Exfoliative and aspiration cytology specimens have long provided the diagnosis of primary and metastatic neoplasms of the respiratory tract. The advent of immunocytochemistry has improved our diagnostic abilities. Transbronchial aspirates of lymph nodes outside of the lung have added staging capabilities. Molecular analyses of cytologic samples contribute therapeutic and predictive data.

Cytologic samples are divided into exfoliative and aspiration types. The former include sputum, washings, lavages, and brushings. Aspirations may be transthoracic (TTFNA) or transbronchial. The levels of diagnostic sensitivity for sputum range from 42-97\% with an average of 66\%. Sensitivity increases with daily numbers of sputum specimens. Sensitivity also depends on the location of the mass: central lesions have a higher sensitivity (71\%) compared with peripheral masses (49\%). Specificity typically approaches 99\%. The ability to distinguish small cell carcinoma (SCCA) from nonsmall cell carcinoma (NSCCA) is at least 95\%, as it is with all other cytologic sample types.

More direct exfoliative cytology was made feasible with the development of the flexible fiberoptic bronchoscope. Specimens include brushings, washings, lavages [and transbronchial aspirations (TBNA)]. The first two are utilized with visualized lesions of the proximal bronchi. Lavages are done for more peripheral abnormalities. The newest modality is TBNA which may be done under endoscopic ultrasound guidance (EBUSNA). For central masses, sensitivity for tissue biopsies, brushings, washings, lavages, and aspirates averages 74\%, 59\%, 48\%, 40\%, and 56\%, whereas for peripheral masses, sensitivity is 46\%, 52\%, 33\%, and 37\%; bronchoscopic aspires are generally not done through the bronchoscope for peripheral nodules. Procurement of multiple different specimen types during the same bronchoscopic session increases diagnostic sensitivity (88\% central lesions; 59\% peripheral lesions). A different approach to a patient with suspected lung cancer is TTFNA. This can be performed for centrally situated masses, but it is more often done for more peripheral lesions. TTFNA possess an average sensitivity and specificity of 89\% and 96\%. In centers which use on-site evaluation, these levels are higher. The newest method is EBUSNA. This has the major advantage of directly visualizing lesions, both within the bronchial wall and in the mediastinum, in real time. Diagnostic sensitivity ranges from 88-95\%; specificity usually exceeds 95\%. One potential pitfall of aspiration diagnoses of lymph nodes is whether the needle samples a node or a mass within or extending from a bronchus. The morphologist should report the presence or absence of lymphocytes to assure that the target was a node and thus accurate staging.

In contrast to histopathology, cytomorphologic criteria to distinguish among primary lung neoplasms are not as well established. With neuroendocrine tumors (NET), the differential diagnosis of SCCA from other family members is uncomplicated. Features of SCCA include small size, uniformity, coarse chromatin, a lack of nucleoli, very high nuclear-to-cytoplasmic ratios and molding. Distinguishing large cell neuroendocrine carcinoma is often relatively straightforward. Smears contain variably dispersed large malignant cells which typically have a single nucleus with a distinct membrane, granular chromatin, and one or more prominent nucleoli. Cytoplasm is volumetrically heterogeneous, although commonly, cytoplasm is abundant and delicate. Cells may be arranged in rosettes and trabeculae; this appearance is probably what is responsible for the fact that these tumors may be misinterpreted as adenocarcinoma. It is probably impossible to determine whether a tumor is a typical or an atypical carcinoid. Another problem is distinguishing a pure SCCA from a combined tumor.
With the major advances in therapy in NCSLC and the recognition of the association of specific tumor cell types with better or worse outcomes and with complications, it is imperative to render diagnoses as specific as possible. In most patients, differentiation of squamous cell carcinoma (SQCA) and adenocarcinoma (ACA) is possible. In addition to keratinization and intercellular desmosomes, other attributes for SQCA are centrally positioned nuclei and polygonal cellular contours. Conversely, eccentrically located nuclei, delicate cytoplasm, and neoplastic acini support a diagnosis of ACA. A small minority of cytologic interpretations remain as NSCLC, NOS. In two studies of preoperative cytologic diagnoses, concurrence of the cytologic and the subsequent histologic diagnoses was 88% and 93% with a better predictive value for ACA.

