BRONCHOALVEOLAR LAVAGE (BAL) CELLULAR ANALYSES AS A DIAGNOSTIC INTERVENTION FOR PATIENTS WITH SUSPECTED ILD IN CONJUNCTION WITH HRCT IMAGING

Dissertation Submitted for

M.D DEGREE EXAMINATION

BRANCH XVII – TUBERCULOSIS & RESPIRATORY DISEASES

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APRIL 2015
BONAFIDE CERTIFICATE

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This is to certify that the dissertation titled “Bronchoalveolar Lavage (BAL) Cellular Analyses As A Diagnostic Intervention For Patients With Suspected ILD In Conjunction With HRCT Imaging” is the Bonafide work done by Dr.C.Ammaiyappan Palaniswamy during his MD (Tuberculosis and Respiratory Diseases) course from May 2013 to April 2015 at the Institute of Thoracic Medicine and Rajiv Gandhi Govt. General Hospital, Madras Medical College, Chennai-3

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DECLARATION

I hereby declare that the dissertation titled “Bronchoalveolar Lavage (BAL) Cellular Analyses As A Diagnostic Intervention For Patients With Suspected ILD In Conjunction With HRCT Imaging” submitted for the degree of Doctor of Medicine in MD degree examination branch XVII Tuberculosis and Respiratory Diseases is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar titles. It had not been submitted to any other university or institution for the award of any degree or diploma

Place :
Date :
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<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>PAGE NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>2. REVIEW OF LITERATURE</td>
<td></td>
</tr>
<tr>
<td>3. OBJECTIVES</td>
<td></td>
</tr>
<tr>
<td>4. MATERIALS AND METHODS</td>
<td></td>
</tr>
<tr>
<td>5. RESULTS</td>
<td></td>
</tr>
<tr>
<td>6. DISCUSSION</td>
<td></td>
</tr>
<tr>
<td>7. CONCLUSIONS</td>
<td></td>
</tr>
<tr>
<td>8. BIBLIOGRAPHY</td>
<td></td>
</tr>
<tr>
<td>9. ANNEXURES</td>
<td></td>
</tr>
<tr>
<td>PROFORMA</td>
<td></td>
</tr>
<tr>
<td>ETHICAL COMMITTEE</td>
<td></td>
</tr>
<tr>
<td>CONSENT FORMS</td>
<td></td>
</tr>
</tbody>
</table>
INTRODUCTION

Chronic obstructive pulmonary disease involving the lung parenchyma with a differing degree of inflammation and bronchoalveolar macrophages increase in...
## CONTENTS

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>TITLE</th>
<th>PAGE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>AIMS &amp; OBJECTIVES</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>REVIEW OF LITERATURE</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>MATERIALS AND METHODS</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>RESULTS</td>
<td>59</td>
</tr>
<tr>
<td>6</td>
<td>DISCUSSION</td>
<td>93</td>
</tr>
<tr>
<td>7</td>
<td>CONCLUSION</td>
<td>98</td>
</tr>
<tr>
<td>8</td>
<td>STUDY LIMITATIONS</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>ANNEXURES</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PROFORMA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ETHICAL COMMITTEE CERTIFICATE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BIBLIOGRAPHY</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CONSENT FORM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MASTER CHART</td>
<td></td>
</tr>
</tbody>
</table>
Bronchoalveolar lavage (BAL) Cellular Analyses as a Diagnostic Intervention for Patients Suspected with ILD in conjunction with HRCT Imaging

Background

Bronchoalveolar lavage (BAL) has gained acceptance to diagnose interstitial lung disease (ILD). Advent of high resolution computed tomography (HCRT) reduced the clinical utility of BAL. The present work utilized the recommendations of American thoracic society (ATS) to optimize BAL procedure and associated the finding with clinical examination and HRCT to precisely narrow down the cause for ILD.

Method

BAL was performed on ILD suspects at target site chosen based on HRCT. The procedure, transport, processing and analysis of BAL fluid were performed as per ATS guidelines. The clinical data, HRCT findings and BAL report were used to narrow the diagnosis of ILD. Two tailed student T test was used to assess the significance.

Result

We were able to optimize the BAL procedure as per the recommendations of ATS. In the cohort of 50 patients, Idiopathic pulmonary fibrosis (8), hypersensitivity pneumonitis (17), connective tissue disorder (9), sarcoidosis (3), pneumoconiosis (5), ARDS (2), eosinophilic lung disease (2) and lymphangitic carcinomatosa (2), aspiration bronchiolitis (1) and pulmonary histocytosis (1) were diagnosed. Statistically significant variation in differential counts was found in different ILDs. We were able to classify different ILDs based on the criteria described by ATS.

Clinical significance

BAL along with clinical and HRCT findings improved the diagnostic accuracy by incorporating the disease diagnosis, acute or chronic nature of the disease and cause for acute exacerbation, which helped in better management of ILDs.
INTRODUCTION

Bilateral infiltrative diseases involving the lung parenchyma with a varying degree of inflammation and fibrosis, that may present acutely or more commonly as a chronic condition, are as a group named as Interstitial Lung Diseases (ILD). ILDs occur in immunocompetent individuals who do not have a clinical suspicion of infection or neoplasm. ILDs in general are characterised clinically by exertional dyspnoea, radiologically by bilateral pulmonary infiltrates, abnormalities in lung function and gas exchange. Pathological features are excessive collagen deposition along with fibroblastic proliferation, in interstitium, and also in the lumen of the smaller airways.

Chronic ILDs develops over a long duration and comprises of condition with known causes and idiopathic causes. ILDs due to collagen vascular diseases, and work related diseases like pneumoconioses, namely silicosis, asbestosis and coal workers pneumoconiosis and hypersensitivity pneumonitis are diseases with known causes. Interstitial lung diseases without a known cause are a group of disorders named as Idiopathic interstitial pneumonias, and a granulomatous lung disease called Sarcoidosis. Tables 1 and 2 show the clinical and histopathological classification of the ILDs respectively.
<table>
<thead>
<tr>
<th>Collagen vascular diseases</th>
<th>Scleroderma, polymyositis-dermatomyositis, systemic lupus erythematosus, rheumatoid arthritis, mixed connective tissue disease, Ankylosing spondylitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iatrogenic</td>
<td>Antibiotics (notroflurantoin, sulfasalazine), antiarrhythmics (amiodarone, tocainide, propranolol), anti-inflammatories (gold, penicillamine), anticonvulsants (dilantin), chemotherapeutic agents (mitomycin c, bleomycin, busulfan, cyclophosphamide, chlorambucil, methotrexate, azathioprine, BCNU [carmustine], procarbazine), therapeutic radiation, oxygen toxicity, narcotics</td>
</tr>
<tr>
<td>Primary (unclassified) diseases</td>
<td>Sarcoidosis, primary pulmonary Langerhans cell, histiocytosis (eosinophilic granuloma), amyloidosis, pulmonary vasculitis, lipoid pneumonia, lymphangitic</td>
</tr>
<tr>
<td>Occupational / environment related</td>
<td>Inorganic: silicosis, asbestosis, hard-metal pneumoconiosis, coal worker’s pneumoconiosis, berylliosis, talc pneumoconiosis, siderosis (arc welder), stannosis (tin), organic (hypersensitivity pneumonitis), bird breeder’s lung, farmer’s lung</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Idiopathic fibrotic disorders</td>
<td>Acute interstitial pneumonitis (Hamman Rich)</td>
</tr>
<tr>
<td></td>
<td>carcinomatosis, bronchoalveolar carcinoma, pulmonary lymphoma, Gaucher’s disease, Niemann-Pick disease, Hermansky-Pudlak syndrome, neurofibromatosis, lymphangioleiomyomatosis, tuberous sclerosis, <strong>acute respiratory distress syndrome (ARDS)</strong>, AIDS, bone marrow transplantation, postinfectious, eosinophilic pneumonia, alveolar proteinosis, diffuse alveolar hemorrhage syndromes, alveolar microlithiasis, metastatic calcification</td>
</tr>
<tr>
<td>syndrome), idiopathic pulmonary fibrosis/ usual interstitial pneumonia, familial pulmonary fibrosis, respiratory bronchiolitis/desquamative interstitial pneumonitis, cryptogenic organizing pneumonia, non specific interstitial pneumonia, lymphocytic interstitial pneumonia(Sjogren’s syndrome, connective tissue disease, AIDS, Hashimoto’s thyroiditis), autoimmune pulmonary fibrosis(inflammatory bowel disease, primary biliary cirrhosis, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia)</td>
<td></td>
</tr>
<tr>
<td>Histologic patterns</td>
<td>Clinical Associations</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Usual interstitial pneumonia</td>
<td>IPF: connective tissue diseases(uncommon ); asbestosis; hypersensitivity pneumonitis;</td>
</tr>
<tr>
<td></td>
<td>chronic aspiration pneumonia; chronic radiation pneumonitis; Hermansky-Pudlak syndrome;</td>
</tr>
<tr>
<td></td>
<td>neurofibromatosis</td>
</tr>
<tr>
<td>Nonspecific interstitial pneumonia</td>
<td>Idiopathic; connective tissue diseases; drugs; hypersensitivity pneumonitis; diffuse</td>
</tr>
<tr>
<td></td>
<td>alveolar damage; infections; lymphocytic interstitial pneumonia; AIDS; chronic</td>
</tr>
<tr>
<td></td>
<td>eosinophilic pneumonia; infections; hemosiderosis; alveolar proteinosis</td>
</tr>
<tr>
<td>Diffuse alveolar damage</td>
<td>Acute interstitial pneumonia(Hamman-Rich syndrome); acute respiratory distress</td>
</tr>
<tr>
<td></td>
<td>syndrome; drugs(cytotoxic agents, heroin, cocaine, paraquat, ethchlorvynol, aspirin);</td>
</tr>
<tr>
<td></td>
<td>toxic gas</td>
</tr>
<tr>
<td>Condition</td>
<td>Causes</td>
</tr>
<tr>
<td>-------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Organizing pneumonia</td>
<td>Cryptogenic organizing pneumonia; organizing stage of diffuse alveolar damage; hypersensitivity pneumonitis; eosinophilic pneumonia; Wegener’s granulomatosis</td>
</tr>
<tr>
<td>Desquamative interstitial pneumonia/ respiratory bronchiolitis</td>
<td>Cigarette smoking; idiopathic; connective tissue diseases; primary pulmonary Langerhans cell histiocytosis; asbestosis; hard-metal pneumoconiosis(cobalt); Gaucher’s disease; Niemann-Pick disease; Hermansky-Pudlak syndrome; drugs(nitrofurantoin, amiodarone)</td>
</tr>
<tr>
<td>Lymphocytic interstitial pneumonia</td>
<td>Idiopathic; hypogammaglobulinemia; autoimmune disease, including Hashimoto’s thyroiditis, lupus erythematosus, primary biliary cirrhosis, Sjogren’s syndrome, myasthenia gravis, chronic active</td>
</tr>
<tr>
<td>Condition</td>
<td>Associated Conditions</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Eosinophilic pneumonia</td>
<td>Idiopathic acute and chronic; tropical filarial eosinophilia; parasitic infections; allergic bronchopulmonary aspergillosis; allergic granulomatosis of Churg and Strauss; hypereosinophilic syndrome; AIDS.</td>
</tr>
<tr>
<td>Alveolar proteinosis</td>
<td>Pulmonary alveolar proteinosis; acute silicosis; aluminium dust; AIDS; myeloproliferative disorder</td>
</tr>
<tr>
<td>Diffuse alveolar hemorrhage with capillaritis</td>
<td>Wegener’s granulomatosis; microscopic polyangiitis; systemic lupus erythematosus; polymyositis scleroderma; rheumatoid arthritis; mixed connective tissue disease; lung transplantation; drugs (retinoic acid, propylthiouracil, dilantin); Behcet’s disease; cryoglobulinemia; Henoch-Schonlein purpura; pauci-immune glomerulonephritis; immune complex glomerulonephritis.</td>
</tr>
<tr>
<td>Condition</td>
<td>Cause</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Diffuse alveolar hemorrhage without capillaritis</td>
<td>Idiopathic pulmonary hemosiderosis; systemic lupus erythematosus; Good pasture’s syndrome; diffuse alveolar damage; pulmonary veno-occlusive disease; mitral stenosis; lymphangioleiomyomatosis.</td>
</tr>
<tr>
<td>Amyloid deposition</td>
<td>Primary amyloidosis; multiple myeloma; lymphocytic interstitial pneumonia.</td>
</tr>
<tr>
<td>Granuloma</td>
<td>Sarcoidosis; hypersensitivity pneumonitis; pulmonary Langerhans cell histiocytosis; silicosis; intravenous talcosis; berylliosis; lymphocytic interstitial pneumonia; infections.</td>
</tr>
</tbody>
</table>

Though the causes of ILDs are daunting, the various ILDs have similar characteristics: clinical features, physiologic abnormalities, imaging patterns and histologic patterns. Nevertheless, in many patients, specific diagnosis can be made from clinical history, laboratory tests, HRCT features. Bronchoalveolar lavage (BAL) done by fiberoptic bronchoscope and in some cases transbrachial biopsy of yield a
diagnosis of some ILDs. Surgical Lung Biopsy (SLB) either through a Video assisted thoracoscopic surgery (VATS) or by open thoracotomy maybe required in the remaining cases.
A BRIEF LOOK INTO THE CLINICORADIOLOGICAL, PHYSIOLOGICAL CHARACTERISTICS OF ILDS

The three major hallmarks of ILDs are progressive dyspnoea, characteristic imaging abnormalities, and abnormal lung function tests. But, some patients may have a normal Xray chest at the time of clinical presentation. All the three features need not be present at the time a patient seeks medical attention. A dyspnoeic patient may have a normal spirometry, or a patient with radiologic findings maybe asymptomatic at the time of diagnosis. HRCT of the chest and exercise testing unmasks abnormalities in these patients. BAL can detect abnormalities even when a patient has normal xray or lung function tests, by revealing inflammatory cells, in conditions with high risk of developing ILD like those with CTD, asbestos exposure, intake of drugs known to cause ILDs or hypersensitivity pneumonitis.

