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1 INTRODUCTION TO LUNG CYTOPATHOLOGY AND SMALL TISSUE BIOPSY

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HISTORY OF RESPIRATORY CYTOPATHOLOGY

Application of the cytologic method to specifically examine respiratory specimens was used first in Europe by the mid-1800s, though exfoliated epithelial cells were described as early as the 18th century. Hampeln's 1897 paper is credited with the first thorough description of normal and non-malignant cellular elements seen in sputum. And, about two decades later, his was one of the first publications to document the use of sputum cytology to diagnose pulmonary carcinoma in a series of 25 cases rather than a single report. Only sporadic and limited publications of respiratory cytology occurred in the early decades of the 20th century. A 3-year study of sputum cytology that culminated in a 1944 publication is considered as one of the most important contributions to clinical cytology of chest diseases in the first half of the 20th century. Nonetheless, it was only with the 1943 seminal publication by Papanicolaou and Traut that a major impetus to apply clinical exfoliative cytology to a variety of body sites really resulted in a renaissance of respiratory tract cytology. The consequence was a profusion of papers (only a handful referenced here) by the early 1950s concentrating in particular on the application of cytology to diagnose various forms of bronchogenic carcinoma, but also documenting the advantages that respiratory cytopathology offered in the recognition of fungal, parasitic, and viral diseases.

Optimal design of the rigid bronchoscope occurred in 1904 when Jackson added a suction channel and a light to its distal end. It was used primarily for removing purulent secretions from the airways, and was the only instrument capable of examining the lower airway up to the late 1960s. However, the exceptional technical skill required to perform needle aspiration with this instrument led to its limited use. The flexible fiberoptic bronchoscope was first introduced by Ikeda et al. With this technical advance, physicians could now visualize and sample segmental and subsegmental bronchi of all lobes, and obtain samples from infants and children. By 1973 its use had become widespread. The flexible bronchoscope allows for passage of brushes, biopsy forceps, and needles through their lumens for diagnostic sampling, and has few complications. Therefore, fiberoptic bronchoscopy ushered in the most common form of cytologic sampling of the respiratory tract in use today.

One of the earliest descriptions of transbronchial needle aspiration to sample mediastinal lymph nodes occurred in 1949, and that same author reported his results with the technique in a series of 83 patients 9 years later. Modern transbronchial fine needle aspiration (TBNA), often referred to as Wang needle after its inventor, was introduced in the late 1970s in the US for diagnosis and staging of bronchogenic carcinoma. Wang and colleagues showed that paratracheal lymph nodes, lesions within the bronchial wall, and masses compressing the tracheobronchial tree could be sampled using esophageal varices needles. This method increased the diagnostic yield over forceps biopsy alone in the detection of submucosal carcinoma. TBNA is typically performed prior to bronchial washing, brushing to minimize contamination of the aspirate with blood and mechanically exfoliated cells. In the last few years endobronchial ultrasound-guided fine needle aspiration (EBUS) has developed as an offshoot of TBNA in that it allows aspiration of mediastinal, hilar lymph nodes as well as pulmonary and mediastinal lesions under direct ultrasound control and in "real-time."

Although reports of percutaneous transthoracic lung aspiration appeared in the late 19th century, these aspiration procedures were employed primarily in patients with suspected pneumonia. Martin and Ellis pioneered the application of percutaneous transthoracic needle lung biopsy (using an 18-gauge needle) to lung cancer by reporting on a series of 41 cases. This was followed by a number of favorable

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Excerpt
More information

reports of large case series by others prior to 1950. However, concern over needle tract seeding by tumor was probably one of the main reasons that the technique did not blossom in the US. Rather, it found favor in Sweden in particular where it was shown to be highly efficacious. With the popularization of small bore needles, most notably at the Karolinska Institute, Stockholm in the mid-1960s, which lessened the risk of serious complications, the modern era of percutaneous transthoracic fine needle aspiration (FNA) began. Usage became more widely accepted, and reports of the success of this technique followed accordingly from the early reports at large academic medical centers to those several decades later from small community hospitals.

RESPIRATORY CELL SAMPLING AND PREPARATION METHODS

The principal methods available for cytologic analysis of the lung are listed in Table 1.1. Many factors influence which cell collection technique is used. These include but are not limited to physician preference, lesion location, lesion size, radiologic interpretation, and severity of patient symptoms. Details of their processing, advantages, and limitations are detailed in the following sub-sections. The majority of pulmonary specimen slides are stained using modifications of the original stain developed over a half century ago by George Papanicolaou. Other stains typically used in North America and Europe are variations of the Romanowsky (Wright-Giemsa, May-Grünwald-Giemsa) type of stain, and the hematoxylin-eosin (H&E) stain. Some textbooks of cytopathology still list the individual stains for these methods; however, in most modern laboratories automated instruments perform routine Papanicolaou staining.

