Minimally Invasive Biopsies of the Lung & Mediastinum in the Era of Personalized Medicine

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Outline

• Introduction
  • Why minimally invasive biopsies?
  • Current Approach to Diagnosis of Mediastinal/Lung Lesions

• Interesting Cases
  • Pitfalls
  • Morphological challenges impacting ancillary studies

• Conclusion

Introduction

• Why minimally invasive techniques?
  • Over half of NSCLC patients present with metastases \(\rightarrow\) Dx & Stage with EBUS-TBNA
  • Minimally invasive biopsies \(\rightarrow\) Shorter length of stay & less cost
  • Need for subtyping and molecular studies/theranostic data, without the need for complete surgical excision

• Advantages of Cytological Specimens
  • Better nuclear & cytoplasmic detail
  • Less fixation artifact
  • Ability to have ROSE for triage & to allocate material for appropriate testing

Imaging & Diagnosis of Mediastinal/Lung Lesions

• Imaging Modalities
  • Chest Xray
  • CT Scan
  • PET CT Scan

• Minimally Invasive Diagnostic Modalities
  • Sputum/BAL/BB/BW/Pl Fl
  • CT-Guided FNA
  • Transbronchial FNA (Wang biopsy)
  • Supernavigational EMN biopsy
  • EBUS & EUS guided FNA
  • Endobronchial biopsy with touch preparation

Small Biopsies of Lung/Mediastinum: The Power of EBUS TBNA

• Advantages:
  • Minimally invasive
  • Image guidance
  • Tissue confirmation of +PET/CT findings & evaluation of LNs <1 cm
  • Broad sampling capability
  • On-site evaluation \(\rightarrow\) triage
  • Lower cost

• Disadvantages:
  • Inability to access all LNs
  • Not universally available
  • Time & experience requirement
  • Non-diagnostic specimens

Among patients with clinical stage IIIA, 40% of patients were down-staged with EBUS-FNA
Gilbert S et al., JTCVS 2009
**Sampling Capability**

LN Stations

**Advantages of Minimally Invasive Small Biopsies**

- Restaging
- Small LNs < 1 cm
- Poor Operative Candidates
- Non-Surgical Diseases
- Biomarker testing

**What is the management?**

EBUS Negative → Mediastinoscopy: Small focus of Metastatic SqCC

**What is new with EBUS TBNA?**

- **Equipment**
  - New needle sizes: 19-22G FNA needle
  - New types of needles: traditional TBNA needle vs Pro-core
  - New designs: superior echogenic design

- **Increased demand**
  - Managing ROSE: Telecytology vs Traditional
  - Weekend and late procedures: Cytology On-Call or Not?
  - Optimizing tissue: Doing more with less

Echotip ProCore™, Image courtesy of Cook Medical
Introduction

- Increase in small biopsies and cytology specimens for lung & mediastinal lesions has led to...
  - New approaches with improved biopsy techniques
  - Variety of different needles to choose from
  - New classification systems for small biopsies & cytology specimens
  - Expanding use for biomarker testing in the era of personalized medicine
    - Not enough to just make a diagnosis anymore
    - Do more with less
      - EGFR, ALK → EGFR, ALK, ROS, PD-L1, and more

Diagnostic Shift in Lung Cancer

PAST

- NSCLC vs. SCC
- Treatment
  - Surgical: NSCLC
  - Non-surgical: SCC

PRESENT

- Need for subtyping and molecular studies
- Theranostic data

Lung Cancer

- SCLC
- NSCLC
- Adenocarcinoma
- Squamous cell carcinoma
- NSCLC NOS
TABLE 1. Proposed Pulmonary Cytology specimen Terminology and Classification Scheme

<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>Risk of malignancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiagnostic</td>
<td>10</td>
</tr>
<tr>
<td>Negative for malignancy</td>
<td>24 (47%)</td>
</tr>
<tr>
<td>Atypical</td>
<td>54</td>
</tr>
<tr>
<td>Neoplastic, benign neoplasm, low-grade carcinoma</td>
<td>NA</td>
</tr>
<tr>
<td>Suspicious for malignancy</td>
<td>82</td>
</tr>
<tr>
<td>Malignant</td>
<td>77 (100%)</td>
</tr>
</tbody>
</table>

