Update in Respiratory Cytology

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Outline

- Specimens and preparations
- Screening for lung cancer
- Intra-operative cytology
- Endoscopic ultrasound guided FNA
- EGFR evaluation
- Pitfalls in effusion cytology
Routine Specimens

- Sputum
  - Productive/induced/post bronchoscopy
- Bronchoscopy specimens
  - Brush/wash/lavage
  - Transbronchial Fine Needle Aspirate
- Percutaneous Fine Needle Aspirate
- Pleural fluid

Conventional Cytology: fixation

- Exfoliative cytology
  - 50% ethanol/Saccamanno fixative
- Fine needle aspirates
  - Direct smear: air dried/alcohol fixed
- Ancillary studies
  - Cell block/flow cytometry/electron microscopy
Conventional cytology: preparations and staining

• Preparations
  • Direct smear
  • Cytospin
  • Cell block/pellet

• Stain
  – Fixed: Papanicolaou
  – Air dried: Romanowsky
  – Hematoxylin and eosin/IHC
Cytologic evaluation of pulmonary carcinoma

Liquid Based Cytology

- Thinprep® and Surepath®
- Suitable for respiratory specimens
- Used alone or in addition to conventional preparations
- Thinprep fixative: Cytolyte®
- Widely used, but literature is scant
Thinprep Performance

- Insufficient quality studies
- At least equal to conventional methods
- Sputum and percutaneous FNA sensitivity increased by 20%*
- Sensitivity improved with the addition of conventional preparations

Thinprep Advantages

• Improved slide quality
  – Decreased obscuring blood, mucus, diathesis, air drying, and smear artifact
  – Better cell preservation and staining
• Increased cellularity and number of diagnostic cells
• Decreased screening time
• Better utilization of personnel
Thinprep Advantages

- Specimen shelf life = 3 weeks
- Additional Papanicolaou slides
- Histochemistry
- Immunohistochemistry
- Cell block preparation
Thinprep Disadvantages

• Technical problems
  – Cellular fluids: central dropout

• Cytologic features altered
  – Loss of background
  – Loss of cell cohesion
  – Cell shrinkage
  – Artifactual clustering

• Increased cost
Thinprep®: small cell carcinoma

- Cleaner background
- Fewer cells
- Single cells
- Loss of spindling
- Decreased molding
- Increased cytoplasm
- Better preserved chromatin

Screening for lung cancer

- Goal: detection of early stage disease
- No appropriate tool available
- Screening with CXR and/or sputum cytology tested in several RCTs in 1970-80
  - Increased detection of early stage cancers
  - Increased surgical rates
  - No impact on frequency of advanced cancers and mortality

Current Investigations

• Low resolution CT imaging of thorax
  – 3 times more sensitive than CXR in detecting small stage I cancers
  – RCT results due after 2009

• Breath Biomarker Assays
  – Volatile organic compounds
  – Microsatellite DNA alterations
Sputum based genetic susceptibility screening

- Moderate dysplasia and higher
  - increased cancer risk
- NSCLC: FISH mapping
  - High levels of deletions
    - 3p22.1 (GC20, RPL 14, CD39A, PMGB)
    - 10q22.3 (SP-A)
      - reflect altered DNA repair

Cancer detection by abnormal sputum cytology and FISH

- Sensitivity:
  - Sputum: 37%
  - Sputum and FISH: 74%

- Specificity:
  - Sputum: 87%
  - Sputum and FISH: 82%
Intra-operative Cytology

• Indications
  – Masses not amenable to pre-operative or intra-operative biopsy

• Used to guide immediate management

• Requirements
  – Rapid
  – Accurate
Intra-operative Cytology

• Preparations
  – Smear
  – LBC

• Fixation and staining
  – Air dried: Giemsa (Diff Quick)
  – Alcohol: Papanicolaou/H&E
4 preparations: interpretative agreements amongst 3 pathologists

DS=direct smear
TP=thinprep®
IOC preparations: turnaround times

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Time (minutes)</th>
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<tbody>
<tr>
<td>DS-H&amp;E</td>
<td>9.2</td>
</tr>
<tr>
<td>DS-Pap</td>
<td>35.2</td>
</tr>
<tr>
<td>TP-H&amp;E</td>
<td>20.4</td>
</tr>
<tr>
<td>TP-Pap</td>
<td>48.5</td>
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</tbody>
</table>
IOC preparations: limited and unsatisfactory specimens

