2nd BOSNIAN TURKISH CYTOPATHOLOGY SCHOOL

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6th-7th May, 2017

ORGANIZED BY:

Turkish Division of the International Academy of Pathology (TDIAP)
Turkish Society of Cytopathology
Department of Pathology, Clinical Centre of the University of Sarajevo, Bosnia and Herzegovina

BREAST FINE NEEDLE ASPIRATION CYTOLOGY

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25 ANOS A DESCOBRIR O CANCRO ipatimup

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PRECLINICAL STUDY

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

Jianyu Dong[1,2] · Amy Ly[1] · Ronald Arpin[1] · Quratulain Ahmed[3] · Elena Brachtel[1]

Table 2 Diagnostic accuracy of breast FNA

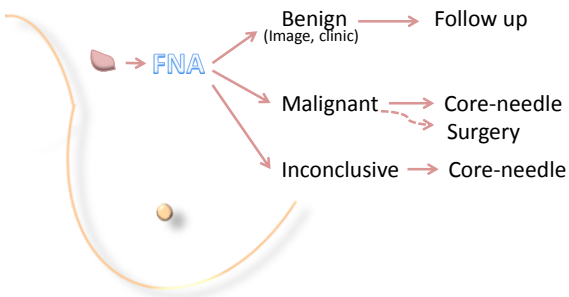
Parameters	Overall (%) $N = 1654$	Primary breast (%) $N = 1602$	Breast reconstruction, chest wall (%) $N = 52$
Nondiagnostic rate	4.8	4.7	5.8
Indeterminate rate	7.1	7.3	0
Absolute sensitivity	74.5	74.4	83.3
Complete sensitivity	91.6	91.7	83.3
Specificity	95.5	95.4	100
PPV of malignant	100	100	100
PPV of suspicious	93.3	93.3	–
NPV of benign	97.5	97.6	97.7
NPV of atypical	59.8	59.8	–
False-positive rate	0	0	0
False-negative rate	7.9	7.8	16.7

PPV positive predictive value, *NPV* negative predictive value

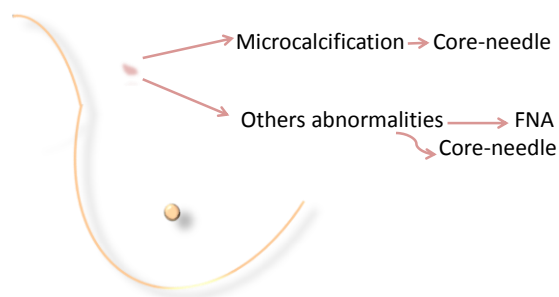
Breast FNAC
Still used in developed countries?

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

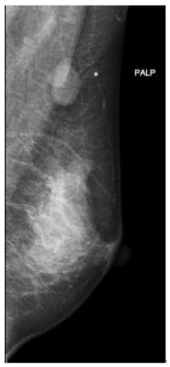
Palpable Lesions

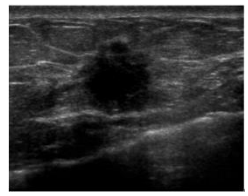


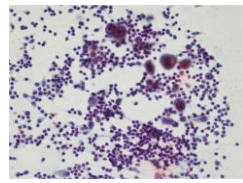
Non-Palpable Lesions



ASSESSMENT OF AXILLARY NODES







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.

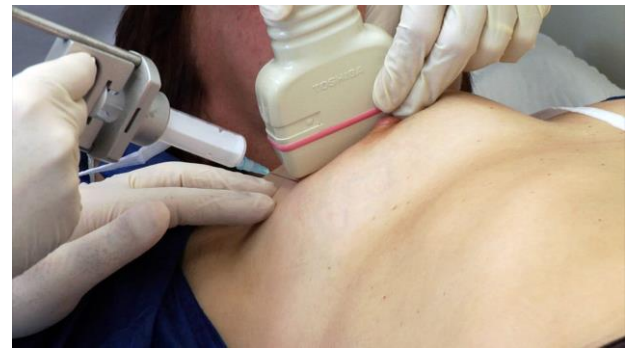
FNAC
Multistep technique

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

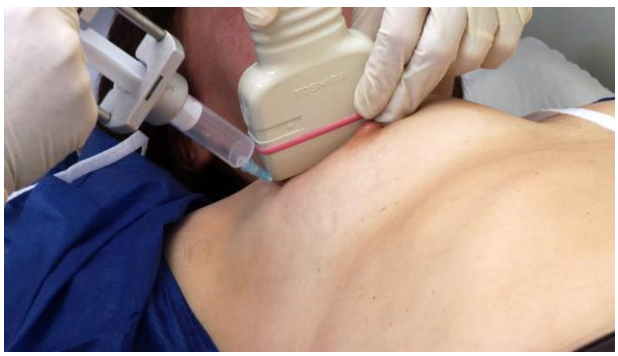
TRIPLE ASSESSMENT APPROACH

BBB: 98% benign – follow up
MMM: 1% error – surgery
Other: biopsy

FNAC Clinic Material



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

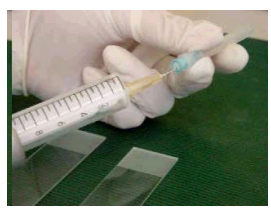


Applying suction while moving the needle helps to pull cells into the needle. A blood-tinged specimen will appear in the hub of the needle. Suction is then released and the needle is withdrawn.

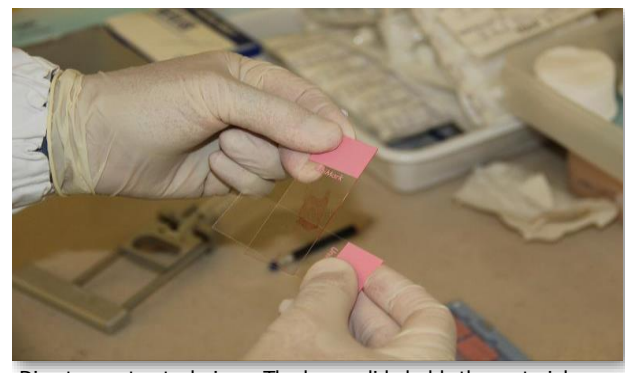
Slide preparation

- 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



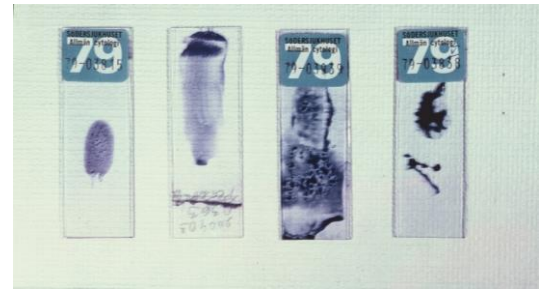
- Sometimes, if the hub of the needle is full, it is possible to tap the hub against the glass and obtain the material directly from there.



Direct one-step technique. The lower slide holds the material, while the upper slide is used as spreader slide. The spreader slide is held at an angle so that its superior edge is poised above the droplet and spread the material.

