President Round 2

Diagnostic Difficulties in Pulmonary Cytology and Proper Use of Ancillary Test

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There are no disclosures necessary.
Diagnostic Difficulties in Pulmonary Cytology and Proper Use of Ancillary Tests

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Epithelial cells of the respiratory tract undergo morphologic changes in response to injuries caused by various agents. Degeneration, necrosis, regeneration and hyperplasia resulting from such insults produce cytologic abnormalities that may mimic cancer. The most common causes of cell injury are infection, chemical agents (i.e., inhalants particularly cigarette smoking, chemotherapeutic drugs) and physical agents (i.e., trauma, irradiation, thermal injury). Knowledge of cytomorphologic changes caused by benign conditions, and good sampling and laboratory preparatory techniques are essential in preventing the disastrous consequences of a false positive cytologic diagnosis. It is also extremely important to correlate the cytologic features with clinical and radiologic findings. For example, the presence of atypical epithelial cells in a bronchoalveolar lavage (BAL) sample of a lymphoma patient on active chemotherapy and with diffuse pulmonary infiltrate is a lot less likely to be from a carcinoma than atypical cells in a bronchial brushing of a patient with a lung nodule and endobronchial lesion.

The most common cells seen in pulmonary cytologic specimens are squamous cells (in the sputum), bronchial epithelial cells (in bronchial washing and brushing specimens) and pulmonary macrophages (in the sputum and BAL). Alveolar lining cells (pneumocytes) are generally absent in cytologic specimens of normal individuals.

**Cellular Response to Injury**

**A. Squamous cells**

The sputum of patients with upper respiratory infections may contain small squamous cells with eosinophilic cytoplasm and dark pyknotic nuclei which may mimic squamous cell carcinoma. These cells which, were observed by Papanicolaou in his own sputum, have been called *Pap cells*. They represent degenerative changes in the epithelial cells of upper airways.

**B. Tracheobronchial cells**

The response of respiratory epithelial cells to injury can be divided into three phases. First is the acute phase immediately following the insult, which is mainly morphologic manifestation of cell degeneration. Loss of cilia is one of the earliest and most common features, and can be seen in such mundane injuries as exposure to anesthetic gases. Other morphologic features of degeneration are vacuolization and eosinophilia of the cytoplasm, pyknosis, karyorrhexis and karyolysis. Vacuolated cells with dark, pyknotic nuclei may mimic adenocarcinoma, and hypereosinophilic cells with pyknotic nuclei in a background of necrotic debris may simulate squamous cell carcinoma. *Ciliocytophthoria*, a term coined by Papanicolaou refers to breakage of the distal (ciliated) end of bronchial cells as a result of injury. The remaining proximal portion of the cell has a high nuclear-cytoplasmic (N/C) ratio and pyknotic nucleus, which may be mistaken for cancer cell. This condition is usually secondary to viral infections, particularly adenovirus.

The second phase of cellular response to injury is reactive and reparative changes. These include nuclear enlargement, prominence of nucleoli and multinucleation. Reactive glandular cells
generally retain their cohesiveness and appear in cytologic preparations as three-dimensional clusters of cells with enlarged nuclei (hence, increased N/C ratio) and prominent nuclei. Features helpful in distinguishing these changes from adenocarcinoma will be discussed below. Multinucleation is a nonspecific response of the bronchial cells to injury. It may be seen subsequent to mechanical trauma (bronchoscopy), irradiation or inhalation of toxic gasses. When cilia are preserved, the cells can be easily recognized. In such cases the columnar appearance of the cell is maintained, but the cell is significantly wider than the adjacent columnar cells. The nuclei are uniform and resemble those of normal cells in the same specimen. These cells are most likely the product of the fusion of adjacent cells. Sometimes the irritated cells form a syncytium, in the shape of a large, round cell with multiple nuclei and without cilia. Herpes virus infection may cause a necrotizing tracheobronchitis with formation of multinucleated, large epithelial cells. The nuclei mold against each other and may contain eosinophilic inclusions. However, more frequently than multinucleated cells, one observes single round cells with enlarged nuclei (high N/C ratio), which exhibit a ground glass appearance and margination of the chromatin at the nuclear membrane. The presence of either single or multinucleated cells affected by herpes virus, combined with necrotic debris in the background, particularly in a poorly preserved or poorly prepared specimen, may be mistaken for cancer. These features may be even more confusing in a patient with history of cancer who has been treated with radiation or chemotherapy.

