BREAST CYTOLOGY

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Breast Cytology

- Minimally invasive, accurate and reliable diagnostic method.

- Follow high-risk cancer patients
- Detect early lesions
- To obtain prognostic and predictive information

Breast FNAC
Still used in developed countries?

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

ASSESSMENT OF AXILLARY NODES

- US-guided FNAC is indicated in a newly diagnosed breast cancer if axillary nodes appear suspicious or clinical examination is equivocal.

- FNAC can be requested when the node is palpable but thought to be reactive.

- Setting of locally advanced breast cancer when neoadjuvant chemotherapy is planned.

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.

Pre-operative diagnostic procedure

The choice of sampling method in any centre should be determined by:

- the sensitivity and specificity of the technique in the centre.
- the diagnostic information required for malignant lesions.
- patient comfort and costs.
- the availability of staff skilled and experienced in using the procedures, particularly FNAC sampling and interpretation.

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.

FNAC Multistep technique

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

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Liquid-Based Cytology in Fine-Needle Aspiration of Breast Lesions: A Review

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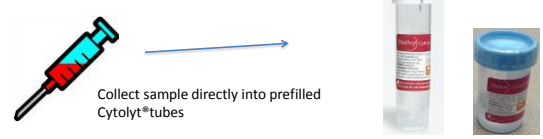

Collect sample directly into prefilled Cytolyt®tubes

HOW REPORT BREAST FNAC ?

Table 1. FNAC breast reporting categories, as listed in the European and USA guidelines

NHSBSP (UK), 2001[5] European Guidelines[16]	NIH recommendations (USA)[15]
C1 Unsatisfactory	Unsatisfactory
C2 Benign	Benign
C3 Suspicious, probably benign	Atypical/indeterminate
C4 Suspicious, probably malignant	Suspicious/probably malignant
C5 Malignant	Malignant

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.

IAC Structured Breast FNAB Cytology Reporting Yokohama 2016

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.

Editorial

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IAC Standardized Reporting of Breast Fine-Needle Aspiration Biopsy Cytology

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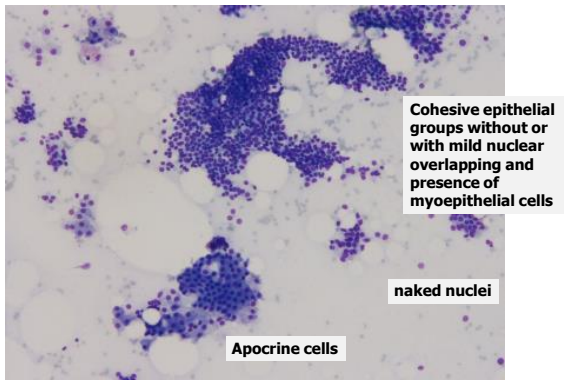
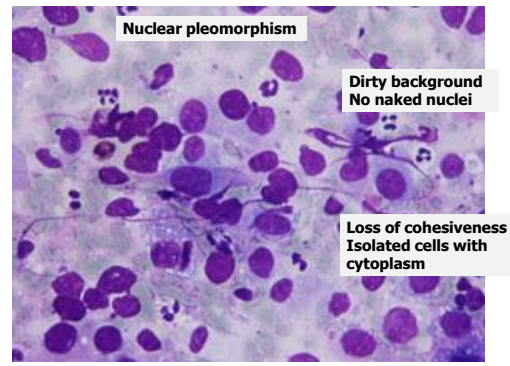
CATEGORIES (PROVISIONAL) FOR REPORTING BREAST FNAB CYTOLOGY

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



CYTOLOGICAL CRITERIA OF MALIGNANCY



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?

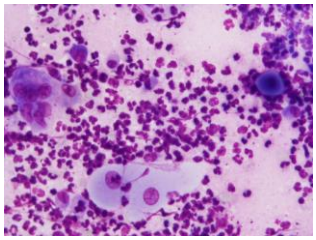
BREAST FNAC: solving problems Benign Lesions

• 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.

