TRANSBRONCHIAL LUNG BIOPSY INTERPRETATION

V. K. VIJAYAN
HENRY. D. TAZELAAR
HELMUT H. POPPER
ULRIKE GRUBER-MOESENBACHER
RITU KULSHRESTHA

VALLABHBHAI PATEL CHEST INSTITUTE
University of Delhi, Delhi- 110007 INDIA
Tel No. Off: +91 11 27402414
Fax No. +91 11 27667420

ISBN No. : 978-81-920546-1-2
978-81-920546-1-2

TRANSBRONCHIAL LUNG BIOPSY INTERPRETATION

TRANSBRONCHIAL LUNG BIOPSY INTERPRETATION

V.K. VIJAYAN
HENRY. D. TAZELAAR
HELMUT H. POPPER
ULRIKE GRUBER-MOESENBACHER
RITU KULSHRESTHA

Published by :
Vidyanilyam Prakashan
RP-19, IInd Floor, Maurya Enclave
Pitam Pura, Delhi-110034
Tel.: 011-27323636

ISBN No. : 978-81-920546-1-2

First Edition : 2010

Printed at :
Neelam Graphics
B-35, 1st Floor, Christian Colony, Main Road
Patel Chest Institute, Near Delhi University
Delhi-110007, Mobile : 9811109750

CONTENTS

CONTRIBUTORS

1. V.K. Vijayan, MD (Med), PhD, DSc, FAMS
   Director
   Vallabhbhai Patel Chest Institute
   University of Delhi, Delhi-110007
   E-mail: vijayanvk@hotmail.com

2. Henry D. Tazelaar, MD
   Professor of Pathology
   College of Medicine, Mayo Clinic
   Department of Pathology and Laboratory Medicine
   Mayo Clinic Arizona, 13400 East Shea Boulevard
   Scottsdale, AZ 85259 USA, 480-301-5530
   E-mail: tazelaar.henry@mayo.edu

3. Helmut H. Popper, MD
   Director, Institute of Pathology, Lab.Molecular Cytogenetics
   Environmental & Respiratory Pathology
   Medical University of Graz, Graz, A-8020, Austria
   Email: helmut.popper@meduni-graz.at

4. Ulrike Gruber-Moesenbacher, IFCAP
   Senior Pathologist, Institute of Pathology
   Universitary Teaching Hospital Feldkirch
   Carinagasse 47, A 6800 Feldkirch, Austria
   E-mail: ulrike.gruber@lkhf.at

5. Kevin O. Leslie, MD
   Professor and Chair, Division of Anatomic Pathology
   Department of Laboratory Medicine and Pathology
   Mayo Clinic Arizona
   Email: Leslie.Kevin@mayo.edu

6. Ritu Kulshrestha, MBBS, DNB
   Assistant Professor, Department of Pathology
   Vallabhbhai Patel Chest Institute, University of Delhi
   Delhi-110007
   E-mail: ritukumar71@yahoo.com

1

Bronchoscopy and Transbronchial Lung Biopsy

V.K. Vijayan

Introduction

Bronchoscopy is a technique of visualising the inside of the airways for diagnostic and therapeutic purpose[1]. The bronchoscope is inserted into the airways, usually through the nose or mouth, or occasionally through a tracheostomy. This allows the Physician to examine the patient's airways for abnormalities such as foreign bodies, bleeding, tumors, or inflammation. Specimens are then taken from inside the lungs and include endobronchial and transbronchial lung biopsies, bronchoalveolar lavage fluid, and endobronchial brushing. The bronchoscopes are of two types: rigid bronchoscope with attached lighting devices and flexible fibreoptic bronchoscopes.

Rigid Bronchoscope

The rigid bronchoscope is a straight, metal tube with an inner diameter of up to one centimeter. It is inserted through the mouth, the patient lying in a supine position and the neck hyper extended. The procedure causes significant di scomfort and is performed under general anesthesia. Rigid bronchoscopy is less often used today, but it remains the procedure of choice for removing foreign materials, as the greater diameter of the rigid bronchoscope allows instruments to be more easily inserted through it. Rigid bronchoscopy also becomes useful when bleeding interferes with viewing the examining area, and allows for more interventions, such as cautery to stop the bleeding.

Flexible (fibreoptic) Bronchoscope

A flexible bronchoscope contains a fibreoptic system that transmits an image from the tip of the instrument to an eyepiece or video camera at the opposite end. It permits visualisation of up to six generations of the bronchial tree in the basal segment and is used for both diagnostic and therapeutic purposes. Flexible bronchoscopy causes less discomfort for the patient than rigid bronchoscopy and the procedure can be performed easily and safely under local anesthesia. It is the technique of choice for most bronchoscopic procedures these days. The bronchi are visualised and the endobronchial lung biopsy is taken. The forceps are then advanced into the distal parenchyma for transbronchial lung biopsy[2].

Transbronchial Lung Biopsy

Anderson et al developed the transbronchial lung biopsy (TBLB) through a rigid bronchoscope in 1965[3]. The introduction of fibreoptic bronchoscope (FOB) in 1967 by Ikeda enabled Levin *et al* to perform the TBLB through the FOB in 1974[4]. Subsequently various workers described the usefulness of TBLB through FOB in both localised and diffuse lung diseases. TBLB, done under fluoroscopic guidance, is a diagnostic alternative to percutaneous transthoracic needle biopsy, thoracoscopy with forceps lung biopsy and thoracotomy with open lung biopsy.

Technique

Preliminary investigations prior to TBLB include pulmonary function tests, blood gas analysis, electrocardiogram, chest radiographs and screening for coagulation disorders (platelet count, prothrombin time, activated thromboplastin time and bleeding time). Contraindications for FOB and TBLB consist of respiratory insufficiency with a P_aO_2 less than 8.0 Kpa, unstable cardiac function with decompensation or malignant arrhythmias and coagulation defects. Premedication includes intravenous diazepam (5-10 mg). Local anesthesia is obtained with 4% lidocaine topical spray in the upper respiratory tract. Oxygen is supplemented through a nasopharyngeal catheter and the patient is monitored with cardiac monitor and pulse oximetry. An intravenous line with 5% dextrose is also achieved.

