Use of Bronchoalveolar Lavage in Humans—Past Necessity and Future Imperative

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Abstract. Limited bronchoalveolar lavage (BAL) as an extension of fiberoptic bronchoscopy has permitted the recovery of airway-alveolar space cells and soluble substances in the extracellular lining fluid that have been used diagnostically and as research specimens in patients with a variety of lung diseases and in normal subjects for the study of lung host defenses. During the past three decades, use of BAL specimens has stimulated immunologic and cellular research of pulmonary diseases, which has provided significant insight into local host immunity, inflammation, fibrogenesis, asthma mechanisms, and infections. From this research new methods of antifibrotic therapy of interstitial pulmonary fibrosis, for example, have followed. Moreover, BAL applications have greatly enhanced professional interest in the field of pulmonary medicine. This review attempts to analyze the history and impact of BAL, appraise its current status, and assess its future usefulness. Understanding the immunopathogenesis of many lung diseases is predicated on obtaining in situ specimens from affected lung tissue and airways. BAL provides a direct sample that can be compared with an endobronchial or transbronchial biopsy tissue specimen and with cellular and immunologic components in the vascular circulation. Thus, the recovery of BAL fluid and its components involved directly with a disease process or contiguous with interstitial tissue permits a much more detailed assessment of new cellular mediators and cytokines participating in the pathologic process. Furthermore, subjecting BAL cells to microarrays of DNA to discern what genes are activated will be one step closer to identifying intracellular processes involved or deranged. Identification of causative factors may solve questions of causation, so that preventive strategies or definitive therapy can be used.

Key words: Bronchoalveolar lavage—Fiberoptic bronchoscopy—Diffuse interstitial lung disease
Introduction

Through the rigid bronchoscope, optimally designed almost a century ago by Dr. Chevalier Jackson (1904) [92], washing a portion of the lungs to remove secretions could be done therapeutically. More extensive lung lavage became treatment for patients with alveolar proteinosis [82] and other respiratory illnesses that featured extensive accumulation of purulent secretions as found with cystic fibrosis, chronic asthmatic bronchitis, and bacterial pneumonia [104]. This method of using a large volume bronchopulmonary lavage (BAL), termed “bronchiolalveolar debridement” [82], was performed through a double-lumen bronchospirometry tube (Carlens tube) [7] with saline fluid and was the established treatment for alveolar proteinosis [104].

To study the physiologic effects of small volume bronchopulmonary lavage, Finley and colleagues [29] lavaged seven healthy volunteers (average age, 27 years) and four patients with obstructive lung disease. A Métras catheter [69], 19F size, was passed into the locally anesthesized airways of awake subjects and anchored in a segmental bronchus under fluoroscopic control. The lung segment was lavaged with 300 mL (100-mL aliquots) of normal saline, after which lung function, arterial oxygenation, and chest film changes were monitored. This study illustrated the feasibility and safety of doing small volume bronchopulmonary lavage in normal subjects. Pratt and colleagues [80] extended the study of endobronchial lavage through a Métras catheter in healthy volunteers, enlisting 16 subjects, equally divided as smokers and nonsmokers. Thereafter, more studies followed [11, 37, 60], using smoker and non-smoker normal subjects, to compare the cellular function of alveolar macrophages in these groups and the yield of surfactant material [28]. Endobronchial lavage was performed through a Métras catheter in these studies. Bronchial washings from intubated surgical patients were aspirated and analyzed for immunoglobulins as well [49, 64].

Clearly, cellular immunology of the lower airways was beginning to be investigated in humans, perhaps stimulated by the innovative work of Myrvik and colleagues [72] to obtain alveolar macrophages with lung lavage in rabbits. This review will summarize first (Fig. 1) past methods that provided access to human airway materials, how these became important in studying respiratory tract host defenses in the context of newly evolving immunologic insights, and how BAL fluid (BALF) and its analysis was first applied to a group of important diseases of the lungs. Then future applications for BAL will be considered.

Retrospectoscope—A Look at the Past

A confluence of several research streams occurred about 30 to 35 years ago: new insights were numerous about cellular and humoral immunity; research emphasis shifted to understanding the host’s response, especially local defense mechanisms of the respiratory tract, as emphasized by Green [36], but that were still based largely on animal model research; and procurement of human respiratory cells was now feasible with safe methods. Thus, explanation of human respiratory immunology in normal subjects was underway, and investigators were poised to take advantage of a powerful technologic advancement—bronchofiberscopy developed by Dr. S. Ikeda and collabo-
rators in Japan [47]. This new instrument would permit routine sampling of cells and secretions from the airways and alveoli of normal research subjects and the affected airway surfaces of patients with various lung diseases.

After introduction of the flexible bronchofiberscope into US and Western European medical centers around 1970 [46, 47], the practice of fiberoptic bronchoscopy (FOB) grew quickly after initial guidelines were described [108, 113]. For research, BAL was used initially to retrieve macrophages from normal volunteers [6, 134] for functional studies, but its use expanded to sample other cellular and soluble components in the fluid [18, 99, 127]. Using volunteers, the impact of cigarette smoking to alter BALF components was examined [129], and the origin of individual substances in BALF such as immunoglobulins, began to be investigated [53]. Analysis of other soluble components was added [58]. As a personal example of exploiting this new technique to readily obtain human BAL cells and protein materials, we had completed an assessment of respiratory host immune responses in a rabbit model [100, 101] and were able to shift immediately into human immunology [95, 98, 99].

