BREAST FINE NEEDLE ASPIRATION CYTOLOGY

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Breast fine needle aspiration continues to be relevant in a large academic medical center: experience from Massachusetts General Hospital

### Still used in developed countries?

- One day clinic
- Palpable lesions
- Axillary nodes
- Metastatic sites

#### Palpable Lesions

- Benign → Follow up
  - (Image, clinic)
- Malignant → Core-needle
- Inconclusive → Core-needle

#### Non-Palpable Lesions

- Microcalcification → Core-needle
- Others abnormalities → FNA
  - Core-needle

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Overall (N = 1404)</th>
<th>Primary breast (N = 992)</th>
<th>Breast reconstruction, chest wall (N = 412)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-biopsy</td>
<td>4.8</td>
<td>5.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Accuracy</td>
<td>71.0</td>
<td>74.4</td>
<td>65.3</td>
</tr>
<tr>
<td>Specificity</td>
<td>96.4</td>
<td>93.7</td>
<td>95.3</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>15.0</td>
<td>14.4</td>
<td>17.2</td>
</tr>
<tr>
<td>PNN of malignant</td>
<td>10.0</td>
<td>10.0</td>
<td>100.0</td>
</tr>
<tr>
<td>PNN of benign</td>
<td>10.0</td>
<td>10.0</td>
<td>100.0</td>
</tr>
<tr>
<td>MP of malignant</td>
<td>10.0</td>
<td>10.0</td>
<td>100.0</td>
</tr>
<tr>
<td>MP of benign</td>
<td>10.0</td>
<td>10.0</td>
<td>100.0</td>
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<tr>
<td>PI of malignant</td>
<td>10.0</td>
<td>10.0</td>
<td>100.0</td>
</tr>
<tr>
<td>PI of benign</td>
<td>10.0</td>
<td>10.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

[Note: PNN = Positive Predictive Value, MP = Negative Predictive Value]
ASSESSMENT OF AXILLARY NODES

- If the result is positive for malignancy, the patient will proceed for full axillary clearance and the SNLB is avoided.
- If the result is negative, the patient will proceed to SNLB as a negative FNAC does not confidently exclude nodal metastasis.
- Failure to visualize all lymph nodes during US, small sized metastasis and preoperative CT are main causes for discrepancy.

BREAST FNAC X CNB

- In terms of pathological diagnosis, both methods are accepted to be highly accurate in the assessment of breast lesion.
- CNB is more used in: non-palpable screen-detected calcifications, borderline lesions and when mammography does not show invasion signs.
- Lack of expertise in cytology is one of the most frequent cause of use CNB.

Accuracy of FNAC

The accuracy of FNAC depends on three main factors:
- a sample that is adequate and representative of the lesion.
- suitable processing and staining without artifact.
- accurate interpretation of the cytological material with a clear report conveyed to the rest of the clinical team.

FNAC
Multistep technique

- Clinical examination
- Image-guided (US)
- Aspiration
- Slide preparation
- Fixation and staining
- Cytological interpretation

TRIPLE ASSESSMENT APPROACH

BBB: 98% benign – follow up
MMM: 1% error – surgery
Other: biopsy
The transducer probe locates the lesion in one of the edges of the US field; the aspirator passes the needle through the skin, in parallel with the transducer probe in the edge where the lesion is located in

Applying suction while moving the needle helps to pull cells into the needle. A blood-tinged specimen will appear in the hub of the needle. Suction is then released and the needle is withdrawn.

Slide preparation

- Material obtained with a fine needle is expelled onto appropriately labelled glass slide.
- This is usually performed by using a 10-ml syringe filled with air, attaching the needle to do it and pushing the contents out of the needle.

Slide preparation

- Sometimes, if the hub of the needle is full, it is possible to tap the hub against the glass and obtain the material directly from there.
Two-step method. Observe the concentration of the material in the middle of the slide that will be after smeared according to the one-step technique.

Quality of the smear

Collect sample directly into prefilled Cytolyt® tubes

IAC Structured Breast FNAB Cytology Reporting Yokohama 2016

The aim is to establish a best practice guideline covering:

i. The indications for breast FNAB cytology.

ii. The FNAB technique, smear making and material handling procedures.

iii. A practical, standardized and reproducible reporting system including report requirements, terminology definitions, descriptive terms and categories, structured reports, checklists and formats.

iv. The appropriate ancillary diagnostic and prognostic tests.

v. Correlation with clinical management algorithms.