Fluoroscopic guidance is employed in order to achieve a correct position of the biopsy forceps. Localised pulmonary lesions should be located both in anterio-posterior and lateral projections. Even though fluoroscopic guidance during TBLB is very useful in the case of localised pulmonary lesions, its utility in diffuse lesions is debatable. TBLB can be performed without fluoroscopic guidance in diffuse lesions, if adequate facilities are not available. TBLB is performed only from one lung to avoid the complication of bilateral pneumothorax. In localised lesions, biopsies are taken within or as close to the lesion as possible. In diffuse bilateral lung disease, TBLBs are taken from periphery of one, two or three lobes.

The biopsy forceps is passed through the FOB to the preselected segmental bronchus. On reaching the periphery of the lung, the forceps is retracted 1-2 cm and the following instructions are given rapidly to the patient and to the assistant. Patient is asked to take a deep breath and the assistant is instructed to open the forceps. The patient is then instructed to let out all air from the lungs. During expiration, the forceps is gently advanced forward 1 cm in order to entrap a small portion of the bronchial wall. Lastly the assistant is asked to close the forceps at the end of expiration and the forceps is then withdrawn completely by the endoscopist. The tip of the bronchoscope is then wedged into the bronchial segment to tamponade any possible bleeding. If there is no bleeding after 2 minutes, the procedure is repeated in an adjacent segment or subsegment collecting four or five samples. When no further biopsies are required, the bronchoscope is wedged for 1-2 minutes in the absence of bleeding or 5-10 minutes if there is bleeding. Since the bronchial arteries are small at the periphery of the lung, there is no significant bleeding. However, the risk of bleeding is increased from pulmonary arterioles and capillaries, if there is pulmonary hypertension.

2

Processing of the Transbronchial Lung Biopsy

Ritu Kulshrestha

Introduction

The introduction of fibreoptic bronchoscopy in 1960's has resulted in an increase in the number of endobronchial and transbronchial lung biopsies being subjected for histopathological examination, worldwide and in India[1]. Optimal specimen handling is essential for the accurate interpretation of biopsies and cytologic material obtained via bronchoscopy and an essential factor influencing the diagnostic yield of the bronchoscopic biopsy. Since the sample size is small, a working knowledge of specimen handling for each procedure ensures the greatest likelihood of success in establishing a specific diagnosis and in the end a rational treatment plan[2].

Specimen Handling of Endoscopic Biopsies

Bronchial and transbronchial biopsies rarely exceed 3 mm diameter and usually 4 to 6 biopsies are submitted for analysis. The diagnostic yield is known to increase with multiple biopsies[3]. The endoscopist directly places all specimens for histological examination into fixative solution. If bacterial, mycobacterial, virus or fungal cultures are required, specimens are sent in saline directly to the corresponding laboratory. The use of gauze or tissue paper is not advised, since tissue may get entwined in them and may make extraction difficult with resultant tissue damage. Caution must also be exerted by endoscopist to avoid prolonged exposure to air which can lead to drying artifact.

Fixation of the Bronchoscopic Biopsy

10% neutral buffered formalin (4% formaldehyde) remains the gold standard for lung biopsy fixation[4]. The biopsies should be fixed in an optimal fixative to specimen volume ratio of at least 10:1. Due to small size of the biopsy, the optimal time for fixation is reduced and varies from 4 to 6 hours. In the laboratory, transferring of the bronchoscopic biopsy from the fixative solution to the cassette is one step where care has to be taken in order to prevent the tearing or crushing of the delicate specimen. Transferring using forceps is to be avoided. Transbronchial lung biopsy (TBLB) can also be fixed in Zenker's or Bouins solutions. These fixatives tend to produce less shrinkage, resulting in enhanced nuclear detail and allow better appreciation of interstitial oedema. However, if the tissue is left in them for a prolonged period it may become brittle.

Grossing of the Bronchoscopic Biopsy

The type of biopsy [endobronchial lung biopsy (EBLB), TBLB] should be noted. The size and number of fragments sampled are subsequently documented. This is important to ensure that all bits have been individually examined histologically. Gently shaking transbronchial biopsy specimens in fixative in the specimen container helps expand the alveolar spaces to avoid the problem of atelectasis. Touch preparations can then be performed on both transbronchial and open lung biopsy specimens[5]. Neoplastic cells and certain infectious agents may be identified in some cases with this method.

Processing and Sectioning of Bronchoscopic Biopsy

This is an important step as altered histological appearance could be caused by events during or immediately after the bronchoscopic procedure, initial fixation, tissue casetting, or subsequent processing. Step sections can be particularly useful for detecting lesions, such as granulomas, in patients with sarcoidosis[6] . Initially serial sections are taken on eight slides in order to prevent refacing and recutting of block repeatedly and hence minimizing the tissue loss. Two slides are stained using the Haematoxylin and eosin stain. Depending on the underlying pathology the special stains, PAS (Periodic acid Schiff), Reticulin, Massons Trichrome, Grams, AFB stain are used. Polarisation microscopy is used to identify birefringent organic and inorganic material (silica, silicates, talc). For anticipated neoplastic infiltrates, as 4-5 micron sections, stained with haematoxylin and eosin at 40 micron intervals additional multiple unstained sections are placed directly on poly-L-Lysine coated slides suitable for immunohistochemistry. For post-transplantation transbronchial biopsies, multiple levels throughout the tissue (complete sampling) is preferred to identify small isolated lesions

Tissue Artifacts of Bronchoscopic Biopsies

The occurrence of artifacts in lung tissue can further complicate the interpretation of TBLB. Artifacts are caused by various alterations in tissue handling and fixation leading to various types and severity of artifacts. These include: atelectasis, bubble artifact, sponge artifact and crush artifact[2]. In most cases one type of artifact predominates but more than one type can be present. Atelectasis consists of collapsed lung with nonexistent intra-alveolar spaces and occasionally prominent interstitial vascular congestion. Atelectasis can be eliminated by fixing the tissue under motion, either by using a magnetic stirrer or forced air bubbles, as opposed to floating on the top of fixative. The bubble artifact consists of irregularly shaped round-to-slightly oval spaces within the lung parenchyma, without evidence of a foreign body giant cell or inflammatory response. The sponge artifact consists of irregularly shaped, variably sized, triangular spaces in lung parenchyma without granulomata or polarizable foreign material. The presence of crush artifact is sometimes considered a useful clue that the tumour is a small cell carcinoma. This is not a reliable indicator as crushed lymphoid follicles and crushed areas of chronic inflammation can have identical appearances. Well-preserved tumour cells with the characteristic nuclear features must be seen for definitive diagnosis.