UPMC EBUS Data (2007-2010)

593 EBUS FNAs from 357 patients
34% with histological follow-up

Table 2. Adequacy Criteria of Rapid On-Site Evaluation Specimens of Endobronchial Ultrasound (EBUS)-Guided Transbronchial Needle Aspiration for the Diagnosis of Lung Cancer

<table>
<thead>
<tr>
<th>FNA Adequacy</th>
<th>Overall Total # (%)</th>
<th>Total with follow-up # (%)</th>
<th>False Negatives # (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsatisfactory</td>
<td>66 (11%)</td>
<td>30 (46%)</td>
<td>7 (23%)</td>
</tr>
<tr>
<td>Less than optimal</td>
<td>107 (18%)</td>
<td>55 (51%)</td>
<td>6 (11%)</td>
</tr>
<tr>
<td>Satisfactory</td>
<td>420 (71%)</td>
<td>118 (28%)</td>
<td>4 (3%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>593</td>
<td>203 (34%)</td>
<td>17 (8%)</td>
</tr>
</tbody>
</table>

UPMC EBUS Data (2007-2010)

593 EBUS FNAs from 357 patients
34% with histological follow-up
**EBUS TBNA & CTG FNA: Quantity & Quality**

<table>
<thead>
<tr>
<th>CTG FNA</th>
<th>EBUS TBNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>Anterior Mediastinum or Lung</td>
</tr>
<tr>
<td>Baseline cellularity (negative case)</td>
<td>Low</td>
</tr>
<tr>
<td>Cells present (negative case)</td>
<td>Few bronchial cells, macrophages &amp; red blood cells. No background mucus present.</td>
</tr>
<tr>
<td>Abnormal case</td>
<td>Qualitative abnormality. Increase in inflammatory cells or tumor cells.</td>
</tr>
<tr>
<td>Screening</td>
<td>Faster (less cells to examine)</td>
</tr>
<tr>
<td>Location</td>
<td>Radiology</td>
</tr>
</tbody>
</table>

**Diagnosis?**

- NE tumor (Carcinoid)
- HG NE tumor (SCLC)
- Squamous Cell Carcinoma
- Adenocarcinoma

**Molecular Testing + PD-L1**

- EGFR
- KRAS
- ALK
- ROS
- BRAF
- RET
- ERBB2
- C-MET
- NTRK

**Biomarker Testing in Lung Cancer**

- Rose with Dedicated passes (FNA) or core biopsies
- Upfront blanks cut to avoid trimming of block
- Unstained charged slides numbered in order
- Limited IHC

**Case 1: CTG-FNA of Lung Lesion**

- 63 year old woman with a history of melanoma in 2012, with metastases in 2015 to the liver
  - Primary was a right lateral vaginal wall tumor in 2012
  - *BRAF* V600E positive, *NRAS* negative, *NF1* unknown
  - Currently on Nivolumab treatment (started 18 months prior to biopsy)
- CT scan: bilateral consolidative pulmonary opacities
- Infection
- CTG FNA of lung mass/opacity sampled
Case Diagnosis

- **Final Diagnosis**
  - Less than optimal- scant cellularity
  - Atypical cells present.
  - Atypical epithelial cells and chronic inflammation, favor reactive.
  - No viral or infectious etiology seen, including special stains.
  - Nivolumab was discontinued.
  - On follow-up (1 month later), most of lung lesions completely resolved.
  - Changes attributed to reactive pneumocyte atypia in the setting of PD-1 inhibitor toxicity (pneumonitis)

Adverse Pulmonary Reactions with PD-1 inhibitors

- Thought to be an autoimmune related pneumonitis
- Seen in 1-14% patients on phase 2 and 3 trials
  - Smaller % with high-grade toxicity
  - Larger % with low-grade toxicity
  - Rare respiratory failure & death (3)
- Sx: Pneumonia-like symptoms with bilateral lung infiltrates (rarely unifocal)
- Onset about 7-24 mo after Tx