- DS-H&E: 38%
- DS-Pap: 28%
- TP-H&E: 56%
- TP-Pap: 4%
Thyroid papillary carcinoma: 4 IOC preparations
Endoscopic ultrasound guided staging of NSCLC

- Stage I/II: surgery
  - Stage I: node negative
  - Stage II: +peribronchial and hilar nodes
- Stage III: combined chemo and XRT
  - +mediastinal nodes
- Mediastinal node assessment determines management

Staging methods

- Cross sectional CT imaging
- PET scanning
- Mediastinoscopy
- Thoracoscopy
- Transbronchial FNA
- EUS guided FNA
EUS FNA

- Introduced in the 1990s
- Evaluates mediastinal lymph nodes, left adrenal and liver
- Requirements
  - Trained endosonographer
  - Linear array echoendoscope
  - Cytopathology personnel
EUS FNA

Indications
- Enlarged or PET + mediastinal nodes and adrenal
- Normal nodes: controversial

Echoendoscope
EUS Cytology

- 22 gauge needle: no suction
- 3-4 passes per node
- On site evaluation
  - Direct smear/Diff quick
- No on site evaluation
  - LBC/other medium
- Specimen adequate if lymphocytes present
- Additional samples in LBC and RPMI
EUS FNA: performance compared to CT and PET

- **Sensitivity**
  - CT: 57%
  - PET: 73%
  - EUS: 94%

- **Specificity**
  - CT: 74%
  - PET: 83%
  - EUS: 71%

- **PPV**
  - CT: 70%
  - PET: 79%
  - EUS: 92%

- **NPV**
  - CT: 62%
  - PET: 75%
  - EUS: 79%
EUS FNA: advantages and limitations

- More sensitive than CT, PET and TB FNA
- More sensitive in posterior node evaluation
- Complications rare
- Less invasive than mediastinoscopy and thoracoscopy
- More cost effective than mediastinoscopy
- Limited in evaluating direct mediastinal spread and anterior nodes
Predictive markers: molecular testing using cytological material

Anti cancer therapy
• inhibits the pathway

Protumorigenic pathway
• activated by mutation or amplification

Cytology samples and genetic alteration testing
• DNA and RNA based
  - alcohol fixation
• FISH based
  - formalin fixation

Indications
• First or second line therapy

Papadopoulos. Nat Biotechnol 2006; 24: 985
EGFR and NSCLC

EGFR
Cell surface protein
Binds EGF
Activates TK

↓

Cell proliferation
Enhanced survival

TK inhibitors
Gefitinib: Iressa
Erlotinib: Tarceva

NSCLC EGFR mutations and amplification

Tumor regression
EGFR and NSCLC

- **TKI responders**
  - 40-50% adenocarcinomas - Asia
  - 20% adenocarcinomas - USA
  - Adenocarcinoma with a BAC component
  - Never smokers
  - Women

- **Non responders**
  - Kras mutations

EGFR mutation analysis

- All adeno/adenosquamous carcinomas
- Fresh FNA sample or paraffin embedded cell block material
- >50% cells = carcinoma
- PCR based assay for exon 19 and 21 deletions and KRAS if negative
- Good concordance between cytology cell block and surgical sample
  - More study needed
Pitfalls in Pleural Cytology

- **False positive results**
  - Benign imposter mimicking malignancy
  - Benign mesothelial cells mimicking malignancy

- **False negative results**
  - Malignant cells mimicking mesothelial cells.