3

Normal Histology, Adequacy and Reporting of the Transbronchial Lung Biopsy

Ritu Kulshrestha

Introduction

Over the past three decades, the perception of transbronchial lung biopsy (TBLB) in the diagnosis of non-neoplastic lung lesions has fluctuated from **'Gold standard' to 'Ailing Gold standard'.** The reasons for this are many fold and range from Inherent 'Sampling Error' in which divergent histopathologic diagnosis in two or more biopsy sites may be found, to, Inter observer variation between pathologists. A review of literature reveals that this discussion is ongoing since Andersen in 1978[1] stressed on "the importance of an interested and experienced pathologist willing to glean every information from tiny bits of TBLB tissue. While the pathologists perspective has been that it is the small specimen size (0.1 – 0.2 cm) which makes TBLB a "histopathologist's nightmare", leading to difficulty in distinguishing different patterns within the spectrum of diffuse parenchymal lung diseases.

Assessment of Specimen Adequacy

Transbronchial Lung Biopsy (TBLB)

TBLB is obtained by flexible fiberoptic bronchoscopy and usually contains alveoli, interalveolar interstitium and often one bronchiole and arteriole. For determination of specimen adequacy, the predominant tissue seen in biopsy specimen is noted: bronchial wall epithelium, subepithelium, alveoli, and cartilage. The specimen is considered to be adequately alveolated if more than 20 alveoli seen[2]. To predict the specimen adequacy at the time of biopsy by the bronchoscopists several factors such as the size of the biopsy, number of samples and 'Float sign' have been considered. Two to four biopsies performed using toothed forceps have been found to be usually adequate[2].

Endobronchial Biopsy

Modern flexible bronchoscopes allow visualisation of the bronchial tree and its mucosal surfaces as far distal as the sixth order bronchi. Biopsy of the visualised mucosal lesion is then commonly performed using the cupped biopsy forceps and called as endobronchial lung biopsy. With this technique the airway epithelium, subepithelial tissue and muscle wall are typically sampled with variable amount of cartilage.

Normal Microanatomy of TBLB

The TBLB can be divided histologically into five compartments: bronchiole, alveolus, interstitium, vascular and pleura. The respiratory bronchiole, in which the alveolus forms part of the wall of the bronchiole is often represented on TBLB. The interstitium of the lung normally contains three stainable types of connective tissue: elastica, collagen and reticulum which vary from fine to coarse fibers on the basis of their location. The normal microanatomy varies somewhat depending upon which lobe or segment is biopsied. Biopsies from the apical segments contain larger alveoli and relatively fewer blood vessels than biopsies from basal segments[3].

Pathological Categorisation on Basis of Anatomic Compartment Involved and Reaction Patterns

While evaluating the TBLB, the pathology needs to categorized on the basis of architecture and anatomic compartment of involvement:

1. Architecture: intact or destroyed.
2. Anatomic compartment: bronchial, alveolar, interstitial, vascular.
3. Intraalveolar space: cells or fibrosis.
4. Interstitium: cells or fibrosis.
5. Vascular: inflammation, thrombosis.
6. Dusts or birefringent particles.

Anatomic Compartment Involved in Few Diseases

Pulmonary Lymphangioleiomyomatosis (PLAM): Diffuse cystic lung disease in women of child bearing age characterized by thin walled cysts with hypertrophy of the smooth muscles in peribronchial, perilymphatic, perivenular areas. These smooth muscle cells (LAM cells) are fusiform, plump and show nucleomegaly when compared to other smooth muscle cells in the lung. They stain positive for HMB- 45 by immunohistochemistry (IHC).

Pulmonary Langerhans Cell Histiocytosis (PLCH): It shows lung parenchyma granulomatous infiltration in **early stage**-composed of CD1a+ Langerhans cells, eosinophils, lymphocytes, macrophages, plasma cells, and fibroblasts, which form nodules centered around the small airways, sometimes with cavitation. In **late stages** of the disease, fibrotic stellate scarring occurs, and **end-stage** PLCH is characterised by this scarring along with cystic spaces and honeycombing. On electron microscopy diagnostic pentilaminar cytoplasmic inclusion bodies (Birbeck granules) seen. S-100 positivity on IHC identifies Langerhans cells in BAL/Biopsy.

Alveolar Proteinosis*:* Rare primary idiopathic disease or associated with other conditions such as infections, malignancy etc which lead to over production of surfactant by type 2 pneumocytes or its impaired clearance. Alveoli are filled with eosinophilic, finely granular, periodic acid schiff (PAS) positive staining material with occasional cholesterol crystals and foamy macrophages and without an inflammatory response. The septal walls are free of

4

Reprinted from Leslie KO, Gruden JF, Parish JM, Scholand MB. Transbronchial Biopsy Interpretation in the Patient with Diffuse Parenchymal Lung Disease. Arch Pathol Lab Med 2007;131(3):407-23 with permission from *Archives of Pathology & Laboratory Medicine.*

Transbronchial Biopsy Interpretation in the Patient With Diffuse Parenchymal Lung Disease

Kevin O. Leslie, MD; James F. Gruden, MD; James M. Parish, MD; Mary Beth Scholand, MD

• ***Context.-*** **The most common lung tissue samples seen by pathologists worldwide are obtained with the flexible bronchoscope. Specimens taken for examination of diffuse or multifocal parenchymal lung abnormalities pose special challenges for the general surgical pathologist, and these challenges are often compounded by high clinical expectations for accurate and specific diagnosis.**

Objective.- **To present and discuss the most common histopathologic patterns and diagnostic entities seen in transbronchial biopsy specimens in the setting of diffuse or multifocal lung disease. Specifically, acute lung injury, eosinophilic pneumonia, diffuse alveolar hemorrhage, chronic cellular infiltrates, organizing pneumonia, alveolar proteinosis, sarcoidosis, Wegener granulomatosis, intravenous drug abuse-related microangiopathy, Langerhans cell histiocytosis, and lymphangioleiomyomatosis are presented.**

Clinical and radiologic context is provided for the more specific diagnostic entities.