Our preliminary study [99] established baseline values for both cells and immunologic components in a specimen of BALF obtained from the lingula or lower lobes of young normal smokers and nonsmokers and from middle-aged smokers and non-smokers undergoing diagnostic evaluation for an isolated upper lobe lesion. Then, we applied this systematic analysis to groups of patients being evaluated for diffuse in-
terstitial lung disease (DILD), especially idiopathic pulmonary fibrosis (IPF) and chronic hypersensitivity pneumonitis (CHP) [16, 97]. For 29 patients with IPF, 19 patients had BALF analysis; for 17 of these 19 patients with IPF, the diagnosis had been established by histopathologic examination of lung biopsy specimens [16, 97, 122]. Of these patients, 7 were untreated and 12 were receiving oral corticosteroids when BAL was performed; 7 were current cigarette smokers. Among BAL cells, a characteristic pattern was noted in patients on corticosteroid treatment of an increased percentage of polymorphonuclear neutrophils (PMN) (mean about 14% with range of 3–40%) and of eosinophils approximately 4%. For untreated patients with IPF, the mean PMN percentage was even greater, but eosinophils were about the same. Low-grade eosinophilia in BALF persisted despite prednisone therapy. The combined finding of more PMNs and eosinophils in BALF from IPF patients was even more striking when compared with values from control smokers and nonsmokers. Other immunologic changes such as a higher ratio of IgG to albumin and more monomeric form of IgA were noted [97].

This pattern of BAL cells with an increased percentage of PMNs and an even higher percentage eosinophils in BALF was also found by Haslam and colleagues [41] in patients with cryptogenic fibrosing alveolitis (CFA). A small number of these patients had a lymphocytosis in BALF. Also, Davis and colleagues [24] described cellular profiles in 16 patients with diffuse interstitial lung disease; they described changes of lymphocytosis in these patients. This abstract appears to have been the first published report of BAL cell profiles in a group of patients with DILD.

In contrast to IPF, BAL cells and protein in fluid from seven patients with chronic hypersensitivity pneumonitis (CHP) [97] disclosed distinctive “foamy” cytoplasmic-appearing alveolar macrophages, a very high percentage of lymphocytes, about 60% of BAL cells, which were predominantly T-cells, a high IgG/albumin ratio, and IgM, an immunoglobulin not detected in BAL fluid of normals or from IPF patients. IgG precipitin antibodies against relevant etiologic microbial antigens could be detected in some patients. In BAL samples from CHP patients, eosinophils and IgE values were not different from controls, nor were values of two components of complement, C4 and C6. Therefore, an active process of cellular and humoral immunity in CHP was evident, which did not include reagin-mediated type I factors found with atopic-allergic respiratory disease [90]. Thus, BAL components provided a distinctive profile of cells and immune factors that helped with disease diagnosis and gave insight into local airway immunopathogenic mechanisms. Whether lavage analysis would “prove useful in following the effect of therapy on these disorders” (IPF, CHP) [97], we were not certain, and this has required critical studies from others, which will be addressed [41, 50, 107, 125, 130].

Although diffuse interstitial pulmonary fibrosis had been well described and often discussed in pathologic and physiologic terms [9, 56, 110], these diseases began to be freshly re-examined by investigators in the Pulmonary Branch, National Heart, Lung, Blood Institute of the National Institutes of Health, Bethesda, Maryland [16] and also at other academic centers [24, 133] with new immunologic and biochemical methods, seeking an origin by dissecting the immunopathology of these (still) enigmatic lung diseases. BAL became part of the diagnostic approach and a means for obtaining
airway-alveolar substances to study in vitro. Fiberoptic bronchoscopy with BAL plus the capability of performing transbronchial biopsies [55] contributed several things:

1. With the reasonably minimally invasive method of fiberoptic bronchoscopy available to visually inspect, sample by BAL, and biopsy endobronchial and parenchymal tissue of patients undergoing diagnostic evaluation, initial and repeated studies could be done for longitudinal monitoring of disease activity. Excellent safety of bronchoscopy and BAL was documented for patients with forms of ILD [120] and mild asthma [85].

2. BAL-retrieved cells and fluid were used for research studies that provided insight into the immunopathogenesis and inflammatory processes of DILD.

3. This combination of an improved method potentially for diagnosis and the recovery of clinical specimens for investigation obtained directly from affected lung airways literally ignited widespread interest worldwide in the study of diffuse interstitial lung diseases. This was evident in the late 1970s and decade of the 1980s from the proliferation of BAL-related research publications (Fig. 2) from medical centers across the United States; in Europe from England, France, Italy, and Germany; and from Japan. As expressed [4], BALF was “a new material in pulmonology.”

The laboratory of Dr. Ronald G. Crystal in the Pulmonary Branch, NHLBI of NIH
Fig. 3. One of the original locations for BAL-oriented investigation of lung diseases was at the National Institutes of Health in Bethesda, Maryland, where patient-directed BALF studies were begun in 1974. This research approach was picked up or adapted, or evolved concomitantly, in many places (●) or was disseminated with members of the Pulmonary Branch, NHLBI, who left to relocate in other medical centers (★) in the decade that followed the clinical application of BAL.

was an original site for lung studies using BAL [16, 97] and contributed to a ripple or radiating effect across the “pulmonary” world. This is documented by the medical staff and trainees who were attracted to his laboratory and subsequently returned to their original center or relocated in other pulmonary groups in the decade that followed BAL’s clinical description and popularization (Fig. 3, location designated by ★ symbol). Many of these pulmonary physicians are readily identified as contemporary leaders in lung medicine worldwide (Appendix I). Others did not come to NIH in Bethesda but were located in other centers that had derived their research methods from the original NIH group, or were made up of other senior scientists who adapted the BAL approach (Fig. 3, ● symbol) (Appendix II). This BAL phenomenon was illustrated by the organization of the first international conference on BAL in 1979 to discuss research and clinical applications [54]. This scientific gathering in Lille, France, featured presentations by investigators from France, Italy, The Netherlands, Canada, Sweden, and the United States.

