Role of Thoracic Cytopathology in the 2015 Molecular Era

Dr Julie McCarthy
Cork University Hospital
And the answer is;

- To make a diagnosis!
- To make an accurate diagnosis
- To collect sufficient material for ancillary studies
- To triage material appropriately for ancillary studies
- To perform molecular tests for common mutations on well preserved material and to preserve material for future testing
- To integrate the results of ancillary studies with morphology and immunocytochemistry so as to inform the patient and the clinician of accurate diagnosis, prognosis, molecular profile and likely response to targeted therapy
Making a diagnosis

- Training in cytology
  Still the most common cause of failing the MRCPath II amongst Irish trainees

<table>
<thead>
<tr>
<th></th>
<th>total</th>
<th>pass</th>
<th>fail</th>
<th>fail cyto</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>2015</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>1</td>
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</table>
Making a diagnosis

- Fellowship style training
- Rotation of senior trainees into cytology for several months at a time
- Competency in several areas signed off
- Including ROSE in bronchoscopy suite
- Sub-specialisation?
Making an accurate diagnosis

- Good quality material (dual responsibility from respiratory clinicians or radiologists and pathologists)
- You can’t make a silk purse out of a sow’s ear
ROSE

- Rapid On Site Evaluation

- “I once had a rose named after me and I was very flattered. But I was not pleased to read the description in the catalogue: no good in a bed, but fine up against a wall.”

Eleanor Roosevelt 1884-1962
Making an accurate diagnosis

- ROSE is ideal, not essential

<table>
<thead>
<tr>
<th></th>
<th>TBNA</th>
<th>ROSE</th>
<th>No ROSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>140/294 (48%)</td>
<td>154/294 (52%)</td>
<td></td>
</tr>
<tr>
<td>Concordance of On Site with Final diagnosis</td>
<td>104/140 (74%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Diagnostic specimen obtained</td>
<td>132/140 (94%)*</td>
<td>138/154 (90% )</td>
<td></td>
</tr>
<tr>
<td>Cell Block Obtained</td>
<td>129/140 (92%)*</td>
<td>136/154 (88% )</td>
<td></td>
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</table>
ROSE

- Consensus is that there are fewer passes and fewer inadequate samples with fewer complications
- Arguably cost effective?
- Draft NCCP Lung Cancer Guidelines June 2015 state
  - “EBUS Rose should be made available whenever resources permit”
- Gives the pathologist “control” of the material, including fresh material for flow cytometry (lymphoma) and culture
Calling a sample adequate

- Danger of reporting very scanty samples as NEGATIVE rather than INADEQUATE
- Improve the Negative predictive value of EBUS from 33-78%
Adequate versus inadequate

Balancing the false negative rate with the true negative and Inadequate rates

Require 3-5 adequate negative passes per site, plateau thereafter

Require 3 granulomatous passes per site for “Sarcoid like adenopathy” in a cancer patient

EBUS false negative rate zero 2014
EUBS inadequate rate 7.8% 2014
Did I tell you the one about the inadequate sample?

The needle was in the node but the node wasn't in the needle…
Definition of adequacy

- Sufficient to satisfy a requirement or meet a need
  *(free dictionary)*
- Representative of the lesion
- No universally agreed criteria for adequacy in a negative sample
- 40 lymphocytes in high power field at EBUS, or clusters of anthracotic laden macrophages are required
- Difficulty in assessing adequacy in a liquid based preparation with pooled passes
- Recently “adequacy” has been redefined- to include sufficient material for molecular studies, USCAP 2015
Photo of anthracotic macrophages in pap
Accuracy of cytological diagnoses

- Correlate with histology; the old fashioned method
- Only see the histology in N1 and some N2 nodal excisions
- Often; patient is diagnosed, staged and managed without corresponding histology
Mom, please put the scissors down

I've had enough of this crap
Accuracy of cytological diagnoses

- EQA; no national scheme for cytology….
  - Z stacking technology will assist for digitised images.

