Present status of bronchoalveolar lavage in interstitial lung disease

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Purpose of review

Sampling the detachable cells and acellular lining secretions of the human respiratory tract by bronchoalveolar lavage (BAL) is a means of obtaining relevant components from the airways and alveolar areas for research use and clinical analysis in normals (controls) and patients with a wide spectrum of interstitial lung diseases (ILDs). This review attempts to discuss recent findings from BAL studies that provide insight into pathogenic mechanisms of ILDs and/or assist in diagnosing disease activity.

Recent findings

BAL analysis and usefulness are reviewed for the major forms of ILDs. In addition, some perspective about this sampling method is given and the context for BAL is provided for the respective disease, either for diagnosis or research use.

Summary

Whereas BAL findings continue to impact on understanding disease pathogenesis and this may be its major use now, BAL fluid components, cells in particular, are not correlated well with activity of disease nor for monitoring disease progress or response to treatment. For a few rarer ILDs, BAL fluid characteristics may strongly support a diagnosis.

Keywords

bronchoalveolar lavage, connective tissue diseases, hypersensitivity pneumonitis, idiopathic pulmonary fibrosis, interstitial lung disease, polymorphonuclear neutrophils, pulmonary alveolar proteinosis, pulmonary Langerhans cell histiocytosis, scleroderma

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Introduction

The Hippocratic Movement of Physicians in Greece that initiated the beginnings of Western Medicine dates to about the fifth century BCE [1]. Contributing to the rationale of how the human body functioned was the interaction of four bodily fluids, termed humors. Phlegm was one and it was associated with old age and winter [1]. How intriguing to consider that respiratory secretions in the elderly might reflect the cumulative effects of environmental exposures such as occupational disease, smoke inhalation, chronic infection, and mucus producing syndromes, whereas winter connotes indoor person-to-person exposure causing acute infections and pneumonitis. Stimulating cough to obtain expectorated sputum and grossly analyzing it has been a mainstay of clinical evaluation. Production of phlegm and perhaps nasal secretions coupled with physical findings in the chest, using palpation, percussion, succussion (commotion), and later indirect auscultation with the stethoscope [2] refined clinical pathologic processes. As this all has created more accurate diagnoses, more sensitive methods to study and monitor lung disease have evolved. Sampling respiratory cells and indigenous proteins and other substances that line or coat normal and diseased 'airways' has led to bronchial lavage and to bronchoalveolar lavage (BAL) [3,4].

The present review is about BAL to retrieve cells and other substances coating the bronchi and lower airways of humans that can be used for research into the immunopathologic mechanisms in respiratory disease, here focusing on interstitial lung diseases (ILDs) with accompanying pulmonary fibrosis. BAL fluid biologicals can be obtained from normals for comparison studies. Also, findings in BAL samples can be contrasted with blood or used themselves for diagnosis and/or monitoring illness. An advantage is that these constituents in BAL fluid are in-situ ones produced directly by the respiratory tract and lungs. But this usage of BAL has not turned out to be as effective as initially hoped, nor the wished for complete blood count ('CBC') clinical test (discussed, [4]). Nevertheless, obtaining components directly from airway surfaces is a first approximation to analyzing and studying tissue directly involved in disease. There is no substitute for this.

Washing the airways has been done since appliances were available to insert into lungs and airways, that is, the rigid bronchoscope (C. Jackson, 1904), balloon-anchored rubber catheters such as a double lumen tube (E. Carlens, 1949) and Metras catheters (H. Metras, 1953), and the flexible bronchofiberscope (S. Ikeda, 1967; reviewed in [3,4]). Lavage of the airways was done to mechanically clear secretions, but also to obtain washings for retrieval of cells and proteins from normals and from patients with lung diseases. As fiberoptic bronchoscopy has proved to be well tolerated in humans and acceptable for experimental use in normals and controls [5,6•], BAL sampling of the lower airways and alveoli has become a common tool of research to obtain cells and proteinacious materials and to describe the milieu of the airways in animals [7] and humans [5]. The popularity of BAL, coupled with fiberoptic bronchoscopy, as a sampling method to obtain airway biological specimens increased quickly (reviewed [4]) and led to the first BAL Conference in Lille, France in 1976 [8]. Over the next 30 years, there have been many more BAL conferences, always interesting and helpful, including the latest in 2008 [9].