Critique of BALF as a Diagnostic Pulmonary Test

Aside from important research insights derived from the study of BAL components, the analysis of BALF seemed to indicate that certain cellular profiles correlated with
several types of lung disease that might be especially helpful in clinically differentiating between forms of DILD. This suggested that a new diagnostic test might be incorporated into the bronchoscopy procedure. Thereafter, several articles presented a methodologic approach for BAL analysis, giving characteristic findings associated with several diseases [22, 44, 131].
### Appendix II

Other initial investigators/proponents BAL

| Country     | Names                                           | City, Country           |
|-------------|-------------------------------------------------|-------------------------|
| Canada      | Yvon Cormier, MD                                | Québec, Canada           |
| Austria     | Heinrich H. Klech, MD                           | Vienna, Austria          |
| England     | Margaret Turner-Warwick, MD, PhD, Patricia L. Haslam, PhD | London, England          |
| France      | M. Perrin-Fayolle, MD                           | Lyon, France             |
|             | Claude Molina, MD                               | Clermont-Ferrand, France |
|             | C. Voisin, MD                                   | Lille, France            |
|             | Andre-Bernard Tonnel, MD                        |                         |
|             | Jean Bousquet, MD                               | Montpellier, France      |
|             | F. B. Michel, MD                                |                         |
|             | Phillippe P. Godard, MD                         |                         |
|             | J. Bignon, MD                                   | Créteil, France          |
|             | Gerard J. Huchon, MD                            | Paris, France            |
|             | F. Basset, MD                                   |                         |
|             | Jacques Chretien, MD                            |                         |
| Germany     | Ulrich Costabel, MD                             | Freiberg, Germany        |
| Italy       | Carlos Albera, MD                               | Torino, Italy            |
|             | Bruno Balbi, MD                                 | Veruno, Italy            |
|             | Venerino Poletti, MD                            | Bologna, Italy           |
|             | Gianpietro Semenzato, MD                        | Padua, Italy             |
| Japan       | Takateru Izumi, MD                              | Kyoto, Japan             |
|             | Sonoko Nagai, MD                                |                         |
| Sweden      | Leif H. Bjermer, MD                             | Umea, Sweden             |
| USA         | Herbert Y. Reynolds, MD                         | Laboratory of Clinical Investigation, National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD |
|             | Harold H. Newball, MD                           | Baltimore, MD            |
|             | William W. Merrill, MD                          | New Haven, CT            |
|             | John A. Rankin, MD                              |                         |
|             | J. Bernard L. Gee, MD                           |                         |
|             | Richard A. Matthy, MD                           |                         |
|             | Yves Sibille, MD                                |                         |
|             | Allan Cooper, MD                                |                         |
|             | Randy K. Young, MD                              |                         |
|             | Robert B. Fick, Jr., MD                          |                         |
|             | Gerald S. Davis, MD                             | Burlington, VT           |
|             | Robert B. Low, PhD                              |                         |
|             | Ronald P. Daniele, MD                           | Philadelphia, PA         |
|             | Milton D. Rossman, MD                           |                         |
|             | James H. Dauber, MD                             |                         |
|             | Robert P. Baughman, MD                          | Cincinnati, OH           |
|             | Talmadge E. King, MD                            | Denver, CO               |
|             | Thomas R. Martin, MD                            | Seattle, WA              |
|             | Ganesh Raghu, MD                                |                         |
|             | Henry M. Yeager, MD                             | Washington, DC           |
From enumeration of cells in BALF, seemingly characteristic patterns were evident, for example, in IPF [16, 40], of more PMN and eosinophils, in hypersensitivity pneumonitis of a very high proportion of T-lymphocytes [16, 97], especially the suppressor subset CD8 [53A]; in sarcoidosis [20, 43, 133] of increased T-helper cells and a high CD4/CD8 ratio, and in both "lone" CFA and collagen-vascular disease with associated lung involvement, a high lymphocyte percentage [41]. All these studies suggested that cellular analysis might be reasonably diagnostic and possibly could supplant other more invasive biopsy procedures.

Although soluble components in BALF seemed distinctive for certain diseases, this aspect of an immediate clinical analysis was not emphasized because measurement of immunologic and biochemical components would be delayed and not as quickly reportable as cell pellet differential counts. Concentration of the specimen and the problem of not having a reliable denominator substance against which to express values for soluble components made interpretation more difficult in the clinical context. In normal subjects or in the absence of tissue inflammation, the concentration of albumin was a fairly reproducible value in BALF [99], but less so when alveolitis and airway inflammation existed. This prompted investigation for a better marker that would estimate the volume of extracellular fluid, accounting for the dilution by the instilled lavage fluid. Many substances have been proposed, and urea [88] was promising, but even its use became problematic [48, 61].

Over the decade after these initial and generally more descriptive diagnostic uses of BALF analysis in the spectrum of interstitial lung diseases [22, 44, 131], an impressive number of subsequent editorial or review-type publications about BAL appeared [13–15, 17, 19, 21, 30–33, 38, 42, 45, 52, 62, 63, 66, 68, 71, 83, 87, 91, 96, 103, 105, 106, 115, 117, 123, 124]. The use of BAL analysis was extended to some rarer lung diseases [38]. Statements from task forces representing the major respiratory societies were published [35, 51]. A book about BAL soon followed [5].