- **Classification error**
  - One type of malignancy mimicking another

Boerner. PCR 2006; 11: 85,
Benign imposters

- Conventional cytology teaching:
  - Foreign = malignant
- Sources
  - Mullerian epithelium
    - Endosalpingiosis
    - Endometriosis
  - Rare cells
    - Megakaryocyte
  - Procedural pickup
    - Skin adnexae
Benign cells: malignant mimicry

- Reactive mesothelium
  - Pneumonia
  - Pulmonary infarct
  - Collagen vascular disease
  - XRT and chemotherapeutic effects
  - Cirrhosis/hepatic failure
  - Renal failure/dialysis
Malignant Mimicry: reactive mesothelium

- 3D clusters
- Large cells
- Cell pleomorphism
- Cytoplasmic vacuoles
- Large nuclei
- Bi and multinucleation
- Hyperchromasias
- Coarse chromatin
- Large nucleolus
- Mitoses
Malignant Cells: benign mimicry

• Feature
  – Single cells
    • Malignant melanoma
  – Very few cells
    • Breast carcinoma
  – Low grade morphology
    • Ovarian serous carcinoma
    • Malignant mesothelioma
Benign Mimicry: metastatic ovarian serous carcinoma

- 3D groupings
  - >3 cells per group
  - cohesive cell borders
- Uniform population of cells
- Irregular enlarged nucleoli
- Necrosis/apoptosis
- Mitoses
Benign Mimicry: mesothelioma

- Cell balls: >20 cells per cluster
- Single cells
- Scalloped border
- Cytoplasmic skirt
- Regular nuclear contours
- Multinucleation
- Prominent nucleoli

Bhatti. PCR 2006; 11: 67.
Immunohistochemistry

- Avoid misclassification error
- Identify the unknown primary site
- Cell block material is preferable
- Antibody selection
  - Guided by morphology
  - Immunoquery®: valuable resource
  - Scant material: judicious selection
Benign versus malignant mesothelial cells

- **XIAP**
  - X linked inhibitor of apoptosis
- **Granular cytoplasmic stain**
- **Focal pattern**
  - 11% benign effusions
  - 80% mesotheliomas
  - 51% adenocarcinomas
- **Malignant performance**
  - Sensitivity 54%
  - Specificity 89%
- **More study needed**

Mesothelioma versus metastatic carcinoma

- Best probability score
- 2 antibody panel
  - Calretinin
  - Ber-EP4
- Other mesothelial markers
  - Mesothelin
  - CK5/6
  - D2-40
  - WTI

Metastatic carcinoma of unknown origin

- 7 most frequent sites
  - Breast, lung, colon, prostate, pancreas, ovary and stomach
- TMA evaluation: 10ABs
- Evaluate in a specific sequence to maximize predictive value
- Site correctly identified in 87% of cases
- Likely higher if clinical and pathology included

# Metastatic carcinoma of unknown origin: tabular approach

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Carcinoma</th>
<th>Se%</th>
<th>Sp%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA</td>
<td>Prostate</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>TTFI</td>
<td>Lung</td>
<td>91</td>
<td>98</td>
</tr>
<tr>
<td>CDX2</td>
<td>Colon</td>
<td>83</td>
<td>96</td>
</tr>
<tr>
<td>CDX2</td>
<td>Colon and stomach</td>
<td>56</td>
<td>98</td>
</tr>
<tr>
<td>CK20</td>
<td>Colon</td>
<td>68</td>
<td>91</td>
</tr>
<tr>
<td>CK20</td>
<td>Colon, stomach and pancreas</td>
<td>36</td>
<td>97</td>
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<tr>
<td>GCDFP-15</td>
<td>Breast</td>
<td>54</td>
<td>96</td>
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<tr>
<td>ER</td>
<td>Breast and ovary</td>
<td>74</td>
<td>95</td>
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<tr>
<td>CA125</td>
<td>Ovary and pancreas</td>
<td>88</td>
<td>88</td>
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<tr>
<td>Mesothelin</td>
<td>Ovary and pancreas</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Stomach and pancreas</td>
<td>65</td>
<td>69</td>
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<tr>
<td>CK7</td>
<td>Stomach and pancreas vs. colon</td>
<td>72</td>
<td>96</td>
</tr>
</tbody>
</table>

Unknown primary site: specificity based decision tree

Negative

- PSA
- TTF1
- GCDFP-15
- CDX2 or CK20
- ER
- CA125
- mesothelin
- lysozyme

Positive

- prostate
- lung
- colon
- breast
- stomach or pancreas
- ovary

Breast or stomach or pancreas

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References


Bavikatty NR and Michael CW. Cytologic Features of Small-Cell Carcinoma on Thin-Prep, Diagn Cytopathol 2003; 29: 8-12.