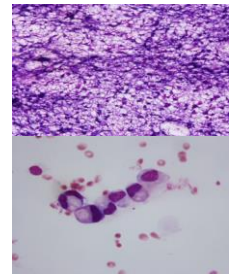
•Keratin and squamous metaplastic cells.

•The identification of giant cells with keratin at cytoplasm is an important clue for the diagnosis.

•Reactive epithelial cells.

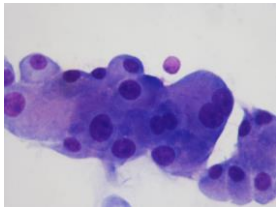
CYTOLOGICAL INTERPRETATION
Inflammatory diseases

BENIGN – FAT NECROSIS



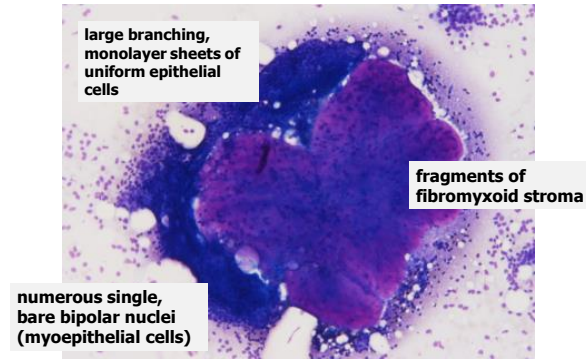
- Moderate to high cellularity.
- Foam cells (sometimes multinucleated)
- Collapsed fat cells.
- Inflammatory cells.
- Sometimes, presence of worrisome nuclei atypical

RULES TO EVALUATE CYSTS



- 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

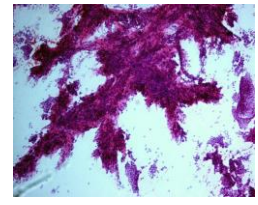
CYTOLOGICAL CRITERIA OF FIBROADENOMA

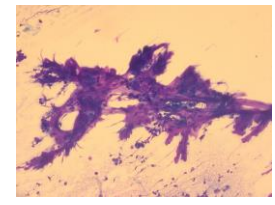


BREAST FNAC: solving problems
"Gray zone"

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

CYTOLOGICAL INTERPRETATION
Papillary Lesions

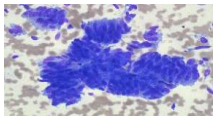


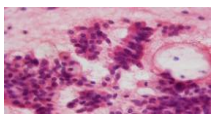


- Cellular smears.
- Papillary three-dimensional arrangements.
- Complex folded and branching sheets of epithelial cells.

CYTOLOGICAL INTERPRETATION
Papillary Carcinoma

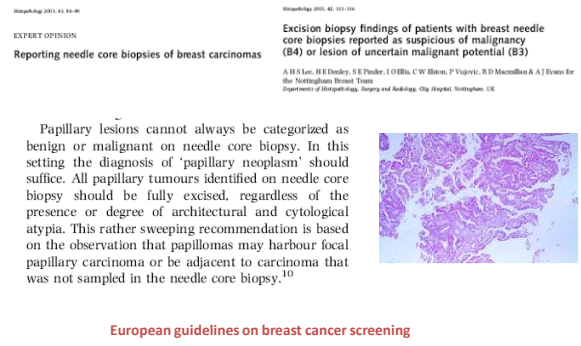
Is it possible to distinguish benign and malignant Papillary breast tumours on FNA ?





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.