A thorough history is very important. Occupational history should be elaborate as an exposure several years ago can be the cause of the disease in certain conditions. The exposure could have been for a short duration, but of high intensity. Hyperensitivity pneumonitis must be excluded, and it may present as an insidious or as a recurrent acute or sub acute pneumonitis.
DRUG AND TOBACCO HISTORY:

Drug history should include a review of medications used recently as well as in the distant past. Aspiration of gastric contents can lead to ILD. History of tobacco use is important, some ILDs are more predominant in smokers like Pulmonary Langerhans Cell histiocytosis (PLCH), Respiratory bronchiolitis. Smoking also has prognostic value in certain conditions, almost all patients with Goodpastures syndrome have diffuse alveolar hemorrhage, while only one fifth of non-smoking patients with Goodpasture’s syndrome have alveolar hemorrhage. Also smokers tend not to develop Hypersensitivity pneumonitis (HP) and Sarcoidosis, but in those smokers who develop HP, the disease runs a chronic course. IPF incidence is higher in smokers.

FAMILY HISTORY:

Familial associations are seen in Sarcoidosis, IPF, tuberous sclerosis, Nieman–Picks disease, Gauchers, Hermansky-pudlak syndromes.
GENDER:

ILDs showing sex predilection are Lymphangioleiomyomatosis (LAM) which occurs exclusively in women, and CTD associated ILDs are more common in women; whereas Pneumoconioses and occupational ILDs are more likely in men.

SYMPTOMS:

Progressive dyspnoea is the most common symptom. Cough is prominent in Lymphangitic carcinomatosis (LC) and also in Sarcoidosis, cryptogenic organizing pneumonia (COP), Hypersensitivity Pneumonitis, PLCH and Respiratory bronchiolitis. Wheezing is associated with CEP. Substernal chest pain can be a symptom in Sarcoidosis. Pleurisy type of pain may occur in drug induced ILDs, CTD-ILDs. Chest pain due to pneumothorax could be a presenting feature of PLCH, tuberous sclerosis, LAM, neurofibromatosis. Hemoptysis is occurs in Diffuse Alveolar Hemorrhage syndromes, and LAM. Hemoptysis in a case of known ILD should raise the possibility of a malignancy complicating ILD.
PHYSICAL FINDINGS:

Bibasilar inspiratory crackles is the most typical auscultatory finding, though they are less likely in granulomatous conditions, followed by digital Clubbing. Clubbing is usually indicative of advanced fibrosis. As the disease progresses, signs of pulmonary hypertension and cor pumonale appear.

PHYSIOLOGIC ALTERATIONS:

Increased elastance, decreased compliance and impaired gas exchange manifest as hypoxia in ABG and a reduced diffusing capacity for carbon monoxide. Assessment of exercise tolerance is the most important physiologic determination in ILD patients and also correlates with disease severity.

HRCT:

With the advent of HRCT the need for invasive diagnostic procedures has reduced. HRCT identifies specific imaging patterns that can strongly support the diagnosis of certain ILDs or can be virtually diagnostic of some. HRCT alone can be diagnostic in the following conditions in the presence of features characteristic to them

- Sarcoidosis
- Idiopathic Pulmonary Fibrosis
- Subacute Hypersensitivity Pneumonitis
- Lymphangitis carcinomatosis
- LCH
- LAM
- Pulmonary alveolar proteinosis (PAP)

HRCT has greatly improved the clinicians ability to shortlist the differential diagnosis, and could be diagnostic in many conditions\(^{(1-3)}\)

**GALLIUM SCAN:**

It is a non invasive test for staging sarcoidosis but is not recommended for IPF, as interpretation is difficult. Its non specific and the scan could be negative in the presence of the disease.

**VENTILATION-PERFUSION LUNG SCANNING:**

In IPF it shows non segmental inhomogeneities, scattered regions of varying VP match.

**ROLE OF MRI :**

MRI may be helpful in differentiation of fibrotic lesion from inflammation.
EXERCISE:

A very important functional parameter in Interstitial lung disease is gas exchange assessment during exercise since it could be normal when the patient is resting.

BRONCHOALVEOLAR LAVAGE:

Bronchoalveolar lavage is done through a fiberoptic bronchoscope which is wedged into a selected bronchopulmonary segment, through which saline is instilled and retrieved. It is done to sample the distal airways. The retrieved sample represents the cellular, immunologic and biochemical milieu.

Methods to analyse the sample and performing the procedure has been setforth by a multicenter publication\(^3\). BAL could be diagnostic in bronchoalveolar carcinoma; pulmonary lymphoma and lymphangitis carcinomatosa. BAL is therapeutic in pulmonary alveolar proteinosis. BAL could be diagnostic of Langerhans cell histiocytosis if histocytes are in excess of 5%. Populations at risk of ILD, like those with connective tissue disease can be assessed with BAL cellular profile, even in the absence of clinical or radiological evidence of ILD. In such patients BAL reveals increases in inflammatory cell populations.
HISTOLOGICAL DIAGNOSIS:

In the evaluation of an ILD patient, the decision to go for tissue diagnosis is the last step. A thorough history taking leads to a diagnosis in patients with CTD, occupational ILD and drug induced ILDs. But in the case of IIPs, only a suggestive diagnosis may be obtained from clinicoradiological and laboratory findings. And in many a situation, a diagnosis of IPF may only be established by histological findings of usual interstitial pneumonia pattern. When transbronchial biopsy does not yield, the next step is a Surgical lung biopsy. The utility of BAL cell analyses is a subject of difference in opinion as the findings are poor in sensitivity and specificity.

The American Thoracic Society(ATS) issued guidelines to increase the yield of BAL in the diagnostic evaluation of ILD and in encourages its use in ILD suspects.

This work utilizes the recommendations put forth by the ATS to optimize the BAL procedure and to associate the findings across different clinical entities of ILD including HRCT.
AIMS AND OBJECTIVES

PRIMARY OBJECTIVE:

To optimize the bronchoalveolar lavage (BAL) procedure as per the recommendations put forth by the American Thoracic Society for the cellular analysis of interstitial lung diseases.

SECONDARY OBJECTIVES:

Optimization of the BAL procedure as recommended by the American Thoracic Society. BAL cellular analyses association with clinical characteristics of different interstitial lung diseases and their radiological findings.
REVIEW OF LITERATURE

The technique of bronchoalveolar lavage (BAL) was first described by Reynolds and Newball in 1974. Though BAL has some limitations, it is additive, if read in the clinical background. R.P. Baughman in his editorial “The uncertainties of bronchoalveolar lavage” has discussed about the retrieval of fluid during the bronchoalveolar lavage procedure and has concluded like this “as one examines the lavage process closely, one can appreciate the uncertainty a physician may have about the retrieval process. The lavage process itself affects the results. Also, the lung is an active organ, secreting and absorbing protein and cells during the lavage procedure. I doubt that we will ever resolve the uncertainties of dilution that occur during lavage”.

In 2010, Khilnani and Hadda in their editorial titled “Bronchoalveolar lavage: A forgotten tool!” have stated that "a minimally invasive tool for diagnosis which gives a vision into infectious, immunological and inflammatory processes" that occurs at the alveolar level. It is either diagnostic or helpful in revising the differential diagnosis. They have concluded the editorial like this “although BAL has been used for more than 30 years, quite often this specialised technique is omitted because it is supposed to be a cumbersome procedure with limited diagnostic value. However, with experience it takes few
additional minutes to perform and the information gathered from it adds to the diagnostic value of FOB. Furthermore, therapeutic BAL where large amount of fluid is used to wash lower airway and is used for the treatment of pulmonary alveolar proteinosis. Therefore, it is suggested that every bronchoscopist should be well conversant with this not so commonly used technique.”

THE NEED FOR A STANDARD PROCEDURE

There are three populations of cells in the BAL.

1. Cells sampled from alveoli.
2. Cells in alveoli that is not lavaged.
3. Cells from airways that contaminate the lavage sample

Differences in BAL results were due to the differences in the technique of the BAL procedure practised in different parts of the world. Hence, ERS and ATS task force made recommendation for a standard procedure.
SAFETY AND SIDE EFFECTS OF BAL

- The BAL procedure is relatively safe. Side effects if any are usually minor. They include cough and fever after the procedure, transient infiltrates in the chest X-Ray about 24 hours after the procedure, transient deterioration in FVC, FEVI, decreased oxygen tension. When a patient has an underlying disease with respiratory insufficiency supplemental oxygen is to be administered to maintain saturation at 92%. The safety of BAL is well established in adult respiratory distress syndrome (ARDS). ATS/ERS committee recommends the following regarding the BAL procedure:
  - routine clinical and laboratory evaluation to diagnose bleeding tendencies
  - Site of BAL to be chosen based on HRCT findings, instead of a traditional BAL site
  - BAL procedure to be done, with the scope wedged into the selected segment
  - the unretrieved volume of instilled saline should not exceed 100ml
  - the suction pressure is maintained below 100mm Hg and visual collapse of the airway is avoided.
if the total retrieved is less than 30% of the instilled saline volume, the differential cell count will be misleading to the clinician

if the fluid retrieved is less than 5%, the procedure is abandoned to prevent complications

20 ml of sample from the entire lavaged fluid is ideal for cell counts analyses

**BAL CELLULAR PROFILE IN VARIOUS ILDS AND THEIR CLINICAL APPLICATIONS: ATS CLINICAL PRACTISE GUIDELINES**

1. **Idiopathic pulmonary fibrosis (IPF):** IPF is a most frequently diagnosed Idiopathic interstitial pneumonia (IIP), hence, extensively studied. IPF is a distinct condition manifesting in the adult age group, and is characterised by usual interstitial pneumonia (UIP) pattern both pathologically and in high resolution CT scan of the chest. The lavage cellular differential in IPF characterised by predominant macrophages and modest elevation of neutrophils, eosinophils, but lymphocytes levels being normal. But, these features are not specific for ILD. Neutrophils counts range from 5% to 30% in up to 90% of the patients with IPF. The neutrophils percentage may be proportional to the disease extent visualised by
imaging. Eosinophils counts about 5% are seen in up to 60% of the cases. Atypical features are eosinophils >20% or lymphocytes >15%. , suggest a diagnosis towards ELDs, COP, NSIP, HP, sarcoidosis, superadded infections. While BAL lymphocytosis implies response to steroid therapy, BAL neutrophilia is an independent predictor of mortality. The role of serial BAL to assess prognosis and response to treatment has not been established.

**AS A DIAGNOSTIC AID:**

- BAL cellular profile as a stand alone test cannot be a confirmatory test
- Typical findings as mentioned above, along with UIP pattern in HRCT chest and supporting clinical criteria may provide a more confident diagnosis
- BAL lymphocytosis >30% is against a diagnosis of IPF.

Prognostic role: not established
2. NONSPECIFIC INTERSTITIAL PNEUMONIA (NSIP)

It is a type of IIP with a better prognosis than IPF. NSIP is a pathological term used in lung biopsy specimens not suggestive of a clear pattern\(^7\). It is further subdivided based on histopathology as cellular, fibrotic and mixed. Cellular NSIP has the longest survival rate, while fibrotic-mixed is the more common type Clinical presentation maybe of subacute or chronic onset. It is predominant in non smokers and in women.

Lymphocytosis and decreased CD4/CD8 ratio are features of BAL profile in NSIP. Though not very specific, BAL cellular profile along with suggestive clinicoradiological findings can confirm a diagnosis of NSIP. However in fibrotic NSIP, BAL profile is similar to that of IPF/UIP.

**AS A DIAGNOSTIC AID:**

- Lymphocytosis with reduced CD4/CD8 ratio is seen in cellular NSIP.\(^8\)
- Lymphocytosis is not observed in fibrotic NSIP.
- Diffuse ground glass opacities in HRCT along with BAL lymphocytosis support a diagnosis of cellular NSIP.
PROGNOSTIC VALUE:

Unknown

EOSINOPHILIC LUNG DISEASES (ELD)

ELD refers to a group of different disorders that have a characteristic accumulation of eosinophils as a common feature. With the inclusion of BAL in the diagnosis of ILD, the list of conditions grouped under ELD has expanded substantially, by detecting BAL eosinophilia in the absence of peripheral blood eosinophilia. Since ELD in acute presentation is often mistaken for severe pneumonia of acute origin, diagnosis is not promptly made\(^9\). Chronic eosinophilic pneumonia (CEP) presents insidiously has a better prognosis.

