Lung Cytology

Pınar Fırat, MD, MIAC

Lung Cytology

Diagnosis of lung cancer
Detection of infections
Evaluation of interstitial diseases
Morphologic features
Approach to small samples

Lung Cancer

- Second most frequent cancer in both sexes
  - Most common reason of cancer deaths
- ~70% diagnosed at advanced stage
  - Small biopsies and cytology: Diagnostic tool
- Differentiating SCLC from NSCLC is important but not sufficient
  - Histologic subtype of NSCLC has clinical relevance;
  - Predicts efficacy and toxicity of some treatments
  - Predicts the likelihood of molecular changes which may lead to specific therapies.

Responsibility of pathologist in lung cancer

- Diagnosis
  - Exfoliative cytology
  - Fine needle aspiration

Lung Cytology

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>Non-invasive, non-expensive</td>
</tr>
<tr>
<td>Brushing</td>
<td>Direct sampling, well preserved cells</td>
</tr>
<tr>
<td>Washing</td>
<td>Sampling wide area, well preserved cells</td>
</tr>
<tr>
<td>Bronchial/alveolar lavage</td>
<td>Alveolar sampling</td>
</tr>
<tr>
<td>Transbronchial FNA</td>
<td>Direct sampling, well preserved cells, on site evaluation, ancillary tests</td>
</tr>
<tr>
<td>Transbronchial FNA (TBNA) with/without EBUS</td>
<td>Very much dependent on technique</td>
</tr>
</tbody>
</table>
Reserve cells

Squamous metaplasia

Reactive changes in alveolar epithelium
Infections
Drug toxicity
Infarction
To avoid false (+) diagnosis ....

- Compare the morphology of atypical cells with native cells
- Do not trust poor preparation
- Never rely on few cells
Transbronchial aspiration-contaminations

For correct diagnosis
• High quality preparation

Classification of Lung Tumors WHO-2015
Epithelial tumors

• Adenocarcinoma
  – Lepidic
  – Acinar
  – Papillary
  – Micropapillary
  – Solid
  – Variants
    • Invasive micropapillary
    • Classic
    • Solid
    • Enteric
  – Minimally invasive
  – Preinvasive lesions
    • Atypical adenomatous hyperplasia
    • Adenocarcinoma in situ
• Squamous cell carcinoma
  – Keratinizing
  – Non-keratinizing
  – Basaloid
  – Preinvasive lesion
• Neuroendocrine tumor
  – Small cell
  – Large cell neuroendocrine
  – Carcinoid tumors
  – Preinvasive lesion
  – Large cell carcinoma
  – Adenosquamous carcinoma
  – Pleomorphic carcinoma, spindle and giant cell carcinoma
  – Carcinosarcoma
  – Pulmonary blastoma
  – Lymphoepithelioma like
  – NUT carcinoma
  – Salivary gland type

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Adenocarcinoma

- Glandular differentiation
  - Glands, papillae, mucin
- Sheets / 3-D groups
- Nucleocytoplasmic polarity (columnar)
- Fine vesicular chromatin
- Nucleoli
- Intracytoplasmic luminas
- Palisating nuclei at the periphery of the groups
Squamous cell carcinoma
**Squamous cell carcinoma**

<table>
<thead>
<tr>
<th>Keratinizing SqCC</th>
<th>Nonkeratinizing SqCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohesion</td>
<td>Single cells</td>
</tr>
<tr>
<td>Cells</td>
<td>Pleomorphic, bizarr</td>
</tr>
<tr>
<td>Cytoplasm (PAP)</td>
<td>Orangeophilic, pink</td>
</tr>
<tr>
<td>Keratinization</td>
<td>Keratin pearls, goast c.</td>
</tr>
<tr>
<td>N/C</td>
<td>/</td>
</tr>
<tr>
<td>Chromatin</td>
<td>Pyknotic</td>
</tr>
<tr>
<td>Nucleoli</td>
<td>Inconspicious</td>
</tr>
</tbody>
</table>

**Morphologic criteria**

- Squamous cell carcinoma
  - Keratinized lamellar
  - Dense opaque
  - Chromatin
  - Pseudosinistral groups
  - Elongated nuclei
  - Pleomorphic-poligonal cells
  - Streaming pattern
  - Cell in cell
  - Keratinized single cells

- Adenocarcinoma
  - Monolayered sheets
  - Nucleocyttoplasmic granular
  - Gland like structures
  - Papillae like structures
  - Intrananuclear inclusions

**Squamous**

- Clusters, streaming, whorling
- Cell in cell pattern
- Central, oval/elongated nuclei
- Course dense chromatin
- Small nucleoli
- Dense cytoplasm
- Distinct cytoplasmic borders
- Keratinized cells