Application of a limited immunocytochemistry (ICC) panel increases the accuracy of tumor subtyping. A large number of antibodies are unnecessary. A major goal is to preserve sufficient sample for molecular analyses. In distinguishing SQCA and ACA, the two most widely used antibodies are probably p63 and TTF-1. If the latter is positive, the cancer is generally considered to be an ACA. If p63 is positive with a negative TTF-1, then squamous differentiation is present. Another antigen which points to glandular differentiation is napsin-A. If one needs to confirm neuroendocrine differentiation, a single antibody eg, CD56 or synaptophysin, may be used. This occurs most often in the diagnosis of LCNEC. A very new arena is the use of microRNAs to determine cell types.

With the advent of personalized thoracic oncology, after a specific diagnostic tumor type is rendered, molecular testing for a variety of mutations may be necessary. Though a greater number of mutations are recognized, three basic evaluations are important currently for therapeutic and predictive purposes: mutations in EGFR, k-ras, and EML4-ALK. Testing may be done on cell blocks, carcinoma scrapped from smears, and tumor cells in an aspirated aliquot dedicated for such tests. For all three mutations, PCR amplification of neoplastic DNA is probably the most widely used. However, for EGFR and ALK mutations, ICC, which is very antibody-dependent, may be of service too. For ALK analyses, FISH probes are directed against commonly translocated chromosomal segments. In several studies, the results of mutational analyses on cytologic material parallels that found in concurrent tissue samples. This is important as cytology specimens may be the only samples with sufficient tumor for such testing.

For several decades, no widely utilized histologic grading system existed for the NSCLC. Recently “grading” has been performed on histologic material from resected tumors wherein the predominate histologic pattern dictates the patient’s course. Histologic patterns of adenocarcinomas are not usually well recapitulated in cytologic smears. Several groups have developed cytologic grading schemes based solely on nuclear features. In one study, three nuclear attributes were included: size, chromatin pattern, and membrane contours. Each was assessed and given a numerical value which were summed to provide a nuclear score. The nuclear scores correlated well with prognosis.
Slide 1

Respiratory Tract Cytology: From Basic Morphology to Advanced Molecular Analyses
Kim R. Geisinger, MD

Slide 2

Introduction

- During the last century, cytology of the lung has progressively improved to form the cornerstone of diagnosis.
- In the last 3-4 decades, ICC has fine-tuned and supported interpretations of specific tumors.
- At the same time, an expansion of FNAB to include staging data.
- Now, advent of molecular analyses of mutations in receptors and oncogenes provide predictive and therapeutic data for individual patients.

Slide 3

Objectives

- Discuss different specimen types with regard to their diagnostic sensitivities & specificities.
- Review specific cytomorphic features of major forms of lung cancer, with emphasis on distinguishing:
  - NETs
  - NSCLCs
  - ICC
  - microRNAs
- Application of cytologic samples to evaluate mutations of EGFR, K-ras, EML4-ALK with PCR, ICC & FISH.
- Need to preserve sufficient tissue & cells.
- Grading.
Slide 4

Methods of Exfoliative Cytology of Lung Lesions

- Sputum: spontaneous, induced, post-bronchoscopy
- Brushings: direct visualization of lesion
- Washings: direct visualization of lesion segments 1-3
- Lavage: segments > 4

Slide 5

Methods of Aspiration Cytology of Lung Lesions

- Transthoracic (percutaneous)
- Transbronchial
  - Conventional
  - Endoscopic ultrasound directed

Slide 6

Indications for Cytology in Thoracic Oncology

Using relatively non-invasive techniques:

- Distinguish benign & malignant lung masses
- Distinguish primary & metastatic cancers
- Distinguish SCCA & NSCLC
- Distinguish among NETs
- Distinguish among NSCLCs
- Stage lung cancers
- Evaluate relevant mutations
Slide 7

Sampling & Cytologic Specimen Type Based On:

- Location of mass
- Size of mass
- History of prior malignancy
- Condition of patient
- Desire to stage
- MD preferences

Slide 8

Performance of Sputum Cytology for Suspected Lung Cancer (Schreiber Chest 2003: 123:115S)

- Problems comparing studies
  - Number of sputum samples
    - Sensitivity with number, with 3-5 optimal
  - Few studies evaluate specificity
    - Most patients have lung cancer
  - Types of sputum samples
    - Very high levels (>95%) for distinguishing SCCA & NSCLC

Slide 9

Performance of Sputum Cytology for Suspected Lung Cancer (Schreiber Chest 2003: 123:115S)

- Levels of Diagnostic Sensitivity
  - Range 42-97%
  - Pooled mean 66%
  - Pooled central 71%
  - Pooled peripheral 49%

- Levels of Diagnostic Specificity
  - Range 68-100%
  - Pooled mean 99%
Slide 10

