ROSE – what does it mean?

Rapid OnSite Evaluation, but...

• To what end?
• Using what methods?
• Performed by whom?
• And, in the literature, reported by whom?
Cellular material obtained by FNA has potentially critical diagnostic value.

Value should be maximised taking account of FNA site and treatment options.
EBUS tissue – (monetary) value

- Single FNA weighs about:
  - 10mg

- NHS tariff for EBUS is:
  - £1276

- Assume 5 passes (50mg), EBUS tissue is worth:
  - £25,520/gram

EBUS tissue - £25,520/gram
Potential benefits of ROSE

Diagnostic

• Adequacy
• Diagnostic yield
  – % of cases with an actual diagnosis
  – May be specified for a particular diagnosis
  – Sensitivity, specificity, PPV, NPV
• Accuracy
  – Comparison with “gold standard”
Potential benefits of ROSE

Process

- Number of passes
- Number of sites
- Procedure time/resources
- Cost
- Repeat procedures
Potential benefits of ROSE

Ancillary tests

• Immunocytochemistry
  – Diagnostic, predictive

• Molecular (mutations, translocations)
  – Predictive, prognostic

• Flow cytometry
  – Diagnostic

• Microbiological
Main sites covered today

• Mediastinum (EBUS/EUS)
• Pancreas (EUS)
• Head and neck
Mediastinum adequacy

Schmidt RL et al
doi:10.1309/AJCPEGZMJKC42VUP

Meta-analysis of 25, 2-cohort, studies with and without ROSE, a total of 12,407 cases

Forest plot shows change in adequacy rate when ROSE used. Analysis is not adjusted for initial adequacy.
Rapid On-site Evaluation of Transbronchial Aspirates in the Diagnosis of Hilar and Mediastinal Adenopathy
Trisolini et al
CHEST 2011; 139(2):395–401

168 patients randomised to conventional TBNA with and without ROSE

Adequacy – “a preponderance of lymphocytes”
Learning endobronchial ultrasound transbronchial needle aspiration – a 6-year experience at a single institution
Sveinung Sørhaug et al
Clin Respir J 2018; 12: 40–47

711 EBUS (855 sites), 299 (368) before ROSE, 412 (487) after ROSE

Adequacy: >40 lymphocytes per x40f
ROSE provided by cytotechnologists
Adequacy in the mediastinum

• Alsharif (Minnesota - 2008)
  – 40 lymphocytes/x40f in most cellular area
  – OR pigmented macrophages
  – OR diagnostic material

• Nayak (New York - 2010)
  – (5 x 100 lymphocytes/x10f AND <2 bronchial cell groups/x10f)
  – OR germinal centre fragments
  – OR diagnostic material
Adequacy in the mediastinum

- x10f has 16 times greater area than x40f
- 40 lymphocytes/x40f = 640 lymphocytes/x10f
- 5 × 100 lymphocytes/x10f = 500 lymphocytes
## Adequacy in the mediastinum


<table>
<thead>
<tr>
<th></th>
<th>Minnesota</th>
<th>Adequate</th>
<th>Unsatisfactory</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate, No. (%)</td>
<td>100 (85)</td>
<td></td>
<td>2 (2)</td>
<td>102 (86)</td>
</tr>
<tr>
<td>Unsatisfactory, No. (%)</td>
<td>0 (0)</td>
<td>16 (14)</td>
<td></td>
<td>16 (14)</td>
</tr>
<tr>
<td>Total, No. (%)</td>
<td>100 (85)</td>
<td>18 (15)</td>
<td>118 (100)</td>
<td></td>
</tr>
</tbody>
</table>

**Simple $\kappa$**

- $\kappa$: 0.931
- Standard error: 0.048
- 95% confidence limits: 0.837-1.000

**McNemar’s Test**

- $\chi^2$: 2.000
- $df$: 1
- $P$: .16

Abbreviation: UAMS, University of Arkansas for Medical Sciences.
Adequacy for physicians


- Criterion 1: Core ≥ 2cm
  - No → Inadequate specimen
  - Yes → Adequate specimen
- Criterion 2: presence of malignant cells
  - No → Inadequate specimen
  - Yes → Adequate specimen
- Criterion 3: presence of MAP
  - No → Inadequate specimen
  - Yes → Adequate specimen
- Criterion 4: LD ≥ 40×40
  - No → Inadequate specimen
  - Yes → Adequate specimen

Accuracy of adequacy of specimen (%)

- Criterion 1: 64.7%
- Criterion 1 and/or criterion 2: 72.0%
- Criterion 1 and/or criterion 2 and/or criterion 3: 95.0%
- Criterion 1 and/or criterion 2 and/or criterion 3 and/or criterion 4: 97.0%

133 patients
300 nodes
Adequacy in the mediastinum

• Does ROSE help?
  – Evidence suggests:
    – yes if the adequacy rate is low (<75%)
    – no if the adequacy rate is ok (>75%)

• Nevertheless, need reproducible criteria
  – We use 40 lymphocytes/x40f or pigmented macrophages or diagnostic material.
Mediastinum
Diagnostic yield and accuracy
Rapid On-site Evaluation of Transbronchial Aspirates in the Diagnosis of Hilar and Mediastinal Adenopathy
Trisolini et al
CHEST 2011; 139(2):395–401

