSIS:

▪ Lymphocytosis is prominent and usually associated with good outcome.

▪ Excessive neutrophils, when present, are associated with persistent disease.

FOR PROGNOSIS: unknown

5.4.4. Dermatomyositis/Polymyositis.

Inreased neutrophils and/or lymphocytes in BAL have been reported by a number of investigators. An earlier, small study showed an increased lymphocyte count in 3 out of 5 patients in whom the procedure was performed (248). Komosci et al (249) found that of eight patients subjected to BAL, four had elevated neutrophils and lymphocytes, while an additional patient had lymphocytosis only. These BAL cell changes were observed despite patients being on treatment that was sufficiently adequate to control the myositis. Schnabel et al (250) screened 63 consecutive patients with polymyositis/dermatomyositis and identified diffuse lung disease in 20 of the 63. The clinical and serological features of the anti-Jo1 syndrome were present in the majority of patients. Ten of the patients with progressive diffuse lung disease had BAL neutrophilia and extensive ground glass opacities on HRCT. An abnormal BAL cell differential was, however, not demonstrated for the remaining 10 individuals without rapidly progressive disease.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for dermatomyositis/polymyositis:

AS AN AID FOR THE DIAGNOSIS:

▪ Increased lymphocytes and neutrophils represent the most common BAL abnormality.

FOR PROGNOSIS:

▪ Increased neutrophils have been associated with disease progression.

***5.4.5. Systemic lupus erythematosus*.**

There have been very few studies of BAL in SLE. Therefore, the committee is unable to make any clear statement on the use of BAL analysis in diagnosis or management of SLE.

***5.4.6. Undifferentiated connective tissue disease*.** Todate, there have been three reports of bronchoalveolar lavage in undifferentiated connective tissue disease (251-253). In a study by Kumanovics (253), 13 patients with signs of lung disease were investigated. Eleven of the 13 had an increase in total cell numbers in BAL. Lymphocytes were increased in six, neutrophils were increased in two, and a mixed BAL cell pattern (lymphocyte and neutrophil increase) was identified in one. No conclusions regarding BAL cellular analysis in undifferentiated connective tissue disease can be drawn.

5.4.7. Conclusions regarding the clinical utility of BAL cellular analyses for all CTD/CVD-associated ILD:

▪ There is a paucity of studies of BAL in CTD-ILD

- Many of the studies combine different rheumatological diseases and very few make any effort to correlate lavage findings with HRCT or lung biopsy, with the exception of SSc
- The prevalence of mixed patterns of disease in the lungs of patients with rheumatological diseases makes any precise interpretation of the predominant pattern of altered BAL cell profiles uncertain
- Subclinical lung inflammation as reflected by abnormal BAL cell patterns is frequently present.
- Eosinophilia appears to be an infrequent finding in BAL.
- BAL lymphocytosis tends to be a predominant finding in Sjögren's syndrome and RA.
- Neutrophilia appears to be more common when the HRCT pattern is suggestive of fibrosis, and the numbers of neutrophils increase with increasing amount of lung involvement on HRCT.
- There is a striking lack of knowledge concerning
  (1) the significance of an abnormal BAL cell profile in subclinical disease
  (2) the progression of different patterns of altered BAL cell profiles and the significance of such changes
  (3) whether BAL reflects disease the specific inflammatory cell types involved in disease pathogenesis
  (4) the relationship BAL findings, HRCT, and/or biopsy for most rheumatological diseases
  (5) the role of BAL cell subtype analysis
  (6) whether BAL cell profiles can be used, together with HRCT, to classify the predominant pattern of lung disease

  Thus, the role of BAL cellular analyses in management of ILD associated with CTD/CVD is unclear.

5.5. Occupational Interstitial Lung Disease

Environmental exposures at work place can cause specific ILD in a genetically predisposed person .Such 'occupation induced ILD' includes the pneumoconioses, a group of chronic lung diseases caused by the accumulation of asbestos, silica, silicate or coal dust in the distal respiratory tract and pulmonary parenchyma combined with the tissue reaction to their presence, metal-induced granulomatous disorders (induced by beryllium, aluminum, titanium, and man-made mineral fibers), hard metal disease (HMD) and, less frequently, fibrotic changes due to exposure to iron, silver, barium and rare earth metals. The diagnosis of coal worker pneumoconiosis (CWP), silicosis and asbestosis is generally based upon a relevant exposure history, a compatible clinical picture and typical roentgenologic abnormalities. Diagnostic criteria for chronic beryllium disease (CBD) include a history of beryllium exposure and a positive response to an *in vitro* peripheral blood or BAL T-cell proliferationtest (Be lymphocyte proliferation test, BeLPT). Tissue examination is usually required for the exclusion of other ILD.

Significant limitations in published reports to date include 1) differences in BAL technical procedures; 2) use of the International Labour Organization (ILO) chest X-ray classification (which has been demonstrated to have low sensitivity and specificity in ILO score when correlated with HRCT studies) to categorize study subjects as exposed or diseased; 3) the potential for study bias because the inclusion and exclusion criteria, recruitment and sampling modalities and current treatments were reported only in a limited number of studies; and 4) the potential for test review bias as most studies did not include blind interpretation of BAL results with respect to the clinical and radiological data that were reported. Data duplication may also have occurred, because many studies that originated from a limited number of research groups. Because of these limitations, the overall level of evidence for the diagnostic accuracy of BAL in dust induced lung diseases is very low.

5.5.1. Coal Workers Pneumoconiosis.

Coal dust inhaled by miners and other coal workers can lead to the development of simple CWP or progressive massive fibrosis (PMF), which are defined by the presence of multiple radiographic shadows smaller or greater than one cm in diameter, respectively. Risk is thought to increase with the cumulative exposure to coal dust. CWP lesions are usually found in the upper lobes with a posterior predominance in the early stages. The pathological hallmark of CWP is a densely pigmented dust macula resulting from accumulation of carbon dust-laden macrophages around the respiratory bronchiole associated with minimal collagenous scarring and focal emphysema. The enlargement and coalescence of adjoining macules lead to nodular lesions, in which clear fibrosis and vascular destruction occur. Nodules may subsequently coalesce into the large opacities that are characteristic of PMF.

BAL cellular profiles of CWP have been described in several studies (254-261) of which six are from the same research groups in Northern France. They report an increase in the total cell counts with increased numbers of alveolar macrophages, lymphocytes and polymorphonuclear leukocytes. An elevated proportion of macrophages that have engulfed numerous black and bright-brown polyhedral and large rectangular particles was reported in one study (257). These changes are seen in current smokers, non-smokers and former smokers, but a dramatic increase in the percentages and total numbers of alveolar macrophages seen in current smokers may obscure the effect of coal dust. Progression to PMF does not seem to modify the BAL cell pattern. A slight increase in lymphocyte percentage has been reported in simple pneumoconiosis versus PMF patients in one study (257). Several studies have reported that exaggerated amounts of macrophage mediators capable of injuring lung parenchimal cells and promoting lung fibrosis are released in the lower respiratory tract of coal workers and CWP affected subjects. Higher levels of interferon-γ have been found in the BAL fluid of both exposed and CWP affected workers, and exaggerated levels of fibronectin, IL-1, MCP-1, PDGF, TGF-β and IGF-1 have

been found in individuals with CWP, but not in exposed, unaffected workers. In summary, BAL cell profiles indicative of excess numbers of macrophages may suggest CWP or PMF in a non-smoking worker.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for CWP:

AS AN AID FOR THE DIAGNOSIS:

▪ BAL cell profiles indicative of increased numbers of macrophages and elevated proportion of coal dust-laden macrophages are suggestive of CWP or progressive massive fibrosis (PMF).