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



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

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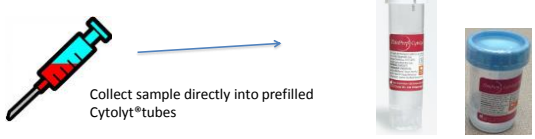

Collect sample directly into prefilled Cytolyt®tubes

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.

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Editorial

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IAC Standardized Reporting of Breast Fine-Needle Aspiration Biopsy Cytology

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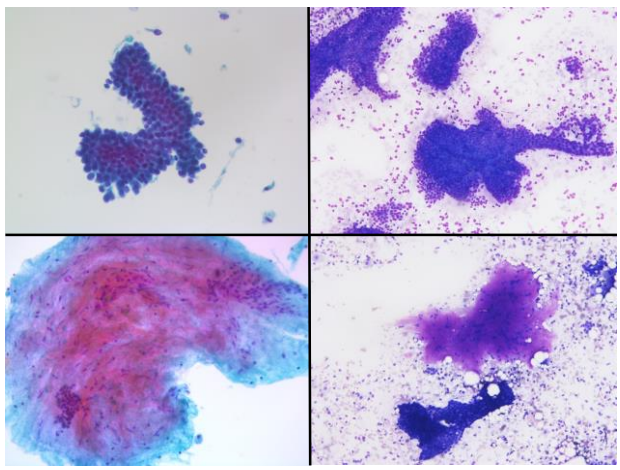
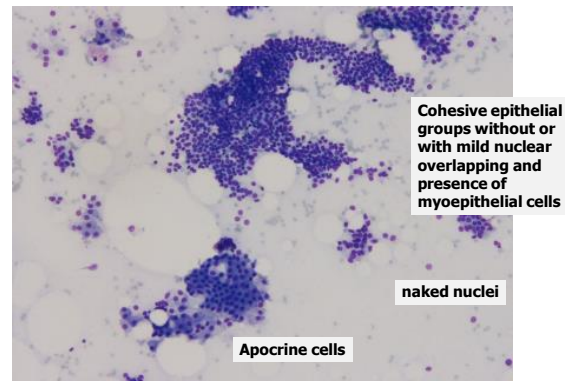
CATEGORIES (PROVISIONAL) FOR REPORTING BREAST FNAB CYTOLOGY

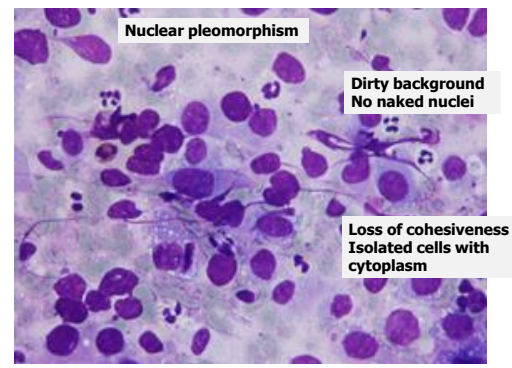
Code 1 – Insufficient material
Code 2 – Benign
Code 3 – Atypical, probably benign
Code 4 – Suspicious, probably in situ or invasive carcinoma
Code 5 – Malignant

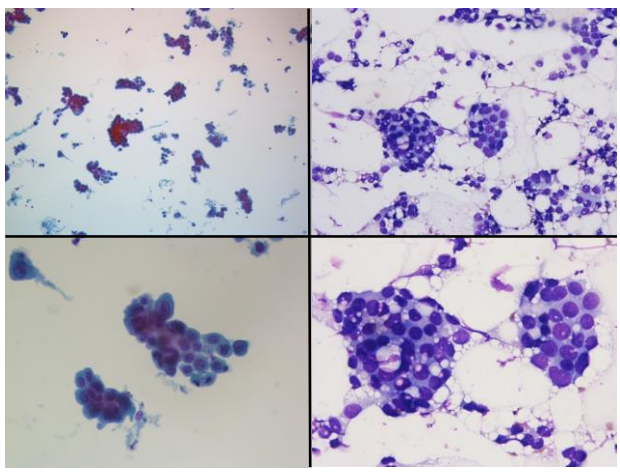


CYTOLOGICAL CRITERIA OF BENIGN LESIONS



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?

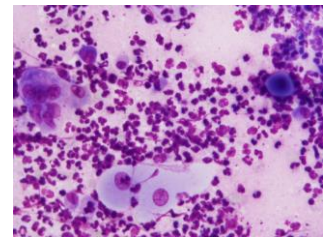
BREAST FNAC: solving problems
Benign Lesions

• 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.

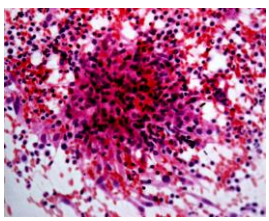
•Keratin and squamous metaplastic cells.

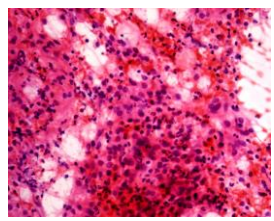
•The identification of giant cells with keratin at cytoplasm is an important clue for the diagnosis.

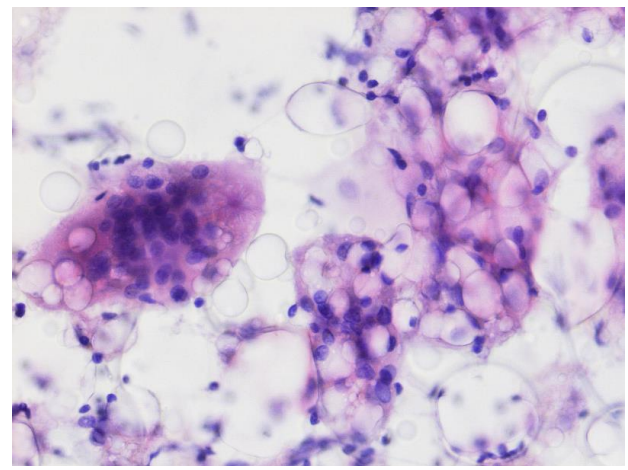
•Reactive epithelial cells.

CYTOLOGICAL INTERPRETATION
Inflammatory diseases

BENIGN – GRANULOMATOUS MASTITIS

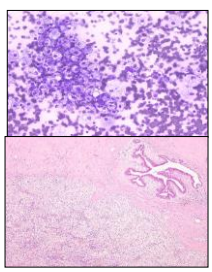






CYTOLOGICAL INTERPRETATION
Inflammatory diseases

BENIGN – FAT NECROSIS



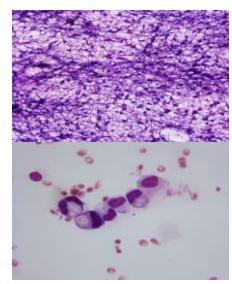
•More frequent in women with large breasts.

•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.