168 patients randomised to conventional TBNA with and without ROSE

Table 2—Results of the Outcome Measures

<table>
<thead>
<tr>
<th>Measure</th>
<th>TBNA (n = 85)</th>
<th>TBNA + ROSE (n = 83)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic yield, No. (%)</td>
<td>64 (75.3)</td>
<td>65 (78.3)</td>
<td>.64</td>
</tr>
<tr>
<td>Adequate samples, No. (%)</td>
<td>109 (86.5)</td>
<td>80 (78.4)</td>
<td>.10</td>
</tr>
<tr>
<td>Number of biopsy sites, median (IQR)</td>
<td>2 (1-2)</td>
<td>1 (1-2)</td>
<td>.0005</td>
</tr>
<tr>
<td>Complication rate of bronchoscopy, No. (%)</td>
<td>17 (20)</td>
<td>5 (6)</td>
<td>.011</td>
</tr>
</tbody>
</table>
Overall diagnostic yield – ROSE 85%, non-ROSE 75%, p=0.23

Table 4. Diagnostic value of EBUS-TBNA for lung cancer

<table>
<thead>
<tr>
<th></th>
<th>ROSE (n = 55)</th>
<th>Non-ROSE (n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>88</td>
<td>86</td>
</tr>
<tr>
<td>Specificity</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>40</td>
<td>63</td>
</tr>
<tr>
<td>Accuracy(^a)</td>
<td>89</td>
<td>89</td>
</tr>
</tbody>
</table>

Data are presented as %. \(^a\) p = 0.95 using \(\chi^2\) test.

Rapid On-Site Cytologic Evaluation during Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration for Diagnosing Lung Cancer: A Randomized Study
Oki et al
Respiration 2013;85:486–492

108 patients randomised to EBUS-TBNA with and without ROSE

Diagnostic yield and diagnostic accuracy for lung cancer secondary endpoints
Impact of Rapid On-Site Cytological Evaluation (ROSE) on the Diagnostic Yield of Transbronchial Needle Aspiration During Mediastinal Lymph Node Sampling: Systematic Review and Meta-Analysis.

Sehgal et al
CHEST 2018; 153(4):929-938

5 studies – 618 subjects – good quality.
No effect of ROSE on diagnostic yield in EBUS or c-TBNA
Diagnostic yield - mediastinum

• Does ROSE help?
  – Evidence suggests:
  – No (even in blind TBNA)
Mediastinum
Process
Table 2—Results of the Outcome Measures

<table>
<thead>
<tr>
<th>Measure</th>
<th>TBNA (n = 85)</th>
<th>TBNA + ROSE (n = 83)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic yield, No. (%)</td>
<td>64 (75.3)</td>
<td>65 (78.3)</td>
<td>.64</td>
</tr>
<tr>
<td>Adequate samples, No. (%)</td>
<td>109 (86.5)</td>
<td>80 (78.4)</td>
<td>.10</td>
</tr>
<tr>
<td>Number of biopsy sites, median (IQR)</td>
<td>2 (1-2)</td>
<td>1 (1-2)</td>
<td>.0005c</td>
</tr>
<tr>
<td>Complication rate of bronchoscopy, No. (%)</td>
<td>17 (20)</td>
<td>5 (6)</td>
<td>.011c</td>
</tr>
</tbody>
</table>

Rapid On-site Evaluation of Transbronchial Aspirates in the Diagnosis of Hilar and Mediastinal Adenopathy

Trisolini et al

CHEST 2011; 139(2):395–401

168 patients randomised to conventional TBNA with and without ROSE

Significant reduction in targeted sites
TABLE 2  ] Procedural Details (per Patient Analysis)

<table>
<thead>
<tr>
<th>Procedural Detail</th>
<th>Overall Population (N = 197)</th>
<th>ROSE (98)</th>
<th>EBUS (99)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration, mean (SD), a min</td>
<td></td>
<td>17.8 (8.34)</td>
<td>17.9 (5.61)</td>
<td>.871</td>
</tr>
<tr>
<td>No. sampled sites b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>76 (55.9)</td>
<td>60 (44.1)</td>
<td>.005c</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>19 (33.3)</td>
<td>38 (66.7)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>3 (75)</td>
<td>1 (25)</td>
<td></td>
</tr>
</tbody>
</table>

Randomized Trial of Endobronchial UltrasoundGuided Transbronchial Needle Aspiration With and Without Rapid On-site Evaluation for Lung Cancer Genotyping
Trisolini et al
CHEST 2015; 148(6):1430-1437

