EBUS-TBNA
Diagnosis and Staging of Lung Cancer

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EUS/EBUS-FNAB: Site Distribution

N = 3,684

Others (30%): GI Tract, Hepatobiliary Tree, Adrenal gland, Spleen, Lung, Kidney

Cytojournal 2012, 9: 14
<table>
<thead>
<tr>
<th>Modality</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>40.3%</td>
</tr>
<tr>
<td>PET</td>
<td>50.0%</td>
</tr>
<tr>
<td>EUS</td>
<td>69.2%</td>
</tr>
<tr>
<td>EUS-FNA</td>
<td>97.1%</td>
</tr>
</tbody>
</table>

N = 104

*Annals of Thoracic Surgery 2005; 79:263-268*
2004

- **Nomenclature**
  - WHO 2004 –
  - Nomenclature based mostly on resected samples
  - Cytology and its role was not very well documented
    - Biopsies and its associated challenges were not taken into account

2011

*IASLC/ATS/ERS J Thorac Oncol. 2011;6(2):244–285.*

- **International Nomenclature**
  - Understanding of the role of new Technologies
  - Improved role of small tissue samples in management
  - Molecular Studies on Rise
  - Personalized therapy became reality
Major Changes in Mind Sets

1. Further classify lung cancer based on morphology as best as possible

1. Make judicious use of additional studies to further characterize lung tumors (not included in 2004)

1. Use of molecular studies for patient management

1. Think forward for a need to perform Molecular studies for Personalizing therapies.
EBUS-FNA
Factors that help Improve Diagnostic Performance

Operator experience
Technique
Lesion Location
Cytopathologist experience
Onsite Adequacy
Communication between pulmonologists and cytopathologists
Type of Needle

How Many Passes and How Many Cells for Flow Cytometry Work Up?

<table>
<thead>
<tr>
<th>Site</th>
<th>Lymphoma</th>
<th>Non-Lymphoid Neoplastic Lesions</th>
<th>Benign and reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of cases (n) / Mean cells (range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Thoracic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcarinal</td>
<td>7 / 5.15 (0.15-14.47)</td>
<td>3 / 0.68 (0.11-1.85)</td>
<td>22 / 10.89 (0.09-212.85)</td>
</tr>
<tr>
<td>Mediastinal*</td>
<td>3 / 0.80 (0.52-1.08)</td>
<td>4 / 3.66 (2.29-5.00)</td>
<td>8 / 2.68 (0.05-8.10)</td>
</tr>
<tr>
<td><strong>Intra-abdominal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celiac</td>
<td>5 / 10.13 (0.56-43.99)</td>
<td>4 / 0.44 (0.08-0.91)</td>
<td>4 / 2.84 (1.30-4.41)</td>
</tr>
<tr>
<td>Peripancreatic</td>
<td>8 / 12.27 (0.12-62.33)</td>
<td>1 / 4.5 (N/A)</td>
<td>21 / 0.94 (0.003-4.79)</td>
</tr>
<tr>
<td>Perigastric</td>
<td>8 / 2.10 (0.17-8.18)</td>
<td>0</td>
<td>6 / 1.46 (0.31-2.53)</td>
</tr>
<tr>
<td>Other†</td>
<td>12 / 3.25 (0.13-13.17)</td>
<td>2 / 156.37 (4.85-307.89)</td>
<td>10 / 1.62 (0.2-3.95)</td>
</tr>
<tr>
<td>All locations</td>
<td>43 / 5.66 (0.12-62.32)</td>
<td>14 / 23.66 (0.02-307.89)</td>
<td>71 / 5.85 (0.003-212.85)</td>
</tr>
</tbody>
</table>

*Review of 1338 lymph node FNA cases. Cytojournal 2012*
Diagnosis and Staging of Carcinoma: A Practical Approach

Neuroendocrine Tumors
- Carcinoid
- WDNET
- Atypical Carcinoid
- PDNET
  - Small Cell
  - Large Cell
  - CA

Non-Small Cell Carcinoma
- Squamous cell CA
- Adeno CA
- Molecular Def
- Others
Non-Small Cell Carcinoma

- Squamous cell CA
  - Sq C C
  - Basaloid Sq C C
- Adeno CA
  - Adeno CA Pattern where possible
  - Favor Adeno Ca
- Others
Male 52 years with 2 cm mass in the right lung. Now with hilar lymphadenopathy.
Squamous Cell Carcinoma

Nuclear Hyperchromasia
Nuclear Membrane variable
Coarse Chromatin
Nuclear Pyknosis
PD Squamous cell ca
(Prominent nucleoli not uncommon)
Cytoplasm with sharp edges
Abnormal cell shapes
Keratin pearls
Necrosis
Neurophilic infiltrate
Giant Cell Response
(occasionally)
BASALOID