FOR PROGNOSIS: Unknown

5.5.2. Silicosis. Silicosis is caused by the exposure to respirable particles of crystalline silica in a variety of occupational settings. The histologic hallmark of silicosis is the silicotic nodule that is formed by dust-laden macrophages (which contain silica particles that can be identified via polarized light microscopy) and hyalinized connective tissue surrounded by fibrous tissue in an onion-like pattern. Silicotic nodules may coalesce and form large conglomerates involving the pulmonary arteries and the airways, ultimately leading to PMF.

BAL cellular data on silicosis patients have been reported in numerous original articles (262-274). The BAL cellular profiles of silica-exposed subjects are characterized by increased numbers of macrophages in non-smoking subjects. This macrophage pattern is associated with moderately increased percentages of lymphocytes and neutrophils in patients with silicosis. Lymphocyte CD4/CD8 ratios of 1.4 and 0.8 were reported in two studies on mixed dust pneumoconiosis (275, 276). Some studies have shown that fibroblast products (e.g. type III procollagen) and macrophage lysosomal enzymes (e.g. alkaline phosphatase) were present at higher levels in the BAL fluid of silicosis patients, and higher levels of immunoglobulins were found both in the exposed and the affected subjects. Some studies have assessed the levels of silica exposure using particle quantification by optical or electron microscopy (265, 266, 270, 272, 274). Although different approaches have been used for light microscopy particle counts, these studies found an increased number of particles within the macrophages in silica exposed and silicosis affected subjects compared to non-exposed controls. However, significant differences in dust burden for exposed individuals versus those with silicosis were not detected. In summary, BAL profiles of silica-exposed workers and workers with silicosis are characterized by an excess of BAL macrophages that is appreciable in non-smokers. Increased numbers of lymphocytes and neutrophils seem to characterize the inflammatory process associated with progression to silicosis.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for silicosis :

AS AN AID FOR THE DIAGNOSIS:

▪ BAL profiles of silica-exposed workers and workers with silicosis are characterized by an excess in BAL macrophages and an increased silica particle burden of macrophages that is appreciable in non-smokers

FOR PROGNOSIS

▪ Increased numbers of lymphocytes and neutrophils have been associated with progression to silicosis.

5.5.3. Asbestosis:

Exposure to fibrous silicates, which have been widely used for industrial purposes including construction of buildings and homes, is the cause of asbestosis. Despite the decline in its use in the past decades, asbestos, due to its environmental persistence, is still a serious health hazard in most industrialized countries. Asbestosis is characterized by a process of interstitial lung fibrosis associated with retention of asbestos fibers and formation of ferruginous bodies. Four grades of severity of asbestosis have been described: starting with (1) the presence of discrete foci of fibrosis limited to the walls of first order respiratory bronchioles, (2) extension of fibrosis into adjacent alveolar ducts and septa, (3) coalescence of fibrotic foci involving the respiratory bronchioles and surrounding alveoli, and (4) the obliteration of the parenchyma with fibrous tissue and honeycombing. The distribution of abnormalities in early or mild disease is subpleural and predominates in lower lung lobes, but it may subsequently spread to the central lung zones and to the upper lung fields.

BAL cellular profile data have been reported in numerous studies (275, 277-312). Although the BAL protocols employed in the published studies varied considerably in terms of lavaged areas and instilled fluid volumes, BAL cellular profiles of asbestos-exposed individuals were characterized by increased numbers of macrophages and, to a lesser degree, increased neutrophils. Individuals with asbestosis tend to have increased macrophages acompanied by a smaller increase in neutrophils and eosinophils. Lymphocyte numbers are also increased in the non-smoking asbestos-exposed subjects and in patients with asbestosis. When lymphocyte CD4/CD8 subpopulations data were reported, the CD4/CD8 ratios ranged from 1.0 to 3.9 in asbestos-exposed subjects and from 0.8 to 6.9 in patients with asbestosis. Quantification of soluble mediators in BAL fluid and in ex vivo BAL cell cultures showed increased levels of oxidants, neutrophil products, proinflammatory cytokines and fibroblast growth-related factors in the lower respiratory tract of asbestos-exposed and subjects with asbestosis. Inhaled asbestos fibers deposited in the respiratory and terminal bronchioles become coated by an iron-rich protein layer and form brownish, club-shaped structures called asbestos bodies (AB) whose thin,

transparent and colorless cores distinguishes them from other ferruginous bodies. Although many studies have been published that measured AB counts in BAL fluid (285, 292, 293, 303-305, 309, 312), these studies have not convincingly demonstrated significant differences in AB concentration between asbestos-exposed and patients with asbestosis.

In summary, non-smoking, asbestos-exposed individuals and patients with asbestosis can have a BAL cell profile that is characterized by a dramatic increase in the numbers of all BAL cell types. Macrophage activation soluble products are increased both in exposed individuals and in subjects with lung disease, while markers related to collagen production are increased in those with lung disease. AB counts in BAL do not discriminate between individuals with significant asbestos exposure versus those with asbestosis.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for asbestosis:

AS AN AID FOR THE DIAGNOSIS:

- BAL profiles in the non-smoking asbestos-exposed individuals and in patients with asbestosis are characterized by an increase in all BAL cell types
- Asbestos body/fiber counts in BAL do not discriminate between exposure and disease

FOR PROGNOSIS: Unknown

5.5.4. Chronic beryllium disease. BAL cellular profiles in beryllium (Be) exposed workers have been reported in numerous manuscripts (313-330). These studies evaluated individuals with beryllium exposure and evidence of sensitization (i.e., with a positive Be-lymphocyte proliferation test (Be-LPT) but no granulomatous histology) or individuals with CBD. BAL profiles in CBD are characterized by a moderate increase in cell counts that can be entirely ascribed to the dramatic expansion of lymphocytes. This lymphocytic BAL cell pattern is associated with the presence of mononuclear cell interstitial infiltrates and granulomas in the lung tissue. A positive correlation has been found between ground glass and nodule extension on HRCT scans and the lymphocyte numbers (317). All the studies analysing T cell subsets reported an increase in the CD4/CD8 ratio that ranged from 3.7 to 7.2.