197 patients randomised to EBUS TBNA with and without ROSE

Significant reduction in targeted sites

<table>
<thead>
<tr>
<th>Number of Biopsy Sites</th>
<th>Non-ROSE (340 Patients)</th>
<th>ROSE (340 Patients)</th>
<th>Difference (Absolute #)</th>
<th>Difference (Proportional)</th>
<th>Significance (P Valuea)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Biopsy Site</td>
<td>122 (35.88%)</td>
<td>231 (67.94%)</td>
<td>109 (47.1%)</td>
<td>0.3206</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>2 or More Biopsy Sites</td>
<td>218 (64.12%)</td>
<td>110 (32.35%)</td>
<td>−108 (49.5%)</td>
<td>−0.3176</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>3 or More Biopsy Sites</td>
<td>113 (33.23%)</td>
<td>22 (6.47%)</td>
<td>−91 (80.5%)</td>
<td>−0.2676</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>4 or More Biopsy Sites</td>
<td>34 (10.0%)</td>
<td>1 (0.29%)</td>
<td>−33 (97.0%)</td>
<td>−0.0971</td>
<td>na^b</td>
</tr>
<tr>
<td>Total Biopsy Sites</td>
<td>709</td>
<td>474</td>
<td>−235 (33.1%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Matched case-control cohorts of TBNA with and without ROSE (340 each).

- **Mean sites/patient** $2.085 > 1.394$
  - **33% reduction in sites biopsied**
- **Mean slides/site** $8.42 > 8.824$
  - **le no significant change**
West Herts

Number of sites sampled per patient - percentage by method

EBUS/EUS with ROSE - 54 patients
TBNA without ROSE - 102 patients

p<0.05
Do any studies show reduction in passes/site?

Table 2. Procedural details

<table>
<thead>
<tr>
<th>Variables</th>
<th>ROSE (n = 55)</th>
<th>Non-ROSE (n = 53)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean puncture number for main target lesion</td>
<td>2.2±0.9 (1–6)</td>
<td>3.1±0.4 (3–5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Additional procedures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBUS-TBNA for other lesions</td>
<td>6</td>
<td>30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TBB for peripheral lesions</td>
<td>2</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>EBUS-TBNA for other lesions and TBB for peripheral lesions</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

108 patients randomised to EBUS-TBNA with and without ROSE

No of needle passes was a secondary endpoint

Rapid On-Site Cytologic Evaluation during Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration for Diagnosing Lung Cancer: A Randomized Study

Oki et al
Respiration 2013;85:486–492
Matched case-control cohorts of TBNA with and without ROSE (340 each).

**29.9% reduction in total slides**

Savings in cytopathologist, BMS, procedure time
Does ROSE help? - Yes

- Good evidence for reduction in sites with ROSE
- Limited evidence for reduction in passes/site
- Latter unsurprising due to
  - Time to stain and examine slides
  - Need for extra passes for ancillary studies

- In finance-driven health economies, may be savings
Mediastinum
Ancillary tests
EGFR

Sharma, 2007
ALK
ROS-1
Figure 2: Cancers cells adapt and exploit immune system to evade immune surveillance by activating PD-L1/PD1 axis. ZEB1 and microRNA200 can regulate this axis. KRAS or EGFR mutation can also influence PD-L1 expression. Blocking PD1 and PD-L1 interaction with checkpoint inhibitor(s) in combination with ROS inducing agent may lead to new approaches to overcome cisplatin resistant lung cancer.
## Diagnostic molecular cytopathology

<table>
<thead>
<tr>
<th>PRE-2004 PARADIGM</th>
<th>POST-2004 PARADIGM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYTOLOGY</td>
<td>CYTOLOGY</td>
</tr>
<tr>
<td>HISTOLOGY</td>
<td>HISTOLOGY</td>
</tr>
<tr>
<td>ARCHITECTURAL FRAMEWORK</td>
<td>ARCHITECTURAL FRAMEWORK</td>
</tr>
<tr>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>CYTOLOGICAL DETAIL</td>
<td>CYTOLOGICAL DETAIL</td>
</tr>
<tr>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>QUALITY OF IHC</td>
<td>QUALITY OF IHC</td>
</tr>
<tr>
<td>++/+++</td>
<td>+++/+++</td>
</tr>
<tr>
<td>EASE OF SAMPLING</td>
<td>EASE OF SAMPLING</td>
</tr>
<tr>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>MOLECULAR TESTING</td>
<td>MOLECULAR TESTING</td>
</tr>
<tr>
<td>+++</td>
<td>++/+++</td>
</tr>
</tbody>
</table>

**CONCLUSION**: In difficult / complex diagnosis, a preliminary cytology diagnosis should be followed by an histological confirmation.

**CONCLUSION**: The molecular result becomes “pathognomonic” for diagnostics, or “final” for therapeutics – the cytology opinion does not need confirmation.

---

Updates from 2016 Molecular Cytopathology meeting, Naples

More Than a Decade of Molecular Diagnostic Cytopathology Leading Diagnostic and Therapeutic Decision-Making
Manuel Salto-Tellez, LMS/MD, FRCPath, FRCPI
Arch Pathol Lab Med—Vol 142, April 2018

---

West Hertfordshire Hospitals NHS Trust
"Cytopathology is an integral part of the whole molecular revolution and, in some areas, such as molecular diagnostics of thyroid neoplasias or the therapeutic pathology of lung cancer, it is a leading application."