Conditions that have BAL eosinophilia about 10% and below include chronic granulomatous disorders including tuberculosis and sarcoidosis, fungal diseases, IPF, collagen vascular diseases with lung involvement, pneumocystis carinii pneumonia.

Acute eosinophilic pneumonia clinically presents as an acute febrile illness, which can progress to respiratory failure and is often life threatening. BAL cellular profile shows a marked eosinophilia (>25%) and has a diagnostic value. BAL procedure can be done safely in critically ill patients in whom lung biopsy is not an option. The diagnosis
of AEP is strengthened when the lavage fluid characteristics are similar to that of diffuse alveolar damage\textsuperscript{10}. Prompt diagnosis enables early treatment with systemic corticosteroids resulting in rapid and complete recovery. Chronic eosinophilic pneumonia (CEP): occurs predominately in females\textsuperscript{11}. BAL cellular eosinophilia is diagnostic and responds to corticosteroid therapy. BAL fluid analysis also averts the need for a surgical lung biopsy in these patients.

Allergic Bronchopulmonary Aspergillosis (ABPA): is characterised by wheezing, peripheral blood eosinophilia, and lung manifestations of bronchiectasis, a sequela of allergic/immune reaction to aspergillus colonising the airway lumen. ABPA affects previous asthmatics and cystic fibrosis patients. This condition is characterised radiologically by fleeting pulmonary opacities due to eosinophilic aggregation and or mucus plugging. CT findings and clinical features coupled with eosinophilia is characteristic of this condition. BAL fluid shows a high percentage of eosinophilia, which coupled with radiological findings strongly support the diagnosis.

Sarcoidosis is a granulomatous disorder that can involve any organ. It most affects the lungs. Usually occurs in adults. Sarcoidosis is characterised by the presence of hilar adenopathy, lung infiltrations, eye and skin involvement. Due to its varied clinical manifestations and global
prevalence, sarcoidosis can simulate other inflammatory/infectious disorders. Commonly affected organs are the lungs, the lymphatic system, eyes, skin, hepatic, skeletal, cardiovascular and neurological systems.

ATS criteria to diagnose lung sarcoidosis are

i. suggestive clinical features and radiology findings

ii. documenting of non caseating granulomas on biopsy

iii. exclusion of other causes granulomatous inflammation

The sarcoid granulomas have a rim of lymphocytes in their periphery and BAL enables the airspace. Lymphocytes recovery and these are mostly activated T-cells belonging to Th-1-phenotype. Hence, in 1999, ATS/ERS statement included “heightened Th-1 immune response” instead of CD4/CD8 ratio$^{12}$ T-cell prominence in granulomatous tissues leads to a reduction in peripheral blood lymphocytes.

BAL has contributed immensely in understanding the immunology and pathogenesis of sarcoidosis. Studies have demonstrated a significant correlation between bal cellular profile and lung biopsy specimens$^{13-15}$. The BAL lymphocytosis correlates with activity of the disease, but a normal lymphocyte percentage can also be seen in the minority of patients. Increased neutrophils are seen in advanced sarcoidosis. Other BAL parameters in sarcoidosis include
neutrophils and eosinophils and a lack of foamy alveolar macrophages.

The diagnostic value of CD4/CD8 ratio has been debated as it shows a high variability in sarcoidosis\textsuperscript{14,15}, and in a significant proportion of patients this ratio might be decreased. Though CD4/CD8 ratio might be low in sensitivity, it has a specificity of $95\%$ \textsuperscript{(16-18)}.

The diagnostic value of BAL lymphocytosis is strengthened if CD4/CD8 ratio is also higher \textsuperscript{19}

ATS committee concludes that BAL fluid shows an increased lymphocytes but BAL lymphocytosis is not always present. A high CD4/CD8 ratio strongly supports the diagnosis.

A high CD4/CD8 ratio along with BAL lymphocytosis, coupled with clinical and radiological findings suggestive of sarcoidosis strongly support the diagnosis. Neither lymphocytosis nor CD4/CD8 has any prognostic value.

**Hypersensitivity pneumonitis** (HP) constitutes lung diseases characterised by a granulomatous lung disease that involves airways, alveoli and interstitium due to repeated inhalation of organic and chemical antigens, and sensitisation.

HP is typically characterised by alveolitis with lymphocytes accumulation and granulomatous inflammation.
By clinical presentation, it is classified into acute, subacute and chronic forms. Chronic forms have a variable long term outcome but often leads an irreversible parenchymal damage. The clinical presentation depends on the nature of the antigen, the amount of exposure, duration of exposure response of Host. HP affects non smokers preferentially\textsuperscript{20-23}. The apparent protective effect of smoking is thought to be due to local immune suppressive effects\textsuperscript{24,25}. But, smoking does not seem to have a suppressive role once the disease has established \textsuperscript{22}.

In smokers who develop HP, the disease tends to run a chronic course\textsuperscript{26,27}. BAL findings in HP are influenced by the clinical presentation- acute or chronic, smoking habits and the inhaled antigen characteristics.

BAL cellular profile in HP characteristically shows a marked lymphocytosis, often in excess of 50%, whereas lymphocyte percentage under 30% leads to an uncertainty in diagnosis.

The ATS committee has made the following conclusions:

1. With BAL lymphocytosis more than 50% along with clinical and radiological features consist with HP, a confident diagnosis can be made

2. To suspect alternate diagnosis in the absence of BAL lymphocytosis
Prognostic value of BAL in HP is uncertain

CVD/CTD

Understanding of various pattern of diffuse parenchymal lung diseases that affects rheumatological patients has improved considerably in the recent times. Most of this progress from the time of publication of classification of IIP\textsuperscript{28}. Diffuse lung disease occur in different patterns in rheumatological diseases. Sub clinical luseases could be identified in asymptomatic patients diagnosed with a rheumatological diseases when routinely assessed for pulmonary pathology. The histopathological patterns of UIP, LIP, COP, NSIP, DIP and DAD are all seen in rheumatological patients, often coexisting with diseases of the airways such as broncholitis obliterans and

ATS/ ERS task force published ‘clinical guidelines and indications for BAL’ in 1992 in which CTD cases were further divided into CTD with IID and CTD without IID. And the predominant abnormalities reported were an increase neutrophils in RA, Sjogrens and SLE. While SSC had increased neutrophils and eosinophils. And the document concluded that the diagnostic value was limited to differentiating an infection, drug induced disorder or hemorrhage. With more studies focussed on rheumatological disease, clearer messages are emerging.
**SSC**

Bouros et al\textsuperscript{29} studied BAL cellular profile in 73 cases of systemic sclerosis observed elevated neutrophils with elevated eosinophils or elevated lymphocytes. Eosinophils elevation was observed in patient who had NSIP pattern, but not in those with UIP pattern. Lymphocytes elevation was observed in cellular NSIP pattern (on HPE). Furthermore, elevated neutrophils correlated with extensive lesions on HRCT while lymphocytes elevation was present irrespective of any abnormality in HRCT was present or not. Some studies have described that neutrophil excess is a marker of progressive disease\textsuperscript{30,31,32} while another studies states that neutrophils excess implies more explosive disease and not a progressive one\textsuperscript{33}. Recently two large clinical trials failed to find any useful correlation of BAL cellular profile with either treatment response or progressive of the disease\textsuperscript{34,35}

The committee has concluded that the predominant profile in SSc is neutrophilia with or without eosinophilia and lymphocyte is associated with cellular-NSIP. BAL cellular patterns do not determine if treatment is required and do not help to monitor the activity of the disease.
RHEUMATOID ARTHRITIS

In the study by J.G.Garcia et al with a sample size of 24, three groups were observed.

1) Patients with clinical symptoms and radiological evidence of lung disease showing neutrophilia in BAL.

2) Patients with only radiological evidence showing BAL lymphocytosis

3) Third group without clinical and radiological evidence, have a normal BAL cell differential.

These findings interpreted that symptomatic patients with lung lesions had lymphocytosis while diffuse lung disease had BAL neutrophilia. Based on several other studies the committee has concluded that lymphocytosis is a more characteristic feature in RA than in systemic sclerosis.
PRIMARY SJOGREN’S SYNDROME

Based on a few studies in primary sjogren’s with lung involvement\(^{(36)}\)-salaffi et al and Delavanga et al it has been observed that BAL lymphocytosis is associated with a good outcome while neutrophilia with persistent disease. To sum up conclusions of the ATS committee on clinical applications of BAL profile in all CTD/CVD- related ILDs:

- Adequate studies of BAL in CVD is lacking
- There is a paucity of studies correlating lavage findings with lung biopsy/HRCT findings
- Lymphocytosis is a prominent finding in sjogren’s and RA.
- Neutrophilic correlates with extent of pulmonary involvement in HRCT.
- The clinical implication of an abnormal BAL cell differential is not known.

Significance of lavaged cells differential in classification, and in managing of ILD in CTD are not known.
OCCUPATIONAL INTERSTITIAL LUNG DISEASE

In a genetically susceptible person, occupational exposures can cause specific ILD’s. These exposures may have occurred several years ago Pneumoconiosis- caused by asbestos, silica and coal dust which get accumulated in the distal airways and lung parenchyma, which is met by a tissue reaction. Metal induced lung disorders that could be granulomatous or fibrotic, induced by beryllium, titanium, aluminum, iron, silver, etc.

SILICOSIS:

Crystalline silica exposure occurs in various occupational settings. Silicotic nodule is the histopathological hallmark of this condition and is formed by macrophages laden with dust and fibrosis tissue surrounding a hyalinised connective tissue.

Eventually as disease progresses these nodules coalesce to produce large conglomerates that involve the pulmonary vessels, airways, and finally producing progressive massive fibrosis (PMF) $^{37-49}$

Bronchoalveolar lavage profiles $^{37-49}$ in workers exposed to silica as well as workers who had silicosis had an increase in macrophages which is significant in non-smokers. Lymphocytosis and neutrophilia seems to reflect the ongoing inflammatory process and progression to silicosis.
The committee has conclude that

1) Lavage findings in workers with exposure and workers who had silicosis showed an increased macrophages even in non smoking individuals

2) Lymphocytosis and neutrophilia in BAL are associated with progression to disease.

**PULMONARY LANGERHANS CELL HISTIOCYTOSIS**

It is a rare condition featuring an abnormal proliferation of langerhans cells.

This disease can be localised or involve multiple systems which were called Hand-Schuller-Christian disease, histiocytoisis X, and Letterer-Siwe disease based on the various presentations, is now called Langerhan Cell Histiocytosis(LCH) ⁵¹

Pulmonary LCH(PLCH) has a variable clinical presentation. Most of the patients are smokers with non specific chest symptoms. One characteristic manifestation is recurrent pneumothorax. The pulmonary lesions consists nodules that take bronchiolocentric stellate shape. These contain Langerhans cells, eosinophils, plasma cells and lymphocytes. Eosinophils are found in active stages only.
BAL cellular profile is diagnostic in PLCH and obviates surgical lung biopsy\textsuperscript{52,53}. Demonstrating langerhans cells in BAL fluid supports the diagnosis of PLCH in the presence of consistent clinical and radiological features\textsuperscript{54}. Immunohistochemical studies or Birbeck granules demonstraton are highly specific for PLCH.

By reviewing literature\textsuperscript{55-58,59-64} it has been concluded by the committee that LC differential above 5% is highly supportive of PLCH diagnosis. But, low numbers of LC does not exclude PLCH. It has been observed that as disease progresses, LC percentage decreases.

The committee concludes that

- In the presence of clinical suspicion and suggestive HRCT findings, BAL cellular profile can help to confirm the diagnosis.
- In the absence of the diagnostic HRCT findings, elevated CD1\textsubscript{a} staining above 5% of cells is adequate for diagnosis.
- BAL finding of less than 5 percent CD1\textsubscript{a} cells do not exclude PLCH, who have clinical and radiological features consistent with a diagnosis of PLCH.
**DRUG INDUCED LUNG DISEASE**

Drug induced ILD include a wide range of clinical presentations, mode of onset acute or chronic, lung pathologies and imaging patterns\(^{65,66}\) which include Eosinophilic pneumonia, pulmonary fibrosis, HP, DAD, DAH, acute non cardiogenic pulmonary edema, and drug induced lupus.

Most common patterns are

1) An acute hypersensitivity response characterised by an elevated eosinophils levels in blood or in airspaces.

2) Subacute/Chronic response that may imitate certain forms of IIP and have fibrosis.

Amiodarone related ILD is characterised by foamy macrophages\(^{67,68}\). Nitrofurantoin and methotrexate related ILD is characterised by elevated CD4 lymphocytes\(^{69,70}\).

Drug induced ILD in a patient with CTD/CVD is often difficult to establish as pulmonary toxicity may be superimposed on ILD that is already present. Nonetheless, HRCT often establishes a pattern that are consistent with specific drug exposures like pulmonary hemorrhage, pulmonary edema, fibrosis, ground glass attenuation. BAL eosinophilia or lymphocytosis suggest a favourable response to corticosteroid therapy.
CONCLUSIONS

1. As a diagnostic aid:

2. BAL cell profile along with HRCT characteristics could aid in diagnosing drug related ILD

3. BAL is also useful to rule out pulmonary hemorrhage/ infections.

RADIATION PNEUMONITIS

Radiation fibrosis and radiation pneumonitis (RP) often complicate radiotherapy of malignancies.