CYTOLOGICAL INTERPRETATION
Inflammatory diseases

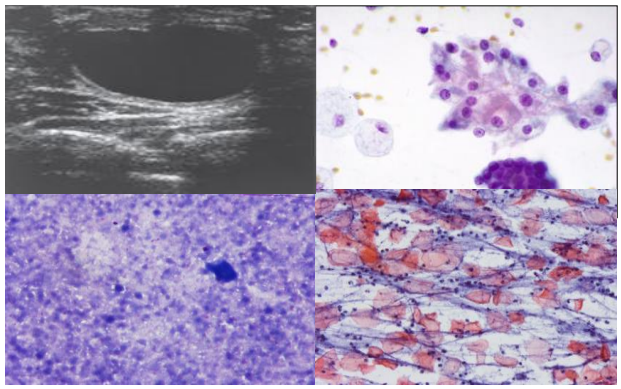
BENIGN – FAT NECROSIS



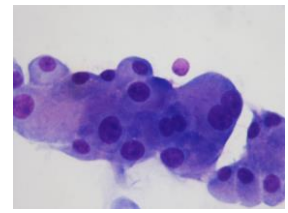
- Moderate to high cellularity.
- Foam cells (sometimes multinucleated)
- Collapsed fat cells.
- Inflammatory cells.
- Sometimes, presence of worrisome nuclei atypical

CYTOLOGICAL INTERPRETATION

BENIGN - CYSTS

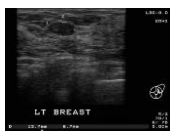


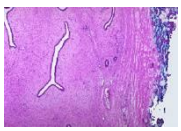
RULES TO EVALUATE CYSTS

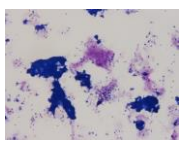


- After complete aspiration of the cyst, it is especially important to re-evaluate the area (US) to determine if a residual breast mass is present.
- If a residual mass is found, a second aspiration should be performed.
- Be careful with apocrine changes

CYTOLOGICAL INTERPRETATION Fibroadenoma

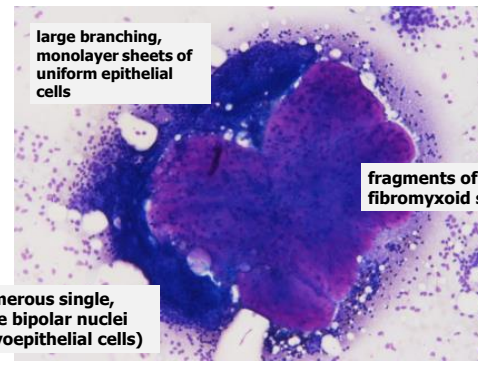




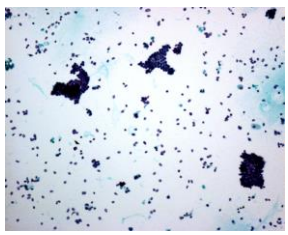


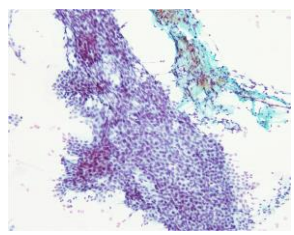
- It is the most common benign tumour of the breast.
- Solitary nodule (most), sometimes multifocal and/or bilateral.
- Usually are non-tender well-circumscribed nodules.
- Biphasic proliferative lesion (epithelial and stromal elements) is similar to the structures of the terminal lobular-ductal unit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

CYTOLOGICAL CRITERIA OF FIBROADENOMA



CYTOLOGICAL INTERPRETATION Fibroadenoma

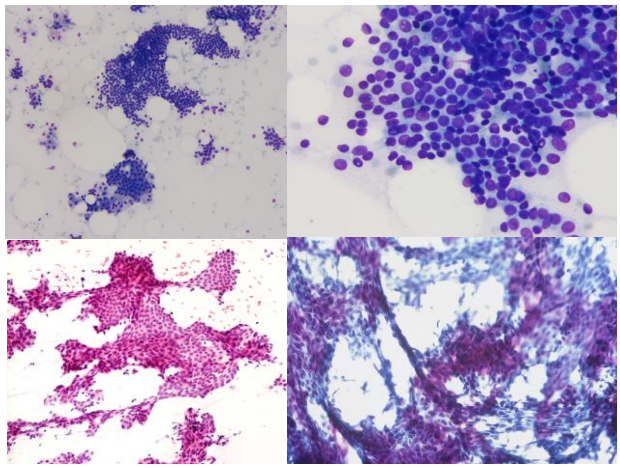




CYTOLOGICAL INTERPRETATION Benign epithelial proliferative lesion

Predominant pattern	
Cystic	Solid
Low cellularity of epithelial cells	Moderate to high cellularity
Foam cells and apocrine metaplasia frequently present	Cohesive epithelial groups without or with mild nuclear overlapping and presence of myoepithelial cells
Fluid background	Heterogeneous 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

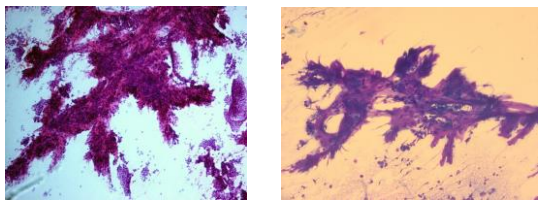
Fine Needle Aspiration Cytology of the Breast
Gary Tse • Puay Hoon Tan
Fernando Schmitt



BREAST FNAC: solving problems
"Gray zone"

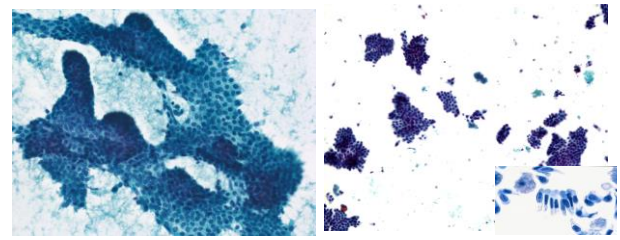
- ✓ Papillary Lesions
- ✓ Epithelial Proliferative Lesions
- ✓ Fibro-epithelial Lesions

CYTOLOGICAL INTERPRETATION
Papillary Lesions

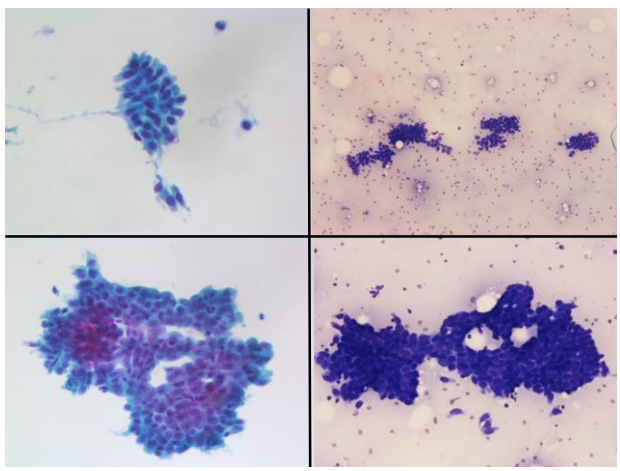


- Cellular smears.
- Papillary three-dimensional arrangements.
- Complex folded and branching sheets of epithelial cells.