Table 1.1 Techniques available for cytologic analysis of lung lesions

- Sputum (induced or spontaneously expectorated)
- Bronchoscopically acquired cells
  - Bronchial brushing
  - Bronchial washing
  - Bronchoalveolar lavage
- Fine Needle Aspiration (FNA)
  - Transbronchial FNA with or without endobronchial ultrasound (EBUS)
  - Percutaneous transthoracic FNA
- Imprint cytology of pulmonary tissue
- Pulmonary artery/capillary sampling

Sputum

Sputum was the mainstay of cytologic sampling of the respiratory tree for much of the early–mid 20th century prior to routine bronchoscopy, and although it remains a useful test, it is less frequently ordered. Many of the early studies relied on the wet film method developed by Dudgeon and Patrick in 1927. Sputum examination has been supplanted in many medical centers in the past few decades by bronchoscopic techniques of washing, brushing, and transbronchial needle aspiration. Nonetheless, it remains a useful modality particularly in symptomatic individuals where it is easy to obtain. Its major advantage rests on the non-invasive nature of specimen collection with little to no inconvenience to the patient. Sputum production depends either on the spontaneous or artificially induced exfoliation of cells. The former is obtained easily from chronic cigarette smokers and those with chronic bronchitis or bronchiectasis. Non-smokers and those without lung disease generally require artificial induction of sputum often by having the patient inhale an aerosolized mist of heated hypertonic saline or water for 30–45 minutes. Some authors feel that induced sputum provides a more representative sample of the lower respiratory tract, and increases the sensitivity in lung cancer detection. Sputum is a mixture of mucus, cell debris, inflammatory cells, and exfoliated cells from the respiratory tract. It must contain either pigmented carbon-bearing pulmonary macrophages ("dust cells") or ciliated columnar cells to be considered satisfactory for interpretation (see below).

The choice of sputum preparation varies among institutions. The optimal collection method is an early morning sample accumulated over a period of several (typically 3) consecutive days. After rinsing the mouth with water, the patient coughs directly into a shallow, wide-mouthed container. Sputum may be sent fresh to the laboratory where it is processed without fixation. Fresh sputum is handled using the so-called "pick and smear" method where the sample is examined grossly for tissue fragments – particularly blood-stained fragments since these will often be the most likely pieces to contain malignant cells. These are picked up with forceps and smeared between two glass slides which are then immediately 95% alcohol fixed. Use of this method is uncommon in many laboratories nowadays; use does vary from region to region. Alternatively, many laboratories

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Excerpt
More information

currently process sputum after it has been pre-fixed. This is carried out by having the patient expectorate directly in 70% ethanol or in Saccomanno fixative. The latter contains 50% ethyl alcohol and 2% polyethylene glycol (Carbowax). With this method, the patient can cough each morning directly into the same container containing fixative over several days. Multiple samples are processed then as a single specimen. Several seconds in a mechanical blender homogenizes the sputum, and is then followed by either cytocentrifugation or smearing of the concentrate to create slides. A biologic safety hood is required with this method because of the risk of infection from aerosolization.

Cell blocks are another means of sputum examination. These are made using a variety of methods including thrombin-plasma clot method with subsequent formalin fixation or by using a coagulating fixative such as Bouin's fixative to create a coagulum that is wrapped in tissue paper and then placed in a cassette. The cassette is mechanically processed in a manner similar to tissue with subsequent paraffin wax embedding and hematoxylin-eosin staining. Some authors praise this form of cytopreparation citing high rates of sensitivity, while others claim this is the worst possible technical approach to respiratory cytology.

Increasingly, many laboratories have switched to liquid-based slides to process sputum because of its technical ease, reduction in the amount of obscuring mucus and blood, reduction in the screening area of the slide that contains cells, and good cell preservation. After sputum is collected it is then placed in a proprietary preservative without pre-fixation for 30 minutes. A mucolytic agent is added if necessary; the specimen is then centrifuged, washed, and the resulting sediment is processed in an automated instrument.

Bronchial washings and brushings

Bronchial washing and brushing is best used to analyze lesions that arise in the 1st through to 3rd order bronchi. Washings are almost always taken in combination with brushings, and sometimes tissue biopsy. The sequence of sampling should begin with the least invasive step, i.e., bronchial washings followed by brushings followed by forceps biopsy if indicated (TBNA should be performed prior to any of these other sampling methods). As one progresses through this sequence, the risk of bleeding increases which tends to dilute any malignant cells that may be present. Unlike sputum, bronchial washings/brushings contain numerous ciliated and mucinous epithelial cells along with a variable number of macrophages, mucus, and inflammatory cells.