Beryllium sensitization is usually detected by performing the Be-LPT on peripheral blood mononuclear cells and routinely used for screening of Be-exposed subjects. Results are expressed as peak stimulation index (peak SI), and higher values of peak SI have been reported for BAL Be-LPT compared to peripheral blood Be-LPT. BAL studies have shown increased levels of TNF-α and IL-6 in the lavage fluids of CBD patients compared to normal controls or non-diseased, sensitized subjects (315, 330). Th1 cytokines are found in Be-stimulated but not in unstimulated BAL cell cultures (329, 330). Soluble BAL

markers of inflammation are increased in CBD compared to Be sensitized subjects, however these determinations are performed only in research settings. Therefore, the immunocytological BAL profile can provide support to a CBD diagnosis and may help with obtaining a firm diagnosis in subjects not undergoing biopsy, and increased soluble mediator levels may be suggestive of active disease. A positive BAL Be-LPT may be diagnostic of CBD in blood Be-LPT negative individuals.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for CBD:

AS AN AID FOR THE DIAGNOSIS:

- A BAL lymphocytosis is the hallmark of CBD
- The immunocytological BAL profile provides support to a CBD diagnosis and may help with obtaining a firm diagnosis in subjects not undergoing biopsy or in whom granulomas are not obtained on TBB.
- A BAL Be-LPT may be positive even when blood Be-LPT testing is negative. A positive BAL BeLPT can replace blood BeLPT as one of the dfining criteria for CBD.

FOR PROGNOSIS: Uunknown

5.5.5. Hard metal disease.

Hard metals include cemented alloys of tungsten carbide with cobalt and other metals such as titanium, tantalum, molybdenum or nickel, used in the production of cutting tools and other machine parts. Sporadically, workers manufacturing or using hard metal tools may develop an occupational asthma syndrome and/or a giant cell interstitial pneumonitis dominated by the infiltration of mononuclear and multinucleated, foreign body type giant cells with aspects of desquamative alveolitis and interstitial fibrosis, defined as hard metal disease (HMD). The pathogenesis of HMD is still unclear, but it may be related to a hypersensitivity reaction to aerosolized cobalt dust. BAL data are available from 30 HMD patients and from 19 HM-exposed subjects reported as case reports (331-342). BAL in HMD patients shows a marked increase in BAL cell numbers, reaching 2500-3000 cells/ml in three patients. The BAL cell pattern is mixed (lymphocytic, neutrophilic, and eosinophilic) with variable proportions of these three cell types. Different BAL cell patterns have been ascribed to different phases of the disease. CD4/CD8 ratios were available for 7 patients and ranged from 0.1 to 1.6 (336-338). Giant cell counts have been published for 20 subjects with a range of 0.1 to 10% (331-333, 337). Although the disease is characterized by high BAL cell counts with giant cells considered to represent the disease hallmark, there is not sufficient evidence to support their diagnostic usefulness in the clinical setting.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for hard metal disease:

AS AN AID FOR THE DIAGNOSIS:

▪ Although giant cells represent the disease hallmark of hard metal disease, current evidence does not support a diagnostic role for detection of giant cells in BAL for diagnosis.

FOR PROGNOSIS: Unknown

5.6. Other Specific Non-IIP Interstitial Lung Diseases

5.6.1. Pulmonary Langerhans Cell Histiocytosis

Langerhans cell histiocytosis (LCH) is a rare, chronic granulomatous disorder involving the monocyte–macrophage cell lineage. Although LCH is one disease, its clinical features vary from an isolated bone lesion to multiple organ involvement (343-346). Localized disease has been previously called eosinophilic granuloma, and multisystem forms were often referred to by eponyms such as Hand-Schüller-Christian disease, histicytosis X and Letterer–Siwe disease, but the terminology has now been simplified to LCH (347). The clinical features of pulmonary LCH (PLCH) are variable. The majority of patients are smoking adults who have nonspecific respiratory symptoms, and recurrent pneumothorax is one of the characteristic manifestations. Immunohistochemical studies are useful in recognizing Langerhans cells (LC), which stain for the S-100 protein, CD1a, and HLA-DR (347-350). Because LCs can be identified in several pathologic pulmonary processes, the mere presence of LCs is not diagnostic of PLCH. Histologic diagnosis of pulmonary LCH in adults rests on the identification of typical lung lesions, coupled with reliable demonstration of increased numbers of LCs (346).

The central histopathological feature of LCH is the abnormal proliferation of LCs (351, 352), which are differentiated cells of monocyte–macrophage lineage that function as antigen-presenting cells. There are two unique morphologic features for the definitive identification of LCs: the presence of Birbeck granules, which appear as pentalaminar (five-layered), tennis racquet-shaped intracellular structures on electron microscopy (343) and the strong presence of the CD1a antigen on the cell surface, which is not observed for other cells of histiocytic origin (346, 349). Langerhans cells are widely distributed in normal tissues including epidermis, interdigitating cells of lymph nodes, thymus, mucosal epithelium of the gastrointestinal systems and the cervix, and lung (353-356). Cigarette smoke causes an increase in LCs in the lungs of patients with PLCH as well as in asymptomatic smokers (353). The origin of the expanded population of LCs is unknown, although locally produced cytokines, such as TNF-α and granulocyte–macrophage colony-stimulating factor (GM-CSF), may have a central role in the recruitment of LCs into the lung (357).

According to the “Histiocyte Society “ the diagnosis of LCH is regarded as “presumptive” when the typical morphological characteristics of Langerhans’ cells are seen with light microscopy, and as

"designated" when additional stains (eg, protein S-100) are positive (358). Diagnosis is "definite" if stains for CD1a antigen are positive and/or intracytoplasmic organelles (Birbeck granules) are seen with electron microscopy (358, 359). Pulmonary LCH is one of the few diseases in which BAL has a high diagnostic value and can replace lung biopsy (132, 360). The BAL cell examination often shows increased numbers of total cells and macrophages with modest elevation of neutrophils and eosinophilis (361-363). These are not diagnostic and may be related to smoking history of most patients, because in nonsmoking LCH patients, the number of macrophages were not increased (363). In the active phase, lymphocyte number also increases with reduction in CD4/CD8 ratio (364). Because LCH is an abnormal proliferation of LC, the demonstration of LC in BAL fluid supports the diagnosis in an appropriate clinicoradiologic setting (361). Even though the demonstration of Birbeck granule is highly specific for LC, the ultrastructural analysis is time consuming and the immunohistochemical study with monoclonal antibody is much easier (365-368). Positive staining for S-100 protein is far less specific for LC (350). However, the presence of LC even with more specific CD1 (OKT6) positivity is not, in itself, diagnostic, because they were found in normal smokers and the patients with other diseases like ILD or bronchoalveolar carcinoma (353, 354, 356, 369). The quantity of LC in BAL fluid is a discriminating feature that differentiates PLCH from other entities, but it is not yet certain how much LC should be present to allow a definitive diagnosis of PLCH to be made on the basis of BAL analysis. It was reported that all patients with PLCH had LC more than 5% of BAL cells in contrast to much less value (< 3.6%) in all other conditions (365). By reviewing all the published literatures (354, 356, 363, 365-368, 370-373) using this 5% as a cutoff value, among 125 normal controls and 571 patients with other types of ILD, only two cases (one with hypersensitivity pneumonitis and the other with chronic bronchitis) had 5% CD1 positive cells (371); all others had a much lower value. Therefore, LC more than 5% of BAL cells is highly suggestive of LCH. Although flow cytometry can be used to quantitate LCs in BAL, autofluorescence of macrophages from smokers can interfere with accurate flow cytometric analysis for positive cells. Smoking cessagion rapidly reduces cell recovery in bronchoalveolar lavage fluid, while alveolar macrophage fluorescence remains high and immunocytochemistry would provide more accurate quantitation (374). However low numbers of LC cannot exclude PLCH, because the lesions are patchy and the number of LC is dependent on the stage and decrease as the disease progresses.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for PLCH:

AS AN AID FOR THE DIAGNOSIS:

- When HRCT and clinical data suggest the diagnosis of PLCH, BAL cellular findings can be supportive and help confirm the diagnosis.

