ENDOSCOPIC ULTRA-SOUND GUIDED FNA OF GI TRACT AND PANCREAS

Prof. Fernando Schmitt
Medical Faculty of Porto University, Porto, Portugal
IPATIMUP
General-Secretary of the International Academy of Cytology
Modern Medicine

- Surgery is less invasive.
- Image guidance is fundamental to achieve the right target.
- Availability of cells or tissues from patients are crucial to identify molecular changes or surrogate markers important for diagnosis, prognosis or therapeutic response.

Cytopathology has a central role in modern medicine.
Endoscopic Ultrasound-guided Fine Needle Aspiration (EUS-FNA)

• EUS-FNA has been increasingly used for the assessment of diverse intra-abdominal and intra-thoracic tumours.

• EUS-FNA is a key component of the evaluation of both extramural and intramural structures of GI tract, allowing determination of origin of the lesion and cytological sampling.
Aspirates of masses in pancreatic head are performed through the duodenum, and those in the body and tail via the stomach.
Gastrointestinal Contamination

- Flat honeycomb pattern of cells
- Sporadically goblet cells

- Small sheets of cells
- Foveolar cells with mucin
Normal pancreas
Histological classification of tumours of the pancreas

### I. Epithelial neoplasms

#### A. Exocrine neoplasms

1. Serous cystic neoplasms
2. Mucinous cystic neoplasms
   - a) mucinous cystadenoma
   - b) mucinous cystadenoma with moderate dysplasia
   - c) mucinous cystic neoplasm with carcinoma in situ
   - d) mucinous cystadenocarcinoma
3. Intraductal papillary mucinous neoplasms
   - e) IPMN-adenoma
   - f) IPMN with moderate dysplasia
   - g) IPMN with carcinoma in situ
   - h) IPMN with an associated invasive carcinoma
   - i) Intraductal oncocytic papillary neoplasm
4. Invasive ductal adenocarcinoma and variants
5. Acinar cell neoplasms

### B. Endocrine neoplasms

1. Adenoma (<0.5cm)
2. Well-differentiated pancreatic endocrine neoplasm of uncertain malignant potential
3. Malignant well-differentiated pancreatic endocrine neoplasm
4. Small cell carcinoma

### C. Epithelial neoplasms (of uncertain differentiation)

1. Solid pseudopapillary neoplasm
2. Pancreatoblastoma

### D. Miscellaneous

1. Teratoma
2. Lymphoepithelial cyst
Ductal Adenocarcinoma
Variants of ductal adenocarcinoma

Foamy adenocarcinoma

Undifferentiated adenocarcinoma

Undifferentiated adenocarcinoma
With osteoclast-like giant cells

Adenosquamous carcinoma
Well-differentiated ductal adenocarcinoma

Nuclear crowding and overlap
Anisonucleosis
Irregular nuclear membrane

“Drunken honeycomb pattern”

Gastric epithelium

Benign glandular epithelium
EUS-FNA
Ancillary Studies

- Special staining.
- Immunocytochemistry.
- Flow cytometry
- Molecular techniques.
Ancillary Studies in Cytology

Challenges

• To select the correct test for a limited sample quantity.

• Avoid to jump from a histological adapted technique directly to cytological material.

• Use appropriate controls for cytological material.
Liquid-Based Cytology in DNA-Based Molecular Research

Viability and Potential Application

Adhemar Longatto Filho, M.Sc., Ph.D., P.M.I.A.C.,
Alberto Emanuel Pinheiro Gonçalves, B.Sc., Olga Martinho, B.Sc.,
Fernando C. Schmitt, M.D., Ph.D., F.I.A.C., and Rui Manuel Reis, Ph.D.