Data Sources.- **The published literature and experience from a consultation practice.**

Conclusions.- **The transbronchial biopsy specimen can provide valuable information for clinical management in the setting of diffuse or multifocal lung disease. Computed tomographic scans are useful for selecting appropriate patients to undergo biopsy and in limiting the differential diagnosis. Knowledge of the clinical context, radiologic distribution of abnormalities, and histopathologic patterns is essential. With this information, the surgical pathologist can substantially influence the diagnostic workup and help guide the clinician to an accurate clinical/radiologic/pathologic diagnosis.**

***(Arch Pathol Lab Med.* 2007;131 :407-423)**

The flexible bronchoscope was introduced in the United States in the late 1960s and allowed pulmonologists and surgeons access to the lung as never before.[1],2 The small biopsy samples obtained with this instrument present special challenges to the general surgical pathologist, not the least of which is knowledge of the clinical and radiologic context that prompted the biopsy. In the case of diffuse or multifocal parenchymal disease, the transbronchial biopsy (TBB) approach is used, often with fluoroscopic guidance. The technique is not perfect, as the efficacy of any biopsy depends highly on the question being asked and the circumstances of the patient. For example, knowledge that the patient is immunocompromised raises the possibility of infection above all others, and requires both the use of special stains to exclude infection and a careful review of every section on the slide(s). In the normal host, knowing whether a radiologic abnormality is localized or diffuse is essential. Mild chronic inflammatory changes in a biopsy specimen taken in the setting of a spiculated mass lesion suspicious for carcinoma may be entirely incidental, whereas those same changes in a patient with diffuse parenchymal abnormalities could be a harbinger of a chronic inflammatory lung disease, or even cellular rejection in the biopsy specimen from a lung transplant recipient. Several excellent reviews of the challenges, strengths, and limitations of the TBB are available for the interested reader3-19 and a recent overview is concisely presented by Churg.[20]

Accepted for publication October 23, 2006.

From the Departments of Laboratory Medicine and Pathology (Dr Leslie), Radiology (Dr Gruden), and Medicine (Dr Parish), Division of Pulmonary Medicine, Mayo Clinic Arizona, Scottsdale; and the Department of Pulmonary and Critical Care Medicine, University of Utah School of Medicine, Salt Lake City (Dr Scholand).

The authors have no relevant financial interest in the products or companies described in this article.

Reprints: Kevin O. Leslie, MD, Department of Laboratory Medicine and Pathology, Mayo Clinic Arizona, 13400 E Shea Blvd, Scottsdale, AZ 85259 (e-mail: leslie.kevin@mayo.edu).

COMPUTED TOMOGRAPHY AND THE TBB

Computed tomography (CT), with scans often performed at high resolution, is very useful in predicting the yield of TBB based on the anatomic distribution and appearance of any abnormalities. In select instances, CT enables a specific diagnosis in the appropriate clinical context, particularly in patients with sarcoidosis, usual interstitial pneumonia, subacute hypersensitivity pneumonitis, acute eosinophilic pneumonia, Langerhans cell histiocytosis (LCH), and lymphangioleiomyomatosis (LAM).') Even when CT is nonspecific, peribronchovascular and central abnormalities are much more amenable to specific TBB diagnosis than is peripheral or nonsegmental disease. Transbronchial biopsy is also often diagnostic in patients in whom the CT findings are those of centrilobular nodules of ground-glass attenuation.[22] Unfortunately, there is still considerable variability in the performance and interpretation of thoracic CT, and the pathologist may receive tissue from a TBB in the absence of CT images or informative reports.

CLINICAL AND PROCEDURAL CONSIDERATIONS The main utility of the TBB rests on the possibility of making a specific diagnosis in a patient with diffuse lung

disease and avoiding a surgical lung biopsy. Although the surgical lung biopsy provides substantially more material for pathologic study, the procedure requires general anesthesia, and one or more days in the hospital with a chest tube in place. Moreover, there have been reports that this surgical procedure may be associated with excess mortality in certain disease states. For example, one study reported that 10 of 60 patients with usual interstitial pneumonia died within 30 days of the surgical lung biopsy.[23] In contrast, bronchoscopy can be done as an outpatient procedure, usually with minimal morbidity and mortality.[24] Even with the limited size of the biopsy specimen obtained with the bronchoscope, the clinician can often combine the information gained with other clinical, radiologic, microbiologic, and cytologic information to arrive at a diagnosis.

The main complication of TBB is bleeding; less frequent complications are pneumothorax, hypoxemia, or cardiac arrhythmias during the procedure. Bleeding occurs to some degree in virtually all TBB procedures and in some cases can be substantial. The risk of bleeding is the main limiting factor in obtaining more or larger biopsy samples. Bleeding is a major concern to the bronchoscopist because of the limited options available to manage excessive bleeding through the flexible bronchoscope. The suction channel of the typical bronchoscope is only 2 mm in diameter, and the volume of blood that can be suctioned through the channel is hence limited. One drop of blood can obscure the small lens of the bronchoscope and eliminate vision through the scope. Moreover, because the entire tracheobronchial tree is only about 150 mL in volume, a relatively small amount of blood can produce major problems with oxygenation.