In retrospect, the initial enthusiasm to use BAL analysis as a diagnostic clinical test was probably premature. Certainly, expectations become too great without an agreed approach for standardization and additional clinical research that would make the “test” more reliable, simpler, and adaptable for general use. As discussed [92], on the basis of preliminary use, BALF analysis was considered at one extreme to represent a liquid biopsy of the lung and at the other end, more modestly, the equivalent of a complete blood count or cerebrospinal fluid analysis obtained from venous blood or a lumbar spinal puncture. Actually, BALF analysis more nearly approximated a microscopic analysis of a random urine or stool specimen. Although both a urine and stool specimen are affected by dilution and/or concentration factors, neither has the complexity of an infused volume being added directly to the potential specimen before being collected or retrieved, as is the case with BALF.

As a “clinical test,” BALF analysis introduced several questions that would need to be resolved before wide-scale application could occur or succeed: First, was BAL sampling reproducible and of sufficient specificity that analysis would be an accurate or sensitive indicator of pathologic events occurring throughout the airways? Restated, would BAL cells, putatively reflecting alveolitis, necessarily correlate with contiguous tissue histopathology contained in transbronchial biopsy specimens taken during the same procedure in areas sampled by lavage, or with tissue obtained later by open lung
biopsy? Second, could laboratory processing of BALF, an unwieldy, large-volume specimen, be simplified by a hospital’s clinical pathology laboratory? Third, what costs were realistically attributable to the analysis, and how much would these increase the technical bill for bronchoscopy? Related also was how much professional charge could be added to the fiberoptic bronchoscopy procedure, and what was a reasonable fee for clinical interpretation of BALF results, such as applied to pulmonary function testing? The issue of costs for BALF analysis was addressed in only a few of the review or editorial articles about BAL [21, 92, 123, 124]. Fourth, did serial BALF results correlate with other indicators of the patient’s response to treatment? For example, the first serial study of BAL in patients with CFA was not published until 1987 [125]. These issues have been variously addressed in the series of perspectives on BALF just listed and by another Task Force report from the European Respiratory Society, published in 1989 [121].

Technical improvements in the procedure of BAL or in processing cells and fluid continued to be offered, and some made lavage more reproducible. Several were significant, such as optimal conditions for the fluid to be infused [79] and a better understanding of the kinetics of cells and soluble substances recovered in sequential lavage fluid aliquots [23, 67, 89], thus how to use (or exclude) the first lavage return specimen. Likewise, the need to standardize the loss of lymphocytes in the distribution of cells during cytocentrifuge preparation was reported [109]. Although many technical suggestions have been made in the review articles, these were particularly well collected and critiqued in the Task Force report edited by Drs. Klech and Pohl [121].

It was important to determine whether the profile of BAL cells did resemble the in situ alveolitis or parenchymal changes found in histopathologic examination of contiguous lung biopsy specimens taken from areas adjacent to the lavage sites. Haslam and colleagues [41] reported 18 patients with CFA, considered to have UIP, who had a BAL in the right lung 2 weeks before an open lung biopsy in the right lower or right middle lobes, areas similar to the site lavaged. Three other patients had asbestos lung disease. A portion of the biopsy specimen was fixed for pathologic review, and in 12 patients with the remaining specimen, cell extraction studies were done with a tissue chopping and pipette dispersion method (no enzyme digestion was used) to recover inflammatory cells. Comparisons between cells in lung washings and biopsy extractions in 12 patients reached statistical significance for neutrophils, and were reasonably good for eosinophils and lymphocytes. Between the extraction cell results and biopsy histologic scores, a significant correlation was found only for lymphocytes and not for inflammatory cells. In an expanded series of 20 patients (12 with lone CFA and 8 with collagen-vascular disease) [38], percentage counts of neutrophils and eosinophils were clearly higher in BALF than biopsy cell extracts; whereas, lymphocytes were higher in the cell extracts (Figure 4). In another study, Haslam [38A] used immunocytochemical staining to identify B and T lymphocytes in CFA biopsies which were of different proportions in tissue infiltrates and BAL; five CFA patients with elevated B-cells in tissue did poorly, clinically.

Hunninghake and colleagues [45] performed a similar kind of analysis in nine patients with IPF, six patients with sarcoidosis, and six nonsmoker control subjects undergoing lung surgery for a solitary lung nodule. To identify inflammatory and immune effector cells, BAL cells were compared with lung biopsy material dispersed
into cell suspensions with a teasing and filtering method. They concluded that the alveolitis of these lung diseases sampled by BAL did reflect the inflammatory and immune effector cells in lung parenchyma. Perhaps, a more conclusive approach to determine whether BAL cells reflected the histologic changes in lung tissue (transbronchial biopsies) was performed by Semenzato and colleagues [112] in patients with sarcoidosis (n = 26) and hypersensitivity pneumonitis (n = 7). BAL cells were compared with immunohistochemical staining of tissue sections with specific monoclonal antibodies. This method avoided the tissue maceration problem and permitted a topographic assessment of immune and inflammatory cells. Generally, the correlation between the percentage of lymphocytes and macrophages in lung biopsy specimens with their respective cell types in BALF was quite good.

Because the BAL technique washes down conducting airways distal to the tip of the wedged bronchoscope into the alveolar units, BALF in the aspirated sample is a composite mixture collected from both distal conducting airways mucosa and alveolar spaces [92]. It has been surprising to me, but reassuring nevertheless, that BAL cellular analysis reflected cellular changes in adjacent tissue as well [40, 45, 112]. This was an important correlation to establish that added more validity to the diagnostic potential of BALF for diffuse interstitial lung disease but also demonstrated a difference between alveolitis and adjacent interstitial tissue cells in some situations.

Because of widespread interest among pulmonary clinicians to include BAL analysis and its interpretation as part of diagnostic FOB, we undertook to determine a fair price for the technical analysis and to evaluate the logistics of providing this laboratory service [84]. BALF was an unwieldy sample to process and one that was obtained infrequently at most hospitals or surgical outpatient centers where elective FOB was done. Moreover, it required some urgency in processing and did not lend itself to automated measurements. There remained a secondary benefit that cells or other lavage components could be used for research if processed readily.