"Formalin-fixed, paraffin-embedded–based molecular testing, following adequate validation, can be applied to most cytopathology samples. Despite early attempts to deny that, it is now part of many national and international guidelines, including those in which cytopathology samples are a large fraction and those in which they may be an exception."
### ROSE – DNA quality from cell blocks

**West Herts cases sent for NGS – January 2015 – March 2016**

<table>
<thead>
<tr>
<th>Type</th>
<th>Subtype</th>
<th>DNA conc’n (ng/µl)</th>
<th>DIN</th>
<th>DIN allocation (cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Cyto</td>
<td>EBUS/EUS (n=22; 21 for DIN)</td>
<td>8.73</td>
<td>0.82 - 40.4</td>
<td>4.29</td>
</tr>
<tr>
<td></td>
<td>FNA (n=8)</td>
<td>4.23</td>
<td>0.76 – 19.8</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>Pleural (n=5)</td>
<td>9.32</td>
<td>0.48 – 10.5</td>
<td>3.86</td>
</tr>
<tr>
<td></td>
<td>Washings (n=2)</td>
<td>1.87</td>
<td>1.47 – 2.26</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>Overall (n=37)</td>
<td>7.47</td>
<td>0.76 – 40.4</td>
<td>3.41</td>
</tr>
<tr>
<td>Histo</td>
<td>Core biopsy (n=14; 13 for DIN)</td>
<td>5.61</td>
<td>0.51 - 11.9</td>
<td>4.40</td>
</tr>
<tr>
<td></td>
<td>Mucosal biopsy (n=8)</td>
<td>5.49</td>
<td>1.04 – 10.1</td>
<td>6.10</td>
</tr>
<tr>
<td></td>
<td>Overall (n=22)</td>
<td>5.57</td>
<td>0.51 – 11.9</td>
<td>4.46</td>
</tr>
</tbody>
</table>
Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors

Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology

1. Any Cytology Sample With Adequate Cellularity and Preservation May Be Tested.—The original recommendation preferred cell blocks over smears. A recent systematic review\textsuperscript{28} identified by the literature search has indicated that numerous studies have been published showing excellent performance of smear preparations, such that this preference is no longer appropriate. It is incumbent upon any laboratory that tests cytopathology specimens to perform appropriate validation studies of these as separate sample types, distinct from tissue and blood samples.
Cytology Smears in the Era of Molecular Biomarkers in Non–Small Cell Lung Cancer

Doing More With Less
Does ROSE help with acquisition of tissue for molecular tests?
Guideline for the Acquisition and Preparation of Conventional and Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration Specimens for the Diagnosis and Molecular Testing of Patients with Known or Suspected Lung Cancer

Erik H.F.M. van der Heijden, Roberto F. Casal, Rocco Trisolini, Daniel P. Steinfort, Bin Hwangbo, Takahiro Nakajima, Birgit Guldhammer-Skov, Giulio Rossi, Maurizio Ferretti, Felix F.J. Herth, Rex Yung, Mark Krasnik

on behalf of the World Association for Bronchology and Interventional Pulmonology Task Force on Specimen Guidelines

Does ROSE influence tissue sampling for molecular analysis?

ROSE is very useful for the confirmation of the presence of tumor cells within the samples. Even though no prospective comparative trials have been published on the possible influence of ROSE on the diagnostic yield of TBNA or EBUS-TBNA for molecular testing, we suggest that ROSE be used when molecular testing is looked for until high-quality trials are available. Currently, an RCT aimed at evaluating the role of ROSE in EBUS-TBNA samples for molecular testing is ongoing (ClinicalTrials.gov identifier: NCT01799382).
Randomized Trial of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration With and Without Rapid On-site Evaluation for Lung Cancer Genotyping
Trisolini et al
CHEST 2015; 148(6):1430-1437

197 patients randomised to EBUS TBNA with and without ROSE
Trend towards greater success in genotyping with ROSE but not statistically significant
Molecular testing on endobronchial ultrasound (EBUS) fine needle aspirates (FNA): Impact of triage

Simon Sung MD¹ | John P. Crapanzano MD¹ | David DiBardino MD² | David Swinarski PhD³ | William A. Bulman MD² | Anjali Saqi MD, MBA¹

_Diagnostic Cytopathology_. 2018;46:122–130.

Retrospective analysis of 100 cases of lung adenocarcinoma in which EBUS with ROSE was utilised. Cases allocated to group A or B according to number and timing of cytology personnel.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Key differences between Group A and B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
</tr>
<tr>
<td>Triage at start of procedure</td>
<td>+</td>
</tr>
<tr>
<td>&gt;1 cytology personnel</td>
<td>+</td>
</tr>
<tr>
<td>Slides prepared by clinical (non-cytology) staff</td>
<td>-</td>
</tr>
</tbody>
</table>
Retrospective analysis of 100 cases of lung adenocarcinoma in which EBUS with ROSE was utilised. Cases allocated to group A or B according to number and timing of cytology personnel.