FP4.122
ASSESSMENT OF DNA, SMALL NCRNA AND MRNA DETECTION OVER TIME IN LIQUID-BASED CYTOLOGY SPECIMENS
J. Wohlschlaeger*, K. Worm*, F. Schmitt†, B. Davidson‡ and M. Tötsch*
*Department of Pathology and Neuropathology, University Hospital of Essen, University of Duisburg-Essen, Germany, †Institute of Molecular Pathology and Immunology and Medical Faculty, University of Porto, Portugal, ‡Division of Pathology, Norwegian Radium Hospital, Rikshospitalet University Hospital, Oslo, Norway
Formalin post-fixed in rehydrated air-dried slides brings reliable and standardized results in ICC.


<table>
<thead>
<tr>
<th>Table 1. Pros and Cons of Cytology Preparation Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pros</td>
</tr>
<tr>
<td>Direct Smear</td>
</tr>
<tr>
<td>May do when no extra material</td>
</tr>
<tr>
<td>No wet material needed</td>
</tr>
<tr>
<td>Can use what available slides were initially obtained (no expense to extra preparations)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Cytospins</td>
</tr>
<tr>
<td>Useful with limited material</td>
</tr>
<tr>
<td>Panels possible</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Monolayer Preparations</td>
</tr>
<tr>
<td>Possibly decreased background</td>
</tr>
<tr>
<td>Extra material frequently available and easily stored</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Cell Block</td>
</tr>
<tr>
<td>Immunohistochemistry laboratory can handle like routine material with proper controls</td>
</tr>
<tr>
<td>Material easily stored</td>
</tr>
</tbody>
</table>
Reduction of variability by using automation, appropriate controls and customized dilution of antibodies according to different samples could improve the quality of ICC and standardize the techniques, along with quality control and quality assurance measures.
Documentation of Immunocytochemistry Controls in the Cytopathologic Literature: A Meta-Analysis of 100 Journal Articles


- 100 articles
- Last 15 years
- 9 most used antibodies

ICC Controls in the Literature

- Absent 54%
- Identical 13%
- Vague 18%
- Other 15%
Molecular cytopathology (MCP) can be defined as molecular studies applied on all types of cytological specimens, namely gynecology cytology, exfoliative non-gyn cytology and fine needle aspirates.
K homology domain containing protein overexpressed in cancer (KOC) is an oncofetal RNA binding protein expressed in the majority of PDA.

- Regulates IGFRII and proliferation.

- ICC study in previous Pap-stained slides showed 100% of specificity for malignancy and negativity for benign ductal epithelium and IPMN cases, including IPMN with high grade dysplasia.
S100P belongs to the family of S100 calcium-binding proteins. It is a 95 a.a protein and was first purified from the placenta.

It is normally present in epithelial cells throughout entire GI tract, but normal pancreatic ductal epithelia and liver usually lack the expression of S100P protein.

The level of S100P expression has been found to increase during the progression from pancreatic intraepithelial neoplasia (PanIN) to invasive adenocarcinoma. Hypomethylation of its gene in PDA is suggested to account for the overexpression of protein.
**Usefulness of S100P in Diagnosis of Adenocarcinoma of Pancreas on Fine-Needle Aspiration Biopsy Specimens**

Hongbing Deng, MD, PhD, Jianhui Shi, MD, PhD, Myra Wilkerson, MD, Steven Meschter, MD, William Dupree, MD, and Fan Lin, MD, PhD

*Am J Clin Pathol* 2008;129:81-88
DOI: 10.1309/5076NDE81E8G545

### Summary of S100P Immunohistochemical Staining Results From Present Study

<table>
<thead>
<tr>
<th>Cytologic Diagnosis</th>
<th>No. of Cases</th>
<th>Type of Specimen</th>
<th>S100P Staining</th>
<th>Follow-up Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CB</td>
<td>Smear</td>
<td>Positive</td>
</tr>
<tr>
<td>PDA</td>
<td>32</td>
<td>26</td>
<td>23</td>
<td>32</td>
</tr>
<tr>
<td>Atypical/&quot;suspicious&quot;</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Benign/reactive</td>
<td>14</td>
<td>10</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Endocrine tumor*</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

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![Image of histological slides](image-url)
EUS-FNA with rescue fluorescence in situ hybridization for the diagnosis of pancreatic carcinoma in patients with inconclusive on-site cytopathology results.