An injury that causes destruction of the respiratory epithelium (e.g., necrotizing infection, mechanical trauma, radiation necrosis, etc.) is followed by a reparative process. Regenerating epithelium appears in cytologic samples as monolayer sheets of medium-sized cells that are generally uniform, and contain regular, centrally placed nucleoli with prominent nucleoli. The chromatin is finely granular and evenly dispersed in the nucleus. Nuclear membrane irregularity is absent or minimal.

The third phase of cell response to injury is seen weeks, months or years after the initial insult. These changes include reserve cell hyperplasia, squamous metaplasia, goblet cell hyperplasia and papillary hyperplasia. Normally, there is a single layer of reserve cells (or basal cells) underneath the ciliated bronchial cells adjacent to the basement membrane. They have a scanty cytoplasm and small nuclei. Basal cells are generally not noticeable in bronchial washing and brushing specimens from normal individuals. A common and nonspecific response to chronic irritation of the bronchus is reserve cell hyperplasia, which is characterized by the presence of several layers of small cells under the superficial layer of columnar cells. This condition is commonly associated with bronchitis. In my experience reserve cell hyperplasia is present virtually in all cigarette smokers. In cytologic samples hyperplastic reserve cells appear as tightly cohesive clusters of small cells with scant cytoplasm and high N/C ratio. Some of the clusters are attached to a layer of ciliated columnar cells. Mitoses and apoptotic cells are generally absent. In well-fixed specimens these cells are easily recognized, but they may be mistaken for small cell carcinoma in poorly prepared samples (see below). Reserve cell hyperplasia is considered a precursor of squamous metaplasia. In persistent chronic irritation, reserve cells gradually transform into squamous cells. There is a spectrum of morphologic changes between the typical reserve cell hyperplasia and mature squamous metaplasia. As the reserve cells gradually gain larger amounts of cytoplasm and differentiate toward squamous cells they pass through the stage of immature metaplasia which may be mistaken for a neoplastic process.
Squamous metaplasia is another common condition of the bronchial mucosa resulting from chronic irritation. The most common cause is tobacco smoking and more than half of smokers contain foci of squamous metaplasia in their bronchi. Other conditions that may cause squamous metaplasia of the bronchial tree include organizing pneumonia, bronchiectasis, tuberculosis, fungal infection and irradiation.

In chronic persistent injury such as cigarette smoking the reserve cells first become hyperplastic. Then the amount of the cytoplasm in reserve cells gradually increases, and the cells progressively resemble squamous cells. Eventually, the ciliated columnar fall off and the bronchial surface is covered by a stratified squamous epithelium. The newly formed squamous cells initially possess small amounts of cytoplasm resulting in high N/C ratio, but gradually the cytoplasm becomes more abundant and the N/C ratio decreases. In cytologic specimens, squamous metaplasia is more readily identified in bronchial washing and brushing samples than in sputum or FNA material. Metaplastic cells are smaller than squamous cells of normal oral mucosa and usually appear in sheets of cohesive, uniform, round to polyhedral cells. Single cells are also seen, but are more difficult to recognize than cell groups. Nuclei are round, centrally placed and have a finely granular chromatin. The nuclear membrane is smooth and regular. Small nucleoli may be evident, particularly in early metaplasia. In some conditions that cause squamous metaplasia, particularly radiation therapy, the metaplastic cells may exhibit marked pleomorphism, abnormalities of chromatin distribution and prominence of nucleoli, which may be easily mistaken for squamous cell carcinoma. Chronic irritation caused by the tracheostomy tube can cause severely atypical squamous metaplasia with keratinization, mimicking carcinoma.

