Current Diagnostic Status of Bronchoalveolar Lavage Studies

V.K. Vijayan

Deputy Director Cardio-pulmonary medicine Unit, TB Research Centre Indian Council of Medical Research

Broncboalveolar lavage using flexible fiberoptic bronchoscopy has emerged as a powerful investigative tool in respiratory medicine. It has helped in better understanding of pathogenesis of various interstitial lung diseases especially idiopathic pulmonary fibrosis and sarcoidosis. It has also been found useful in the diagnosis of various infectious and non-infectious diseases of lung particularly in immunodeficient patients. Even though this technique is used all over the world, it is still a research tool and more studies are required to exactly assess its value as a diagnostic fool in clinical medicine. BAL is a safe procedure.

Introduction

Bronchoalveolar lavage (BAL) using a flexible fiberoptic bronchoscope has emerged as an important investigative tool in pulmonology during the last decade[1]. BAL is a technique that allows the recovery of both cellular and noncellular components from the epithelial surface of the lower respiratory tract and differs from bronchial washings which refer to the aspiration of either secretions or small amounts of instilled saline from the large airways[2]. The first bronchoalveolar lavage was performed by Cantrell and colleagues in 1973 in normal volunteers[3]. Subsequently it has become an acceptable procedure to sample the contents of the lower respiratory tract[4]. The analysis of cellular and molecular components of the alveolar epithelial lining fluid has helped to elucidate the pathogenetic mechanisms and also to assess the activity of interstitial and other lung diseases[5]. This review deals mainly with the methods and the role of bronchoalveolar lavage in the diagnosis and management of various pulmonary diseases.

Method of bronchoalveolar lavage[2,6]

Bronchcalveolar lavage is performed with a flexible fiberoptic bronchoscope (FOB) usually under local anesthesia[2,6]. It can also be performed in patients with an oral or nasotracheal tube in position by inserting the FOB through the tube, provided its diameter is greater than 6mm[6]. Patients can be pre-medicated with atropine (0.6mg IM). Local anesthesia is accomplished by spraying of the nasal, oral, pharyngeal and laryngeal areas with 4% lidocaine. The trachea, carina and bronchi can be anesthetized by direct instillation of 1.5-2% lidocaine. BAL is a safe procedure. Mild side-effects such as coughing during lavage, fever and chills some hours after lavage and transient alveolar infiltration 24 hours after the procedure can occur in a proportion of subjects. Supplemental oxygen by nasal prongs, ear oximetry and ECG-monitoring are advised in patients with severe underlying disease or other critical conditions.

In localized disease, the involved segment is lavaged. However, in diffuse lung disease, the middle lobe or lingula has been most commonly used as standard sites; occasionally a lateral or anterior segment of a lower lobe is also used. The bronchoscope is wedged in fourth or fifth order bronchi, taking care not to "overwedge" which may result in less fluid recovery. BAL is usually performed with buffered or unbuffered, pyrogen-free saline (isotonic 0.9% NaCl) at room temperature or warmed to body temperature (37°C). The total volume instilled per site is 100 to 150ml, and it is usually instilled as five 20-ml aliquots or two or three 50-ml aliquots. The maximum total volume used (all sites combined) is usually no more than 300 ml[7]. After each aliquot is instilled, it is aspirated either by hand suction into the syringe or by normal clinical suction apparatus using negative pressures of 25-100mm Hg into a specimen trap. There is no special time for lavage fluid to dwell in the lung before it is removed. However, the patient can be encouraged to take one or two normal breaths before the fluid is withdrawn. The recovery from first aliquot is generally poor. Thereafter the return from subsequent aliquots is 60 to 70% of the instilled volume in normal subjects and the overall recovery is above 50 to 60%. Recovery of fluid is decreased in smokers and in most patients with diseased lungs, especially if airway obstruction exists. After bronchoscopy, the subject should be observed for 4 to 6 hours until the effects of local anesthesia have worn away.