- National Quality Improvement Programme established to monitor KPIs and KQIs in Histo and Cytopathology (including TAT, Intradepartmental consultation rate, MDT correlation and amended report rates etc)
Background to the QI Programme

Need for Formal Measures of Quality Improvement In Histopathology

- High Profile cancer misdiagnosis cases in 2007 & 2008
- No formal measures to reassure the public that Irish Histopathology Laboratories provide a quality service to the highest international standards
- No set national standards or benchmarks for key aspects of diagnostic service
Vision of National QI Programme

A patient centred Quality Improvement framework within each department, which routinely reviews performance and drives improvement, in key quality areas against intelligent targets.
### At a Glance – Intradepartmental Consultation

<table>
<thead>
<tr>
<th>Category</th>
<th>% cases</th>
<th>Snapshot % cases</th>
<th>Trend % cases</th>
<th># cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases with intraD consult. (Q006)</td>
<td>6.26</td>
<td></td>
<td></td>
<td>154</td>
</tr>
<tr>
<td>Histology cases with intraD consult. (Q006)</td>
<td>5.81</td>
<td></td>
<td></td>
<td>168</td>
</tr>
<tr>
<td>Cytology cases with intraD consult. (Q006)</td>
<td>12.59</td>
<td></td>
<td></td>
<td>21</td>
</tr>
</tbody>
</table>
Targets in progress – Multi Disciplinary Team Meetings (Tumor Boards) 2013/14
Non Gynaecological Cytology FNA (P06) TAT Percentage Completed by day 5 - per month
All Hospitals (Inc. CC/NonCC Split)

National NQAIS Report 2014
Accuracy of cytological diagnosis

- Locally; concordance rates for cytology samples with concomitant histology or excision samples are audited periodically.

- Audit should assist with identification of methods to preserving tissue and avoidance of duplication of ICC stains, ensuring good communication.

Cost and tissue saving
Cleveland Clinic Ohio algorithm 2015

Determining Tumor Type in Small Specimens

Non-small cell carcinoma

Morphologic features present

Adenocarcinoma

Squamous cell carcinoma

NSCLC, NOS
TTF-1, Napsin A, p63, CK5/6, (p40)

TTF-1, Napsin A +

NSCLC favor Adenocarcinoma

p63, p40, CK5/6 +

NSCLC favor Squamous cell carcinoma

No further testing

EGFR mutational analysis and ALK-1 testing
Algorithm for immunocytochemistry

Malignant cells NOS

- AE1AE3, LCA, SOX10, S100, Calretinin (CD34, CD30, OCT3/4)

Carcinoma confirmed

- Neuroendocrine markers
  - P40 (rarely CK5/6)
  - TTF-1 (rarely Napsin A)

Non small cell ca
- Squamous cell ca by ICC

Non small cell ca
- Adenocarcinoma by ICC
Tissue sparing immunocytochemistry
Other tumours
Melanoma at
Sarcoma at
Lymphoma at
<table>
<thead>
<tr>
<th>2013 modality</th>
<th>number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchoscopy NOS</td>
<td>68 (26%)</td>
</tr>
<tr>
<td>CT biopsy</td>
<td>61 (24%)</td>
</tr>
<tr>
<td>EBUS EUS TBNA</td>
<td>48 (19%)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>14 (5%)</td>
</tr>
<tr>
<td>VATS</td>
<td>21 (7%)</td>
</tr>
<tr>
<td>FNA superficial LN</td>
<td>14 (5%)</td>
</tr>
<tr>
<td>Sputum</td>
<td>9 (4%)</td>
</tr>
<tr>
<td>Other biopsy</td>
<td>7 (3%)</td>
</tr>
<tr>
<td>Clinical (no tissue)</td>
<td>17 (7%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>259</strong></td>
</tr>
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</table>
## Pleural fluid cytology 2013 (N=320)

<table>
<thead>
<tr>
<th></th>
<th>inadequate</th>
<th>benign</th>
<th>atypical</th>
<th>suspicious</th>
<th>malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 (4%)</td>
<td>225 (70%)</td>
<td>12 (4%)</td>
<td>7 (2%)</td>
<td>64 (20%)</td>
</tr>
</tbody>
</table>

**Malignant subtypes**
- 74% Adenocarcinoma
- 8% Malignant NOS
- 8% Squamous carcinoma
- 6% Melanoma
- 3% Lymphoma
- 1% Small cell carcinoma
Cytology specimen; **Exfoliative/Effusions**

**advantages**
- Minimally invasive procedure
- Broad sampling (good for screening/staging)

**disadvantages**
- May have low percentage of tumour cells
- May have numerous interfering non-tumour cells (e.g. mesothelial cells).
## Sputum 2013

<table>
<thead>
<tr>
<th>Outcome</th>
<th>C5</th>
<th>C4</th>
<th>C3</th>
<th>C2</th>
<th>total</th>
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</thead>
<tbody>
<tr>
<td>Malignant</td>
<td>27</td>
<td>8</td>
<td>10</td>
<td>14</td>
<td>59</td>
</tr>
<tr>
<td>Benign</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>49</td>
<td>57</td>
</tr>
<tr>
<td>total</td>
<td>27</td>
<td>10</td>
<td>16</td>
<td>63</td>
<td>116</td>
</tr>
</tbody>
</table>
sputum