HOW REPORT BREAST FNAC?

<table>
<thead>
<tr>
<th>C1 Unsatisfactory</th>
<th>Unsatisfactory</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2 Benign</td>
<td>Benign</td>
</tr>
<tr>
<td>C3 Suspicious, probably benign</td>
<td>Atypical/indeterminate</td>
</tr>
<tr>
<td>C4 Suspicious, probably malignant</td>
<td>Suspicious/probably malignant</td>
</tr>
<tr>
<td>C5 Malignant</td>
<td>Malignant</td>
</tr>
</tbody>
</table>

In FNAB cytology of breast there will be:

• A statement of whether the lesion is completely benign, such as “No malignant cells are seen”.

• A statement of cellularity which in some ways is a measure of the adequacy of the material.

• A cytological description including any diagnostic criteria or check list of features and a brief discussion of the features which support various possible diagnoses.

• A conclusion or summary with a standardized descriptive diagnosis of the lesion which should be as specific a diagnosis as possible.

• A code or category can be placed in the body of the report but not in the conclusion.
IAC Standardized Reporting of Breast Fine-Needle Aspiration Biopsy Cytology

Categories (Provisional) for Reporting Breast FNAB Cytology

Code 1 – Insufficient material
Code 2 – Benign
Code 3 – Atypical, probably benign
Code 4 – Suspicious, probably in situ or invasive carcinoma
Code 5 – Malignant

Cytological criteria of benign lesions
- Cohesive epithelial groups without or with mild nuclear overlapping and presence of myoepithelial cells
- Naked nuclei
- Apocrine cells

Cytological criteria of malignancy
- Loss of cohesiveness
- Isolated cells with cytoplasm
- Nuclear pleomorphism
- Dirty background
- No naked nuclei

Breast FNAC: solving problems

- Current evidence indicates that the use of non-operative diagnosis substantially reduces the number of unnecessary operations performed both for benign disease and for malignancy, with reduced discomfort and inconvenience to the patient.

- What is the role of breast FNAC in benign lesions, malignant lesions and “gray zone” lesions?
**BREAST FNAC: solving problems**

**Benign Lesions**

*FNAC is a useful and reliable tool in the evaluation and management of benign breast lesions, such as:*

- Inflammatory conditions
- Cysts
- Fibroadenoma

**CYTOLOGICAL INTERPRETATION**

**Inflammatory diseases**

**BENIGN – SUBAREOLAR ABSCESS**

- A high yield of inflammatory cells and multinucleated giant cells.
- Keratin and squamous metaplastic cells.
- The identification of giant cells with keratin at cytoplasm is an important clue for the diagnosis.
- Reactive epithelial cells.

**BENIGN – GRANULOMATOUS MASTITIS**

**CYTOLOGICAL INTERPRETATION**

- Moderate to high cellularity.
- Foam cells (sometimes multinucleated)
- Collapsed fat cells.
- Inflammatory cells.
- Sometimes, presence of worrisome nuclei atypical

**BENIGN – FAT NECROSIS**

- More frequent in women with large breasts.
- In general there is a history of recent severe trauma, surgery or radiotherapy, although that does not exist in some cases.
- There may present as a palpable nodule or just a focal area of pain.
After complete aspiration of the cyst, it is especially important to re-evaluate the area (US) to determine if a residual breast mass is present.

If a residual mass is found, a second aspiration should be performed.

Be careful with apocrine changes.

**Rules to Evaluate Cysts**

**Cyto logical Interpretation**

**Fibroadenoma**

- It is the most common benign tumour of the breast.
- Solitary nodule (most), sometimes multifocal and/or bilateral.
- Usually are non-tender well-circumscribed nodules.
- Biphasic proliferative lesion (epithelial and stromal elements) is similar to the structures of the terminal lobular-ductal unit (TLDU).