The technique of bronchial washing entails the installation of 3–10 ml of sterile saline into bronchi that is immediately re-aspirated, collected in a specimen trap, and then submitted to the laboratory in an unfixed state. Bronchial brushing results from the bronchoscopist using disposable or reusable stiff bristle-like brushes to brush in a back and forth motion over the surface of a visible mucosal lesion thereby dislodging cells. In the past it was usual for the bronchoscopist (once the brush was removed from the patient) to roll it over the central portion of a glass slide(s) and then immediately immerse the slide(s) into 95% ethanol. Unstained slides are sent to the laboratory for staining. This method however is highly dependent on the technical skill of the person smearing the glass slides. Avoiding any delay in ethanol fixation is vital so that air-drying artifact does not occur. Thus, due to several factors including convenience and those advantages listed under sputum preparation, most bronchial wash/brush specimens are currently processed using either cytocentrifugation or a liquid-based method. Both methods have the advantages of (1) concentrating cells in a more or less monolayer, (2) on a small area of the slide, and (3) lysing potentially obscuring red blood cells, thus allowing for more efficient screening by increasing the probability that abnormal cells are not missed. For either cytocentrifugation or liquid-based preparations the end of a disposable brush needs merely to be cut and dropped in a small amount of balanced salt solution or commercial fixative, respectively, then transported to the laboratory. Papanicolaou staining is the most common method used in the US for these specimens.

Histochemical staining for infectious organisms is commonly performed also on bronchial washing and brushing specimens. This is accomplished using either unstained slides if additional sample is available or de-staining stained slides. Staining for fungi and acid-fast bacilli is typically performed using Gomori-methenamine and Zeehl-Nielsen methods respectively; however, other staining methods are also available.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) is used to obtain cellular material from the 5th–6th order bronchi by wedging a bronchoscope against the bronchial lumen. The distal lung is then irrigated with 20–50 ml aliquots of warm or room temperature sterile normal saline to a usual limit of 100–300 ml. After each fluid instillation the bronchus is then aspirated with suction kept at low pressure (usually

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Excerpt
More information

< 60 mmHg) to avoid visible airway collapse and the cell-rich fluid is retrieved. This series is rapidly repeated 4–5 times in succession, and the pooled contents submitted to the laboratory in a fresh state. BAL causes exfoliation primarily of pneumocytes and macrophages with lesser numbers of ciliated bronchial cells compared with bronchial washing/brushing. In contrast to bronchial brushing/washing that concentrates on centrally located lung lesions that are directly visualized, BAL is primarily used to evaluate peripheral alveolar-based lung disease that is not visible to the bronchoscopist including infectious disease and interstitial lung disease. If requested, cell counts should be made from unfiltered, unwashed samples. BAL is a safe procedure, and not associated with an increased risk of complications over that seen with bronchoscopy alone; it thus may provide useful information in those patients unable to tolerate open lung biopsy. The liquid obtained with BAL must be transported to the laboratory as soon as possible. Processing is done in a similar manner to bronchial washings/brushings.

Transbronchial fine needle aspiration

From its initial reports in the early 1980s transbronchial needle aspiration (TBNA) has become a useful procedure in select situations for the diagnosis and staging of bronchogenic carcinoma. Indications for TBNA are listed in Table 1.2. Its value is enhanced because it is practical to couple it simultaneously with diagnostic bronchoscopy. TBNA is a safe procedure with few complications. Needles ranging from 18 to 23 gauge are passed through the suction channel of the bronchoscope with the needle retracted into the catheter. When the area for aspiration is identified, the needle is inserted into the lesion. Back and forth movements are made for 5–10 seconds with suction at the other end of the needle. Suction is released prior to withdrawing the entire needle from the bronchoscope. Its tip is held close to a set of glass slides, and then an air-filled syringe expels the cellular material onto the slides from which direct smears are made. Alternatively, the material is squirted into a preservative or a balanced salt solution for processing as either liquid-based or cytocentrifuged slides, respectively.

Table 1.2 Indications for TBNA

• Endobronchial and intrapulmonary masses
• Lymph node staging in lung cancer: subcarinal, hilar, mediastinal nodes
• Mediastinal masses
• Pancoast tumors
• Peribronchial masses from the inner one-third of the lung

Endobronchial ultrasound (EBUS) evolved after the development of miniaturized ultrasound probes with radial scanning capability that could be inserted through a bronchoscope's working channel. EBUS with an integrated linear array ultrasonic bronchoscope allows for a resolution of < 1 mm and a depth of penetration of 4–5 cm, thus providing for excellent assessment and differentiation of the layers of the airway wall, lymph node, tumor masses, vascular structures, and other parabronchial structures. Unlike conventional TBNA where needle aspiration occurs without actual visualization but using only landmarks based on static CT scan images, EBUS-TBNA using the new convex-probe apparatus allows for direct visualization of the lesion and of the needle path. This is rapidly changing the management algorithm for interventional pulmonology where in the past mediastinoscopy was necessary to sample select N1 and N2 lymph nodes. Cytology laboratories can expect to see an increase in aspiration specimens using this technology not only for staging of mediastinal nodes in lung cancer but also for the diagnosis of lymphoma in the future.