- For all specimens, sensitivity depends on tumor location
- Level of Diagnostic Sensitivity (%)

<table>
<thead>
<tr>
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<th>Washing</th>
<th>Lavage</th>
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<tbody>
<tr>
<td>Central</td>
<td>74</td>
<td>55</td>
<td>40</td>
<td>40</td>
<td>56</td>
<td>89-96</td>
</tr>
<tr>
<td>Peripheral</td>
<td>46</td>
<td>52</td>
<td>33</td>
<td>37</td>
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- Procurement of multiple specimen types increases sensitivity: central 88%; peripheral 59%
- False positive uncommon

Slide 11

- Mean diagnostic sensitivity: 89%
- Mean diagnostic specificity: 96%
- On-site evaluation increases levels of both, especially sensitivity
- Although applicable for central lesions, more often used for peripheral masses & nodes
- Most common cytologic procedure for identification of metastases

Slide 12

- 59yo male with asymptomatic long term cigarette smoking history
- CXR: 4cm speculated mass in RL, UL
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Slide 16

Slide 17

TBFNA: sampling relies on landmarks & correlation with CT
EBUSNA: sampling uses direct visualization of target in real time
Results of EBUSNA are superior to TBFNA & comparable to surgical mediastinal node staging
Specificity 95-100%
Caveat for aspirates: is needle sampling node or mass in or extending from bronchus
Report lymphocytes present

Slide 18

Cytologic Staging of Lymph Nodes

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Slide 19

Neuroendocrine Tumors

- Histologic classification based on cell size, mitotic figure counts & the presence & the amount of necrosis
- Creates challenges for cytologic diagnosis of certain specific NETs.
  - Impossible to distinguish in an cytologic sample typical & atypical carcinoids as
    - MF counts are not an established validated procedure
    - MF counts do not have an accurate cytologic counterpart
    - Presence of small volume of necrosis difficult to identify
  - Potential problem of pure SCCA or combined tumor

Slide 20

Neuroendocrine Tumors

GOOD NEWS!

- Able to diagnose accurately & precisely SCCA
- Able to identify LCNEC in many cases
- Able to diagnose carcinoid tumor with high degree of certainty

Slide 21

Cytomorphologic Features of SCCA

Architectural
- Single cells & aggregates
- Classic molding & rosettes

Cellular
- Uniformly small cell size
- Single nucleus with granular to smudged hyperchromatic chromatin
- Inconspicuous nucleoli
- Very high N/Cs
- Cytoplasmic blue bodies

Background
- Crush
- Necrosis
- MFs & apoptotic debris
Cytomorphologic Features of LCNEC

Architectural
- Single cells & 3D aggregates with palisading
- Stepped nuclei

Cellular
- Large cell size with pleomorphism
- Solitary large nuclei with irregular membranes, granular chromat & nucleoli
- Scant to abundant delicate cytoplasm with generally low N/Cs
- No molding

Background
- Necrosis

Ancillary
- ICC usually needed
Cytomorphologic Features of Carcinoid Tumors

Architectural
- Single cells & aggregates
- Aggregates include palisaded sheets, acini, trabeculae & vascularized tissue fragments

Cellular
- Single cells may have plasmacytoid appearance
- Cells are round, oval, and spindled
- Smooth round-oval nucleus with fine distinct chromatin & inconspicuous nucleoli
- Nuclear membranes delicate & blend with chromatin
- Scant to moderate basophilic granular cytoplasm

Background
- Clean to granular
Slide 36

- 33yo female with hx of asthma and current short term smoking history
- Imaging: 6.5cm mass in LL, LL
- TTFNA
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In 9%, IHC with 100% concordance

Cytology has better predictive value for ACA

How well do we do in distinguishing SqCCA & ACA?

Cytomorphologic Features of Adenocarcinoma

Architectural
- Single cells but especially flat to 3D aggregates
- 3D groups include spheres & papillae
- Flat groups include sheets with acini & glands

Cellular
- Cytoplasm: delicate, granular to finely vacuolated
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- Nuclei: eccentrically located solitary large structures with slightly irregular membranes (grooves, INIs), finely granular chromatin, well developed nucleoli
Architectural
- Single cells & flat aggregates
- Pearls

Cellular
- Polygonal, oval & irregular shapes, including spindled & tadpole contours
- Cytoplasm is dense
- Keratinization
- Central nuclei with coarse to pyknotic hyperchromatic chromatin & oval to rectangular shapes
- Nucleoli with high grades