Evaluation of the patient prior to the procedure is crucial. Even though inspection of the airways and possibly bronchoalveolar lavage can be done in patients with bleeding abnormalities or in those receiving anticoagulation therapy such as warfarin, TBB is contraindicated in the presence of bleeding abnormalities. An international normalized ratio (INR) greater than 1.5 is an absolute contraindication; successful TBB can be done if the INR is less than 1.5. If a patient is taking warfarin, it can usually be withheld for 4 to 5 days to allow the INR to slowly decrease to a safer range. If the warfarin cannot be withheld—for example, if the patient has a mechanical heart valve—then TBB will likely not be possible. Fresh-frozen plasma can be administered to reverse warfarin anticoagulation more quickly. Transbronchial biopsy is also contraindicated if the platelet count is less than 50 000/μL but the platelet count can be increased relatively quickly with platelet transfusions prior to the procedure. Also, platelet dysfunction is a relative contraindication to biopsy. There are insufficient data on antiplatelet agents such as clopidogrel, but most bronchoscopists are hesitant to perform a TBB if the patient is using this agent. Other contraindications for bronchoscopy and TBB are severe hypoxemia, uncontrolled cardiac arrythmias, unstable angina, and severe asthma or exacerbation of chronic obstructive pulmonary disease. The patient on mechanical ventilation support is at very high risk of pneumothorax, and TBB is not done in this circumstance, nor when extensive bullous disease, such as emphysema, is present.

In the hands of an experienced bronchoscopist, the procedure is quite tolerable for most patients. Following informed consent, conscious sedation is achieved, typically with a short-acting benzodiazepine and a narcotic, such as fentanyl or meperidine. A topical anesthetic, such a lidocaine, is applied to the upper airway mucosa. The bronchoscope can be passed through the nose or the mouth, depending on the preference of the bronchoscopist, and then through the vocal cords into the trachea. An endotracheal tube can be passed over the bronchoscope and into the trachea to achieve control of the airway. Topical lidocaine is administered to the bronchial mucosa during the procedure to reduce coughing. A complete endobronchial inspection of all segments of both lungs is performed to exclude significant endobronchial abnormalities. Often, bronchoalveolar lavage is obtained for culture and cytology specimens prior to the TBB.

The TBB is almost always done with fluoroscopic guidance as the rate of pneumothorax is reduced when fluoroscopy is used. The bronchoscope is directed to the segment desired for the biopsy and is wedged into that segmental bronchus as far as the scope will go. This "wedge" technique then isolates that segment in the case of bleeding, and also allows tamponade of that segment. Biopsy samples are then obtained repeatedly from this segment without removing the scope. A forceps is passed through the bronchoscope and into the segment as far as it will pass. The patient is asked to inhale and the forceps are opened. The patient is then asked to exhale and, at end-expiration, the forceps jaws are closed. If the patient experiences pain at this point, the forceps is opened and withdrawn because the only pain-sensitive structure in the area is the visceral pleura. Another location is selected. Approximately four to six biopsies are ideal (Figure 1). The bronchoscope is held in the wedge position until no further blood is visualized through the suction tubing. Biopsies from two different segments from the same lung can be obtained, but it is contraindicated to obtain biopsy specimens from both lungs because of concern for bilateral pneumothorax.

Transbronchial biopsies are obtained most commonly for the evaluation of mass lesions or localized infiltrates, in which neoplasm or infection leads the differential diagnosis. Patients with diffuse parenchymal lung disease (more than one lobe involved, and often bilateral abnormalities) undergo biopsy by this method, hoping to secure a definitive diagnosis, thus avoiding more invasive diagnostic procedures as discussed previously. Neoplasm and infection are still considerations when infiltrates are diffuse, but more often, inflammatory parenchymal disease is the primary clinical concern, and the TBB is the nonsurgical biopsy method of choice. The samples derived are typically only 2 to 3 mm in size but they hold a wealth of potentially useful information for the care of the patient.

A helpful general approach to the interpretation of the TBB is presented in Figure 2. Knowledge of the patient's immune status is an essential early key discriminator. For diffuse parenchymal diseases, histopathologic patterns of inflammatory disease are very helpful. Included in these patterns are (1) acute or subacute injury (infection, drug toxicity, systemic autoimmune disease); (2) chronic interstitial inflammation; (3) granulomatous inflammation and its differential diagnosis; (4) vascular diseases (including vasculitis/diffuse alveolar hemorrhage, pulmonary hypertension, and intravenous drug abuse microangiopathy); and (5) alveolar-filling processes such as alveolar proteinosis. In addition, rare distinctive lesions such as pulmo-

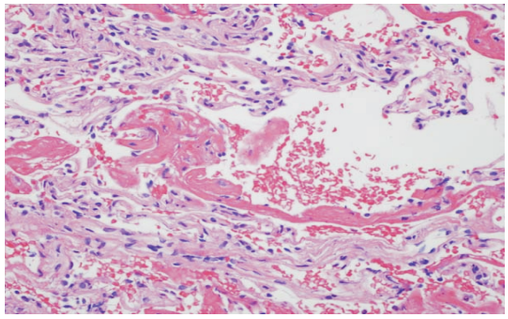
5

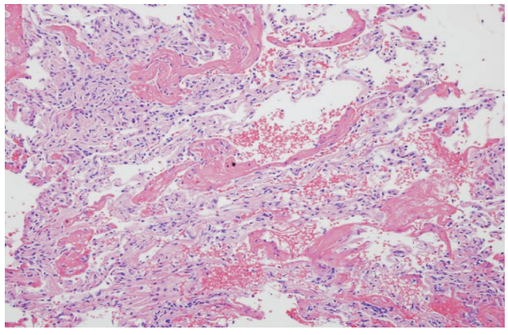
Non-neoplastic Lung Lesions

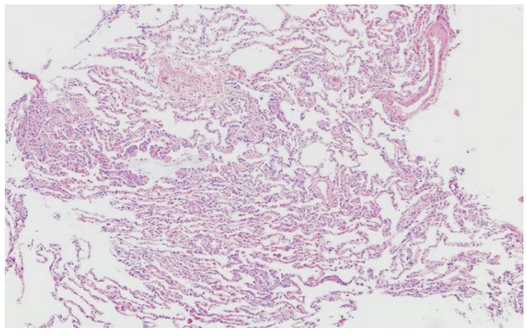
Henry D. Tazelaar

Case 5. 1

History: The patient is a 67-year-old man who was previously healthy. He developed severe respiratory distress over the course of 12 days and underwent transbronchial biopsy. Following this he became ventilator dependent.