As a service for pulmonologists located within a geographically contained area (ie, about 50 miles to the most distant hospital or a one hour maximum transport time), who were also associated through an affiliated hospital network around New Haven, Connecticut, a central laboratory processing and analysis protocol was established [84]. The objective was to use a standard approach to BAL analysis that would provide reliable information in a timely manner to clinicians (results communicated within 2–24h after the lavage sample received) that would help with subsequent diagnostic decisions for patients with an unknown form of DILD. The impact of this analysis on the pulmonologists’s subsequent management of the patient was also assessed [119].

The logistics of transporting the BAL specimens and standard analysis of them that followed revealed several interesting things about lung cells in the aspirated BAL fluid samples. BAL cells were hardy and survived well, even ingesting the few nasopharyngeal-contaminating microbes that inevitably are in lavage fluid [99], so that adding an antibiotic to the specimen was not necessary for bacteriostasis if processed within a reasonable period (4 h). Enough glucose was washed off/out of the airways and concentrated in BAL to provide sufficient nutrients for cells (approximately 40 mg/mL glucose in unconcentrated lavage fluid), such that good viability of cells was maintained for up to 4h. This facilitated subsequent use of the cells for in vitro research cell cultures. The initial cost for cell counts, cellular analysis of T-lymphocyte subsets, and
soluble liquid phase components (protein, immunoglobulins, and lipids) was about $110 plus labor costs of about $275. This total of just less than $400, reflecting costs of reagents and technical labor in the early to mid 1980s [84], would be more now. There was no billable professional fee for the lavage procedure added to bronchoscopy. At present, this fee for basic FOB is $598 at The Milton S. Hershey Medical Center, and an additional $60 can be added for bronchial lavage; for transbronchial biopsies another $113 can be charged. The technical charge for a diagnostic bronchoscopy is $422. Thus, added costs for lavage and the BALF analysis are within reason and justifiable if the results would contribute to better diagnostic accuracy and more cost-effective patient management [21]. An important outcome of BALF analysis would be if it helped with clinical diagnostic reasoning and with monitoring disease activity or progression.

The impact of BALF analysis on pulmonary clinicians’ diagnostic evaluation of patients with DILD was assessed by Stoller and colleagues [119]. For pulmonologists submitting BAL specimens to a central laboratory for processing [84], 93 of these specimens were accompanied by a questionnaire completed by the referring physician when the BALF was initially submitted to the laboratory, and another questionnaire was completed immediately after results were returned, which was within a 2- to 24-h period. Results of other diagnostic tests done at FOB, such as microbial cultures and pathologic interpretation of transbronchial biopsy specimens, would not yet have returned; thus, a specific final diagnosis would rarely be available on the basis of other laboratory data before this second questionnaire was completed by the pulmonologist. The objective of the study was to judge whether the clinician’s diagnostic reasoning was affected or subsequent approach modified. How would a test such as BALF analysis affect the relative likelihood of several contending diagnoses that the clinician had generated in his/her differential diagnosis? Admittedly, BALF differential cell counts alone do not identify conclusively a specific DILD, because most single laboratory tests are not pathognomonic unless a specific cytologic or serologic value is present that definitively defines a disease as in some rarer diseases [38]. However, would the analysis of differential cell counts alter or reinforce diagnostic impressions or add certainty? Seventy-eight of 93 paired questionnaires (84%) were evaluated and three findings were noted [119]: (1) when the first and second questionnaires were compared, in 59% (n = 48/78 patients) at least one diagnostic change had occurred; (2) the type of diagnostic change made was in the level of confidence given to a particular diagnosis (38 of 77 or 49%); and (3) the diagnostic change recorded was usually considered appropriate in relation to the final diagnosis. In 24 patients in whom the diagnostic change was considered to be appropriate, several changes were significant, especially in patients suspected of having sarcoidosis. In two patients BALF results precluded the need for planned surgical lung biopsy procedures, and another patient with presumed sarcoidosis actually had acquired immunodeficiency syndrome and an opportunistic lung infection that gave a diffuse interstitial appearance on chest imaging studies. This was a small and perhaps modest study but was an example of the kind of prospective impact BALF assessment might have on clinicians who were evaluating patients with unknown forms of DILD [117].

Two contemporary studies [73, 81] that assessed the accuracy of clinicians’ diagnosis of patients with DILD, using results of high-resolution lung scans and expert
clinical acumen, indicated that clinical diagnosis is still not very good. Reliance on high-resolution computed tomography (HRCT) for a correct diagnosis among the top three choices overall was about 60% [73]. BAL analysis was available to help ascertain the diagnosis for only 22 of 134 cases (16%) [73]. Combining expert clinical evaluation and HRCT, approximately 30% of patients were not accurately diagnosed with new-onset IPF and still had to undergo a selective open lung biopsy [81]—still a very invasive procedure. BAL analysis was not included in the assessment with this protocol [81], because these investigators believed that the usefulness of a BALF cellular profile was controversial as a diagnostic tool and was not done routinely in their geographic area; this was based on their published studies also. But could the use of BAL results have added confidence to clinicians’ reasoning, as found by Stoller [119]? Future clinical studies that include BALF analysis with new diagnostic modalities such as HRCT scan might decrease the necessity for mandatory lung biopsies.