**Adenoid**

- Sheets, papillary structures
- Acini
- Exantric, round/oval nuclei
- Granular chromatin
- Large nucleoli
- Pale/lacy cytoplasm
- Indistinct cytoplasmic borders
- Mucin secretion
Small cell carcinoma

- Size (<3 lymphocyte)
- Neurosecretory granules
- Absence of nucleoli
- Hyperchromatic but powdery chromatin
- Scanty cytoplasm
- Paranuclear blue bodies, crush artifact, molding, necrosis
Atypical carcinoid is a resection diagnosis

Carcinoid tumor
Leiomyosarcoma
Adenocarcinoma
Distinguishing Carcinoid Tumor From Small Cell Carcinoma of the Lung

- 26 carcinoid cases, 1100 interpretations
- Frequently misclassified 19 cases (>20% of responses) were reviewed
- Patterns:
  - Poorly preserved pale staining cells with pale chromatin and suggestion of molding
  - Numerous large spindle shaped cells
  - Numerous cells varying markedly in size and shape, some are smudgy and degenerated
  - Hypocellular specimen
  - Blood obscuring cells
  - Tumor cells predominantly in groups, few isolated cells

Neuroendocrine Tumors of the Lung

- High grade neuroendocrine neoplasm
- Morphologic and clinical features are close to small cell carcinoma rather than non-small cell

Neuroendocrine features in cytology

- Salt&pepper (fine&coarse granular) chromatin
- Single cells, loose groups
- Loss of cytoplasm, naked nuclei
- If present cytoplasm is delicate and scanty
- Nuclear molding
- Nucleoli variable
- Cells attached to capillaries

Large cell neuroendocrine carcinoma (LCNEC)

- Necrosis +++ +
- Groups +++ ++
- Rosettes ++ +
- Molding ++ +++
- Nuclear size >3L <3L
- Salt&pepper chro. +++ +++
- Nucleoli + -
- Cytoplasm ++ -
Large cell neuroendocrine carcinoma

Original cytological diagnosis given to LCNEC

- Non-small cell carcinoma
- Poorly differentiated carcinoma
- Undifferentiated carcinoma
- High grade neuroendocrine carcinoma
- Small cell carcinoma
- Large cell neuroendocrine carcinoma
Differential Dx of LCNEC

- LCNEC vs NSCLC:
  - immunohistochemical reactivity with neuroendocrine markers accompanied by some cytologic features suggestive of neuroendocrine morphology.
- LCNEC vs SCLC:
  - tight cellular clusters, nuclear size, identifiable cytoplasm and presence of nucleoli
Pulmonary hamartoma

Transthoracic aspirations
Non-diagnostic

Entities cannot be diagnosed/subtyped by small samples
- In situ or minimally invasive adenocarcinomas
- Large cell carcinomas
- Carcinomas with more than one component
  - Adenosquamous carcinoma
  - Pleomorphic carcinoma

BX: Squamous Ca
Resection: Pleomorphic carcinoma

NSCLC with spindle and giant cells, could represent pleomorphic carcinoma (mention if sqcc or aden component if present)
The 2015 World Health Organization Classification of Lung Tumors

Impact of Keratin, Glucose and Histochemical Assays

The WHO 2015 Edition

Table 5: Diagnostic Significance of Small Biopsy and Cytologic Specimens in Small Cell Carcinoma, LCNEC, Adenocarcinoma, and Metastatic Carcinoma

<table>
<thead>
<tr>
<th>WHO 2015</th>
<th>IASLC/ATS/ERS - 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Cell</td>
<td>Non-small Cell</td>
</tr>
<tr>
<td>Morho.</td>
<td>IHC</td>
</tr>
<tr>
<td>Squamous diff</td>
<td>Adenoid diff</td>
</tr>
</tbody>
</table>

Approach to small biopsies and cytologic samples

- Subtyping is needed!
- But saving the tissue is also important!

- Is it small cell?  YES
- Immunohistochemistry, sign out

University of Istanbul

Subtyping of Non-small Cell Lung Carcinoma
A Comparison of Small Biopsy and Cytology Specimens

Carlo S. Neyel, MD*; Andrea L. Morita, MD, PhD*; William D. Travis, MS*; Halicien P. Zahnoda, MD*; Robert H. Grinnell, MS; and Natasha Kollin, MD, PhD*

J Thorac Oncol Oct 2016; 11: (868-876)

- The value of cytology and small biopsies for subtyping of NSCLCs is equivalent (directly diagnosing or favoring a subtype)
- Concordance between cytology and histology is 93%
- If used together, at least one gives a clear-cut diagnosis in 84% of the cases, if favoring diagnosis is also added, untyped NSCLCs reduces to 4%