**TABLE 1  Key differences between Group A and B**

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triage at start of procedure</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>&gt;1 cytology personnel</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>Slides prepared by clinical (non-cytology) staff</td>
<td>−</td>
<td>+/−</td>
</tr>
</tbody>
</table>

There was a difference in availability of sufficient tissue for MT on cell blocks between Group A and Group B. One case from Group A (n = 1/22; 4.5%) and 20 from Group B (n = 20/78; 25.6%) had insufficient malignant cells in cell block(s) for MT. Because the smallest expected cell count in the resulting contingency table is smaller than 5, the classic Pearson-Fisher χ²d test is not recommended for these data. Instead, following the recommendations of Campbell, the “N−1” χ²d test was used and showed that the difference between the rate of failure for MT in Group A and the rate of failure for MT in Group B is statistically significant with P values = 0.033.¹¹
Does ROSE help with acquisition of tissue for molecular tests?

Yes, probably
Can you diagnose lymphoma at EBUS?

Endobronchial Ultrasound and Lymphoproliferative Disorders: A Retrospective Study

Seher Iqbal, MD, Zachary S. DePew, MD, Paul J. Kurtin, MD, Anne-Marie G. Sykes, MD, Geoffrey B. Johnson, MD, Eric S. Edell, MD, Thomas M. Habermann, MD, Fabien Maldonado, MD

The Annals of Thoracic Surgery
Volume 94, Issue 6, Pages 1830-1834 (December 2012)

- Mayo Clinic: 2006-2011
- Retrospective study cross-referencing lymphoma + EBUS databases
- 65 patients
- Sensitivity 29%
  - 21G needle
  - No ROSE – min 3 passes, unless 2 passes produced visible core
  - No flow cytometry
Can you diagnose lymphoma at EBUS?

Diagnosis and Subtyping of De Novo and Relapsed Mediastinal Lymphomas by Endobronchial Ultrasound Needle Aspiration
Mufaddal T. Moonim, Ronan Breen, Paul A. Fields, and George Santis
Am J Respir Crit Care Med Vol 188, Iss. 10, pp 1216–1223, Nov 15, 2013

• 100 cases of suspected lymphoma in 5 years
  – ROSE service
  – Flow cytometry + cytogenetics etc available

• Correct diagnosis of
  – 48/51 de novo lymphoma (88%)
  – 15/15 relapsed lymphoma (100%)
  – 32/34 non-lymphoma (96%)

• Sensitivity/specificity = 89%/97%

• Sensitivity of sub-typing
  – HGL – 90%
  – LGL – 100%
  – HD – 79%

• EBUS result enough for clinical mgt in 84/100 (84%)
Yes, but there needs to be

- Good (ie abundant) cell block material
- Appropriate material for flow cytometry, if necessary
- A good relationship with the Haematopathology service, wherever that is
  – Specialist Integrated Haematological Malignancy Diagnostic Service
- And the sensitivity for HD and HGNHL may be a challenge
ROSE – specimen management

- Adequate aspirate
  - Granulomatous
  - Microbiology
  - Malignant
  - Cell block
  - ?Reactive/?LG lymphoma
  - Flow cytometry
Advantages
- Instant (actually 2-3 minute) feedback for endoscopist
  - Adequacy and provisional diagnosis
- Specimen management and triage
  - Solid tumour/high grade lymphoma – cell block
  - ?Reactive node/?low grade lymphoma – flow cytometry
  - Granulomas – microbiology
- Reduction of sites/patient (?passes/site)

Disadvantages/reasons not more utilised
- BMS and/or consultant time and resource
- May be out of comfort zone for either
- Potential specimen compromise – endoscopist’s fear of slides+++++/insufficient material for molecular
Pancreas
Pancreatic EUS – key differences

• Generally only one target
  – Though possible to sample lymph nodes as well

• Clear division into
  – Solid and cystic lesions
  – Different sample handling and implications

• For the majority of solid pancreatic lesions (i.e., pancreatic ductal carcinoma), diagnosis is morphological
Pancreatic EUS – key challenges

- GI epithelial contamination is a major issue
- Benign inflammatory lesions are a problem (IgG4, chronic pancreatitis)
- Specimens may be paucicellular
• So, is there any point doing ROSE, if
  – You can’t lower the number of targeted sites, and
  – Ancillary tests are less used?

• Well, there’s always adequacy, diagnostic yield, process etc.
The Influence of Rapid Onsite Evaluation on the Adequacy Rate of Fine-Needle Aspiration Cytology. A Systematic Review and Meta-Analysis. Schmidt RL et al
Am J Clin Pathol. 2015;139(3):300-308. doi:10.1309/AJCPEGZMJKC42VUP

Meta-analysis of 25, 2-cohort, studies with and without ROSE, a total of 12,407 cases

Forest plot shows change in adequacy rate when ROSE used. Analysis is not adjusted for initial adequacy.

Schmidt RL et al

doi:10.1309/AJCPEGZMJKC42VUP

Meta-analysis of 25, 2-cohort, studies with and without ROSE, a total of 12,407 cases

Analysis adjusted for initial adequacy.