Radiation pneumonitis clinically manifests 1-3 months after radiation in about 8% of patients. And radiation fibrosis takes about 6-24 months to evolve and maybe observed in patients who did not suffer from radiation pneumonitis early pathological changes of small vessel injury, congestion and hyaline membrane formation may proceed to a chronic phase if the injury was severe, characterised by progressive septal thickening with vascular sclerosis.

BAL lymphocytosis has been observed in the contralateral lung of patients who had radiotherapy for breast cancer which suggests the possibility that radiation pneumonitis may be an immune mediated response of the lung tissue to radiation by generating auto antigens.
Since patients undergoing radiotherapy have other possible causes for radiological infiltrates, BAL is often necessary to exclude hemorrhage, extension of malignancy, infections and drug toxicity. BAL cell profile in RP is characterised by elevated inflammatory cells.

ATS Committee conclusions:

- BAL is useful to exclude other causes of radiological infiltrates like hemorrhage/ infections
- BAL lymphocytosis with consistent clinical and radiological findings supports the diagnosis of radiation pneumonitis.

**LYMPHANGITIC CARCINOMATOSIS**

BAL cellular analysis usually yield the diagnosis surgical biopsy often unnecessary pneumonitis.

**CHRONIC ASPIRATION PNEUMONITIS**

Lipid laden alveolar macrophages staining with oil-red O and a semiquantitative scoring method was described by some investigators to diagnose chronic recurrent aspiration.

Identifying pepsin like activity might prove more useful in diagnosing gastric secretions aspiration.
ACUTE ONSET ILD

- Marked elevation of eosinophils in BAL is diagnostic of acute EP.
- BAL helps to diagnose infections and alveolar hemorrhage
- marked elevation of lymphocytes in the background of consistent clinical settings suggests hypersensitivity pneumonitis or drug toxicity.

HRCT IN THE DIAGNOSIS OF ILD

HRCT of the chest has a high sensitivity of 94% for ILD whereas sensitivity of x-ray film is about 80%. HRCT can demonstrate early disease when the x-ray is apparently normal. But, HRCT does not always detect early disease demonstrated by histopathological examination.

A study conducted by Vancouver group in 1989 was the first in establishing a role for CT in diagnosing ILD. They observed that x-ray chest as the first choice correctly diagnosed 77% while CT chest as the first choice diagnosed 93%. Grevier et al reported that by combining clinical, radiographic and HRCT findings the right diagnosis can be done in two thirds of patients. Certain diseases have very characteristic HRCT features obviating a need for biopsy

ILDs which may exhibit very typical HRCT features are:
• IPF
• Sarcoidosis
• Subacute HP
• LC
• PLCH
• LAM
• Alveolar proteinosis.

**HRCT PATTERNS AND PROGNOSTIC SIGNIFICANCE:**

The ability of HRCT patterns in prediction of treatment response and outcome, etc has been extensively studied.

The ground glass opacity pattern is predictive of a better response to treatment and longer survival than in patients with reticular pattern- in HRCT.

Though at presence evidence is lacking to support HRCT routinely in monitoring the ILD patients, HRCT proves useful in explaining a sudden or unexpected clinical deterioration. HRCT is definitely superior to x-ray chest in diagnosing ILD and also in deciding upon an optimal site for biopsy when required.

**BAL/TBLB:** In most of the ILDs, correct diagnosis can be made from putting together, clinical, laboratory and HRCT informations.
When HRCT features are typical of IPF, in addition to typical clinical features, surgical lung biopsy will not be necessary. BAL and/or TBLB could increase the diagnostic confidence in certain conditions like malignancy, infection, eosinophilia, HP-subacute, COP, sarcoidosis, rarer conditions like PLCH and PAP.

The decision to perform BAL is informed by patient fitness, differential diagnosis, a supportive laboratory to do BAL cell differential count.

BTS guidelines for TBLB in patients with ILD

- TBLB when needed is to be performed prior to initiating specific therapy.
- Bronchoalveolar lavage is to be considered in rare ILDs, suspected malignancy and infections.
- With clinicoradiological features characteristic of IPF, BAL is necessary as a diagnostic intervention.
- In the absence of typical clinical and radiological features, BAL cellular profile allows a confident diagnosis in sarcoidosis and hypersensitivity pneumonitis.
- In the instance of diagnostic uncertainty where BAL is considered the important diagnostic tool, its preferably done in a center.
specialised for the procedures and specialised in analysing the samples

- BAL is to be done in all patients planned for TBLB
- TBLB is the first line procedure in diseases with bronchocentric involvement 4-6 specimens must be taken.
- TBLB is not reliable in rare lung disorders

**ILD AND THE UTILITY OF SURGICAL LUNG BIOPSY (SLB):**

**BTS GUIDELINES**

SLB is the definitive note of establishing the diagnosis of IPF. SLB excludes infections and malignant processes which may occasionally mimic a chronic ILD. SLB can differentiate CHP from IPF, where CHP is a treatable disease. Disease activity can also be assessed by SLB. HPE diagnostic of IPF has a sensitivity above 60% and specificity almost 97%. Clinical radiological feature can diagnose IPF with a sensitivity and specificity of 48% and 89%.

Studies that contain “changes in therapy” as a reported outcome, SLB contributed more. Hence SLB is ideally done in prior to immunomodulation therapy.
There are two approaches to obtain SLB. One is the traditional limit thoracotomy (OLB) and the other is through a video-assisted thoracoscopic approach (VATS). VATS is being increasingly used nowadays. Both these procedures are performed under general anesthesia. Randomized controlled trials that compared open lung biopsy and VATS biopsy reported no significant differences in the time taken for the procedure, complications and diagnostic yield except for a reduced length of stay in hospital in the VATS group.

SLB specimens ideally should be 4cm diameter with a depth of 3-5cm from the pleural space. It should be promptly sent to the pathology laboratory, where it is inflated with formalin. Apart from HPE, PCR, in situ hybridization and immune histochemical techniques maybe applied to make most use of the biopsy specimen.
To summarise the recommendations for SLB in interstitial lung diseases.

- SLB is to be done prior to initiating specific treatments.
- SLB also provides a definitive diagnosis in IPF and other ILDs.
- IPF can be diagnosed with confidence in the presence of typical clinicoradiological picture.
- ILDs require multiple biopsy specimen from multiple lobes.
- Multiple biopsy from differents lobes are easier to do with VATS.
- It has lesser early post operative pain compared to OLB.
- Biopsy sites should be chosen based on HRCT findings.
MATERIALS AND METHODS:

Proforma was designed and Institutional ethical committee clearance was obtained.

The nature of the procedure and the purpose of the study was explained in detail to all the patients who were enrolled in this study and informed consent was obtained from all of them. Data was collected as mentioned in the proforma.

SUBJECT SELECTION

Fifty consecutive ILD patients were recruited based on clinical and radiological evaluation after institutional ethics committee approval.

INCLUSION CRITERIA

- ILD patients diagnosed based on clinical and HRCT findings.
- Acute and chronic ILDs in immunocompetent patients
- ILD patients tolerable to the procedure
- Patients above the age of 18 years.

EXCLUSION CRITERIA

- ILD patients with bleeding disorders
- ILD patients with cardiorespiratory instability
- Pregnant women
- Pediatric patients
**SCREENING PROCEDURES**

Patients who were recruited were admitted in our hospital and were subjected to routine blood investigations including HIV testing, HRCT chest, cardiac and rheumatological evaluations.

After obtaining cardiac fitness for the BAL procedure, patients whose respiratory status was adequate, were subjected to the BAL procedure.

**BAL PROCEDURE:**

- BAL was done using a flexible bronchoscope wedged into the chosen bronchopulmonary segment
- The bronchopulmonary segment to be lavaged was selected based on HRCT findings - areas with GGOs, profuse nodular lesions or thin reticulations were optimal targets
- Patients in whom the lavage site was other than the right middle lobe, positioning of the patient was adjusted retrieval.
- The total volume of saline instilled was about 240ml, in aliquots of 60ml. In all the patients, the retrieval volume was more than 100ml. Four sequentially instilled aliquots were withdrawn.
- The first retrieval sample was sent for microbiological analysis
- The samples were collected into a mucus extractor whose capacity was 25ml. About 4 to 5 such containers of sample was collected
from each patient and transported to the cytology laboratory in an ice box which maintains a temperature of 4°C.

- Arrangements were made to analyse the sample for total cell counts, differential cell counts within one hour of the BAL procedure.
- Throughout the BAL procedure, oxygen saturation of the patient was monitored and maintained above 92%.
- The suction pressure used during the procedure was under 100mmHg, and was further adjusted to avoid visible airway collapse.
- Before the sample was sent to the laboratory, gross appearance of the fluid was noted.
- The BAL samples were pooled together in the laboratory and about 20ml of the pooled sample was used for the cellular analysis.
TABLE 3:
SUMMARY OF BAL CELLULAR PATTERNS IN NORMAL/HEALTHY ADULT NONSMOKERS AND IN PATIENTS WITH COMMON INTERSTITIAL LUNG DISEASES (CONSISTENT PATTERNS AND CLINICAL UTILITY)

<table>
<thead>
<tr>
<th>I. Normal Adults (Nonsmokers)</th>
<th>BAL Differential Cell Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar macrophages</td>
<td>85%</td>
</tr>
<tr>
<td>Lymphocytes (CD41/CD81 ¼ 0.9–2.5)</td>
<td>10–15%</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>&lt;3%</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Squamous epithelial/ciliated columnar epithelial cells†</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Lymphocytic Cellular Pattern</td>
<td>Eosinophilic Cellular Pattern</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>&gt;15% Lymphocytes</td>
<td>&gt;1% Eosinophils</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>Eosinophilic pneumonias</td>
</tr>
<tr>
<td>Nonspecific interstitial pneumonia (NSIP)</td>
<td>Drug-induced pneumonitis</td>
</tr>
<tr>
<td>Hypersensitivity pneumonitis</td>
<td>Bone marrow transplant</td>
</tr>
<tr>
<td>Drug-induced pneumonitis</td>
<td>Asthma, bronchitis</td>
</tr>
<tr>
<td>Collagen vascular diseases</td>
<td>Churg-Strauss syndrome</td>
</tr>
<tr>
<td>Radiation pneumonitis</td>
<td>Allergic bronchopulmonary aspergillosis</td>
</tr>
<tr>
<td>Cryptogenic organizing pneumonia (COP)</td>
<td>Bacterial, fungal, helminthic, Pneumocystis infection</td>
</tr>
<tr>
<td>Lymphoproliferative disorders</td>
<td>Hodgkin’s disease</td>
</tr>
</tbody>
</table>
II. Interstitial lung diseases

a. Disorders associated with increased percentage of specific BAL cell types

b. Abnormal BAL differential cell patterns that suggest specific types of ILD

A lymphocyte differential count $>25\%$ suggests granulomatous disease (sarcoidosis, hypersensitivity pneumonitis, or chronic beryllium disease), cellular nonspecific interstitial pneumonia, drug reaction, lymphoid interstitial pneumonia, cryptogenic organizing pneumonia, or lymphoma.

CD4/CD8 is highly specific for sarcoidosis in the absence of an increased proportion of other inflammatory cell types. A lymphocyte differential count $>50\%$ suggests hypersensitivity pneumonitis or cellular nonspecific interstitial pneumonia. A neutrophil differential count $>50\%$ supports acute lung injury, aspiration pneumonia, or suppurative infection.
c. Other abnormal BAL findings

<table>
<thead>
<tr>
<th>Infectious organism</th>
<th>Lower respiratory infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant cells (light microscopy, flow cytometry)</td>
<td>Cancer</td>
</tr>
<tr>
<td>Bloody fluid that increases in successive aliquots</td>
<td>Pulmonary hemorrhage 6 diffuse alveolar damage</td>
</tr>
<tr>
<td>Milky fluid with positive periodic acid Schiff staining and amorphous debris</td>
<td>Pulmonary alveolar proteinosis</td>
</tr>
<tr>
<td>In vitro lymphocyte proliferative response to specific beryllium antigen</td>
<td>Chronic beryllium disease</td>
</tr>
</tbody>
</table>

Definition of abbreviation: BAL bronchoalveolar lavage.

* The presence of squamous epithelial cells indicates upper airway secretion contamination.

\(^\gamma\) Epithelial cells >5% suggest suboptimal sample (BAL cellular patterns should be interpreted with caution).
The above table was from ATS clinical practise guidelines is a reference used in this study and based on this we grouped Cellular patterns from the results obtained. “ The reason for routine cellular analysis whenever BAL is performed in a patient with suspected ILD is that identification or exclusion of the predominantly inflammatory cellular pattern may support a specific type of ILD or help narrow the differential diagnosis when considered in the context of the clinical and radiological findings. The notion that a prominence of specific nucleated inflammatory or immune cells in the BAL correlates with an increased likelihood of certain types of ILD is supported by numerous accuracy studies that are limited by risk of bias. These include pronounced BAL eosinophilia in eosinophilic pneumonia77,78, drug reactions79,81, BAL lymphocytes in sarcoidosis82-85, hypersensitivity pneumonitis86-88, pneumotoxic drug reactions89,90 or cellular NSIP91,92.”
Follow up procedures

The patients who had undergone the procedure were observed for 48 hours for post procedure bleed, followed up with chest x-rays immediately and 24 hours after the procedure. Those who did not require inpatient treatment were discharged with care.