Bronchoscopy and bronchoalveolar lavage: getting started

Although technical training in flexible or rigid bronchoscopy is provided for pulmonary and surgical residents and specialty fellows, and is part of the skill set of faculty and private specialty care physicians, an occasional review and updating of available equipment and the technique is worthwhile. Many articles have been published previously about bronchoscopy and BAL technique [3,4]. A recent review of bronchoscopy by Drs Yoneda and Morrissey is recommended [10•]. The spectrum of flexible bronchoscopy procedures has expanded and comments about preprocedure screening and participant consent are helpful. Recently, in the summary of a National Heart, Lung, & Blood Institute/National Institute of Allergy and Infectious Diseases (NHLBI/NIAID) workshop [6•], recommendations were made about special use of pharmacologic agents or physical stimuli to induce nonspecific airway responsiveness in patients with asthma and chronic obstructive pulmonary disease (COPD); safety issues about research in children and adolescents and informed consent are given in the Summary or in Appendix material (from [6•]).

For technical recommendations about BAL, including standard procedures, selecting areas of the lung to sample, processing the retrieved lavage fluid for acellular and cellular elements, and analyzing cell characteristics, the article by Baughman [11•] is excellent. He also compares recommendations for the BAL procedure from several prior US and European cooperative and consensus groups (Table 1, from [11•]). In a similar fashion, Meyer [12•] provides an insightful assessment of BAL as a diagnostic tool. It is important to emphasize that BAL is an important adjunct for establishing a diagnosis in a large variety of respiratory diseases, including adult and pediatric ones. As a sequel for this review, use of BAL in diffuse ILDs, a detailed list is provided (Table 4, BAL cellular distinctive changes associated with diffuse lung diseases [12•]) for the major detachable airway cells recovered in BAL fluid.

Finally, focusing on common ILDs with a broad view from English-origin thoracic groups (British Thoracic Society, Thoracic Society of Australia and New Zealand, and the Irish Thoracic Society), Wells and Hirani [13••] have produced a pertinent ILD guideline, providing levels of evidence and grades of recommendations. Several rare conditions, Langerhans cell histiocytosis, lymphangioleiomyomatosis, pulmonary vasculitis, and alveolar proteinosis are considered in an orphan lung disease project [13••]. Guidelines for BAL (along with transbronchial lung biopsy) are given (page V6, [13••]) with generally C or D grades of recommendations.

Connective tissue diseases and interstitial lung disease

Among the common connective tissue diseases (CTDs), rheumatoid arthritis, scleroderma (SSc), lupus erythematosus, polymyositis/dermatomyositis, all can have associated ILD and other thoracic cavity manifestations such as pleural effusion, pleuritis and pulmonary vascular disease (hypertension). Whereas rheumatological symptoms and related findings in skin, joints, eyes, muscular-skeletal system, and mucous membranes are usually obvious, respiratory ones may not be the most prominent or mentioned early on by the patient, so an index of suspicion is needed. Or, sometimes, an apparent respiratory localized illness indicating an ILD has developed, but only later do the joint and arthritic symptoms occur, revealing that rheumatoid arthritis is blossoming. The CTDs affect female patients disproportionally than male patients, roughly a $2:1$ ratio. We noted among a spectrum of patients (the author updated in 2002, $n = 264$ patients) presenting with ILD that 34 (13%) were found to have an associated CTD [14].

Lung disease in rheumatoid arthritis

In addition to joint disease, extraarticular manifestations are frequent, including respiratory symptoms from the pleuropulmonary thoracic area. The common respiratory manifestations are pleuritis, pleural effusions, rheumatoid lung nodules, and ILD. Focusing on rheumatoid arthritis-associated ILD, about a third of asymptomatic rheumatoid arthritis patients in fact have ILD when sensitive detection methods are used. Lung disease

may precede the overt presentation and diagnosis of rheumatoid arthritis in 20% or so of patients [15,16]. The pathology of the ILD can be variable, including nonspecific interstitial pneumonia (NSIP) with a lymphocytic infiltrate pattern, termed cellular NSIP, or the usual interstitial pneumonia (UIP) pattern [13••,15,16]. For this review, comments given about the usefulness of BAL are pertinent [15,16]. BAL in the work up of patients with rheumatoid arthritis is not as useful for assessing ILD as in other forms of CTD. BAL fluid cellularity and using this for predicting response to therapy or for prognosis is not established [15,16].