EXPERT OPINION

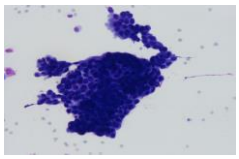
Reporting needle core biopsies of breast carcinomas

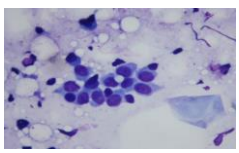
Excision biopsy findings of patients with breast needle core biopsies reported as suspicious of malignancy (B4) or lesion of uncertain malignant potential (B3)

Papillary lesions cannot always be categorized as benign or malignant on needle core biopsy. In this setting the diagnosis of 'papillary neoplasm' should suffice. All papillary tumours identified on needle core biopsy should be fully excised, regardless of the presence or degree of architectural and cytological atypia. This rather sweeping recommendation is based on the observation that papillomas may harbour focal papillary carcinoma or be adjacent to carcinoma that was not sampled in the needle core biopsy.[10]

European guidelines on breast cancer screening

CYTOLOGICAL INTERPRETATION
Epithelial proliferative lesions

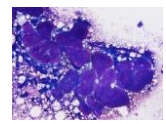


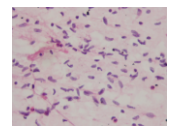


- Moderate to high cellularity.
- Epithelial cell groups with overlapping and without or w/ few myoepithelial cells.
- Bipolar naked nuclei in the background absent or in few numbers.
- Less cell cohesively in the borders of the cell groups with occasional isolated epithelial cells with preserved cytoplasm.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

CYTOLOGICAL INTERPRETATION
Fibroepithelial lesions

PHYLLODES TUMOUR



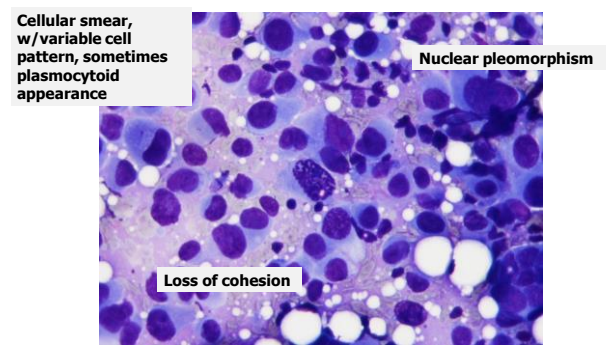


- Biphasic proliferative lesion (epithelial and stromal elements) similar to fibroadenoma but with predominance of the stroma over the epithelium
- Fibromyxoid stromal fragments are larger than those seen in fibroadenomas and are highly cellular with fibroblastic spindle cells.
- The presence of isolated stromal cells with spindle nuclei and abundant pale cytoplasm is suggestive of PT.

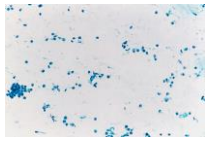
BREAST FNAC: solving problems
Malignant Lesions

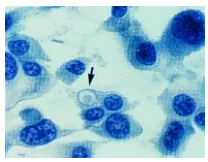
- Definitive surgery for carcinoma can be planned preoperatively using the triple approach or radiological imaging, clinical examination and FNAC (or CNB). This permits treatment for many malignant lesions in a one-stage operation.

CYTOLOGICAL CRITERIA OF INVASIVE DUCTAL CARCINOMA

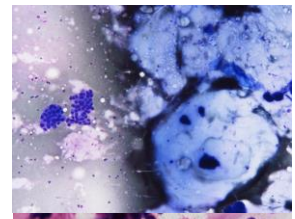


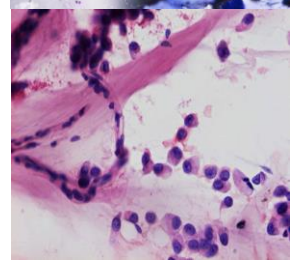
CYTOLOGICAL INTERPRETATION
Invasive lobular carcinoma





- 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.

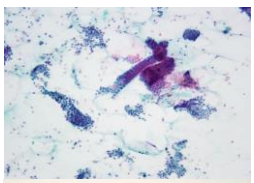


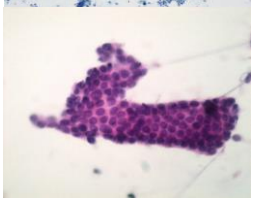


CYTOLOGICAL INTERPRETATION
Mucinous carcinoma

- 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.