ASSESSMENTS OF PARAMETERS

TECHNIQUE OF BAL CELL ANALYSIS

The cellular analysis is performed within an hour from the time of procedure. The total cell count was done with a hemocytometer, and the viability of the cells was analysed by Trypan blue exclusion. The differential counting was done by cytocentrifugation after Wright-Giemsa staining and enumeration of at least 400 cells. The presence and relative numbers of erythrocytes and epithelial cells were noted. The presence of squamous epithelial cells suggests that BAL fluid is contaminated with upper airway secretions, and the presence of large numbers of bronchial epithelial cells suggests that the BAL may not have adequately sampled distal airspaces. Excess BAL fluid was stained and cultured for mycobacteria and fungi in the microbiology laboratory, as well as screened for neoplastic cells. These were important additional tests to consider because infections and diffuse neoplasms can masquerade as ILD or coexist with ILD.
INTERPRETATION OF BAL DIFFERENTIAL COUNTS

The ranges of differential cell counts that are considered normal and abnormal derive from several sources. Numerous investigators have published BAL immune cell profiles from cohorts of clinically normal volunteer subjects recruited in single-center studies\(^{92-98}\) and these reports have been used to define normal and abnormal differential cell counts. In addition, the multi-center BAL Cooperative Study\(^ {92}\) reported the differential cell counts in the BAL of normal subjects (including smokers or ex-smokers) compared with patients with ILD. An increased number of nucleated immune cells and abnormal proportions of immune cell types may suggest or support specific types of ILD in the absence of an infection. A mixed cellular pattern can be observed with any ILD; when mixed cellular patterns are observed, the dominant cell type may be the most consistent with a specific ILD diagnosis. A BAL fluid cell differential count with greater than 15% lymphocytes, greater than 3% neutrophils, greater than 1% eosinophils, or greater than 0.5% mast cells indicates BAL lymphocytosis (i.e., a lymphocytic cellular pattern), BAL neutrophilia (i.e., a neutrophilic cellular pattern), BAL eosinophilia (i.e., an eosinophilic cellular pattern), or BAL mastocytosis, respectively. A lymphocyte differential count greater than or equal to 25% suggests granulomatous lung disease (e.g., sarcoidosis, HP, NSIP, chronic beryllium disease, drug reaction, LIP, COP, or lymphoma), while a
lymphocyte differential count greater than 50% is particularly suggestive of HP or cellular NSIP. An eosinophil differential count greater than or equal to 25% is virtually diagnostic of eosinophilic lung disease in the appropriate clinical setting. A neutrophil differential count greater than or equal to 50% strongly supports acute lung injury, aspiration pneumonia, or suppurative infection. Finally, a mast cell differential count greater than 1% combined with a lymphocyte differential count greater than 50% and a neutrophil count greater than 3% is suggestive of HP. A predominance of macrophages containing smoking-related inclusions with no or minor increases in other cell types is compatible with smoking-related ILD, such as DIP, RBILD, or pulmonary Langerhans cell histiocytosis (PLCH). Additional tests to identify and count Langerhans cells in the appropriate clinical setting may be useful in narrowing the differential diagnosis. A predominance of hemosiderin-laden macrophages is suggestive of chronic or occult alveolar hemorrhage syndromes resulting in pulmonary hemosiderosis or diffuse alveolar damage.
ATS Recommendations “For patients with suspected ILD in whom BAL is performed, the lymphocyte subset analysis NOT be a routine component of BAL cellular analysis. The lymphocyte subset analysis (by cytometry or immunocytochemistry) will not be performed routinely, but rather would be performed if a lymphocytic disease is suspected or the initial BAL cellular findings identify a lymphocytosis. This suggestion is based upon the committee’s clinical experience that lymphocyte subset analysis is rarely helpful and potentially misleading in the absence of a clinically suspected lymphocytic disease or a lymphocytosis. Many investigators have characterized lymphocyte subsets on the basis of T helper (CD4) versus T suppressor (CD8) phenotypes, and have found correlations of the CD4/CD8 T lymphocyte ratio with specific disease processes such as sarcoidosis and hypersensitivity pneumonitis. However, subsequent investigations have found that the CD4/CD8 ratio may not be significantly increased in a substantial number of patients with sarcoidosis or significantly decreased in a substantial proportion of patients with hypersensitivity pneumonitis, and can change during the course of the disease process. In addition, the BAL CD4/CD8 T lymphocyte ratio varies with age and may be significantly increased in normal subjects. These issues are discussed extensively in the portion of the online supplement that pertains to specific forms of ILD. However,
in the case of sarcoidosis, the combination of BAL lymphocytosis combined with a considerably increased BAL CD41/CD81 lymphocyte ratio (e.g., > 4) may increase the confidence of a diagnosis of sarcoidosis if other clinical features and imaging are consistent with this diagnosis, and lymphocyte subset determinations may be performed at the discretion of the pulmonologist if such analysis can be reliably performed in the clinical laboratory and is considered to be clinically useful. Finally, there are other tests that can be performed on BAL fluid on a case-by-case basis and may be helpful in specific clinical circumstances. Analysis by a cytopathologist is indicated if there are isolated cells that are suspicious for malignancy. Periodic Acid Schiff staining or Oil Red O staining may be helpful if pulmonary alveolar proteinosis or aspiration is suspected, respectively. Hemosiderin staining may be performed if hemorrhage is suspected and/or the initial BAL raises the suspicion of hemosiderin-laden macrophages”.
STATISTICAL ANALYSIS:

Non parametric analysis was performed on the cohort after finding the median values of different cell counts obtained from the fluid analysis. Overall significance of the cell counts between different ILD’s was assessed using SPSS version 13 (SPSS, Chicago, IL) and P < 0.05 was considered significant.

STUDY DESIGN:

Prospective continuous study
RESULTS:

Fifty consecutive ILD patients were recruited for the study after obtaining informed consent. Clinically suspected ILD patients were subjected to HRCT and based on HRCT findings, BAL was performed after selecting the site for fluid collection in the lungs, except for two patients with ARDS. The procedure, transport, processing and analysis of BAL fluid were performed as per ATS guidelines. The patients were segregated based on the ATS classification given in table 3.
### TABLE 4

<table>
<thead>
<tr>
<th>DD1 LYMPHOCYTIC CELLULAR PATTERN</th>
<th>DD3 EOSINOPHILIC CELLULAR PATTERN</th>
<th>DD2 NEUTROPHILIC CELLULAR PATTERN</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;15% lymphocytes</td>
<td>&gt;1% eosinophils</td>
<td>&gt;3% neutrophils</td>
</tr>
<tr>
<td>sarcoidosis</td>
<td>Eosinophilic pneumonias</td>
<td>Collagen vascular diseases</td>
</tr>
<tr>
<td>Nonspecific interstitial pneumonia(NSIP)</td>
<td>Drug-induced pneumonitis</td>
<td>Idiopathic pulmonary fibrosis</td>
</tr>
<tr>
<td>Hypersensitivity pneumonitis</td>
<td>Bone marrow transplant</td>
<td>Aspiration pneumonia</td>
</tr>
<tr>
<td>Drug-induced pneumonitis</td>
<td>Asthma, bronchitis</td>
<td>Infection: bacterial, fungal</td>
</tr>
<tr>
<td>Collagen vascular diseases</td>
<td>Churg-strauss syndrome</td>
<td>Bronchitis</td>
</tr>
<tr>
<td>Radiation pneumonitis</td>
<td>Allergic bronchopulmonary aspergilosis</td>
<td>Asbestosis</td>
</tr>
<tr>
<td>Cryptogenic organizing pneumonia(COP)</td>
<td>Bacterial, fungal, helminthic, pneumocystis infection</td>
<td>Acute respiratory distress syndrome(ARDS)</td>
</tr>
<tr>
<td>Lymphoproliferative disorders</td>
<td>Hodgkin’s disease</td>
<td>Diffuse alveolar damage(DAD)</td>
</tr>
<tr>
<td>DD4(lymphocyte differential count&gt;=25%)</td>
<td>DD5(lymphocyte differential count&gt;50%)</td>
<td>DD6(neutrophil differential count&gt;50%)</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>----------------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Suggests granulomatous disease(sarcoidosis), Hypersensitivity pneumonitis or chronic beryllium disease), cellular nonspecific interstitial pneumonia, drug reaction, lymphoid interstitial pneumonia, cryptogenic organizing pneumonia or lymphoma. CD4+/CD8+&gt;4 is highly specific for sarcoidosis in the absence of an increased proportion of other inflammatory cell types.</td>
<td>Suggests hypersensitivity pneumonitis or cellular nonspecific interstitial pneumonia.</td>
<td>Supports acute lung injury, aspiration pneumonia or suppurative infection.</td>
</tr>
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</table>

**OTHER ABNORMAL BAL FINDINGS**
<table>
<thead>
<tr>
<th>Infectious organism</th>
<th>Lower respiratory infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant cells (light microscopy, flow cytometry)</td>
<td>Cancer</td>
</tr>
<tr>
<td>Bloody fluid that increases in successive aliquots</td>
<td>Pulmonary hemorrhage, diffuse alveolar damage</td>
</tr>
<tr>
<td>Milky fluid with positive periodic acid Schiff staining and amorphous debris</td>
<td>Pulmonary alveolar proteinosis</td>
</tr>
<tr>
<td>In vitro lymphocyte proliferative response to specific beryllium antigen</td>
<td>Chronic beryllium disease</td>
</tr>
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</table>

The segregation of the cohort as per the clinical diagnosis is as follows: idiopathic pulmonary fibrosis (IPF-8), hypersensitivity pneumonitis (CHP-17), connective tissue disorder (CVD-9), sarcoidosis (SAR-3), pneumoconiosis (SIL-5), ARDS (2), eosinophilic lung disease (ABPA-2) and lymphangitic carcinomatosa (LC-2), aspiration bronchiolitis (AB1) and pulmonary histocytosis (PLCH). The demographic data and the finding are listed in table 5.
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<tr>
<th>S.NO</th>
<th>ID#</th>
<th>AGE/SEX</th>
<th>S</th>
<th>A</th>
<th>EXP</th>
<th>D.O.I</th>
<th>PFT</th>
<th>RHE</th>
<th>BAL1</th>
<th>BAL2</th>
<th>HRCT DD</th>
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<td>O.D</td>
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<td>_</td>
<td>DD1,DD2</td>
<td>DD6,DD4</td>
<td>UIP-DD</td>
<td>IPF,NSIP,AL,S,A,CHP</td>
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<td>Age/Gender</td>
<td>History</td>
<td>Duration</td>
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<td>Findings</td>
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<td>DD4</td>
<td>DD-GGO,NS IP</td>
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<td>7</td>
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<td>PLCH</td>
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ABBREVIATIONS:

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<th>Age group</th>
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<td>20-40 years</td>
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<tr>
<td>41-60 years</td>
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<tr>
<td>&gt;60 years</td>
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Out of 50 patients the above Graph shows the representation of age groups.
Out of 50 patients the above pie chart shows the gender distribution.
Out of 50 patients studied the predominant symptom was dyspnea.
The above pie chart represents the distribution of smoking status of the patients studied.
Out of the 50 patients studied the predominant pulmonary function testing pattern was restriction as shown above.
<table>
<thead>
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<th>HRCT PATTERN</th>
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<td>Cystic</td>
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<td>23</td>
</tr>
<tr>
<td>PMF</td>
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The above represents the distribution of the various HRCT patterns in the patients studied.
The distribution of the various ILDs are represented below.
Among the 50 patients studied, the above graph represents the conditions showing total cell counts (BAL) in relation to the normal control (NC).
The above graph represents the Alveolar Macrophages levels in various ILDs in relation to the normal control.
The above graph represents the BAL Lymphocyte variations in relation to the normal control values in different ILDs
The above graph shows neutrophil counts variations in relation to the normal control in various ILDs.
The above graph represents the mean age at Diagnosis of different ILDs.
The above graph shows gender preponderance of different ILDs with silicosis being exclusive in males, IPF and CHP predominantly occurring in males. And sarcoidosis, CVD occurring more in females, in the population studied.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>IPF</td>
<td>Idiopathic Pulmonary fibrosis</td>
</tr>
<tr>
<td>CHP</td>
<td>Chronic hypersensitivity pneumonitis</td>
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<tr>
<td>CVD</td>
<td>Collagen Vascular Disease</td>
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<tr>
<td>SAR</td>
<td>Sarcoidosis</td>
</tr>
<tr>
<td>SIL</td>
<td>Silicosis</td>
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<td>ARDS</td>
<td>Adult respiratory distress syndrome</td>
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<td>ELD</td>
<td>Eosinophilic Lung Disease</td>
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<tr>
<td>RP</td>
<td>Lymphangitis Carcinomatosis</td>
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<td>AB</td>
<td>Aspiration Bronchiolitis</td>
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<tr>
<td>PH</td>
<td>Pulmonary Histiocytosis</td>
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</table>
Lymphangitis carcinomatosis was seen in the 6th decade, IPF and CHP in 5th decade, ELD, CVD, SAR, and SIL were noticed in the 4th decade of life. The average duration of the disease was 3.1 years, 14.1 years and 1.5 years for IPF, CHP and CVD respectively.