- Multiprobe FISH to study the ploidy status for chromosomes 3, 7, 17 and deletion of the 9p21 locus

Kubiliun N et al. Gastrointest Endosc 2011
EUS-FNA with rescue fluorescence in situ hybridization for the diagnosis of pancreatic carcinoma in patients with inconclusive on-site cytopathology results.

69 inconclusive cases

- 54 Malignant
- 15 Benign

FISH detected an additional 13 cases of pancreatic adenocarcinoma missed by cytology.

Sensitivity for malignancy

- Cytology: 61%
- FISH: 74%
- Both: 85%

Kubiliun N et al. Gastrointest Endosc 2011
# Pancreatic solid cellular neoplasms

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Pancreatic endocrine neoplasm</th>
<th>Acinar cell carcinoma</th>
<th>Solid pseudopapillary neoplasm</th>
<th>Pancreatoblastoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40–60 years old M = F; 50%</td>
<td>Adults in their 60s ≫ children; M:F 20–30 years old F ≫ M = 4:1; lipase hypersecretion syndrome in some</td>
<td>2–3 years old ≫ 40 years old M = F; 50% Asian</td>
<td></td>
</tr>
<tr>
<td>CT/EUS features</td>
<td>Round, generally small (1–2cm) well-defined and circumscribed solid, occasionally cystic masses</td>
<td>Well-defined and circumscribed, often lobulated homogeneous solid, occasionally cystic masses, avg. 10cm</td>
<td>Well-defined and circumscribed solid and cystic masses, avg. 10cm; often in the tail</td>
<td>Well-defined and circumscribed masses, avg. 10cm</td>
</tr>
</tbody>
</table>
Pancreatic solid cellular neoplasms

Acinic cell carcinoma

Squamoid corpuscle

Pancreatoblastoma

Pancreatic endocrine neoplasm
Diagnostic approach to pancreatic tumors with the specimens of endoscopic ultrasound-guided fine needle aspiration.

<table>
<thead>
<tr>
<th></th>
<th>CK 7</th>
<th>CDX 2</th>
<th>NE markers</th>
<th>KRAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>NE tumors</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Acinic cell tumors</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
</tr>
</tbody>
</table>

Hosoda W et al. Pathol Int 60: 358, 2010
<table>
<thead>
<tr>
<th>Likely Diagnosis</th>
<th>CA 19-9</th>
<th>CEA</th>
<th>Chromogranin</th>
<th>α-Chymotrypsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ductal adenocarcinoma</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acinar cell</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Mucinous LMP</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Islet cell type</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Solid pseudopapillary type</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Microcystic adenoma</td>
<td>?</td>
<td>?</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Benign pancreatic cysts (if benign cells present)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>?</td>
</tr>
</tbody>
</table>
Solid pseudopapillary pancreatic tumour
Pancreatic Cystic Lesions

• Pseudocysts

- Non-mucinous
  - Serous cystoadenoma (VHL)
  - Cystic endocrine tumours
  - Rare lesions

• Cystic neoplasms.

- Mucinous
  - Benign
  - IPMN
  - Malignant
Pancreatic Cystic Lesions

• The clinical management of these lesions is evolving as we learn more about the biologic behaviour of these cysts.

• Management is based on the preoperative distinction of nonmucinous and mucinous cysts in general and of benign and malignant in particular.

• Treatment is increasingly nonsurgical even when neoplasia is confirmed.
  
  ➢ So, accurate preoperative diagnosis is critical to proper patient management.
  