After measuring the recovered volume, the bronchoalveolar lavage fluid is strained through a sterile gauze to remove the mucous particles. Total cell count is done on a haemocytometer in the unconcentrated lavage fluid and expressed both as the absolute number of cells recovered by lavage; or as the concentration of cells per ml of recovered lavage fluid. Cell viability can be performed by the Trypan blue exclusion test. Total cell counts can also be done after centrifugation. However, this may result in loss of cells. Differential cell count can be done from millipore filter preparations, which can be stained with a modified hematoxylin - eosin method or with a PAP technique. Differential ceil counts can also be made from a cytocentrifuged cell preparation stained with Wright-

Correspondence may be addressed to:
Dr. V. K. Viayan,
Cardio-pulmonary Medicine Unit
TB Research Centre
I.C.M.R.
Madras 600 031

Giemsa. However, this method underestimates the percentage of lymphocytes, especially if their proportion is increased. The differential counts are made by viewing with a light microscope, employing objectives of x40 x 100. At least 300-500 cells should be counted. The cells recovered from a normal non-smoking individual are alveolar macrophages, lymphocytes, neutrophils, eosinophils, mast cells and occasionally plasma cells. A high percentage of epithelial cells (>5%) is indicative of contamination with bronchial inflammatory cells. Special immunofluorescent and immunocytochemical techniques using monoclonal antibodies directed against antigens present on the ceil surface of lymphocytes and/or monocytes/ macrophages can be applied for better characterization of the nature of the cells recovered by the BAL.

The saline used for lavage significantly dilutes the epithelial lining fluid. Therefore, the soluble components in BAL cannot be quantitated because a completely reliable denominator or reference substance is not yet available. Even though albumin, potassium, methylene blue and urea were tried previously, no ideal. denominator or method of calculation exists to quantitate the dilution factor.

BAL in healthy volunteers

Cellular and acellular constituents in bronchoalveolar lavage fluid (BALF) in non-smoking adults 8 are given in Table 1. In smokers, the cell yield is 3 to 4 times greater. An increased number of alveolar macrophages is recovered from smokers and there may also be a slight increase(1-4%) in neutrophils. In healthy smokers, the proportion of BAL T-helper cells was lower, T-suppressor cells higher and therefore, the helper - suppressor ratio is lower than that in non-smokers. BALF IgG was greater in smokers than in never-smokers[9].

1. Diagnostic value of BAL[2,4,5]

Bronchoalveolar lavage is diagnostic in some of the infectious and non-infectious pulmonary diseases. In some others BAL findings may contribute to the diagnosis, although they are not diagnostic.

(a) Diseases in which BAL findings are diagnostic

(i) Infectious diseases

In immunodeficient patients (eg. patients receiving immunosuppressive drugs and with advanced human immunodeficient virus infection) with pulmonary infiltrates suggesting lower respiratory tract infection, BAL is a safe procedure for making definitive aetiologic diagnosis. In patients with HIV disease and suspected *Pneumocystis carinii* pneumonia, an induced sputum examination should precede BAL. The isolation of pathogenic microorganisms in lavage fluid can be considered diagnostic only if the isolated organism is not known to colonize the respiratory tract. BAL also can be useful in immunocompetent patients especially with nosocomial pneumonia. BAL fluid should be analyzed for bacterial, fungal, opportunistic and viral infections. Lavage differential cell determination shows a lymphocytic alveolitis in patients with HIV infection. Infectious diseases that can be diagnosed by isolation of microorganisms from BAL fluid are shown in Table 2.

(ii) Non-infectious disease

Even though the major contribution of BAL was in understanding the pathogenesis of lung injury, repair and fibrosis especially in interstitial lung disease, BAL findings are diagnostic in a few of the following non-infectious respiratory disorders (Table 2).