**Cytological Criteria of Fibroadenoma**

- Large branching, monolayer sheets of uniform epithelial cells
- Fragments of fibromyxoid stroma
- Numerous single, bare bipolar nuclei (myoepithelial cells)
- Predominant pattern: Solid
- Low cellularity of epithelial cells
- Coherent epithelial groups without or with mild nuclear overlapping and presence of myoepithelial cells
- Heterogenous cell population: mild variation in the size and shape of the nuclei (oval, round, or spindle)
- Inflammatory cells can be present
- Bipolar naked nuclei in the background
- Foam cells, apocrine metaplasia, and stroma fragments can be observed

**Cyto logical Interpretation**

**Benign epithelial proliferative lesion**

Fine Needle Aspiration Cytology of the Breast
Gary The + Puyu Hoon Tan
Fernando Schmitt
BREAST FNAC: solving problems
“Gray zone”

- Papillary Lesions
- Epithelial Proliferative Lesions
- Fibro-epithelial Lesions

CYTOLOGICAL INTERPRETATION
Papillary Lesions

- Cellular smears.
- Papillary three-dimensional arrangements.
- Complex folded and branching sheets of epithelial cells.

CYTOLOGICAL INTERPRETATION
Papillary Lesions

- Columnar cells in rows, palisades and single.
- Variable nuclear atypical
- Epithelial cells with cytoplasm vacuoles
CYTOLOGICAL INTERPRETATION
Papillary Carcinoma
Is it possible to distinguish benign and malignant Papillary breast tumours on FNA?

Cytological findings favouring malignant
- Higher cellularity
- Papillary three-dimensional arrangements without a central fibro vascular core (cell balls)
- Tall columnar cells frequent.
- Isolated cells with cytoplasm.
- Absence of bare nuclei, apocrine metaplasia and rare macrophages.

PAPILLARY LESIONS: CNB helps?

European guidelines on breast cancer screening

CYTOLOGICAL INTERPRETATION
Epithelial proliferative lesions
- Moderate to high cellularity.
- Epithelial cell groups with overlapping and without or w/ few myoepithelial cells.
- Bipolar naked nuclei in the background absent or in few numbers.
- Less cell cohesively in the borders of the cell groups with occasional isolated epithelial cells with preserved cytoplasm.
- 20% are malignant at biopsy.
PHYLLODES TUMOUR

• Biphasic proliferative lesion (epithelial and stromal elements) similar to fibroadenoma but with predominance of the stroma over the epithelium.

• Fibromyxoid stromal fragments are larger than those seen in fibroadenomas and are highly cellular with fibroblastic spindle cells.

• The presence of isolated stromal cells with spindle nuclei and abundant pale cytoplasm is suggestive of PT.

FIBROADENOMA

Myxoid changes

Atypia

BREAST FNAC: solving problems

Malignant Lesions

• Definitive surgery for carcinoma can be planned preoperatively using the triple approach or radiological imaging, clinical examination and FNAC (or CNB). This permits treatment for many malignant lesions in a one-stage operation.
CYTOLOGICAL CRITERIA OF INVASIVE DUCTAL CARCINOMA

- Cellular smear, w/variable cell pattern, sometimes plasmocytoid appearance
- Nuclear pleomorphism
- Loss of cohesion

CYTOLOGICAL INTERPRETATION
Invasive lobular carcinoma

- Variable cellularity. In some cases very poor cell yield.
- Cells single and in small clusters, short single files common.
- Epithelial cells have small dark nuclei with scanty cytoplasm. The lack of pleomorphism can be cause of a false-negative diagnosis.
- Intracytoplasmic lumina/vacuoles.

A most valuable clue on ILC is the tendency to form small chains of cells in the aspirates

CYTOLOGICAL INTERPRETATION
Invasive lobular carcinoma

- Well defined and circumscribed tumour (similar to fibroadenoma).
- Abundant background mucinous, atypical cells in small solid aggregates, single files or isolated. The mucin stains violet to blue with MGG or pink on HE staining.

CYTOLOGICAL INTERPRETATION
Mucinous carcinoma

- Variable cellularity (moderate to intense). At low magnification a pattern somewhat similar to fibroadenoma.
- Cells arranged mostly in tubular structures with comma-like pattern.
- Epithelial cells are uniform and bland. The lack of pleomorphism can be cause of a false-negative.
- Bare nuclei are present in rare cases.