Apart from its value as an aide to diagnosis, BAL analysis was anticipated to help with management as a prognostic indicator or to monitor a response to treatment. However, these issues are controversial and unresolved. What bedeviled BAL cellular analysis early on and singey had the most impact on largely discrediting it as a clinical laboratory test for monitoring patients’ responsiveness to corticosteroid therapy for IPF (or CFA) was the enumeration of PMNs in an initial or serial BALF samples. Originally, the distinctive cellular findings in BALF from IPF (lone CFA) patients were the combination of an elevated percentage of PMNs and of eosinophils noted in several studies [16, 39A, 41, 97]. Of all the cells to be identified readily and counted on the stained cell (cytocentrifuged) sample, PMNs are the easiest to recognize; this has been realized and reported as the neutrophilic alveolitis characteristic of IPF [15]. The counterpart in the blood cell differential of an elevated PMN count or leukocytosis, especially with a left shift to more immature cell forms, is ingrained as a sensitive parameter to monitor for systemic or local organ infection and for subsequent change with antibiotic or other therapy. So it was with the BALF cell PMN percentage. Certainly, patients already on oral prednisone as therapy for IPF at the time of an initial BAL did have lower PMN percentages than some untreated patients and a higher percentage than control smokers (Fig. 1, Ref. 97); the percentage of eosinophils was unaffected by corticosteroids. Likewise, for 36 patients with CFA (most with “lone” fibrosing alveolitis among the group receiving treatment), 24 patients received BAL before treatment. The neutrophil counts (as percentages) tended to be lower in the responder patients, especially in those receiving prednisolone, although not attaining statistical significance (Fig. 3, Ref. 107). However, the most important findings were that lymphocyte counts were significantly higher in patients responding to prednisolone, while eosinophils in BALF were much higher in the non-responders, confirming earlier findings [41].

Keogh and colleagues [50] treated the alveolitis in a group of mid-stage IPF patients for 6 months with a low dose of oral prednisolone (0.25 mg/kg daily, n = 8 patients) contrasted with high-dose therapy (oral regimen plus 2 g/weekly of methylprednisolone intravenously, n = 5 patients). Results indicated that four of the patients receiving high-dose treatment had a reduction in the BALF percentage of PMN, a mean of 46% below their baseline values. Other cells, lymphocytes and macrophages, and total cells did not change significantly between the groups. Eosinophils were not
reported. Thus, the neutrophil component of the alveolitis responded to a high dose of corticosteroid treatment that included oral and parenteral administration.

These prior studies were point in time [41, 97] or seemed to be preliminary [50], and a more detailed longitudinal study was required to clarify the issue whether inflammatory cells, especially neutrophils, in the BAL cellular profile would decrease as clinical improvement occurred, and if the initial lavage cell counts would predict patient progress or response to therapy. Drs. Turner-Warwick and Haslam [125] prepared a valuable report about serial BAL analyses and clinical progress in 32 patients with CFA (27 patients had lung biopsy confirmation), of whom 26 had “lone” fibrosing alveolitis and no connective tissue disorder. The patient treatment regimens were with prednisolone at a high dose, orally 60 mg/day, or a low-dose 20 mg orally on alternate days plus cyclophosphamide, 100 to 120 mg daily. Importantly, 23 of 26 patients with “lone” fibrosing alveolitis were untreated at the time of the first lavage; follow-up lavages could occur at 3 to 6 months and at 12 to 18 months, but a mean of three lavages were done (range, 2–5) for each of the 32 patients. Patient improvement, classified into four groups at 1 year follow-up, was assessed clinically and by a breathlessness questionnaire, chest radiographs, and lung function tests. For predictability of BAL cellular results, Group 1 of the responders with lone CFA to prednisolone (n = 4) or cyclophosphamide (n = 4) therapy were of note, between an initial lavage and follow-up. For those receiving prednisolone, the total percentage of all inflammatory cells (lymphocytes, neutrophils, and eosinophils) decreased from an initial percentage of 48 to 14.5, with selective reductions in the individual percentages of lymphocytes (13.5–4.0%), neutrophils (23.5–6.0%), and eosinophils (4.0–1.0%). These cellular changes did not occur for the cyclophosphamide group. Overall, the conclusions reached for the predictive value of BAL cellular profiles and patient response were conservatively presented and are repeated briefly. Characteristics of the alveolitis were confirmed, as in prior findings [41, 107], that some patients had an initial increase in lymphocytes (8 of 32 patients) and most of these (7 of 8 patients) responded or had an initial response to therapy. The cellular pattern of an increase of both neutrophils and eosinophils (17 of 32 patients) was found, and the therapeutic response was less good, but better with cyclophosphamide. As a comment, the role of the eosinophil is perhaps a more important ingredient in the alveolar inflammatory response than originally considered. After the serial changes in inflammatory cell profiles from lavages were analyzed in several different ways, conclusions showed “a trend of return towards normal in those improving compared with those in the other groups” [125]. However, many patients with an increased percentage of neutrophils failed to respond to therapy so “the raised initial neutrophil count does not clearly distinguish patients who will respond well to treatment from those who will not” [125].

Another well-conducted study by Watters and colleagues [130] that involved 26 patients with IPF made comparisons between pretreatment BAL cellular analysis sampled from the right middle lung lobe, histologic interpretation in open lung biopsy specimens obtained 3 weeks after lavage from upper and lower lobes of the same lung, and subsequent response to prednisone therapy. Lung biopsy specimens were analyzed from stained tissue sections and not by a cellular extraction method [40, 45]. Results indicated that the neutrophilia among BALF cells did not correlate with the histologic changes nor was this a predictor of clinical improvement. Lymphocytes could be increased in some patient’s BAL cells, and this increase did correlate with moderate to
severe alveolar septal inflammation. For five of seven patients with BAL lymphocytosis before treatment, clinical improvement was found after 6 months of prednisone therapy, whereas only three of nine patients with BAL neutrophilia improved with therapy. Eosinophils were increased in BALF of patients with more severe overall clinical impairment. A general BALF pattern of more neutrophils (mean 23% in count), more eosinophils (mean 17%), and low lymphocytes (6% mean) was found in patients with the most severe clinical impairment (highest clinical-radiographic-physiologic score). In contrast, BALF lymphocytosis was associated with alveolar septal inflammation but little honeycombing change and more likely indicated improvement from corticosteroids, perhaps reflecting an earlier, cellular stage of IPF. This study also confirmed earlier results [47, 107]. Clearly, the lavage neutrophil alveolitis pattern reported by Keogh [50] was not found.