Keratinizing SCC
Male 54 years with history of hemoptysis
Differential Diagnosis

- Basaloid Squamous cell carcinoma
- Neuroendocrine Carcinoma, poorly differentiated
- Lymphoma
## Cytologic Features

<table>
<thead>
<tr>
<th>Basaloid Sq Cell Ca</th>
<th>Small Cell Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Tightly cohesive clusters</td>
<td>• Cellular with small groups</td>
</tr>
<tr>
<td>• Single Cells</td>
<td>• May be single cells</td>
</tr>
<tr>
<td>• Palisading</td>
<td>• Crush artifact</td>
</tr>
<tr>
<td>• Crush artifact</td>
<td>• Hyperchromasia</td>
</tr>
<tr>
<td>• Hyperchromasia</td>
<td>• Nuclear molding</td>
</tr>
<tr>
<td>• Focal nuclear molding</td>
<td>• No/ Inconspicuous Nucleoli</td>
</tr>
<tr>
<td>• Inconspicuous nucleoli</td>
<td>• Scant cytoplasm</td>
</tr>
<tr>
<td>• Scant Cytoplasm</td>
<td>• Apoptosis</td>
</tr>
<tr>
<td>• Necrosis</td>
<td></td>
</tr>
<tr>
<td>• Apoptosis</td>
<td></td>
</tr>
<tr>
<td>• squamous differentiation</td>
<td></td>
</tr>
</tbody>
</table>

*Diagn Cytopathol. 2011 Feb;39(2):92-100*
FNA Features on Cytology
Immunohistochemical Stains

**Basaloid Squamous Cell Ca.**
- p63 (+),
- High molecular weight cytokeratin (+),
- CK5/6 (may be focal)
- TTF-1 (-)
- Chromogranin (focal)

**Small cell Carcinoma**
- P63 (usually negative)
- TTF1 (usually positive)
- Chromogranin (positive)
- Synaptophysin (positive)
- CD56 (positive)
## HPV and Squamous Cell CA

<table>
<thead>
<tr>
<th>Histology</th>
<th>Basaloid Sq Cell CA</th>
<th>Keratinized Sq Cell Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 inactivated by E 6</td>
<td>p53 inactivated by mutation</td>
<td></td>
</tr>
<tr>
<td>Rb inactivated by E7</td>
<td>Rb inactivated by cyclin D1 amplification</td>
<td></td>
</tr>
<tr>
<td>p16 over-expressed</td>
<td>Inactivation of p16</td>
<td></td>
</tr>
</tbody>
</table>
Non - Small Cell Carcinoma

- Squamous cell CA
  - Sq C C
  - Basaloid Sq C C

- Adeno CA
  - Adeno CA Pattern where possible
  - Favor Adeno Ca

- Others
Adenocarcinoma

Adenocarcinoma
Describe pattern as possible

Adenocarcinoma with lepidic pattern (Bronchioloalveolar pattern)

Minimally Invasive
Adenocarcinoma with or without mucinous features
Adenocarcinoma: Patterns of Cells on EBUS
In the era of Personalized Care
Case

- Male 58 years with right sided peri-hilar lung mass/ lymph node.
- **Prior attempts to obtain tissue diagnosis**
  - Including 2 CT guided bx - Diagnosis remained inconclusive
- Bronchoscopy was performed
- BAL was performed
- EBUS – FNA of perihilar lung mass/lymphnode performed.
- **Patient Management**: Awaiting tissue diagnosis
Primary Tumor
Adenocarcinoma
Diagnosis

Lung, perihilar mass, EBUS-FNA:
Poorly differentiated carcinoma with glandular differentiation, see note.

IHC Performed
+ve for CK7, TTF-1, Napsin-A and CK5/6
-ve for CK20
-ve for P63

- Molecular testing performed on the cell block
“Dr. Jhala,

Just got the cytology report in my inbox and I see immunohistocheical stains are pending. Please note that we already know that this patient has EGFR activating mutation positive recurrent tumor. Therefore, the priority is to get local CPD testing here looking for T790 activating mutation. IHC is not a high priority. I also want to make sure that there is sufficient material for CPD testing....”
Processing Cell Blocks
Keep 3 unstained between level 1 and another level, use 3 micron sections

Table 1. All cases with cell blocks (n=221, p=0.52)

<table>
<thead>
<tr>
<th>Type of Cut</th>
<th>Number (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1=2 (First and second cuts equally representative)</td>
<td>102 (46.1%)</td>
</tr>
<tr>
<td>1&gt;2 (First cut more representative than second cut)</td>
<td>59 (26.7%)</td>
</tr>
<tr>
<td>2&gt;1 (Second cut more representative than first cut)</td>
<td>60 (27.1%)</td>
</tr>
</tbody>
</table>