- When the HRCT is nondiagnostic, the finding of elevated levels of CD1a staining (>5 percent of cells) in the BAL fluid strongly supports the diagnosis.
- Non diagnostic BAL findings (<5% CD1 cells) do not exclude PLCH in otherwise clinically suspected cases and surgical lung biopsy should be considered in such situations for establishing or eliminating the diagnosis of PLCH.

FOR PROGNOSIS: Unknown

5.6.2. Pulmonary Alveolar Proteinosis

Pulmonary alveolar proteinosis (PAP) is a rare disease in which alveoli are filled with phospholipid-rich proteinacious material. More than 90% of cases occur as a primary acquired disorder of unknown etiology, but occasionally it occurs in association with other conditions, and congenital PAP is a distinct familial disorder that is transmitted in an autosomal recessive manner (375-379). Secondary PAP has been described in association with (1) lung infections (377, 378), (2) hematologic malignancies and other conditions altering a patient's immune status (376), and (3) exposure to inhaled chemicals or minerals, such as silica, aluminum dust, insecticides, and titanium (380-383). Most patients are men (male:female ratio 2.65:1.0) and smoke, and the age distribution at diagnosis closely resembles that of the population as a whole (377). PAP usually presents with the gradual onset of progressive dyspnea, a minimally productive cough, or fatigue which are variably associated with weight loss and low-grade fever. Marked fever usually indicates the presence of a complicating infection. Chest X-ray findings are suggestive of the "butterfly" or "bat wing" appearance of pulmonary edema without other radiographic signs of left heart failure (384). HRCT scanning has a characteristic appearance of patchy or geographic air-space "ground-glass" opacities or consolidation with some thickening of the interlobular septa, often in polygonal shapes, resulting in a "crazy-paving" appearance (385). Hypoxemia is common, and elevated serum LDH has been reported (386). PAP is a disease in which BAL has one of the highest diagnostic yields, making surgical lung biopsy unnecessary in most cases (387). Additionally, lung lavage has a major value in the treatment.

A recent major advance is the discovery of animal models of PAP based on deletion of genes for GM-CSF itself or the GM-CSF receptor. Mice lacking either GM-CSF (GM-/-) or ßc (ßc-/-) receptor have impaired surfactant clearance by alveolar macrophages, leading to a condition that resembles human PAP (388, 389). Bone marrow transplant with the ßc-chain-repleted stem cells corrected the PAP in the ßc-deleted mice (390). Thus deficiency of GM-CSF is suspected as a pathogenetic mechanism for acquired primary PAP in adults. In the patients with PAP, GM-CSF level in the BAL fluid was actually higher than normal (391, 392) and the demonstration of neutralizing anti–GM-CSF antibodies in the serum and

BAL fluid has suggested that the disease may have an autoimmune pathogenesis (391, 393-395) with the pulmonary disease resulting from a blockade of endogenous GM-CSF.

When BAL is performed, the retrieved fluid is grossly cloudy or milky and contains dense, white flocculent material that settles out fairly rapidly when left to stand (66, 396). Stained cytospins show pauci-cellular fluid with an amorphous basophilic granular background material and the characteristic (although not specific) dense, globular structures, which stain pink with PAS but do not stain with Alcian Blue, differentiating them from mucins (66, 397-400). Morphologically abnormal "foamy" macrophages engorged with diastase-resistant PAS-positive intracellular inclusions are also present. Ultrastructurally, the BAL sediment is identical to the intra-alveolar material seen in biopsy specimens: abundant lamellar bodies and cellular debris are present (401). The alveolar macrophages are usually enlarged with abundant intracellular bodies and lipid droplets or empty vacuoles. The major constituent of the lavage fluid is lecithin, a major phospholipid component of surfactant (402, 403). Also increased concentration (10- to 50-fold) of surfactant protein A (SP-A) and surfactant protein D was found in BAL fluid and serum (404-407) . These findings in conjunction with electron microscopic features and sequential reports demonstrating the restoration of normal surface activity after ethyl alcohol extraction confirm that the accumulated alveolar material is surfactant-derived (377). Biochemical analysis of BAL fluid has shown a higher protein and phospholipid concentration with qualitative abnormalities in phospholipid composition (408). Various tumor markers, including carcinoembryonic antigen and CA 19-9 have been detected in the BAL fluid and serum of patients with PAP (409). However, they were also found in other pulmonary diseases (410) and the specificity of these findings for PAP compared to other diffuse lung disorders remains to be determined. One recent study suggested that elevated pulmonary SP-D levels in BAL fluid compared to levels in the serum could be highly specific for PAP (406). Such tests, however, are currently experimental and their clinical value needs to be confirmed in larger populations. Cells are usually sparse in BAL fluid, and the differential cell count of the lavage fluid is usually not helpful in the diagnosis (383); decreased percentage and absolute number with defective function of macrophages was reported. However, both a lymphocyte-predominant pattern (411) and a macrophage-predominant pattern (407) have been reported in small series of patients. The CD4/CD8 ratio among the lymphocytes in the BAL fluid was similarly variable and both low and high ratios found (411, 412).

A number of other conditions may be considered for differential diagnosis of PAP. Exogenous lipoid pneumonia frequently has opaque/milky BAL fluid and *Pneumocystis carinii* is a fairly common potential cause of a false-positive diagnosis of PAP (66). Special stains such as lipid staining, Gomori's methenamine silver (GMS) or fluorescent stain for *P. carinii* should be done. Amiodorone and chlorphenteramine toxicity may show lamellated inclusion bodies within macrophages, which mimic PAP (399, 413, 414). Although rare, amyloid has reported to be found in BAL fluid, but it can be easily

differentiated and identified by special stains such as Congo red and electron microscopy showing parallel and interlacing arrays of fibrils (415). Sometimes resolving pneumonia with collections of eosinophilic globular extracellular material, which represented fibrin, can lead to a false-positive diagnosis of PAP. Surfactant may accumulate in conjunction with tuberculosis(416), *Pneumocystis carinii* (417), or endogenous lipid pneumonia associated with an obstructed bronchus (418). Thus in the absence of large amounts of surfactant and supporting clinical and radiologic findings, the diagnosis of PAP should be made with caution.