<table>
<thead>
<tr>
<th>Equation Factor</th>
<th>Coefficient</th>
<th>Value</th>
<th>ROSE Impact</th>
<th>95% CI</th>
<th>t^b</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ROSE adequacy rate, $X_i$</td>
<td>$\beta$</td>
<td>−0.67</td>
<td></td>
<td>−0.82, −0.51, −8.91</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Tissue effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>$\alpha_1$</td>
<td>0.00</td>
<td>Low</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>$\alpha_2$</td>
<td>0.14</td>
<td>High</td>
<td>0.05, 0.23, 3.26</td>
<td>.004</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>$\alpha_3$</td>
<td>0.14</td>
<td>High</td>
<td>0.07, 0.22, 3.78</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td>$\alpha_4$</td>
<td>0.15</td>
<td>High</td>
<td>0.02, 0.28, 2.42</td>
<td>.02</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>$\alpha_5$</td>
<td>0.14</td>
<td>High</td>
<td>0.07, 0.21, 4.07</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Multiple</td>
<td>$\alpha_6$</td>
<td>0.12</td>
<td>High</td>
<td>0.03, 0.20, 2.81</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>Mediastinum</td>
<td>$\alpha_7$</td>
<td>0.01</td>
<td>Low</td>
<td>−0.15, 0.16, 0.12</td>
<td>.90</td>
<td></td>
</tr>
<tr>
<td>Soft tissue</td>
<td>$\alpha_8$</td>
<td>0.03</td>
<td>Low</td>
<td>−0.12, 0.18, 0.44</td>
<td>.66</td>
<td></td>
</tr>
<tr>
<td>Head and neck</td>
<td>$\alpha_9$</td>
<td>0.15</td>
<td>High</td>
<td>0.05, 0.24, 3.28</td>
<td>.004</td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>$\kappa$</td>
<td>0.53</td>
<td></td>
<td>0.40, 0.65, 9.00</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>
The presence of a cytopathologist increases the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration cytology for pancreatic adenocarcinoma: a meta-analysis

S. Hébert-Magee*, S. Bae†, S. Varadarajulu‡, J. Ramesh‡, A. R. Frost*, M. A. Eloubeidi‡ and I. A. Eltoum*

*Division of Anatomic Pathology, Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA,
†Division of Preventive Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA,
‡Division of Gastroenterology and Hepatology, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

Cytopathology 2013, 24, 159–171

Table 5. Predefined subgroup analysis with multivariate meta-regression showing only cytopathology is statistically significant

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>RDOR 95% (CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>1.00 (1.00–1.01)</td>
<td>0.1329</td>
</tr>
<tr>
<td>On-site cytopathology</td>
<td>5.95 (2.15–16.45)</td>
<td>0.0012</td>
</tr>
<tr>
<td>Reference standard</td>
<td>4.91 (0.62–38.92)</td>
<td>0.1264</td>
</tr>
</tbody>
</table>

Meta-analysis of 34 studies (3644 patients) some with, some without ROSE. No effect on adequacy, but diagnostic accuracy improves with ROSE.

Fanyang Kong¹, Jianwei Zhu¹, Xiangyu Kong¹, Tao Sun¹, Xuan Deng², Yiqi Du¹⁺*, Zhaoshen Li¹⁺*  

1 Department of Gastroenterology, Shanghai Hospital, Second Military Medical University, Shanghai, China, 2 Shanghai Medical College of Fudan University, Shanghai, China

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>ROSE Events</th>
<th>ROSE Total</th>
<th>Without ROSE Events</th>
<th>Without ROSE Total</th>
<th>Weight</th>
<th>Risk Difference M-H, Random, 95% CI</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alsaibani2009</td>
<td>14</td>
<td>22</td>
<td>14</td>
<td>22</td>
<td>7.2%</td>
<td>0.00 [-0.28, 0.28]</td>
<td>2009</td>
</tr>
<tr>
<td>Garcia2011</td>
<td>92</td>
<td>95</td>
<td>67</td>
<td>87</td>
<td>22.2%</td>
<td>0.20 [0.10, 0.29]</td>
<td>2011</td>
</tr>
<tr>
<td>Cernak2012</td>
<td>124</td>
<td>167</td>
<td>162</td>
<td>214</td>
<td>23.1%</td>
<td>-0.01 [-0.10, 0.07]</td>
<td>2012</td>
</tr>
<tr>
<td>Nayar2013</td>
<td>83</td>
<td>97</td>
<td>73</td>
<td>82</td>
<td>21.9%</td>
<td>-0.03 [-0.13, 0.08]</td>
<td>2013</td>
</tr>
<tr>
<td>Wani2015</td>
<td>114</td>
<td>121</td>
<td>108</td>
<td>120</td>
<td>25.5%</td>
<td>0.04 [-0.03, 0.11]</td>
<td>2015</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>502</td>
<td>525</td>
<td>100.0%</td>
<td></td>
<td></td>
<td>0.04 [-0.04, 0.13]</td>
<td></td>
</tr>
<tr>
<td>Total events</td>
<td>427</td>
<td>424</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.01; Chi² = 14.65, df = 4 (P = 0.005); I² = 73%  
Test for overall effect: Z = 0.99 (P = 0.32)

Fig 4. Forest plot displaying the Risk Difference and 95% CIs of each study for the diagnosis yield.