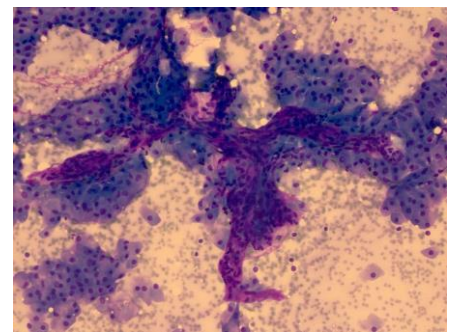
CYTOLOGICAL INTERPRETATION
Papillary Lesions



- Columnar cells in rows, palisades and single.
- Variable nuclear atypical
- Epithelial cells with cytoplasm vacuoles

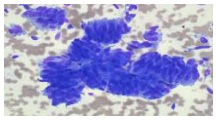


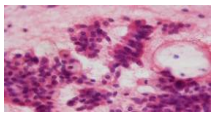
CYTOLOGICAL INTERPRETATION
Papillary Lesions



CYTOLOGICAL INTERPRETATION
Papillary Carcinoma

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

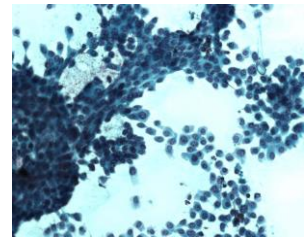


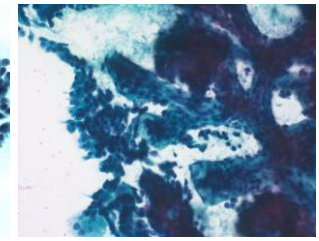


Cytological findings favouring malignant

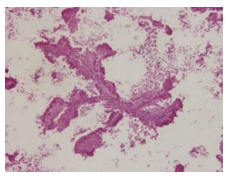
- Higher cellularity
- Papillary three-dimensional arrangements without a central fibro vascular core (cell balls)
- Tall columnar cells frequent.
- Isolated cells with cytoplasm.
- Absence of bare nuclei, apocrine metaplasia and rare macrophages.

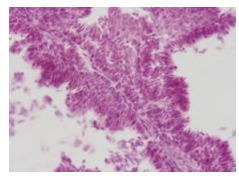
CYTOLOGICAL INTERPRETATION
Papillary Carcinoma

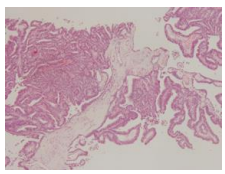


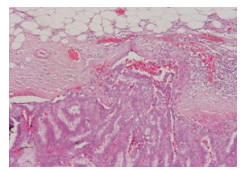


PAPILLARY LESIONS: CNB helps ?









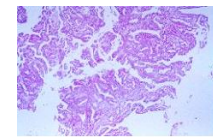
EXPERT OPINION

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)

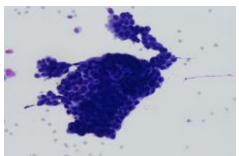
A H S Lee, H E Denley, S E Pinder, I O Ellis, C W Elston, P Vujovic, R D Macmillan & A J Evans for the Nottingham Breast Team

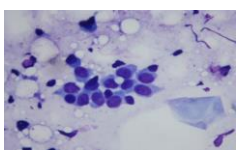
Papillary lesions cannot always be categorized as benign or malignant on needle core biopsy. In this setting the diagnosis of 'papillary neoplasm' should suffice. All papillary tumours identified on needle core biopsy should be fully excised, regardless of the presence or degree of architectural and 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]



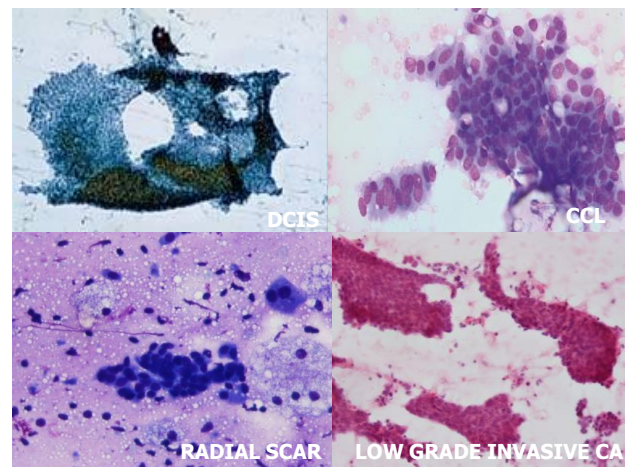
European guidelines on breast cancer screening

CYTOLOGICAL INTERPRETATION
Epithelial proliferative lesions



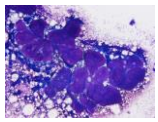


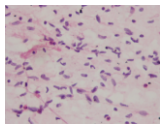
- Moderate to high cellularity.
- Epithelial cell groups with overlapping and without or w/ few myoepithelial cells.
- Bipolar naked nuclei in the background absent or in few numbers.
- Less cell cohesively in the borders of the cell groups with occasional isolated epithelial cells with preserved cytoplasm.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



CYTOLOGICAL INTERPRETATION
Fibroepithelial lesions

PHYLLODES TUMOUR

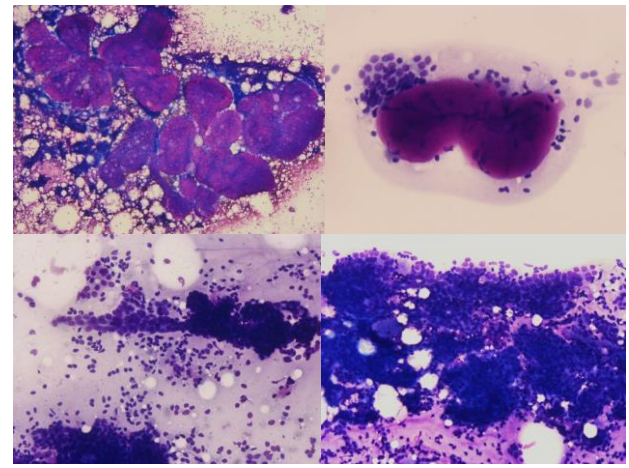


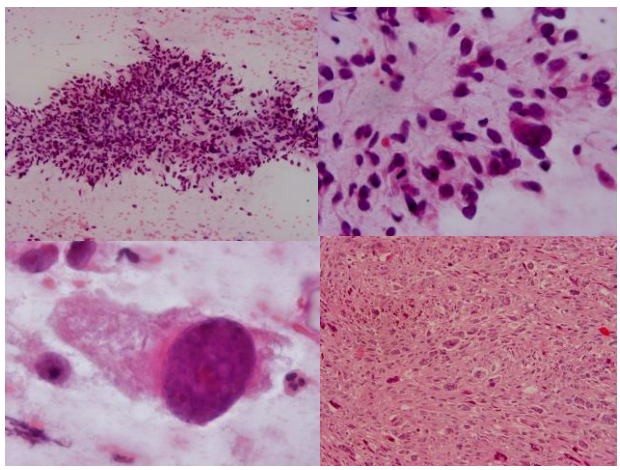


•Biphasic proliferative lesion (epithelial and stromal elements) similar to fibroadenoma but with predominance of the stroma over the epithelium

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

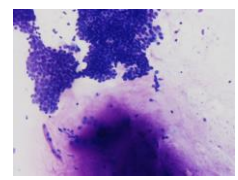
• The presence of isolated stromal cells with spindle nuclei and abundant pale cytoplasm is suggestive of PT.