Scleroderma interstitial lung disease

SSc, or systemic sclerosis, is an autoimmune CTD affecting the microvasculature with fibrosis involving the skin and many visceral organs; as many as 90% of patients have pulmonary fibrosis and the mortality rate among patients with severe ventilatory restriction [forced vital capacity (FVC) <50%] is about 40% within 10 years of the patient's first recall of experiencing Raynaud's phenomenon [17•,18]. A clinical trial to assess the effects of oral cyclophosphamide in symptomatic patients with SSc-ILD was reported [17•]; modest benefits were found on lung function for 24 months and on some secondary features. For enrollment into the study, patients had to have evidence of active alveolitis on examination of BAL fluid [either >3 polymorphonuclear neutrophils (PMNs) or eosinophilia >2%, or both], or have ground-glass opacity in their lungs on a thoracic high resolution computerized tomography (HRCT) scan [17•]. Among 267 patients who underwent screening, 144 patients, about 71%, whose BAL fluid results could be evaluated cytologically met criteria for alveolitis.

Further refinement of BAL data in these SSc-ILD patients was reported by Strange *et al.* [19••]. Of the 267 patients screened, 201 were lavaged and none had a serious adverse event. Lavage was done in the right middle lobe (RML). They reported that of the 158 patients randomized to the trial [19••], 141 were lavaged. The object was to see whether BAL fluid inflammatory cells (3% PMNs and/or 2% eosinophils) would predict responsiveness to cyclophoshamide (CYC) therapy. Among the 158 patients randomized to the study, 141 had BAL and 101 of these cases had abnormal cellularity (71.6%). The mean age of the SSc-ILD patients undergoing BAL was 49 years, two thirds were female patients, and their duration of SSc disease was about 5 years. Those with positive BAL cellularity had worse lung function, and on lung HRCT more ground glass and fibrosis were evident. This prospective study of BAL cellularity as a predictor of patient's responsiveness to oral CYC treatment for 1 year to affect progression of ILD found that positive BAL cellularity was not a helpful predictor of either the course of disease, measured by FVC % predicted, or lung activity of SSc-ILD in the placebo group or in the CYC-treated patients after 12 months of therapy. This trial did address issues of assessing ILD activity raised by previous studies (see [19••]). BAL should rarely be performed for clinical care in SSc-ILD unless infection is suspected. Although not helpful in predicting the course of ILD, the use of BAL to obtain cells and epithelial lining fluid for further investigation of the pathogenesis of ILD in SSc does remain a helpful tool. Finally, if BAL is used in further studies of SSc-ILD, multilobar sites of sampling should be considered instead of only a RML location.

The important study by Strange *et al.* [19••] has spawned other related assessments about the role of BAL analysis in SSc-ILD [18,20•,21]. These bring into perspective prior studies in the literature, dating to 1984 [22], on the use of BAL in SSc-ILD. Finally, in a group of 25 patients studied between May 1992 and November 2003 with SSc-ILD treated with CYC [23], patients underwent a pretreatment BAL for cell counts and differential, with BAL performed in the right middle, right lower, and right upper anterior lobes with 100 ml infused into each lobe; fluid was pooled for cellular analysis. Following CYC treatment, which lasted a median duration of 1.4 years (ranged from 1 month to 4.8 years), a follow-up BAL was done. In about half (13 of 24) of the patients treated with CYC, there was an improvement in the BAL percentages of PMNs and eosinophils; for six patients treated for a longer interval with CYC (average 5 months to 4.8 years, mean of 2.2 years), BAL counts normalized. However, overall for the study population, there was no correlation between the change in the percentage of PMNs or eosinophils in BAL nor a significant decline in lung function [% predicted FVC and diffusing capacity for carbon monoxide – a lung function test (DLCO)]. Thus, it was concluded that persistently abnormal results on BAL fluid cellular analysis following CYC treatment (occurred in 76%) was a common finding and did not predict a subsequent decline in lung function [23]. As a final comment about the clinical relevance of using BAL cellularity for alveolitis in SSc-ILD, its utility and significance clinically is in doubt [24].