Thus, in summary, reliance on a single type or limited number of cells in a profile of BALF-recovered cells was too simplistic, or perhaps too good to be true, because this single laboratory parameter did not necessarily reflect the complexity of the alveolitis among this group of poorly understood idiopathic diseases, DILD. This as much as any factor torpedoed widespread acceptance of BAL analysis as a routine, helpful laboratory test in clinical pulmonology, and rightly so, relegating BAL to a clinical research status. A secondary impact was not to have BAL qualify for a professional fee charge or the cellular analysis to generate an interpretative fee such that the procedure would be used routinely in clinical practice. Where did this leave the status of BAL about a decade ago at the end of the 1980s?

Scorecard on BAL

Unquestionably, BAL permitted recovery of normal human airway-alveolar cells and soluble substances in the epithelial lining fluid for in vitro research that has helped explain local host responses such as antibody formation and immunologic components involved with the induction and regulation of inflammation, fibrogenesis, surfactant production and clearance, and the cellular kinetics of certain cytokine driven T-lymphocyte responses, characterized as TH1 and TH2 [1, 39]. This research has greatly improved concepts of immunopathogenesis and provided a basis for investigating many forms of lung disease. Clinically, BAL has been used extensively to sample affected airways–alveolar spaces of patients with various conducting airway diseases, such as asthma and bronchitis, with lower tract infection resulting from disease-related immunosuppression, such as HIV, or from medically induced immunosuppression related to organ transplantation or cytotoxic chemotherapy, and with air exchange surface abnormalities reflecting alveolitis and diffuse interstitial inflammation. Acute lung injury and occupational lung diseases have been studied. Even a minimal review of the substantial research literature about normal and abnormal disease-related changes in BALF is beyond the scope of this article but is addressed in many of the reviews about BAL. A recent update about BAL has appeared from the European BAL Task Force [summarized, 39]. Several other perspectives bridge the last 10 years well, specifically related to interstitial pulmonary fibrosis [12, 76, 128], which has remained important as a clinical problem requiring innovative and better forms of therapy [34, 65]. Still a curious dichotomy exists between the delineation of immunopathogenic changes that suggest new kinds of anti-inflammatory or antifibrotic therapy and lack of
a good correlation for BALF cellular profiles, especially for PMN, with patient responsiveness to therapy or prognosis of IPF (CFA) [125, 130]. Patients with IPF, however, have been found to have the pro-inflammatory cytokine IL-8 present in BALF and IL-8 mRNA was expressed in alveolar macrophages; the level of mRNA for IL-8 correlated with the percent or number of PMN in BALF [8, 59, 75, 116]. Mechanisms of neutrophilic alveolitis should be resolved in the future with more sophisticated scrutiny of cells and other BALF components retrieved from affected airways and alveoli.

As illustrated in Figure 2, the use or interest in BAL was reflected by the striking number of publications that began to appear. A few years after the initial human BAL studies with FOB appeared in the mid 1970s, a 40-fold increase occurred in published human lavage studies, which plateaued about 15 years later, but paper output remains constant. BAL animal model research reflected a similar pace. However, just the number of publications does not reveal the evolution and change in the use and application of BAL. Whereas early on human BAL reports emphasized findings from or application pertaining to diffuse alveolar/interstitial diseases from prominent lung investigating groups, publications in the late 1980s indicated some important changes, perhaps healthy ones. Because BAL analysis had not become a routine test in pulmonary clinical practice for lack of a good correlation with prognosis or treatment outcome in patients with DILD, BAL use widened to other more useful applications, such as microbial recovery in lung infections, especially in HIV immunocompromised patients with *Pneumocystis carinii*, and to other groups of diseases. Illnesses increasingly studied were asthma [114]; occupational inhalation diseases (asbestos, organic antigen exposure, and airborne metals); lung cells in HIV-infected patients [135]; acute lung injury resulting in adult respiratory distress syndrome [118]; or complications of organ transplantation, including lung, bone marrow, and even liver transplantation. However, many studies each year continued to be published about sarcoidosis, perhaps because BALF changes are usually distinctive in this and other granulomatous lung diseases. Although BAL had been performed in children usually with lung illness, the first reports of BALF analysis in healthy children without parenchymal lung involvement appeared in the mid 1990s [70, 86, 102], extending the spectrum of the procedure. Inevitably, some of the early and most enthusiastic proponents of BAL research phased out or redirected interests to other lung problems or avenues of therapy, such as gene delivery to the lungs. Yet another generation of colleagues continues, and the biannual conferences on BAL flourish—the 7th Conference on BAL was held June 28–July 1, 2000, in Krakow, Poland.

As noted, the overall number of human BAL-related publications persists at about 400 articles/y, although the peak occurred in the 1989–90 span. Articles also reflect increasingly a higher proportion of lower impact scientific journals for about 25–30% of the articles began to be published in secondary journals, beginning in 1988 continuing throughout the 1990s. This illustrates that BAL findings or its application have pushed out into a more local environment, as occurs with medical technology once it moves beyond the academic centers. However, many of the prominent investigators, particularly in Europe, have authored BAL reviews in native language publications.

