

Diagnostic challenges in lung cancer cytology

Neda Kalhor, MD

Assistant Professor, Departments of Pathology and Laboratory Medicine,
The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA

Lung cancer is the number one cause of cancer related deaths in men and women in the United States and the world. Cytology is a major diagnostic modality in initial evaluation and follow up of patients with lung cancer. Moreover, cytologic material provides tissue for molecular profiling of tumor, which is becoming a crucial step in treatment of non-small cell carcinoma. Various sampling techniques available for lung specimen procurement include exfoliative (sputum, thoracentesis), abrasive (bronchial wash, brush and lavage) and fine needle aspiration (transbronchial, transthoracic CT scan-guided and Endobronchial Ultrasound-guided and transesophageal ultrasound guided). It is critical to be familiar with the potential mimics and pitfalls to avoid in evaluation of lung cytologic material since cytology very often serve as the only available diagnostic tools and false diagnosis may have significant impact in the patient management and morbidity and mortality.

FNA has a sensitivity of 85% and specificity of 90%, comparable to the yield of core needle biopsy, in diagnosis of lung lesions. The accuracy is significantly higher when the two modalities (FNA and core needle biopsies) are both applied. ¹ False negative results are often attributed to sampling errors.

Some of the most common diagnostic dilemmas in pulmonary cytology include squamous reactive atypia vs. squamous cell carcinoma, reactive type 2 pneumocyte hyperplasia and reactive bronchial epithelial cells vs. well differentiated adenocarcinoma, particularly well differentiated bronchioloalveolar type, small cell carcinoma vs. non-small cell carcinoma, grading of neuroendocrine carcinoma and subclassification of poorly differentiated non-small cell carcinoma.

Reactive Squamous Atypia vs. Squamous Cell Carcinoma

Squamous epithelial cells can be seen in all types of lung cytologic specimens including sputum, bronchial washings, brushings, lavage and FNAs. They may represent contamination by the upper respiratory tract, squamous metaplasia due to infectious/inflammatory process or cellular injury due to smoking. They may also represent respiratory epithelium that has undergone dysplastic process. The latter can be particularly difficult to distinguish from invasive squamous cell carcinoma in exfoliative specimens such as bronchial washings, brushings and lavage.

Presence of atypical squamous epithelium can be encountered in non-neoplastic conditions such as infection (TB and Aspergillus presenting as cavitary lesions), prolonged tracheostomy and non-infectious pneumonitis, diffuse alveolar damage and infarcts. The smears will show clusters and single squamous epithelium with high N:C

ratio, smudged chromatin, mild to moderate nuclear irregularity and orangeophilic cytoplasm in a background of inflammation and necrotic cellular debris that can be mistaken for squamous cell carcinoma.

There is extensive overlap between the cytologic features seen in atypical squamous cells in non-neoplastic conditions and squamous cell carcinoma. Cytologic features seen in well differentiated squamous cell carcinoma include single cells and cohesive sheets of malignant cells with high N:C ratio, containing nuclei with irregular nuclear contour, smudged chromatin and dense cytoplasm. Marked necrosis and granulomatous inflammation may be observed. Abundant single cells with tad-pole or spindled and angulated shapes may be seen.

Cytologic features that are useful in distinguishing reactive squamous atypia from squamous cell carcinoma include high cellularity, abundance of cells with marked nuclear irregularity (tad pole, spindled and pointed process) and unequivocal malignant features such as increased nuclear size, marked nuclear pleomorphism and abundant abnormal mitotic figures. Therefore, distinction of a malignant process from non-neoplastic process in specimens that are suboptimal for evaluation due to paucity of tissue can be extremely difficult and an unequivocal diagnosis should be rendered only in aspirates with adequate tissue. Clinical and radiologic correlation to ensure accurate diagnosis in such settings is of utmost importance.

Poorly differentiated squamous cell carcinoma may be indistinguishable from poorly differentiated adenocarcinoma. High cellularity, three dimensional cell clusters and discohesive tumor cells with high N:C ratio, nuclear pleomorphism and variable chromatin and prominent nucleoli can be seen in both types of tumor. Without histochemical studies or immunohistochemistry, it may be impossible to subclassify these poorly differentiated carcinomas.

Reactive type 2 pneumocyte or bronchial cells vs. BAC type well differentiated adenocarcinoma

Distinguishing BAC type adenocarcinoma from type 2 pneumocyte hyperplasia and reactive bronchial cells may pose difficulty in cytology specimens. Some of the inflammatory processes that can lead to pneumocyte type 2 hyperplasia include infection, interstitial pneumonitis, pulmonary fibrosis, infarct and chemotherapy and radiation associated changes. Aspirate from such lesions may contain reactive type 2 pneumocytes singly or in groups, demonstrating increased nuclear size, nuclear pleomorphism, hyperchromasia, prominent nucleoli and vacuolated cytoplasm, that may be misinterpreted as adenocarcinoma. Such pitfall may also occur in surgical core biopsies, particularly when the biopsy is limited.