CYTOLOGICAL INTERPRETATION
Tubular carcinoma

- Is an invasive ductal carcinoma with metaplastic changes: squamous cells, spindle cells, osteoid or chondroid.
- Smears can show different cell types: ductal, spindle or squamous.
- Sometimes we can observe multinucleated giant cells and myxoid material.
- Can be cystic at aspiration and with necrotic material.
**CYTOLOGICAL INTERPRETATION**

Invasive micropapillary carcinoma

- Highly cellular smears composed by angulated small groups of cohesive cells with papillary configurations without fibro vascular cores.
- Cells showing nuclear atypical, irregular nuclear contours and prominent nucleoli.
- Cytoplasm vacuoles are rarely seen.
- Background is clean with rare isolated neoplastic cells.

Apocrine carcinoma

- Malignant cells have a large dense eosinophilic granular cytoplasm with large nuclei with prominent nucleoli.
- Neoplastic cells are isolated, sometimes without cytoplasm and in small aggregates.
- Necrosis is frequent.

Breast carcinoma with osteoclast-like giant cells

- Cellular smears composed by cohesive groups of epithelial cells, with low grade of atypia.
- Groups of plump spindle cells as well as isolated atypical epithelial cells.
- Presence of osteoclast-like multinucleated cells at periphery of the epithelial cells or in the background of the smears.

Adenoid cystic carcinoma

- Highly cellular
- Pattern of large tissue fragments, consisting of cells with poorly defined cytoplasm, minimal cytological atypia and myoepithelial cells.
- Background may have dispersed bare nuclei and/or dispersed intact atypical cells.
- Hyaline spherules, varying from mucinous to collagenous

**METASTATIC MALIGNANCY**

- Metastatic melanoma
- Metastatic ovarian carcinoma

**OTHER MALIGNANCY**

- Non-Hodgkin lymphoma
BENIGN – INTRAMAMMARY LYMPH NODE

35-year old female presented with a 25 mm well-defined nodule in the right breast. Mammography and US are compatible with fibroadenoma. There is no family history of breast cancer.

Well-circumscribed mass (similar to fibroadenoma).

High cellular smears with irregular groups and single atypical cells. The cells are large, pleomorphic with prominent nucleoli. Background rich in lymphocytes.

Definitive diagnosis requires the demonstration of well defined borders.

They are frequently associated with germinal mutation of BRCA-1.

CYTOLOGICAL INTERPRETATION

Inflammatory diseases

Medullary carcinoma

33-year old female presented with a 15 mm ill-defined nodule in the right breast. Mammography and US are compatible with carcinoma.

Imaging typically shows a dense mass with stellate margin, simulating malignancy.

High cellular yield.

Cells showing moderate atypia with intact, abundant and granular cytoplasm.

Breast cancer is not a single disease at molecular level

Benign Granular Cell Tumour

Imaging typically shows a dense mass with stellate margin, simulating malignancy.

High cellular yield.

Cells showing moderate atypia with intact, abundant and granular cytoplasm.
Histological types of breast carcinoma

Cytological types of breast carcinoma

Breast cancer classification

ORIGINAL ARTICLES

Cytological Criteria to Predict Basal Phenotypes of Breast Carcinomas

Breast cancer classification

Russnes et al. JCI 2011
In breast cancer, molecular cytopathology can be used

- In primary tumours
- In metastatic tumours

ALK amplification is related to worst prognosis and high proliferative index

There is a good correlation between FIS, RT-PCR and IHQ.

INFLAMMATORY BREAST CARCINOMA (IBC)
MOLECULAR STUDIES

NGS results in 76 cases of LABC

INFLAMMATORY BREAST CARCINOMA (IBC)
MOLECULAR STUDIES and RESPONSE TO QT

METASTATIC DISEASE? AND NOW?
Be sure to treat the present disease

Table 5. Proportion of Women with a Change in Originating Targeted Therapy by Prognostic Group

| Prognostic Group | Number (N) | Test of Independence
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HER-2 positive</td>
<td>30 (44.1)</td>
<td></td>
</tr>
<tr>
<td>HER-2 negative</td>
<td>34 (55.9)</td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Distribution of Survival by Prognostic Group