NORMAL DESMOPLASIA (preTx)

TUMOR REGRESSION (postTx)

Cottrell TR et al, Annals of Oncology 2018
61 yo man with Hx of Melanoma, s/p Pembrolizumab, now LAD. EBUS FNA (subcarinal)

- Treatment: Steroids may not be required (systemic steroids needed in about 40% cases).
- Removal of the PD-1 checkpoint inhibitor (e.g., Nivolumab) usually causes granulomatous lesions to regress.
- No cases have been refractory to treatment, thus far.

64 yo woman with history of DLBCL s/p R-CHOP, now with residual mesenteric LAD, moderately FDG avid on PET scan.

- Benign/reactive epithelial cells
- Therapy-related atypia (chemotherapy, radiation, medication)
- Metaplastic changes (goblet cell or squamous metaplasia)
- Mesothelial hyperplasia
- Granulomatous inflammation/epitheloid histiocytes
- Cellular granulomas
- Contamination of the FNA needle
- Primary luminal dysplasia (Barrett’s esophagus) in EUS-FNA
Take Home Messages

- In the era of personalized medicine with new therapeutic agents, think about treatment related changes
  - Expanding number of protocols & targeted or other therapies
  - Neoadjuvant therapy for down-staging prior to resection
  - PD-1 inhibitors (Lung cancer, Melanoma, Other)
  - Other radiation/chemotherapy-related changes
  - Avoid False Positive Diagnoses
  - Cytology findings: pneumocyte/squamous atypia, granulomatous, fibrotic, inflammatory, or necrotic changes in the lymph node and lung

- Important to consider drug-induced toxicity for treatment
  - Test of time: Withdrawal of agent leading to improvement
  - Sometimes the best medicine is no medicine

Case 2: Lung EMN biopsy

- 40 year old woman with right lung mass
  - Non-smoker
  - 3.0 cm lung mass identified at an OSH
  - Previously biopsied at an OSH and called squamous cell carcinoma
  - Clinicians requested repeat biopsy given that she is non-smoker
  - EMN biopsy of lung mass
Case Diagnosis

- Final Diagnosis:
  - Satisfactory for interpretation.
  - Positive for malignant cells.
  - Non-small cell carcinoma.

- Comment: There are squamous and glandular features.
- Histology: Adenosquamous lung carcinoma
- Material submitted for molecular testing.
  - EGFR mutation +
- Pitfall: Diagnosis of squamous cell carcinoma could exclude patient from molecular testing and potential targeted therapies.

Diagnosis Matters

![Diagram showing diagnosis options and molecular testing]

**Table 1.95** Diagnostic terminology for small biopsy specimens comparing the new IASLC/ATS/ERS terms with 2015 WHO terms in parentheses (2014, 2016).

- Small biopsyology: IASLC/ATS/ERS classification
- 2015 WHO classification

- Small cell carcinoma
- Large cell neuroendocrine carcinoma
- Adenosquamous carcinoma (if both components ≥ 10%)
- Adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma or large cell carcinoma with unclear immunohistochemical features.
- Pleomorphic, spindle cell, and/or giant cell carcinoma

**Original Article**

Analysis of Major Known Driver Mutations and Prognosis in Resected Adenosquamous Lung Carcinomas

- Ad1100C
- Ad1003C
- Ad1103C
- Ad1203C
- Ad1303C
- Ad1403C
- Ad1503C
- Ad1603C
- Ad1703C
- Ad1803C
- Ad1903C
- Ad2003C
- Ad2103C
- Ad2203C
- Ad2303C
- Ad2403C
- Ad2503C
- Ad2603C
- Ad2703C
- Ad2803C
- Ad2903C
- Ad3003C
- Ad3103C
- Ad3203C
- Ad3303C
- Ad3403C
- Ad3503C
- Ad3603C
- Ad3703C
- Ad3803C
- Ad3903C
- Ad4003C
- Ad4103C
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- Ad4303C
- Ad4403C
- Ad4503C
- Ad4603C
- Ad4703C
- Ad4803C
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- Ad6703C
- Ad6803C
- Ad6903C
- Ad7003C
- Ad7103C
- Ad7203C
- Ad7303C
- Ad7403C
- Ad7503C
- Ad7603C
- Ad7703C
- Ad7803C
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- Ad8003C
- Ad8103C
- Ad8203C
- Ad8303C
- Ad8403C
- Ad8503C
- Ad8603C
- Ad8703C
- Ad8803C
- Ad8903C
- Ad9003C
- Ad9103C
- Ad9203C
- Ad9303C
- Ad9403C
- Ad9503C
- Ad9603C
- Ad9703C
- Ad9803C
- Ad9903C