There are several cytologic features that are useful in distinguishing reactive type 2 pneumocyte hyperplasia from adenocarcinoma. First, cellularity is often limited in non-

neoplastic process; whereas, the cytologic specimens from adenocarcinoma show high cellularity. 2nd is the nuclear pleomorphism which is more common in reactive process. The pneumocytes in inflammatory process often show a spectrum of nuclear pleomorphism. In contrast, FNA of BAC type adenocarcinoma often shows a monotonous population of epithelial cells that are distinct from background benign epithelium (two distinct cellular populations). 3rd is the background which is often clean in well-differentiated adenocarcinoma, as opposed to abundant inflammation in reactive/inflammatory process. Irregular nuclear contour is more often encountered in adenocarcinoma.

Other cytologic features such as cellular size, chromatin details, nuclear inclusions and cytoplasmic vacuolization have extensive overlap between the neoplastic and non-neoplastic process and are more meaningful when used as constellation of criteria with above mentioned features. Given the overlapping cytologic features between reactive pneumocyte hyperplasia and well-differentiated adenocarcinoma, several factors should be kept in mind to avoid false positive diagnosis. First, adequacy of the specimen; extreme caution is warranted in establishing malignancy if the aspirate is limited and realizing that the specimen may not be sufficient for an unequivocal diagnosis. Clinicians are familiar or should be educated, if not already familiar, about the limitation of the tissue aspirate or biopsy. In such cases that an unequivocal diagnosis is not rendered, a repeat biopsy or close clinical and radiologic follow up may be warranted, depending on the clinical scenario. 2nd, close clinical and radiologic correlation is essential while evaluating the specimen.

Small Cell Carcinoma vs. NSCLC

Distinguishing small cell carcinoma from non-small cell carcinoma is critical due to therapeutic and prognostic implications. Cytologic features of small cell carcinoma are well established in the literature. Smears are often cellular and demonstrate abundant single cells and loosely cohesive groups of tumor cells. The tumor cells are often small, relatively monotonous with round to spindled shape and demonstrate nuclear molding. Occasional larger cells may be noted. The cells have high N:C ratio, scant to moderate cytoplasm and contain nuclei with homogenous and fine chromatin. Nucleoli are absent or inconspicuous. Apoptotic cells, single cell necrosis and mitotic figures are frequent. The cytologic materials are preferred to transbronchial biopsies for establishing the diagnosis of small cell carcinoma due to better cellular preservation and lack of crush artifact.

The most common pitfall in diagnosis of small cell carcinoma is with non-small cell carcinoma, particularly large cell neuroendocrine carcinoma. Large cell neuroendocrine carcinoma, by definition is a non-small cell carcinoma with neuroendocrine morphology and differentiation supported by ultrastructural analysis or immunohistochemistry. The tumor cells are often pleomorphic with abundant cytoplasm and contain nuclei with fine chromatin and prominent nucleoli. The tumor often has high mitotic activity (more than

10 mitoses per 10 high power fields). Diagnosis of large cell neuroendocrine carcinoma is often made only in surgical resections rather than cytologic material or surgical biopsies due to required pathologic criteria that may be difficult to assess in such limited specimens. The presence of occasional larger cells with more abundant cytoplasm and some tumor cells with nucleoli may lead to misclassification of small cell carcinoma as large cell neuroendocrine carcinoma. It is important to recognize that small cell carcinoma, despite the name designation can demonstrate some degree of cellular pleomorphism and size variation as was pointed in the first description by Bernard in 1926. Such findings, if focally present, may be seen in small cell carcinoma. However, if these findings are diffusely present in the aspirate, they may warrant a mixed/combined small cell carcinoma/non-small cell carcinoma, a combination that is reported to occur in more than 20% of small cell carcinoma tumors.

Basaloid squamous cell carcinoma is another potential pitfall in the differential diagnosis of SCLC. Aspirate of these tumors show abundant tumor cells with relatively monotonous appearance, often in cohesive cell groups and some singly with occasional nuclear molding. The tumor cells demonstrate high N:C ratio, medium to large nuclei with coarse chromatin and occasional prominent nucleoli. Necrotic tumor cells and mitotic figures are often frequent. Despite subtle cytologic differences with SCLC, the distinction may be very difficult. Immunohistochemical studies can be helpful in such settings. Basaloid squamous cell carcinoma stains with high molecular weight cytokeratin (CK5/6, CK903) and p63 and is often negative for TTF-1 and neuroendocrine markers (synaptophysin, chromogranin and CD56).