- 90% of positive samples were from stage IV patients
- 51% of positive cases were squamous cell ca, 43% adenoca, 6% small cell ca
- 2 samples underwent EGFR testing- wild type
- Not surprisingly- inadequate for ALK
sputum

- PPV of C5 is 100%, C4 90%, C3 63%
- Low sensitivity, may be improved by multiple samples (3-5)
- Worth collecting in frail high stage patients
- Suitable for molecular testing if cellular
Purifying samples for molecular tests

LymphoPrep for lymphocytic fluids

Commercial kits
HIGH QUALITY DNA FROM ANY SAMPLE
Including challenging samples like urine, plant, and soil

Genomic DNA Isolation Kit

For the isolation of genomic DNA from animal tissues, cells, bodily fluids and swabs

- Isolate genomic DNA from animal tissues, cells, bodily fluids and swabs
- No phenol required for isolation
- Rapid and convenient spin column procedure
- Purified DNA is of the highest quality and integrity for sensitive downstream applications

Protocol Version: Protocol versions are based on the kit label. To download the correct version of the protocol, click here to view options.

PROTOCOL
Genomic DNA Isolation Kit Bar code Version - Protocol
PDF version
Genomic DNA Isolation Kit No Barcode Version - Protocol
PDF version

PRODUCT INFORMATION SHEETS
Genomic DNA Isolation Kit - Product Information Sheets
PDF version

MSDS
Genomic DNA Isolation Kit - MSDS
Cytology specimen: FNA Aspiration

**Advantages**
- Typically high percentage of tumour cells
- Low proportion of interfering non-tumour cells

**Disadvantages**
- Occasionally difficult to access
Suitability of cytology preparations for molecular studies

- Better quality DNA as less denatured if not formalin fixed
- Suitable samples include
  - air dried slides
  - ThinPrep slides
  - Cytospins (air dried for CISH, FISH)
  - Cell blocks

*Note; caution when using alcohol fixation for FISH*
Preparation type: Cell block

**Advantages:**
- Suitable for all sample types
- Easy to transport (formalin fixed block)
- Assessment of presence and quality of tumour in tested sample

**Disadvantages:**
- May be low tumour percentage (esp. fluids, tumour enrichment required)
- Degradation of nucleic acids due to formalin fixation
- Partial nuclei in standard 4-5 um sections
- Heterogeneous distribution of tumour and non-tumour
- Variable methods for preparation

*Favoured by guidelines*
Preparation Type: Liquid Based

Advantages:
- Ease of use
- Can use preservative designed for optimal preservation of nucleic acids
- Nuclei whole – results in higher nucleic acid yields

Examples:
- HPV analysis by FISH on cervical cytology
- Other sites: thyroid, urine, pancreatic

In Practice:
Used to augment cytology diagnosis not replace, requires validation of every type
Preparation Type: Direct Smear

**Advantages:**
- High quality of nucleic acids (Romanowsky stain > Pap stain)
- Immediate assessment for tumour adequacy
- May be highly cellular
- Assessment of presence and quality of tumour in tested sample

**Disadvantages:**
- Requires technical skill to prepare (ROSE)
- May not be suitable/validated for some tests (e.g. FISH)
- Must sacrifice the slide for testing: loss of slide from the diagnostic archive, consider digital archive
Cytology samples for EGFR testing

traditional cell block material

- 340 specimens sent for testing (2009-2012)
  - 155 were cytology and 185 were histology

- 28 cases showed mutations (8%)
  - 12 were cytology, 14 histology, 2 not recorded

- 19 cases were “insufficient” for testing
  - 10 were cytology and 9 were histology
Cytology samples for ALK testing

*traditional cell block material*

- Limitations in the amount of material for ALK immunocytochemistry/FISH when this is requested subsequent to EFGR and ICC

- Significantly more “insufficient” cytology samples than histo; related to sample type (sputum and washings) and ICC (average number 3.6 cut)

- Challenging for future markers ie ROS1, Met etc
Electromagnetic guidance systems to aid bronchoscopy have shown a diagnostic sensitivity of 60%, specificity of 91%, PPV of 95%, NPV of 42% and overall accuracy of 67% (NICE, 2011). Electromagnetic guidance used in conjunction with EBUS had a higher diagnostic yield (88%) compared to EBUS alone (69%) (Eberhardt et al., 2007)

New driver mutations ROS1 and RET for adenocarcinoma will require more tissue, or better use of tissue
Multiple options & methods

- We only require the skill to know what to do and how to do it……

- That is the role of the Cytopathologist in the 2015 Molecular era!
end