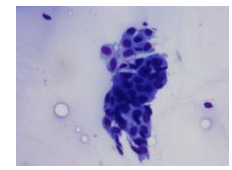


CYTOLOGICAL INTERPRETATION
Fibroepithelial lesions

FIBROADENOMA

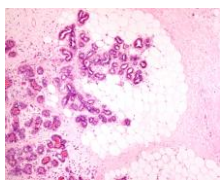


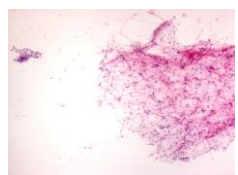
Myxoid changes

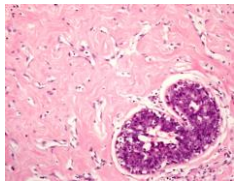


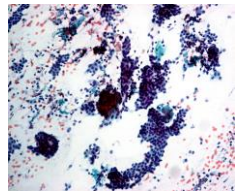
Atypia

CYTOLOGICAL INTERPRETATION
Fibroepithelial lesions





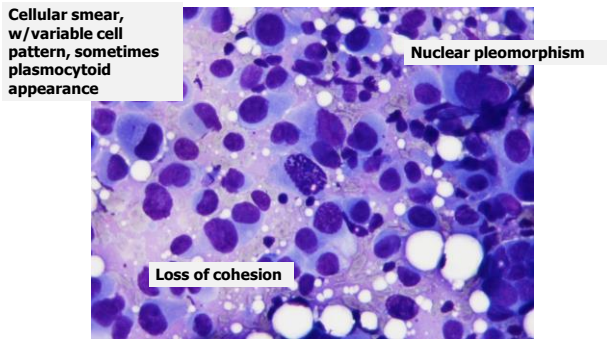




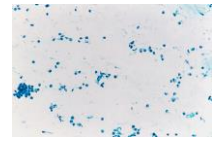
BREAST FNAC: solving problems
Malignant Lesions

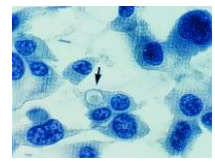
• Definitive surgery for carcinoma can be planned preoperatively using the triple approach or radiological imaging, clinical examination and FNAC (or CNB). This permits treatment for many malignant lesions in a one-stage operation.

CYTOLOGICAL CRITERIA OF INVASIVE DUCTAL CARCINOMA



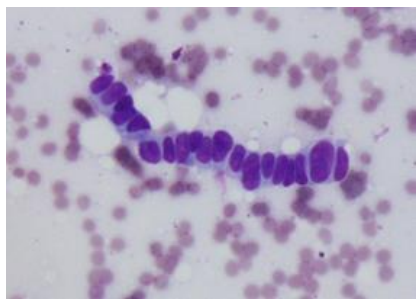
CYTOLOGICAL INTERPRETATION Invasive lobular carcinoma





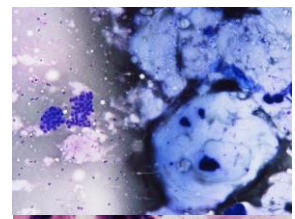
- Variable cellularity. In some cases very poor cell yield.
- Cells single and in small clusters, short single files common.
- Epithelial cells have small dark nuclei with scanty cytoplasm. The lack of pleomorphism can be cause of a false-negative diagnosis.
- Intracytoplasmic lumina/vacuoles.

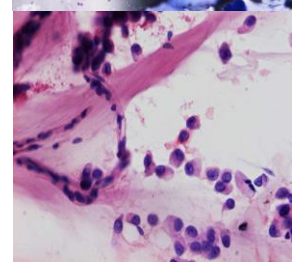
CYTOLOGICAL INTERPRETATION Invasive lobular carcinoma



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

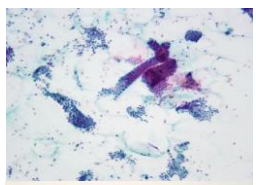
CYTOLOGICAL INTERPRETATION Mucinous carcinoma

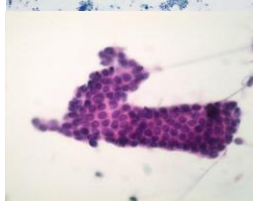




- Well defined and circumscribed tumour (similar to fibroadenoma).
- Abundant background mucinous, atypical cells in small solid aggregates, single files or isolated. The mucin stains violet to blue with MGG or pink on HE staining.

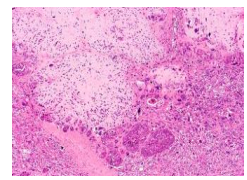
CYTOLOGICAL INTERPRETATION Tubular carcinoma

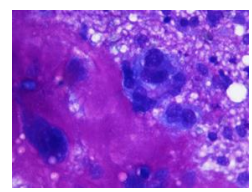




- Variable cellularity (moderate to intense). At low magnification a pattern somewhat similar to fibroadenoma.
- Cells arranged mostly in tubular structures with comma-like pattern.
- Epithelial cells are uniform and bland. The lack of pleomorphism can be cause of a false-negative.
- Bare nuclei are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

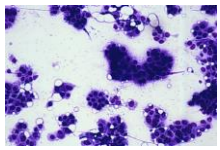
CYTOLOGICAL INTERPRETATION Metaplastic carcinoma

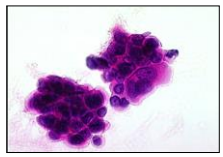




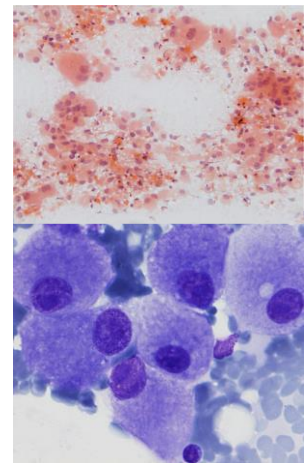
- Is an invasive ductal carcinoma with metaplastic changes: squamous cells, spindle cells, osteoid or chondroid.
- Smears can show different cell types: ductal, spindle or squamous.
- Sometimes we can observe multinucleated giant cells and myxoid material.
- Can be cystic at aspiration and with necrotic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

CYTOLOGICAL INTERPRETATION
Invasive micropapillary carcinoma





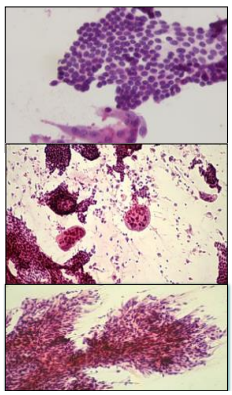
- Highly cellular smears composed by angulated small groups of cohesive cells with papillary configurations without fibro vascular cores.
- Cells showing nuclear atypical, irregular nuclear contours and prominent nucleoli.
- Cytoplasm vacuoles are rarely seen.
- Background is clean with rare isolated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



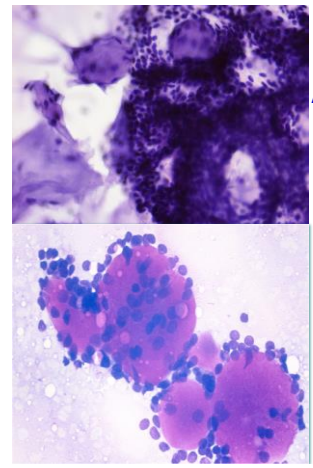
CYTOLOGICAL INTERPRETATION
Apocrine carcinoma

- 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.