101 simultaneous cytology & biopsy cases, examined separately (blinded)
**Approach to small biopsies and cytologic samples**

- **Is it small cell?**
  - NO
- **Squamous or adenocarcinoma?**
  - YES: Sign out
  - I AM NOT SURE: IHC
Kappa values

Intercellular bridges!
Single cell keratinization!
Intracytoplasmic vacuoles!
Giant cells

Reevaluation and Reclassification of Resected Lung Carcinomas Originally Diagnosed as Squamous Cell Carcinoma Using Immunohistochemical Analysis

6.5% misdiagnosed
4.2% adenocarcinoma

Immun markers for subtyping

Squamous
- P40
- P63
- CK 5/6
- Desmocollin

Adenocarcinoma
- TTF-1
- Napsin A
- CK 7
- Mucin stains

Immune markers for subtyping

Squamous
- P40
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- CK 5/6
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Adenocarcinoma
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- Napsin A
- CK 7
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Histopathology

Large-scale comparative analysis of immunomarkers for diagnostic subtyping of non-small-cell lung cancer biopsies

- 1103 cases, TMA
- Untyped NSCLC 10%

<table>
<thead>
<tr>
<th>ADC</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>SGCC</th>
<th>Specificity</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK7</td>
<td>0.79</td>
<td>0.96</td>
<td>CK9v6</td>
<td>0.07</td>
<td>0.94</td>
</tr>
<tr>
<td>TTF-1</td>
<td>0.68</td>
<td>0.87</td>
<td>p40</td>
<td>0.87</td>
<td>0.93</td>
</tr>
<tr>
<td>Napsin A</td>
<td>0.99</td>
<td>0.73</td>
<td>Desmocollin</td>
<td>0.99</td>
<td>0.84</td>
</tr>
</tbody>
</table>

>1%
Immunohistochemical Subtyping in Large Cell Lung Carcinomas

A retrospective study with cases diagnosed between 2000-2013

- 91 resection specimen
- 47 LCC, 25 solid/solid predominant Adeno, 20 poorly dif. squamous

Cut-off 30%\\nCut-off 10%

<table>
<thead>
<tr>
<th></th>
<th>p40</th>
<th>SK5/6</th>
<th>TTF-1</th>
<th>Napsin-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>84%</td>
<td>79%</td>
<td>60%</td>
<td>56%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Unpublished Data

TTF-1

2 different clones
- SPT24: Sensitive but not specific enough
- 8G7G3/1: Specific but less sensitive
How to use IHC in small samples?

One squamous, one (p40, TTF-1)
- p40(+), TTF-1(-): NSCLC, favor squamous Ca
- p40(-), TTF-1(+): NSCLC, favor adeno Ca
- p40(+), TTF-1(+), different cells: NSCLC, might be adenosquamous Ca
- p40(+), TTF-1(+), same cells: NSCLC, favor adeno Ca
- p40(-), TTF-1(-): NSCLC, NOS
Applying IHC to small samples?

- If any doubt in defining squamous or adenoid differentiation, then IHC is needed
- Threshold for squamous differentiation should be high (dense eosinophilic cytoplasm, distinct intercellular borders? Not enough! pseudosquamous adenocarcinoma)
- Use limited IHC to save the tissue for molecular tests, 1 squamous 1 adenoid marker
- TTF-1 specific
- P40 is more specific than p63

How to prepare cell blocks

- Clots and tissue fragments directly into formalin
- Cell suspensions
  - Saline, PBS, formalin, 50% ethanol, commercial fixative solutions...
- Centrifuge- tissue process for the cell pellet-
  - Commercial systems
  - Ethanol, ethanol/formaline
  - Plasma & thrombin/thromboplastin
- Original methodologies
Utility of the Thromboplastin- Plasma Cell-Block Technique for Fine-Needle Aspiration and Serous Effusions

- Rinse the needle in PBS/tamponated solution
- Centrifuge
- Add few drops of pooled plasma on the sediment, mix well, than add few drops of thrombin/ thromboplastin, mix again
- Wait for 5 min
- Remove the resultant clot, wrap, cassette and store in formalin till tissue processing

Reagent used for prothrombin time test

Questions to answer at ROSE

- Is the specimen representing the target?
  - Clinical features/ radiology
- Is the amount and quality of cells sufficient for a satisfactory morphologic evaluation?
  - What is the preliminary diagnosis ?
- Any ancillary test needed either for diagnosis or for therapy?
  - How should I handle the specimen?

Good material… for diagnosis, for therapy

- Shared responsibility:
  - pulmonologist
  - radiologist
  - pathologist/ cytopathologist

Goal is to reach maximum biologic information by minimum material

The most suitable sampling technique should play a pivotal role in pathologic classification, molecular categorization and staging

for the patient

for the ancillary tests