**Molecular Testing** + PD-L1
- EGFR, KRAS, ALK, ROS
- BRAF, RET, ERBB2, C-MET, NTRK
Pitfall: Combined tumors

- Should I suggest adenosquamous lung carcinoma, or is one component benign/reactive?
  - Look at nuclear grade

- Combined tumors are rare (<5%), but could have important implications for testing
  - If you falsely assume an adenosquamous cell carcinoma is a squamous cell carcinoma, then you could exclude the patient from important targeted therapies if you did not send it for molecular testing
  - When in doubt...
    - Check history
    - Err on the side of calling NSCLC, NOS to initiate molecular testing

Misclassification of Lung AdenoCa

- Benign/Reactive changes
  - Goblet cell metaplasia, Treatment-related changes

- Low-to-intermediate grade neuroendocrine tumors

- Poorly differentiated NSCLC without material for cell block/IHC
  - SqCC, LCNEC

- Salivary gland type tumors
  - Mucoepidermoid carcinoma

- Metastatic non-pulmonary carcinomas

- Metastatic melanoma
Final Diagnosis: Neuroendocrine tumor, favor low-intermediate grade

Pitfall
Neuroendocrine tumors can have:
- Pseudoglandular spaces/rosettes
- Intranuclear inclusions
  - Seen in 2/227 (0.9%) of pulmonary NETs
  - Usually higher-grade NETs

NSCLC with giant cell/pleomorphic features
Lung Biopsy with touch prep: ROSE was Adenocarcinoma

TTF1
p40, Syn
Final Diagnosis

Squamous cell carcinoma on Lung Biopsy with touch prep

**Basaloid SqCC**

**Large cell neuroendocrine Ca**

**Mucoepidermoid Carcinoma in the Lung**

(ROSE was Adenocarcinoma)

**EBUS TBNA: Metastatic Urothelial Carcinoma with Squamous differentiation**

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**Take Home Messages**

- Subclassification can be difficult in a subset of lung tumors
  - Obtain good clinical history (age, smoking status)
  - Look for history of prior malignancies
  - Use IHC to help

- Avoid unnecessary testing & loss of material
  - Morphological details can help guide selection
  - Do just enough to be correct
Case 3: CTG FNA and Core Bx with TP

- 82 year old woman with incidental well-circumscribed lesion in right upper lobe of the lung
- Prior CTG FNA showed features of a pulmonary hamartoma, but lesion was growing on imaging.
- CTG FNA and core biopsy with touch preparation.

Case Diagnosis

- Final Diagnosis:
  - Satisfactory for Interpretation
  - Positive for neoplasm
  - Salivary gland-type tumor, favor Epithelial-Myoepithelial Carcinoma.

- Challenges:
  - Biphasic lesions in the lung: not always hamartoma
  - Salivary gland-type tumors: primary versus metastatic
  - Lung tumors that do not fall into SCLC vs NSCLC are challenging

Follow-up

- No primary salivary gland lesion identified on CT-PET scan.
- Lobectomy showed a well-circumscribed, lobulated lung lesion grossly.
- Resection showed:
  - Carcinoma ex Pleomorphic adenoma
  - Carcinomatous component was an Epithelial-Myoepithelial carcinoma
Carcinoma ex Pleomorphic Adenoma in Lung