Idiopathic pulmonary fibrosis (cryptogenic fibrosing alveolitis)

Among the many forms of diffuse ILD, idiopathic pulmonary fibrosis (IPF; UIP) is a common diagnosis, but it has to be differentiated from NSIP, which has a different and distinctive histopathological pattern [25]. Mixed in is the cryptogenic fibrosing alveolitis (CFA) diagnosis with IPF, especially used in prior epidemiological studies as discussed [13••]. A thorough discussion of BAL use in IPF was produced by Nagai *et al.* [26]. Dr Nagai has long

been an active investigator of BAL and a proponent, as has been Dr Izumi [26]. Under the idiopathic interstitial pneumonia classification, seven subtypes are given based on histologic patterns; each has distinctive cellular profiles in BAL fluid [26]. Information about genetic profiles of BAL cells is reviewed for IPF, NSIP, and hypersensitivity pneumonitis; and acellular components in BAL fluid are given, mainly for IPF. This investigative use of BAL is encouraging as genetic defects in surfactant proteins A2 can be found in IPF and also in lung cancer [27]. Other findings of blood biomarkers metalloproteases MMP7 and MMP1 in patients with IPF are revealing. Analysis of BAL fluid for MMP7 and MMP1 in 23 patients with IPF and 14 control lungs lavaged found BAL fluid concentrations and gene expression for these two metalloproteases to be significantly overexpressed in IPF [28]. Other studies have found various cytokine and chemokine levels to be increased in BAL fluid in IPF [29] and levels of several CC chemokines, CCL 2, CCL 17 and CCL 22, in BAL fluid suggested a poor outcome in IPF patients [30].

By recent classification criteria, the diagnosis of IPF can be established largely by HRCT without the need for surgical biopsy or routine BAL analysis. But for 74 patients so diagnosed with IPF by 2002 American Thoracic Society/European Respiratory Society (ATS/ERS) consensus recommendations, the finding of lymphocytosis among BAL fluid cells (>30%) was a clue to proceed further with diagnosis [31]. Six of the 74 patients had another diagnosis other than IPF made; three patients each had NSIP and extrinsic allergic alveolitis. BAL helped prevent a misdiagnosis.

Desquamative interstitial pneumonia

Desquamative interstitial pneumonia (DIP) is a form of ILD that is related to respiratory bronchiolitis-ILD and is considered a smoking-related interstitial disease. A detailed study of 20 patients (19 male patients and 17 current cigarette smokers) was conducted to clarify the clinical features, lung disorder, imaging, and laboratory data; included were BAL findings from 17 patients [32]. BAL total cell counts were increased, eosinophils were quite elevated (18% mean, 9% median), and neutrophils were increased also (11%, 7%). Peripheral blood eosinophils were also increased. Thus, BAL eosinophilia is considered a finding of DIP. Another case of DIP was reported [33] with a marked increase in BAL fluid eosinophils, representing 62% of cells. Further investigation with BAL samples may help elucidate the immunopathogenesis of this form of diffuse interstitial disease (DLD). In this manner, Papakosta *et al.* [34] have found that the increased eosinophils in BAL fluid from 27 patients with eosinophilic pneumonia may interrelate with a natural killer cell subpopulation of lymphocytes.

Interstitial lung disease with a granulomatous component

ILD can have granulomatous changes reflecting a delayed type host immunity response involving dendritic and/or macrophage antigen presenting cells [35] and T helper (Th1) lymphocytes. For the common diseases such as hypersensitivity pneumonitis, which is an occupational or hobbyist exposure illness, an inciting microbial antigen or bird-pet-related protein antigen may be defined [36], or for sarcoidosis, this likely involves a mycobacterial antigen in some patients [37]. Particulate inhalation of silica or metallic substances such as beryllium can cause similar granulomatous tissue changes.