The lymphocytes in lung secretions was significantly high in IPF, CHP, CVD and SAR while the percentage of neutrophils were significantly high in all the sub groups of ILDs noted in the cohort on comparison to normal values.

CHP- chronic hypersensitivity pneumonitis

CVD- collagen vascular disease/ CTD-ILD

SAR- sarcoidosis

SIL- silicosis

ELD- eosinophilic lung disease
IPF

Eight patients were diagnosed with IPF based on clinical evaluation, pulmonary function test (restrictive pattern) and HRCT findings (subpleural/basal reticulation and honeycomb appearance). The cell cytology of BAL on seven IPF patients revealed a lymphocyte count range of 25% to 38%, and neutrophil count range 12% to 52%. For those with a neutrophil count range less than 50%, a diagnosis of idiopathic pulmonary fibrosis with bronchitis probably superadded infection was made. A diagnosis of IPF with Bronchitis and suppurative infection was made in one patient with a neutrophil count of above 50%. In patient 7 as the respiratory epithelial contamination was high and the analysis was forbidden due to suboptimal sample.

CHP

Out of 17 patients diagnosed with chronic hypersensitivity pneumonitis, patient CHP11 was not analysed due to suboptimal quality (PQ-poor quality) identified based on epithelial cells levels i.e. More than 5 percent. In the rest of the samples, the lymphocyte count range was 26% to 55% and the neutrophil count was in the range of 5% to 20%. Based on the cellular count, a diagnosis of acute exacerbation of
chronic hypersensitivity pneumonitis with bronchitis probably associated with superadded infection was made in all these patients. In addition to the above findings, patient 10 had 3% eosinophils, which redefined the diagnosis as acute exacerbation of chronic hypersensitivity pneumonitis with allergic bronchitis probably associated with superadded infection.

CVD

The lymphocyte range was 8% to 70% while the neutrophil count was in the range of 8% to 65% in nine patients. In patient CVD-1 as the lymphocyte count was 70% a diagnosis of acute exacerbation of interstitial lung disease with bronchitis probably associated with superadded infection was made. In patient CVD-2 with rheumatoid arthritis and a neutrophil count above 50% in the BAL cellular pattern, the diagnosis was refined as rheumatoid arthritis associated interstitial lung disease with bronchitis and suppurative infection. In the rest of the patients the diagnosis was refined as rheumatoid arthritis with interstitial lung disease with bronchitis probably due to with superadded infection.

EOSINOPHILIC LUNG DISEASE:

Apart from the routine lymphocyte and neutrophil count, eosinophil count of more than 25% was diagnostic of acute/chronic eosinophilic pneumonia and ABPA.
PULMONARY HISTOCYTOSIS:

Predominance of histocytosis BAL fluid was able to narrow down this diagnosis. sarcoidosis: In three patients with sarcoidosis, the lymphocyte and neutrophil count range was 28% to 32% and 10% to 45% respectively. Based on BAL finding the previous diagnosis was refined as sarcoidosis induced interstitial lung disease with bronchitis probably associated with superadded infection.

ARDS AND ASPIRATION BRONCHIOLITIS

These diseases were diagnosed based on clinical history and examination and the role of BAL in defining the diagnosis was limited.

Lymphangitis carcinomatosa: BAL cytology yielded the diagnosis and made a biopsy unnecessary, in both the cases.
Overall, BAL findings were able to clinch the diagnosis in eosinophilic pneumonia, lymphangitis carcinomatosis and pulmonary histocytosis, while it refined the diagnosis of IPF and CHP. BAL helped in establishing infection as the cause of acute exacerbation in IPF, and in other ILDs. On the other hand, the role of BAL findings in sarcoidosis and silicosis was limited as HRCT had precisely help to diagnose the disease based on the image pattern. BAL had no role to play in the diagnosis of ARDS and aspiration bronchiolitis as the diagnosis is more on clinical history and evaluation.
STATISTICAL SIGNIFICANCE:

Statistically significant increase in the different cell counts were obtained for the following ILDs after comparison with normal cell counts; ARDS: alveolar macrophages ($p=0.018$), CHP: neutrophils ($p=0.01$), ELD: mast cells ($p=0.000$). A statistically significant decrease in the lymphocytes was noticed in ARDS ($p=0.001$).
DISCUSSION

Usefulness of the clinical diagnostic test is graded based on its sensitivity, specificity, invasiveness, reproducibility and its contribution to the diagnosis of the disease. Based on these factors, new diagnostic tests for disease are invented and the old test wane of with time. Interestingly, as the pathogenesis of disease unwinds with time there may be need of multiple clinical tests to make precise diagnosis and to improve the management strategy. Under these situations, it may be difficult to conceive that an old test for a particular disease that has been used in the past may have new potential uses in modern medicine.

Bronchoalveolar lavage (BAL) is now widely accepted as a safe procedure to sample alveolar secretions for studying cellular and acellular components for diagnostic purpose. Bronchoscopy and BAL was once thought to hold a great scope in diagnosing and managing ILD. With time it came to understand, that though BAL immune cell features often had characteristics that were highly consistent with several forms of ILD, BAL cell profiles or soluble components could not reliably make a definite diagnosis in several forms of ILD if used as a standalone test of diagnosis. The evaluation of interstitial lung disease based on BAL findings was typically nonspecific, being consistent with or suggestive of a given condition, rather than pathognomonic, BAL data were subject to
considerable variability, and the potential number of diseases are much more than the number of safely discernible cellular patterns. This, only in rare instances, the data lead to a unique conclusion; in the majority of cases, BAL cell differentials are only able to render some diagnoses more likely and to exclude others with some probability. This uncertainty, in combination with differences in clinical setting and experience, resulted in different opinions about the diagnostic value of BAL among clinicians.

In early 1990s, HRCT came into widespread clinical use. As HRCT imaging patterns were consistent with specific forms of ILD such as IPF or sarcoidosis, the likelihood of making a diagnosis was high. Despite the acceptance of obtaining HRCT scan during the initial stages of patients with ILD, many patients with new onset ILD may not have the characteristic patterns that allow a diagnosis to be made with a high level of confidence by HRCT imaging alone. However, when clinical informations and HRCT findings were combined with BAL fluid analysis, confident diagnosis may emerge that obviate the need for surgical lung biopsy. Although the BAL cell pattern can provide useful information of the specific ILD diagnosis, this would be possible only if the bronchoscopist uses the appropriate technique to obtain the fluid, and the differential cell count is performed according to good clinical laboratory practice by personnel with adequate experience BAL
cytological analysis and interpreted by an expert familiar with the diverse spectrum of specific forms of ILD\textsuperscript{114,115}. With this in mind the present study utilized the recommendations and guidelines of ATS concerning the use of BAL in the diagnosis and management of patients with suspected ILD. Based on the cellular pattern obtained we were able to fit the differential diagnosis of 47 patients within the classification provided by ATS. We were also able to find inappropriate BAL procedure in rest of the three patients. Later we made diagnosis of different ILDs based on clinical examination, HRCT findings and BAL cell pattern separately. And then with all the three modes of assessment. When a clinical diagnosis was combined with HRCT the differential diagnosis was narrowed down to minimum of two diseases. Considering BAL cellular pattern along with clinical examination and HRCT findings not only narrowed the type of ILD but also enhanced the diagnosis for better management strategy. The enhancement in the diagnosis included stability of the disease, acute or chronic nature of the disease and probable cause for acute exacerbation. This leaves the physician to take an appropriate decision to treat the cause of acute exacerbation along with the primary aetiology or to treat the primary cause alone. Thus, this multiple assessment strategy was able to treat patients appropriately with much more precision and accuracy.
In patient 1 an IPF suspected patient HRCT had provided four differential diagnosis of IPF, CHP, asbestosis, sarcoidosis and CVD based on honeycoomb appearance. The clinical findings and BAL analysis suggested four differential diagnosis namely IPF, NSIP, acute lung disease and suppurative infection. Combining clinical, HRCT and BAL findings, the diagnosis was refined as acute exacerbation of idiopathic pulmonary fibrosis with bronchitis and probably associated with superadded infection. This had helped the clinician to treat the infection to control the acute episode. In patient 8, HRCT listed the differential diagnosis similar to that of patient 1 based on cystic changes honey comb appearance, but BAL clearly demonstrated predominance of histocytes in the lung secretion to narrow down the diagnosis as pulmonary histocytosis. This changed the modality of treatment in this patient.

In patient 10, with a clinical diagnosis as ILD, HRCT revealed bilateral upper lobe fibrosis with traction bronchiectasis. Adding BAL input redefined the diagnosis as sub acute exacerbation of CHP associated with non infectious bronchitis based on the lymphocyte and neutrophil counts. On the other hand, patient 12 had a neutrophil count more than 50% in the cellular pattern that gave a diagnosis of acute exacerbation of CHP with bronchitis and suppurative infection. In eosinophilic
pneumonitis BAL played a very important role in assisting clinical and HRCT findings to clinch the diagnosis. In some of the ILDs such as the sarcoidosis, silicosis, ILD associated with collagen vascular diseases HRCT stands as the important test along with clinical examination in determining the diagnosis. In all these diseases BAL was helpful to predict the acute/ sub acute or chronic nature of the disease and the association of infection. Whereas in ARDS the clinical criteria along with x ray chest was adequate to make a diagnosis.

Thus the present study was able to demonstrate that BAL done as per the guidelines of ATS may act as an important test along with clinical and HRCT findings for a proper diagnosis in some ILDs, while in others, HRCT was found to be very successful in predicting the diagnosis, BAL assist in predicting the acute/chronic nature of the disease and gives the hint on the superadded infection status that would help in proper management. Thus BAL may be a routine test in eligible ILD patients along with clinical evaluation and HRCT.
CONCLUSIONS

- BAL cellular profile obviates the need for surgical lung biopsy in certain ILDs namely lymphangitis carcinomatosis, Eosinophilic lung diseases and Langerhans cell histiocytosis.

- BAL cellular profile could be supportive of diagnosis in the presence of clinical and radiological features typical of a specific ILD.

- BAL plays an important role in establishing infection as the cause of an acute exacerbation of ILD. If infection is not established, guides the clinician to look for an alternate cause such as a thromboembolic event or a left heart failure.
BAL cellular analysis has documented the presence of inflammatory cells in asymptomatic patients with HRCT evidence of early ILD, which could predict an exacerbation in the near future.

- BAL is a relatively safe procedure, and maybe a routine test in ILD (in the absence of contraindications to bronchoscopy), along with clinical evaluation and HRCT
STUDY LIMITATIONS

- Sample size was small
- This study did not include all types of ILDs, namely Cryptogenic Organising Pneumonia, Acute Interstitial Pneumonias, Drug induced ILDs to name a few.


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1. Title

**Bronchoalveolar lavage (BAL) Cellular Analyses as a Diagnostic Intervention for Patients with Suspected ILD in conjunction with HRCT Imaging**

**Principle Investigator**

Name: Dr. C. Ammaiappan Palaniswamy

Designation: Post Graduate in M.D. TB&RD

Address:

**Guide Prof. Dr. D. Ranganathan, M.D., D.T.C.D, D.N.B (Chest)**

Name:

Designation: Director, Institute of Thoracic Medicine, Chetpet, Chennai,

Address: an Prof. and Head, Dept. of Thoracic Medicine, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai
2. OBJECTIVE (S) / AIM

Primary Objective

To optimize the Brochoalveolar Lavage (BAL) procedure as per the recommendations put forth by the American Thoracic Society for the cellular analysis of interstitial lung diseases.

Secondary Objectives

- Optimization of the BAL procedure as per the recommendations of the American Thoracic Society
- BAL cellular analysis association with clinical characteristics of different interstitial lung diseases and their radiological/pathological findings.

3. INTRODUCTION

Bilateral parenchymal infiltration of lung tissue is called interstitial lung disease (ILD). It can present as an acute or chronic condition with variable degree of inflammation and fibrosis. The condition is immune mediated where infection and neoplasm do not play a role in the etiology of the disease. ILDs are generally characterized clinically by exertional dyspnea, bilateral pulmonary infiltrates on thoracic imaging, abnormal pulmonary physiology, and abnormal gas transfer. They are characterized pathologically by an accumulation of inflammatory and immune effector cells that is often accompanied by abnormal extracellular matrix in the distal airways, alveolar walls, and interstitium.
Chronic ILDs usually evolve over months to years and include disorders of both known and unknown causes. Among the ILDs with known causes or associations are the pneumoconioses, ILD associated with connective tissue disease (CTD-ILD), and hypersensitivity pneumonitis (HP). Among the ILDs of unknown cause are sarcoidosis and idiopathic interstitial pneumonias (IIP). Idiopathic interstitial pneumonia (IIP) includes idiopathic pulmonary fibrosis (IPF), nonspecific interstitial pneumonia (NSIP), desquamative interstitial pneumonia (DIP), respiratory bronchiolitis with interstitial lung disease (RBILD), acute interstitial pneumonia (AIP), cryptogenic organizing pneumonia (COP), and lymphoid interstitial pneumonia (LIP).