Carcinoid tumors (typical and atypical) vs. high grade neuroendocrine carcinoma

Neuroendocrine carcinomas of low or intermediate grade (typical carcinoid and atypical carcinoid, respectively) comprise 1-2% of all pulmonary malignant neoplasms. The correct diagnosis of carcinoid tumors and their distinction from high grade neuroendocrine carcinomas is crucial because of their therapeutic and prognostic implications. Carcinoid tumors are treated primarily by surgical resection; whereas chemotherapy and radiation is often the mainstay of treatment for high grade neuroendocrine carcinomas. Cytologic findings include cellular smears consisting of abundant single cells as well as loose aggregates, occasionally arranged along thin walled vessels. Tumor cells may be round, spindle, or plasmacytoid. Cytoplasm is small to moderate and often stripped from the nucleus. The nuclei are often small and contain finely granular (salt and pepper) chromatin with absent or inconspicuous nucleoli. Mitotic figures and necrosis are uncommon. The distinction from small cell carcinoma is often easy; however, several overlapping cytologic features with SCLC such as discohesive tumor cell, finely granular chromatin pattern, nuclear molding and crush artifact may pose difficulty in making the distinction. The error rate would be higher in aspirates with limited cellularity and poor cellular preservation. In this context, ki67 may be useful in separating the carcinoid tumors from high grade neuroendocrine carcinoma. Ki67 index

by immunohistochemical analysis is less than 25% in carcinoid tumors; whereas, the index is almost always higher than 50% in high grade neuroendocrine carcinoma.

Subclassification of non-small cell carcinoma

With recent advances in personalized oncology; there has been a paradigm shift in diagnosis of non-small cell carcinoma. A non-committal diagnosis of non-small cell carcinoma is no longer adequate and subclassification of non-small cell carcinoma has become imperative. Cytology is a valuable method for diagnosis and subclassification of the non-small cell carcinoma. Cytologic features of adenocarcinoma vs. squamous cell carcinoma are well-recognized in the literature (table 1)

Although in majority of cases, a line of differentiation can be identified by morphology, difficulty arises in a subset of cases which are poorly differentiated.

Immunohistochemistry is useful in characterizing the line of differentiation as adenocarcinoma versus squamous cell carcinoma in cases that cannot be classified on morphologic ground. Fine needle aspiration smears and cell blocks can be used for immunohistochemical analysis. A panel of TTF-1, Napsin-A, CK5/6 and p63 can often help with subtyping the tumor. TTF-1 is expressed in 70-80% of pulmonary adenocarcinoma. Although it is relatively specific for pulmonary neoplasms, it is expressed in thyroid tumors, high grade neuroendocrine carcinomas of thoracic and extrathoracic origin and rarely in tumors of Mullerian origin. Napsin-A, is also relatively specific for pulmonary adenocarcinoma. It may also be positive in nephrogenic tumors. CK5/6, a high molecular weight cytokeratin, is a highly sensitive marker for SCC (96%). The expression may be focally present in adenocarcinoma (4%). p63 is a highly sensitive marker for squamous cell carcinoma (100%); however, it can be expressed in up to 30% of the adenocarcinomas as well. Using an immunohistochemical panel in addition to the cytologic findings can help with accurately subclassifying the great majority of non-small cell carcinomas.

Table 1. Cytologic features of adenocarcinoma vs. squamous cell carcinoma

	Adenocarcinoma	Squamous cell carcinoma
Cell group arrangement	3-dimensional, papillary, acinar, cell balls Flat sheath in BAC type	Large sheaths and cohesive 3-dimensional in PD SCC
Individual Cells	Round, cuboidal, columnar	Polygonal
Cytoplasm	Vacuolated	Dense
Chromatin	Open	Coarse
Nucleoli	Present, often prominent	Micro-nucleoli, may be prominent in PD SCC
Cell border	Vague	Distinct
Mucin	Present	Absent
keratin	Absent	Present

References

1. Gong Y, Sneige N, Guo M, Hicks ME, Moran CA. Transthoracic fine-needle aspiration vs concurrent core needle biopsy in diagnosis of intrathoracic lesions: a retrospective comparison of diagnostic accuracy. *Am J Clin Pathol*. 2006 Mar;125(3):438-44.
2. Naryshkin S, Young NA. Respiratory cytology: a review of non-neoplastic mimics of malignancy. *Diagn Cytopathol*.1993;9:89 –97.
3. DeMay RM. Respiratory cytology. In: *The art and science of cytopathology. Volume 1*. Chicago: ASCP Press, 1996: 207–56.
4. DeMay RM. Lung. In: *The art and science of cytopathology. Volume 2*. Chicago: ASCP Press, 1996: 947–98.
5. Silverman JF. Inflammatory and neoplastic processes of the lung: differential diagnosis and pitfalls in FNAB biopsies. *Diagn Cytopathol*. 1995;13:448–62.
6. Johnston WW. Type II pneumocytes in cytologic specimens. A diagnostic dilemma. *Am J Clin Pathol* 1992;97:608 –9.
7. Zaman SS, van Hoesen KH, Slott S, Gupta P. Distinction between bronchioloalveolar carcinoma and hyperplastic pulmonary proliferations: a cytologic and morphometric analysis. *Diagn Cytopathol* 1997;16:396–401.