Although the gross apperance and the microscopic features of the BAL fluid are very charcteristic, in the future, additional tests on the BAL fluid, particularly for the presence of specific pulmonary surfactant apoproteins such as SP-D, may further increase the specificity in diagnosing PAP. Serologic diagnosis by demonstration of autoantibodies against GM-CSF has been reported to be by highly sensitive (100%) and specific (98%) (419). Gene therapy may be a future treatment option for patients with specific genetic defects and for other adult patients with possible autoimmune-mediated PAP, and future pathophysiologic insights will likely lead to targeted therapeutic strategies.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for PAP:

AS AN AID FOR THE DIAGNOSIS:

- BAL fluid retrieved from patients with PAP is typically cloudy or milky in nature and contains dense, white flocculent material that rapidly settles with gravity. This amorphous material, particularly its globular structures, stain positive with PAS staining but negative with Alcian Blue, and cytospins usually show relatively few inflammatory cells.
- BAL fluid analysis in combination with typical HRCT findings and clinical presentation can strongly support a diagnosis of PAP.
- Lung biopsy may not be required for a confident diagnosis.

FOR PROGNOSIS: Unknown

5.6.3. Alveolar Hemorrhage Syndromes

Diffuse alveolar hemorrhage (DAH) refers to a distinct subset of pulmonary hemorrhage with widespread bleeding into distal airspaces, presumably because of microvascular injury associated with an immunologic or autoimmune disorder (420-422). It can occur in association with a variety of conditions or as a disease isolated to lung (420-428). Because extensive DAH can be life threatening, early recognition of DAH is critical and may decrease the likelihood of respiratory failure and end-stage renal disease. Unfortunately, the diagnosis is frequently delayed due to the absence of hemoptysis (429, 430).

Furthermore, alveolar hemorrhage is not an uncommon problem in immunocompromised patients, including bone marrow or stem cell transplantation (431-436), in whom invasive diagnostic procedures are risky because of the high likelihood of the presence of a bleeding diathesis. In this situation, BAL has the advantage of not only being less invasive and less risky, but also being very effective in the diagnosis of infections, which were often identified in these patients (433, 435, 437).

No clear definition of DAH has been unanimously accepted from BAL data, but several definitions have been proposed in the literature. One definition holds that grossly bloody BAL fluid in all aliquots, or progressively bloodier BAL fluid with each instilled aliquot from at least three different samples identifies DAH (438), and this has been confirmed pathologically at autopsy in some patients (432). However, discrepancies have been found between this gross finding of bloody BAL fluid and the histology (433, 439). In a study that examined 77 patients who died of pulmonary complications, 4 among 8 patients with confirmed DAH at autopsy had grossly normal BAL fluid, but 7 of 13 patients without DAH also had hemorrhagic BAL fluid (439). Additionally, no correlation of the bloody appearance of BAL fluid or the presence of large numbers of RBCs in BAL specimens with either an elevated hemosiderin score or the presence of alveolar hemorrhage in tissue specimens was reported (440). Although bloody BAL fluid is suggestive, it is not in itself a definite sign of DAH, especially in the presence of thrombocytopenia or other coagulopathy. Furthermore, BAL fluid that is clear and appears free of significant hemorrhage does not exclude the possibility of DAH.

Hemosiderin has been used as a key marker to define DAH, and the presence of numerous hemosiderin laden macrophages is probably more specific, or at least a reasonable marker for AH. However, the absence of hemosiderin in alveolar macrophages does not exclude the possibility of recent (less than 48 hour) or remote (greater than 12 days) alveolar hemorrhage (441), because animal experiments and human studies have shown that it requires at least 48–72 hours after the onset of hemorrhage into the airspaces for hemosiderin (a hemoglobin degradation product) to be detected in BAL macrophages (442-444). Total clearance of hemosiderin is possible within 2 to 4 weeks after an acute hemorrhage, but it may not be cleared for up to 2 months (444). In chronic bleeding the siderophages tend to accumulate in the alveolar spaces for a longer time and can be observed in BAL fluid for several months after bleeding ceases (441). Furthermore, the mere presence of siderophages without their quantification is insufficient to diagnose acute hemorrhage, because normal individuals, especially smokers (up to 3 %) and patients with interstitial lung diseases may have up to 5% siderophages in their alveoli (427, 445, 446). In order to quantify the alveolar macrophage (AM) hemosiderin content in BAL samples, Golde and colleagues proposed a numerical scale based on the blue intensity of AM on an iron stain (Prussian blue or Perls) graded from 0-4 (0 - no color; 1 - faint blue or trace of hemosiderin in a minor portion of cytoplasm; 2 - deep blue in a minor portion of the cell or intermediate color throughout

the cytoplasm; 3 - deep blue in most areas of cytoplasm; 4 - deep blue throughout the cell or macrophages filled with hemosiderin). The mean score for 100 AM was calculated (Golde score) (430, 447). The efficacy of this Golde scale was validated 12 years later by Kahn in 51 immunosuppressed and 8 non-immunocompromised patients with new pulmonary infiltrates. It correlated closely with the histologic severity of DAH in 26 patients from whom a lung biopsy or autopsy specimens were available. Golde score greater than 100 correlated with severe DAH and 20-100 with moderate hemorrhage (440). Similar validation via correlations with histologic diagnosis was provided by others (430, 448). Although the quantification of the hemosiderin content of AM improved the specificity of the diagnosis of DAH by BAL (446), this laboratory technique is very time-consuming and difficult for routine use. However, good correlation between the percentage of siderophages and the Golde score has been reported (429), and a percentage of siderophages equal or greater than 20% is sufficient and is an easier determinant of the diagnosis of DAH than the Golde score; in normal individuals, the siderophage count is always lower than 10% of BAL AM. However, smoking can lead to increased numbers of iron-positive macrophages (429), and a minority (less than 5%) of the patients with collagen vascular disease and vasculitis had iron-positive AM (427). However special consideration is needed in case of thrombocytopenia (< $50{,}000/mm^3$), coagulopathy or drugs (e.g. heparin or aspirin), renal failure, and heart transplantation due to chronic heart failure (447, 448).

Most patients with DAH present with dyspnea and bilateral diffuse alveolar infiltrates on chest roentgenogram. Hemoptysis is usually present, but not always. Anemia that may be a feature of low-grade, chronic alveolar hemorrhage further supports the diagnosis; however anemia is usually not present in acute DAH. Therefore, any diffuse bilateral alveolar infiltrate on chest roentgenogram should prompt the consideration of DAH, and diagnostic bronchoscopy and BAL should be done early. The progressively bloodier fluid retrieved in each aliquot is suggestive, even though not definitely diagnostic in itself. BAL fluid should be sent for the microbiologic studies for possible infection and cytospin slides should be stained for iron (Prussian blue or Perls). A siderophage count more than 20% of BAL cells or Golde (hemosiderin) score more than 100 is indicative of significant AH. However, Maldonado and colleagues (449) have reported that >20% hemosiderin-laden macrophages can be seen in some patients with diffuse alveolar damage (DAD), and DAD must be considered in the differential diagnosis. Similarly, increased numbers of hemosiderin-laden macrophages have been observed in biopsy specimens from patients with IPF accompanied by secondary pulmonary hypertension, although an increase in hemosidering-laden macrophages in BAL from such patients has not yet been reported (450). Additionally, interpretation should be performed cautiously in cases with a bleeding tendency. Once the diagnosis of AH is established, careful clinical evaluation is essential for the differential diagnosis of underlying disease, especially renal diseases, collagen-vascular diseases, and systemic vasculitis, with

serologic studies including anti –GBM antibody and ANCA. However, the results of these serologic tests are generally too late for early decision-making, and tissue biopsy results (including direct immunofluorescence studies) may be more quickly available. Furthermore, histopathologic diagnoses lend justification to the long-term immunosuppression required for many patients with DAH.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for Alveolar hemorrhage syndromes:

AS AN AID FOR THE DIAGNOSIS:

- The finding of progressively bloody BAL fluid with sequential aliquots, especially if accompanied by the identification of a large number of BAL macrophages containing hemosiderin, supports a diagnosis of DAH.
- BAL cannot identify specific causes of DAH.