Bronchoalveolar lavage has been used to evaluate patients with suspected ILD to identify the specific type of ILD. Advent of high resolution computed tomography (HCRT) has reduced the clinical utility of BAL. HRCT can noninvasively identify specific imaging patterns that may be virtually diagnostic or strongly support certain forms of ILD. This has greatly improved the clinician’s ability over the past decade to narrow the differential diagnosis. As a result, a likely diagnosis is determined in the majority of cases (1–3). The widespread use of HRCT to evaluate patients with ILD has reduced the need for invasive diagnostic procedures, although sampling is still performed to confirm or secure an accurate diagnosis. Diagnostic sampling is also performed when there is ongoing clinical suspicion of ILD despite a normal HRCT (i.e., occasionally patients whose HRCT was interpreted as normal have evidence of ILD on BAL or lung biopsy). Following the initial clinical and radiographic evaluation of patients presenting with suspected ILD, BAL cellular analysis may be a useful adjunct in the diagnostic evaluation of individuals who lack a confident usual
interstitial pneumonia (UIP) pattern on high-resolution computed tomography (HRCT) imaging of the thorax.

BAL is one sampling technique. It samples the cellular and acellular components of distal bronchioles and gas exchange units. BAL analysis is seldom diagnostic by itself, but BAL cell pattern results may support a diagnosis and/or narrow the differential diagnosis when considered in the context of the medical history (e.g., occupational and environmental exposures, drug ingestion, prior radiation therapy), physical examination (e.g., extrapulmonary abnormalities), and radiologic findings (e.g., HRCT findings). The usefulness of BAL cell profiles is the subject of ongoing debate and controversy because its findings are hampered by poor sensitivity and specificity (4). In addition, a normal BAL differential cell profile does not exclude the presence of microscopic abnormalities in lung tissue. The clinical utility of BAL in identifying the cause of ILD is proven, provided the technique is performed correctly, BAL fluid be handled and processed properly. Recently, the American Thoracic Society (ATS) clinical practice guideline provides a comprehensive, conceptually balanced, and evidence-based perspective on the clinical utility of BAL cellular analysis for the evaluation of suspected ILD. These guidelines will increase the utility of BAL in the diagnostic evaluation of ILD and promote the use of BAL in clinical of ILD so that future guidelines may be based upon higher quality evidence.

The present work would utilize the recommendations put forth by the ATS to optimize the BAL procedure and associate the finding across different clinical entities of ILD including HRCT. In addition, the study would also associate BAL findings with lung tissues procured through transbronchial biopsy in subset of patients for
whom biopsy is warranted (5). This may facilitate future clinical studies in patients with suspected ILD, which investigate potential biomarkers in BAL that may predict prognosis and response to therapeutic interventions for ILD.

4. METHODOLOGY (MATERIALS & METHODS)

Subject Selection

Seventy five ILD patients will be enrolled for the study based on clinical and radiological evaluation after institutional ethics committee approval. 15 ml of peripheral blood would be collected from the patients after obtaining informed consent for the study. The blood samples will be utilized for routine blood investigations, disease specific investigations and for genetic analysis.

Inclusion Criteria

- ILD patients diagnosed based on clinical and HRCT findings
- Acute and chronic ILDs in immunocompetent patients.
- ILD patients tolerable to the procedure.
- Patients above 18 years of age.
- Patients with ability and voluntarily signs the informed consent.

Exclusion Criteria

- ILD patients without HCRT diagnosis
- ILD patient with immunodeficiency
- ILDs associated with infection or neoplasm
- ILD patients with bleeding tendency.
- Pediatric patients
- Pregnant women
Screening Procedures / Visits:

1. BAL is performed with the fiberoptic bronchoscope in a wedge position within the selected bronchopulmonary segment. The total instilled volume of normal saline should be no less than 100 ml and should not exceed 300 ml. Three to five sequentially instilled aliquots are generally withdrawn after each aliquot instillation.

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

3. A minimal volume of 5 ml of a pooled BAL sample is needed for BAL cellular analysis. The optimal volume is 10 to 20 ml. It is acceptable to pool all aliquots of the retrieved BAL fluid for routine analyses (including the first retrieved aliquot).

4. BAL cell differential counts with greater than 15% lymphocytes, greater than 3% neutrophils, greater than 1% eosinophils, and greater than 0.5% mast cells represent a lymphocytic cellular pattern, neutrophilic cellular pattern, eosinophilic cellular pattern, and mastocytosis, respectively. Each has diagnostic implications.

5. A predominance of macrophages containing smoking-related inclusions with no or minor increases in other cell types is compatible with smoking-related ILD such as desquamative interstitial pneumonia (DIP), respiratory bronchiolitis interstitial lung disease (RBILD), and Langerhans cell histiocytosis.

I. PRE-PROCEDURE PREPARATION

Patients with suspected ILD for whom the clinician is considering BAL should undergo routine clinical evaluation before the procedure. This evaluation, which
includes inquiry and appropriate testing for bleeding tendencies, is intended to minimize the likelihood of procedure-related complications by identifying potential risk factors that can be corrected or mitigated in advance. Once it is confirmed that the patient is a suitable candidate for BAL, the procedure may be scheduled.

**Recommendation 1.**

For patients with suspected ILD in whom it has been decided that a BAL can be tolerated and will be performed, we suggest that the BAL target site will be chosen on the basis of an HRCT performed before the procedure, rather than choosing a traditional BAL site (i.e., the right middle lobe or lingula). HRCT can be useful for identifying target areas of the lung that are most likely to provide diagnostic specimens when sampling via BAL. Generally, areas of alveolar ground glass opacity, more prominent nodular profusion, or fine reticulation are likely to provide optimal targets. Target areas as well as characteristics of parenchymal abnormalities may change with time and, therefore, the HRCT should not be performed too far in advance of the BAL procedure. Although there are no controlled clinical trials that have compared whether BAL sites identified by HRCT yield more useful information than traditional BAL sites (i.e., easily accessible sites that provide a good volume of return such as the right middle lobe or lingula), some reports suggest that HRCT may be useful for choosing a site of lavage.

II. THE BAL PROCEDURE

During standard flexible bronchoscopy, the bronchoscope is placed in a wedge position within the selected bronchopulmonary segment. Normal saline (at room temperature) is instilled through the bronchoscope, with a total volume that is between 100 and 300 ml and divided into three to five aliquots. After the instillation of each aliquot, instilled saline is generally retrieved using a negative suction pressure less than 100 mm Hg. The negative suction pressure should be adjusted to avoid visible airway collapse. The minimal total volume retrieved should be greater than or equal to 5% of the instilled volume (optimal sampling retrieves >30%). If less than 5% of each instilled aliquot volume is recovered during the procedure due to retention of most of
the fluid in the lung, the procedure should be aborted to avoid increased risk to the patient. A minimal volume of 5 ml of a pooled BAL sample is needed for BAL cellular analysis (the optimal volume is 10–20 ml); it is acceptable to pool all aliquots of the retrieved BAL fluid for routine analyses. Occasionally, the gross appearance of the BAL fluid will provide diagnostic clues. For example, grossly bloody BAL fluid that returns with increasing intensity in sequential aliquots indicates acute diffuse alveolar hemorrhage, while grossly cloudy (i.e., milky or light brown-beige color) BAL fluid that returns with flocculent material that settles by gravity to the bottom of the container within 15 to 20 minutes of fluid retrieval is highly suggestive of pulmonary alveolar proteinosis (PAP).

III. HANDLING OF THE BAL FLUID

The BAL fluid should be collected in containers that do not promote cell adherence to container surfaces (e.g., silicone coated glass or polypropylene or other plastics that are designed for suspension tissue culture). Its method of transport then depends upon how long it is anticipated that it will take to reach the analytical laboratory. The specimens will be transported at 4°C (i.e., on ice). If a delivery time greater than 1 hour is anticipated, then transport in the original lavage saline is discouraged. Instead, the cells should be centrifuged at a speed that maintains cellular integrity (e.g., 250–300 g for 10 min) and then resuspended in a nutrient-supplemented medium (e.g., MEM125 mM HEPES or RPMI 1640125 mM HEPES) and stored at 4°C, where they may remain for up to 24 hours. If a centrifuge is not available, MEM or RPMI may be added to the pooled lavage sample with subsequent storage at 4°C for up to 12 hours, but the sample will be transported to the laboratory as soon as possible and a prolonged interval between BAL fluid retrieval and laboratory processing is discouraged. BAL fluid will not be frozen or transported with dry ice.

IV. PROCESSING

Prompt processing of the BAL fluid or cell suspension once it reaches the laboratory provides optimal results. Labware will be used that does not promote cell
adherence to container surfaces. Specimens with gross mucus can be strained through loose gauze, or small amounts of mucus can be dissolved with dithiothreitol, if necessary. The specimen will then be centrifuged at an appropriate speed, resuspended, and analyzed. BAL fluid that is not going to be analyzed immediately will be centrifuged, the cell pellet resuspended in a nutrient supplemented medium, and then refrigerated at 4°C for up to 24 hours. Cells that were already suspended in a nutrient supplemented medium due to delayed transport can simply be refrigerated at 4°C. Specimens obtained more than 24 hours before are not suitable for analysis.

V. BAL CELLULAR ANALYSIS IN THE DIAGNOSIS OF SPECIFIC ILD

A variety of diagnostic studies may be performed on BAL fluid. In patients with suspected ILD, typical diagnostic studies are a differential cell count, microbiological studies (to screen for mycobacterial and fungal disease), and cytopathology.

**Recommendation 2.**

For patients with suspected ILD who undergo BAL, a differential cell count be performed on the BAL fluid. This includes lymphocyte, neutrophil, eosinophil, and mast cell counts. The remaining sample may be used for microbiological, virological, and/or malignant cell cytology laboratory testing, if clinically indicated. The reason for routine cellular analysis whenever BAL is performed in a patient with suspected ILD is that identification or exclusion of a predominantly inflammatory cellular pattern (increased lymphocytes, eosinophils, and/or neutrophils) may support a specific type of ILD or help narrow the differential diagnosis, when considered in the context of the clinical and radiological findings. The notion that a prominence of specific nucleated inflammatory or immune cells in the BAL correlates with an increased likelihood of certain types of ILD is supported by numerous accuracy studies that are limited by risk of bias. These include pronounced BAL eosinophilia in eosinophilic pneumonia (7, 8) or drug reactions (9–11), and BAL lymphocytosis in
sarcoidosis (12–15), hypersensitivity pneumonitis (16–18), pneumotoxic drug reactions (19, 20), or cellular NSIP (21, 22).

Follow up Procedures / Visits:

The patient will be observed for 48 hours for post procedure bleed at the in-patient ward before discharged with care.

Assessments of Parameters

TECHNIQUE OF BAL CELL ANALYSIS

The cellular analysis will be performed within 1 hour if the BAL fluid is in nutrient-poor media (e.g., saline) or within 2 to 3 hours for optimal results if the BAL fluid is in a nutrient-supplemented medium. The total cell count (nucleated immune cells) is usually obtained via a hemocytometer, and cell viability is determined by Trypan blue exclusion. Differential cell counts will be performed via cytocentrifugation with staining (Wright-Giemsa or May-Grunwald-Giemsa) and enumeration of at least 400 cells. The presence and relative numbers of erythrocytes and epithelial cells should be noted. The presence of squamous epithelial cells suggests that BAL fluid is contaminated with upper airway secretions, and the presence of large numbers of bronchial epithelial cells suggests that the BAL may not have adequately sampled distal airspaces. Excess BAL fluid will be stained and cultured for mycobacteria and fungi in the microbiology laboratory, as well as screened for neoplastic cells. These are important additional tests to consider because infections and diffuse neoplasms can masquerade as ILD or coexist with ILD.

INTERPRETATION OF BALL DIFFERENTIAL COUNTS

The ranges of differential cell counts that are considered normal and abnormal derive from several sources. Numerous investigators have published BAL immune cell profiles from cohorts of clinically normal volunteer subjects recruited in single-center studies (22–28) and these reports have been used to define normal and abnormal differential cell counts. In addition, the multi-center BAL Cooperative Study (22) reported the differential cell counts in the BAL of normal subjects
(including smokers or ex-smokers) compared with patients with ILD. An increased number of nucleated immune cells and abnormal proportions of immune cell types may suggest or support specific types of ILD in the absence of an infection. A mixed cellular pattern can be observed with any ILD; when mixed cellular patterns are observed, the dominant cell type may be the most consistent with a specific ILD diagnosis. A BAL fluid cell differential count with greater than 15% lymphocytes, greater than 3% neutrophils, greater than 1% eosinophils, or greater than 0.5% mast cells indicates BAL lymphocytosis (i.e., a lymphocytic cellular pattern), BAL neutrophilia (i.e., a neutrophilic cellular pattern), BAL eosinophilia (i.e., an eosinophilic cellular pattern), or BAL mastocytosis, respectively. A lymphocyte differential count greater than or equal to 25% suggests granulomatous lung disease (e.g., sarcoidosis, HP, NSIP, chronic beryllium disease, drug reaction, LIP, COP, or lymphoma), while a lymphocyte differential count greater than 50% is particularly suggestive of HP or cellular NSIP. An eosinophil differential count greater than or equal to 25% is virtually diagnostic of eosinophilic lung disease in the appropriate clinical setting. A neutrophil differential count greater than or equal to 50% strongly supports acute lung injury, aspiration pneumonia, or suppurative infection. Finally, a mast cell differential count greater than 1% combined with a lymphocyte differential count greater than 50% and a neutrophil count greater than 3% is suggestive of HP. A predominance of macrophages containing smoking-related inclusions with no or minor increases in other cell types is compatible with smoking-related ILD, such as DIP, RBILD, or pulmonary Langerhans cell histiocytosis (PLCH). Additional tests to identify and count Langerhans cells in the appropriate clinical setting may be useful in narrowing the differential diagnosis. A predominance of hemosiderin-laden macrophages is suggestive of chronic or occult alveolar hemorrhage syndromes resulting in pulmonary hemosiderosis or diffuse alveolar damage.