CYTOLOGICAL INTERPRETATION
Breast carcinoma with osteoclast-like giant cells



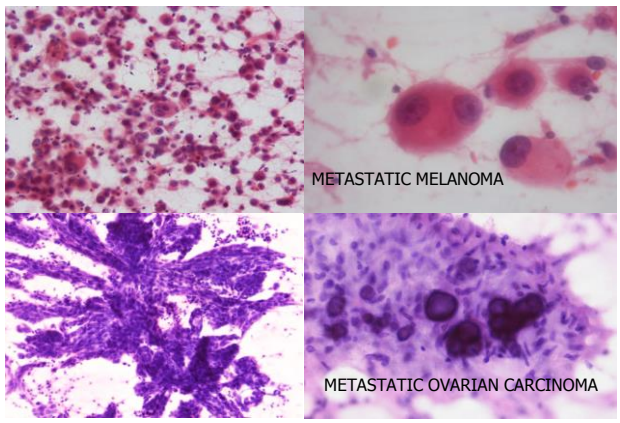
- Cellular smears composed by cohesive groups of epithelial cells, with low grade of atypia.
- Groups of plump spindle cells as well as isolated atypical epithelial cells.
- Presence of osteoclast-like multinucleated cells at periphery of the epithelial cells or in the background of the smears.



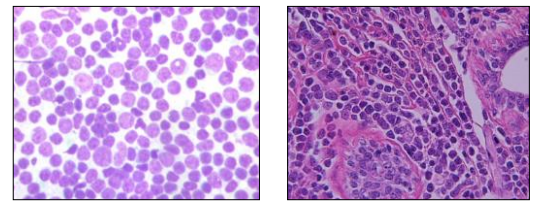
CYTOLOGICAL INTERPRETATION
Adenoid cystic carcinoma

- 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



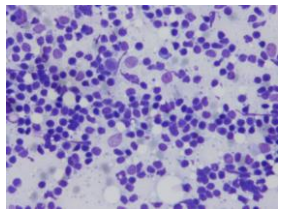
CYTOLOGICAL INTERPRETATION
Other malignancy



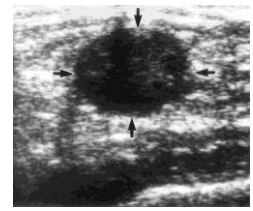
NON-HODGKIN LYMPHOMA

CYTOLOGICAL INTERPRETATION
Inflammatory diseases

BENIGN – INTRAMAMMARY LYMPH NODE

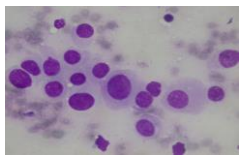


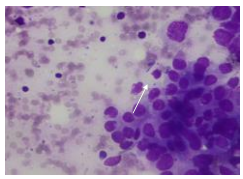
BREAST FNAC: TRIPLE DIAGNOSIS



- 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.

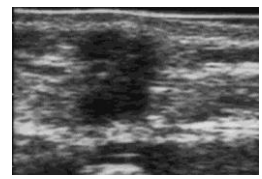
CYTOLOGICAL INTERPRETATION
Medullary carcinoma





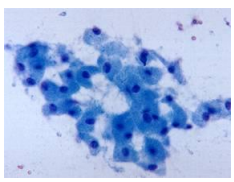
- Well-circumscribed mass (similar to fibroadenoma).
- High cellular smears with irregular groups and single atypical cells. The cells are large, pleomorphic with prominent nucleoli. Background rich in lymphocytes.
- Definitive diagnosis requires the demonstration of well defined borders.
- They are frequently associated with germinal mutation of BRCA-1.

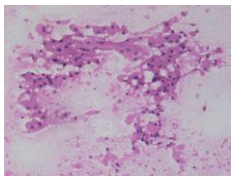
BREAST FNAC: TRIPLE DIAGNOSIS



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

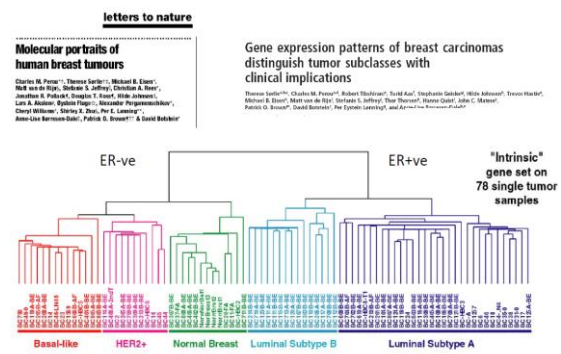
Benign Granular Cell Tumour



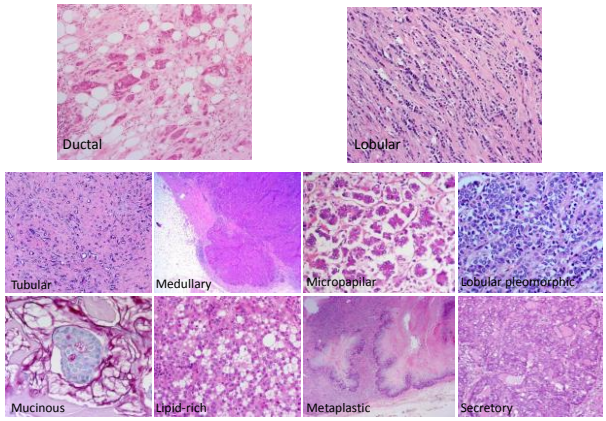


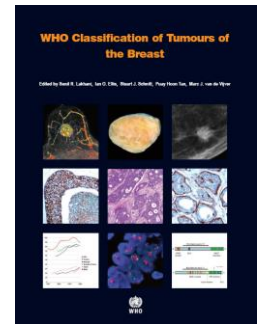
- Imaging typically shows a dense mass with stellate margin, simulating malignancy.
- High cellular yield.
- Cells showing moderate atypia with intact, abundant and granular cytoplasm.