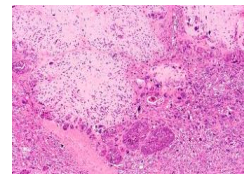
CYTOLOGICAL INTERPRETATION
Tubular carcinoma

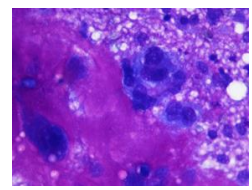




- Variable cellularity (moderate to intense). At low magnification a pattern somewhat similar to fibroadenoma.
- Cells arranged mostly in tubular structures with comma-like pattern.
- Epithelial cells are uniform and bland. The lack of pleomorphism can be cause of a false-negative.
- Bare nuclei are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

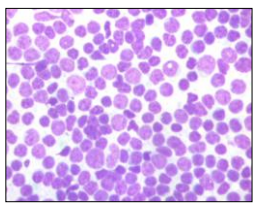
CYTOLOGICAL INTERPRETATION
Metaplastic carcinoma

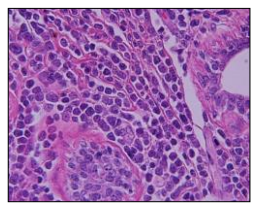




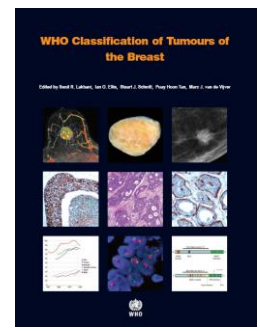
- Is an invasive ductal carcinoma with metaplastic changes: squamous cells, spindle cells, osteoid or chondroid.
- Smears can show different cell types: ductal, spindle or squamous.
- Sometimes we can observe multinucleated giant cells and myxoid material.
- Can be cystic at aspiration and with necrotic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

CYTOLOGICAL INTERPRETATION
Other malignancy





NON-HODGKIN LYMPHOMA

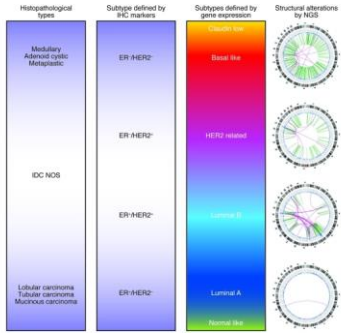


1-1H

Classification using needle-core biopsy and fine-needle aspiration

F. Schmitt
N. Sneige
A. Lee

Breast cancer classification



Russnes et al. JCI 2011

ORIGINAL ARTICLES

Cytological Criteria to Predict Basal Phenotype of Breast Carcinomas

Rozany Mucha Dufloth, M.D., Ph.D.,[1] Jacy Maria Alves, M.S.,[1] Diana Martins, B.Sc.,[2] Daniella Serafin Couto Vieira, M.D., M.Sc.,[1] Horácio Chikota, M.D.,[1] Luiz Carlos Zeferino, M.D., Ph.D.,[1] and Fernando Schmitt, M.D., Ph.D., FIAC[3]

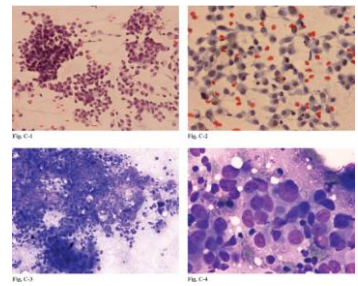
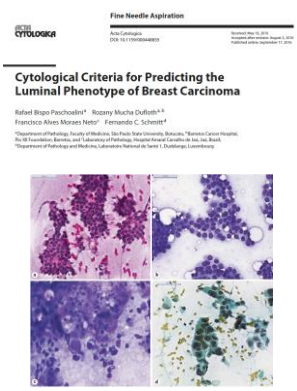