<table>
<thead>
<tr>
<th>Prognostic Group</th>
<th>Survival (In Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER-2 positive</td>
<td>11.8 (n = 30)</td>
</tr>
<tr>
<td>HER-2 negative</td>
<td>19.6 (n = 34)</td>
</tr>
</tbody>
</table>

Park K et al. (2015) J Oncotarget

Be sure to treat the present disease.
Multinational study of oestrogen and progesterone receptor immunocytochemistry on breast carcinoma fine needle aspirates

29/04/2017

Antigen retrieval

ER/PR ASSESSMENT IN BREAST FNAs

Estimation of Hormone Receptor Status in Fine-Needle Aspirates and Paraffin-Embedded Sections From Breast Cancer Using the Novel Rabbit Monoclonal Antibodies SP1 and SP2

Multinational study of oestrogen and progesterone receptor immunocytochemistry on breast carcinoma fine needle aspirates

- Cytospins and monolayer preparations were superior to direct smears for the evaluation.
- Methods of fixation and antigen retrieval were the key points in the staining process.
- While it was not possible to prove the superiority of a single fixation protocol, the usefulness of antigen retrieval (heat-induced) was clearly demonstrated.

Multinational study of oestrogen and progesterone receptor immunocytochemistry on breast carcinoma fine needle aspirates

- Air-dried fixed in 4% formaldehyde for IHC, discrepancies of 9%ER, 7.5%PR and 32.8% for Ki-67.
- Differences in ER (4-histo e + cytology and 4+cytology and 4-hist).
- Ki-67 not standardized!
ISH FOR HER2 IN BREAST FNA

- ISH can be performed successfully in the majority of cases on archival cytological slides, and the results are reliable and accurate.
- Good concordance between HER-2 amplification in FNA samples and whole histological sections, using single or dual probes.

FISH studies comparing primary breast cancers and their matched distant metastases

<table>
<thead>
<tr>
<th>Source</th>
<th>Patients (n)</th>
<th>Discordance between primary and metastases</th>
<th>Type of Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gancberg et al., 2002</td>
<td>68</td>
<td>7%</td>
<td>Histology</td>
</tr>
<tr>
<td>Bozetti et al., 2003</td>
<td>14</td>
<td>0</td>
<td>Histology</td>
</tr>
<tr>
<td>Houssanni et al., 2011</td>
<td>105</td>
<td>7.6%</td>
<td>Histology</td>
</tr>
<tr>
<td>Wilking et al., 2011</td>
<td>147</td>
<td>9.5%</td>
<td>FNA</td>
</tr>
<tr>
<td>Schmitt et al., 2012</td>
<td>30</td>
<td>10%</td>
<td>FNA</td>
</tr>
</tbody>
</table>

Experts' opinion: Recommendations for retesting breast cancer metastases for HER2 and hormone receptor status

Frédéric Pinaud-Hecq, Renata A. Czukay, Yevdii M. Hanna, Robert X. Osamura, Josef Ritschel, Giuseppe Viale

- ISH can be performed successfully in the majority of cases on archival cytological slides, and the results are reliable and accurate.
- Good concordance between HER-2 amplification in FNA samples and whole histological sections, using single or dual probes.

NCCN Guidelines Version 1.2012 Breast Cancer

- biopsy documentation of first recurrence if possible, and determination of hormone receptor status (ER and PR) and HER2 status should be repeated, especially if unknown, originally negative or not over-expressed.

And now?

- Emergence of Constitutively Active Estrogen Receptor-α Mutations in Pretreated Advanced Estrogen Receptor–Positive Breast Cancer

- HER2 status should be repeated, especially if unknown.
Breast FNAC
How not to be washed away?

QUALITY

Technical factors

False-positives
- Bad quality of smears
- Fixation artefacts

False-negatives
- Operator dependent
- Characteristics of the lesion:
  - Size of the lesion
  - Size of the breast
  - Location
  - Histological type

Breast FNAC
How not to be washed away?

Aspiration should be direct to a define target.

FNAC is a multi-step procedure and to obtain a good material is essential for the diagnosis.

The cytological diagnosis should be done only with the knowledge of the clinical context and preferential in a multidisciplinary environment.

Negative results can not solve the patient problem.