Percutaneous transthoracic fine needle aspiration

Transthoracic FNA is performed using radiologic guidance, most often by CT scan but also using ultrasound; the latter is suitable only for lung nodules with pleural contact since ultrasound cannot penetrate aerate lung. Historically, fluoroscopic guidance was the principal method used. Indications for its use are listed in Table 1.3. Virtually any lung or mediastinal mass is amenable to examination using this technique. The advantages are numerous and listed in Table 1.4. Morbidity, mortality, and cost associated with thoracotomy are markedly lessened. The principal hazard with this technique is the likelihood of pneumothorax, the incidence of which is dependent on lesion size, lesion texture, experience of the operator, number of passes into the lesion, and location within the lung. In most examples, pneumothoraces are small and do not require chest tube placement. Older studies using larger needles showed up to one-third of patients developing a clinically significant pneumothorax, but reports that are more recent show only 2–10% of patients developing pneumothorax, and far fewer requiring chest tube placement. Tumor implantation, air embolism, hemopericardium (mediastinal FNA), and pulmonary

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Excerpt
More information

Table 1.3 Indications for percutaneous transthoracic FNA of a lung nodule

• Solitary lung mass: distinction between primary and metastatic neoplasm
• Distinction between non-neoplastic condition (hamartoma, unresolved pneumonia, granuloma) and neoplasm
• Multiple lung masses in cancer patient: documentation of suspected metastatic neoplasm
• Suspected lung cancer that is inoperable
• Suspected infectious process unresponsive to antifungal or antibiotic therapy (either immunocompromised or immunocompetent patient)
• Patient who refuses exploratory thoractomy
• Medically unstable patient (non-surgical candidate)
• Patient who is not a candidate for any therapy may wish to have a diagnosis for purposes of determining prognosis
• Patient who is a candidate for neoadjuvant chemotherapy, or pre-operative radiation

Table 1.4 Advantages of percutaneous transthoracic FNA

• Saves time, expense, and patient discomfort compared to other invasive methods of sampling lung lesions, i.e., speed, safety, cost (can be performed as an outpatient procedure)
• Accuracy
• Sampling of multiple lesions
• Readily repeatable
• Application in patients that refuse surgery
• Application in non-surgical candidates prior to chemoradiation treatment

Table 1.5 Potential complications of percutaneous transthoracic FNA

• Pneumothorax (usually transitory)
• Pneumomediastinum
• Hemoptysis
• Clinically significant pulmonary hemorrhage
• Air embolization
• Pyothorax
• Tumor implantation of needle tract
• Cardiac arrest (very rare)

hemorrhage are rare. Short-lived hemoptysis is more likely. Other very uncommon to rare complications are shown in Table 1.5. Using a 22-gauge needle and avoiding multiple passes into the mass are associated with fewer complications. Relative contraindications for transthoracic FNA are listed in Table 1.6.

With this technique, the skin is anesthetized locally and a 20–22-gauge needle trocar is inserted to prevent contamination with cells through which the needle passes. After localization into the lesion, the stylet is removed and the needle moved back and forth relatively rapidly for several seconds. A vacuum is created during this phase usually by pulling back on a 10 ml syringe that is attached to the needle. Prior to removing the needle, suction is discontinued, the needle quickly removed and then passed to an individual to expel the cellular material onto waiting glass slides where direct smears are made. The optimal situation is to have the slides stained and examined immediately by a pathologist, pathology fellow, or cytotechnologist for adequacy assessment (and possible preliminary interpretation) using a rapid Papanicolaou stain, an air-dried Romanowsky-type stain, an H&E stain, or a combination of these in the radiology suite thus permitting a repetition of the procedure if diagnostic cellular material has not been obtained. Needle rinsing (after smears are made) in a balanced salt solution allows for the capture of more cells. This gives one the flexibility to perform a variety of ancillary studies on this rinsed cellular material including cell block technique, submission to flow cytometry for immunophenotyping, and fluorescence in situ hybridization (FISH).

Formalin-fixed cell blocks are a particularly important cytologic method for analyzing transthoracic FNA-procured neoplastic cells as they allow one to treat a cytologic specimen similar to a tissue specimen. The advantages include the ability to cut multiple sections (dependent on quantity of cells obtained), to discern spatial relationships on occasion that are not obvious from conventional direct smears, and to apply several different histochemical and immunocytochemical stains to cells. This latter advantage enhances the probability to more correctly and confidently subtype a particular neoplasm. Immunocytochemistry in particular is extremely efficacious in helping to determine whether a malignancy is primarily of the lung, or represents metastatic disease in many instances. Several methods exist for the creation of cell blocks as alluded to in the discussion on sputum processing. Among the more popular is the thrombin–plasma clot, the collodion bag, and the cytoblock™ methods. Specific methodological details are found in texts

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Excerpt
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Table 1.6 Relative contraindications of TBNA and percutaneous transthoracic FNA

• Severe pulmonary hypertension
• Advanced emphysema
• Uncooperative patient
• Uncontrollable cough
• Prior pneumonectomy
• Suspected vascular lesion
• Suspected echinococcal cyst (cyst rupture → possibility of anaphylactic shock)
• Uncorrectable coagulopathy
• Patient scheduled for surgery regardless of cytology results

on this subject. The most recent method for transforming cellular samples into those by means of which they are processed in paraffin is the Cellient™ Automated Cell Block System (http://www.cellientsystem.com). To our knowledge, a large-scale study using pulmonary specimens with this technique does not yet exist. Immunocytochemical staining is now routine whenever neoplasms require subclassification; specific antibody panels are discussed in subsequent chapters. Immunochemistry and histochemistry are also applicable for further analysis of suspected infectious organisms on any of these specimen types. FISH analysis is feasible from slides made either by cytocentrifugation, or from cells captured in a cell block.