(a) Alveolar proteinosis

BAL fluid in alveolar proteinosis is

Table 1

Cellular and soluble constituents in BAL fluid in non-smoking adults

	Mean ± SD)
Cells	
Total	10 to 15 x10^6 cells/dl of BAL fluid.
Macrophages %	84±1
Lymphocytes %	11 ±1
T cells	6 2 ± 2
Helper (CDA)	4 6 ± 3
Suppressor (CDA)	2 5 ± 2
B cells	5 ± 2
Neutrophils %	<1
Eosinophils %	<1
Basophils %	< 1
Soluble factors	
IgG, IgA	+
IgM	-
C4, C3, factor B	+
C5	

(Daniel RP et al. Annals of Internal Medicine. 1985; 102: 93-108).

opaque and/or milky in appearance. Microscopic evaluation reveals large acellular eosinophilic bodies against a background of small eosinophilic granules and amorphous debris. On combined Alcian blue - periodic acid-schiff (PAS) stain, there is predominant PAS staining of the proteinaceous material with a lack of significant Alcian blue staining. Small lamellar bodies of wavy or regular periodicity, tubular myelin structures and myelin-like multilamellated structures with electron dense central region are characteristic findings in electron microscopy.

(b) Histiocytosis X (Langerhan's cell granulomatosis)

Total 2 counts are increased in BALF and 90% of the patients with Langerhans cell granulomatosis (LCG) are smokers The differential cell count reveals a high percentage of alveolar macrophages, and a slight increase of neutrophils and eosinophils. The diagnostic feature is the demonstration of Langerhans' cells. These cells contain characteristic pentalaminar plate-like cytoplasmic organelles and cytoplasmic inclusions variously referred to as Langerhans' granules, Birbeck's granules or histiocytosis X bodies which can be demonstrated by electron microscopy. Langerhans' cells also express CD a(T6) surface antigen. BAL samples can be diagnostic if there are greater than 5% of cells that react with OKT6 antibody.

(c) Pulmonary malignancies

Bronchoalveolar lavage of affected segments may permit a cytological diagnosis of cancer. Bronchial and alveolar specimens can be processed in any one of the following procedures: smears, cytocentrifuge preparations, membrane filter preparations and cell pellets embedded in paraffin. Processed samples canl be stained with Papanicolaou, Wright-Giemsa or haematoxylin-eosin stains.

Special stains using monoclonal antibodies for tumor markers can also be employed. The diagnostic yield of BAL is higher for bronchoalveolar cell carcinoma and lymphatic spread of metastatic cancer. The diagnostic yield had varied from 14 - 69% in various studies. A number of tumor markers have been studied, but none had proved to be diagnostic.

Non-infectious diseases in which BAL may be helpful in diagnosis

(i) Pulmonary haemorrhage

BAL fluid may be either bloody or orange - pink in colour in patients with pulmonary haemorrhage (PH) and haemosiderosis. The total cellular count and alveolar macrophages are increased in PH. Presence of haemosiderosis laden alveolar macrophages may aid in diagnosis. Free red blood cells and red blood ceils in macrophages can also be observed in patients with PH.

(ii) Eosinophilic lung disease

Eosinophilic infiltrates in the lung can

Table 2

Infectious diseases in which isolation from BALF is diagnostic

1. *Pneumocystis carinii*
2. *Toxoplasma gondii*
3. Stronglyoides
4. Legionella
5. Histoplasma
6. *Mycobacterium tuberculosis*
7. Mycoplasma
8. Influenza
9. *Respiratory syncytial virus*

Non-infectious diseases in which analysis of BALF is diagnostic

1. Alveolar proteinosis
2. Langerhans' cell granulomatosis (Histocytosis X).
3. Pulmonary malignancies

Non-infectious diseases in which BALF finding may be helpful in the diagnosis

1. Pulmonary haemorrhage
2. Eosinophilic lung diseases
3. Occupational lung diseases (berylliosis, asbestosis)
4. Hypersensitivity pneumonitis
5. Idiopathic pulmonary fibrosis
6. Sarcoidosis

(Modified from American Thoracic Society Statement, Am Rev Respir Dis 1990; ,142: 481-486)

occur in a variety of disorders such as allergic broncho-pulmonary aspergillosis, tropical eosinophilia and eosinophilic pneumonia. Since eosinophils are not seen in lavage fluid of normal controls, any increase in eosinophils in BALF suggests a pathologic process. The highest eosinophil counts are usually present in tropical eosinophilia and eosinophilic pneumonia. A 5-10 fold increase in total cells with a very high eosinophil count in BALF in our country may suggest tropical eosinophilia [10,11].