FOR PROGNOSIS: Unknown

5.6.4. Drug-Induced Interstitial Lung Disease

A large number of drugs are potentially pneumotoxic and capable of inducing radiologic infiltrates. Drug-induced ILD syndromes, which can range from acute to chronic in onset and clinical presentation, encompass a wide range of pulmonary pathologies (451) and associated radiologic patterns (452) that include acute noncardiogenic pulmonary edema, alveolar hemorrhage, diffuse alveolar damage, eosinophilic pneumonia, hypersensitivity pneumonitis, drug-induced lupus, and pulmonary fibrosis. However, there are no generally accepted criteria that one can use to absolutely determine if radiologic infiltrates are caused by exposure to a drug, and drug-induced pneumotoxicity is often an idiosyncratic reaction. The two most common patterns of drug-induced ILD are that of (1) a relatively acute hypersensitivity response that may be associated with compartimentalized airspace or peripheral blood eosinophilia or (2) a subacute to chronic form that may mimic some forms of IIP and have some degree of fibrosis (453, 454).

Numerous published studies have examined BAL cell profiles in drug-induced ILD (455-464). Although total cell count may be normal, it is usually increased. Increased numbers of neutrophils, eosinophils, and/or lymphocytes may be present in BAL, and mast cells and/or plasma cells may also be identified. Lymphocytes have frequently been examined for some ILD induced by drugs such as methotrexate, amiodarone, nitrofurantoin, or bleomycin, and a low CD4 to CD8 T lymphocyte ratio is frequently present, even if total lymphocytes/ml BAL fluid is not elevated (459, 462). However, a predominance of CD4 T lymphocytes has been reported with drugs such as methotrexate or nitrofurantoin (460, 465). The inflammatory cells that flux into the lung and that are retrieved in BAL fluids likely

reflect the type of inflammation induced by a particular drug combined with pro-inflammatory gene expression (i.e. cytokines and chemokines) of a given individual in response to a toxic drug reaction in the lung. Amiodarone causes characteristic changes in the alveolar macrophage population with foamy intracytoplasmic alterations corresponding to a form of phospholipidosis (462, 466).

The use of prescribed and over-the-counter medications needs to be carefully examined when patients are evaluated for ILD. The differential diagnosis is frequently complicated by the presence of disorders such as connective tissue diseases that can cause ILD, and pneumotoxicity may be superimposed on ILD that is already present. Nonetheless, radiologic evaluation including HRCT scanning can establish a pattern that suggests acute inflammation (ground glass attenuation), pulmonary edema, pulmonary hemorrhage, or chronic fibrotic changes caused by specific drug exposure. Bronchoalveolar lavage and BAL fluid analysis can establish the presence of pulmonary hemorrhage and/or lower tract infection. It can also detect an expansion of inflammatory cell populations that supports the diagnosis of a pneumotoxic drug reaction. The detection of large numbers of eosinophils or lymphocytes suggests inflammation that may respond particularly well to anti-inflammatory therapy.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for DILD:

AS AN AID FOR THE DIAGNOSIS:

- BAL cell profiles (e.g. BAL lymphocytosis or eosinophilia) in an appropriate clinical setting, especially when combined with HRCT findings, can support a diagnosis of drug-induced ILD.
- BAL analysis can be used to rule out other potential entities such as pulmonary hemorrhage or infection.

FOR PROGNOSIS: UNKNOWN

5.6.5. Radiation Pneumonitis

Therapeutic irradiation of thoracic malignancies can be complicated by radiation pneumonitis and radiation fibrosis. Symptomatic radiation pneumonitis usually manifests after 1 to 3 months of completion of radiotherapy and is observed in approximately 7 to 8 percent of patients (467, 468). Radiation fibrosis usually evolves over a 6 to 24 month period and may occur in patients who did not have manifestations of acute pneumonitis (467). Early histopathologic changes following lung irradiation, which generally occur within the first 2 months, are characterized by capillary and small vessel injury, vascular congestion, and increased capillary permeability with hyaline membrane formation (469). An intermediate stage at 2 to 9 months is characterized by pulmonary capillary obstruction and interstitial fibrosis with septal thickening which can subside with mild radiation injury. A chronic phase may arise, typically after nine months, with severe injury and is characterized by progressive septal

thickening and vascular sclerosis (470). Endothelial cell damage is thought to play a key role in pulmonary radiation injury, and host factors, such as impaired production of plasminogen activator by endothelial cells, may predispose to radiation fibrosis (471). Animal models of radiation pneumonitis generally show increased neutrophils and lymphocytes in BAL fluid (472, 473), and a study in patients demonstrated increased lymphocytes and eosinophils in BAL fluid from those who developed radiation pneumonitis (474). Increased BAL lymphocytes have been found in the controlateral lung following thoracic irradiation for breast cancer (475), suggesting that radiation pneumonitis may be, at least in part, immunologically mediated, perhaps as a response to autoantigens generated by the effect of ionizing radiation on lung tissue.

Because individuals who receive thoracic irradiation may have other causes of radiologic infiltrates, bronchoscopy with BAL may be necessary to exclude other causes of symptomatic pulmonary dysfunction such as pulmonary hemorrhage, diffuse spread of malignancy, pulmonary infection, or drug toxicity. The presence of increased inflammatory cells and exclusion of the aforementioned entities strongly supports radiation pneumonitis if the appropriate temporal association with thoracic irradiation is present. However, patients given radiotherapy often receive drugs that can induce lung inflammation and damage with associated increases in BAL inflammatory cells, making it difficult to determine the specific cause of perturbations in BAL cell differential counts.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for Radiation Pneumonitis:

AS AN AID FOR THE DIAGNOSIS:

- BAL can be useful in evaluating patients with ILD that may be linked to radiation injury by excluding confounding entities such as hemorrhage or infection.
- The presence of significantly increased numbers of lymphocytes cells combined with an appropriate clinical setting and HRCT findings can lend support to the diagnosis.

FOR PROGNOSIS: unknown

7. OTHER ILD

Various other disorders can alter lung parenchyma and cause diffuse changes on thoracic imaging, but these entities are relatively uncommon causes of ILD. These include lipoid pneumonia, chronic aspiration pneumonitis, lymphangitic or bronchoalveolar carcinoma and lymphangioleiomyomatosis (LAM). Diffuse pulmonary infiltrates may also be associated with, amyloidosis, pulmonary alveolar microlithiasis, bronchiolitis, giant cell pneumonitis, various metabolic

disorders (e.g. lysosomal storage diseases or Hermansky-Pudlak syndrome), or gastrointestinal disease (e.g. inflammatory bowel disease). Although BAL studies have been performed in patients with these latter entities, the data are relatively limited, and BAL analysis appears to be of limited diagnostic value.