Pulmonary artery/capillary wedge sampling

Pulmonary microvascular cytology is an exceedingly uncommon specimen. Since these patients already have a pulmonary artery catheter in place there is generally no risk in acquiring cells. A standard heparinized or EDTA coated tube is used to prevent clot formation, and a buffy coat blood smear is prepared using a Ficoll-Hypaque gradient. It should be remembered that circulating tumor cells make up as few as 1 cell per 1×10^9 hematologic cells in the peripheral blood of patients with metastatic cancer.

GENERAL REPORTING OF RESPIRATORY CYTOPATHOLOGY

Diagnostic terminology

Unlike gynecologic cytopathology wherein the Bethesda system has largely standardized diagnostic nomenclature with actual phrasing of diagnostic terms, no such system exits for pulmonary cytopathology. As in much of non-gynecologic cytopathology, diagnoses are placed under five general categories as listed in Table 1.7. The inconclusive non-committal term "atypia" deserves a descriptive explanation when used to guide the physician(s) managing the patient. Some laboratories include such phrases as "atypia secondary to epithelial repair," "reactive atypia," and "radiation-induced atypia" under the benign category to eliminate any confusion for the clinician. For most malignant diagnoses the pathologist is able to classify the neoplasm as either a carcinoma, lymphoma, or sarcoma, and further subtype carcinomas into non-small cell carcinoma, and small cell neuroendocrine carcinoma. Specific diagnostic terminologies for the various neoplasms that are encountered in the lung are discussed in the appropriate chapters. The Papanicolaou Society of Cytopathology guidelines make a distinction between the terms "adequate" and "non-diagnostic" when applied to transthoracic FNA. Their recommendation is to designate an FNA composed of benign respiratory epithelium, macrophages, and inflammatory cells in the presence of a lesion clinically or radiologically suspicious for malignancy as "non-diagnostic" rather than "adequate" despite the presence of abundant cellular material, thus implying that further investigation should be considered in that patient because the clinical concern has not been answered by the FNA findings. Terminology for infectious organisms uses generic names only (e.g., *Candida*) with speciation left to the microbiology laboratory.

Table 1.7 Diagnostic categories for pulmonary cytopathology

• Benign/negative for malignant cells
– if infectious or inflammatory, specify organism when present or type of inflammation
– if benign neoplasm, specify if possible
• Atypical cells
– descriptive explanation
• Suspicious for malignancy
• Malignant/positive for malignant cells
– specify type
• Unsatisfactory
– specify reason

Adequacy

It goes without saying that if malignant cells are identified, the specimen is adequate regardless of how it was acquired. Adequacy as defined for sputum specimens requires the presence of pulmonary macrophages or ciliated respiratory

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Excerpt
More information

epithelium. Though most cytopathologists expect to see several macrophages to consider it a "deep cough" specimen, no numerical standard exists, and thus even a single carbon-bearing macrophage is theoretically sufficient to avoid a designation of completely unsatisfactory. Greenberg stated that the adequacy of a sputum sample was directly proportional to the number of alveolar macrophages present. Moreover, the presence of squamous cells and/or inflammatory cells alone indicates that the sample is saliva, and is unsatisfactory for interpretation. Ciliated cells are actually very uncommon in spontaneously expectorated sputum; they are encountered more often in post-bronchoscopy sputum or lesions that have eroded the bronchi.

For bronchial washings and bronchial brushings, bronchial epithelium must be present to be considered adequate. The Papanicolaou Society of Cytopathology has stated that washings or brushings containing few bronchial cells, and heavily contaminated and obscured by large numbers of oral squamous cells, abundant blood, inflammatory cells, oral saprophytes, or air-drying artifact, be deemed unsatisfactory. An adequate BAL specimen should contain numerous pulmonary macrophages – typically more macrophages than bronchial cells. Some have advocated the presence of 10 or more alveolar macrophages per high-power field, or a greater number of macrophages (> 25 per high-power field) when excessive ciliated cells, or a mucopurulent exudate is present in order for a BAL to be considered satisfactory for interpretation. TBNA specimens of peribronchial and mediastinal lymph nodes should require the presence of lymphocytes to be considered adequate although no numerical standard exists and the pathologist must use judgment. It is best if one can see a range of lymphocyte sizes to be assured a lymph node has been sampled rather than merely lymphocytes circulating within the peripheral blood. Percutaneous transthoracic FNAs must contain pneumocytes, alveolar macrophages, and/or bronchial epithelial cells. Aspirates with only cutaneous squamous cells, skeletal muscle and connective tissue, or mesothelial cells or a combination of these are inadequate for evaluation.