- Rare in the lung, but arises from the bronchial glands
- Must exclude a head and neck primary
- Considered a low-grade malignancy with long interval to recurrence or metastasis
- Most common carcinomas in this setting:
  - Poorly differentiated adenocarcinoma
  - Salivary duct carcinoma
  - Epithelial-myoepithelial carcinoma
- Gross: well circumscribed, pushing border in an endobronchial location

Carcinoma ex Pleomorphic Adenoma in Lung

- Histologically: Malignant myoepithelial cells and duct-like structures in benign chondromyxoid stroma
  - No mature cartilage
  - Biphasic cell population:
    - Large, clear myoepithelial cells (myoepithelial cells +S100, p63, SMMH, vim)
    - Small, dark ductal cells (epithelial cells +CK, EMA, +/- S100)
- Cytomorphology:
  - Cellular aspirates with cellular chondromyxoid-type material
  - Naked nuclei due to fragile clear cytoplasm of myoepithelial cells
  - Atypia
  - No mature cartilage

Differential Diagnosis

- Benign: Granuloma, Amyloidoma
- Hamartoma
- Mesenchymal tumor (e.g. solitary fibrous tumor, sarcoma)
- Metastatic spindle cell tumor with myxoid change (e.g. GIST)
- Salivary gland-type tumor
  - Primary (arising from the bronchial glands) vs. Metastatic
  - Benign (pleomorphic adenoma) vs. Malignant (epithelial-myoepithelial carcinoma)
  - Variable subtypes: pleomorphic adenoma, epithelial-myoepithelial carcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, basal cell neoplasm
- Primary lung carcinoma with desmoplastic stroma or mucin (Adenocarcinoma, Basaloid squamous cell carcinoma, Carcinosarcoma)

Pulmonary Hamartoma

- Scant cellularity
  - Due to dense nature of the lesion
  - Rubber eraser-like effect
- Clean Background
  - No necrosis or inflammation
  - Reactive bronchial cells
- Cartilaginous or Fibromyxoid fragments (metachromatic)
- Recurrent clonal rearrangements of HMGI(Y) gene on chr.6p21

Chromosomal abnormalities in salivary gland-like tumors that can be detected with FISH studies in small biopsies and cytology specimens.

*Note: These salivary gland tumors have only rarely been reported in the lung, and primarily are seen as metastases.

<table>
<thead>
<tr>
<th>Salivary gland-type tumor</th>
<th>Gene(s)</th>
<th>Chromosome(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary analogue secretory carcinoma*</td>
<td>ETV6-NTRK3</td>
<td>t(12;15)</td>
</tr>
<tr>
<td>Mucoepidermoid carcinoma</td>
<td>MECT1-MAML2</td>
<td>t(11;19)</td>
</tr>
<tr>
<td>Adenoid Cystic Carcinoma</td>
<td>MYB-NFIB</td>
<td>t(6,9)</td>
</tr>
<tr>
<td>Salivary duct carcinoma*</td>
<td>Her2/neu</td>
<td>17q</td>
</tr>
<tr>
<td>Hyalinizing clear cell carcinoma*</td>
<td>EWSR1-ATF1</td>
<td>t(12;22)</td>
</tr>
</tbody>
</table>
Take Home Messages

- Pulmonary hamartomas typically do not grow rapidly.
  - Increased growth on serial imaging is a **RED** flag.
- Think of SGTTs in the lung when you see a biphasic tumor with chondromyxoid material and basaloid or myoepithelial-type cells.
  - Atypical features to look for in a fibromyxoid lesion in the lung: high cellularity, atypia, bilayered glandular structures, and lesional growth
- Although SGTTs can occur as a primary in the lung (from the bronchial glands), a metastatic tumor should be excluded.
- FISH studies are becoming increasingly helpful in SGTTs for definitive classification.

Conclusions

- Minimally invasive biopsies & new FNA techniques have changed the way that thoracic & mediastinal lesions are approached
- New Classification Systems for small biopsies provide a framework for how we should be formulating diagnoses
- Biomarker testing is crucial & growing
- Small biopsy & cytology diagnoses can be challenging

Thank you!