Meta-analysis of 7 studies (1299 patients)
Rapid On-Site Evaluation for Endoscopic Ultrasound-Guided Fine-Needle Biopsy of the Pancreas Decreases the Incidence of Repeat Biopsy Procedures

Brian T. Collins, MD¹; Faris M. Murad, MD²; Jeff F. Wang, MD¹; and Cory T. Bernadt, MD, PhD¹

**TABLE 2. ROSE EUS FNA Biopsy in Repeat Procedures: Proportional Difference**

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>Repeat Patients/All Patients</th>
<th>Proportional Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ROSE service</td>
<td>22/377</td>
<td>0.0584</td>
</tr>
<tr>
<td>ROSE service</td>
<td>11/379</td>
<td>0.029</td>
</tr>
<tr>
<td>Difference</td>
<td>50% difference</td>
<td>−0.0293 (P value &lt; .024)</td>
</tr>
</tbody>
</table>

Abbreviations: EUS FNA, endoscopic ultrasound-guided fine-needle aspiration; ROSE, rapid on-site evaluation.

**TABLE 4. ROSE EUS FNA Biopsy in Repeat Procedures: Definitive Categorization After Second Biopsy**

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>Definitive Diagnosis on Second Biopsy/All Patients</th>
<th>Proportional Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ROSE service</td>
<td>6/22 (27%)</td>
<td>0.273</td>
</tr>
<tr>
<td>ROSE service</td>
<td>7/11 (64%)</td>
<td>0.636</td>
</tr>
<tr>
<td>Difference</td>
<td>37% higher rate of positivity on ROSE second biopsy Twice as likely to have a definitive positive using ROSE than non-ROSE service</td>
<td>0.364 (P value &lt; .044)</td>
</tr>
</tbody>
</table>

Case-controlled cohort study, 377 non-ROSE, 379 ROSE
Does ROSE help with pancreatic EUS?

Maybe (with adequacy and diagnostic yield)
Pancreas – ancillary tests
• **Immunocytochemistry**
  – For the minority of solid lesions that are not pancreatic ductal carcinoma, immuno may be crucial – ie cell blocks needed

• **Molecular - currently**
  – No guideline role for molecular testing in solid pancreatic lesions
  – However, there is a role for KRAS testing in cystic pancreatic lesions – distinguishes lesions of mucinous origin

• **Other ancillary tests**
  – CEA/amylase in cyst fluid in ddx of pseudocyst/mucinous cyst
Head and neck

Schmidt RL et al
doi:10.1309/AJCPEGZMJKC42VUP

<table>
<thead>
<tr>
<th>Equation Factor</th>
<th>Coefficient</th>
<th>Value</th>
<th>ROSE Impact</th>
<th>95% CI</th>
<th>( t^b )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ROSE adequacy rate, ( x_i )</td>
<td>( \beta )</td>
<td>-0.67</td>
<td></td>
<td>-0.82 to -0.51</td>
<td>-8.91</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Tissue effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>( \alpha_1 )</td>
<td>0.00</td>
<td>Low</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>( \alpha_2 )</td>
<td>0.14</td>
<td>High</td>
<td>0.05 to 0.23</td>
<td>3.26</td>
<td>.004</td>
</tr>
<tr>
<td>Lung</td>
<td>( \alpha_3 )</td>
<td>0.14</td>
<td>High</td>
<td>0.07 to 0.22</td>
<td>3.78</td>
<td>.001</td>
</tr>
<tr>
<td>Lymph node</td>
<td>( \alpha_4 )</td>
<td>0.15</td>
<td>High</td>
<td>0.02 to 0.26</td>
<td>2.42</td>
<td>.02</td>
</tr>
<tr>
<td>Thyroid</td>
<td>( \alpha_5 )</td>
<td>0.14</td>
<td>High</td>
<td>0.07 to 0.21</td>
<td>4.07</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Multiple</td>
<td>( \alpha_6 )</td>
<td>0.12</td>
<td>High</td>
<td>0.03 to 0.20</td>
<td>2.81</td>
<td>.01</td>
</tr>
<tr>
<td>Mediastium</td>
<td>( \alpha_7 )</td>
<td>0.01</td>
<td>Low</td>
<td>-0.15 to 0.16</td>
<td>0.12</td>
<td>.90</td>
</tr>
<tr>
<td>Soft tissue</td>
<td>( \alpha_8 )</td>
<td>0.03</td>
<td>Low</td>
<td>0.12 to 0.16</td>
<td>0.44</td>
<td>.66</td>
</tr>
<tr>
<td>Head and neck</td>
<td>( \alpha_9 )</td>
<td>0.15</td>
<td>High</td>
<td>0.05 to 0.24</td>
<td>3.28</td>
<td>.004</td>
</tr>
<tr>
<td>Constant</td>
<td>( \kappa )</td>
<td>0.53</td>
<td></td>
<td>0.40 to 0.65</td>
<td>9.88</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Meta-analysis of 25, 2-cohort, studies with and without ROSE, a total of 12,407 cases

Analysis is adjusted for initial adequacy.
2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer

“The largest studies of preoperative molecular markers in patients with indeterminate FNA cytology have respectively evaluated a seven-gene panel of genetic mutations and rearrangements (BRAF, RAS, RET/PTC, PAX8/PPARγ), a gene expression classifier (167 GEC; mRNA expression of 167 genes), and galectin-3 immunohistochemistry (cell blocks).”
[A17] AUS/FLUS cytology

■ RECOMMENDATION 15

For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery....