DIAGNOSTIC PERFORMANCE OF RESPIRATORY CYTOPATHOLOGY

Because sputum cytology has failed to show a mortality-reducing effect from bronchogenic carcinoma when it was combined with chest X-ray in a large-scale randomized screening project from the early 1970s, it is not officially recommended as a screening procedure in asymptomatic individuals. The sensitivity and specificity for sputum cytology with regard to neoplasms is dependent on several factors including specific subtype of neoplasm, size of neoplasm, location of neoplasm, and number of samples submitted. In a study of over 600 cases, sensitivity for a centrally located neoplasm was 77% versus 47% for peripherally located ones. Accurate recognition of specific subtype occurred with squamous cell carcinoma, large cell carcinoma NOS, small cell carcinoma, and adenocarcinoma in 80%, 73%, 64%, and 57% of cases, respectively. The highest sensitivity for cancer detection using sputum cytology seems to be with centrally located tumors that are exposed to the bronchial lumen whereby cells are shed directly into bronchial secretions in contrast to those restricted to the submucosa or more peripherally located. Although many studies of sputum accuracy found in the literature are now several decades old, their results are still valid. Repeated submission of sputum specimens is somewhat analogous to the "magic" of compounding interest seen in the financial markets. This is illustrated in a study from 1970 of 107 lung cancers where sensitivity for recognizing cancer in a single sputum examination was as low as 41%, but rose to 82% with three specimens, and 91% with 5 specimens. A sensitivity of 89% emerged in a study of 149 tissue proven lung cancers when three or more specimens were examined; it dropped to 60% from examining only the first specimen. Similar numbers were shown in a much larger study of 1889 patients that was repeated over 20 years later. Accuracy is also affected by cytologic method. A comparison study of almost 1000 cases made between liquid-based preparations (LBPs) and conventional direct smears of sputum found a false-negative rate of 50% for the former and 70% for the latter method, and the unsatisfactory rate was almost three times *lower* for the LBP.

Most reports on the topic of performance characteristics for sputum cytology fail to mention the indication(s) for such testing, which of course affects the results. A recent paper pooling 16 large studies from years 1948 to 1992 that amounted to 28 477 patients showed the results listed in Table 1.8. This confirms prior smaller studies indicating a very high specificity, but much lower sensitivity for sputum cytology in the recognition of bronchogenic carcinoma.

Bronchial washings show similar findings to those discussed for sputum cytology. Overall accuracy was 74% in a large study of 276 consecutive cases. Positive cytology (for malignant cells) decreased from 84% to 30% as the lesion site moved from the major bronchus to a peripheral bronchiole, from 82% in carcinomas > 5 cm to 15% in those

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Table 1.8 Performance characteristics (28 477 patients) of sputum cytology for diagnosis of suspected lung cancer

• Sensitivity	
range	42–97%
pooled	66%
for central lesion (1077 patients)	71%
for peripheral lesion (1077 patients)	49%
• Specificity	
range	68–100%
pooled	99%
• False-positive rate	9%
• False-negative rate	6%

Table 1.9 Sensitivity of flexible bronchoscopic procedures for central lung cancer (3754 patients, 30 studies)

• Endobronchial biopsy	74%
• Bronchial brushing	59%
• TBNA	56%
• Bronchial washing	48%
• All methods (14 studies)	88%

Table 1.10 Sensitivity of flexible bronchoscopic procedures for peripheral lung cancer (4136 patients, 30 studies)

• Bronchial brushing	52%
• BAL	43%
• Bronchial biopsy	46%
• All methods (12 studies)	69%

< 2 cm, and from 92% with two washings to 68% when only one washing was examined. The literature review of Schreiber et al found 30 studies after 1970 that examined sensitivity of various cytologic techniques obtained using flexible bronchoscopy. For centrally located carcinoma, these showed endobronchial biopsy provided the highest sensitivity followed by TBNA, bronchial brushing, and bronchial washing (Table 1.9). However, for carcinoma beyond the visible segmental bronchi brushings provided the highest sensitivity (Table. 1.10). TBNA had a sensitivity of 67% but only 6 of 30 studies used this technique. These authors also uncovered eight studies (341 patients) where lesion size was indicated. Sensitivity for using bronchoscopy in peripheral lesions < 2 cm diameter was 33% while for those lesions > 2 cm diameter it was 62%. Notable in these latter two tables is the additive effect resulting in increased diagnostic sensitivity when a combination of cytologic sampling methods is submitted from the same patient. Therefore, it cannot be overemphasized that establishing a diagnosis at a single procedure by combining bronchial washing, brushing, BAL, and if indicated, TBNA has a profound effect on clinical management. Early reports of TBNA using the EBUS method have demonstrated excellent results. A study of 572 mediastinal lymph nodes showed a diagnostic yield of 94%. EBUS is also being combined with transesophageal endoscopic ultrasound to sample virtually all mediastinal nodes with high levels of success.