In normal bronchial epithelium mucus producing goblet cells are scattered among the ciliated columnar cells. Chronic bronchitis causes an increase in number of goblet cells and hyperplasia of mucous glands. This condition is very common in cigarette smokers. Goblet cell hyperplasia is recognized in bronchial brushing and washing samples by an increase in the ratio of goblet cells to ciliated cells. Frequently, the cytoplasm of the goblet cells is disrupted during the smearing process and mucus is released, resulting in the appearance of naked nuclei floating in a pool of basophilic mucus. Another feature is the formation of goblet cell clusters which may mimic adenocarcinoma.

In chronic bronchitis, asthma and some other chronic conditions the respiratory epithelium of the bronchus may become hyperplastic and form papillary infolding into the lumen. These papillary structures have a fibrovascular core and are lined by a mixture of ciliated columnar cells, basal cells and goblet cells. Papillary hyperplasia is easily recognized in biopsy specimens, but the cytologic diagnosis can be difficult. The cells exfoliate in three-dimensional clusters that may mimic adenocarcinoma in sputum samples. Naylor and Railey who first reported this entity in asthmatic patients called these clusters Creola bodies, named after one of their patients. Features helpful in distinguishing these structures from adenocarcinoma will be discussed below.

C. Alveolar cells (Pneumocytes)

Generally, the alveolar lining cells (pneumocytes type I and type II) are not recognized in cytologic specimens from normal individuals, but reactive and hyperplastic forms may be found. Pneumocytes are damaged by a variety of insults, the most common of which are infections (i.e., bacterial pneumonia, granulomatous inflammations caused by mycobacteria or fungi, viral
pneumonia), diffuse alveolar damage (caused by various factors including shock, oxygen therapy, drugs, aspiration, radiation therapy, chemotherapy), autoimmune disorders, ischemia (infarct), inhaled particles (pneumoconiosis) and idiopathic conditions (various types of interstitial pneumonia). In acute injury the pneumocytes undergo degeneration and necrosis. This stage is usually followed by a reparative phase during which the type II pneumocytes proliferate and line the alveolar spaces. These metabolically active cells have enlarged nucleoli and prominent nucleoli. Although the nuclei of these reactive cells are usually bland and uniform, in some cases, particularly in radiation pneumonia significant variation in size, hyperchromasia and abnormality of chromatin distribution may be seen. Chemotherapy (particularly with busulfan) and radiation induced changes are characterized by pulmonary fibrosis and the presence of large pneumocytes which posses large nuclei with dark, smudged chromatin and sometimes prominent nucleoli. In the sputum and BAL specimens single, large atypical cells are seen. Although the cells have very large nuclei, the N/C ratio is not increased due to the abundance of the cytoplasm. In some chronic conditions proliferating pneumocytes form papillary or pseudo-papillary nests of cells that appear as three-dimensional clusters of cells in the sputum, BAL or FNA samples mimicking bronchioloalveolar carcinoma (see below for discussion of differential diagnosis).

D. **Pulmonary macrophages**

Pulmonary macrophages are best seen in deep cough sputum and BAL samples. Acute injury causes degenerative changes in these cells, characterized by dark, pyknotic nuclei. In reaction to injury, macrophages may exhibit multinucleation and/or prominence of nucleoli. Multinucleation is a nonspecific response and should not be considered an indication of granulomatous inflammation. Vacuolization of pulmonary macrophages occurs as a response to specific conditions. In *lipid pneumonia* enlarged macrophages are seen that contain multiple vacuoles. If necessary, fat stains can be employed on air-dried smears to demonstrate the presence of lipid. The affected cells contain single or multiple nuclei which are of normal size or may show reactive changes. Another condition that is associated with vacuolization of the pulmonary macrophages is *amiodarone* toxicity. This antiarrhythmic drug may cause pulmonary infiltrates with fibrosis and accumulation of numerous large, foamy macrophages. The cells have one or two nuclei, which may show slight atypia and prominent nucleoli. Hemosiderin-laden pulmonary macrophages seen in congestive heart failure and pulmonary hemorrhage (e.g., Goodpasture syndrome, idiopathic pulmonary hemosiderosis) are easily recognized in cytologic specimens, and the presence of iron can be demonstrated by histochemical stains in unusual or confusing circumstances.