Final Diagnosis: **Diffuse alveolar damage**

6
Lung Neoplasms and Their Differential Diagnosis

Ulrike Gruber-Moesenbacher

Overview and Clinical Aspects

Transbronchial biopsy technique is the method of choice for the histological diagnosis of tumors of the lung, situated extraluminal concerning bronchi, in medium and/or peripheral parts of the lung, until 3 to 5 cm towards the subpleural region. These lesions can be reached by a forceps through the flexible bronchoscope. In contrast to diffuse lesions in the lung, the yield of more centrally located tumors is high, between 80 – 100%, in peripheral lesions the success rate of endo-transbronchial biopsy techniques is 40 -80%[1]. Localisation of these lesions requires chest X-ray or CT. For tumors less than 20 mm ultrasound guidance could be of advantage[2]. For small peripheral lesions transthoracic biopsies (TTB) are superior, which is shown in a comparison of methods with sensitivity rates of 62% for TBB of peripheral tumors larger than 2 cm and 33% for smaller tumors, whereas TTB had a sensitivity of 90%[3].

For staging purposes transbronchial and transtracheal biopsies in my experience 2 – 3 needle biopsies are sufficient, if needle aspirates are used, at least 4 – 5 needle passes at lymphnode stations critical for staging of lung cancer should be performed[4].

Before the aera of targeted therapy, diagnosis of malignancy, discerning lymphomas and soft tissue tumors from carcinomas, and discrimination of small cell lung carcinoma (SCLC) and non-small cell carcinoma (NSCLC) was sufficient for therapeutic purposes. Nowadays the knowledge of the influence of histological subtype, mutations, amplification of genes requires a subclassification of lung carcinomas, at least of non-small cell tumors (NSCLC) into squamous and non-sqamous carcinomas, finally also into adenocarcinoma and large cell carcinoma, respectively. Morphological preselection for molecular analysis requires further subtyping of adenocarcinomas. Because a large proportion of peripheral lung carcinomas are still diagnosed in advanced stages, the diagnostic possibilities are restricted to small biopsies or even aspiration cytology specimen out of a frequently heterogeneous tumor.This requires a size of tumor particles for at least 7-10 consecu tive sections : minimum 3 H&E sections and sections for an immunohistochemical panel of: CK5/6, p63, TTF1 and D-PAS[5].

The following descriptions focus on entities, which are shown in the slide seminar and are not intended to be a complete list of lung tumors.

Malignant Lymphomas in the Lung

Malignant lymphomas affecting primarily the lung are:

- Marginal zone B-cell lymphoma of the MALT type,

- Diffuse large B-cell lymphoma,
- Lymphomatoid granulomatosis,
- Langerhans cell histiocytosis.

Those lesions are rare, but they have to be considered in the differential diagnosis of epithelial tumors with of small cells. 0,4% of all non Hodgkin lymphomas are lymphomas in the lung, they comprise 3, 6% of e•tranodal lymphoma s and 0,5-1% of all primary lung neoplasms.

70-90% of primary pulmonary lymphomas are marginal zone B-cell lymphomas of MALT type, 5-20% of primary pulmonary lymphomas are diffuse large B-cell lymphomas (DLBCL).

MALT lymphomas develop from mucosa associated lymphoid tissue (MALT), which is not found in the normal bronchial mucosa. They are frequently associated with Sjögren`s syndrome. MALT lymphomas are localized periphe rally as solitary nodules, nodular infiltrates with aerobronchograms, or as diffuse bilateral disease. 5-year survival is more than 80%.

Diagnosis can be made by bronchoscopic or transbronchial biopsy although not infrequently a surgical lung biopsy will be required. Bronchoalveolar lavage and fine-needle aspiration biopsy specimens can be diagnostic of lymphoma if a clonal B-cell population can be demonstrated, but the specific type of lymphoma can rarely be diagnosed by these techniques[6].

Differential diagnosis in resection specimen are:

- Inflammatory myofibroblastic tumor,
- Lymphoid interstitial pneumonia,
- Follicular bronchiolitis,
- Extrinsic allergic alveolities (EAA).

In small biopsies they have to be discerned from:

- "Small blue round cell" tumors, and from
- Small cell carcinoma, which also can form intraepithelial lesions.

If cytologic features are preserved in the biopsies, which often have crush artifacts, the nuclear size of 12 - 14µ in MALT-lymphomas is helpfu l in differentiating them from the 17 - 23µ measuring nuclei of small cell carcinoma. Immunohistochemically MALT-lymphoma is positive for the B-cell marker CD20 and monoclonality should be proven.

Immunhistochemically light chain restriction can be shown by staining for kappa and lambda light chains.

7

Infectious Pneumonias

Ritu Kulshrestha

Introduction

Infectious pneumonias (IP) caused by bacteria, viral, fungal infectious agents are frequently encountered by the pulmonary pathologists in bronchoscopic lung biopsies. Their definitive diagnosis requires identification of the organism by its morphological and staining characteristics, culture and/or identification of the histological pattern of tissue reaction. The pathology of lung infection is a reflection of a composite of host pathogen interactions. The distortion in pulmonary anatomy, decreased mucociliary clearance, etc., predisposing to pulmonary infection needs to be considered before making a final diagnosis.

Classification

Traditionally, clinicians have classified pneumonia by duration/clinical characteristics, into acute pneumonias (of less than three weeks duration) and chronic pneumonias. Acute pneumonias are further divided into the classic bacterial bronchopneumonias (such as *Streptococcus pneumoniae*), the atypical pneumonias (such as the interstitial pneumonitis of *Mycoplasma pneumoniae* or *Chlamydia pneumoniae*), and the aspiration pneumonia syndromes. Chronic pneumonias tend to be either non-infectious, or mycobacterial, fungal, or mixed bacterial infections caused by airway obstruction. Chronic pneumonias, mainly include granulomatous pneumonias (*Mycobacterium tuberculosis* and atypical mycobacteria, *Histoplasma capsulatum* and *Coccidioides immitis*), *Nocardia*, *Actinomyces* and *Blastomyces dermatitidis*[1].

Role of Transbronchoscopic Biopsies in Diagnosis of Infectious Pneumonias

Despite being limited by the size of the biopsies, transbronchoscopic biopsies have a high diagnostic yield in identifying infection when pulmonary infiltrates are diffuse, e.g., in diffuse alveolar damage due to viral pneumonia, but are far less reliable in the diagnosis of localised parenchymal infections[2]. Proper handling of the biopsy obtained is critical for obtaining the highest diagnostic yield from tissue sample. The scarcity of microorganisms in tissue biopsies creates a diagnostic challenge for the pathologist. In addition, previous antibiotic treatment can sterilize the inflamed tissue making it difficult to identify the original cause of the infection *in situ*. For these reasons the surgical pathologist must be prepared to review multiple sections in routine assessment of infection.