For non-neoplastic conditions – in particular for pulmonary infiltrates in the immunocompromised host – BAL is the diagnostic procedure of choice due to its relatively high diagnostic yield, the possibility of obtaining a large sample volume thus allowing for a variety of laboratory studies, and its relatively low risk. One series of immunosuppressed patients showed sensitivity for diagnostic BAL of 82% and specificity of only 53%. Another showed a similar sensitivity, but higher specificity (80% and 100%, respectively) when BAL was used to diagnose tuberculosis (TB) in a population where the prevalence of TB is very high. A review of 11 studies examining 841 immune suppressed patients showed the percentage of final diagnoses obtained by BAL to range from 38 to 92%. The mean diagnostic yield from 10 separate studies (667 bronchoscopies) analyzing BAL in bone marrow transplant patients was 53%. The utility of BAL in the diagnosis of ventilator-associated pneumonia (VAP) seems to be secure. A review of 23 case series analyzing the diagnostic performance of BAL in VAP patients reported mean values of 73 ± 18% and 82 ± 19% for sensitivity and specificity, respectively.

The American Thoracic Society and European Respiratory Society endorse the use of transthoracic FNA as the procedure of choice in patients in whom the benign nature of the solitary pulmonary nodule cannot be established by clinical criteria and in whom surgery cannot be undertaken. One of the largest studies analyzing the utility of transthoracic FNA was performed using the College of American Pathologists database. The results from 11 922 satisfactory aspirates shown in Table 1.11 demonstrate a highly reliable and accurate test. Importantly, these results were garnered primarily from non-university medical centers in North America demonstrating the widespread applicability of this test, and also that the false-negative rate was primarily one of sampling error since the sensitivity of FNA diagnosis was higher than that of FNA performance

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Table 1.11 Performance characteristics (11 922 cases) of transthoracic FNA for diagnosis of suspected lung cancer

• Sensitivity	89%
• Specificity	96%
• Positive predictive value	98%
• Negative predictive value	70%
• False-positive rate	0.8%
• False-negative rate	8%

(99% vs. 89%). A summary of 21 separate studies (6305 patients) addressing the ability of various cytologic modalities to differentiate non-small cell carcinoma from small cell neuroendocrine carcinoma showed the following results: accuracy 98%, false-positive rate 9%, and false-negative rate 2%. Fifty-seven percent of these patients (57%) had a transthoracic FNA, 28% sputum cytology, 11% bronchial brushing, and 4% TBNA. Fifty-three cases from the Early Lung Cancer Action Project (all with histologic confirmation) tested the reliability of transthoracic FNA in patients with lesions having a mean diameter of only 13 mm (range 0.4–40 mm). The sensitivity of making an outright malignant diagnosis was 93%, and was 100% if one included the four cases diagnosed as suspicious for malignancy (atypical bronchioloalveolar proliferation). For the subclassification of non-small cell carcinoma as adenocarcinoma, the positive predictive value was 92% illustrating the exceptional diagnostic accuracy that is possible with transthoracic FNA even with very small pulmonary nodules.

SMALL TISSUE BIOPSY OF LUNG AND MEDIASTINAL LESIONS

Introduction

A multitude of techniques and innovative technologies have improved the diagnosis of pulmonary diseases. Especially noteworthy are flexible fiberoptic bronchoscopy along with various imaging modalities such as video bronchoscopy, auto florescence-bronchoscopy, narrow band imaging, and linear esophageal endobronchial ultrasound. These techniques have been introduced in clinical practice and are revolutionizing the diagnosis and management of pulmonary disease. This is especially relevant for the diagnosis, staging, and management of lung cancer. Factors affecting the diagnostic yield include lesion, location, size, endobronchial vs. submucosal locations, and central or peripheral placement.

Biopsy procedures vary with location of lesions and consist of:

1. **Bronchoscopic forceps biopsy** performed under direct visualization for central lesions
2. **Bronchoscopic brush biopsy** yielding a highly cellular cytologic brushing
3. **Bronchoscopic bronchoalveolar lavage** performed by instilling 150–180 ml of saline into the airways. This technique is especially useful for studying pathology of the distal airways
4. **Transbronchial needle aspiration (TBNA)** or "Wang" biopsies.

First introduced by Wang and Terry in 1983, TBNAs have good diagnostic results and the yield is higher than endobronchial biopsy. Using a combination of techniques improves the diagnostic yield. It is not uncommon to perform forceps and brush biopsies, Wash/BAL, or TBNA in a single setting, either for central or peripheral lesions. Although the diagnostic yield for peripheral lesions is less.

With the advent of smaller gauge core needle biopsies and proliferation of various imaging techniques, the cytopathologist is at the forefront of pulmonary diagnosis. With increasing demand for **rapid on-site examination (ROSE)** cytopathologists can receive and process the tissue in appropriate ways and with varying cyto-preparatory techniques to provide the best diagnosis for the patient. In today's practice the cytopathologist has the ability to review punch biopsies and core needle biopsies and can process small tissue fragments by various cell block techniques in order to provide both cytologic and architectural contrasts for a complete diagnosis. Moreover, these small tissue fragments can provide adequate material for performing adjunctive studies such as immunohistochemistry, or special stains to arrive at the best diagnosis. The small biopsy samples from the lung have few complications. Some routine complications encountered during these procedures are hemorrhage, pneumothorax, etc.