Exogenous lipoid pneumonia, which is most commonly associated with mineral oil aspiration, can cause diffuse radiographic infiltrates and may be associated with granulomatous or fibrotic histopathology (476). Fat globules in alveolar spaces and AM are typically seen, and BAL will usually retrieve AM that have vacuolated cytoplasm and stain positive for fat with Sudan or oil red O stains (476, 477).

Pulmonary fibrosis has been associated with chronic, subclinical aspiration of gastric contents that occurs in patients with esophageal problems such as gastroesophageal reflux and/or hiatal hernia (476), and gastroesophageal reflux has been identified in many patients with IPF (478). Although semiquantative scoring of BAL AM via staining with oil red O has been described and associated with chronic aspiration (479), the use of this technique and scoring of staining intensity has not been validated. Although some investigators have reported a lipid-laden AM scoring method as useful to identify repetitive aspiration in children (480, 481), others have found such scoring to be non-specific and of limited utility in identifying children with chronic silent aspiration (482). Other markers, such as pepsin-like activity, may prove more valuable in the diagnosis of aspiration of gastric secretions (483). However, no studies have validated the utility of BAL pepsin concentrations in the diagnosis of gastric aspiration.

Bronchoalveolar carcinoma, lymphangitic carcinoma, or infiltration of the lung with bone-marrow-derived malignant cells can all give interstitial patterns on chest radiographic imaging. Bronchoalveolar carcinoma can be diagnosed via sputum cytology, examination of BAL fluid, or transbronchial lung biopsy, and surgical lung biopsy is usually unnecessary (476, 484). Lymphangitic carcinomatosis, which is usually caused by metastasis of adenocarcinomas from various organs, typically gives an interstitial pattern on chest radiography and often causes considerable thickening of interlobular septae on HRCT (485). Bronchial brushings, BAL analysis, or bronchoscopic lung biopsies usually show positive cytopathology or histopathology (486).

Lymphangioleiomyomatosis (LAM), which is either sporadic or associated with tuberous sclerosis and occurs almost exclusively in females, is a rare disorder characterized by smooth muscle proliferation leading to progressive respiratory failure due to cyst formation throughout the lungs (487). A presumptive clinical diagnosis of LAM can be made on the characteristic appearance of cysts on HRCT, particularly when extrapulmonary manifestations of LAM or angiomyolipoma are present (487, 488). The diagnosis can be unequivocally demonstrated by surgical lung biopsy or transbronchial biopsy that shows characteristic histologic changes combined with positive staining of smooth muscle cells with

the monoclonal antibody, HMB45 (487, 489, 490). Bronchoalveolar lavage does not contribute to the diagnosis and appears to have no role when LAM is clinically suspected, although it can be used to rule out other possibilities.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for other ILD:

AS AN AID FOR THE DIAGNOSIS:

Lipoid pneumonia

- BAL will usually retrieve AM that have vacuolated cytoplasm and stain positive for fat with Sudan or oil red O stains.

Chronic aspiration pneumonitis

- Semiquantative scoring of BAL AM via oil red O staining has been described and associated with chronic aspiration, but the use of this technique and scoring of staining intensity has not been validated.
- Some investigators have reported a lipid-laden AM scoring method as useful to identify repetitive aspiration in children, but others have found such scoring to be non-specific and of limited utility in identifying children with chronic silent aspiration.
- Other markers, such as pepsin-like activity, may prove more valuable in the diagnosis of aspiration of gastric secretions.

Bronchoalveolar carcinoma

- Can be diagnosed via sputum cytology, examination of BAL fluid, or transbronchial lung biopsy.
- Surgical lung biopsy is usually unnecessary for diagnosis.

Lymphangitic carcinomatosis

- Bronchial brushings, BAL analysis, or bronchoscopic lung biopsies usually show the malignant cells.

Lymphangioleiomyomatosis

- BAL does not contribute to the diagnosis and appears to have no role when LAM is clinically suspected.
- BAL can be helpful in ruling out other possible diagnoses.

Amyloidosis

- BAL does not provide diagnostic cell patterns.

Pulmonary alveolar microlithiasis

- BAL may be cloudy and clear only with low-speed centrifugation.
- Microliths may be seen in the BAL cell pellet.

Bronchiolitis

- BAL does not provide diagnostic cell patterns.

FOR PROGNOSIS: Unknown

8. CLINICAL UTILITY OF BAL CELLULAR ANALYSIS IN THE EVALUATION OF ACUTE ONSET ILD

Bronchoalveolar lavage findings can assist in the diagnosis of acute onset ILD. Acute onset of ILD was defined by the committee 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 ARDS (e.g. sepsis, trauma, aspiration). Diagnostic considerations include infection, non-infectious ILD (AIP, acute EP, DAH, acute HP, acute COP, pneumotoxic drug reactions, or radiation injury), 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 acute HP or drug toxicity, especially if accompanied by plasma cells and an appropriate exposure history. Lung injury due to severe pneumonia or DAD is associated with desquamation of type II pneumocytes (491), and reactive type II pneumocytes in BAL are commonly observed with drug reactions, respiratory infection, or other forms of acute lung injury (492, 493). Bronchoscopy with BAL at the time of acute presentation may facilitate diagnosis and minimize procedural risk if a diagnosis can be made that obviates the need to progress to a surgical lung biopsy.

The committee concludes the following regarding the clinical utility of BAL cellular analyses for diagnostic evaluation of acute onset ILD:

- Marked BAL eosinophilia supports the diagnosis of acute EP.
- BAL is useful in detecting alveolar hemorrhage syndromes or infection.
- Marked BAL lymphocytosis suggests HP or drug toxicity as the etiology in the appropriate clinical setting.

9. FUTURE DIRECTIONS:

Since its introduction into clinical investigation in the 1970's, it is clear that BAL has been an extremely valuable research tool for the retrieval and study of cells involved in lung inflammation, lower respiratory tract infections, and extra-cellular components in epithelial surface liquid. Research utilizing BAL has not only generated extensive data concerning the identity and functions of immune cells in the peripheral airspaces, but also identified mediators and other products derived from inflammatory cells and diseased lung tissue that play important roles in disease pathogenesis. Unfortunately, only a small portion

of this mass of research information has yet been shown to be of value to the practicing clinician in the diagnosis or management of ILD.

The fact that methods are still lacking to accurately assess disease activity, stage various diseases and render a prognosis, aid the selection of optimal approaches to treatment, or monitor response to therapeutic interventions constitutes a major problem that needs to be resolved through additional clinical investigation. Identifying specific and sensitive markers that can address these problems remains an elusive but vitally important target. Universal adoption of a reasonably standardized BAL procedure will undoubtedly improve the chance 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 indicate clinical disease activity, detect disease progression, provide a prognosis, and guide treatment strategies.