Sarcoidosis

Sarcoidosis is a multisystem disease (reviewed in [38]) that affects the respiratory tract in most patients, and very often the skin, various locations of lymph nodes, and the eyes, heart, abdominal organs, especially the liver, spleen and kidneys, and the neurological system. An acute often self-limited form is named Löfgren's syndrome for which recent genetic patterns have revealed predilection and outcome for patient groups, especially female patients [39]. Pulmonary sarcoidosis also was one of the first respiratory diseases to be studied with BAL fluid cellular analysis dating to the mid 1970s; many reports describe the finding of lymphocyte patterns in BAL fluid (reviewed in [40]). More findings continue. Drent *et al.* [41•] provide a detailed review of BAL in sarcoidosis, emphasizing the features of BAL cells, the various chemokines found, and the foreign body components that can cause or represent a misdiagnosis of sarcoidosis. Also this review discusses how the lavage fluid profile can vary with early or advanced disease and be impacted by current cigarette smoking that might lessen the inflammatory profile. Whereas BAL analysis is helpful in sorting out the differential diagnosis of pulmonary sarcoidosis, monitoring alveolitis with repeated sampling to guide prognosis or disease outcome is not recommended [13••,41•].

In contrast to using BAL cellular profiles to clinically monitor a patient's lung status in sarcoidosis, using retrieved cells for investigative insight into the control of granulomatous inflammation is fruitful. An example is the regulatory action of the nuclear cell transcription factor peroxisome proliferator-activated receptor gamma (PPARγ), which potentially regulates inflammation and is expressed in cells of the monocyte-macrophage lineage [42]. In the almost 20 years since PPARs were described as ligand-activated transcription factors that belong to the nuclear hormone receptor super family, they have been found to help regulate many physiological processes. The PPARγ isotype is expressed in adipose tissue and other

types in which it regulates genes involved in cellular differentiation and growth, inflammation, apoptosis, and angiogenesis [43]; it is considered a novel molecular target in lung disease. PPARγ has been reported to be deficient in alveolar macrophages from patients with alveolar proteinosis [44] and in these cells in pulmonary sarcoidosis [42,45]. Further research in sarcoidosis continues to find PPARγ regulation involved [45].

As microbial causes of sarcoidosis continue to be sought [46,47], BAL specimens are helpful in this pursuit. Study of propionibacterial DNA in BAL cells has been reported [48].

Hypersensitivity pneumonitis

Hypersensitivity pneumonitis, also known as extrinsic allergic alveolitis, is one DLD that is included in the broader category of occupational lung diseases resulting from inhalation of organic and inorganic substances and various particles, fumes, gases, and infectious agents. The pneumoconioses can also be included in this group of DLD. Hypersensitivity pneumonitis is an inflammatory and granulomatous ILD occurring after reported inhalation of organic dusts. Over 200 different antigens have been associated with hypersensitivity pneumonitis; the main causes are listed in [49]. Dramatic among the BAL cellular profile is the alveolitis with an increase in overall cell count and the high percentage of lymphocytes often 50% or higher and the preponderance of CD4 cells causing an inversion of the CD4/CD8 cell ratio. Descriptions of BAL findings in these two diseases are well presented by Cordeiro *et al.* [49]. Hypersensitivity pneumonitis can be difficult to diagnose as its exposure source can be unsuspected or obscure if a hobbyist or one's recreational activity is related to the inhalational exposure instead of an obvious workplace environment, or a farming job such as pitching moldy hay to livestock [50]. As the classification of hypersensitivity pneumonitis (CHP) continues to be confusing, it needs better definition [51]. This has been attempted recently by a hypersensitivity pneumonitis study group of distinguished colleagues [52•]. Rather than continuing the classification of acute, subacute, and CHP, a cluster analysis of 168 patients favored two groups, one with more recurrent systemic symptoms and normal chest films, and another with more DLD features such as clubbing, hypoxemia, restrictive pattern of pulmonary function, and fibrosis on HRCT. An elevated percentage of lymphocytes on BAL analysis was useful diagnostically for both groups. Most CHP patients had avain exposure and were considered to have pigeon breeder's or bird fancier's disease.

Further study of a group of 86 patients with bird fancier's lung (BFL) was done to establish clinical characteristics [53]. The BFL patients were subjected to a consistent protocol for workup, including bronchoscopy with BAL. From BAL fluid analysis, lymphocytosis (>20%) was found in 83% of cases regardless of the stage of BFL; inversion of the CD4/CD8 lymphocytic ratio was found in 62% of patients. Lymphocytosis in BAL fluid persisted in the chronic phase of the disease.