**Differential Diagnosis in Pulmonary Cytology**

In the following sections the most common benign conditions that may be confused with malignant neoplasms in cytologic preparations are discussed. These entities are divided into four categories: mimics of squamous cell carcinoma, adenocarcinoma, small cell carcinoma and malignant lymphoma. In general, two of the most important factors in avoiding erroneous diagnoses are knowledge of clinical and radiologic findings and specimens of good quality. In the absence of obvious and undisputed malignant features, the pathologist should discuss the findings with the clinician, and if necessary request better samples. Proper fixation, preparation and staining are essential in accurate interpretation of specimens.
I. Mimics of Squamous cell carcinoma

- Squamous metaplasia and dysplasia of bronchial epithelium
  - Secondary to inhalants (cigarette smoking)
  - Secondary to infections (TB, fungal infections)
  - Associated with bronchiectasis

- Radiation induced changes

- Cells originating from tracheostomy site

- Atypical squamous cells originating in oral cavity lesions (i.e., “leukoplakia”)

- Pemphigus vulgaris

- Vegetable cells

As previously discussed, squamous metaplasia of the bronchial epithelium is a common occurrence and is readily recognized in cytologic preparations. However, in chronic infections and bronchiectasis the metaplastic cells may show significant degree of atypia, which may be mistaken for squamous cell carcinoma. Generally, metaplastic squamous cells are more cohesive and less prone to necrosis than cancer cells. Keratinization is uncommon in metaplastic epithelium, except when it is caused by a tracheostomy tube. Therefore, the presence of a large number of single cells with marked pleomorphism and keratinization in a necrotic background is highly in favor of carcinoma. Cells originating in tracheostomy sites are sometimes very difficult to distinguish from squamous cell carcinoma. With a knowledge of the clinical history and source of the specimen (tracheal aspirate) caution should be exercised and a higher threshold be used for a definitive diagnosis of malignancy. In cases of atypical squamous metaplasia usually more typical metaplastic cells are also present and frequently a spectrum of changes can be seen between the benign appearing cells and the most atypical forms. This is particularly true in the bronchial brushing which is often the most cellular sample of bronchial epithelium. In the sputum, bronchial washings and FNA specimens the number of atypical cells may be smaller and the diagnosis could be more difficult.

Another difficult diagnosis in pulmonary cytology is squamous dysplasia of bronchial epithelium. Metaplastic squamous epithelium usually goes through progressive degrees of dysplasia to in situ carcinoma prior to the development of invasive squamous cell carcinoma. Dysplastic cells morphologically resemble their counterpart in uterine cervix. The cells from severe dysplasia or carcinoma in situ which are characterized by large, hyperchromatic nuclei and coarse chromatin may be difficult to distinguish from invasive squamous cell carcinoma, particularly in limited samples. A helpful feature is the absence of necrosis in dysplasia and its presence in invasive carcinoma. While dysplasia is frequently present in the bronchi adjacent to an invasive squamous carcinoma, it may be seen in the absence of invasive carcinoma, particularly in tobacco
Smokers. Therefore, it should be kept in mind that it is possible to have a lung mass caused by a benign condition such as granuloma or organizing pneumonia associated with atypical squamous metaplasia or dysplasia of the bronchus. A bronchoscopically obtained sample may be misinterpreted as squamous cell carcinoma leading to an unnecessary surgical resection. When a bronchial sample contains few atypical cells or when unequivocal cytologic criteria of malignancy are absent an FNA sample should be requested to determine the nature of the mass.

Squamous metaplasia caused by irradiation may exhibit marked atypia, which may be mistaken for carcinoma. Marked variation in cell size and bizarre nuclei, as well as abnormalities of chromatin distribution and prominent nucleoli may be seen. Helpful features to recognize radiation-induced atypia include a proportionate increase in size of the cell and nucleus, hence low N/C ratio, smudginess of chromatin and presence cytoplasmic and nuclear vacuoles. If a mass is present, FNA can be helpful in the differential diagnosis. It is highly unlikely to obtain a large number of atypical metaplastic cells by FNA, since unlike bronchial brushing, the needle can only sample small areas of bronchial tree. Therefore, the presence of a large number of atypical squamous cells in an FNA favors carcinoma.