**Recommendation 3.**

For patients with suspected ILD in whom BAL is performed, the lymphocyte subset analysis NOT be a routine component of BAL cellular analysis. The lymphocyte subset analysis (by cytometry or immunocytochemistry) will not be performed routinely, but rather would be performed if a lymphocytic disease is
suspected or the initial BAL cellular findings identify a lymphocytosis. This suggestion is based upon the committee’s clinical experience that lymphocyte subset analysis is rarely helpful and potentially misleading in the absence of a clinically suspected lymphocytic disease or a lymphocytosis. Many investigators have characterized lymphocyte subsets on the basis of T helper (CD41) versus T suppressor (CD81) phenotypes, and have found correlations of the CD41/CD81T lymphocyte ratio with specific disease processes such as sarcoidosis and hypersensitivity pneumonitis (16,17,29,30). However, subsequent investigations have found that the CD41/CD81 ratio may not be significantly increased in a substantial number of patients with sarcoidosis (31,32) or significantly decreased in a substantial proportion of patients with hypersensitivity pneumonitis (33,34), and can change during the course of the disease process. In addition, the BAL CD41/CD81T lymphocyte ratio varies with age and may be significantly increased in normal subjects (35). These issues are discussed extensively in the portion of the online supplement that pertains to specific forms of ILD. However, in the case of sarcoidosis, the combination of BAL lymphocytosis combined with a considerably increased BAL CD41/CD81 lymphocyte ratio (e.g., >4) may increase the confidence of a diagnosis of sarcoidosis if other clinical features and imaging are consistent with this diagnosis, and lymphocyte subset determinations may be performed at the discretion of the pulmonologist if such analysis can be reliably performed in the clinical laboratory and is considered to be clinically useful. Finally, there are other tests that can be performed on BAL fluid on a case-by-case basis and may be helpful in specific clinical circumstances. Analysis by a cytopathologist is indicated if there are isolated cells that are suspicious for malignancy. Periodic Acid Schiff staining or Oil Red O staining may be helpful if pulmonary alveolar proteinosis or aspiration is suspected, respectively. Hemosiderin staining may be performed if hemorrhage is suspected and/or the initial BAL raises the suspicion of hemosiderin-laden macrophages.
5. RESULTS

Statistical Analysis Plan

Cellular analysis will be separately compared between different types of ILDs using the Mann–Whitney U test. Associations between phenotypic characteristics will be studied by Pearson's coefficient of correlation. All analyses will be performed with SPSS version 14.0 (SPSS Inc., Chicago, IL) and p<0.05 was considered significant.
6. APPENDIX

Reference


INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No. ECR/270/Inst./TN/2013
Telephone No: 044 25305301
Fax: 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. C Ammaiappan Palaniswamy,
PG in Thoracic Medicine,
Institute of Thoracic Medicine,
Madras Medical College, Chennai-3

Dear Dr. C. Ammaiappan,
The Institutional Ethics Committee of Madras Medical College,
received and discussed your application for approval of the proposal entitled “Broncholaveolar
lavage (BAL) cellular Analyses as Diagnostics Intervention for patients with suspected ILD
in Conjunction with HRCT Imaging” No 33032014

The following members of Ethics Committee were present in the meeting held on 11.03.2014
conducted at Madras Medical College -3

1. Dr. C Rajendran, M.D
2. Dr. R Vimala, M.D,
   Dean, MMC, Ch-3
3. Prof. Kalaiselvi, M.D
   Vice Principal, MMC, Ch-3
4. Prof. Nandhini, M.D,
   Inst. of Pharmacology, MMC, Ch-3
5. Prof. Bhavani Shankar, M.S,
   Prof & HOD
6. Prof V Padmavathi, M.D,
   I/C Director of Pathology, MMC, Ch-3
7. Thiru S Govindasamy, BABL
8. Tmt. Arnold Saulina, MA, MSW
9. Thiru S Ramesh Kumar,
   Administrative Officer, MMC, Ch-3

   -- Chairperson
   -- Deputy Chairperson
   -- Member Secretary
   -- Member
   -- Member
   -- Member
   -- Lawyer
   -- Social Scientist
   -- Lay Person

We approve the proposal to be conducted in its presented form

Sd/Chairman &Other Member

The institutional Ethics Committee expects to be informed about the progress of the Study,
and SAE occurring in the course of the study, any changes in the protocol and
Patients information/ informed consent and asks to be provided a copy of the final report.

[Signature]
Member Secretary
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
அட்ராங்கி போலவே கோல்

அட்ராங்கி போலவு:

ILD தோற்றம் (லோகல் பார்வோயில் தோற்றம்) ஐட்ராங்கியுடன் ஒத்துக்கொண்டு அட்ராங்கியில் காரணியை ஒத்துக் கொண்டு இலோகல் பார்வோயில் போலவுண்டும் அட்ராங்கியின் மூலம் ஒத்துக் கொண்டு இலோகல் பார்வோயில் போலவுண்டும் காரணி குறிப்பிட்டுள்ளது.

முதல்வரைய அட்ராங்கி போலவு பணியாளர்கள்:

1. குறிப்பிட்டுள்ளது

அட்ரங்கி போலவு விழா வருகை:

1. குறிப்பிட்டுள்ளது

அட்ரங்கி போலவு பரிசோதனைகள்:

1. குறிப்பிட்டுள்ளது

HRCT காண்பிட்டும் பார்வோயில் தோற்றம் தங்களுடன் இலோகல் பார்வோயில் பரிசோதனை பெற்றுக் கொண்டுள்ளது;

1. குறிப்பிட்டுள்ளது

2. குறிப்பிட்டுள்ளது (Bronchoalveolar Lavage).
நூற்றாண்டு தலைமுறை:
1. புதைக்குறிட்டு
2. குறுக்கு மறை

நூற்றாண்டு தலைமுறை உரை:

தொழில் மாற்றம் குறிப்பிட்டு கூறிக் கொண்டு ஆலோசிக்கும் உரையாக அறிவித்து கூறினார்.

இதுவரை மொத்தம் கலைப்பிணைப்பால் கூட்டப்பட்டுள்ள எண்ணிக்கையில் மிகுந்தும், தொழில்நுட்பப்பிணைப்புகளின் உயர்ந்துவருவதும் வகுப்பில் நூற்றாண்டு தலைமுறையின் காலத்தில் நேர்ந்து எடுக்கப்பட்டது.

இதுவரை மொத்தம் பாதுகாப்பியியலில் பிள்ளிக்குழப்பிலிருக்கும், பாதுகாப்பு கூறிக்கொள்ளும் கிளைக்குழப்பிலும் நூற்றாண்டு புரேப்பு அறிவிக்கப்பட்டது குறிப்பிட்டு போன்றுள்ளன.

இதுவரை மொத்தம் தொழில்நுட்பக் குழுவில் பதிவிடப்பட்டு விளக்கும் உள்ளேந்து சிறியும் பாதுகாப்பு, தொழில்நுட்பம் பாதுகாப்பு மற்றும் மண்டலத்தைப் பாதுகாப்பிற்கு இடைவெளியாக லைத்து பாதுகாப்பிலிருந்து.

ஆனால் இங்கு இந்த மொத்தம் பாதுகாப்பில் தாக்கமிட்டு பிரிக்கும், மேலும் யாரும் ஒருவருக்கும் நோக்கு லார்க்கை காரணமாக நிலைக்கு அனுமானமாக காண லும் ஆய்வில் இன்றைய கோட்டை.
INFORMATION TO PARTICIPANTS

Title:

Bronchoalveolar lavage cellular analyses as a Diagnostic Intervention for patients with suspected ILD in conjunction with HRCT imaging.

Principal investigator:

Dr. C AMMAIYAPPAN PALANISWAMY

Guide:

Prof. Dr. D. RANGANATHAN

Co-Guide:

Dr. V. SUNDAR

You are invited to take part in this study. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns.

What is the purpose of this study?

Interstitial lung disease is a progressive disease of the lungs. To optimize the BAL- Bronchoalveolar lavage procedure as per recommendations put forth by the American Thoracic Society for the cellular analyses of Interstitial lung diseases, which aids to diagnose the subtypes of ILD.

Study design and procedures:

A prospective study, which involves the BAL procedure after obtaining fitness for the procedure from concerned specialists, and blood investigations, which involves prick with a needle and syringe, of about 15ml of blood.

Possible Risks:

The procedure has some risks of hemorrhage from the lavage site.
Possible benefits to you:

In helping diagnose the subtype of the ILD, which might help us tailor the treatment required / and in prognosis.

Possible benefits to other people:

The results of the study might provide benefits to the society in terms of advancement of medical knowledge and / or Therapeutic benefits to future patients.

Confidentiality of the information obtained from you:

You have the right to confidentiality regarding the privacy of your personal information. The information from this study if published or presented in scientific journals / meetings, your identity will not be revealed.

How will your decision in not to participate in the study affect you?

Your decision not to participate in this study will not affect your medical care or your relationship with the investigator or the institution.

You will not lose any benefits to which you are otherwise entitled.

Can you decide stop participating in the study once you have agreed?

The participation in this study is purely voluntary and you have the right to withdraw from this study at anytime during the course of this study without giving reasons.

Signature of the Investigator

Signature of the Participant

Date:

Place:
முப்புற காலம்

எல்லை தொடர்பில் அதிகம் நீர்ப்பொருள் குழியும் நூல்களில், அனைத்து
நேசனேலியன் அல்லது கனவின் சூழ்ப்பொருள் குழியும் நூல்களில் நூல்கள்
பிரித்தல் விளக்கம் பிறந்து அதன் பட்டியல், பார்வைத்தள பொி தமிழில் விளக்கம் பிரித்தல் விளக்கம் பதிவு செய்யப்பட்டுள்ளது.

இப்பகுதி
CONSENT FORM

I have been told about the bronchoalveolar lavage procedure and the possible side effects. I am willing to undergo this procedure.
<table>
<thead>
<tr>
<th>Measure</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>2023</td>
<td></td>
</tr>
<tr>
<td>Month</td>
<td>Jan</td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

**Measure Details**

- **Total Revenue**: $1,200,000
- **Net Profit**: $100,000
- **Gross Profit**: $1,100,000

**Financial Analysis**

- **Revenue Growth**: 10%
- **Profit Margin**: 8%

**Operational Highlights**

- **Inventory Management**: Improved by 20%
- **Customer Satisfaction**: Increased by 5%

**Key Performance Indicators**

- **Sales Volume**: 10,000 units
- **Customer Retention**: 90%

**Strategic Goals**

- **Market Expansion**: New product launch in Q2
- **Technology Integration**: Implement AI in operations by year-end
<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Location</th>
<th>Event Description</th>
<th>Duration</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 August</td>
<td>10:00</td>
<td>Museum</td>
<td>Visit to the exhibit</td>
<td>2 hours</td>
<td>None</td>
</tr>
<tr>
<td>28 August</td>
<td>12:00</td>
<td>Restaurant</td>
<td>Lunch with friends</td>
<td>1 hour</td>
<td>None</td>
</tr>
<tr>
<td>28 August</td>
<td>18:00</td>
<td>Theater</td>
<td>Watch the play</td>
<td>2 hours</td>
<td>None</td>
</tr>
<tr>
<td>29 August</td>
<td>08:00</td>
<td>Zoo</td>
<td>Visit to the animals exhibit</td>
<td>3 hours</td>
<td>None</td>
</tr>
<tr>
<td>29 August</td>
<td>12:00</td>
<td>Picnic</td>
<td>Enjoy a picnic</td>
<td>2 hours</td>
<td>None</td>
</tr>
<tr>
<td>29 August</td>
<td>16:00</td>
<td>Beach</td>
<td>Relax on the beach</td>
<td>4 hours</td>
<td>None</td>
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<tr>
<td>30 August</td>
<td>09:00</td>
<td>Museum</td>
<td>Visit to the exhibit</td>
<td>2 hours</td>
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</tr>
<tr>
<td>30 August</td>
<td>12:00</td>
<td>Restaurant</td>
<td>Lunch with friends</td>
<td>1 hour</td>
<td>None</td>
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<td>None</td>
</tr>
<tr>
<td>31 August</td>
<td>08:00</td>
<td>Zoo</td>
<td>Visit to the animals exhibit</td>
<td>3 hours</td>
<td>None</td>
</tr>
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