  ➢ Only (cyto)morphology has low sensitivity
  
  ➢ We need ancillary tests
Optimal Preparation of EUS-FNAB of Cystic Masses

• **Direct Smears** (if thick and of sufficient quantity)
  - Alcohol fixed
  - Air dried

• **Fresh Fluid: triage volume dependent**
  - Molecular Analysis (0.5cc)
  - Cyst Fluid Analysis (1cc)
  - Cytospins
    • 1 Pap
    • 2 mucin stains: mucicarmine and Alcian Blue pH2.5
  - LBC (ThinPrep and SurePath)
Cytology of Neoplastic Mucinous Cysts (MCN or IPMN)

Thick “colloid-like” mucin on cytology defines a neoplastic mucinous cyst.
Pancreatic Cystic Lesions
Ancillary tests

- Amylase level.
- Tumour markers (CEA, CA 125, KOC).
- Molecular techniques (K-Ras, P53, LOH)
Amylase Levels

• High levels should be seen in
  – Pseudocysts

• Low levels should be seen in
  – Serous cysts
  – LEC

• Variable levels in
  – IPMN
  – MCN
**TABLE 1. Characteristics of cystic neoplasms of the pancreas**

<table>
<thead>
<tr>
<th>Location</th>
<th>Cytology</th>
<th>Viscosity</th>
<th>Cyst fluid CEA, ng/mL</th>
<th>Cyst fluid amylase level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>Evenly distributed</td>
<td>Low</td>
<td>&lt;0.5</td>
<td>Low</td>
</tr>
<tr>
<td>Mucinous</td>
<td>Tail</td>
<td>Increased</td>
<td>&gt;200</td>
<td>Low</td>
</tr>
<tr>
<td>IPMN</td>
<td>Head</td>
<td>High</td>
<td>&gt;200</td>
<td>High</td>
</tr>
<tr>
<td>Pseudocyst</td>
<td>Evenly distributed</td>
<td>Low</td>
<td>&lt;200</td>
<td>High</td>
</tr>
</tbody>
</table>

- CEA cyst fluid analysis is considered the most accurate predictor for the diagnosis of a mucinous cyst with 79.2% accuracy using a threshold level of 192 ng/mL.
Historically, the treatment for all neoplastic mucinous cyst has been surgical excision, however many of them are not malignant and have excellent prognosis.
<table>
<thead>
<tr>
<th>Symptoms, DD, MN or positive cytology</th>
<th>For malignancy</th>
<th>For invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sensitivity</td>
<td>specificity</td>
</tr>
<tr>
<td>AEC only(^1)</td>
<td>0.83</td>
<td>0.57</td>
</tr>
<tr>
<td>CEA &gt;2,500 ng/ml only(^2)</td>
<td>0.50</td>
<td>0.82</td>
</tr>
<tr>
<td>AEC or CEA &gt;2,500 ng/ml</td>
<td>0.93</td>
<td>0.57</td>
</tr>
</tbody>
</table>
Serous Cystadenoma

Head and tail of elderly m or w
Microcystic
CEA and amylase is low

Grossly large and well circumscribed
Multilobulated with fibrous septae

Watery, scant, non-mucinous fluid
Monolayered sheets with bland cells
Association with haemosiderin-laden macrophages

Small cysts lined by glycogen-rich cuboidal epithelial cells
Lymphoepithelial Cyst

- Lymphoepithelial cysts are rare benign cysts lined by squamous epithelium with subepithelial non-neoplastic lymphoid tissue.
- Much more common in men. Mean age of 56 years.
- Are benign with no reported cases of malignant transformation.
- Amylase and CEA are low.
Mucinous Cysts of the Pancreas
(AFIP Classification in 4th series fascicle)

• Mucinous cystic neoplasm
  ➢ Mucinous cystic neoplasm with low grade dysplasia
  ➢ Mucinous cystic neoplasm with moderate dysplasia
  ➢ Mucinous cystic neoplasm with high grade dysplasia (CIS)
  ➢ Mucinous cystic neoplasm with invasive carcinoma