In summary, there is currently no single optimal molecular test that can definitively rule in or rule out malignancy in all cases of indeterminate cytology, and long-term outcome data proving clinical utility are needed.

[A19] Suspicious for malignancy cytology

■ RECOMMENDATION 17

(B) After consideration of clinical and sonographic features, mutational testing for BRAF or the seven-gene mutation marker panel (BRAF, RAS, RET/PTC, PAX8/PPARγ) may be considered in nodules with SUSP cytology if such data would be expected to alter surgical decision-making.
• Molecular testing in thyroid disease is not yet mandated but...

• It would be wise to make sure you have a robust mechanism ready for molecular testing of your thyroid specimens in the future...
• Reasonable evidence that ROSE improves adequacy and diagnostic yield
• Ancillary tests similar to other sites – immuno for selected cases, flow and immuno for possible lymphoma, micro for possible infection
• Molecular testing in thyroid a fast-developing field
ROSE in West Herts – preparations

• 3 slides per pass
  – one air-dried - rapid-stained for ROSE
  – one fixed for later Pap stain
  – one “spreader” air-dried – later MGG
• Solid material into formalin for cell block
• “Bloody” material into saline for cell block later
• Micro – sterile saline
• Flow – saline flush then into EDTA tube

• If ROSE team cannot attend – all into ThinPrep (LBC) unless lymphoma/infection suspected
• 49 year old woman
• Aug 2016 – G3 IDC, ER 0, PR 3, HER2 3+
  – Rx primary chemo + Herceptin
• MRI – 3 x residual foci of carcinoma
• Mastectomy April 2017
• February 2018 – cough
• CT showed R hilar mass - EBUS
ROSE at West Herts
ROSE at West Herts
ROSE at West Herts
ROSE at West Herts
ROSE at West Herts
ROSE at West Herts
ROSE at West Herts
ROSE at West Herts
ROSE PROCEDURE CHART FOR EBUS/EUS

Date of procedure: 3/4/18
Initials: cr

<table>
<thead>
<tr>
<th>Pass</th>
<th>Site</th>
<th>Cyto - direct slides</th>
<th>PBS</th>
<th>Cell block</th>
<th>Micro</th>
<th>Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4R</td>
<td>✔️  ✔️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4R</td>
<td>✔️  ✔️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4R</td>
<td>✔️  ✔️</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4R</td>
<td>✔️  ✔️</td>
<td></td>
<td>✔️</td>
<td>✔️</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ROSE at West Herts
ROSE at West Herts
ROSE at West Herts

40 lymphocytes/x40 field
ROSE at West Herts
ROSE at West Herts
ROSE at West Herts

Cell block 1
ROSE at West Herts

Cell block 1

GATA3

TTF-1
ROSE at West Herts

Cell block 2
ROSE – who’s in the team?

• At West Herts (and most places in UK where service available)
  – Cytopathologist
  – Biomedical scientist

• Around the world
  – May have purely cytotechnologist (BMS) teams
  – Aarhus University Hospital – kappa coefficient for diagnosis 0.99  (Schacht et al. Cytopathology 2016;27(5):344-350)
ROSE – availability

- 2014
- Telephone survey of 147 respiratory MDTs
- 73 currently using EBUS
- 15 have ROSE
- (11 using EUS in addition to EBUS)
- Most MDTs unaware of ROSE as a technique
ROSE – who could/should be the team?

- Cytopathologist
- Biomedical scientist/cytotechnologist
- Endoscopist
- Radiologist
- Combinations of the above
Sample assessment for adequacy for reporting
Certain NGC samples are taken by specific clinical procedures (e.g. mediastinal EBUS, FNA of many sites) by clinical teams or by Pathologists. An opinion as to sample adequacy and sometimes a diagnosis can be offered by a Pathologist at the time the sample is taken. In most settings though, resources do not allow for this. A comment on sample adequacy (Rapid on-site evaluation – ROSE) may be offered by a biomedical scientist. If the biomedical scientist has suitable experience based on competency and service needs and appropriate training/qualifications they may also be able to offer a preliminary opinion mainly for triage of the sample material rather than for patient management as well as ROSE.
Possible competency framework for ROSE
Summary

• The main benefit of ROSE is
  – Specimen management
  – Making the best use of valuable material

• Depending on site targeted and non-ROSE adequacy rate, may be beneficial for
  – Adequacy, diagnostic yield, efficiency of process

• In my view, best done by members of Cytology team, but not necessarily pathologists
Thanks to the West Herts ROSE team

- Winnie Tang, band 7 BMS and lead
- Claire Kiepura, band 6 BMS
- Claire Plank, band 6 BMS
- Maureen Grosso, cytoscreener
- Sharon Bunting, cytoscreener
Thank you