Considerable discussion already has reviewed evidence that a BALF cellular analysis made during the initial evaluation of patients with DILD, particularly with IPF or CFA, has not always predicted response to corticosteroid treatment nor prognosis
well, especially when the PMN percentage is the primary indicator. When other inflammatory cells in BALF are considered, the profile is more indicative, especially if lymphocytes are increased in IPF (CFA); elevated eosinophils portend a poor response to corticosteroids usually. Moreover, the alveolitis as sampled by BAL may not reflect the histologic findings in lung biopsy specimens or cellular changes in alveolar septa/interstitial tissue for IPF [38A]; however, correlations between BAL cells and tissue cell extractions are better for lymphocytic alveolitis and granulomatous lung disease.

This lack of diagnostic specificity of BALF differential cells allowed another imaging modality to become popular, which has greatly altered the evaluation and monitoring of patients with DILD, namely HRCT lung scans [27]. From HRCT scans, patterns of lung involvement suggest specific diagnoses, findings of ground-glass appearance are equated with parenchymal cellularity, and serial scans can monitor patient progress [74, 132]. Dependence on HRCT scans has become integral to patient management. However, as found in several reports [73, 81], combining expert clinical acumen and HRCT scan interpretation still does not provide a confident clinical diagnosis for a significant percentage of patients, and tissue verification with open lung biopsy [81] is still needed. Would incorporation of BALF analysis be helpful then in changing the clinician’s confidence in a diagnosis [119] or altering subsequent evaluation?

**Future Role for BAL Sampling**

The technical aspects of BAL have been assembled and reviewed thoroughly [121] and updated recently [39]; consensus appears good about its indications, optimal ways to perform lavage, and how to analyze the cellular and soluble components. However, unless a clinical laboratory is prepared to process BALF samples frequently, the analysis remains time consuming, and verification can be problematic. The use of computer-assisted software for comparisons with other laboratories’ databases [25, 26], or atlas-type reference material of cytopathic appearances of BALF cells prepared by cytopathologists may be useful [10], if the application includes a broad variety of lung diseases. Improving the quality of cytopathic analysis and reproducibility of results [3] is still needed. With more confidence in reported laboratory results, BAL differential cell counts may be more helpful and acceptable for clinical diagnostic reasoning. As already mentioned, it is likely that BALF analysis will remain of added value for improving diagnostic accuracy in the evaluation of patients with DILD and prove to be complementary to clinical acumen and HRCT lung scans [73, 81].

Increasingly, more elderly patients are being encountered with perhaps milder or an insidious form of IPF [93] in whom more-limited or less-invasive evaluation can be performed or is clinically appropriate. In healthy, elderly volunteers (>60 y of age), preliminary BAL results compared cellular changes that might provide clues to altered immune processes (CD4 accumulation of lymphocytes) that possibly contribute to illness (UIP) in older patients [2]. Other analytic modalities such as specimens of condensed exhaled air from the lungs [111], local nasal mucosal washings, or induced fresh sputum specimens will be used also to quantitate inflammation or relevant cytokine expression.

Something of a renaissance for BALF analysis is predicted, but within the confines of clinical research protocols. New or different applications of BAL will continue and...
become more innovative, especially as the technique is used to examine different lung
diseases. As mentioned for Figure 2, published research that initially reflected DILD
has shifted during the past 15 years to study many other important diseases, such as
asthma, lung infection in immunocompromised patients, bronchitis/COPD, organ trans-
plantation, acute lung injury, etc. [92]. Potentially useful insights for all these diseases,
especially allergic, occupational inhalation exposure(s), and bronchitis, will derive
from study of mucosal–alveolar space fluids obtained discreetly from the nasopharynx,
conducting airways, and alveolar microenvironment and all contrasted. Sensitive new
techniques are needed to sample discrete areas [57] as with minute absorptive pledgets
applied to the airway lining surface. Bronchitis/COPD associated with cigarette smok-
ing is an obvious choice. Because the entire respiratory tract is exposed and at risk for
illness, selective sampling of portions of the airways, including BAL for alveolar-
emphysema mechanisms, and interrelating results will provide new insights. Integrating
surfactant and its proteins into the host defense scheme is an active research area
[77, 78] for which BAL recovery is essential and has been a longstanding product of
BAL research already [28, 80, 99]. Moreover, surfactant dysfunction is involved with
ARDS [39], an injurious process that is receiving more research attention, including
with BAL.

As manipulation of mucosal surfaces to resist infectious agents becomes more
feasible by regulation of cytokines [94], study of antigen-presenting cells, such as
dendritic cells and airway macrophages, is focusing on the lungs. BAL is a method to
retrieve these dendritic cells [126]. Moreover, BAL cells are obtained from the distal
airways and alveolar surface affected by a disease process. By subjecting these cells to
an array of DNA sequences representing portions of human genes, a microscan for
activated genes or products can be made that will give information directly about what
genes are involved or are contributing to understanding the cause of many lung diseases
that remain without a defined origin.

In conclusion, the procedure of BAL retrieves cells and soluble substances from
the lining fluid of the distal airways and alveolar units, containing immunologic com-
ponents of the lung’s epithelial surface. In many instances it was assumed that BALF
also reflected the milieu of the interstitium or parenchyma as well, and this is not
always the case. Alveolar surface changes in disease (alveolitis) are not necessarily a
continuum of an interstitial process, although the compartments are contiguous; they
may remain separate or unique. Nonetheless, lavage sampling of the airway and al-
veolar surfaces provides a specimen that is involved with a disease process or in close
approximation to it. This approach will continue to be helpful as more details of the
normal lung are found and pathogenesis of diseases are explored.

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