Table IV. Correlation Between Cytological Criteria Comparing the Basal Phenotype and Other Phenotypes of Breast Carcinoma-Defined Immunohistochemically

Cytological criteria	p*	OR (CI 95%)
Cellularity (scant vs. moderate/abundant)	0.0257	
Basal × HER2-overexpressing		6.48 (0.29–146.54)
Basal × luminal A		18.03 (0.96–337.81)
Basal × luminal B		15.00 (0.50–449.55)
Basal × nonbasal[b]	0.0247	12.38 (0.69–222.95)
Cell pattern (dissociated vs. fairly equal representation of clustered and dissociated cells /clustered)	0.3968	
Basal × HER2-overexpressing		0.28 (0.05–1.69)
Basal × luminal A		0.37 (0.08–1.58)
Basal × luminal B		0.76 (0.09–6.45)
Basal × nonbasal[b]		0.36 (0.10–1.29)
Necrosis (present)	0.0151	
Basal × HER2-overexpressing		2.75 (0.66–11.54)
Basal × luminal A		6.97 (1.72–28.25)
Basal × luminal B		0.61 (0.05–7.24)
Basal × nonbasal[b]		3.80 (1.16–12.38)
Nucleoli (inconspicuous vs. present/present and prominent)	0.0013	
Basal × HER2-overexpressing		0.23 (0.01–5.22)
Basal × luminal A		8.18 (1.51–44.21)
Basal × luminal B		2.50 (0.17–37.26)
Basal × nonbasal[b]		3.61 (0.72–18.19)
Nuclear atypia (mild vs. moderate/severe)	0.0230	
Basal × HER2-overexpressing		1.13 (0.02–60.37)
Basal × luminal A		12.30 (0.64–234.91)
Basal × luminal B		3.89 (0.05–224.24)
Basal × nonbasal[b]	0.1703	6.07 (0.32–113.83)

Diagnostic Cytopathology, 2009



Cytological Criteria for Predicting the Luminal Phenotype of Breast Carcinoma

Rafael Bispo Paschoalini[1] · Rozany Mucha Dufloth[2,3]
Francisco Alves Moraes Neto[2] · Fernando C. Schmitt[4]

Cytological criteria	p value	OR (95%CI)
Cellularity (mild/moderate vs. abundant)		
Luminal × non-luminal	0.019	7.12 (1.61–31.52)
Luminal A × non-luminal	0.012	6.78 (1.51–30.45)
Luminal B × non-luminal	0.009	8.11 (1.61–39.49)
Luminal A × luminal B	0.637	0.84 (0.40–1.76)
Luminal A × other (including luminal B)	0.263	1.46 (0.75–2.84)
Cell cohesion (mild vs. moderate/intense)		
Luminal × non-luminal	0.087	0.44 (0.17–1.13)
Luminal A × non-luminal	0.043	0.37 (0.14–0.97)
Luminal B × non-luminal	0.524	0.70 (0.23–2.10)
Luminal A × luminal B	0.095	0.53 (0.25–1.12)
Luminal A × other (including luminal B)	0.009	0.46 (0.24–0.88)
Necrosis (present vs. absent)		
Luminal × non-luminal	0.935	0.00 (0.00–999.99)
Luminal A × non-luminal	0.934	0.00 (0.00–999.99)
Luminal B × non-luminal	0.955	0.00 (0.00–999.99)
Luminal A × luminal B	0.187	0.56 (0.26–1.30)
Luminal A × other (including luminal B)	0.003	0.32 (0.15–0.68)
Nucleoli (inconspicuous/present vs. prominent)		
Luminal × non-luminal	0.001	8.31 (2.36–29.19)
Luminal A × non-luminal	0.000	11.12 (3.12–39.94)
Luminal B × non-luminal	0.061	3.73 (0.94–14.82)
Luminal A × luminal B	0.005	2.99 (1.39–6.41)
Luminal A × other (including luminal B)	0.000	4.43 (2.24–8.77)
Nuclear atypia (mild/moderate vs. intense)		
Luminal × non-luminal	0.002	8.42 (1.90–37.25)
Luminal A × non-luminal	0.003	9.93 (2.22–44.44)
Luminal B × non-luminal	0.042	5.25 (1.06–25.88)
Luminal A × luminal B	0.103	1.89 (0.88–4.07)
Luminal A × other (including luminal B)	0.002	2.96 (1.48–5.93)