Whereas the small tissue biopsies provide an important tool and adjunct to cytology diagnosis, there are certain limitations which are associated with the limited nature of the material obtained, non-sampling of the lesion, and very small samples from the edge of the lesion which may not be sufficient for diagnosis. Other limitations include crush artifacts.

In this chapter some of the salient histologic features of commonly encountered lesions on small biopsy material will be described.

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Lung

In the current practice, no longer is it sufficient to identify just malignancy. Identification of various subtypes of lung carcinoma have added significance because of the implication in prognosis and the prediction of behavior of certain subtypes as well as the treatment differences.

Previously in cytopathology practice, classification was simplified and categorized to small cell carcinomas vs. non-small cell carcinoma; thus identifying the surgical nature of the latter. In current practice it is important to follow the WHO classification of lung tumors, importantly and especially further categorization of non-small cell carcinoma, adenocarcinoma, and squamous cell carcinoma is necessary.

Squamous cell carcinoma

This generally presents as large central masses with hilar and peri-hilar lymphadenopathy. These tumors can present as cystic, oftentimes necrotic masses and can be approached by various techniques, transbronchial and transthoracic. Even in small biopsy and small tissue fragments in cell blocks the features of squamous cell carcinomas are distinctive. The diagnosis of squamous cell carcinoma is based on the presence of keratin and intercellular bridges (Fig. 1.1). Keratin pearls may be seen, and mucin vacuoles are often noted. Commonly the tumors are necrotic and may be accompanied by foreign body giant cell granulomatous reaction initiated by keratin debris. This finding is more commonly observed in metastasis where occasionally the lymph node parenchyma is replaced by keratin-induced granulomas. Therefore on small biopsy samples, occasionally a definitive diagnosis of squamous cell carcinoma may be difficult even though it is strongly suspected. In poorly differentiated tumors separation from small cell undifferentiated carcinomas may present problems and appropriate immunohistochemical stains should be employed when necessary. Squamous cell carcinoma can have clear cell, spindled, or basaloid features. Whenever possible the tumor should be graded into well, moderately, or poorly differentiated tumors based on the amount of keratinization. Reactive squamous atypia in the setting of cavitary lesions may present problems in differential diagnosis and especially on small biopsies.

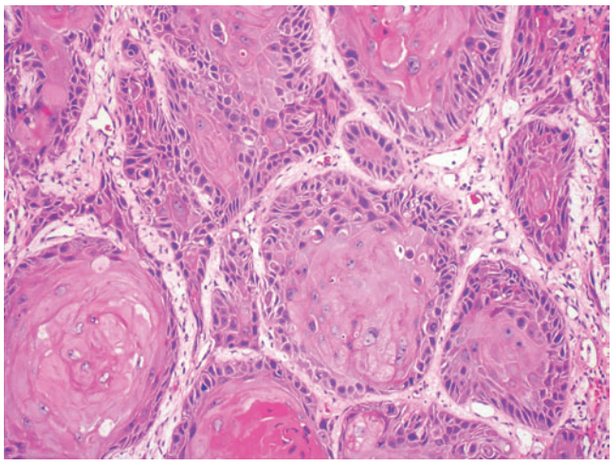

Figure 1.1 Invasive squamous cell carcinoma. The carcinoma shows prominent intercellular bridges and keratin pearl formations.

Adenocarcinomas

This most commonly occurs in the peripheral location but occasionally can present as a central mass. Adenocarcinoma may exhibit various architectural patterns and therefore a mucin stain is extremely helpful to identify intracytoplasmic mucinous vacuoles (Fig. 1.2). For poorly differentiated tumors, immunohistochemical stains may be necessary to differentiate metastatic carcinomas from other organs. Primary lung adenocarcinomas are generally TTF1, Napsin-A, and carcino-embryonic antigen (CEA) positive.

Bronchioloalveolar carcinoma (BAC)

BAC is regarded as a well-differentiated adenocarcinoma arising from Clara cells of alveoli and predominantly exhibits an alveolar (lepidic) pattern of growth (Fig. 1.3). It is important to note that this pattern of growth may be seen in other tumors as well and therefore it is not possible to make a firm diagnosis of BAC on small biopsy samples. These cases are best referred to as adenocarcinoma with BAC features recommending further confirmation.

Large cell undifferentiated carcinoma

This by definition does not exhibit squamous or glandular differentiation. Therefore it may not be feasible to diagnose large cell undifferentiated carcinoma in small biopsy samples since differentiation may be present focally. In this circumstance it is best to refer to these tumors as non-small cell carcinoma, not otherwise specified. These tumors are composed of large nuclei with prominent nucleoli and no evidence of cytoplasmic mucin or intercellular bridges and keratin (Fig. 1.4).

Approximately 10% of the lung tumors have spindled growth pattern and are referred to as sarcomatoid carcinomas by WHO classification (Fig. 1.5). Other tumors occasionally encountered in lungs are adenoid cystic carcinoma and muco-epidermoid carcinoma.

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