Table 2. Intrathoracic lymph nodes (n=192, p=0.065)

<table>
<thead>
<tr>
<th>Lymph Node Sites (all EBUS-FNA)</th>
<th>1=2 (First and second cuts equally representative)</th>
<th>1&gt;2 (First cut more representative than second cut)</th>
<th>2&gt;1 (Second cut more representative than first cut)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcarinal</td>
<td>28 (42.2%)</td>
<td>19 (28.7%)</td>
<td>19 (28.7%)</td>
</tr>
<tr>
<td>Left hilar</td>
<td>21 (52.5%)</td>
<td>11 (27.5%)</td>
<td>8 (20.0%)</td>
</tr>
<tr>
<td>Right hilar</td>
<td>18 (47.4%)</td>
<td>12 (31.6%)</td>
<td>8 (21.1%)</td>
</tr>
<tr>
<td>Left parastracheal</td>
<td>9 (52.9%)</td>
<td>4 (23.5%)</td>
<td>4 (23.5%)</td>
</tr>
<tr>
<td>Right parastracheal</td>
<td>11 (39.5%)</td>
<td>7 (22.6%)</td>
<td>13 (41.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>87 (45.3%)</td>
<td>53 (27.6%)</td>
<td>52 (27.1%)</td>
</tr>
</tbody>
</table>

Figure 1. Example of case with 1st cut (A) more representative than 2nd cut (B).
Molecular testing guideline for selection of lung cancer patients for EGFR and ALK Tyrosine Kinase Inhibitors (from CAP, IASLC, AMP)
Benefits of NGS

• Many targets in one assay
• Same amount of starting material can address many questions, compared to sequential testing that keeps requiring more DNA and also prolongs TAT (turn around time)
• Quicker TAT- 7 to 10 days to get results on 10s to 100s of genes
• Cost is cheap, so you can also target rare mutations
Solid Tumor Sequencing Panel

- Sequence analysis of 47 genes
- ABL1, AKT1, **ALK**, APC, ATM, BRAF, CDH1, CSF1R, CTNNB1, **EGFR**, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK2, JAK3, KDR, KIT, **KRAS**, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, **PIK3CA**, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL.
Guidelines

• **4.2 Recommendation:** Expert consensus opinion: Cytologic samples are also suitable for EGFR and ALK testing, with cell blocks being preferred over smear preparations.

• **8.1 Recommendation:** If a laboratory performs testing on specimens from patients with acquired resistance to EGFR kinase inhibitors, such tests should be able to detect secondary EGFR T790M mutation in as few as 5% of cells.---Role of Next gene Sequencing for TAT and detection of T790M!!
Lung cancer differentiation and metastasis

LKB1 modulates lung cancer differentiation and metastasis

Hongbin Ji1,4,17, Matthew R. Ramsey10,12,17, D. Neil Hayes11, Cheng Fan10, Kate McNamara1,4, Piotr Kozlowski5, Chad Torrice11, Michael C. Wu3, Takeshi Shimamura1, Samantha A. Perera1,4, Mei-Chih Liang1,4, Dongpo Cai1, George N. Naumov8, Lei Bao13, Cristina M. Contreras14, Danan Li1,4, Liang Chen1,4, Janakiraman Krishnamurthy10,11, Jussi Koivunen1, Lucian R. Chiriac6, Robert F. Padera6, Roderick T. Bronson9, Neal I. Lindeman6, David C. Christiani2, Xihong Lin3, Geoffrey I. Shapiro1,2, Pasi A. Jänne1,7, Bruce E. Johnson1,7, Matthew Meyerson1,15, David J. Kwiatkowski8, Diego H. Castrillon14, Nabeel Bardeesy16, Norman E. Sharpless10,11,12 & Kwok-Kin Wong1,7
EGFR Leu858Arg Mutation POSITIVE

EGFR Exon 19 deletion: Negative
EGFR Leu858Arg mutation: Positive

Patient will respond to Erlotinib or Gefitinib

--personalized medicine----
Adenocarcinoma with lipedic pattern

Mucinous

K- Ras Mutation

Non – Mucinous

EGFr Mutation
What Did we Learn

• NGS requires special cutting (cannot cross contaminate)

• Block has to be cut at the time of request—cannot use older slides (DNA degrades)

• How Much to Cut:
  – NGS – 10 SIDES

• Alcohol based transport medium for cytology samples often provide a more useful information
Take Home Points

• Morphology is the Key
• Understand your clinical teams well
• Judicious utilization of IHC for sample preservation
• Utilization of Powerful molecular techniques will help clinical teams tailor therapies for their patients.