In the sputum atypical, sometimes keratinized squamous cells may be seen which are originated in the oral mucosa. These cells which arise from leukoplakia or the epithelium irritated by the denture are generally few in number and lack full criteria of malignancy. Proper collection technique (adequate mouth wash) decreases the likelihood of such contamination. When necessary, bronchoscopic specimens should be obtained to investigate the possible origin of the cells in lower respiratory tract. Another potential source of confusion in cytology is food contamination. Vegetable cells may have large, dark nuclei and mimic malignant cells, particularly in poorly preserved or poorly stained smears. These cells, however, can be recognized by the presence of very thick cell membrane, refractile cytoplasmic granules and a lack of chromatin detail.

Pemphigus vulgaris may rarely affect the oropharyngeal mucosa, shedding atypical cells in cytologic specimens. This condition is characterized by the loss of cohesiveness of squamous cells. This results in the appearance of single or loosely cohesive sheets of cells, which have enlarged nuclei and prominent nucleoli. The cells, however, are uniform and the nuclei have bland chromatin and smooth nuclear membrane. Correlation of cytologic features with clinical history and radiologic and bronchoscopic findings is essential in correct interpretation.
II. Mimics of Adenocarcinoma

- Reactive bronchial cells
- Repair/Regeneration of bronchial or alveolar epithelium
- Hyperplasia of bronchial or alveolar epithelium
- Atypical epithelial cells secondary to chemotherapy
- Acute radiation changes
- Viral pneumonia
- Amiodarone toxicity
- Vegetable cells

Reactive and regenerating epithelial cells are sometimes very difficult to distinguish from well-differentiated adenocarcinoma. An extremely helpful feature in differential diagnosis is the presence of a range of abnormalities in reactive conditions. With careful examination a spectrum of changes can be found ranging from obviously benign ciliated bronchial cells with slight nuclear enlargement to the most atypical cells which may have variation in size and prominent nucleoli. The chromatin in reactive cells is finely granular and evenly dispersed. The nuclear membrane is generally smooth and regular. In contrast, in adenocarcinoma a transition between atypical cells and normal cells cannot be established. Cell clusters are composed purely of atypical cells that are distinctly different from normal ciliated or goblet cells in the background. Good fixation and staining methods are extremely important for the study of nuclear details. Even the most well differentiated adenocarcinomas exhibit irregularity of the nuclear membrane which can be seen best under high magnification. In the case of a lung mass with equivocal cytologic findings in bronchoscopic specimens, an FNA of the lesion can be very helpful for establishing a correct diagnosis. As discussed previously, it is unlikely to obtain a large number of cells from bronchial epithelium by FNA and therefore, an abundance of atypical cells in FNA is in favor of a neoplasm.

Creola bodies, which are the cytologic manifestation of papillary hyperplasia of the bronchial epithelium, can be mistaken for adenocarcinoma. Under careful, high magnification examination cilia or brush borders can be observed in at least some of the cells. In addition to the columnar cells, scattered goblet cells and layers of basal cells may be seen in the same clusters. This mixture of different population of cells is characteristic of benign proliferation. The clinical history of asthma or other chronic pulmonary diseases, combined with the absence of radiologic evidence of a mass enables the pathologist to arrive at a correct diagnosis. On the other hand, if a mass exists on chest x-ray further investigation of the lesion by bronchoscopic samples or FNA is warranted.

Pulmonary infiltrates in cancer patients who are treated with chemotherapy raise the possibility of recurrence/metastatic cancer versus drug toxicity or an opportunistic infection.
Cytologic evaluation of bronchoscopic specimens plays an important role in determining the etiology. In reaction to cytotoxic drugs, highly atypical pneumocytes may line the alveolar spaces. These cells are best seen in BAL specimens and usually appear as single cells with abundant cytoplasm and enlarged, hyperchromatic nuclei. Features helpful in distinguishing chemotherapy effect from malignancy are the scarcity of atypical cells, proportionate increase in cell and nuclear size resulting in low N/C ratio, and smudginess of the chromatin. Additionally, squamous metaplasia of the bronchial mucosa may be present. Correlation with clinical and radiologic findings and comparison with the patient’s original tumor are helpful in avoiding a false positive diagnosis.