• Intraductal papillary mucinous neoplasm
  ➢ Intraductal papillary mucinous neoplasm with low grade dysplasia
  ➢ Intraductal papillary mucinous neoplasm with moderate dysplasia
  ➢ Intraductal papillary mucinous neoplasm with high grade dysplasia (CIS)
  ➢ Intraductal papillary mucinous neoplasm with invasive carcinoma: tubular type or colloid carcinoma
Genetic alterations in Pancreatic Neoplasia

1. hit

K-ras
Telomerase

2. hit

p16
p53
DPC4
others

3. hit

E-cad
β-cad

years few months? weeks?
K-Ras mutations

EGFR overexpression:
• CRC (27–77%)
• Pancreatic cancer (30–50%)
• Lung cancer (40–80%)
• NSCLC (14–91%)

EGFR mutation:
• NSCLC (10%)
• Glioblastoma (20%)

K-Ras mutation:
• CRC (30–50%)
• Pancreatic cancer (90%)
• Papillary thyroid cancer (60%)
• NSCLC (30%)

B-Raf mutation:
• CRC (10%)
• Melanoma (70%)
• Papillary thyroid cancer (50%)

MAPK
Pancreatic Cystic Lesions
Ancillary tests

Pancreatic cyst fluid DNA analysis in evaluating pancreatic cysts: a report of the PANDA study

Asif Khalid, MD, Maliha Zahid, MD, Sydney D. Finkelstein, MD, Julia K. LeBlanc, MD, Neeraj Kaushik, MD, Nuzhat Ahmad, MD, William R. Brugge, MD, Steven A. Edmundowicz, MD, Robert H. Hawes, MD, Kevin M. McGrath, MD

Pittsburgh, Philadelphia, Pennsylvania, Indianapolis, Indiana, Boston, Massachusetts, St Louis, Missouri, Charleston, South Carolina, USA

- K-ras gene mutation appears to be the best discriminator between mucinous cysts and nonmucinous cysts, but is also present in nonmalignant lesions.
Molecular Analysis of Pancreatic Cyst Fluid

A Comparative Analysis With Current Practice of Diagnosis

Jian Shen, MD, PhD; William R. Brugge, MD; Christopher J. DiMaio, MD; and Martha B. Pitman, MD

Cancer Cytopathology  June 25, 2009

• K-ras
• LOH
• DNA quantity/quality

Molecular Diagnosis

Benign nonmucinous
Benign mucinous
Malignant (in situ or invasive carcinoma)

Criteria

1) DNA quantity/quality low to moderate; AND 2) k-ras gene point mutation not present; AND 3) LOH, <2 genomic loci present

1) DNA quantity/quality: high; OR 2) k-ras gene point mutation present; OR 3) LOH, ≥2 genomic loci present

1) k-ras gene point mutation, high amplitude (>75%); OR 2) ≥2 more LOH, high amplitude (>75%)
Molecular Analysis of Pancreatic Cyst Fluid

A Comparative Analysis With Current Practice of Diagnosis

Jian Shen, MD, PhD; William R. Brugge, MD; Christopher J. DiMaio, MD; and Martha B. Pitman, MD

Cancer Cytopathology  June 25, 2009

- Molecular analysis of pancreatic cyst fluid adds diagnostic value to the preoperative diagnosis with high sensitivity, specificity and positive predictive value for the major cysts classification of malignant, benign mucinous and benign nonmucinous.

- A combination of different ancillary tests is critical for a more accurate diagnosis.
Mucinous Cystic Neoplasm

- Middle aged females
- Body and tail
- 20% have a rim of calcification

- Cysts do not communicate with pancreatic ductal system

- Cysts lined by mucinous, generally non-papillary epithelium

- Associated with a subepithelial “ovarian-like stroma” (not seen in cytology)
- Heterogenous atypia
MCN with invasive carcinoma

- Invasive component can be very focal
- Diagnosis cannot be made from FNA of cyst alone
- Invasion suggested by combination of cytology and EUS features
Intraductal Papillary Mucinous Neoplasm (IPMN)

Main duct IPMN  Branch duct IPMN  Combined IPMN
Intraductal Papillary Mucinous Neoplasm (IPMN)

- Older patients M>F
- Generally in pancreatic head
- Single or multiple cysts
- Cysts do not communicate
- Mostly papillary mucinous epithelium with variable atypia.
- No association with ovarian-like stroma under the epithelium
- LGD (adenoma)
- MD
- HGD (CIS)
In total, we found that GNAS mutations were present in 66% of IPMNs and that either KRAS or GNAS mutations could be identified in 96%.