Molecular biology and cytopathology. Principles and applications

Cytopathologie moléculaire. Outils et applications

Fernando C. Schmitt[a,*], Philippe Vielh[b]

Annales de pathologie (2012) 32, e57–e63

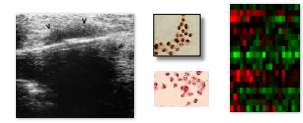
Possible use and role of molecular techniques in fine-needle aspiration cytology (FNAC) practice

Fernando Schmitt
Helena Barroca

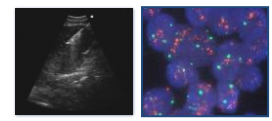
DIAGNOSTIC HISTOPATHOLOGY 17:7

In breast cancer, molecular cytopathology can be used

- ***In primary tumours***

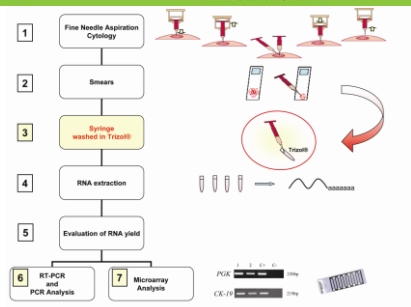


- ***In metastatic tumours***



High-Throughput Molecular Analysis from Leftover of Fine Needle Aspiration Cytology of Mammographically Detected Breast Cancer[1,2]

Laura Annaratone*, Caterina Marchiò*, Tommaso Renzulli†, Isabella Castellano*,‡, Daniela Cantarella†, Claudio Isella†, Luigia Macrì*,‡, Giovanna Mariscotti‡,§, Davide Balmativola*, Elisabetta Cantanna*, Cristina Deambrogio*, Francesca Pietribiasi‖, Riccardo Arisio#, Fernando Schmitt**, Enzo Medico† and Anna Sapino*,‡



Translational Oncology

VOLUME 30 · NUMBER 21 · JULY 20 2012
JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Clinically Used Breast Cancer Markers Such As Estrogen Receptor, Progesterone Receptor, and Human Epidermal Growth Factor Receptor 2 Are Unstable Throughout Tumor Progression

Linda Sofie Lindström, Eva Karlsson, Ulla M. Wilking, Ulla Johansson, Johan Hartman, Elisabet Kerstin Lidbrink, Thomas Hatschek, Lambert Skoog, and Jonas Bergh

Assessing changes from primary to relapse:

ER (459 patients) => 32,4% (McNemar's test P .001),
PR (430 patients) => 40.7% (P .001),
HER2 (104 patients) => 14.5% (P .44).

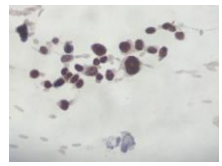
Assessing changes between subsequent relapses:

ER (119 patients) => 33.6%,
PR (116 patients) => 32.0%
HER2 (32 patients) => 15.7%

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

Guillerma Cano, M.D.[1] Fernanda Milanezi, M.D.[2] Dina Leitão, B.Sc.[2,3] Sara Ricardo, B.Sc.[2] Maria José Brito, M.D.[1] and Fernando Carlos Schmitt, M.D., Ph.D.[2,3]*



Diagn. Cytopathol. 2003;29:207–211.