In acute irradiation bronchial and alveolar cells undergo necrosis, followed by reparative changes and squamous metaplasia. The presence of variable-sized cells with bizarre nuclei, cytoplasmic vacuoles and necrotic background may give rise to an erroneous diagnosis of malignancy in cytologic specimens. With careful examination, it is noted that despite marked nuclear enlargement most cells have maintained a low N/C ratio because of an increased amount of cytoplasm. While the presence of cytoplasmic vacuoles may mimic adenocarcinoma, the vacuoles have a “punched out” appearance without the bluish hue produced by mucus and without causing an indentation on the nucleus that is commonly seen in secretory vacuoles. Additionally, similar vacuoles are seen in the nucleus. The chromatin may appear smudged in degenerating cells, while large nucleoli are seen in regenerating cells. Another helpful feature is identification of atypical large cells that have cilia or brush borders and are recognized as reactive bronchial cells.

In viral pneumonia the presence of multinucleated cells, intranuclear inclusions resembling prominent nucleoli and cytoplasmic vacuoles may be mistaken for adenocarcinoma. Clinical and radiologic correlation is extremely important, as is good preparatory technique to better visualize the inclusions and margination of the chromatin.

Large, vacuolated macrophages with atypical nuclei seen in amiodarone toxicity may be confused with adenocarcinoma. However, with careful search dust particles are usually found in at least some of the cells. Vacuoles are small and foamy-appearing rather than large vacuoles that are seen in adenocarcinomas. The absence of mucin can be demonstrated by special stains.

Three-dimensional clusters of vegetable cells with dark nuclei and sometimes with vacuoles may resemble adenocarcinoma, particularly in poorly stained cytologic preparations. However, thick cell membrane and lack of nuclear detail provide clues for correct identification of the cells.

### III. Mimics of Small Cell Carcinoma

- Reserve cell hyperplasia
- Degenerated bare nuclei
- Lymphocytes
  - Follicular bronchitis (in bronchial washing/brushing specimens)
  - Benign or malignant lymphocytes from lymph node (in needle aspirates)
It is of utmost importance to avoid a false positive diagnosis of small cell carcinoma, since this tumor is often not treated surgically and radiation and chemotherapy are instituted on the basis of a definitive cytologic diagnosis. In fact this tumor is often easier to diagnose in cytologic preparations since in biopsies tumor cells are frequently crushed. Because of the scant cytoplasm and dark nuclei, reserve cell hyperplasia may mimic small cell carcinoma. The distinction can particularly be difficult in limited or poorly fixed samples. In well-prepared specimens, however, these two entities are easily distinguished. In reserve cell hyperplasia the cells always appear in tightly cohesive clusters. The nuclei are small and compact. Necrosis or mitoses are not present. In contrast, small cell carcinoma cells are seen in large clusters, as well as small groups and single cells. They have larger nuclei than reserve cells, which show some variation in size and frequent mitoses. Necrotic debris is commonly present and individual cell apoptosis is often seen in cell clusters.

Extreme caution should be exercised in interpretation of scanty cellular samples. In such smears distorted benign bronchial or basal cells may form small clusters of naked nuclei, mimicking small cell carcinoma. I have also seen rare cases of carcinoid tumor misinterpreted as small cell carcinoma in scant and poorly fixed samples. If the cells are few or poorly preserved, the pathologist should not hesitate to request a better sample by bronchoscopy or FNA.