Histochemical Stains: Microbes that can be identified with the standard haematoxylin and eosin (H&E) stains include cytopathic viral infections, most fungal infections and all parasites. For identification of bacteria tissue Gram stain (Brown-Hopps and Brown-Brenn) is used in tissue sections. The Gram positive organisms stain a deep magenta while the Gram negative bacteria are pale pink. The Gomori silver methenamine (GMS) is the stain of choice for

identifying fungi in tissue sections. Both fungal yeast and hyphae stain intensely with GMS. The PAS (Periodic acid Schiff) stain is used primarily to complement GMS and not as a first line screening tool. Ziehl Neelsen (ZN) stain and its modifications (Fite-Farraco) are used in the identification of M. tuberculosis and atypical mycobacteria/Nocardia respectively

Histological Tissue Reaction Patterns in Pneumonia: The histological tissue reaction patterns in pneumonia have been classified on the basis of anatomic compartment primarily involved into airway disease, alveolar disease, and interstitial disease[3]. These patterns when combined with a panel of special stains including, ZN stain, GMS stain, and Grams stain can be useful in the diagnosis of suspected cases of pneumonia which have failed detection by microbiological techniques[4].

Pathological patterns and agents of pulmonary infection[3]

Pattern	Most Common Agent
Airway disease	
- Bronchitis/Bronchiolitis	Virus, bacteria, mycoplasma
- Bronchiectasis	Bacteria, mycobacteria
Alveolar disease	
Acute exudative pneumonia	
- Purulent-neutrophilic	Bacteria
- Lobular-bronchopneumonia	Bacteria
- Confluent-lobar pneumonia	Bacteria
- With granules	Botryomycosis, actinomycosis
- Eosinophilic	Parasites
- Foamy alveolar cast	Pneumocystis
Chronic pneumonia	
- Histiocytic	Mycobacteria
- Fibroinflammatory	Bacteria, mycobacteria
- Eosinophilic	Parasite
Nodular/ Necrotizing pneumonia	Fungi, mycobacteria
Interstitial pneumonia	
- Granulomatous	Mycobacteria
- Perivascular lymphoid	Virus
- eosinophilic	Parasite

Adapted from the Chapter of Rosati AL, Leslie KO. Lung infections, In Leslie KO, Wick MR (eds). Practical Pulmonary Pathology: A Diagnostic Approach. Churchill Livingstone 2005. pp 97-180.

Bronchiolitis: It indicates epithelial ulceration and necrosis of bronchiolar mucosa with submucosal oedema and inflammation and intraluminal accumulation of cells and necrotic debris[5]. Acute bronchiolitis is typically seen in *Haemophilus influenza* and *Hemophilus pertussis* infection, evoking a shirtsleeve like peribronchiolar lymphocytic infiltrate. Bronchiolitis may progress to bronchopneumonia or may result in bronchiolitis obliterans (Luminal obliteration).

Acute Exudative (Purulent) Pneumonia: This is the classical bacterial pneumonia which results in intraalveolar filling with fibrin and neutrophils. Necrosis of the interalveolar septa depends on the type of organism, host defense and duration of disease. Grams stain reveals the presence of Gram positive cocci or Gram negative bacilli in untreated cases, but not after antibiotic therapy.

Tubercular Pneumonia: Tuberculous pneumonia occurs particularly in persons with poor host resistance. The direct AFB smears of respiratory specimens are negative in at least 50% cases[6]. Invasive procedures like the transthoracic needle aspiration biopsy and transbronchial lung biopsy are then used to confirm the diagnosis of mycobacterial infection in these patients. Histologically, the alveoli are filled with neutrophils and histiocytes and tuberculous granulomas are poorly formed or not present at all. Tuberculous bronchitis/bronchiolitis and vasculitis can also be identified in few cases.

Histiocytic Pneumonia: A predominantly histiocytic intraalveolar pneumonia is seen in Legionnaires disease, *Mycoplasma pneumoniae*, cryptococcosis, amoebiasis, etc. In untreated cases bacteria are intracellular as well as extracellular. They are difficult to identify in treated cases.

Viral Pneumonia: Virtually all viruses cause diffuse alveolar damage. The acute phase is characterised by acute alveolar wall damage with hyaline membrane formation (composed of an extravascular fibrin coagulum admixed with necrotic alveolar cell debris)[7] interstitial and intraalveolar oedema and haemorrhage. In the later phase alveoli are filled with neutrophils and histiocytes. Cytopathic change is seen in bronchiolar epithelium. This viral associated cytopathic effect seen on H&E stained slides or highlighted by immunohistochemical change are the hallmark of viral pneumonias[8].

Fungal Pneumonia: Inflammatory patterns of fungal lung injury range from acute bronchopneumonia to granulomatous inflammation (necrotising and nonnecrotising) to diffuse alveolar damage[9]. Diagnosis is confirmed by identification of the fungal pathogen by GMS stain/fungal culture, in cytological samples like sputa, bronchoalveolar lavage and transthoracic needle aspirates[8].

Actinomycosis/Botryomycosis: Sulphur granules are characteristic and found within the center of the abscess. Histologically a sulphur granule shows central collection of organisms surrounded by neutrophilic reaction- the Splendore Hoeppli phenomenon[10]. Special stains required for definitive diagnosis include- AFB, PAS, GMS and Grams stain. The staphylococcal sulphur granules usually result from aspiration and are found in abscesses or bronchiectasis and called as botryomycosis.

References

1. Medlar EM: The behaviour of pulmonary tuberculous lesions. A pathological study. *Am Rev Tuberc*1955; 71: 1-244.

2. Kradin R. The pathology of pulmonary infection. In: *Fishman's Pulmonary Diseases and Disorders*. Fishman AP, Elias JA, Fishman JA, Grippi MA, Senior RM, Pack AI. Eds 2008 McGraw Hill publications Vol 2: pp 2031-2050.