Table III. Accuracy, Sensitivity, and Specificity of Estrogen Receptor (ER) Immunocytochemical Assay Using the Rabbit Monoclonal Antibody SP1 in Fine-Needle Aspirate Specimens (FNA) and Formalin-Fixed Specimens (FF) Compared With ER Detection Using the Mouse Monoclonal Antibody 6F11 in Formalin-Fixed Specimens (FF)

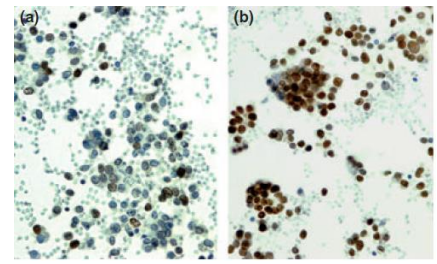
	FF-6F11 N (%)		
Reaction	*Accuracy*	*Sensitivity*	*Specificity*
FNA-SP1	38/40 (95)	22/24 (91.7)	16/16 (100)
FF-SP1	40/40 (100)	24/24 (100)	16/16 (100)

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

Ž. P. Marinšek*, N. Nolde*, I. Kardum-Skelin†, R. Nizzoli‡, B. Önal§, T. Rezanko¶, E. Tani**, K. T. Ostović††, P. Vielh‡‡, F. Schmitt§§ and G. Kocjan¶¶

Cytopathology 2012

Antigen retrieval



Histopathology

Fine-needle aspiration cytology samples: a good source of material for evaluating biomarkers in breast cancer

DOI: 10.1111/his.12439

Stalhammar G, Rosin G, Fredriksson I, Bergh J, Hartman J. Low concordance of biomarkers in histopathological and cytological material from breast cancer. *Histopathology* 2014; **64**: 971–980.

- 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!

Fernando Schmitt[1]
Philippe Vielh[2,3]

[1]Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto, Toronto, ON, Canada, [2]Department of Pathology, University Health Network, Toronto, ON, Canada, and [3]Department of Medical Biology and Pathology, Translational Research Laboratory and Biobank, Gustave Roussy Comprehensive Cancer Centre, Villejuif, France

FISH studies comparing primary breast cancers and their matched distant metastases

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

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

Frédérique Penault-Llorca[a,b,*], Renata A. Coudry[c], Wedad M. Hanna[d], Robert Y. Osamura[e], Josef Rüschoff[f], Giuseppe Viale[g]

Recommendations for sample type and testing methods

Fine-needle and core biopsies are acceptable for testing, providing they are feasible, formalin-fixed, and safe to obtain

- Bone samples are not acceptable for immunohistochemistry as decalcification is required for testing, and methods must be validated
  - Bone samples are acceptable after decalcification when *in situ* hybridization is used
- Only core-needle biopsies, not fine-needle aspirates, are acceptable if primary tumor samples are not available
  - To allow an ample number of definitive tumor cells for the detection of target molecules by immunohistochemistry and/or *in situ* hybridization
- When the primary tumor is heterogeneous, as many samples as are feasible should be taken from the metastatic site

***In situ* hybridization should be used**

- Fine-needle aspirate samples may have compromised cell membranes, which are unsuitable for immunohistochemistry

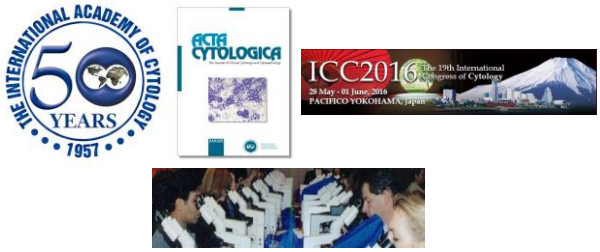
Repeat staining of the primary tumor (if available) and of the metastases should be performed in the same test run to minimize technical artifacts

- Before reporting any change in receptor status in the metastasis (particularly receptor-positive to -negative changes)

The Breast 22 (2013) 200–202

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.



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