In eight cases, we could investigate invasive adenocarcinomas that developed in association with IPMNs containing GNAS mutations. In seven of these eight cases, the GNAS mutations present in the IPMNs were also found in the invasive lesion.

GNAS mutations were not found in other types of cystic neoplasms of the pancreas or in invasive adenocarcinomas not associated with IPMNs.
Cytology of Neoplastic Mucinous Cysts (MCN or IPMN)

- Neoplastic mucinous cysts are heterogenous, so cytologic sampling may not be representative and often under-represents the final grade of the neoplasm.
- Most of neoplastic mucinous cysts are currently excised because of the lack of FNA specificity.
- Cytology can suggest subclassification of mucinous cysts.
- Recognition of malignancy most important, but invasion can only be diagnosed by FNAB of cyst wall masses.
Pancreatic Cystic Lesions

- Thick colloid-like mucin even if acellular is sufficient to diagnose a neoplastic mucinous cyst
- Cytology may under-estimate the final histology of mucinous cysts
- Multiple cyst septations, thick cyst wall, mural nodule and background necrosis support a carcinoma
- Cyst fluid without a high amylase is very unlikely to be a pseudocyst
- CEA > 200 ng/ml supports mucinous cyst with very high levels often correlating with carcinoma
- Mucinous cysts with a high amylase supports IPMN over MCN but is there is overlap
- Mucin stains help to highlight thin background mucin
- Molecular analysis may provide helpful diagnostic information for cyst aspirates, especially aspirates of very scant fluid where cytology and cyst fluid analysis are not likely to be processed.
EUS-FNA AND MALIGNANT LYMPHOMAS
# Diagnosis of deep-seated lymphomas by endoscopic ultrasound-guided fine needle aspiration combined with flow cytometry

A. Stacchini*, P. Carucci†, D. Pacchioni‡, G. Accinelli‡, A. Demurtas*, S. Aliberti*, M. Bosco‡, M. Bruno†, A. Balbo Mussetto†, M. Rizzotto†, G. Bussolati§ and C. De Angelis†

<table>
<thead>
<tr>
<th>Sample:</th>
<th>Screening Panel</th>
<th>Additional Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI 1640 medium 10% FCS</td>
<td>CD3, CD4, CD5, CD8, CD10, CD19, CD20, CD45, Kappa, Lambda</td>
<td>CD22, CD23, CD30, CD38, CD43, CD79b, CD103, CD138, IgA, IgD, IgG, IgM, BCL 2</td>
</tr>
</tbody>
</table>

Adequacy: 2000 events in the lymphocytic gate
Diagnosis of deep-seated lymphomas by endoscopic ultrasound-guided fine needle aspiration combined with flow cytometry

A. Stacchini*, P. Carucci†, D. Pacchioni‡, G. Accinelli‡, A. Demurtas*, S. Aliberti*, M. Bosco‡, M. Bruno†, A. Balbo Mussetto†, M. Rizzetto†, G. Bussolati§ and C. De Angelis†