3. Rosati AL, Leslie KO. Lung infections, In: *Practical Pulmonary Pathology: A Diagnostic Approach*. Leslie KO, Wick MR (eds). Churchill Livingstone 2005. Chapter No 6: pp 97-180.

4. Chandler FW: Approaches to the pathologic diagnosis of infectious diseases. In: *Pathology of Infectious Disease*. Connor D, Chandler FW, Schwartz DA, Manz HJ, Lack EE, Eds.1997, Appleton and Lange, Stamford, CT, pp 3-7.

5. Mark EJ. Bacteria and protozoa. In: *Lung Biopsy Interpretation*. Williams and Wilkins (Baltimore/London). 1984, Chapter 4: pp 31-44.

6. Gardiner DF, Beavis KG: Laboratory diagnosis of mycobacterial infections. *Semin Respir Infect* 2000; 15: 132-43.

7. Johnston WW, Elson CE: Respiratory tract. In: *Comprehensive Cytopathology*, Bibbo M, Eds.1991, WB Saunders: Philadelphia, pp 340-52.

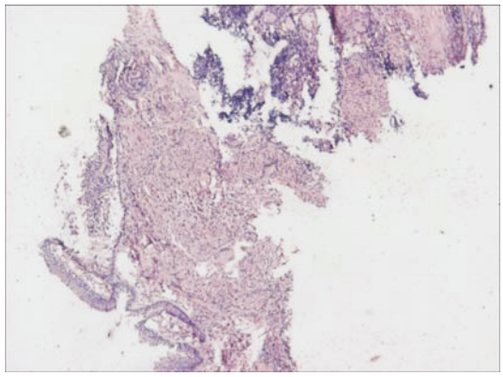
8. Kradin R: Pulmonary immune response, In: *Immunopathology of the Lung.* Kradin RLK, Robinson BWS (eds). Boston, Butterworth-Heinemann, 1996, pp 1-13.

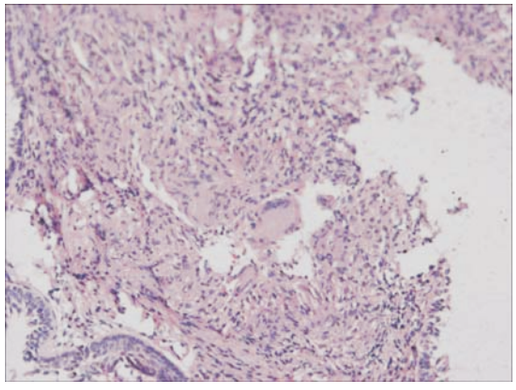
9. Harkin TJ, Ciotoli C, Addrizo-Harris DJ, *et al*. Transbronchial needle aspiration in patients infected with HIV. *Am J Respir Crit Care Med* 1998; 157: 1913-9.

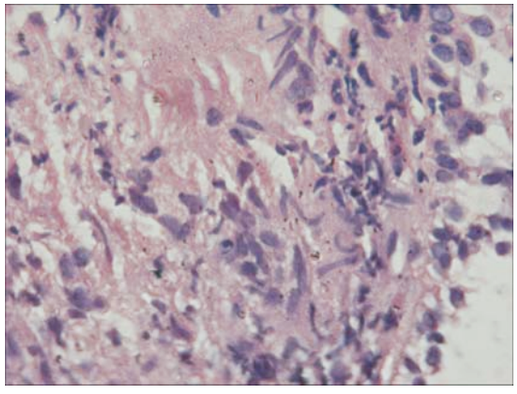
10. Mckenna R Jr, Mountain C, McMurtey M. Open lung biopsy in immunocompromised patients. *Chest* 1984; 86: 671.

Case 7.1

History: A 44 year old female patient with fever and breathlessness since 9 months, on Antitubercular therapy since 3 months. Previous investigations; radiological -bilateral nodular lesions on CT scan, fine needle aspiration biopsy of left cervical lymphadenopathy- necrotic background with polymorphonuclear infiltrate, suggestive of Cold abscess, Mantoux Test-Positive. Presently showing elevated Serum Angiotensin enzyme levels. Differential diagnosis: ?Tuberculosis ? Sarcoidosis







Final Diagnosis: **M. Tuberculosis**

8

Present Facts / Future Directions in Transbronchial Lung Biopsy Interpretation: What can/cannot be Diagnosed by TBLB?

Helmut H. Popper and Ulrike Gruber-Mosenbacher

The probability of a definitive diagnosis depends on localization and distribution of the process. Centrally located lesions can be reached by bronchial biopsies, lesions which are located in the lung parenchyma up to the 9th generation of bronchi less than 3 cm from the pleural surface can be reached by forceps and cryotechnique, which can acquire large transbronchial specimen. If the process is circumscribed, radiological or ultrasound guidance is necessary. If the process is distributed along bronchovascular bundles or disseminated, probability to reach the lesion is high. Subpleurally situated patchy processes in the lower lobes are a domain of surgical wedge resection. Transthoracal biopsy technique can be helpful in radiologically or by ultrasound detectable peripheral tumors.

Localisation	**Disease**	**Possibility of Diagnosis by TBLB**
Tend to be peribronchial	Infectious diseases	Mostly
Along bronchovascular bundles	Granulomatous pneumonias	Mostly e.g., sarcoidosis, additionally BAL recommended
Subpleural, patchy	Idiopathic interstitial pneumonias	Rarely Exception: DAD (diffuse alveolar damage)
Peribronchial/peribronchiolar distribution	Smoking induced lung diseases RB-ILD LHCH DIP COPD	Mostly
Diffuse in parenchyma	Rare interstitial lung diseases (LAM)	Sometimes
Peribronchial/peribronchiolar	Inhalation induced (environmental) lung diseases	Mostly Additionally BAL recommended
Mostly peribronchial Peribronchial and in parenchyma In lung parenchyma	Major types of lung cancer SCLC SCC AC	Mostly Because of heterogeneity of AC: predominant pattern impossible, grading not recommended
	Rare types of cancer and benign tumors	Nearly impossible may even be misdiagnosed (e.g., sclerosing hemangioma vs. AC)