Bronchoalveolar lavage findings in rarer forms of interstitial lung disease

BAL for diagnosis and to help describe lung tissue changes has been used in many so-termed 'rare' ILDs; characteristic findings of these were reviewed by Costabel *et al.* [54]. Rare ILD is also addressed in a guidelines paper edited by Wells and Hirani [13••]. Included in this ILD group are several infrequently seen but distinctive entities –pulmonary Langerhans cell histiocytosis (PLCH; previously designated as histiocytosis X and eosinophilic granuloma) and pulmonary alveolar proteinosis (PAP). In both PLCH and PAP, findings in BAL related to cells and fluid can confirm the diagnosis, such as the cytoplasmic organelles (X-bodies on Birbeck granules) in histiocytes seen on electron microscopy, and in PAP the milky turbid BAL fluid and foamy alveolar macrophages [54]. Other lung diseases reviewed are diffuse alveolar hemorrhage, drug-induced ILD in which BAL is helpful in excluding other diseases, and radiation-induced lung disease. ILD associated with collagen vascular disease, especially with SSc, and eosinophilic pneumonia has been reviewed separately.

Bronchoalveolar lavage in lung malignancy

Although lung cancer often presents as a single abnormal structure seen on imaging studies, or as nodular metastases or endobronchial lesions found at bronchoscopy, malignancy commonly affects the lung through metastases, perhaps creating a diffuse infiltrative appearing lung disease. BAL can be helpful in the diagnostic evaluation, and recovered cells obviously can have defining cytological properties. The review by Poletti *et al.* [55] provides an excellent perspective on how malignancy can appear in the lungs and how biomarkers in BAL may be useful. A further association between lung cancer cells and regulation of cellular differentiation is the PPARs, in particular involving PPARγ [56]. PPARγ has been implicated in the proliferation of cells in sarcoidosis [42] and PAP [44].

Bronchoalveolar lavage is not the only way now to sample the airways

The intent of this review is to focus on BAL components that assist in defining the milieu of the normal respiratory tract and the diseased one, and perhaps helping in clinical

diagnosis. Increasingly, this is but one of the ways now to sample respiratory constituents. Having utilized expectorated secretions and sputum since antiquity, by special washing of airways for the past half century with the rigid bronchoscope, then through various anchored tubes, and mainly in conjunction with fiberoptic bronchoscopy for the past 40 plus years, different ways are now emerging to sample the airways [57].

First, however, induced sputum from patients with ILD is still helpful and may substitute for BAL sampling as a noninvasive means to identify T-lymphocyte subsets [58]. The main technique is to analyze exhaled breath condensate (EBC). Various constituents in exhaled air samples have been measured and correlations attempted with disease states. As examples, exhaled carbon monoxide has been measured in inflammatory lung diseases in smokers as a marker of oxidative stress, which contributes to tissue damage in diffuse lung diseases. Exhaled carbon monoxide was found to be increased in sarcoidosis patients but was not considered to be of diagnostic usefulness [59]. Hydrogen peroxide (H_2O_2) production results from oxidative stress in the lungs of patients with different DLDs. Samples of EBC were compared with BAL fluid supernatants for H_2O_2 [60]. Whereas relative concentrations of H_2O_2 were similar in EBC and BAL, other correlations with cell counts et cetera were not found. Nitric oxide exhaled values were measured in patients with diffuse pulmonary microvascular dilatation from intrapulmonary shunts due to the hepatopulmonary syndrome [61]. Exhaled nitric oxide was greater in these patients than in controls, indicating alveolar capillary increased nitric oxide production. Extracellular superoxide dismutase (EC-SOD), an antioxidant enzyme that inhibits inflammation and may affect lung fibrosis, can be measured in BAL fluid from patients with IPF and normals [62]. This may provide insight into how syndecan-1 is involved in alveolar epithelial wound healing and subsequent fibrosis. Perhaps there is a way to go still before this testing is routine or finds its place in the diagnostic test hierarchy, but the future for noninvasive methods to sample the airways is intriguing and bright.

Acknowledgements

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