Breast cancer is not a single disease at molecular level



Histological types of breast carcinoma



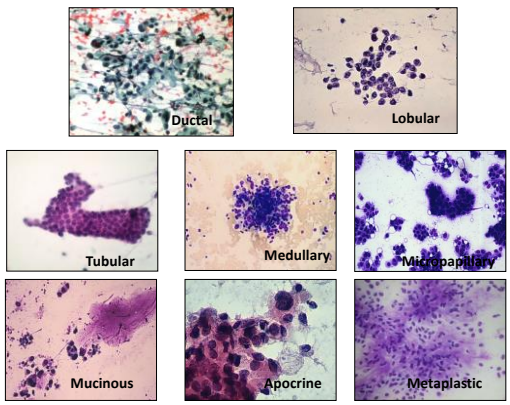


1-1H

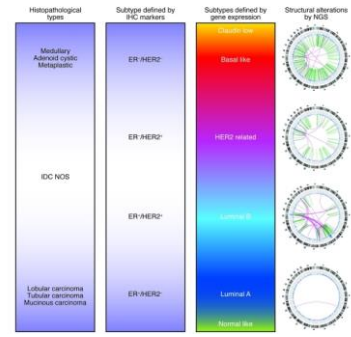
Classification using needle-core biopsy and fine-needle aspiration

F. Schmitt
N. Sneige
A. Lee

Cytological types of breast carcinoma



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.,[1] Daniella Serafin Couto Vieira, M.D., M.Sc.,[1] Horácio Chikota, M.D.,[1] Luiz Carlos Zeferino, M.D., Ph.D.,[2] and Fernando Schmitt, M.D., Ph.D., F.I.A.C.[1]

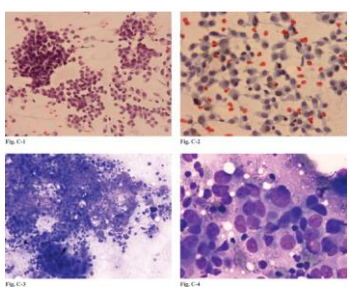


Table IV. Correlation Between Cytological Criteria Comparing the Basal Phenotype and Other Phenotypes of Breast Carcinoma-Defined Immunohistochemically

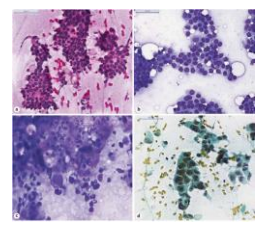
Cytological criteria	p*	OR (CI 95%)
Cellularity (scant vs. moderate/abundant)	0.0257	
Basal × HER2-overexpressing		6.48 (0.29–146.54)
Basal × luminal A		18.03 (0.96–337.81)
Basal × luminal B		15.00 (0.50–449.55)
Basal × nonbasal[a]	0.0247	12.38 (0.69–222.95)
Cell pattern (dissociated vs. fairly equal 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[a]	0.1228	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[a]	0.0004	3.80 (1.16–12.38)
Nucleoli (inconspicuous vs. present/present and prominent)	0.0013	
Basal × HER2-overexpressing		0.23 (0.05–5.22)
Basal × luminal A		8.18 (1.51–44.21)
Basal × luminal B		2.50 (0.17–37.26)
Basal × nonbasal[a]	0.0521	5.61 (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[a]	0.1703	6.07 (0.32–113.83)

Diagnostic Cytopathology, 2009

Fine Needle Aspiration

Cytological Criteria for Predicting the Luminal Phenotype of Breast Carcinoma

Rafael Bispo Paschoalini[a] Rozany Mucha Dufloth[a,b]
Francisco Alves Moraes Neto[c] Fernando C. Schmitt[b]



Cytological criteria	p value	OR (95%CI)
Cellularity (mild/moderate vs. abundant)		
Luminal × non-luminal	0.010	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)	0.002	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	0.145	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

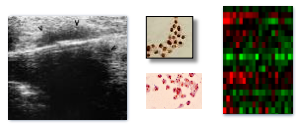
Possible use and role of molecular techniques in fine-needle aspiration cytology (FNAC) practice

Fernando Schmitt
Helena Barroca

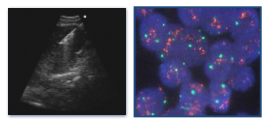
DIAGNOSTIC HISTOPATHOLOGY 17:7

In breast cancer, molecular cytopathology can be used

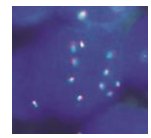
- ***In primary tumours***

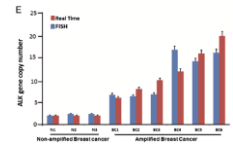


- ***In metastatic tumours***

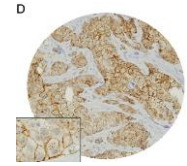


ALK alteration is a frequent event in aggressive breast cancers



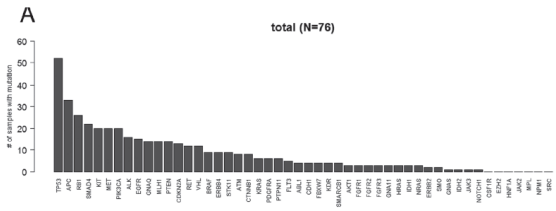


- ALK is amplified in 13.5% of breast carcinomas
- 75% of IBC have ALK amplification
- ALK amplification is related to worst prognosis and high proliferative index
- There is a good correlation between FIS, RT-PCR and IHQ



Siraj *et al. Breast Cancer Research* (2015) 17:127

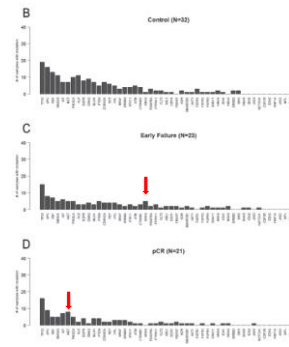
INFLAMMATORY BREAST CARCINOMA (IBC) MOLECULAR STUDIES



- NGS results in 76 cases of LABC

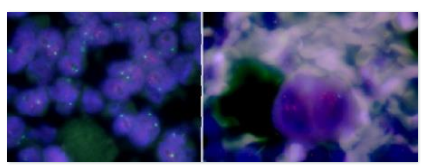
Park K et al. J Oncontarget 2015

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



Park K et al. J Oncontarget 2015

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



Primary BC
HER-2 negative

FNA from liver metastases
HER-2 positive

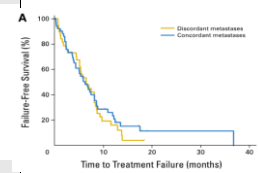
Schmitt FC, 2013

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Prospective Study Evaluating the Impact of Tissue Confirmation of Metastatic Disease in Patients With Breast Cancer

Table 2. Proportion of Women With a Change in Originally Planned Therapy by Subgroup

Subgroup	No.	%	Test of Interaction *P*
All patients	17	14.0	
Lines of therapy			
Newly metastatic	7	12.5	.72
1 prior line of therapy in metastatic setting	2	9.5	
≥ 2 prior lines of therapy in metastatic setting	8	18.2	
2 lines (n = 14)	2	14.3	
3 lines (n = 8)	1	12.5	
4 lines (n = 4)	1	25	
5 lines (n = 4)	1	25	
≥6 lines (n = 14)	3	21.4	
Duration from primary breast cancer diagnosis and biopsy			
First quartile (<35 months)	4	11.4	.15
Second quartile (36-67 months)	4	15.4	
Third quartile (68-118 months)	7	24.1	
Fourth quartile (>118 months)	2	6.5	