<table>
<thead>
<tr>
<th>Final diagnosis (EUS-FNA and FC)</th>
<th>Number of cases (n = 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive lymphoadenopathy</td>
<td>20</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Small lymphocytic lymphoma</td>
<td>2</td>
</tr>
<tr>
<td>B-cell lymphoma, not otherwise specified</td>
<td>3</td>
</tr>
<tr>
<td>Metastatic carcinoma</td>
<td>13 (5)</td>
</tr>
<tr>
<td>Granulomatous lymphadenopathy</td>
<td>9 (1)</td>
</tr>
<tr>
<td>Mediastinal extramedullary haemopoiesis</td>
<td>1</td>
</tr>
<tr>
<td>Suspicious for lymphoma (cytology and flow)</td>
<td>1</td>
</tr>
<tr>
<td>but not confirmed on molecular analysis</td>
<td></td>
</tr>
<tr>
<td>Reactive cytology, inadequate material for FC</td>
<td>1</td>
</tr>
</tbody>
</table>

n, histological or molecular confirmation.
EUS-guided biopsy for the diagnosis and classification of lymphoma

Afonso Ribeiro, MD, Denise Pereira, MD, Maricer P. Escalón, MD, Mark Goodman, MD, Gerald E. Byrne, Jr, MD

**TABLE 2.** Results of FNA + flow cytometry (FC) and Trucut biopsy (TCB) for diagnosis and classification of lymphoma

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Lymphoma</th>
<th>Correct classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUS-FNA</td>
<td>23</td>
<td>13*</td>
<td>0</td>
</tr>
<tr>
<td>EUS-FNA + FC</td>
<td>19</td>
<td>18 (94.7%)</td>
<td>14 (73.6%)†</td>
</tr>
<tr>
<td>EUS-TCB</td>
<td>22</td>
<td>16 (73%)</td>
<td>16 (72.7%)</td>
</tr>
<tr>
<td>EUS-FNA + FC + TCB</td>
<td>24</td>
<td>19 (79.1%)</td>
<td>16 (66.6%)</td>
</tr>
</tbody>
</table>

*Suspicious or suggestive of Hodgkin or non-Hodgkin lymphoma.
†Results with combined TCB analysis.
EUS-FNA
OESOPHAGUS AND GI TRACT
The advantages of cytology

• Survey of large mucosa areas
• Investigation of the cardiac region, not always accessible to endoscopic biopsy
• Exploration of large ulcers
Oesophagus non-neoplastic lesions
Oesophagus neoplastic lesions
Gastric lesions
Intestinal lesions
DIAGNOSIS

PROGNOSIS

THERAPEUTIC TARGETS
Gastrointestinal Stromal Tumors

• GISTs are frequently discovered on endoscopy performed for other reasons and are characterized by a bulging of the GI wall with intact, normal, overlying mucosa.

• EUS allows identification of the tumor and collection of material for diagnosis and molecular analysis by EUS-guided FNA.

• GISTs are characterized by the presence of activating mutations of CKIT and PDGFR TK receptors. Detection of these mutations are useful for diagnosis confirmation and to predict likelihood of Imatinib response.
GI ST is the ideal model for treatment with TK inhibitors

- There is a relevant oncogenetic mechanism involving a TK receptor
- Present in most tumours
- Demonstrable in diagnostic tumour tissue
- Targetable in vitro and in vivo
GIST: KIT Mutation Predicts Imatinib Responsiveness

- KIT mutations are predictive of response to imatinib mesylate
- Exon 11 mutants respond best
Gastrointestinal stromal tumour treated with neoadjuvant imatinib

Pre-imatinib mesylate  4 weeks of imatinib mesylate

GISTs
Cytological findings

- The tumour cells are uniform and spindling with ill-defined cytoplasmic border.

- The nuclei are spindle and sometimes embedded in a fibrillary background.