(iii) Occupational lung diseases

Berylliosis and asbestosis are two occupational lung diseases in which BAL may be helpful in the diagnosis. Berylliosis is a granulomatous lung disease that is clinically and histologically similar to sarcoidosis, but the inciting antigen is known in berylliosis. BALF shows macrophage-lymphocyte alveolitis and most of the lymphocytes are helper T calls. Proliferative response of broncho-alveolar lymphocytes especially CD4 + T cell subset to beryllium salts can be utilized as a diagnostic test. BAL in asbestosis is characterized by several fold increases in the numbers of alveolar macrophages and by mild-to-moderate increases in neutrophils and lymphocytes. There is a good correlation between asbestos bodies in BALF and in lung parenchyma. One asbestos body per mil. of lavage fluid is shown to be associated with more than 1000 asbestos bodies per gram of dried lung parenchyma.

(iv) Hypersensitivity pneumonitis

Hypersensitivity pneumonitis (HP), also known as extrinsic allergic alveolitis, is an interstitial lung disease associated with repeated exposure to a wide range of inhaled organic dusts and related occupational allergens. BAL studies reveal an increase in the proportion and number of lymphocytes and majority of lymphocytes* are CD8+ lymphocytes resulting in a low CD4/CD8 ratio. The presence of BAL lymphocytes characterised by CD3+/CD8+/CD56+ CD57/CD16 phenotype is highly suggestive of Hp.

(v) Idiopathic pulmonary fibrosis

Alveolitis of active idiopathic pulmonary fibrosis (IPF) is characterized by three to four fold increase in total numbers of lung effector cells and the cells comprising alveolitis are mainly alveolar macrophages and neutrophils with smaller numbers of lymphocytes and eosinophils. The combination of increased neutrophils and eosinophils occur in about two-thirds of patients with IPF. Patients with lavage lymphocytosis represent an earlier cellular stage in inflammatory process of IPF and improves with corticosteroids. However, lavage eosinophilia is associated with advanced IPF and is unresponsive to treatment and lavage neutrophils do not reflect response to treatment.

(vi) The alveolitis in sarcoidosis is characterized by an increase in the number of T-lymphocytes and macrophages in the alveolar structures. T-lymphocytes are mainly of helper/ induced type, resulting in an elevation of helper to suppressor T-cell ratio. In an appropriate clinical setting, BALF T-cell lymphocytosis and CD4 : CD8 ratio more than 3.5 can be consistent with a diagnosis of sarcoidosis. More than 20% 01*/07* macrophages in lavage fluid may also be helpful in the diagnosis if combined with other parameters. An increase in mast cells in BALF may suggest initiation of fibrosis and thus is a prognostic sign.

2. Therapeutic applications[5]

Whole lung lavage (WLL) has been found to be useful in the treatment of alveolar proteinosis. Mucus plugs can be removed in patients with status asthmaticus through a bronchosccpe after the instillation of saline or acetylcysteine. But, this procedure is associated with a high risk benefit ratio. WLL has been used to remove the irritating dust in the acute form of silicosis in order to prevent irreversible damage. WLL has also been proposed in the treatment of alveolar microlithiasis and exogenous lipoidisis.

Although BAL has helped in understanding the pathogenesis of various interstitial lung diseases and has been found to be useful in the dianogsis of certain pulmonary disorders, it is still a research tool and further studies are required before it can be recommended for general clinical use.

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