Clinical management of breast cancer heterogeneity

Dimitrios Zardavas, Alexandre Irrthum, Charles Swanton and Martine Piccart

Table 4 | Clinical implications of tumour heterogeneity in breast cancer

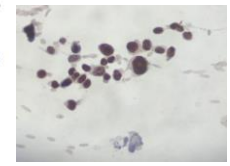
Type of heterogeneity	Clinical implications	Potential solution
Intertumour	Need for patient stratification	High-throughput molecular profiling technique Molecular classifiers
	Need for therapy selection/clinical development of targeted agents	Innovative trial designs: Master protocols Basket trials Adaptive trial design N-of-1 studies
Intratumour	Need to define the phenotype of the recurrent disease	Metastatic biopsy
	Molecular evolution of the disease	Repeated tumour biopsies Geographically separated biopsies Liquid biopsies
	Identification of driver events	Next-generation sequencing Bioinformatic tools and algorithms Systems biology Animal models/functional validation
	Identification of predictive biomarkers	Deep sequencing Single-cell sequencing
	Emergence of treatment resistance	Combination of targeted agents Exploiting passenger events Eradicating the 'lethal clone' Adaptive therapy Targeting the tumour microenvironment Cancer immunotherapy

Zardavas, D. *et al. Nat. Rev. Clin. Oncol.* **12**, 381–394 (2015)

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

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

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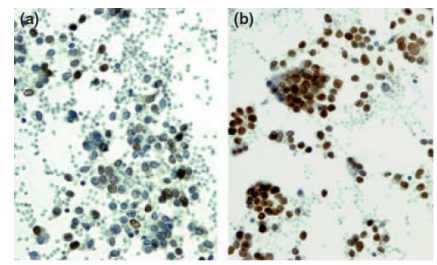
Antigen retrieval



- 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.

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*

Breast Cancer Res Treat (2016) 158:297–305
DOI 10.1007/s10549-016-3846-0

PRECLINICAL STUDY

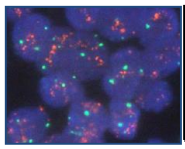
Breast fine needle aspiration continues to be relevant in a large academic medical center: experience from Massachusetts General Hospital

Jianyu Dong[1,2] · Amy Ly[1] · Ronald Arpin[1] · Quratulain Ahmed[1] · Elena Brachtel[1]

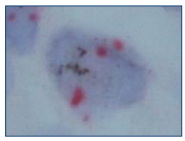
Cell block IHC	Tissue block IHC		Total	Concordance rate (%)	Kappa	*P* value
	Positive	Negative				
ER				98.2	0.951	<0.001
Positive	43	0	43			
Negative	1	13	14			
Total	44	13	57			
PR				100	1.000	<0.001
Positive	32	0	32			
Negative	0	23	23			
Total	32	23	55			

IHC immunohistochemistry, *ER* estrogen receptor, *PR* progesterone receptor

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.

Gu M *et al.* Acta Cytol 2005
Ricardo S *et al.* J Clin Pathol 2007

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)

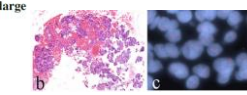
The Breast 22 (2013) 200–202

Breast Cancer Res Treat (2016) 158:297–305
DOI 10.1007/s10549-016-3886-9

PRECLINICAL STUDY

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

Jianyu Dong[1,2] · Amy Ly[1] · Ronald Arpin[1] · Quratulain Ahmed[1] · Elena Brachtel[1]



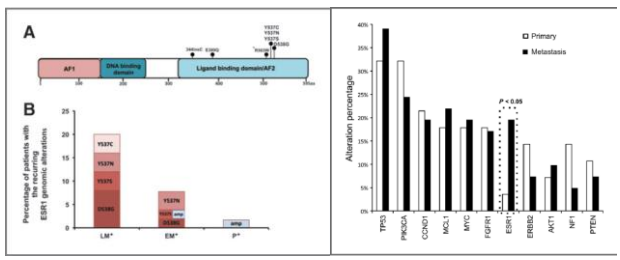
HER-2	Tissue block IHC			Total	Concordance rate	Kappa	*p* value
Cell block IHC	0/1+	2+	3+				
0/1+	32	4	1	37			
2+	4	18	1	23			
3+	0	1	4	5			
Total	36	23	6	65	83.1 %	0.694	<0.001
HER-2	Tissue block FISH			Total	Concordance rate	Kappa	*p* value
Cell block FISH	Amplified	Not amplified					
Amplified	7	0		7			
Not amplified	3	36		39			
Total	10	36		46	93.5 %	0.785	<0.001

IHC immunohistochemistry, *FISH* fluorescence in situ hybridization

Human Cancer Biology
See related article by Segal and Dowsett, p. 1724

Clinical Cancer Research

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



And now?

clinical practice guidelines

Annals of Oncology 23 (Supplement 7): vii11–vii19, 2012
doi:10.1093/annonc/mds232

Locally recurrent or metastatic breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†

F. Cardoso[1,2], N. Harbeck[3], L. Fallowfield[4], S. Kyriakides[5] & E. Senkus[6], on behalf of the ESMO Guidelines Working Group*

and HER2 status should be repeated, especially if unk

Estrogen, progesterone and HER-2 receptors of the metastatic lesion should be obtained at least once in the evolution of the disease, if technically possible, and particularly if not available from the primary tumor

Breast FNAC
How not to be washed away ?

QUALITY

QUALITY

QUALITY

QA objectives	Outcome measurements	Standards	Outline methods of achieving objectives
4. To minimise the number of unnecessary surgical operations[c]	(a) Benign biopsy rate	(a) See radiology standards[a]	(a) Monitoring performance using the biopsy QA and cytology QA systems and addressing deficiencies
	(b) Unsatisfactory samples from cancer-bearing breasts and core biopsy miss rate (B1 + B2) from cancer-bearing breasts	(b) For needle core biopsy Minimal < 15% Achievable < 10% For cytology Minimal < 10% Achievable < 5%	(b) Ensuring that clinical staff are able to obtain satisfactory samples and check the quality of the preparations
	(c) False positive rate[d]	(c) For needle core biopsy Minimal < 0.5% Achievable < 0.1% For cytology Minimal < 1% Achievable < 0.5%	(c-i) Produce guidelines on reporting non-operative diagnosis specimens (c-ii) Run national educational courses on non-operative diagnosis specimen interpretation (c-iii) Development of EQA scheme for needle core biopsy using telepathology
	(d) False negative rate[d]	(d) For needle core biopsy, use miss rate (b) above For cytology Minimal < 5% Achievable < 4%	(d) Provide guidance in breast needle core biopsy and FNAC technique

NHSBSP, 2011

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.

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