Another important clinical problem is the evolving importance of histopathologic patterns within the spectrum of IIP to define subgroups that constitute distinct disease entities that indicate different management approaches. Because of differences in prognosis and treatment, a large number of patients with possible IIP may need to undergo definitive surgical lung biopsy unless a reasonably confident diagnosis can be reached via other diagnostic testing. Under these circumstances, it is clearly important, whenever possible, to identify other diagnostic groups, such as those related to known etiologic agents, using less invasive procedures that avoid the use of the higher risk procedure of surgical lung biopsy. To minimize risk further, an important objective for research is to seek less invasive approaches to differentiate the distinct subgroups within the idiopathic forms of ILD. 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. 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 and provide information that can guide effective therapy.

Breakthroughs in knowledge that can lead to a better understanding of pathogenetic mechanisms in ILD will undoubtedly be facilitated by the identification of full genomic sequence information by the human genome project. 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. These cutting edge methods may eventually prove extremely useful in making an accurate diagnosis, identifying useful biomarkers that determine patients response to therapeutic interventions and predict clinical course, 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 being aided by the recent development of DNA microarray and other technologies that identify and monitor expression patterns of vast numbers

of genes (494, 495). In addition, recent advances in proteomics that allow mapping and identification of multiple protein expression patterns by 2D gel electophoresis, image analysis, protein spot transfer and mass spectrometry may allow the identification and quantatitation of products of genes linked to disease pathogenesis (496).

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 (497, 498), 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 (499, 500). Rottoli et al. (501) used protein mapping via 2-dimensional electrophoresis to construct protein maps from BAL fluid that could profile differences among IPF, systemic sclerosis, and sarcoidosis, and Sabounchi-Schüutt et al. (502) have used similar techniques to identify inflammatory serum markers of patients with sarcoidosis and speculated that proteins in BAL fluid and/or peripheral blood may eventually provide disease-specific markers. 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. 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 (503).

A global analysis approach has identified many genes with increased expression in IPF(495), and certain gene products that may play an important role in IPF pathogenesis and/or serve as disease biomarkers, such as pigment epithelium-derived factor (504) matrilysin (505), and osteopontin, have been identified using these methods. Thonhofer et al. (506) examined gene expression by stimulated BAL cells from patients with sarcoidosis and showed that over a thousand genes were up- or down-regulated, 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. Validation of this concept has been provided by Selman et al. (507), 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 (508). In lung transplantation, although the diagnosis of chronic rejection currently depends upon spirometry as a

surrogate marker to detect and grade it (509), techniques to diagnose chronic rejection via microarray analysis of BAL cell gene expression (508) or BAL fluid protein analysis via MALDI-TOF profiles (510) 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 acute cellular rejection (511).

In addition to profiling of lung tissue and/or 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 pharmacologic 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 pro-survival profile, while NFkB is up-regulated in sarcoidosis patents with progressive disease (512). Additionally, Rutherford et al. (513) have demonstrated specific peripheral blood gene expression patterns that correlate with self-limited disease. Such studies, especially when combined with examination of biopsy specimens and/or BAL cells, may allow the identification of circulating biomarkers, such as osteopontin in peripheral blood (514) that may eventually prove useful in clinical practice.

Despite significant diagnostic potential, very few patients have been investigated so far using gene and protein mapping approaches. The applications of the powerful techniques of gene and protein mapping to the study of interstitial lung diseases are still at a very early stage. To exploit their full potential in the future will require access to large collections of well-characterized clinical samples. Collections of blood, biopsies and BAL samples can all make important contributions to our understanding and differentiation of various forms of ILD.

Standardization of the clinical procedure of BAL will undoubtedly contribute greatly to the use of BAL in the diagnosis of specific ILD and allow comparison of BAL data among centers around the world. The Committee strongly encourages the adoption of the technical aspects of performing and analyzing BAL fluid for cellular analyses outlined in this statement by pulmonologists and laboratories worldwide.

BRONCHOALVEOLAR LAVAGE CELLULAR ANALYSIS REQUEST FORM

Patient Information:
Name: _____
Med. record #: _____
Date of Birth: _____
Age: ___ Gender: M F
Current smoker: Yes No
Ex-smoker (quit >2 yrs): Yes No
Remote smoker (quit >20 yrs): Yes No
Never smoker: Yes No
Bird exposure? Yes No
Coagulopathy? Yes No

Other Required Information:
A. Date of procedure _____
B. Time of procedure _____
C. Name of physician ordering BAL_____
D. Ordering physician contact information_____

A. Additional Information:
1. Pertinent clinical features: _____
2. Radiographic/CT pattern: _____
3. Preliminary diagnosis: _____
4. Drug-induced? _____
5. Lung (R or L) and area lavaged (e.g. RML, lingula, etc.):
□ Right Lung □ RML □ RLL □ RUL
□ Left Lung □ Lingula □ LLL □ LUL (not lingula)

6. Total volume instilled (mL): _____
7. Total volume of BAL fluid retrieved (mL): _____
8. Volume of BAL fluid sent to laboratory: _____

B. BAL cell analysis is optimal if an adequate volume (e.g. 10-20 mL) is available for testing. If BAL volume is limited, please identify tests that **must** be performed and prioritize your requests.

____ Cell count and differential (Wright-Giemsa stain)
____ T lymphocyte subsets by flow cytometry
____ Hemosiderin stain
____ Other testing (specify): _____

C. Tests for infectious agents: use separate microbiology forms as needed

D. Malignancy suspected? Send aliquot to pathology

D. Specimens must be promptly transported to the laboratory at room temperature and must not be frozen. If not analyzed immediately, BAL cell suspensions can be kept at 4°C for up to 2 hours in nutrient-poor media (normal saline). Specimens for BAL cellular analysis can be maintained in nutrient-supplemented media for up to 24 hrs.

BRONCHOALVEOLAR LAVAGE CELLULAR ANALYSIS FINAL REPORT FORM

General Information:

Patient name: _____
Med. record #: _____
Date of Birth: _____
Date of procedure: _____
Requesting physician name: _____

1. Volume of original BAL fluid received and analyzed: _____
2. Appearance of original BAL fluid (color and turbidity): _____
3. BAL cell profile:

Viability of nucleated cells (% of total):	_____
Total nucleated cell count (per μL)	_____
Macrophages (% of differential)	_____
Lymphocytes (% of differential)	_____
Neutrophils (% of differential)	_____
Eosinophils (% of differential)	_____
Epithelial cells (% of differential)	_____
- Type: squamous ___ bronchial ___	
Mast cells (% of differential)	_____
Other cell type (% of differential)	_____
- Type: _____	

Flow cytometry:	% CD4 positive	_____
	% CD8 positive	_____
	CD4/CD8 ratio	_____

BAL cell profile reference values:		
	% Macrophages	[80-100]
	% Lymphocytes	[0-15]
	% Neutrophils	[0-3]
	% Eosinophils	[0-1]
	% Epithelial cells	[0-5]
	CD4/CD8 ratio	[0.9-2.5]

BAL cell profile interpretation:

Sample adequate? Yes No

Additional comments:

BAL cell profile interpretation provided by: _____

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