- Necrosis is absent.
Cell-block reveals many tissue fragments of a spindle cell neoplasm with dissecting fascicles.
Molecular Analysis of c-Kit and PDGFRA in GISTs Diagnosed by EUS

Ana L. Gomes, BSc, Ricardo H. Barrales, MD, Fernanda Milanezi, MD, Rui M. Reis, PhD, and Fernando Schmitt, MD, PhD

Table 2: Mutation analysis results for c-Kit gene in cell-blocks obtained from aspirates

<table>
<thead>
<tr>
<th>Tumours</th>
<th>c-Kit status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>GIST (n = 33)</td>
<td></td>
</tr>
<tr>
<td>non-GIST (n = 18)</td>
<td></td>
</tr>
</tbody>
</table>
KRAS and BRAF as Predictive Factors in Metastatic Colorectal Cancer

- K-RAS and BRAF are negative predictors of response to anti-EGFR monoclonal therapies for patients with mCRC.

<table>
<thead>
<tr>
<th>KRAS wt / BRAF wt</th>
<th>Cetuximab YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS wt / BRAF mut</td>
<td>Cetuximab NO</td>
</tr>
<tr>
<td>KRAS mut</td>
<td>Cetuximab NO</td>
</tr>
</tbody>
</table>
KRAS status in primary and metastatic site can be different

- 252 sporadic MSS CRC
- 132 LN neg
- 120 LN pos
- PCR, SSCP, Sequencing
- 29 LN Metastases
- 17% K-ras mutation

Oliveira C et al. Oncogene 2007
**KRAS and BRAF mutation analysis can be reliably performed on aspirated cytological specimens of metastatic colorectal carcinoma**

Table 2: Clinical findings and summary of comparative mutation results

<table>
<thead>
<tr>
<th>No.</th>
<th>Race, gender and age</th>
<th>Cytology specimen type and location</th>
<th>Source of paired histological sample</th>
<th>Location of paired histological sample</th>
<th>Mutation analysis of KRAS codons 12, 13 and 61 and BRAF codon 600</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chinese male/68</td>
<td>FNA lung</td>
<td>Primary tumour</td>
<td>Sigmoid colon</td>
<td>Cytology – WT for KRAS</td>
</tr>
<tr>
<td>2</td>
<td>Chinese female/71</td>
<td>FNA lung</td>
<td>Primary tumour</td>
<td>Rectal</td>
<td>Cytology – WT for KRAS and BRAF</td>
</tr>
<tr>
<td>3</td>
<td>Chinese female/69</td>
<td>FNA lung</td>
<td>Primary tumour</td>
<td>Rectal</td>
<td>Cytology – WT for KRAS and BRAF</td>
</tr>
<tr>
<td>4</td>
<td>Chinese male/72</td>
<td>FNA lung</td>
<td>Primary tumour</td>
<td>Sigmoid colon</td>
<td>Cytology – G12D for KRAS</td>
</tr>
<tr>
<td>5</td>
<td>Chinese female/91</td>
<td>FNA lung</td>
<td>Primary tumour</td>
<td>Rectal</td>
<td>Cytology – G12D for KRAS</td>
</tr>
<tr>
<td>6</td>
<td>Chinese female/53</td>
<td>FNA liver</td>
<td>Primary tumour</td>
<td>Ascending colon</td>
<td>Histology – G12D for KRAS</td>
</tr>
<tr>
<td>7</td>
<td>Chinese female/79</td>
<td>FNA intra-abdominal mass</td>
<td>Resected metastasis</td>
<td>Peritoneal nodule</td>
<td>Histology – WT for KRAS and BRAF</td>
</tr>
<tr>
<td>8</td>
<td>Chinese male/58</td>
<td>FNA supraclavicular lymph node</td>
<td>Primary tumour</td>
<td>Sigmoid colon</td>
<td>Cytology – WT for KRAS and BRAF</td>
</tr>
</tbody>
</table>

FNA, fine needle aspiration; WT, wild type.
Ancillary Studies in Cytology

Conclusions

• Results of ancillary tests can only be interpreted in the context of an informed, carefully considered clinical and cytological diagnosis.

• In many cases, a single test are unlikely to provide a specific diagnosis even within a limited differential diagnosis.

• Cytological material is an excellent alternative to study therapeutic targets with minimal discomfort for the patients and high accuracy.
EUS-FNA