Background
- Cellular debris

Cytomorphologic Features of SqCCA
Slide 58

Slide 59

Slide 60

Immunocytochemistry

- Prefer cell block over direct smears
- Improves accuracy of NSCL subtyping
  - Glandular differentiation: TTF-1, napsin-A, CK7
  - Squamous differentiation: p63, CK5/6
  - TTF-1 trumps p63
- Helps identify LCNEC: synaptophysin, CD56, chromogranin
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- Preserve sufficient sample for molecular analyses!
Slide 61

• Based on morphology, preop cytologic dx (n=192) in agreement with histology in 93%
• IHC used in 9% of cases
  This made cyto/histo concordance 100%
• If TTF-1 positive, classed as ACA, even if p63 & HMWCK co-expressed

Slide 62

• Direct smears (unstained; air dried) of 25 cases
• ICC using TTF-1, napsin-A & p63
• In 83% ACAs, TTF-1 & napsin-A positive & p63 negative or focally positive
• In 3 ACAs, all 3 negative
• In 100% SqCCA, diffusely positive for p63 & negative for both TTF-1 & napsin-A

Slide 63

• Panel of 4 miRNAs to detect ACA
  Sensitivity 81%, specificity 92%
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  Sensitivity 78%, specificity 96%
• Proposed that miRNAs might provide early detection of NSCLC in sputum samples
Slide 64

43 yr old woman with SOB
- Imaging: 3.2cm peripheral mass abutting pleura and associated with pleural thickening and small effusion
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Slide 65

Slide 66
• Mutational analyses of EGFR, Kras, & EML4-ALK provide important therapeutic & predictive data
• Most studies have used histologic tumor tissue as substrate
  Cytologic samples have been used only sparingly
• Analytic methods include PCR-based analyses, FISH & IHC
• In some patients, cytology is only tumor sample available
  Preserve sufficient sample: it is for more than diagnosis
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- 37 Pap stained FNAs, effusions, brushings & washings
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EGFR Mutations in Cytologic Specimens with NSCLC
(Tsuta AJCP 2006;126:608)

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- Sensitivity & specificity 100%

EGFR Mutations in FNA of Adenocarcinomas
(Smith AJCP 2008;61:487)

Slide 72
• 128 cell blocks of NSCLC submitted
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• 75% FNAs, 23% pleural effusions
• EGFR mutation in 31 (25%)
• Kras mutation in 25 (20%)
• Pap stained monolayers of 73 FNAs of ACA
  Each previously graded histologically
• Evaluate 3 nuclear features: size, chromatin pattern & membrane irregularities
  Each individually scored & then summed for NG
• NG divided into low and high grades
• NG correlated with survival
• Good IOV

Nuclear Grade
(Sigel Cancer 2010;118:370)
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• Desire to stage
• MD preferences
• Problems comparing studies
  • Number of sputum samples
    Sensitivity with number, with 3-5 optimal
  • Few studies evaluate specificity
    Most patients have lung cancer
  • Types of sputum samples

• Very high levels ($\geq 95\%$) for distinguishing SCCA & NSCLC
• Levels of Diagnostic Sensitivity
  Range  42-97%
  Pooled mean  66%
  Pooled central  71%
  Pooled peripheral  49%

• Levels of Diagnostic Specificity
  Range  68-100%
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• For all specimens, sensitivity depends on tumor location

  Level of Diagnostic Sensitivity (%)

<table>
<thead>
<tr>
<th>Specimen Type</th>
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<th>Peripheral</th>
</tr>
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<tbody>
<tr>
<td>Biopsy</td>
<td>74</td>
<td>46</td>
</tr>
<tr>
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• 75% FNAs, 23% pleural effusions
• EGFR mutation in 31 (25%)
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• 24 D-Q stained FNA smears
• 12 ACAs, 10 NSCLC, NOS, 1 SqCCA, 1 SCCA
• Tumor enriched areas macrodissected (scaped)
• DNA suitable in 100%
• EGFR mutation in 1 ACA
• Kras Mutations in 7 ACAs & 3 NOS
• Grading system is relevant if it correlates with prognosis

• For decades, no widely used, standard histologic grading scheme for lung ACA

• Recent work suggests predominant histologic pattern in ACA associates with prognosis

• Difficult to identify specific histologic ACA patterns in cytologic samples

• Attempts to develop cytologic grading schemes
• Pap stained monolayers of 73 FNAs of ACA
  Each previously graded histologically

• Evaluate 3 nuclear features: size, chromatin pattern & membrane irregularities
  Each individually scores & then summed for NG

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