While it is often difficult to distinguish between lymphocytes and small cell carcinoma in small crushed biopsies, this problem is uncommon in cytologic specimens. Bronchial washings and brushings of patients with chronic bronchitis usually contain no or few lymphocytes which do not present a diagnostic problem. However, in cases of follicular bronchitis, which is characterized by massive infiltration of bronchial mucosa by lymphocytes, cytologic sample may contain numerous lymphocytes. Another source of lymphocytes in pulmonary cytologic specimens is mediastinal lymph nodes that can be sampled by transbronchial or percutaneous needle aspirations. In well-fixed smears lymphocytes are readily distinguished from small cell carcinoma, since lymphocyte are always single and round, whereas carcinoma cells form cohesive groups with molding and irregular shapes. Cell blocks sometimes are more difficult to interpret than smears as they may show artifacts similar to biopsies. However, if the smears are not diagnostic and differential diagnosis in the cell block is between small cell carcinoma and lymphocytes, a simple panel of immunocytochemical stains (LCA and Cytokeratin) can solve the problem.

### IV. Mimics of Malignant Lymphoma

- Follicular bronchitis (in bronchial washing/brushing specimens)
- Benign lymphocytes from lymph node (in needle aspirates)

The diagnosis of malignant lymphoma by sputum, bronchial washing and brushing or BAL is very difficult. Low grade (small cell) lymphomas cannot be distinguished from benign lymphocytic pulmonary infiltrates (i.e., follicular bronchitis, lymphocytic interstitial pneumonia, pseudolymphoma) without the help of cell marker studies and bronchoscopic samples seldom yield adequate cells for such studies. Large cell lymphomas on the other hand can be diagnosed by
morphologic criteria, but may be difficult to distinguish from poorly differentiated carcinoma. Immunocytochemical stains are useful in this differential diagnosis. Fine needle aspiration is often employed to diagnose mediastinal masses. Adequate samples can be obtained by FNA to allow a definitive diagnosis of lymphoma by a combination of morphologic criteria and cell marker studies by flow cytometry.

While for many years classification of lung cancer into small cell and non-small cell carcinoma was sufficient for instituting appropriate treatment, the advent of targeted therapies for lung cancer in recent years has led to a need for accurate sub-classification of non-small cell lung carcinomas into adenocarcinoma (AC) and squamous cell carcinoma (SqC). While the distinction between well-differentiated AC and SqC is readily made on the basis of morphologic criteria in cytologic samples and small biopsies, this task is more challenging, and at times impossible, in poorly differentiated carcinomas due to the overlap of cytomorphic features. However, there are distinct differences in the immunocytochemical profile of AC and SqC, which has led to a significant increase in the use, and in some laboratories the routine application of immunocytochemical stains (ICC) in pulmonary cytology. A number of antibodies have been reported helpful in this context, including CK5/6, CK7, 34βE12, napsin A, p63 and TTF-1. In our experience, a small panel of p63 and TTF-1 is adequate in most instances (Kimbrell, et al: Acta Cytol 2012, 56:419-424). Positive staining (moderate to diffuse) for p63 with negative TTF-1 is indicative of SqC. Negative staining for p63 with positive TTF-1 indicates adenocarcinoma. Focal staining for p63 can be seen in AC and should not be considered a positive result. CK7, while positive in practically all adenocarcinomas, is also positive in most squamous carcinomas and therefore is not helpful in this differential diagnosis. CK5/6 usually parallels p63. 34βE12 is positive in SqC and many of adenocarcinomas, and therefore, we do not use this stain in this context. Because of the heterogenous staining in some tumors and inconsistent immunostaining of large cell carcinomas, in a small percentage of cases a definitive subclassification of non-small cell carcinoma cannot be made despite the immunocytochemical stains.

Another area of difficulty is the differential diagnosis of basaloid carcinoma versus small cell carcinoma (SCC). In most cases of basaloid carcinoma, foci of squamous differentiation are found upon careful examination of the slides. However, occasionally an overlap of morphologic features makes this distinction very difficult in cytologic samples and small biopsies. In this scenario a panel of ICC consisting of neuroendocrine markers (particularly synaptophysin and CD56, as chromogranin is frequently negative in SCC), TTF-1 and p63 solves the problem. Basaloid carcinoma is positive for p63 and negative for TTF-1 and neuroendocrine markers, whereas SCC is positive for neuroendocrine markers and TTF-1 and negative for p63.

Selected References:


