IAC
Hong Kong Cytology Tutorial 2023

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CYTOLOGY OF LUNG

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RISE Health Research Network From the Lab to the Community

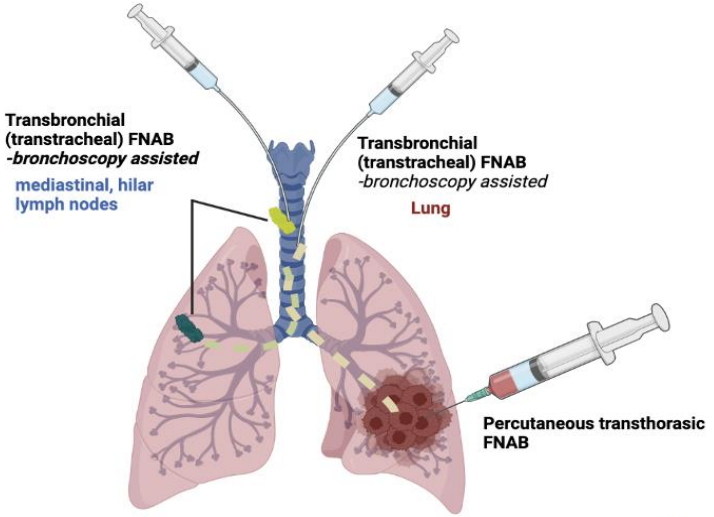
U.PORTO FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

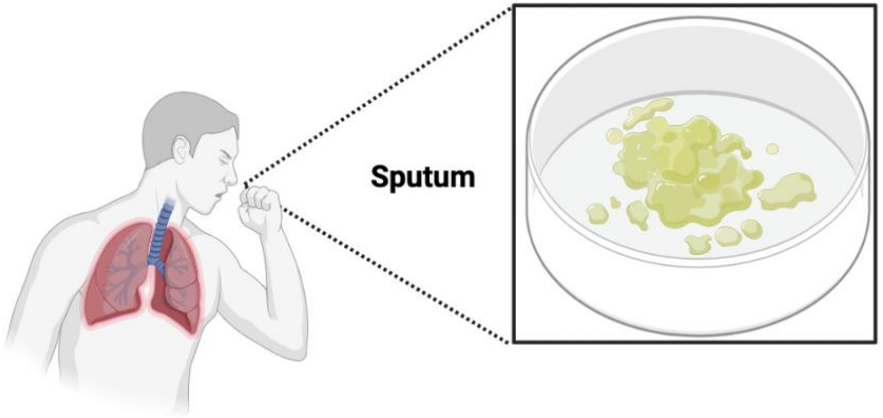
LUNG CYTOLOGY

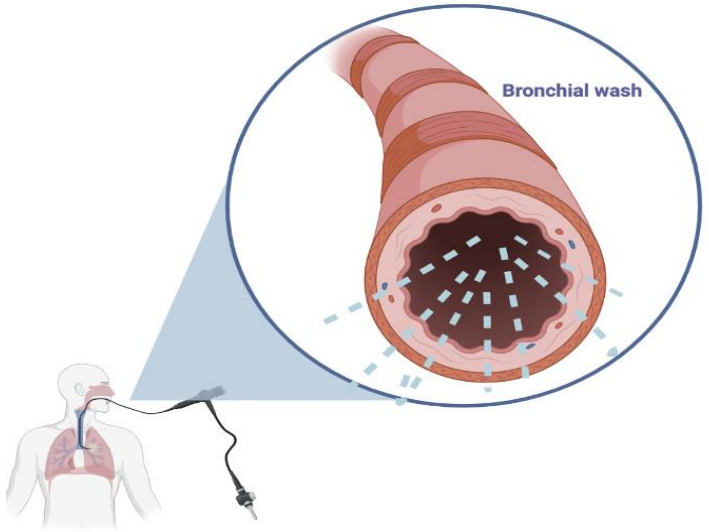
- Diagnosis of lung cancer
- Detection of infections
- Evaluation of interstitial diseases.

LUNG CYTOLOGY

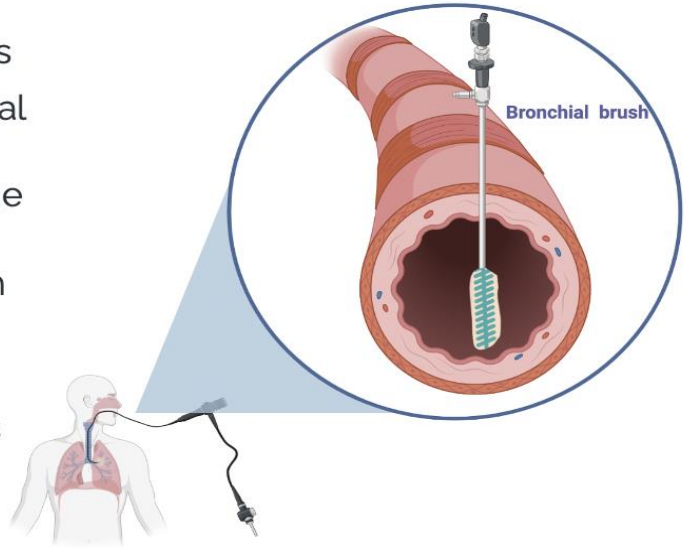
Sampling







- FNAB
  - Percutaneous
  - Transbronchial
- Bronchial lavage
- Bronchial brush
- Sputum
  - Spontaneous
  - Induced



LUNG CYTOLOGY

Sensitivity and Specificity of Sampling Cytological methods for diagnosis of malignancy

Sampling technique	Sensitivity (%)		Specificity (%)	
	average	range	average	range
Sputum	66	34-97	99	68-100
Bronchioalveolar lavage (BAL)	22.9	7.1-76	-	-
Bronchial wash/brush	52	35-83	-	-
Transbronchial needle aspiration (TBNA)	89	87-97	100	-
Transthoracic needle aspiration (TTNA)	90	88-96	97	96-98

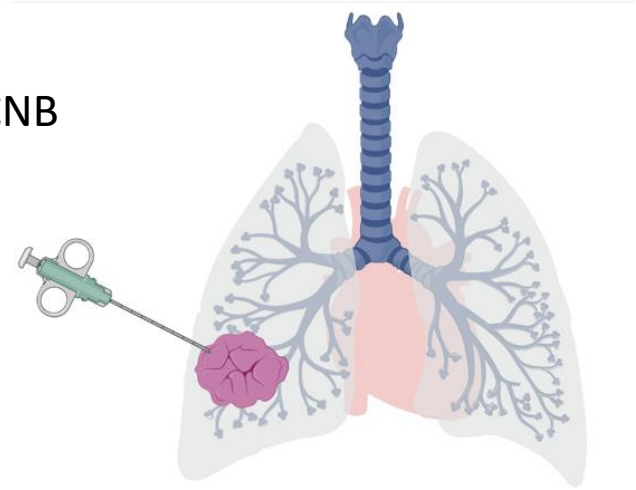
Sensitive and specificity has been reported to increase with the use of cell block and combination of sampling modalities

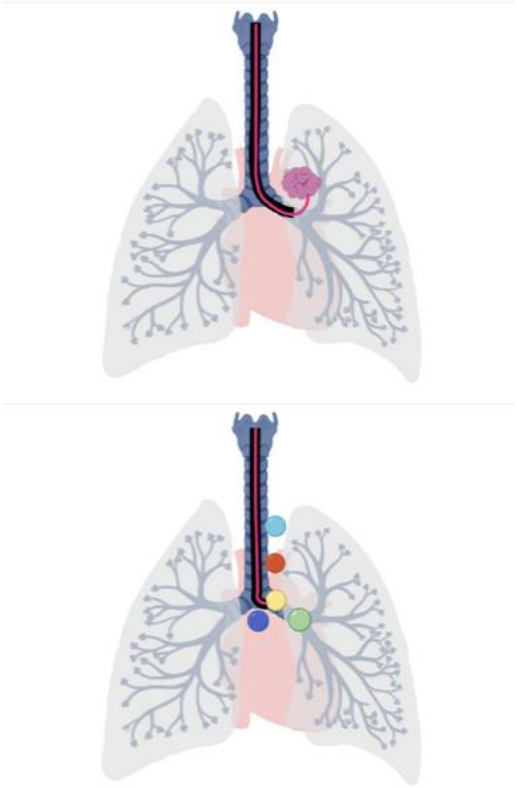
Source: Andre Moreira, Chapter 2: International System for Reporting Lung Cytopathology, 2022

LUNG CYTOLOGY SAMPLING
How the procedure type is determined ?

- PERIPHERAL:

CT-guided FNA or CNB
Diagnosis





- CENTRAL:

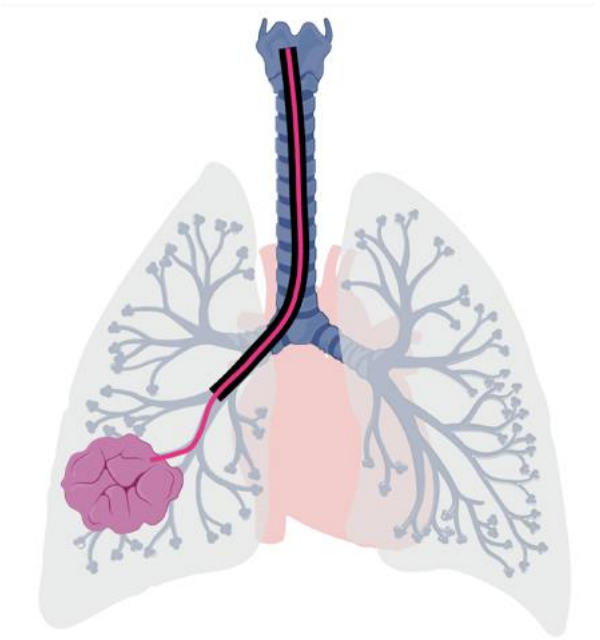
EBUS FNA or CNB
Diagnosis
Staging (Lymph nodes)

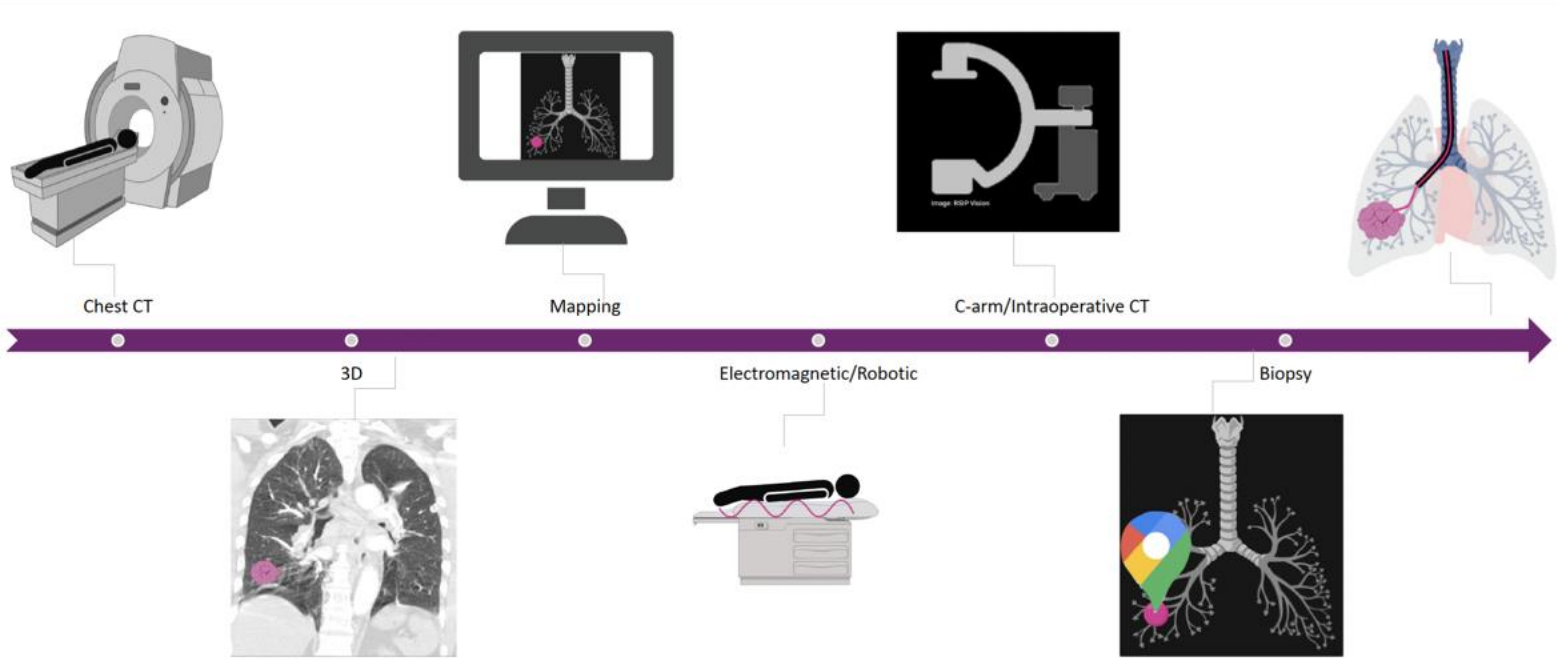
Among other factors, lesion locate affects the procedure

LUNG CYTOLOGY SAMPLING
How the procedure type is determined ?

- PERIPHERAL:

Navigational bronchoscopy





CELL PREPARATION METHODS

Molecular/biomarker testing in lung cytology: A practical approach

Fernando Schmitt MD, PhD, FIAC[1,2,3] | Maria D. Lozano MD, PhD, MIAC[4,5,6]

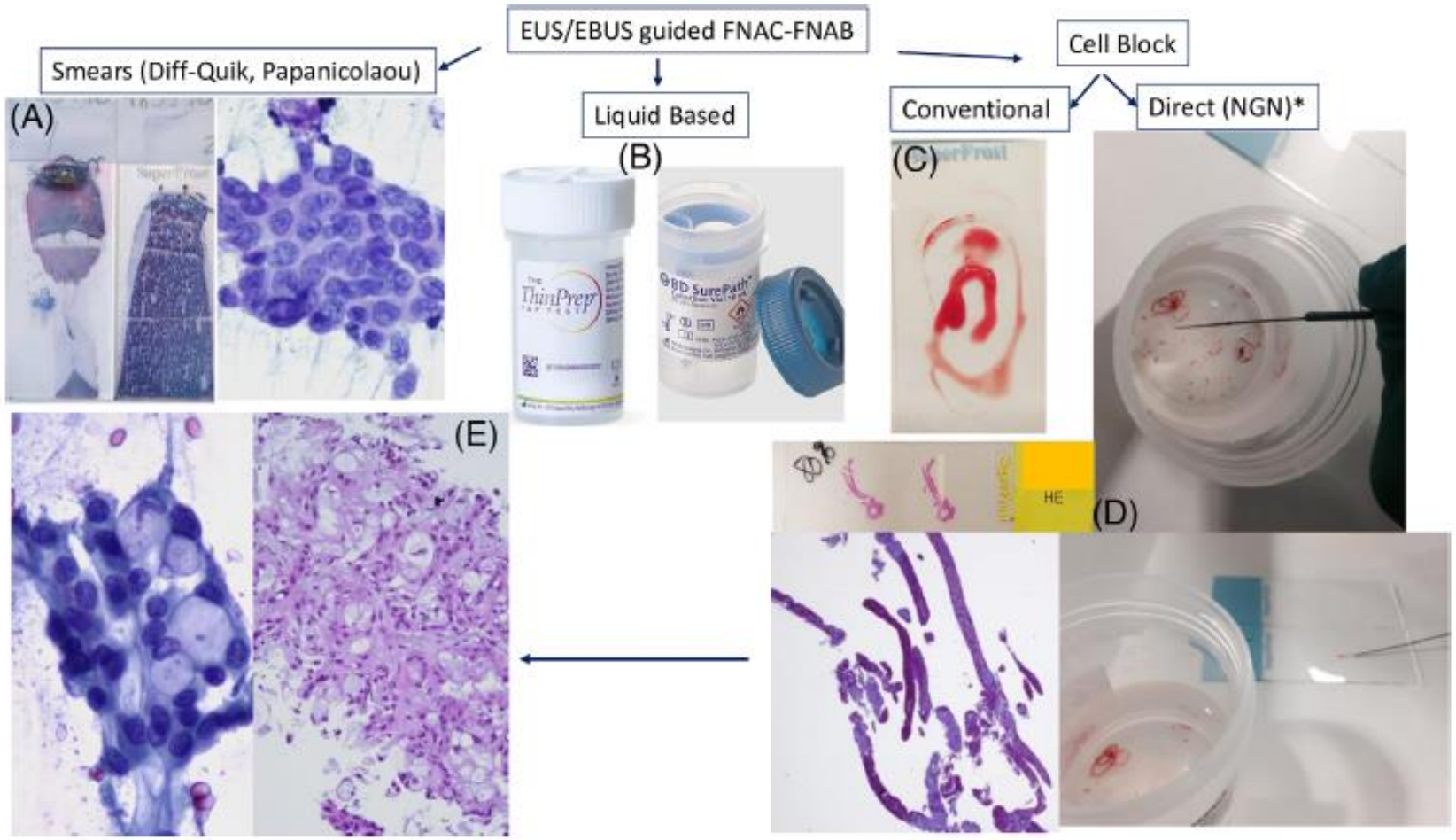
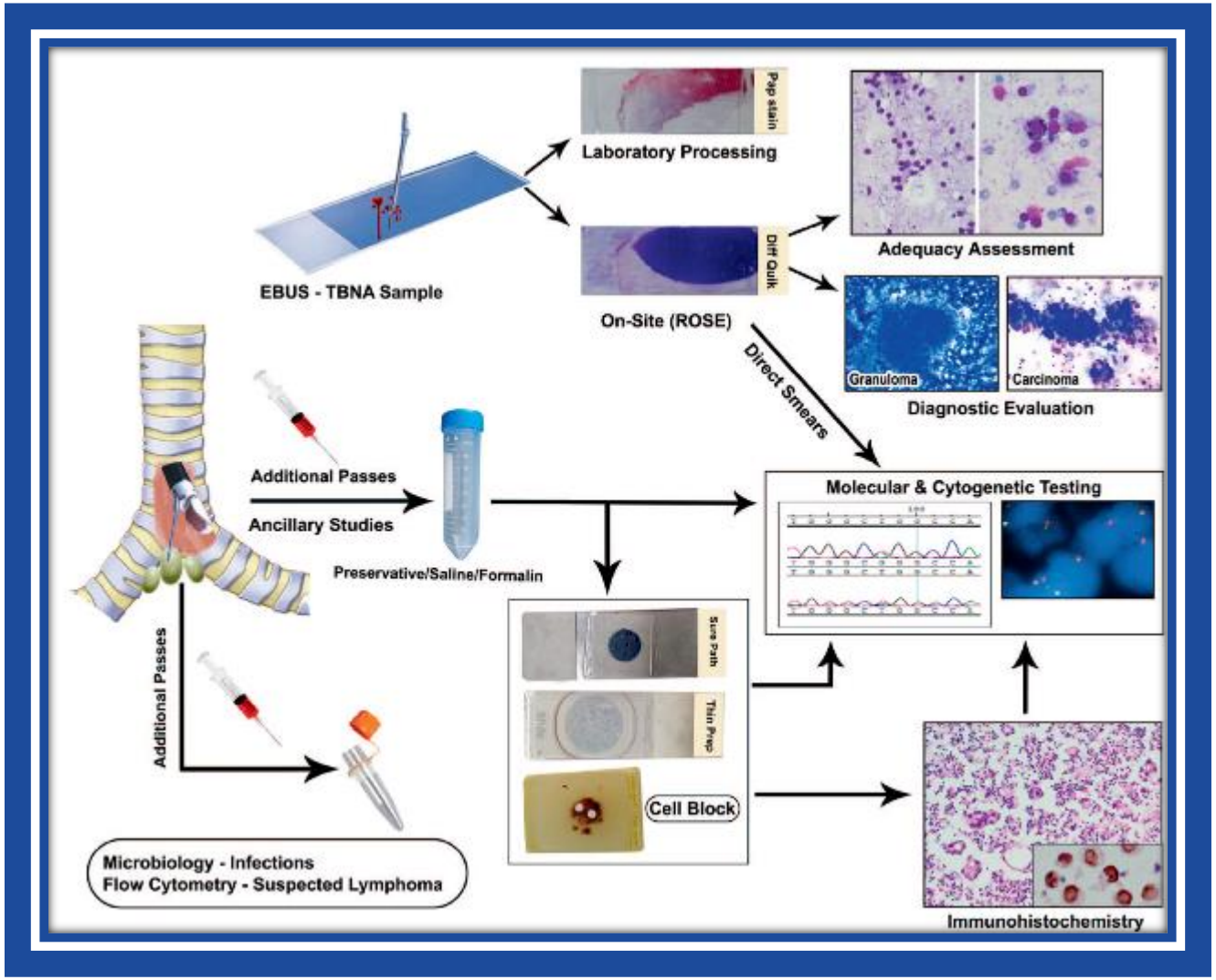


FIGURE 1 Diversity of types of cytological samples obtained from minimally invasive procedures. (A) Smears; (B) liquid based cytology; (C) conventional cell block; (D) direct cell block using new generation needles*; (E) cyto-histological correlations

TABLE 1 Type of cytology samples, fixatives, and ICC results and efficiency

Type of cytological samples	Fixative	Results
Cellblock	Formalin	Comparable results to surgical samples/ biopsies
Pap-stained smears	Alcohol 96°	Comparable results to surgical samples/ biopsies
Unstained smears	Alcohol 96°	Slightly lower but OK
DQ and air-dried smears	No fixative	High rate of false—Low intensity of immunostaining
Liquid based	Methanol-based fixatives	High rate of false—Low intensity of immunostaining

WORKFLOW OF ROSE



Jain D, et al. *Arch Pathol Lab Med* 2018;142:253-62.

Adequate at rapid on-site evaluation (ROSE), but inadequate on final cytologic diagnosis: Analysis of 606 cases of endobronchial ultrasound-guided trans bronchial needle aspirations (EBUS-TBNA)

5%

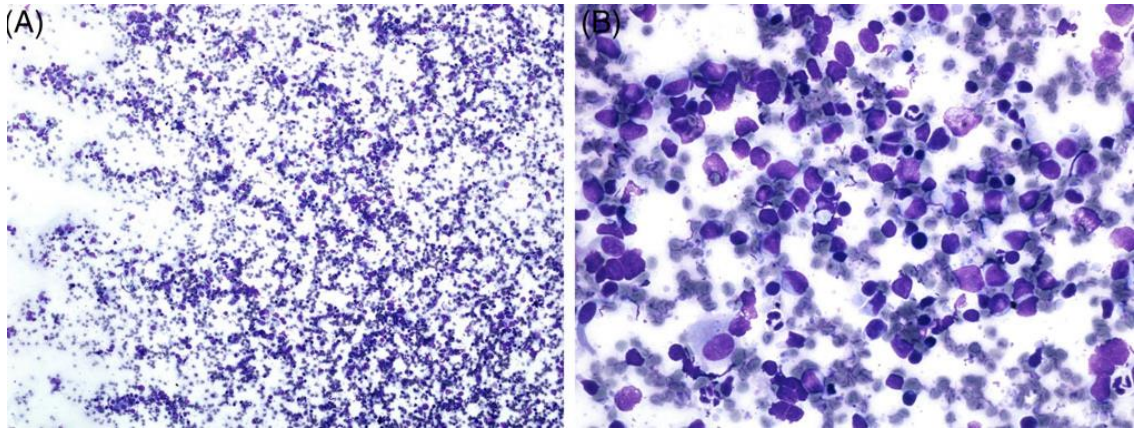


TABLE 1 Distribution of all cases

Final cytologic diagnosis	ROSE-adequate	ROSE-inadequate	Total cases
Positive	203 (55%)	18 (7.6%)	221
Negative	149 (40.4%)	69 (29%)	218
SUS	6 (1.6%)	4 (1.7%)	10
ATY	7 (1.9%)	26 (11%)	33
ND	4 (1.08%)	120 (50.6%)	124
Total cases	369	237	606

Diagnostic Cytopathology. 2019;47:367–373.

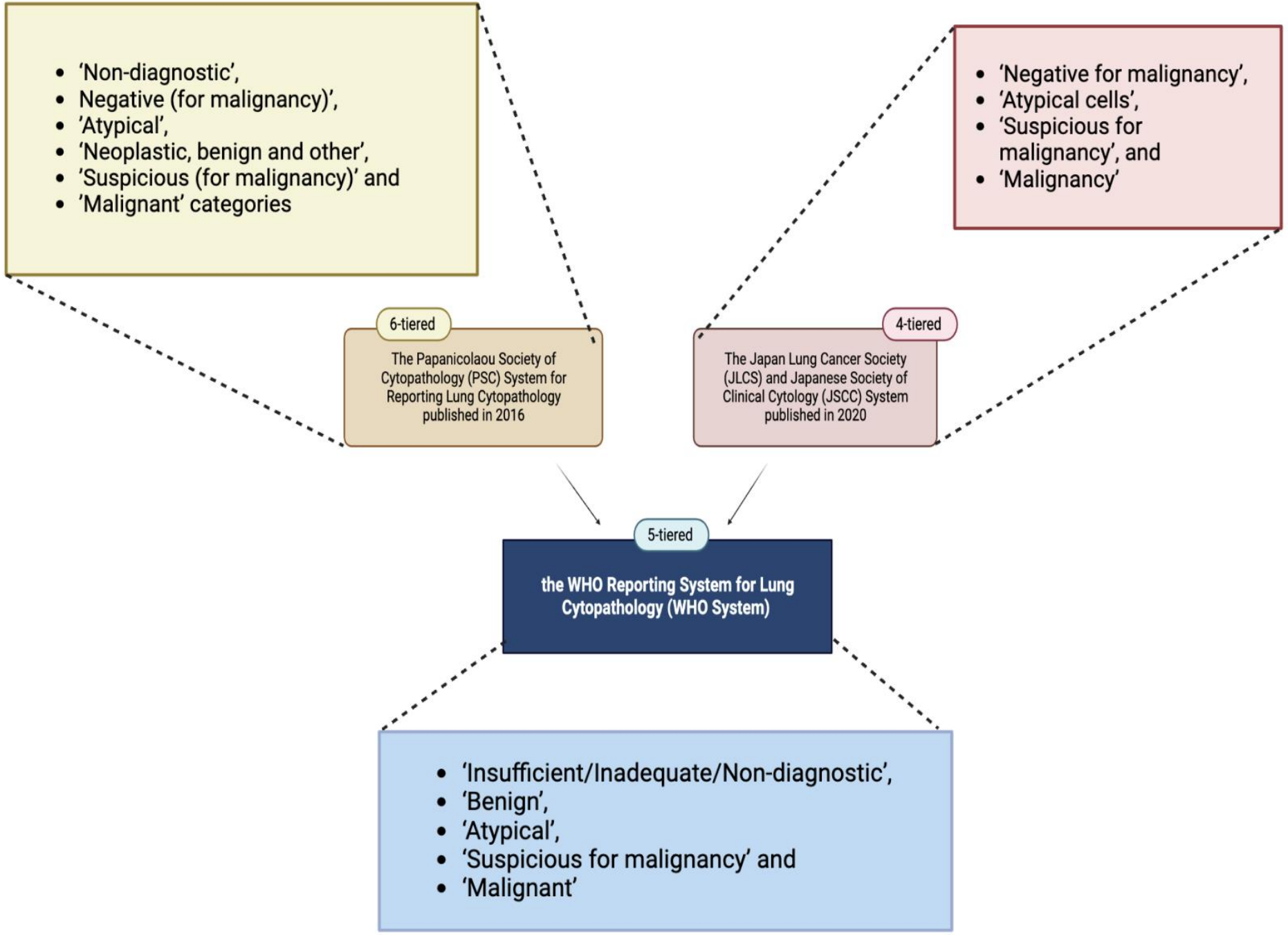
Introduction

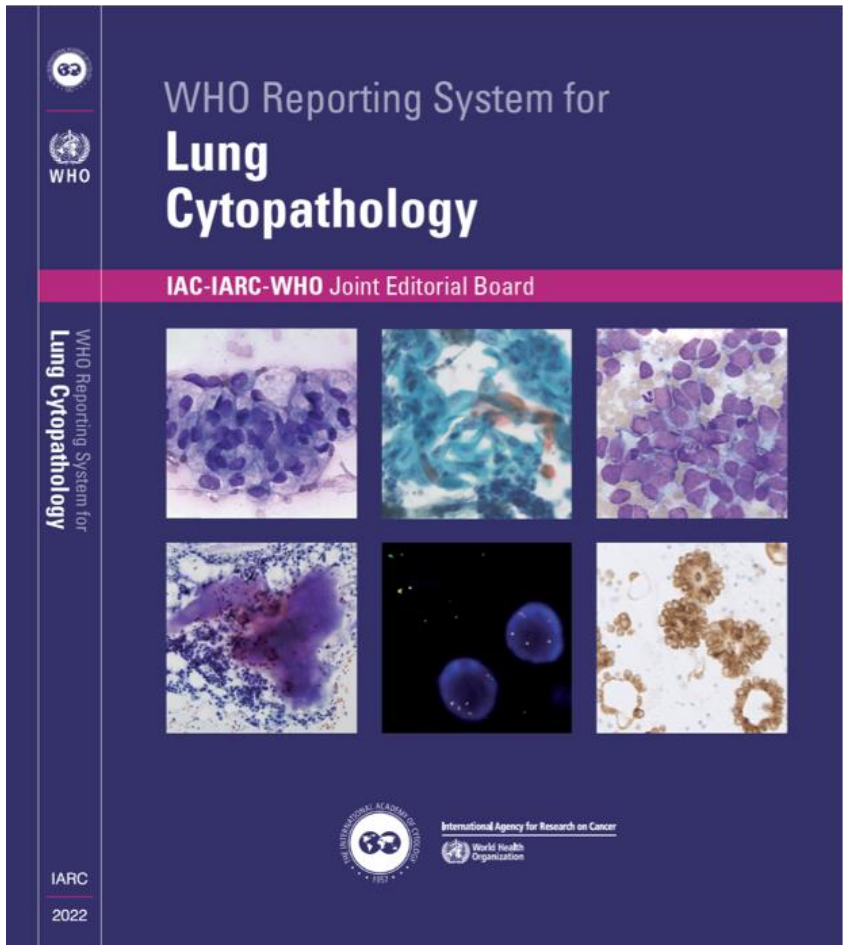
- **Lung cytology** is a simple, non-invasive to minimally invasive and simple technique for detecting **malignancy** and **infectious** processes in the respiratory tract.

- **Samples** can be obtained from sputum, bronchoalveolar lavage, bronchial washing, bronchial brushing, endobronchial ultrasound–guided transbronchial and transthoracic fine-needle aspiration biopsy.

- **Ancillary testing is possible** with these sampling techniques, allowing for the frequent diagnosis of lung carcinoma by cytopathology alone.

- A solid knowledge of the **wide range of morphological findings** in lung cytopathology is key.


'Non-diagnostic',
Negative (for malignancy)',
'Atypical',
'Neoplastic, benign and other',
'Suspicious (for malignancy)' and
'Malignant' categories
'Negative for malignancy',
'Atypical cells',
'Suspicious for malignancy', and
'Malignancy'
6-tiered
The Papanicolaou Society of Cytopathology (PSC) System for Reporting Lung Cytopathology published in 2016
4-tiered
The Japan Lung Cancer Society (JLCS) and Japanese Society of Clinical Cytology (JSCC) System published in 2020
5-tiered
the WHO Reporting System for Lung Cytopathology (WHO System)
'Insufficient/Inadequate/Non-diagnostic',
'Benign',
'Atypical',
'Suspicious for malignancy' and
'Malignant'



WHO REPORTING SYSTEM FOR LUNG CYTOPATHOLOGY

International Academy of Cytology – International Agency for Research on Cancer – World Health Organization Joint Editorial Board. WHO Reporting System for Lung Cytopathology. Lyon (France): International Agency for Research on Cancer; 2022. (WHO cytopathology reporting systems series, 1st ed).

WHO Reporting System for Lung Cytopathology

- Five diagnostic categories
  - Insufficient/Inadequate/Non-diagnostic (ND)
  - Benign (B)
  - Atypical
  - Suspicious for malignancy (SFM)
  - Malignant (MAL)

Not very different from current reporting practices
Criteria and management for each category

- Each should provide useful, inherent information
  - Clear communication with clinicians
  - Appropriate clinical management and follow-up
- Each with a risk-of-malignancy (ROM)
  - Based on recent literature
  - Should be refined as experience increases
  - May vary between individual practices

Current Topics and Practical Considerations of Cytology Practice in Lung Cancer: Reflexions from the Lung Symposium at the 42nd European Congress of Cytology, Malmö, 2019

Sule Canberk[a–c] Verena Tischler[d] Marianne Engels[e]

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a

PSC guidelines diagnostic categories	ROM of PSC guidelines, %	ROM of all cytology samples, %	ROM of BAL/wash, %	ROM of brush, %	ROM of sputum, %	ROM of FNA, %	Distribution of all cytological samples (n = 1,290)
ND	40	64.01	81	75	100*	53	16% (n = 206)
NM	24–43	48.27	42	38	42	64	53% (n = 684)
A	54	59.09	86	100	0	55	5.4% (n = 69)
N-B-LG	n/a	100.00	–	–	–	100	0.4% (n = 5)
SM	82	90.00	100	100	–	88	2.1% (n = 27)
M	77–100	89.74	100	100	–	88	23.1% (n = 299)

b

Sampling technique	Sensitivity, % (positive test = SM + M)	Specificity, % (positive test = SM + M)	Diagnostic accuracy (positive test = SM + M)
BAL/wash	23	100	
Brush	63	100	68.13
FNA	74	59	

* This ratio is explained by having only a few corresponding histology samples for 82 sputum cases which all were malignant. ROM, risk of malignancy; ND, nondiagnostic; NM, negative for malignancy; A, atypical; N-B-LG, benign neoplasm or low-grade carcinoma; SM, suspicious for malignancy; M, malignant; BAL, bronchoalveolar lavage; FNA, fine needle aspiration.

WHO Reporting System for Lung Cytopathology

Table 1. The World Health Organization International System for Reporting Lung Cytopathology on FNAB: implied risk of malignancy and clinical management options by diagnostic category

Diagnostic category	Estimated risk of malignancy , %	Clinical management options
Insufficient/Inadequate/Non-diagnostic	43–53	Correlate with CLIN-IMG-MICRO, ideally discuss at a MDT meeting, and perform repeat FNAB with or without CNB
Benign/negative for malignancy	19–64	Correlate with CLIN-IMG-MICRO, and if these confirm benign diagnosis, then routine follow up at 3–6 months. If no correlation, then perform repeat FNAB with or without CNB
Atypical	46–55	Correlate with CLIN-IMG-MICRO, and ideally discuss at a MDT meeting. If all show a benign diagnosis, then routine follow up at 3–6 months. If no correlation, then perform repeat FNAB with ROSE with or without CNB
Suspicious for malignancy	75–88	Correlate with CLIN-IMG-MICRO, and ideally discuss at a MDT meeting. If all support a diagnosis of malignancy, consider definitive treatment. If no correlation that lesion is Malignant, perform repeat FNAB with ROSE with or without CNB
Malignant	87–100	Correlate with CLIN-IMG-MICRO, and ideally discuss at a MDT meeting. If all support a diagnosis of malignancy, provide definitive treatment. If no correlation that lesion is Malignant, consider repeat FNAB with ROSE with or without CNB

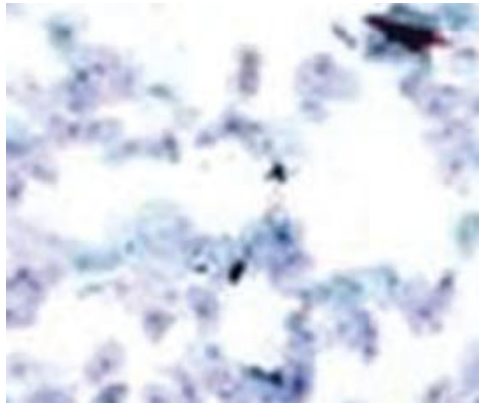
FNAB, fine needle aspiration biopsy; CLIN, clinic; IMG, imaging; MICRO, microbiology; CNB, core needle biopsy; MDT, multidisciplinary team; ROSE, rapid on-site evaluation.

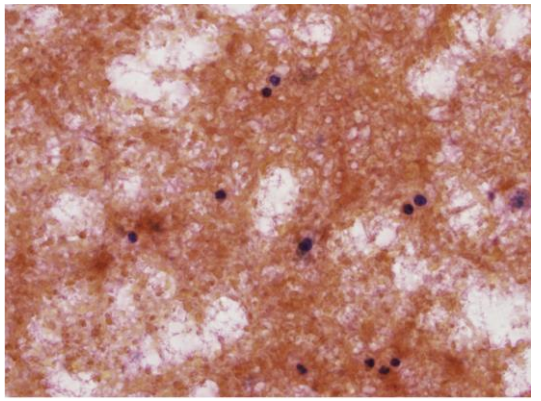
Acta Cytologica 2023;67:1–12
DOI: 10.1159/000527580

Categories For The WHO Reporting System for Lung Cytopathology

Insufficient/Inadequate/Non-diagnostic

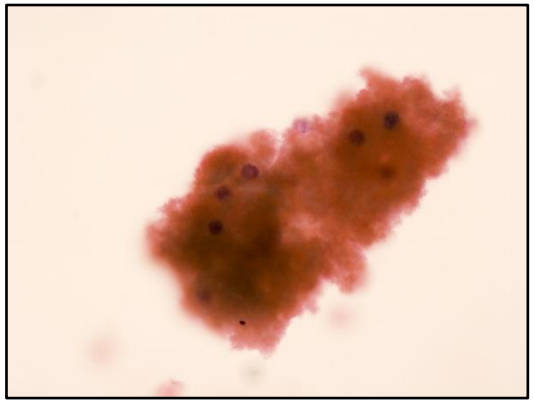
- Provides no useful diagnostic information (in a specific clinical context)
  - ✓ Insufficient cellularity
  - ✓ Cellular degeneration
  - ✓ Hemorrhagic samples
  - ✓ Bad preservation of cells



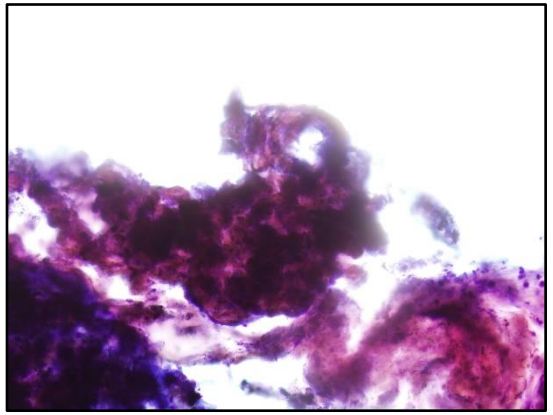


- Any atypia should be reported as such and put under the atypical or “suspicious” category.
- Incidence: around 16% (few studies)
- Reported ROM: 43-53% (few studies, different samples)

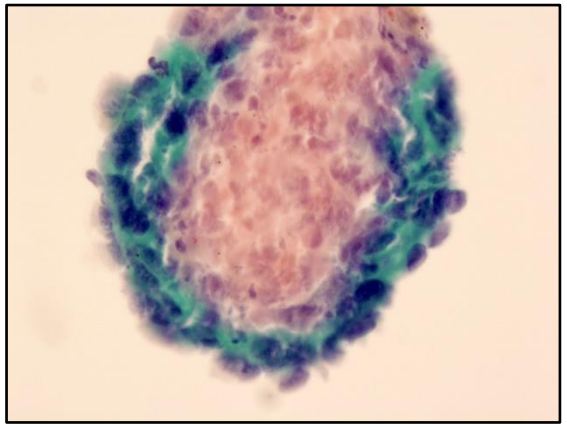
Insufficient/Inadequate/Non-diagnostic



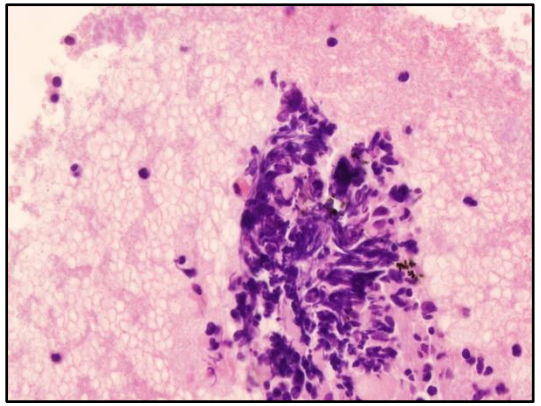
Presence of obscuring blood.



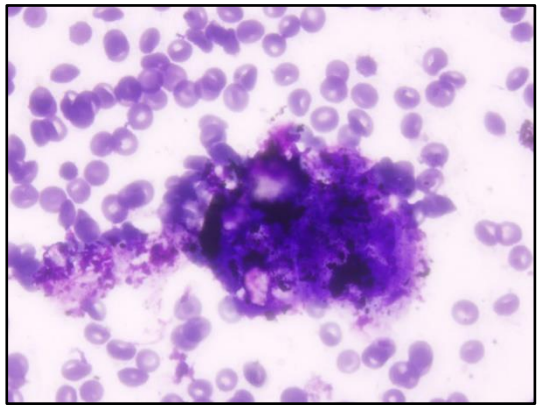
Cells trapped in thrombi are poorly visualized.



Markedly poor preservation and staining.



Severe crushing artifact.



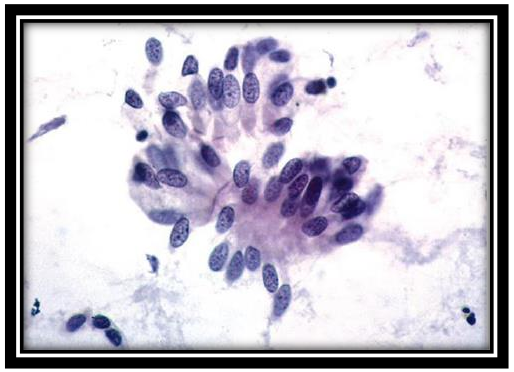
Contamination by non-specific debris.

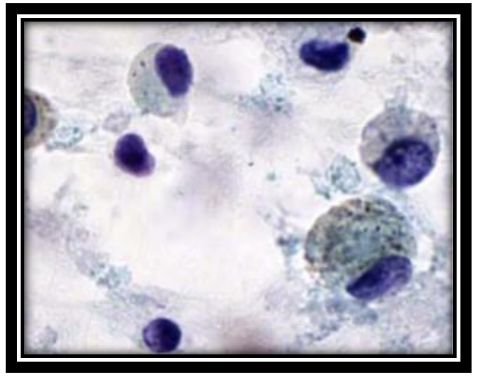
Categories For The WHO Reporting System for Lung Cytopathology

Insufficient/Inadequate/Non-diagnostic

- Adequacy criteria is disputable and none require a specified number of cells or cell types
  - ✓ Sputum: samples should include at least a few alveolar macrophages and ciliated columnar cells
  - ✓ Bronchial brush (BB) - should include abundant bronchial epithelial cells. Macrophages may be present.
  - ✓ Bronchial wash (BW)/bronchial alveolar lavage (BAL) - should include readily identified alveolar macrophages (for BAL > 10 alveolar macrophages/high-power field).

✓ Fine needle aspiration biopsy (FNAB) –should include some alveolar (pulmonary) macrophages, which generally contain pigment (carbon and/or hemosiderin) and may include tissue fragments. A TBFNA/EBUS should include reactive bronchial ciliated columnar cells or lymphocytes (≥40 lymphocytes in the area of highest cellularity)





Categories For The WHO Reporting System for Lung Cytopathology

Insufficient/Inadequate/Non diagnostic

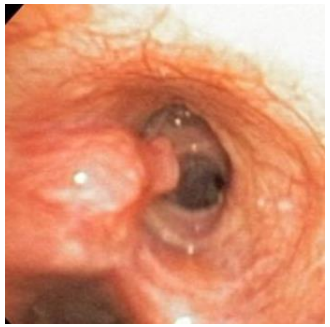
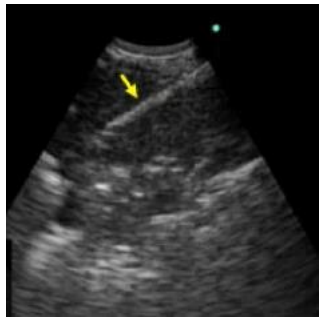
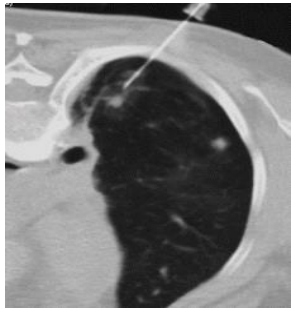
An inadequate/insufficient/non-diagnostic specimen demonstrates

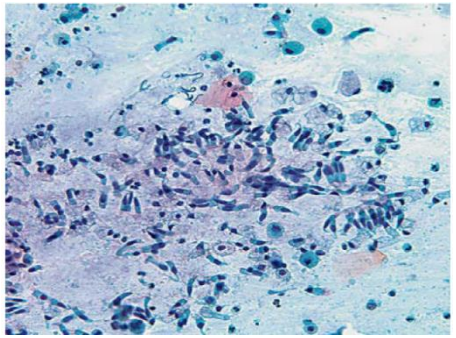
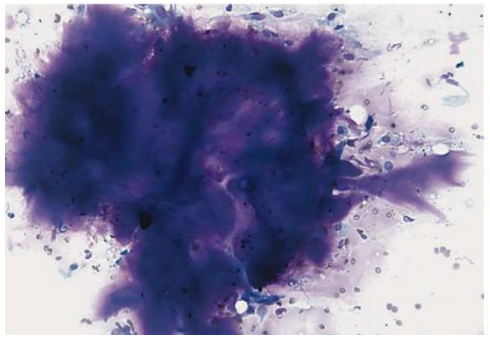
- No pathologic infectious microorganisms (bacterial, fungal elements, parasites).
- No nuclear or cytoplasmic viral cytopathic effect.
- No abundance of inflammatory cells to suggest an inflammatory or allergic reaction.
- No granulomatous inflammation.
- No diagnostic matrix or acellular material such as fat, amyloid, clean mucin, proteinaceous or aspirated foreign material.
- Pulmonary macrophages that do not contain hemosiderin pigment, melanin, fatty vacuoles or other pathological substances suggest a pathological condition.
- No cytological atypia.
- Sputum devoid of any pulmonary macrophages or insufficient material to prepare two smears.

Source: Wendy Raymond, Chapter 4: International System for Reporting Lung Cytopathology, 2022

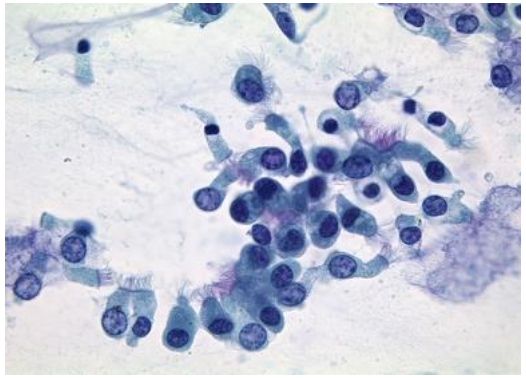
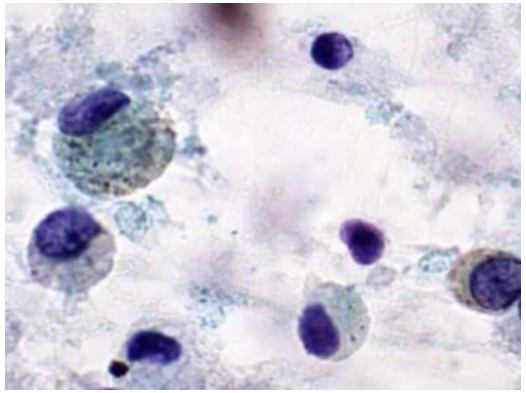
Categories For The WHO Reporting System for Lung Cytopathology

Insufficient/Inadequate/Non diagnostic

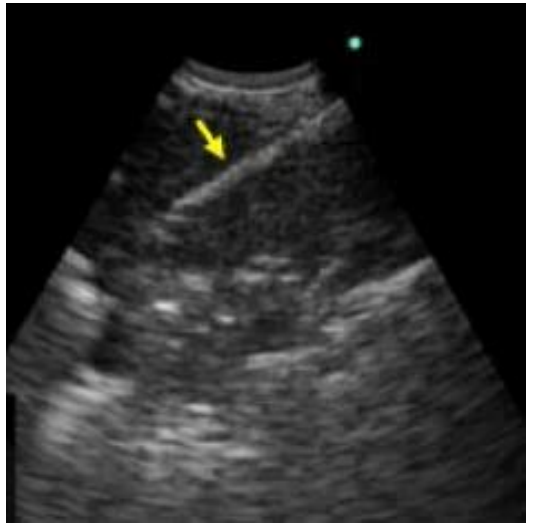




68 y-old man with pulmonary lesion/nodule

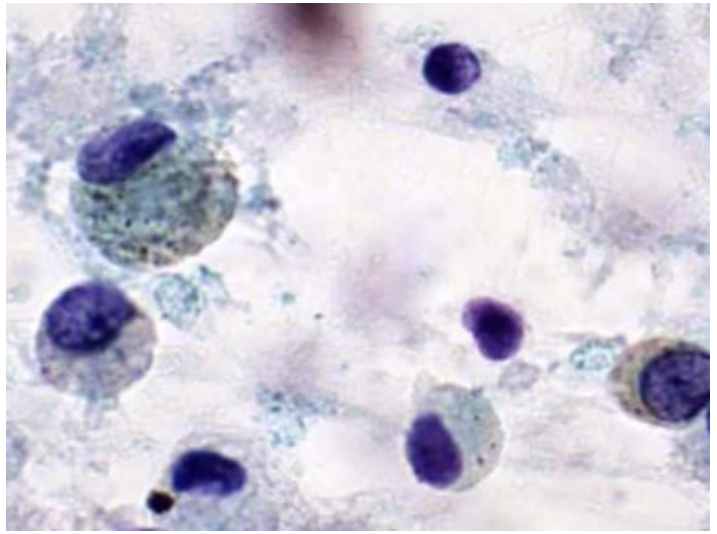



Example Report
2 cm well round mass in the lung



- Insufficient/Inadequate/Nondiagnostic
- Only macrophages (see note)

Note: The biopsy does not explain a well-defined lung mass.



Categories For The WHO Reporting System for Lung Cytopathology

Benign

- *A specimen categorized as 'Benign' demonstrates unequivocal benign cytopathological features, which may or may not be diagnostic of a specific process or benign neoplasm.*

- INCIDENCE: around 50% * (Few studies)
- Reported ROM: 19-64% (few studies, different samples)
- MAIN CAUSES: inflammatory/infectious diseases/benign neoplastic lesions
- MANAGEMENT: Correlate with CLIN-IMG-MICRO, and if these confirm benign, routine follow-up 3-6 months. If no correlation consider new sampling.

Categories For The WHO Reporting System for Lung Cytopathology

Benign

5.0: Diagnostic category: Benign

- 5.0.0.1: Introduction
- 5.0.0.2: Definition
- 5.0.0.3: Discussion and background
- 5.0.0.4: Risk of malignancy and management recommendations

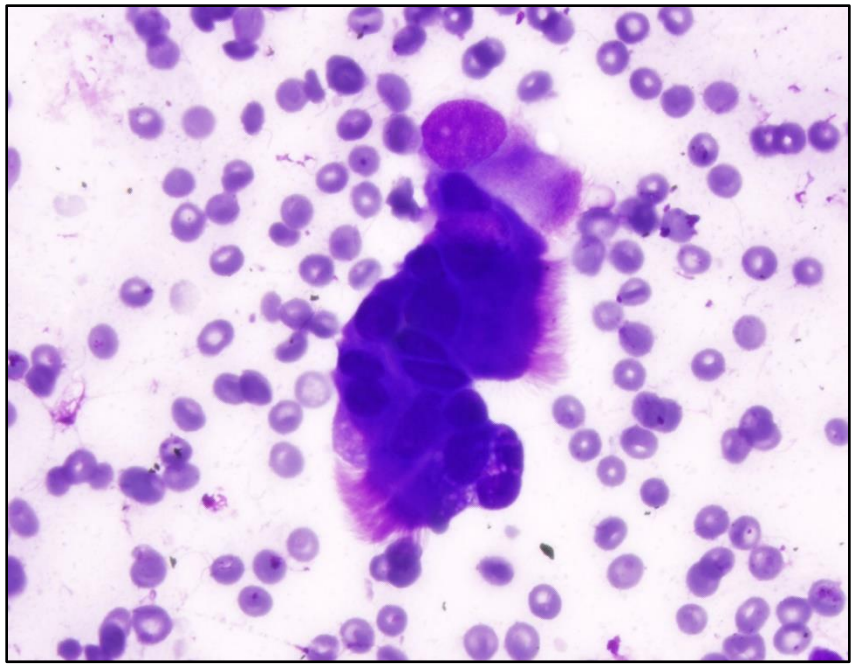
5.1: Inflammatory processes

- 5.1.0.1: Acute inflammation and suppuration
- 5.1.0.2: Histiocytic, lymphocytic, and eosinophilic inflammatory patterns
- 5.1.0.3: Granulomatous disorders
- 5.1.0.4: Inflammatory and reactive changes in glandular cells and squamous cells

5.2: Benign neoplastic lesions

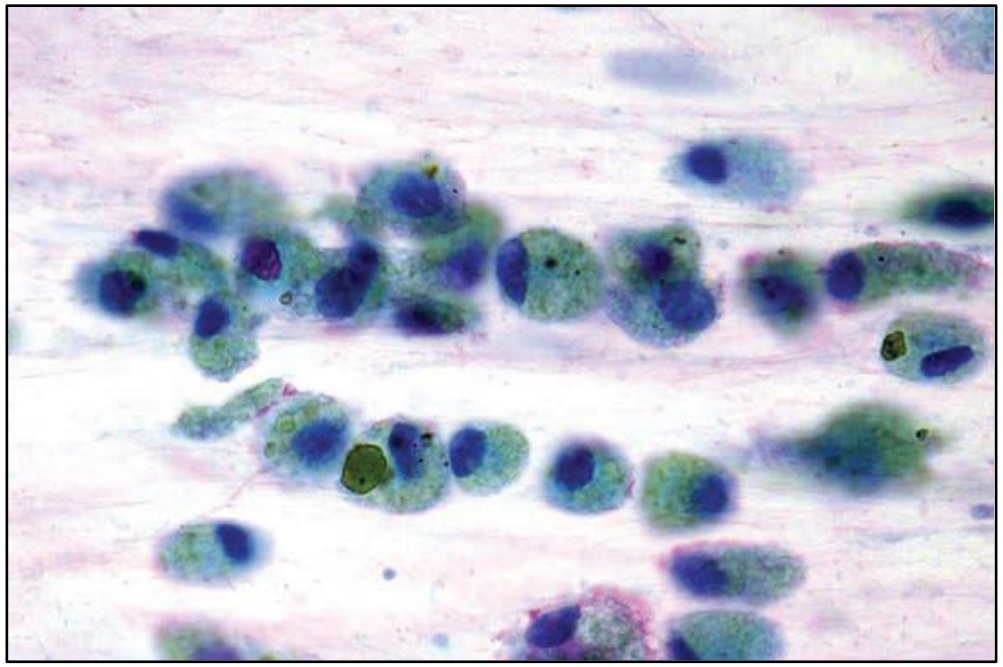
- 5.2.0.1: Pulmonary hamartoma
- 5.2.0.2: Sclerosing pneumocytoma
- 5.2.0.4: Bronchial papillomas
- 5.2.0.5: Salivary gland tumours
- 5.2.0.8: PEComa
- 5.2.0.9: Spindle cell tumours
- 5.2.0.10: Meningiomas
- 5.2.0.11: Granular cell tumour
- 7.0.1.3: Ectopic thyroid and parathyroid tumours
- 5.2.1: Sample reports

Benign – Normal



Benign bronchial cells

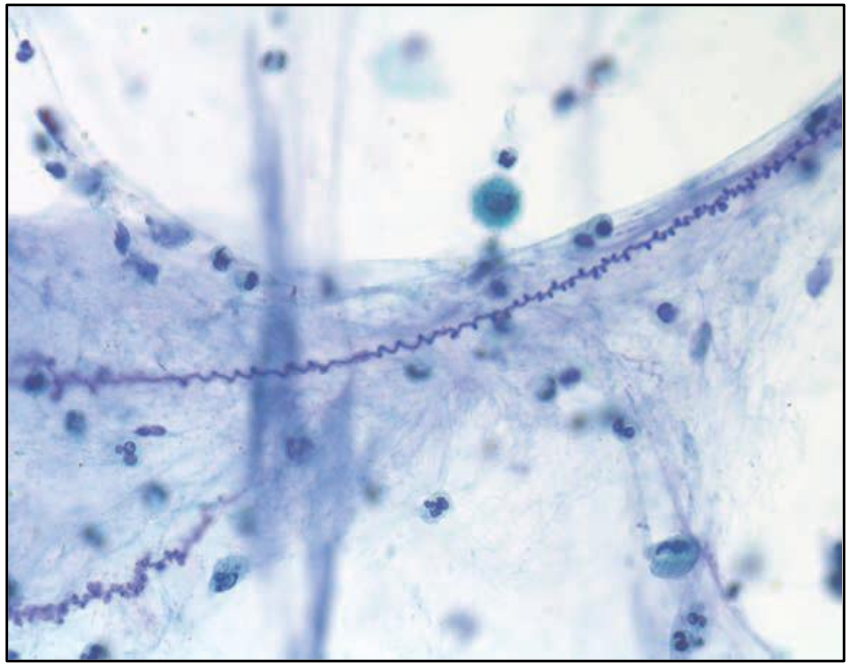
- Uniform cuboidal or columnar cells with a terminal bar (darker band at the luminal aspect of the cell)
- Round to oval nuclei with smooth nuclear membranes and inconspicuous nucleoli
- Cilia may or may not be seen (presence of cilia favors benignity)



Pulmonary macrophages

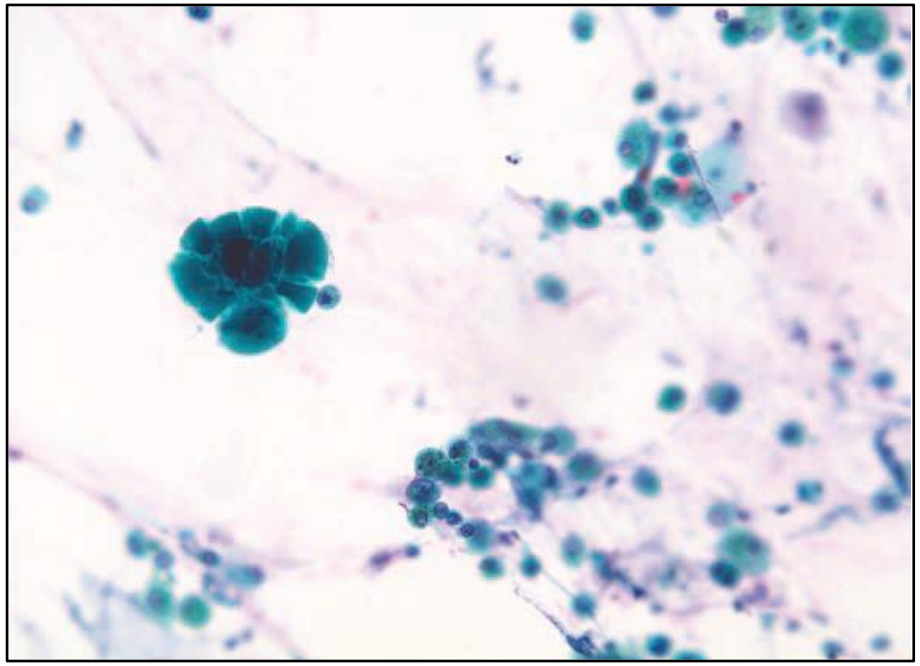
- Large cells with bubbly green/brown cytoplasm and eccentric reniform nucleus.
- May contain brown cytoplasmatic pigment.

Benign – Normal



Curschmann's Spirals

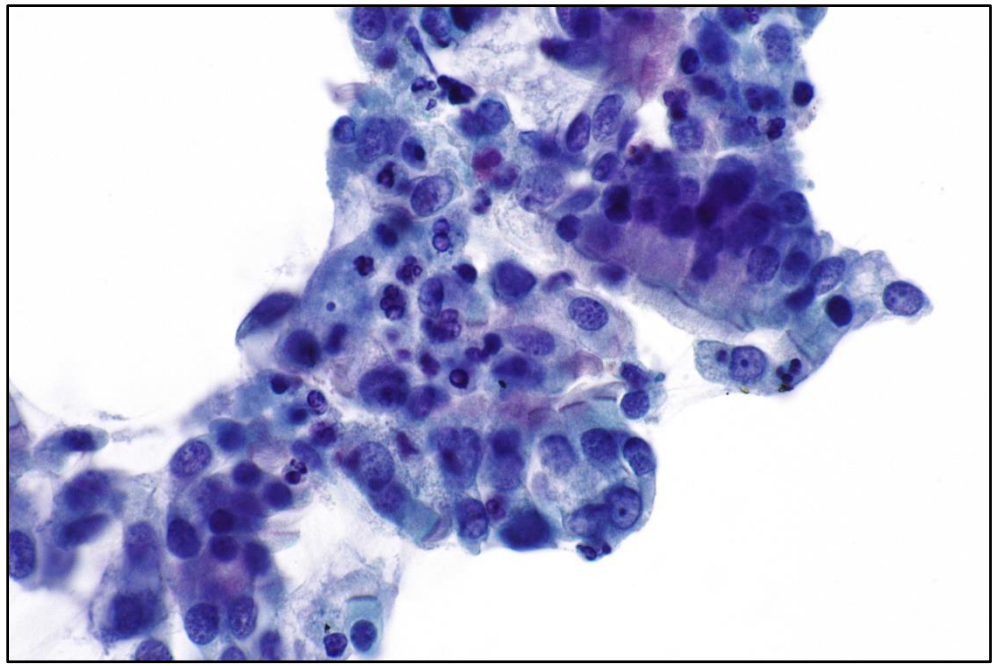
- Spirally coiled, purple-coloured mucus casts.
- Formed as secretions become inspissated to form a cast of the encasing airway



Corpora Amylacea

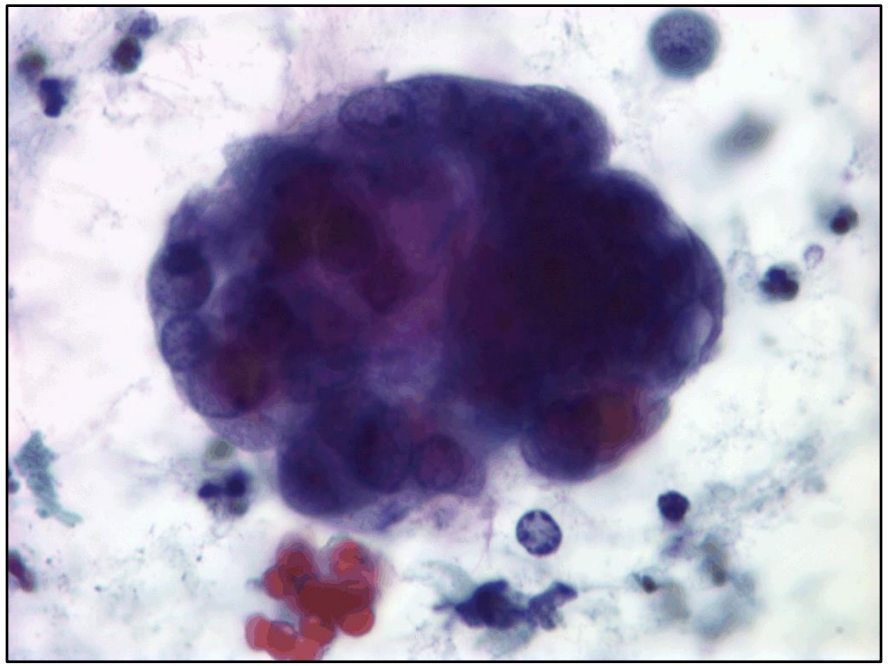
- Dense, lamellated concretions found within scattered alveoli.
- May have cracking artifacts around the edges.
- These are incidental findings and have no known significance.

Benign – Reactive



Reactive epithelial cells

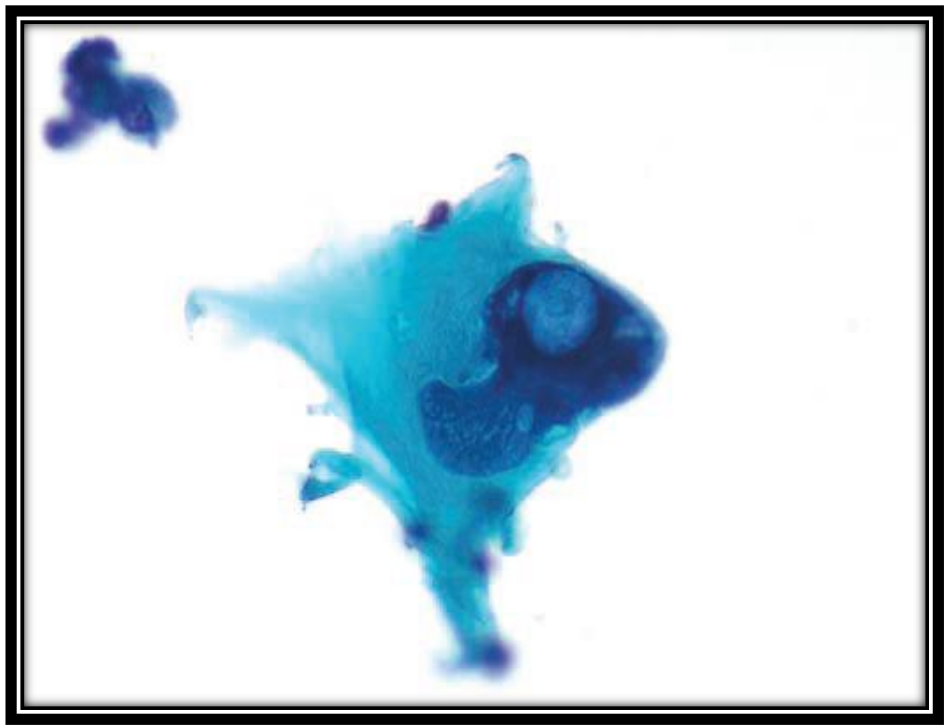
- Some cells are round while other cells have a columnar or flask-like appearance.
- Nuclear-to-cytoplasmic ratios vary tremendously.
- Presence of cilia or a terminal bar favors reactive changes over malignancy.



Creola bodies

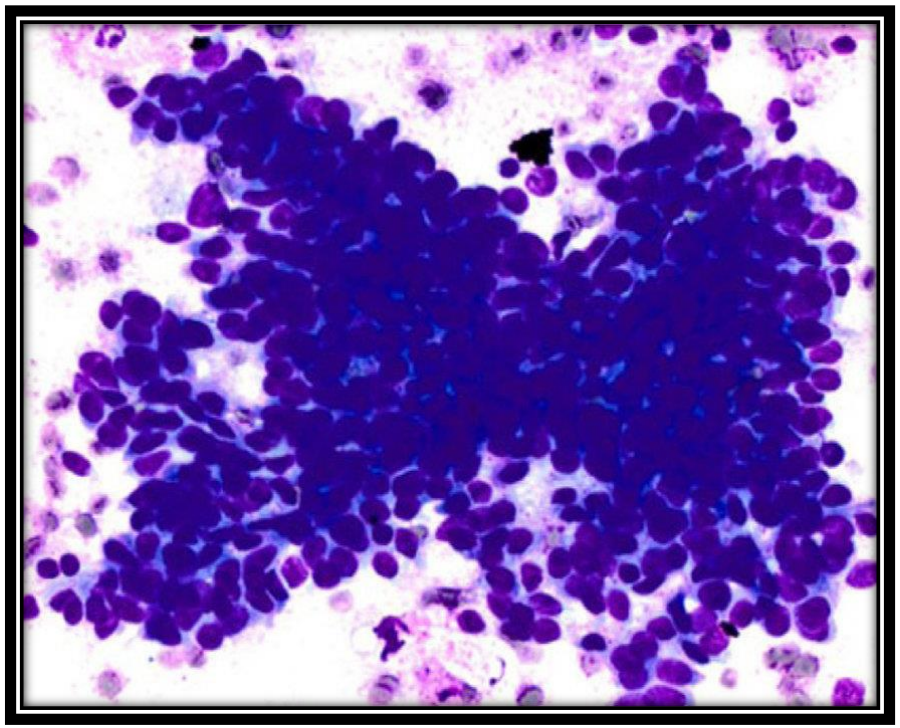
- Three-dimensional clusters of sloughed-off reactive bronchial cells are known as Creola bodies and can be seen in chronic airway diseases such as asthma.

Benign



Reactive changes to therapy

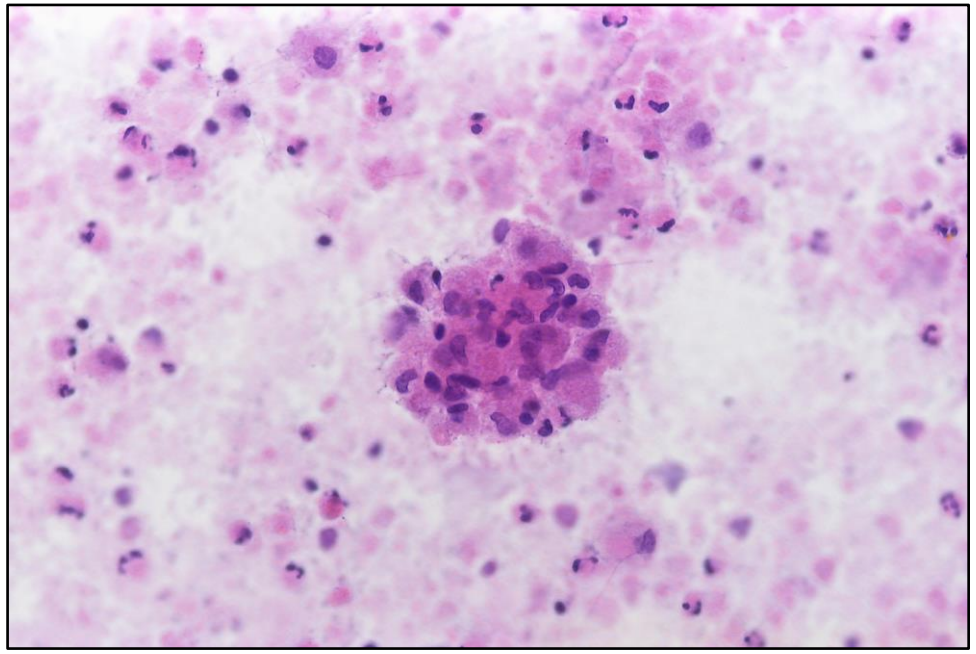
- Cellular changes that mimic carcinoma (e.g., increased N/C ratio, multinucleation, and prominent nucleoli) can be seen after administration of chemo and radiation therapy.

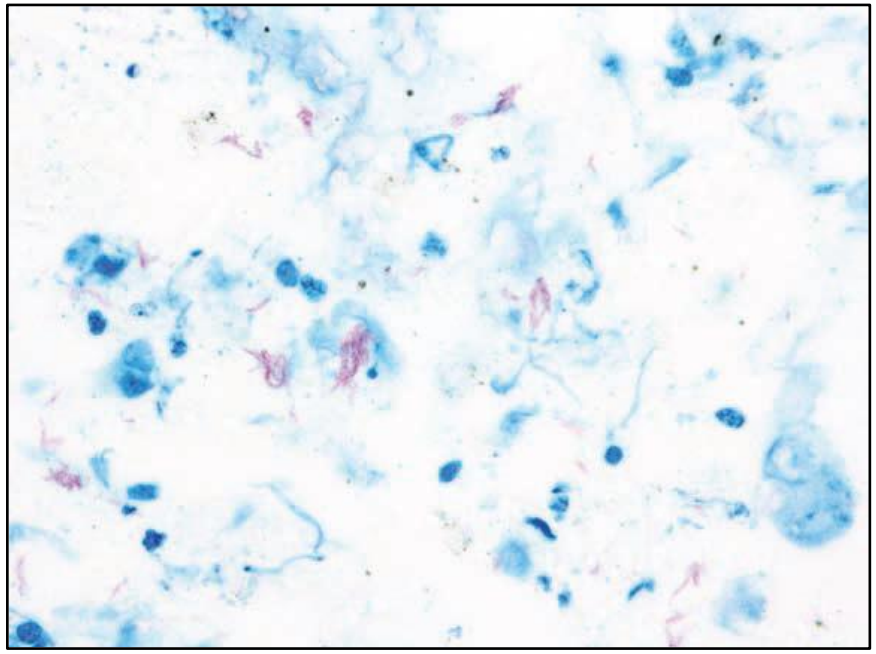


Bronchial reserve cell hyperplasia

- Reserve bronchial cells are in tightly packed, cohesive groups of small cells.
- Approximately the size of an erythrocyte and show nuclear moulding, scant cytoplasm, and dark, smudgy chromatin.
- Associated with surface epithelium injury.

Benign – Bacterial and mycobacterial infections

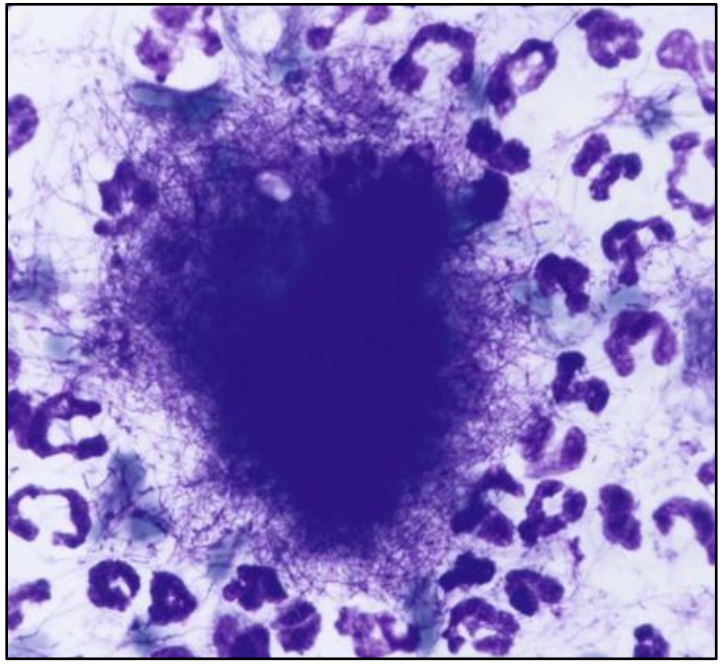




Tuberculosis

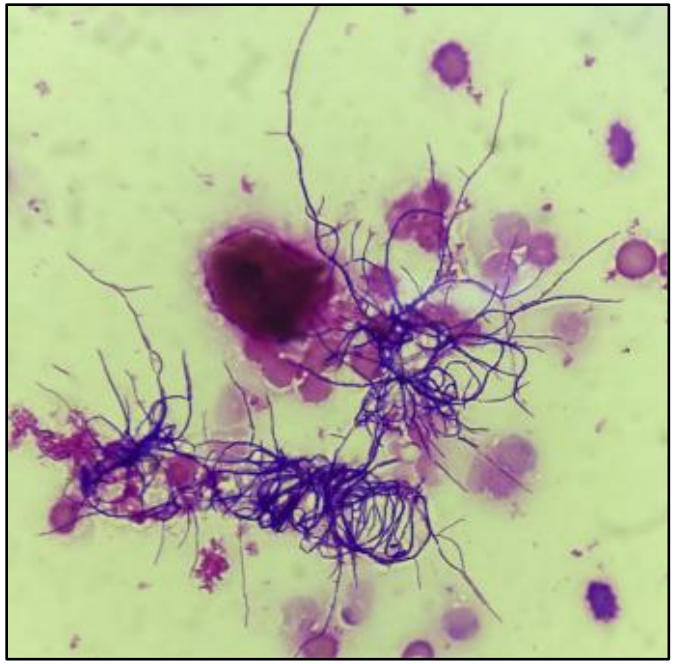
- Can show granulomatous inflammation, seen as tight aggregates of epithelioid histiocytes, lymphocytes, and Langhans giant cells.
- Histiocytes show syncytial arrangement with indistinct cell borders, with curved or elongated nuclei.
- A variable amount of necrosis and inflammation may be present.
- Mycobacterial organisms can be identified using Ziehl-Neelsen and auramine-rhodamine stains.

Benign – Bacterial and mycobacterial infections



Actinomyces

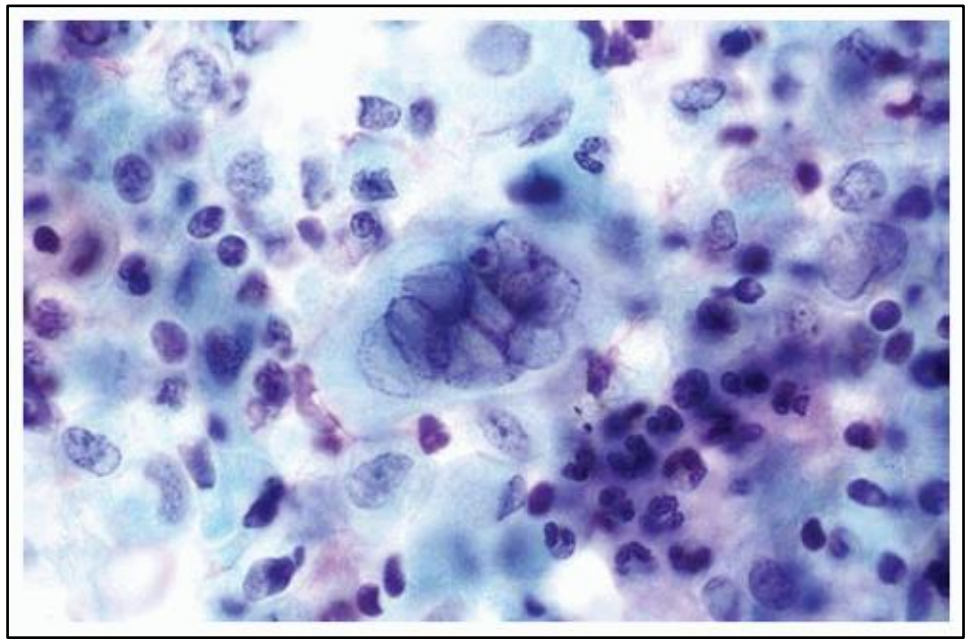
- The radiating filaments of Actinomyces colonies appear as blue cotton balls on lung cytology.
- Actinomycetes pneumonia shows bacterial colonies associated with neutrophilic inflammation.



Nocardia

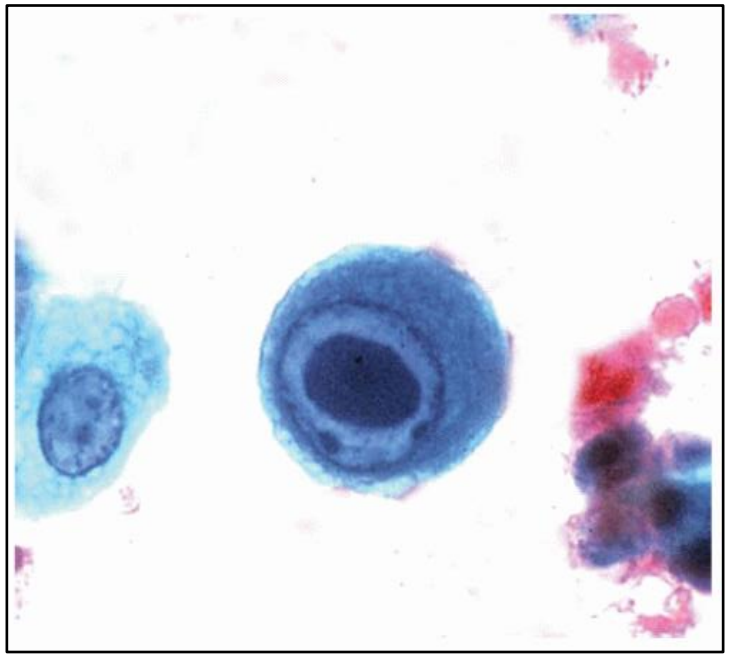
- Nocardial organisms are thin, filamentous organisms that show a beaded appearance with right-angle branching (Chinese letter pattern) in a background of neutrophils.
- These are gram-positive, weakly acid-fast, and can be highlighted with silver stains.

Benign – Viral infections



Herpes simplex virus

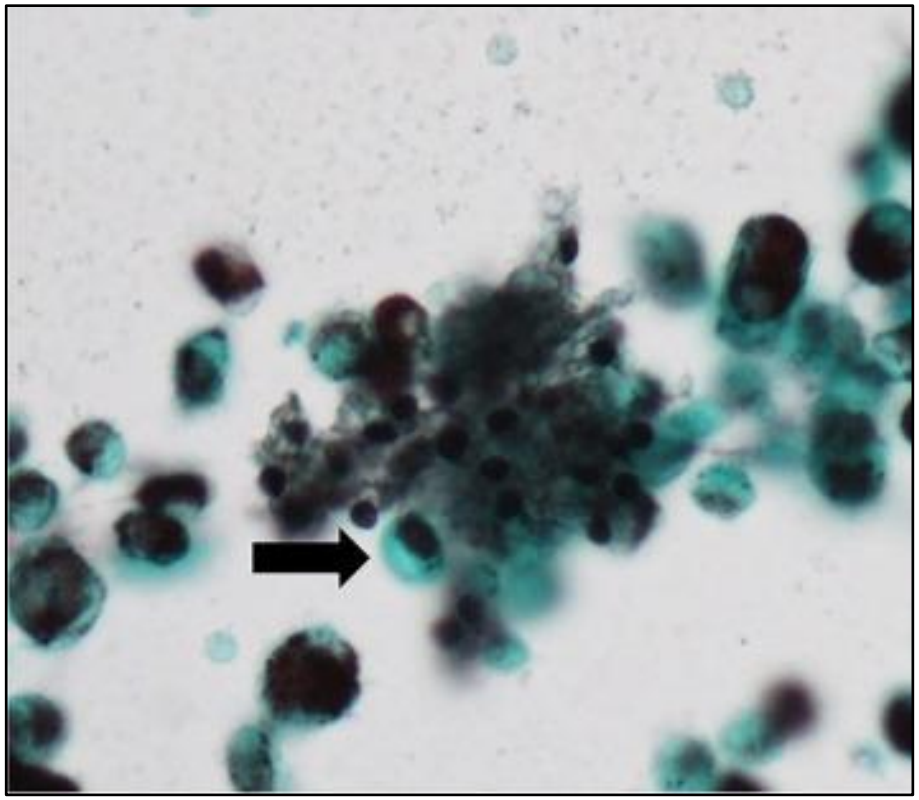
- Characteristic features of cells infected by HSV are multinucleation, nuclear moulding, ground-glass nuclei and margination of chromatin.
- Cells show large, eosinophilic intranuclear inclusions (Cowdry type A).



Cytomegalovirus

- Infected cells show nuclear and cytoplasmic enlargement and large, basophilic nuclear inclusions with a halo (can be highlighted with PAS and GMS).
- Smaller basophilic inclusions can also be identified within the cytoplasm of affected cells.

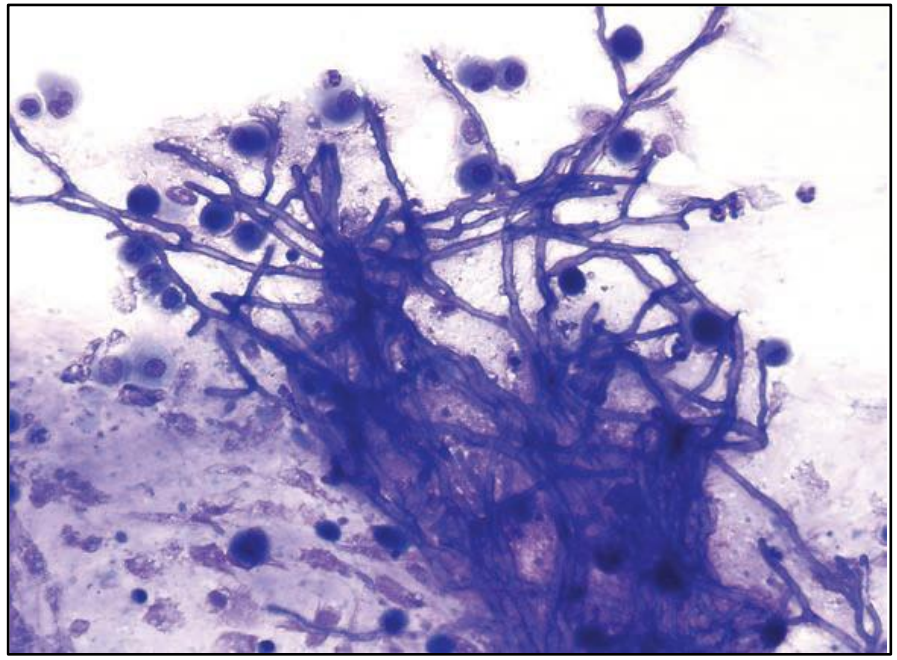
Benign – Fungal infections



Pneumocystis jirovecii

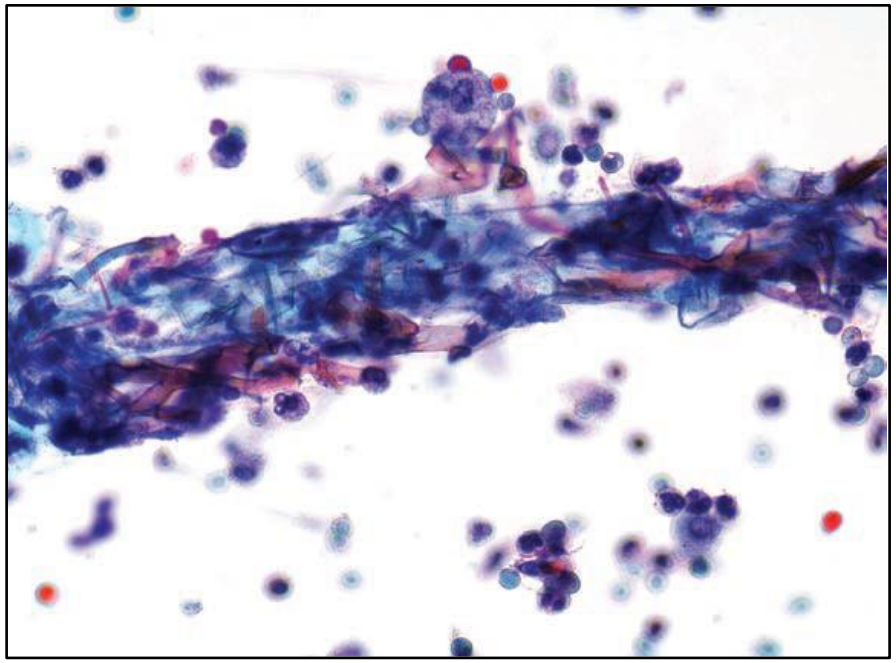
- Cytology can show green, foamy alveolar casts, which are masses of organisms within proteinaceous material and cell debris.
- Cysts are cup-shaped or boat-shaped, 5-7 microns in diameter, and often display a central dark zone.
- Gomori Methenamine Silver stain (GMS) highlights cysts with ovoid morphology and prominent central dot.

Benign – Fungal infections



Aspergillus

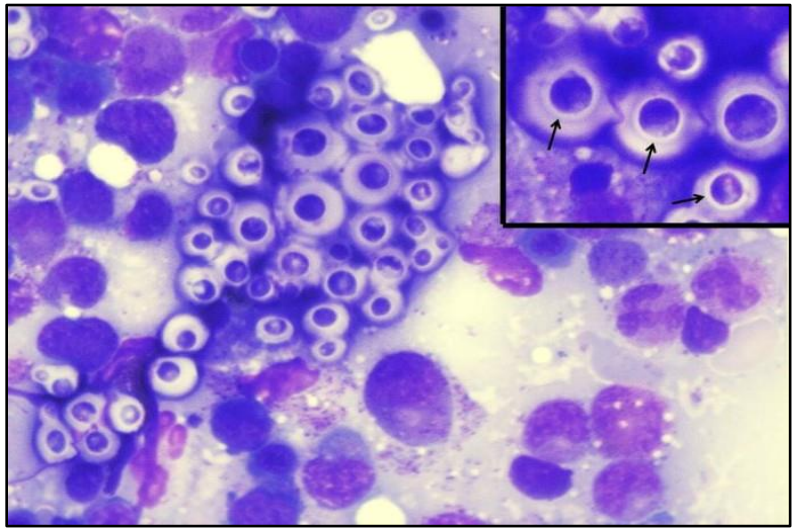
- Septate hyphae (10-30 micron wide) showing acute angle branching, frequently associated with neutrophils and pulmonary alveolar macrophages.
- Characteristic “fruiting head” is seen in cavitary lesions; these may be associated with polarizable calcium oxalate crystals.



Mucor

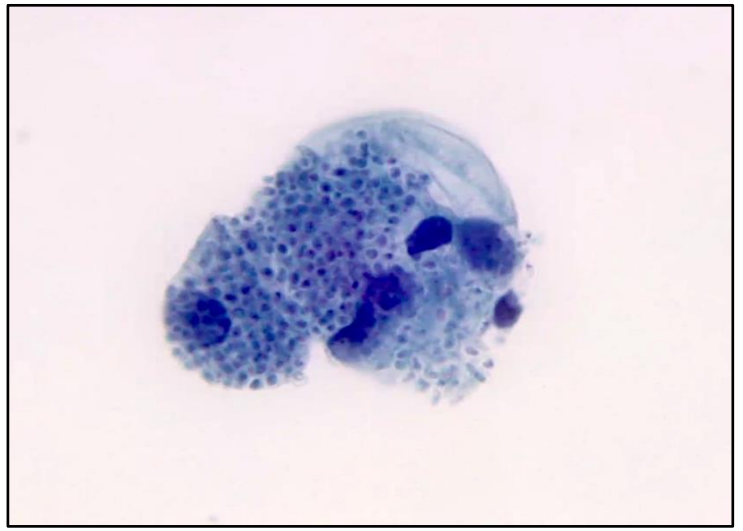
- Variably sized, pauci-septate, ribbon-like hyphae (10-30 microns wide) with right angle branching.
- In the background are typically scattered alveolar macrophages, neutrophils, and red blood cells.

Benign – Fungal infections



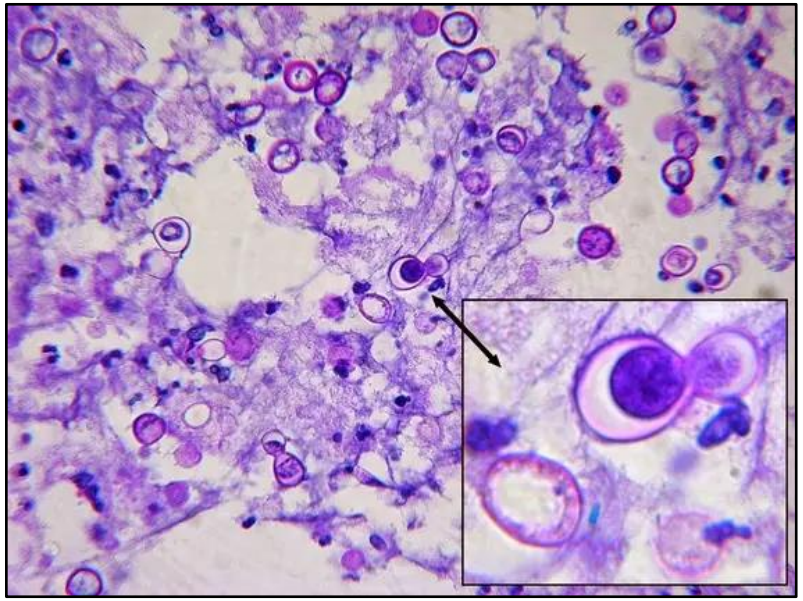
Cryptococcus neoformans

- Narrow budding, thin-walled yeast with mucin capsule and refractile center.



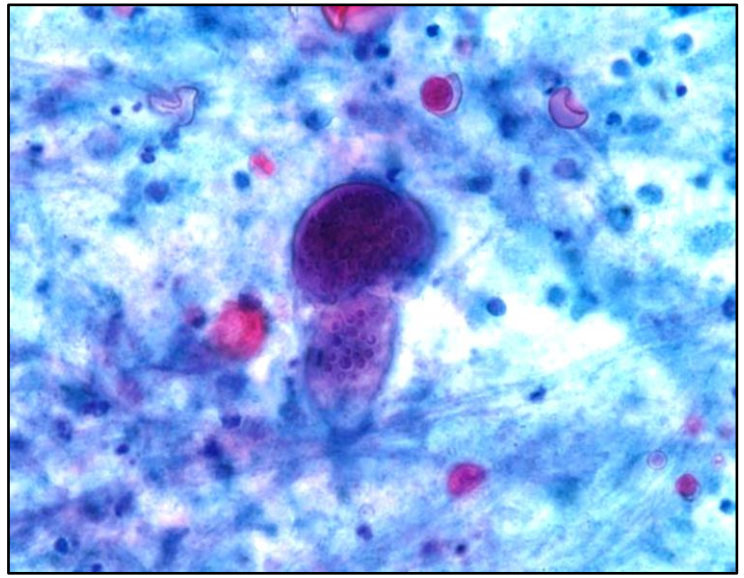
Histoplasma capsulatum

- Budding intracellular yeasts.



Blastomyces dermatitidis

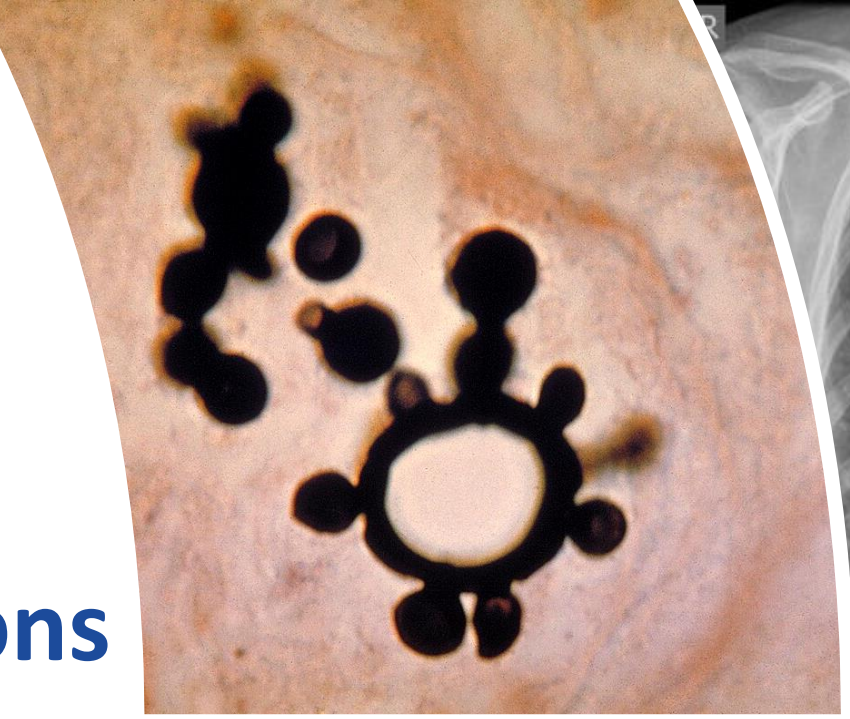
- Broad-based yeast with thick cell wall.

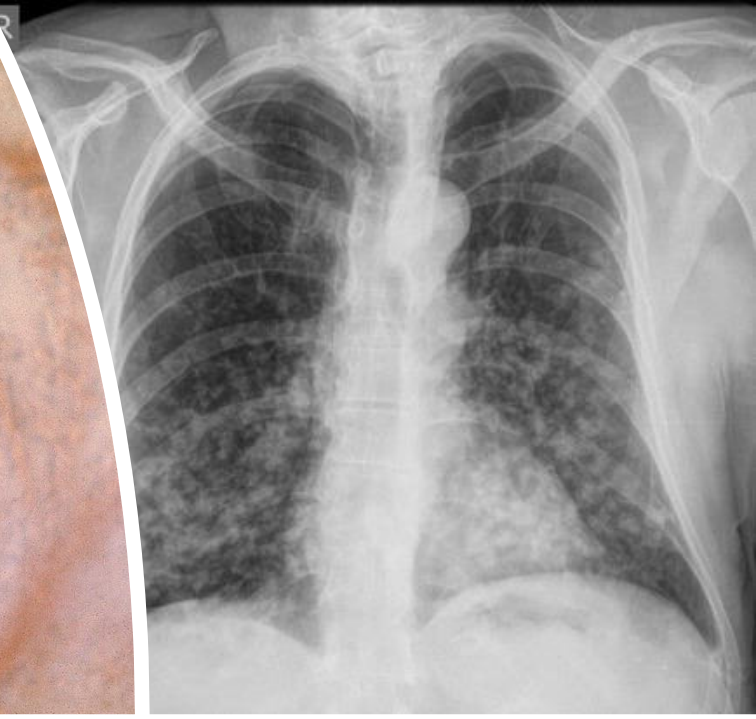


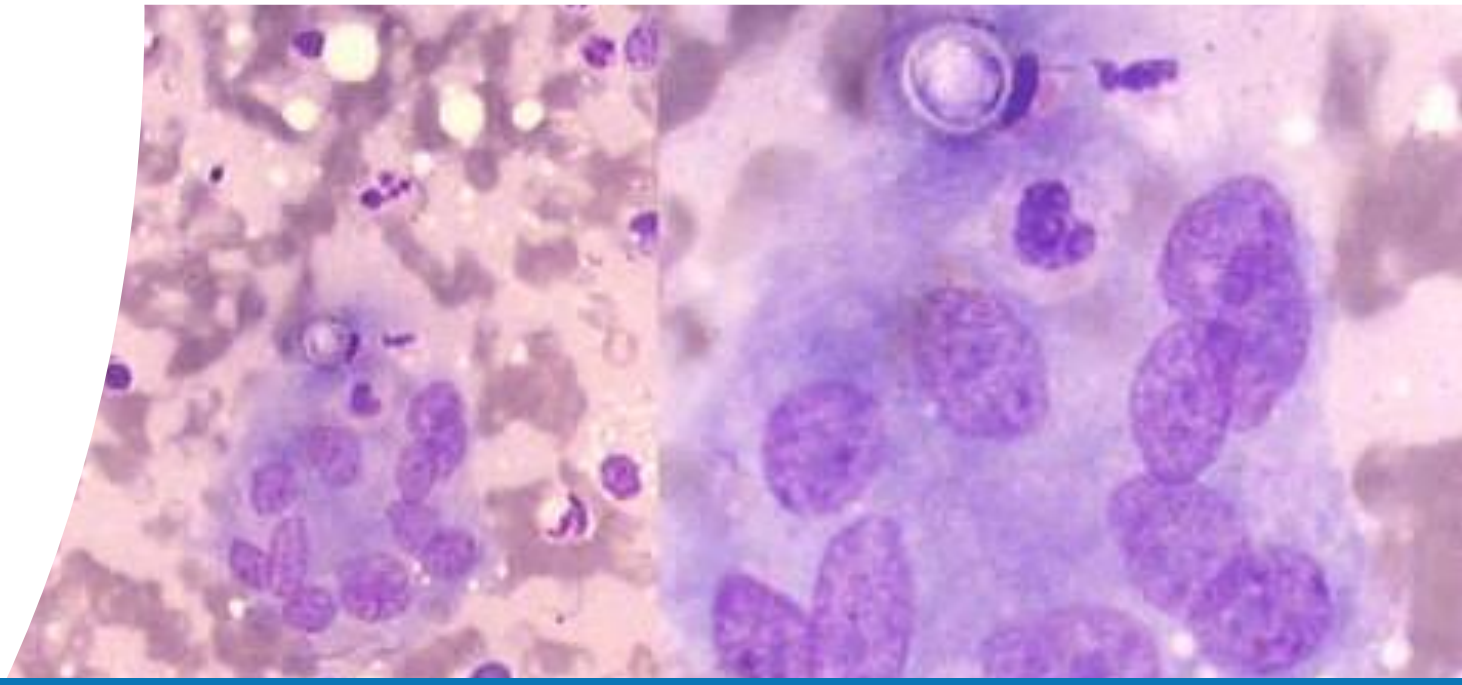
Coccidioides immitis

- Mature or immature spherules (15-60 microns) with a "broken ping-pong ball" appearance.

Benign – Fungal infections



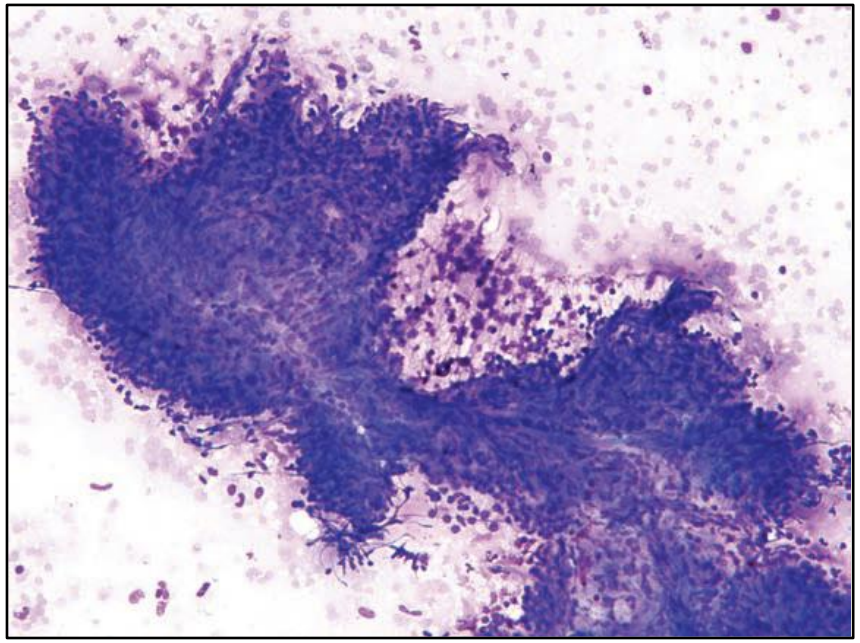


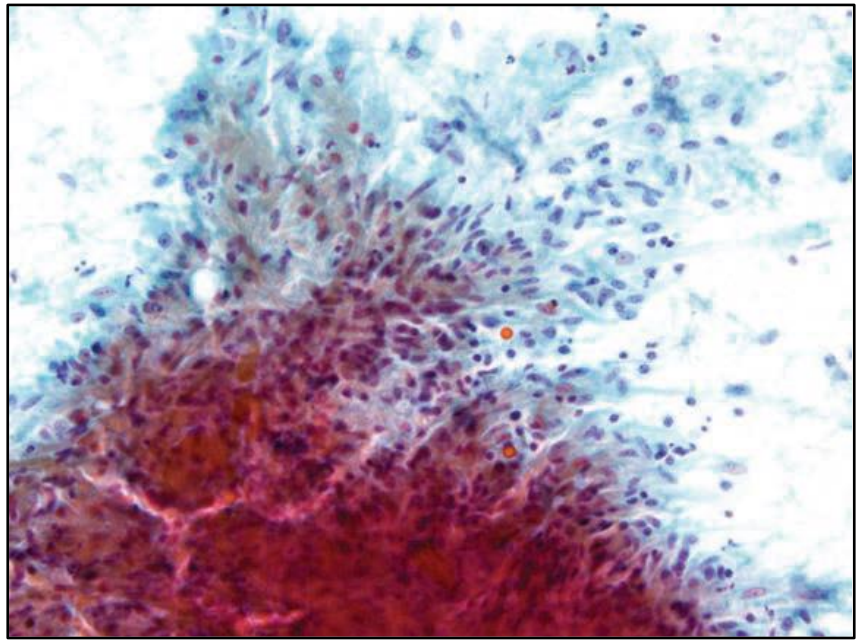


Benign – Sarcoidosis

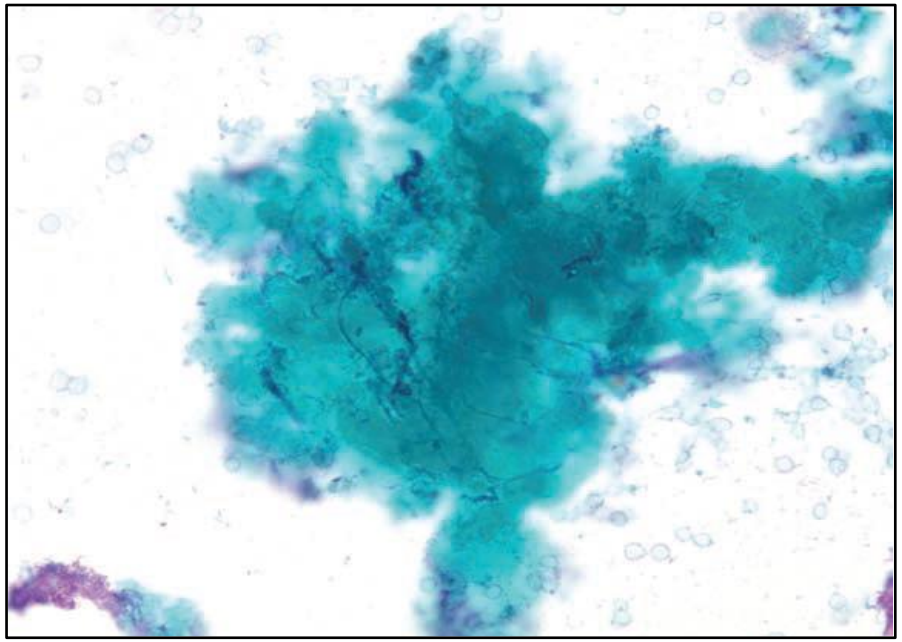
- Noncaseating, well-formed granulomas comprising of epithelioid histiocytes and lymphocytes, with or without multinucleated giant cells.

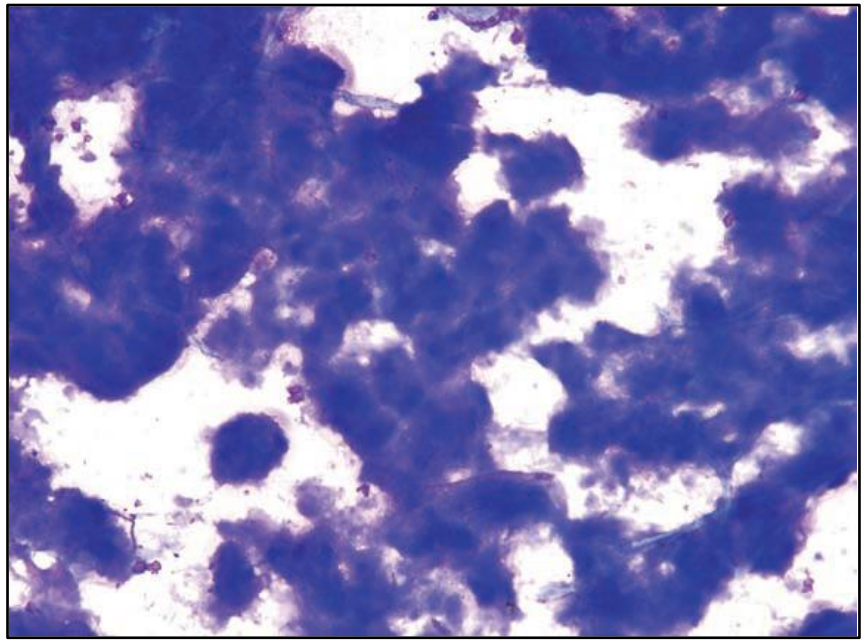
- The epithelioid histiocytes are arranged in a pseudo syncytial pattern and show round, curved, spindle-shaped nuclei with vacuolated cytoplasm.





Benign – Amyloidosis





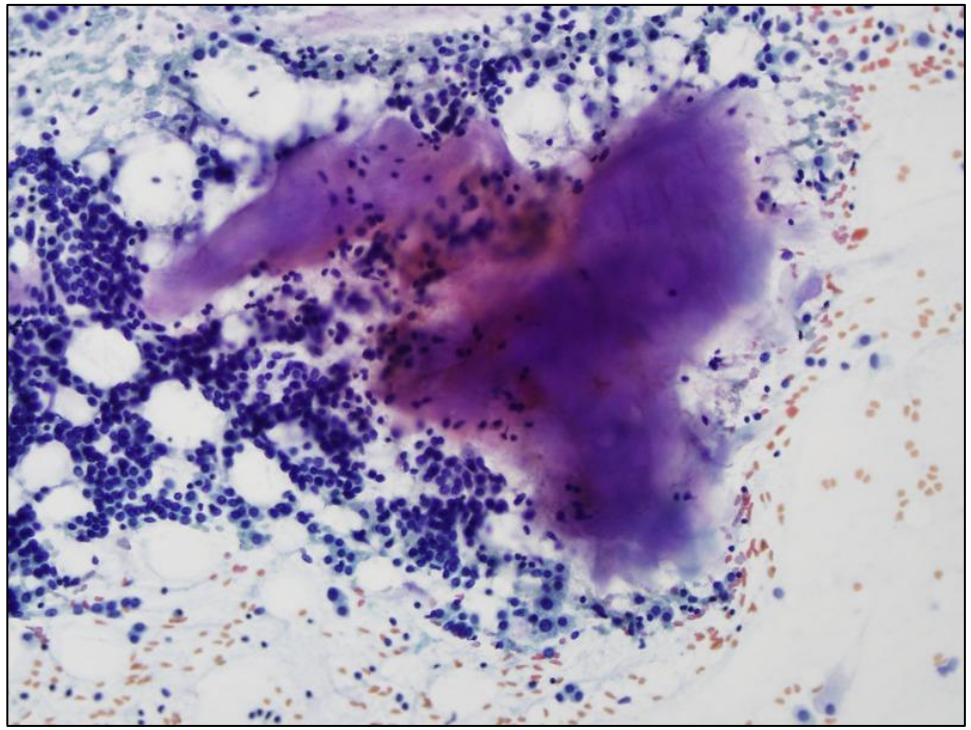
- Cytology of limited value and cellular yield is usually scant.
- FNA shows amorphous, waxy material that appears blue-green on pap stains.
- Calcification, ossification, and multinucleated giant cells can be present.

Benign – Benign neoplastic lesions

Example Report

Female 40y-old, 1.5 cm well-round mass in the lung periphery

- ❑ Satisfactory for Evaluation
- ❑ Benign
- ❑ Pulmonary hamartoma (consistent with)

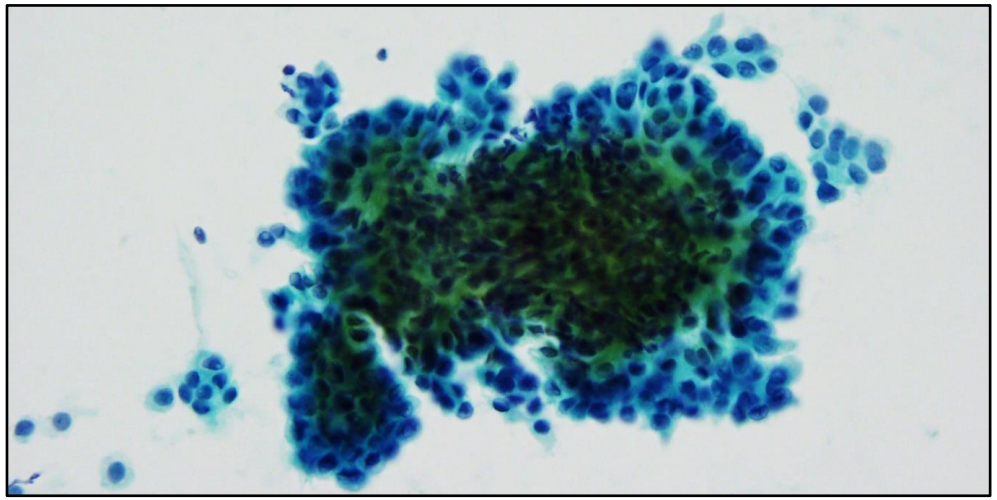


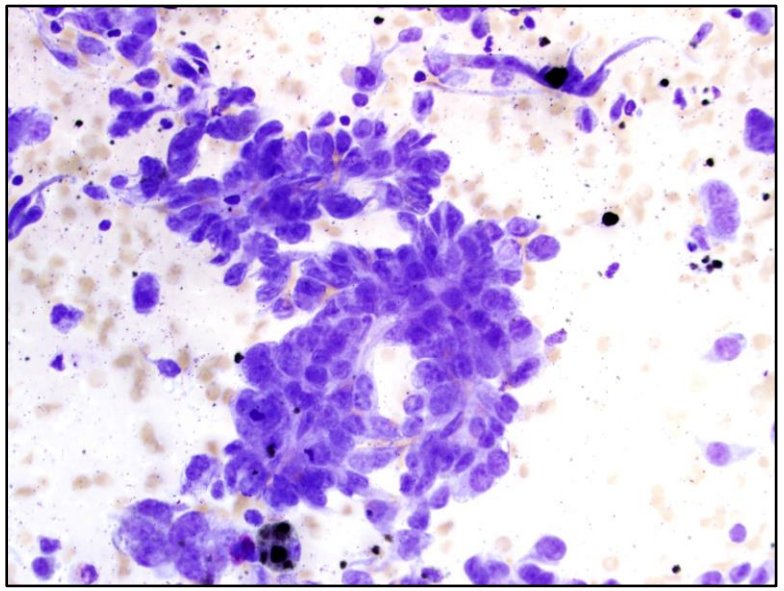
Source: Longwen Cheng and Matthew Zarka, Chapter 5: International System for Reporting Lung Cytopathology, 2022

Benign – Benign neoplastic lesions

Sclerosing pneumocytoma

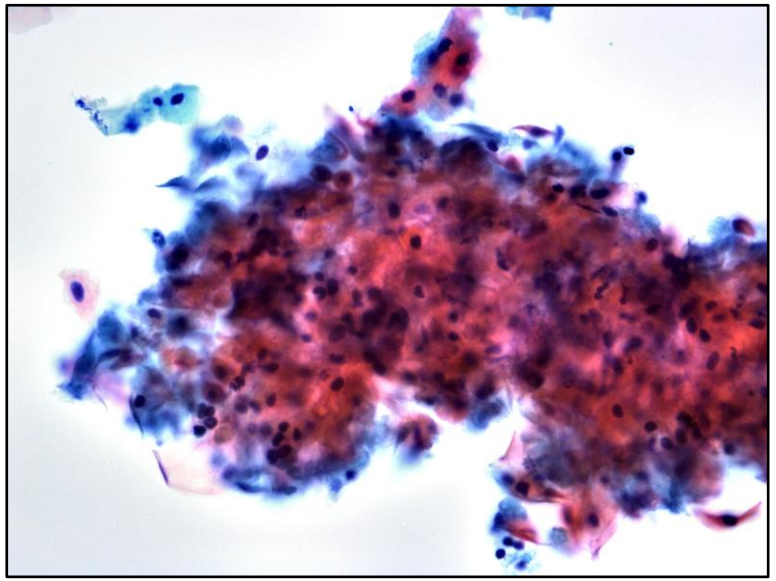
- Cellular specimens, showing two populations of cells:
- Monomorphic, ovoid stromal cells (predominant population) that have pale cytoplasm, round nuclei, smooth nuclear borders, finely textured chromatin, and one or more small nucleoli;
- Cuboidal surface cells (pneumocyte-like cells).

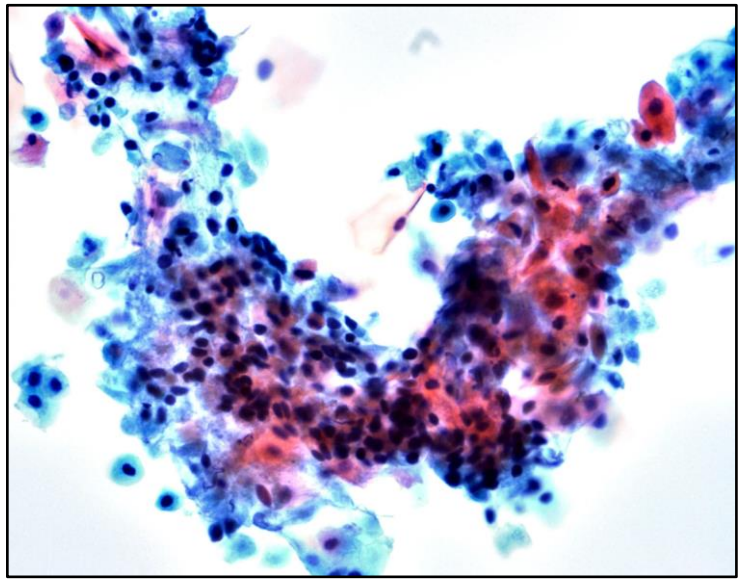




- Papillary microarchitecture with pneumocyte-like cells lining a core of round stromal cells.
- Can show a degree of pleomorphism, mild nuclear atypia, intranuclear pseudoinclusions, and prominent nucleoli.

Benign – Benign neoplastic lesions



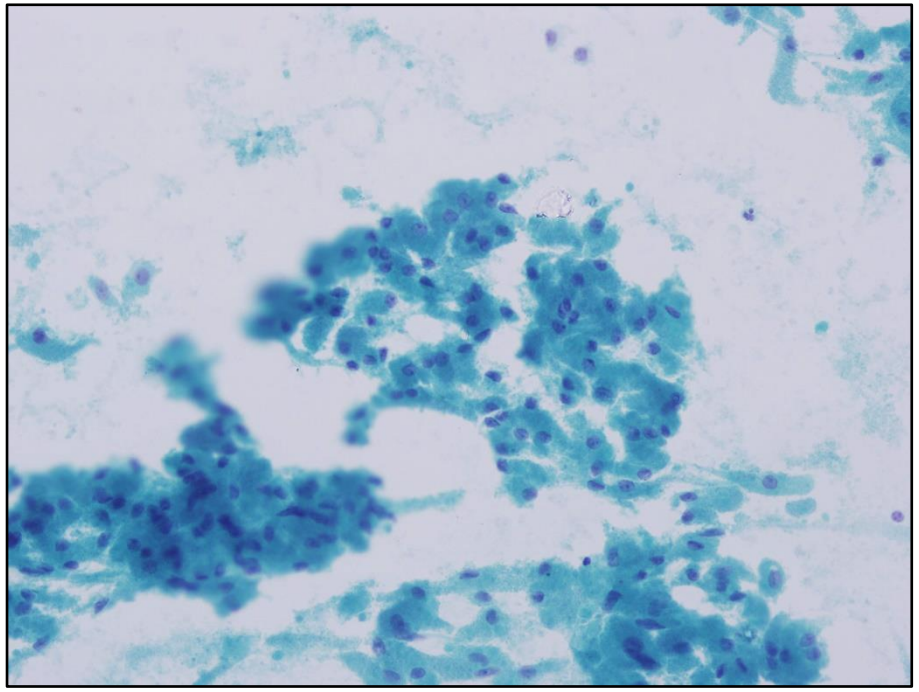


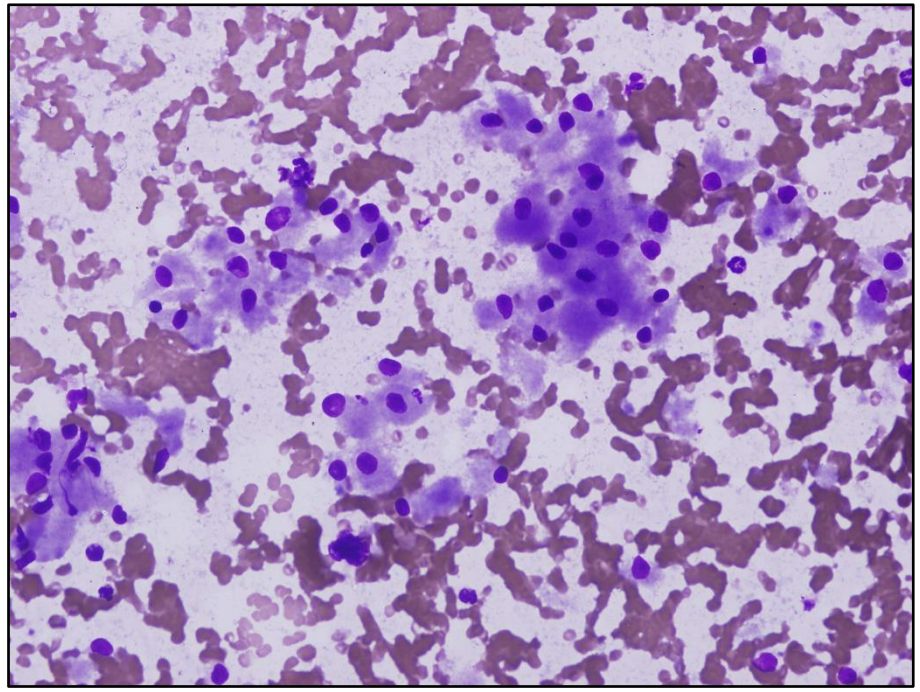
Papilloma

- Cellular specimens with small, dark, pyknotic nuclei showing varying degrees of reactive atypia.
- Most cases lack mitosis.
- The type of epithelial cells seen depends on the histology of the papilloma (mature squamous, mucinous glandular, ciliated)

Benign – Benign neoplastic lesions

- ✓ **Granular cell tumour**
- ✓ Variably cohesive clusters of monotonous, epithelioid tumour cells.
- ✓ Tumor cells show characteristic abundant, granular, eosinophilic cytoplasm, and small, round to oval, uniform nuclei.

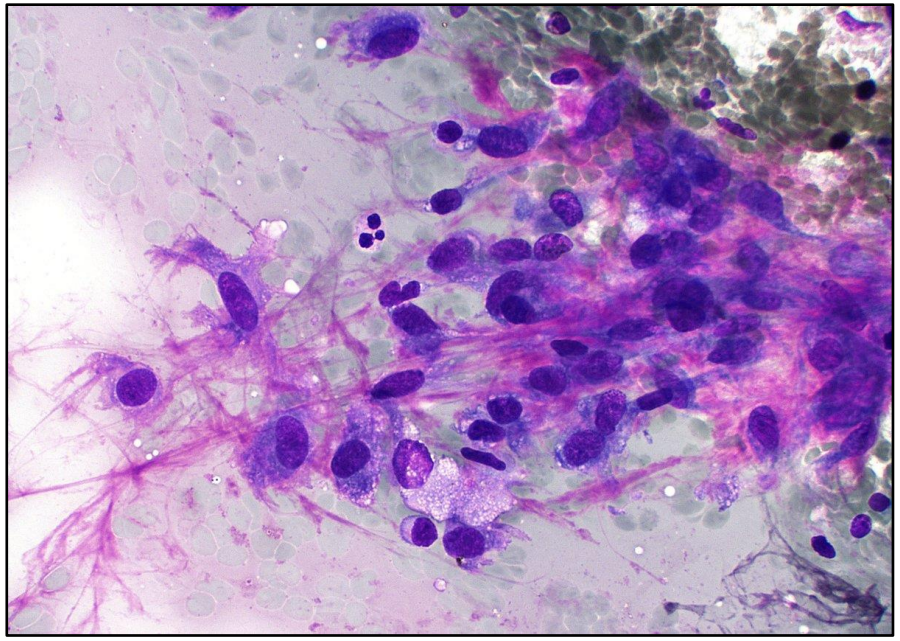


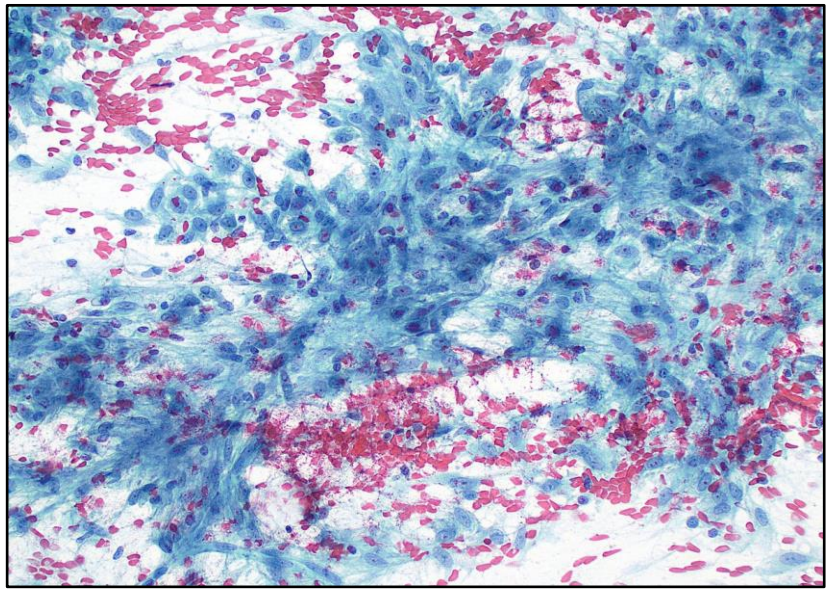


Benign – Benign neoplastic lesions

Inflammatory myofibroblastic tumor

- Mainly composed of spindle cells admixed with a significant polymorphous inflammatory infiltrate.
- Bland-appearing spindle to stellate cells show minimal pleomorphism with rare mitoses.





- Inflammatory infiltrate can be marked, showing lymphocytes, plasma cells, histiocytes, and Touton-type giant cells.
- A minimal amount of necrosis is not incompatible with this diagnosis but is usually absent.

Categories For The WHO Reporting System for Lung Cytopathology

Atypical

- *A specimen categorized as 'Atypical' demonstrates features predominantly seen in benign lesions and minimal features that may raise the possibility of a malignant lesion but with insufficient features either in number or quality to diagnose a benign or malignant lesion.*

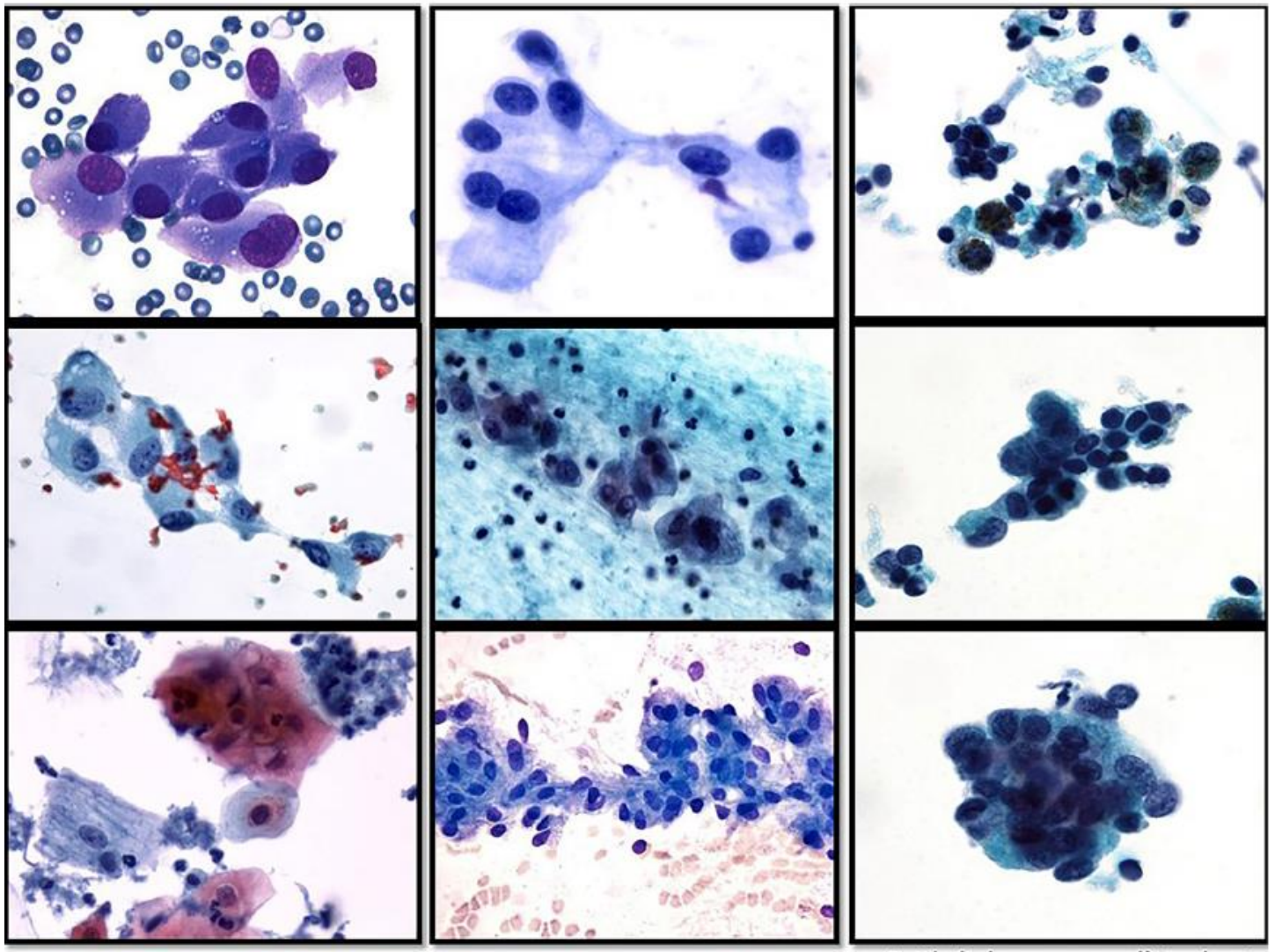
- INCIDENCE: around 5% (few studies)
- Reported ROM: 46-55% (few studies, different samples)
- MAIN CAUSES: reactive changes (metaplasia, hyperplasia), infectious (viral), post-therapy changes
- MANAGEMENT: Correlate with CLIN-IMG-MICRO; if these are benign, repeat in case of exfoliative cytology or follow-up at 3-6 months after MDT in case of FNAB. If the clinical or image is atypical or suspicious for malignancy, then perform BB/BW or FNAB with or without CNB.

Reactive vs Malignant Lung Cytology

- Equivocal lung cytology in ≈2% of the lung patients
- WHO reporting system: atypical – suspicious
- Main DD:
  - Repair/regeneration of bronchial epithelium
  - Atypical type-II alveolar cells.
- Clinical & radiological context
- Clinical consequences (re-intervention, follow-up, resection, systemic treatment)

Atypia in pulmonary cytology: Morphologic spectrum and causes

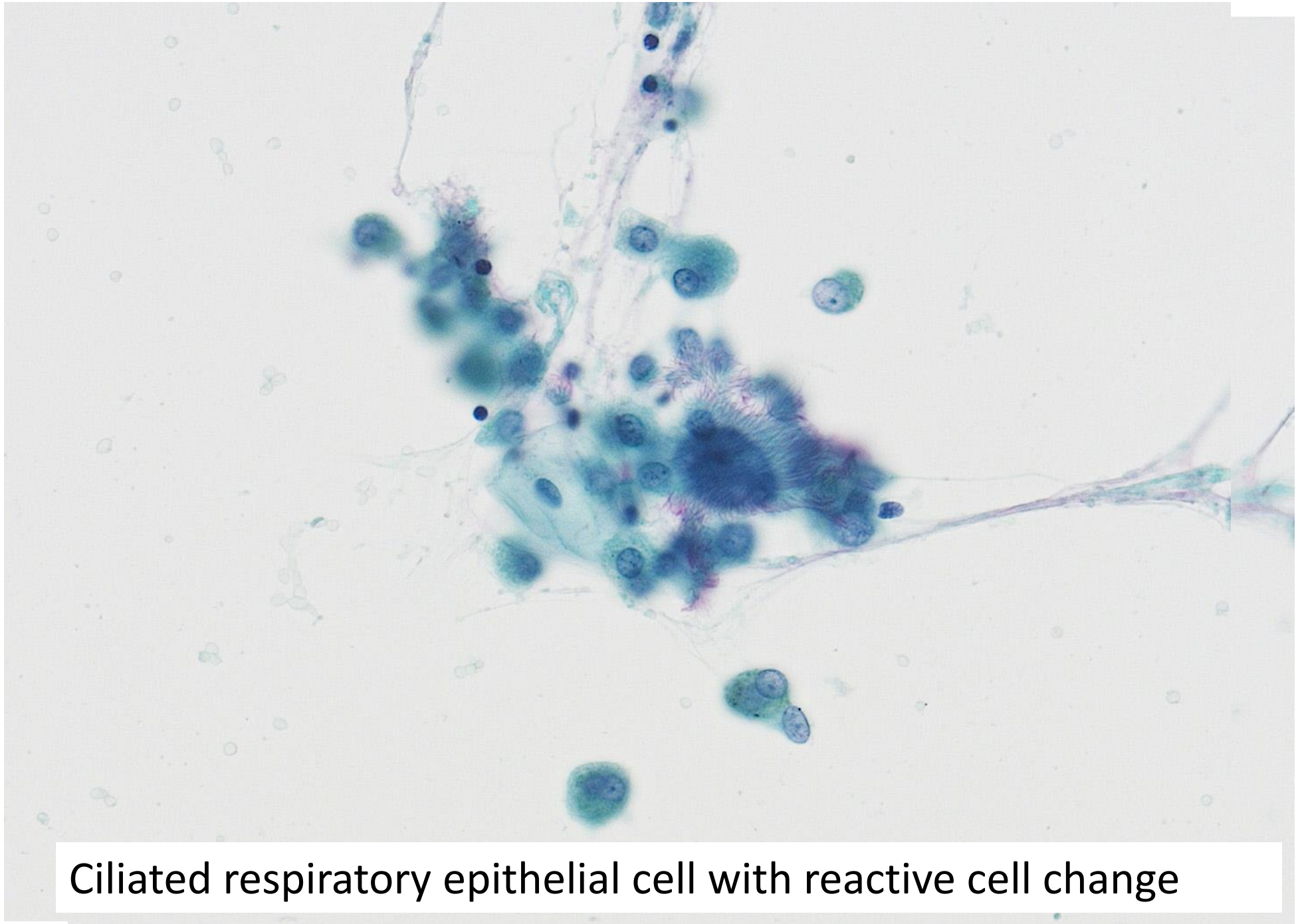
Lester J. Layfield MD[1] | Zubair Baloch MD, PhD[2]



Squamous Metaplasia with atypical nuclei

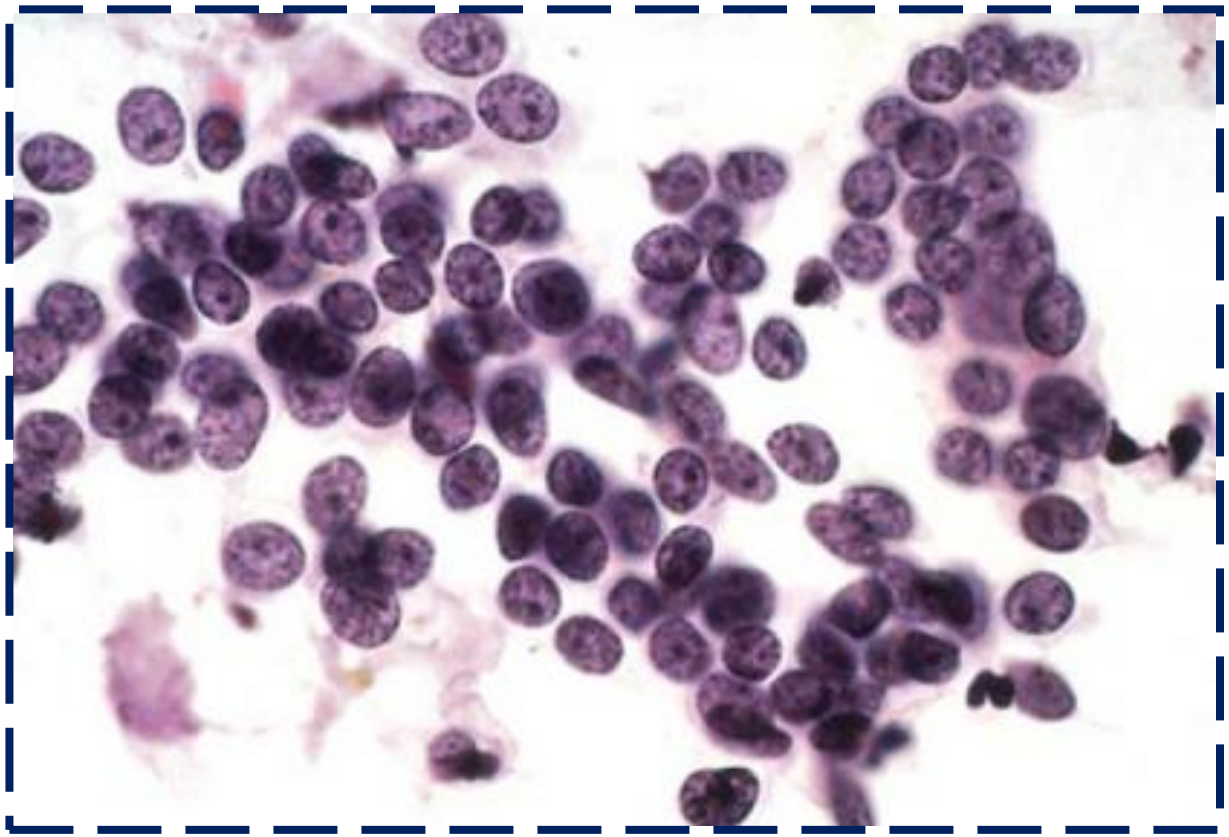
Epithelioid cells with clear/foamy cytoplasm

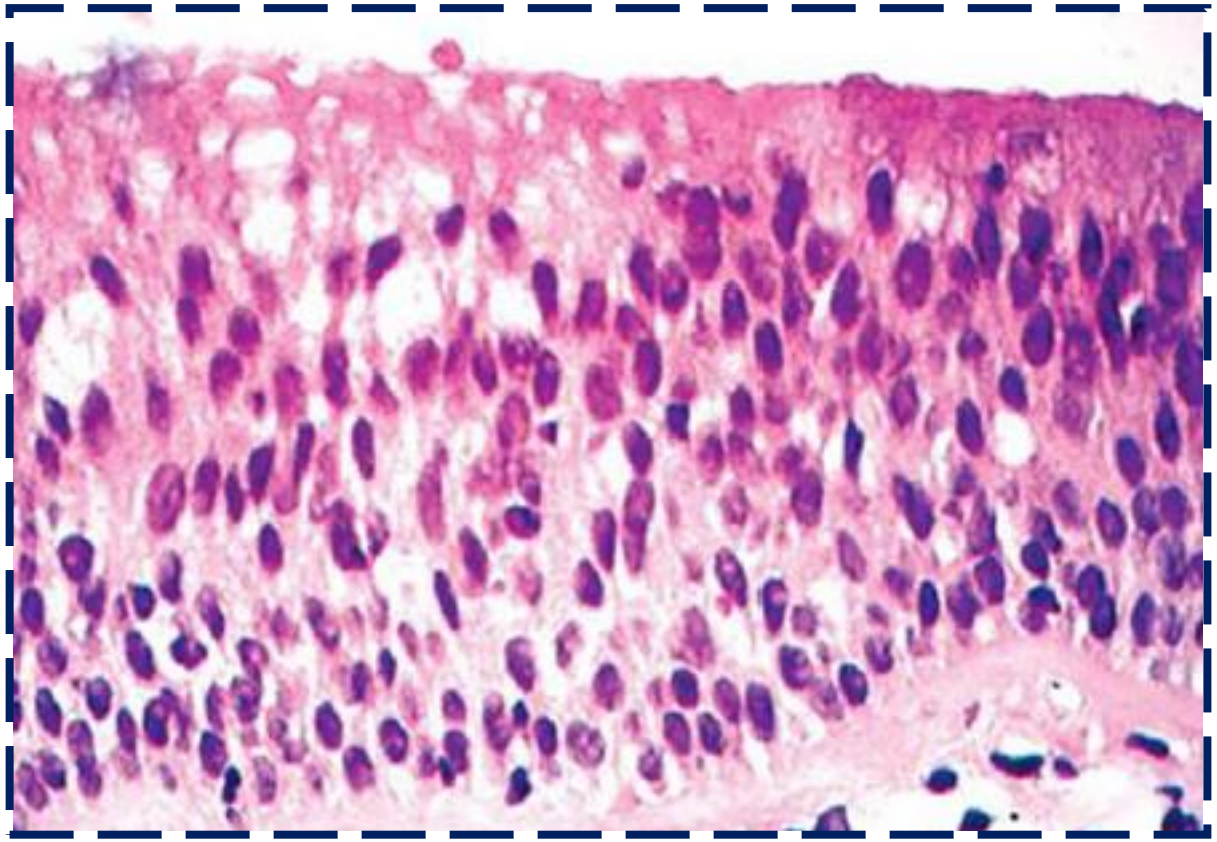
Epithelial or reserve cells in sheets and three dimensional clusters

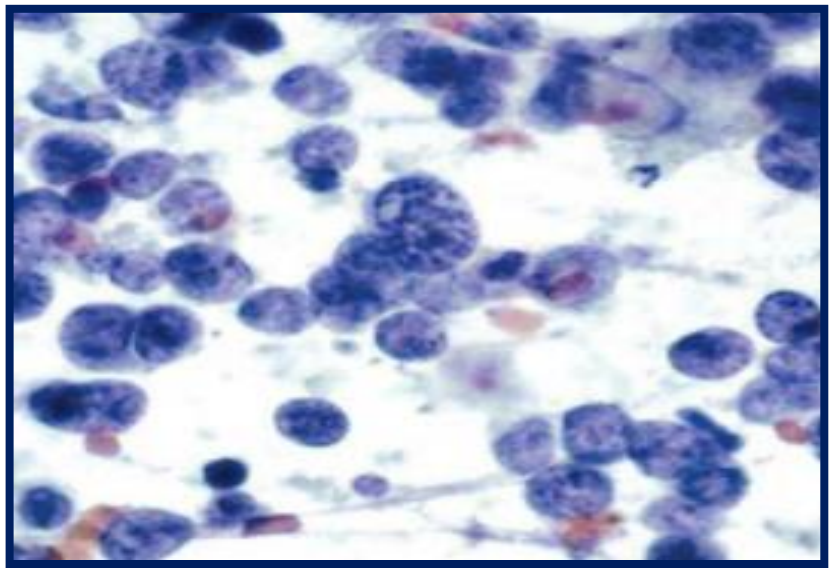


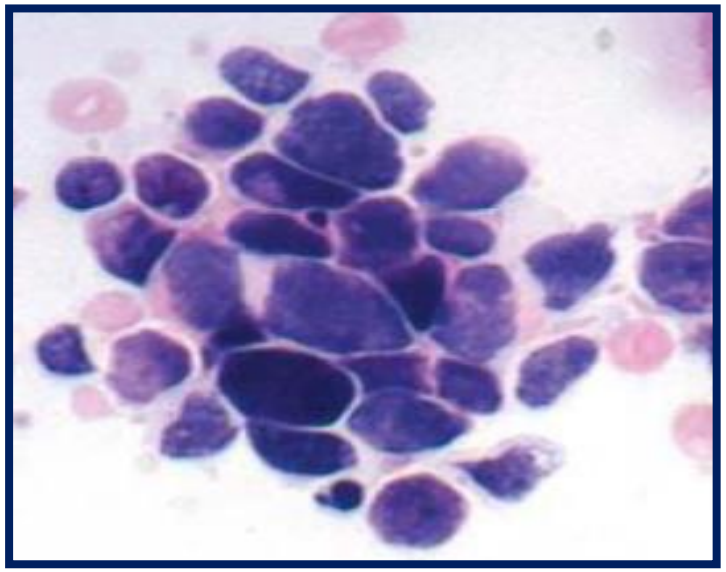
Ciliated respiratory epithelial cell with reactive cell change

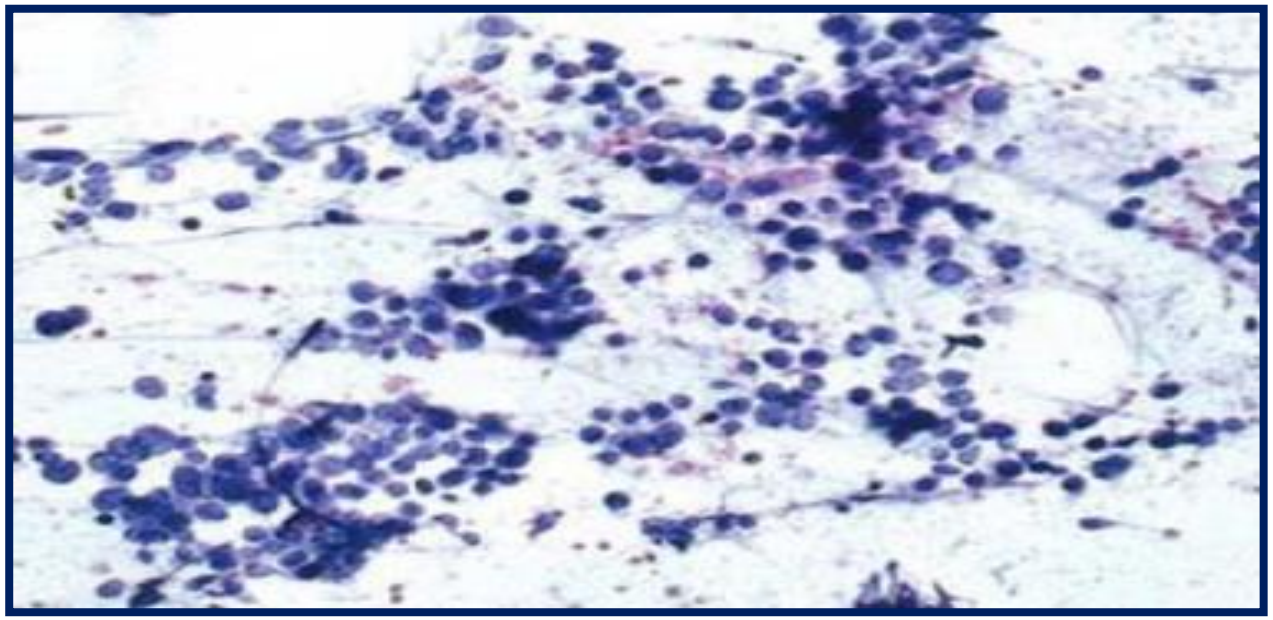
Source: Prof Lukas Bubendorf

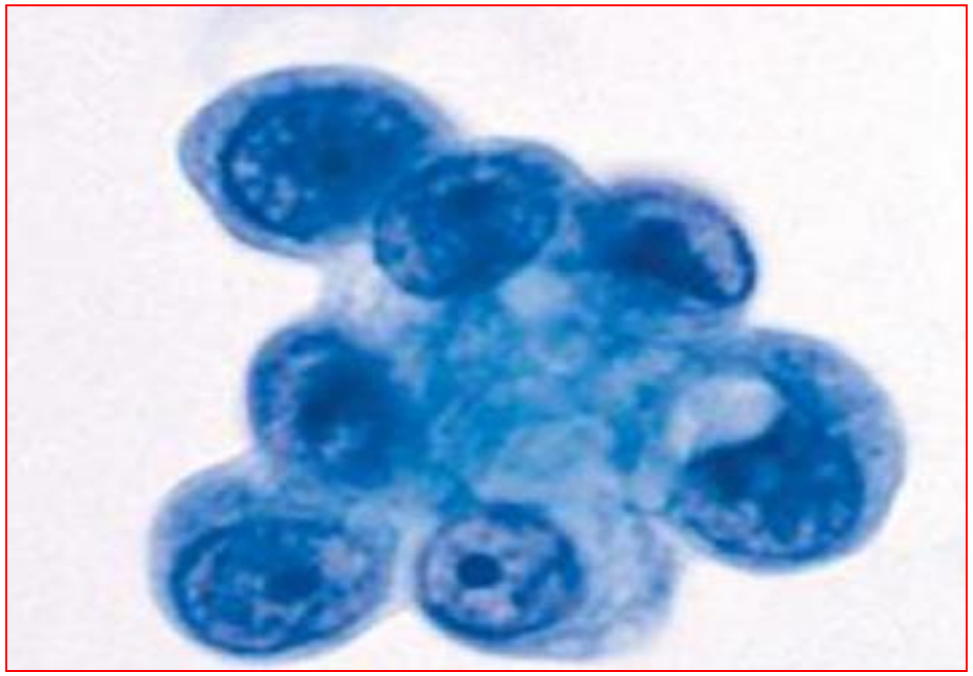




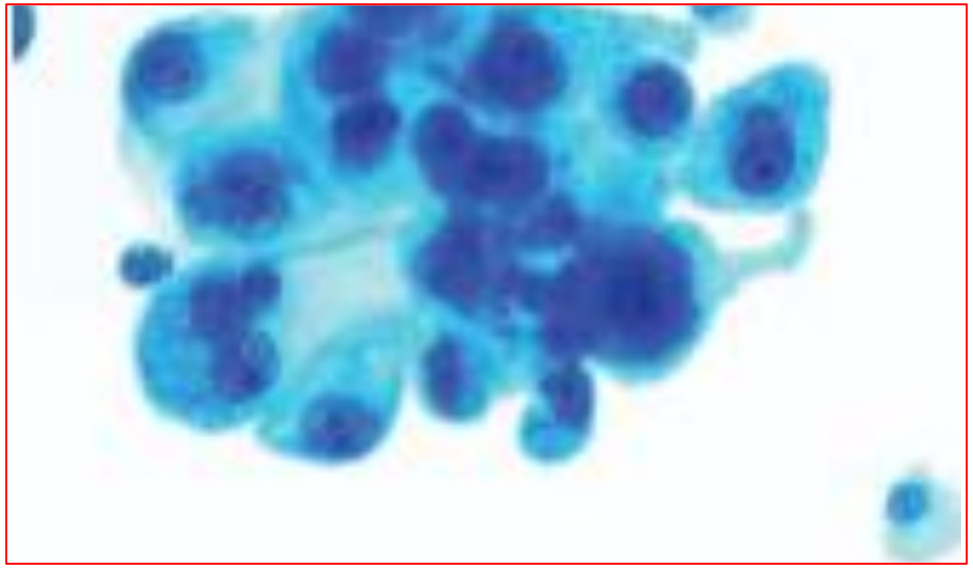


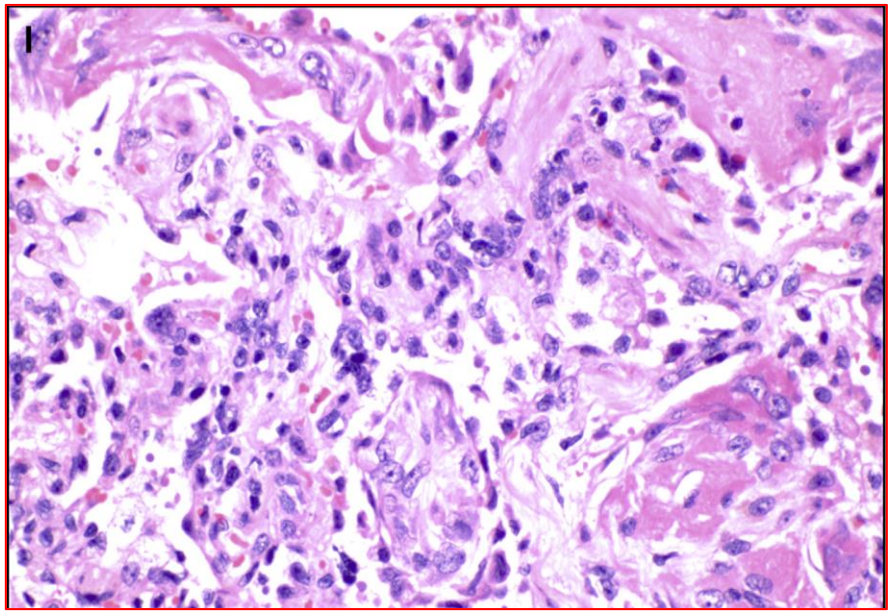




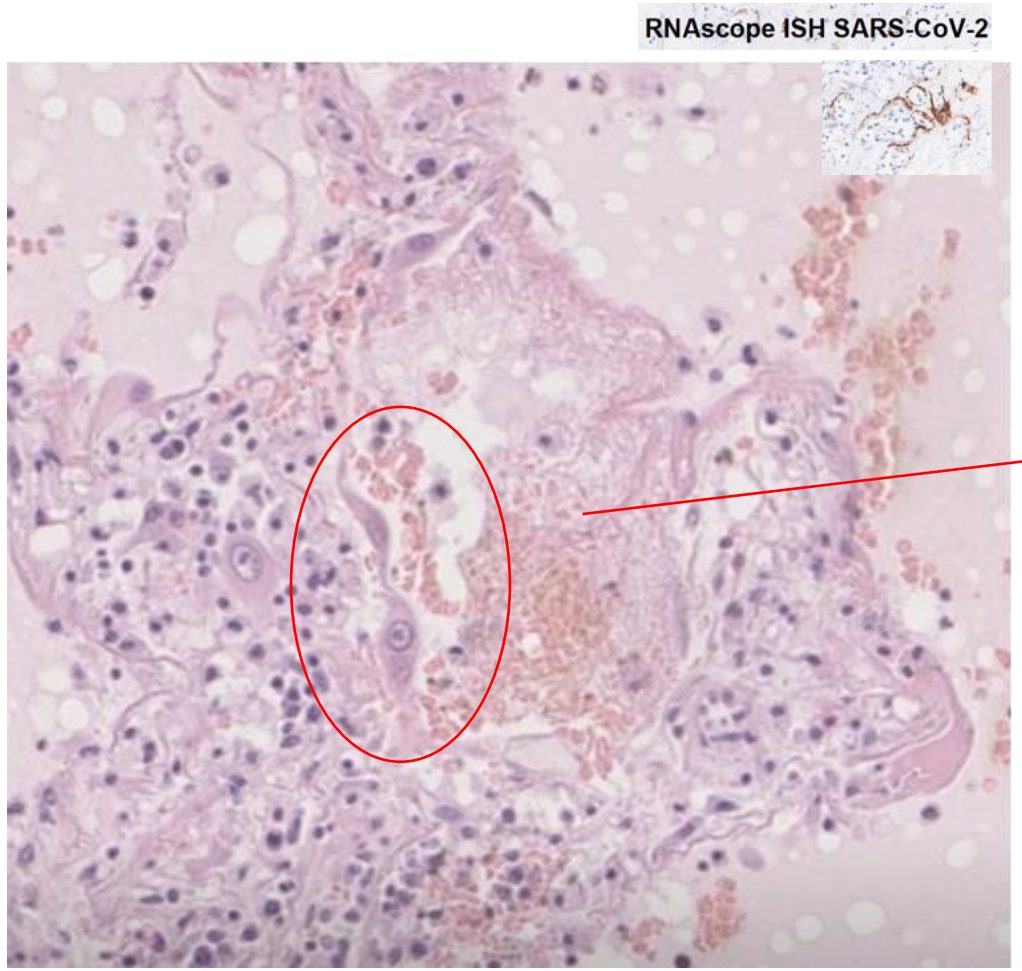


Reactive alveolar atypia in DAD

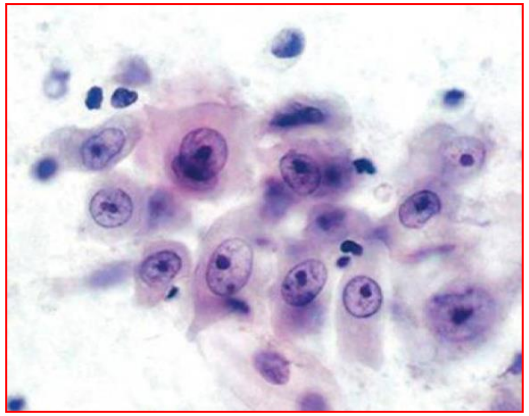


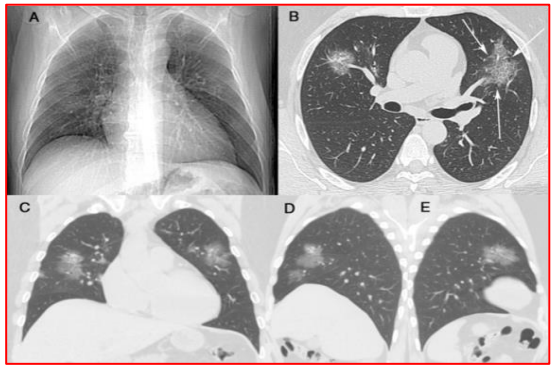


Reactive changes in alveolar epithelium



- ✓Infections (including SARS-COV2
- ✓Drug toxicity (amiodarone
- ✓Infarction





Covid 19 pneumonia

Schmitt W et al. Pulmonology J 2020

Algorithm for evaluation of lung cytology and potential pitfalls

Clinical Presentation

- *GENERAL RULE:* Cancer patients usually do not present acutely ill!
- *Be wary of making a malignant diagnosis without a mass*
- Lepidic carcinoma may sometimes present as pneumonic-like consolidation – often treated as pneumonia without resolution.

Int J Clin Exp Pathol 2010;3(4):367-385

Algorithm for evaluation lung cytology and potential pitfalls

Cellularity

- *GENERAL RULE:* Be wary of making a malignant diagnosis in a low cellularity specimen, unless there are unequivocal features of malignancy.
- Benign or reactive lesions may occasionally be hypercellular.
- Attention to cytomorphologic features critical

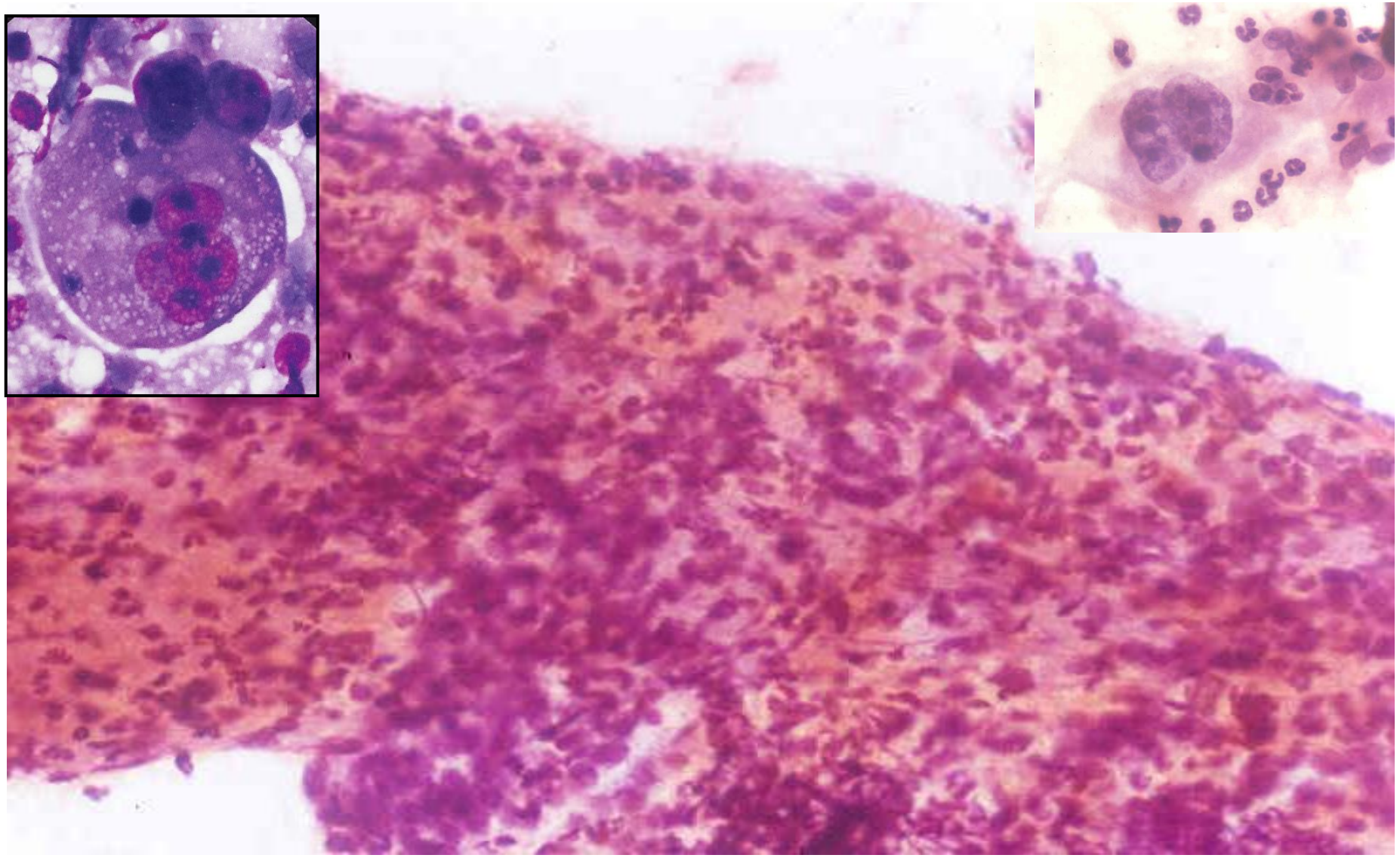
Idowu, M. O., & Powers, C. N. International journal of clinical and experimental, 2010

Algorithm for evaluation lung cytology and potential pitfalls

Background

- *GENERAL RULE:* Be wary of making a malignant diagnosis with a background with inflammation.
- Both benign and malignant lesions may have inflammatory background.

Idowu, M. O., & Powers, C. N. International journal of clinical and experimental, 2010



Pulmonary infarction

BENIGN | REACTIVE | ATYPICAL FEATURES

- MORPHOLOGIC
  - The presence of cilia can be
    particularly l
    a benign pro
  - Malignancy i
    when there i
    cause and a
    transition fr
    malignant ce
  - A close comp
    nuclear features is the key to distinguish between benign versus malignant cells

- IHC
  - IHC stains to separate benign
    has
    are no
    ant”

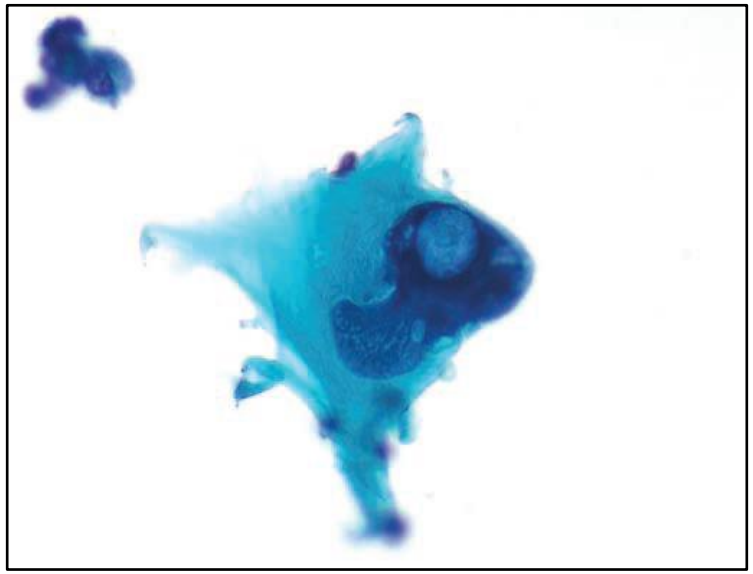
Benign reactive atypia

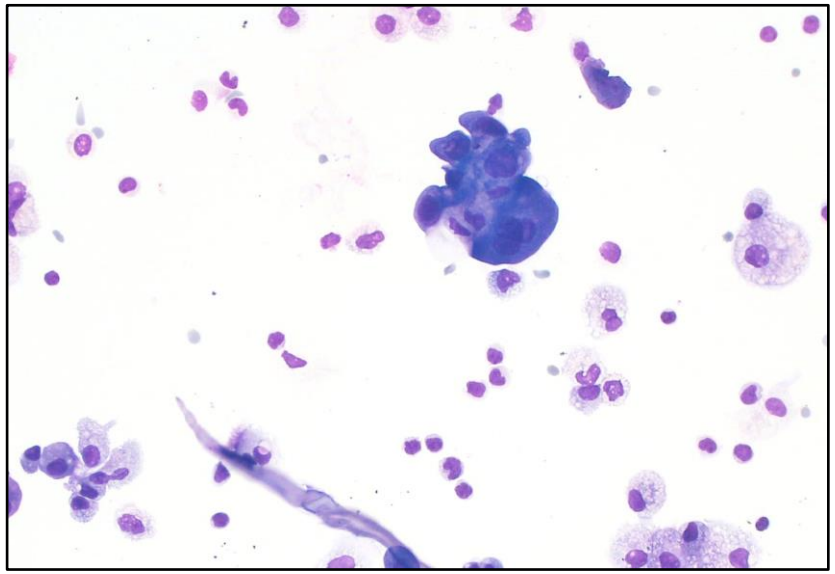
- Repair/regeneration (e.g.Tbc)
- Pulmonary infarction
- Diffuse alveolar damage
- Chemotherapy
- Radiotherapy
- DD: adenocarcinoma (lepidic)

Atypical

What morfological findings should prompt the “atypical” diagnostic category?

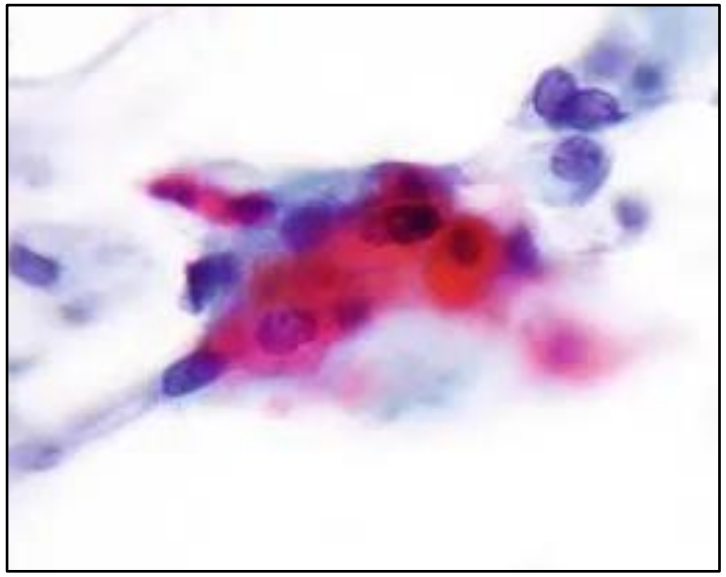
1) “**Atypical** bronchial or other epithelial cells showing **extreme** reactive and reparative cellular changes, which may **mimic malignancy**, occurring in a **setting** of concurrent inflammatory or infective changes, radiation therapy, or chemotherapy.”

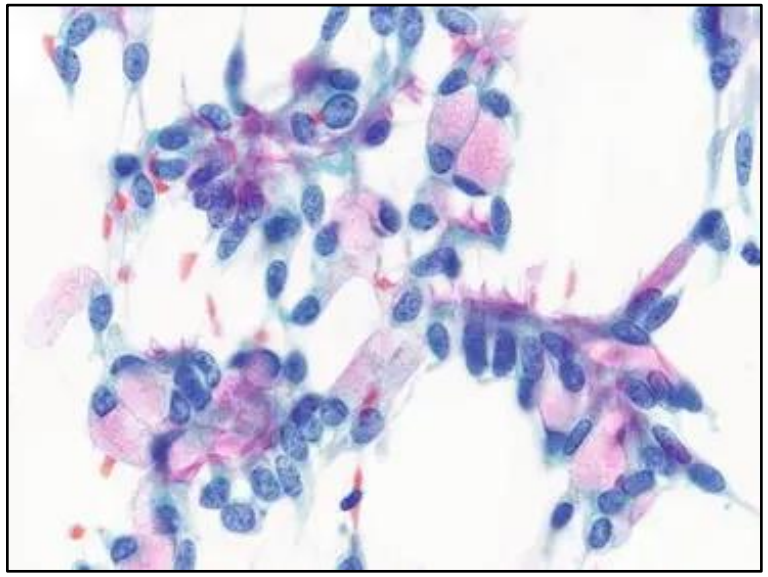




Atypical

What morfological findings should prompt the “atypical” diagnostic category?

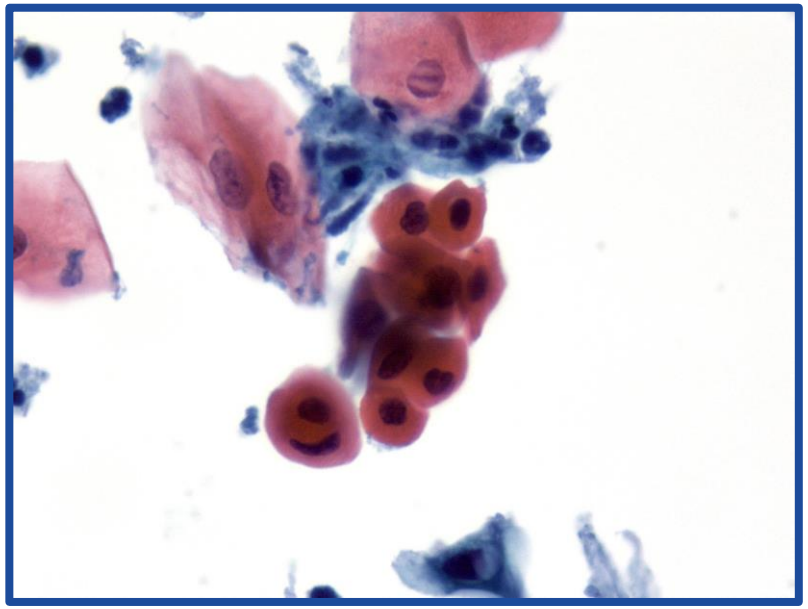




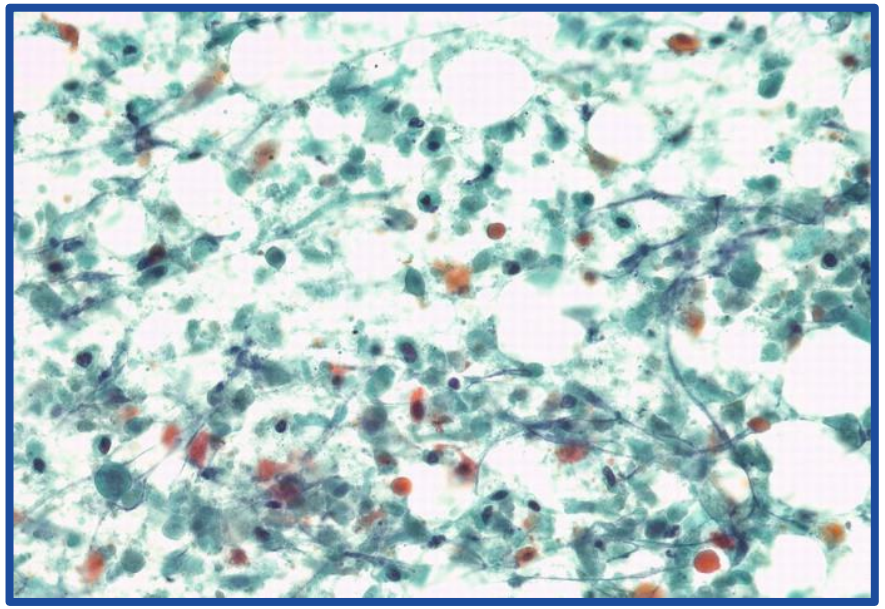
2) “**Squamous** or other **metaplastic** changes that cannot be distinguished from a neoplasm; for example, **goblet cell hyperplasia**, which can mimic mucinous adenocarcinoma.”

Atypical

What morfological findings should prompt the “atypical” diagnostic category?



3) “**Low cellularity** resulting in only scant cells, with **some cells** showing **cytopathological features** usually seen in an epithelial malignancy or a lymphoproliferative disorder.”

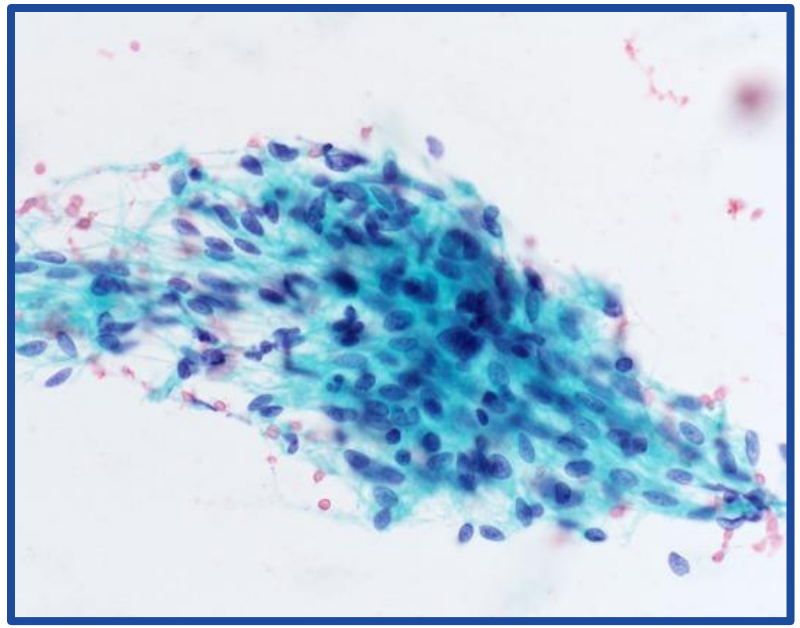


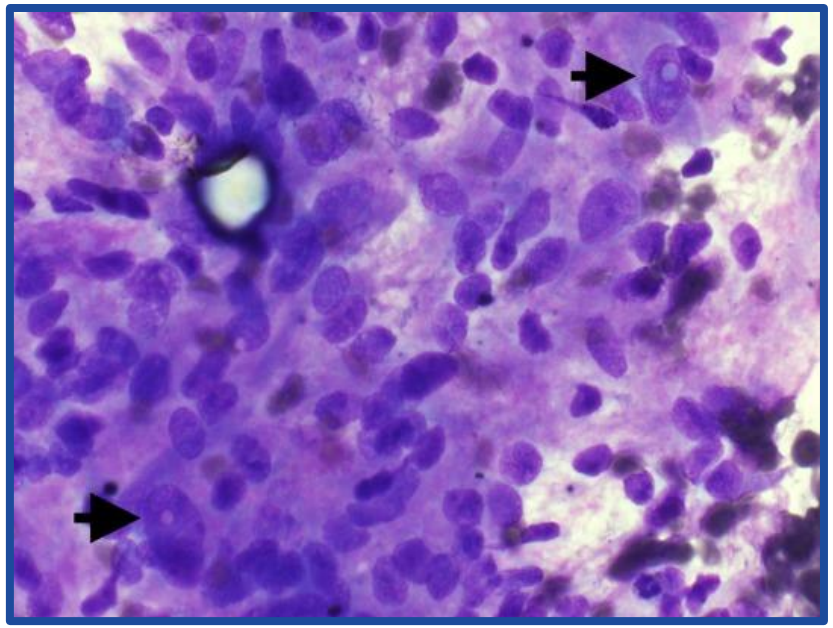
4) “Specimens with **background** elements **suggestive of a neoplasm**, such as necrotic or keratinous debris, thick mucin, or apoptotic cells.”

Atypical

What morfological findings should prompt the “atypical” diagnostic category?

5) “Spindle cell lesions with bland cytopathology that are likely to be initially assessed as “Atypical” until ancillary tests are able to provide a specific diagnosis.”





Categories For The WHO Reporting System for Lung Cytopathology

Suspicious for Malignancy

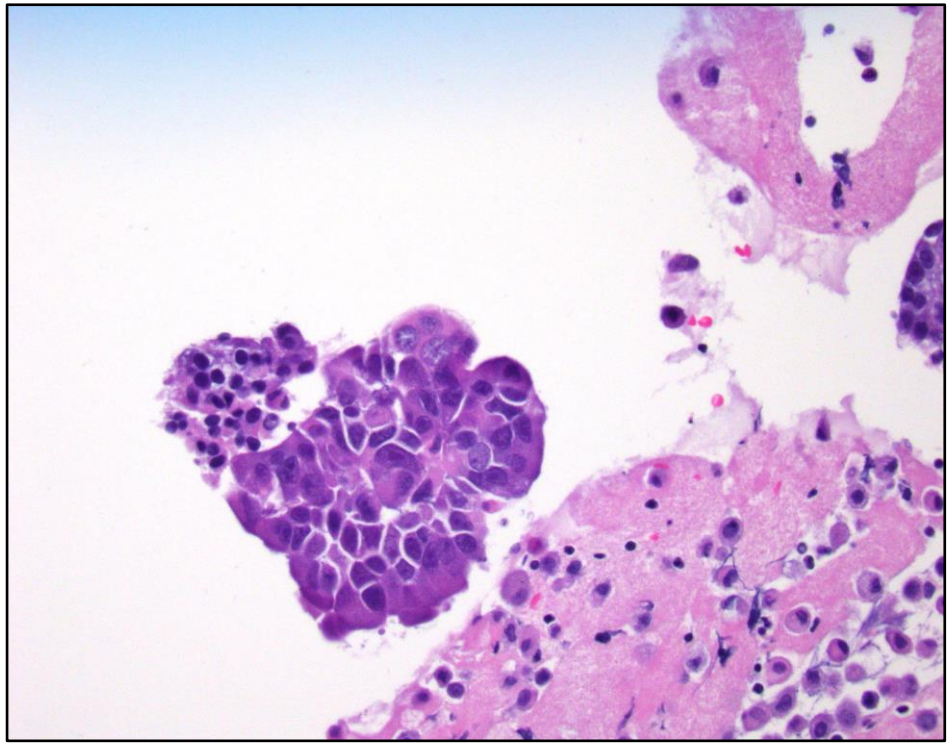
- *This diagnostic category applies to samples that demonstrate some features suggestive of malignancy but insufficient either in number or quality to make an unequivocal diagnosis of malignancy.*

- INCIDENCE: around 5% (Few studies)
- Reported ROM: 75-88% (few studies, different samples)
- MAIN CAUSES: intrinsic characteristics of the tumour (low-grade), extreme reactive atypia.
- MANAGEMENT: Correlate with CLIN-IMG-MICRO and ideally discuss at a MDT meeting. If there is no correlation that lesion is malignant, perform repeat FNAB with ROSE with or without CNB.

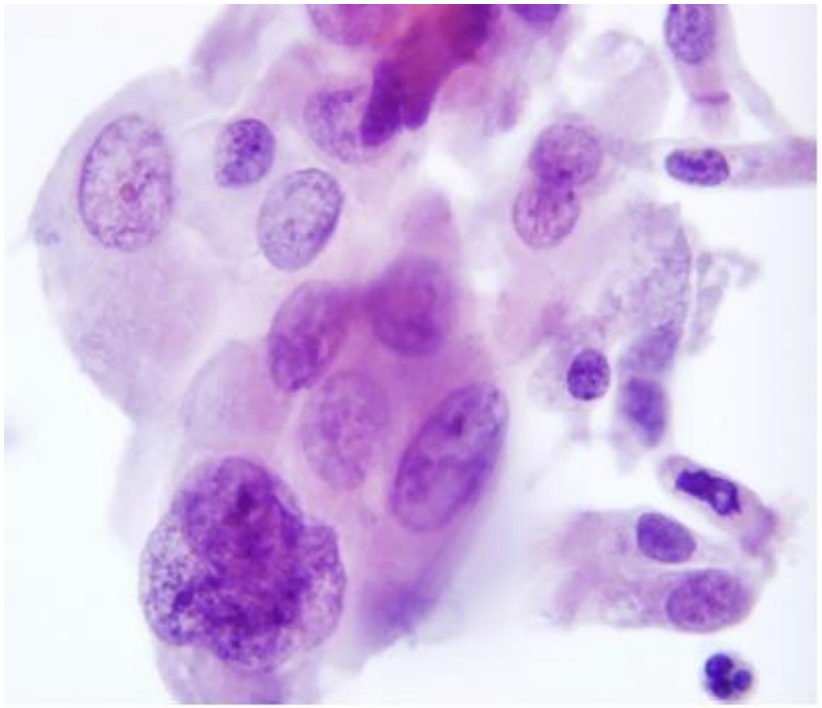
Suspicious for malignancy

What about "suspicious for malignancy"? When should it be used?

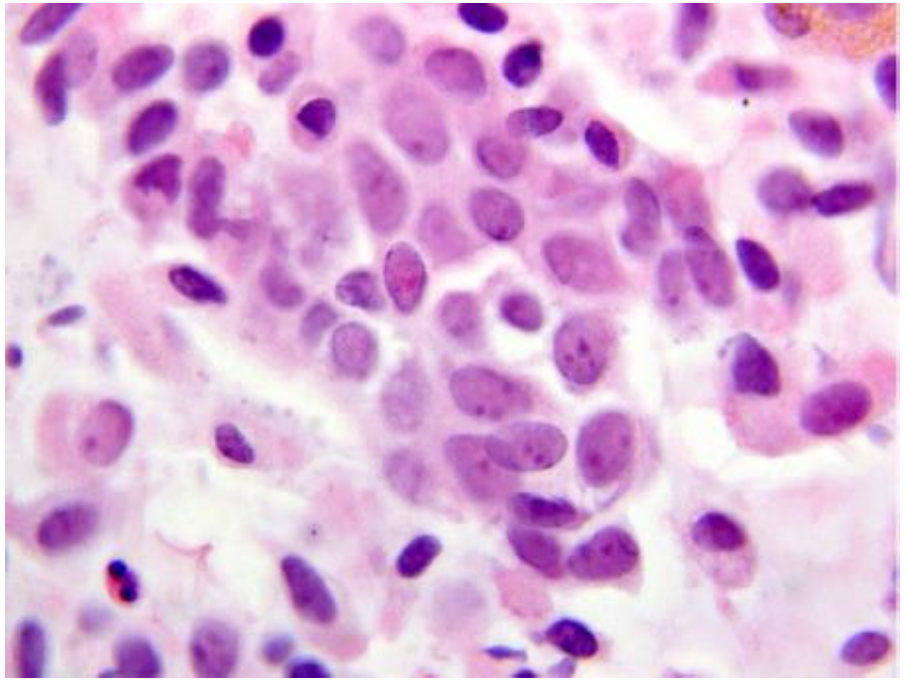
"usually applied in cases where there is **significant cytopathological atypia** (including *nuclear enlargement*; *anisonucleosis*; *irregular nuclear membrane*; *coarse or hyperchromatic chromatin*; and *prominent, often large or irregular nucleoli*) along with **loss of architectural orientation or polarity**, *nuclear crowding* and *moulding*, and *variability in cell size and shape*, but where **there are only a small number of cells** showing these features or the qualitative features are not definitive for a diagnosis of malignancy.



LUNG CYTOLOGY
SFM



Anisonucleosis



Increase N/C ratios

Source: Layefield L, Baloch Z. Diagnostic Cytopathology 2021; 1-8.

A specimen categorized as “Suspicious for malignancy” demonstrates some cytopathological features suggestive of malignancy but has insufficient features either in number or quality to make an unequivocal diagnosis of malignancy

ROM 75–100% for exfoliative samples

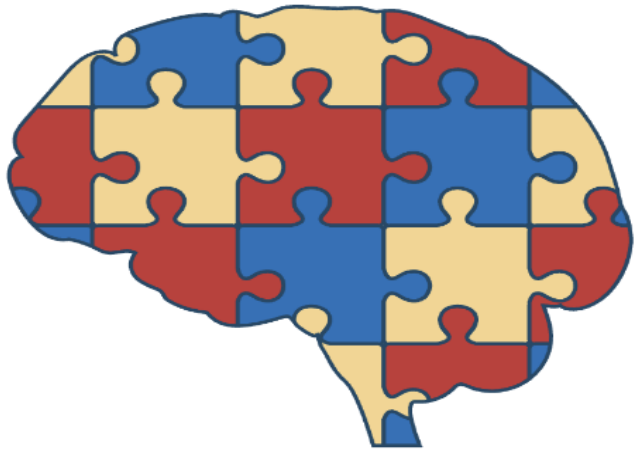
ROM 75–88% for FNAB

Although the ROM is high...

- ***the cytopathological categorization should not be used alone*** as a substitute for a malignant diagnosis, and ***further biopsy*** is recommended !!!

whenever this is correlated with suspicious clinical and imaging findings in ***a multidisciplinary setting***, there may be situations where pulmonary ***multidisciplinary teams*** proceed to specific treatment.

The categorization of a case as “Suspicious for malignancy” is NOT equivalent to a final diagnosis of "malignancy"



- TRIPLE TEST
- TUMOUR BOARD
- SECOND OPINION

Categories For The WHO Reporting System for Lung Cytopathology

Malignant

- *A specimen classified as "Malignant" demonstrates unequivocal cytomorphologic features for malignancy. An attempt should be made to further subclassify the neoplasm based on cytomorphology and, if necessary, by ancillary tests.*

- INCIDENCE: around 20% * (Few studies)
- Reported ROM: 87-100% (few studies, different samples)
- MAIN CAUSES: primary and second malignancies.
- MANAGEMENT: Correlate with CLIN-IMG-MICRO, and ideally discuss at a MDT meeting. If all FOUR support a diagnosis of malignancy, provide definitive treatment. If no correlation that lesion is malignant, consider repeat FNAB with ROSE with or without CNB

ROLE OF THE CYTOPATHOLOGIST IN LUNG CANCER

Diagnosis

- 2nd most frequent cancer and with higher mortality
- 70% is diagnosed in advanced stage: small biopsy/cytology is the main diagnostic tool

Subtyping

- not important only distinguish SCLC and NSCLC
- histological subtype of NSCLC has clinical relevance

Molecular Tests

- Fundamental for tailored therapy

Malignant

8.1: Specific malignant lesions

- 8.1.1: Non-small cell carcinomas
  - 8.1.1.1: Adenocarcinoma of the lung
  - 8.1.1.2: Squamous cell carcinomas
  - 8.1.1.3: Non-small cell carcinoma NOS
- 8.1.2: Other specific carcinomas
  - 8.1.2.1: Salivary gland-type carcinomas
  - 8.1.2.2: Adenosquamous carcinoma
  - 8.1.2.3: Pleomorphic carcinoma
  - 8.1.2.4: Pulmonary blastoma
  - 8.1.2.5: Carcinosarcoma
  - 8.1.2.6: NUT carcinoma
  - 8.1.2.7: Thoracic SMARCA4-deficient undifferentiated tumour

8.2: Neuroendocrine neoplasms

- 8.2.1: Neuroendocrine tumours
  - 8.2.1.1: Carcinoid/neuroendocrine tumours of the lung
- 8.2.2: Neuroendocrine carcinomas
  - 8.2.2.1: Small cell lung carcinoma
  - 8.2.2.2: Large cell neuroendocrine carcinoma

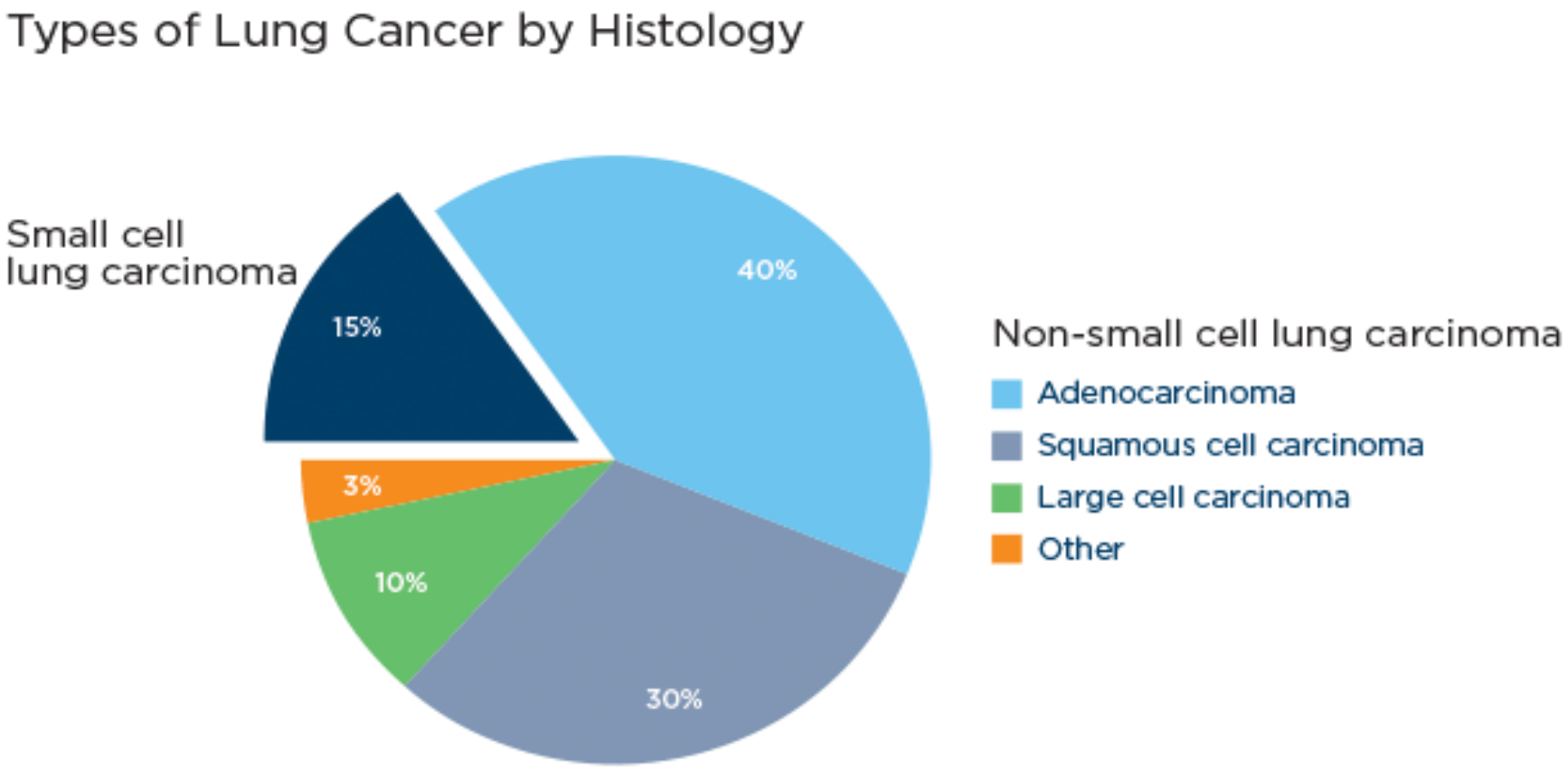
8.3: Lymphoproliferative diseases

- 8.3.0.1: Lymphomas
- 8.3.0.2: Pulmonary Langerhans cell histiocytosis
- 8.3.0.3: Erdheim-Chester disease

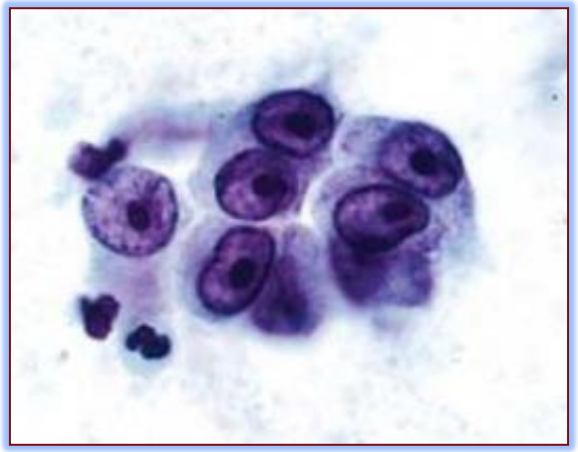
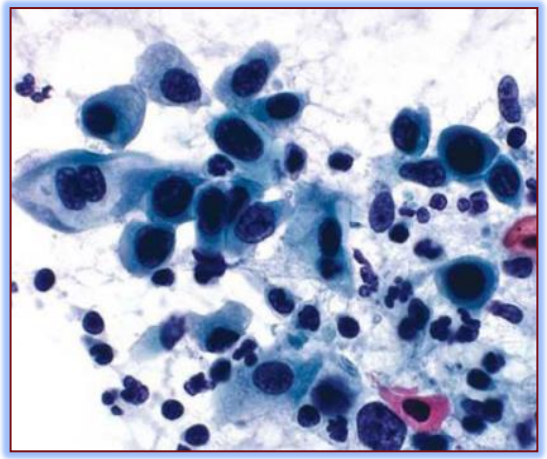
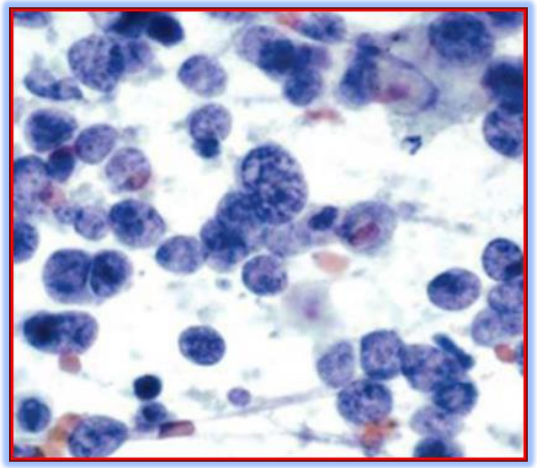
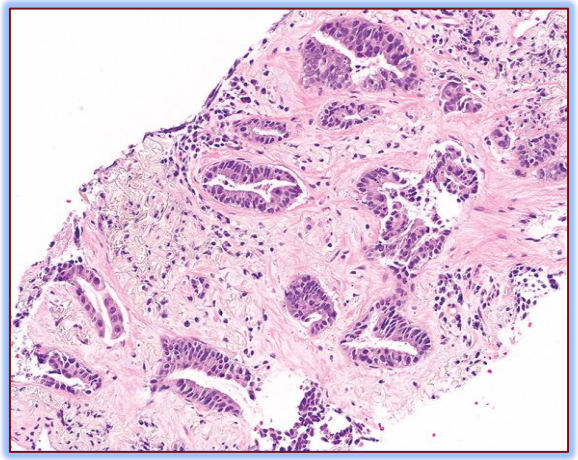
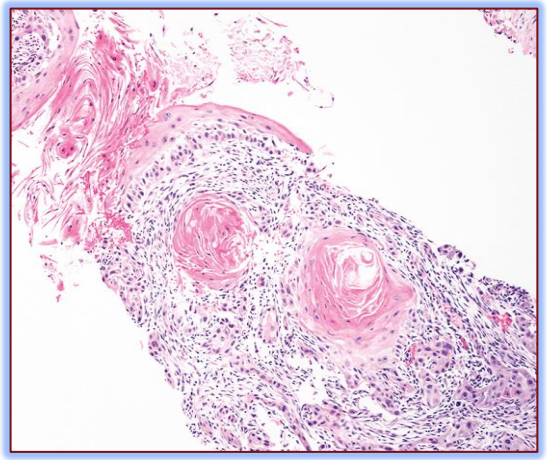
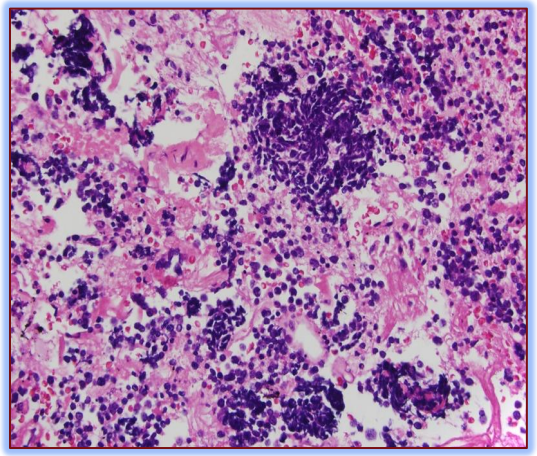
8.4: Other malignancies

- 8.4.0.1: Spindle cell tumours
- 5.2.0.7: Paraganglioma
- 8.4.0.2: Diffuse pleural mesothelioma
- 8.4.0.3: Primary germ cell tumours of the mediastinum
- 8.4.0.4: Pulmonary and thoracic metastases
- 8.4.0.5: Angiosarcoma
- 8.4.1: Sample reports

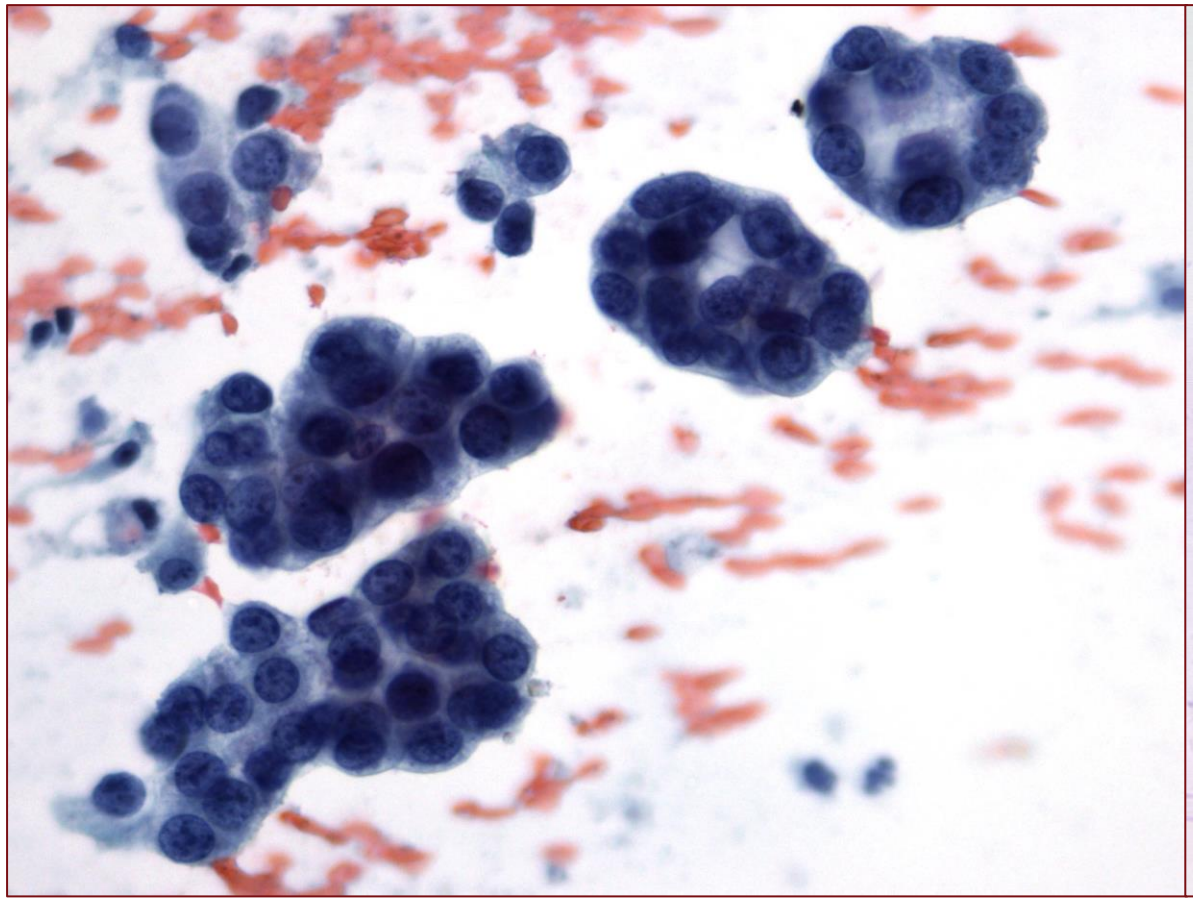
LUNG CANCER
Histological subtypes

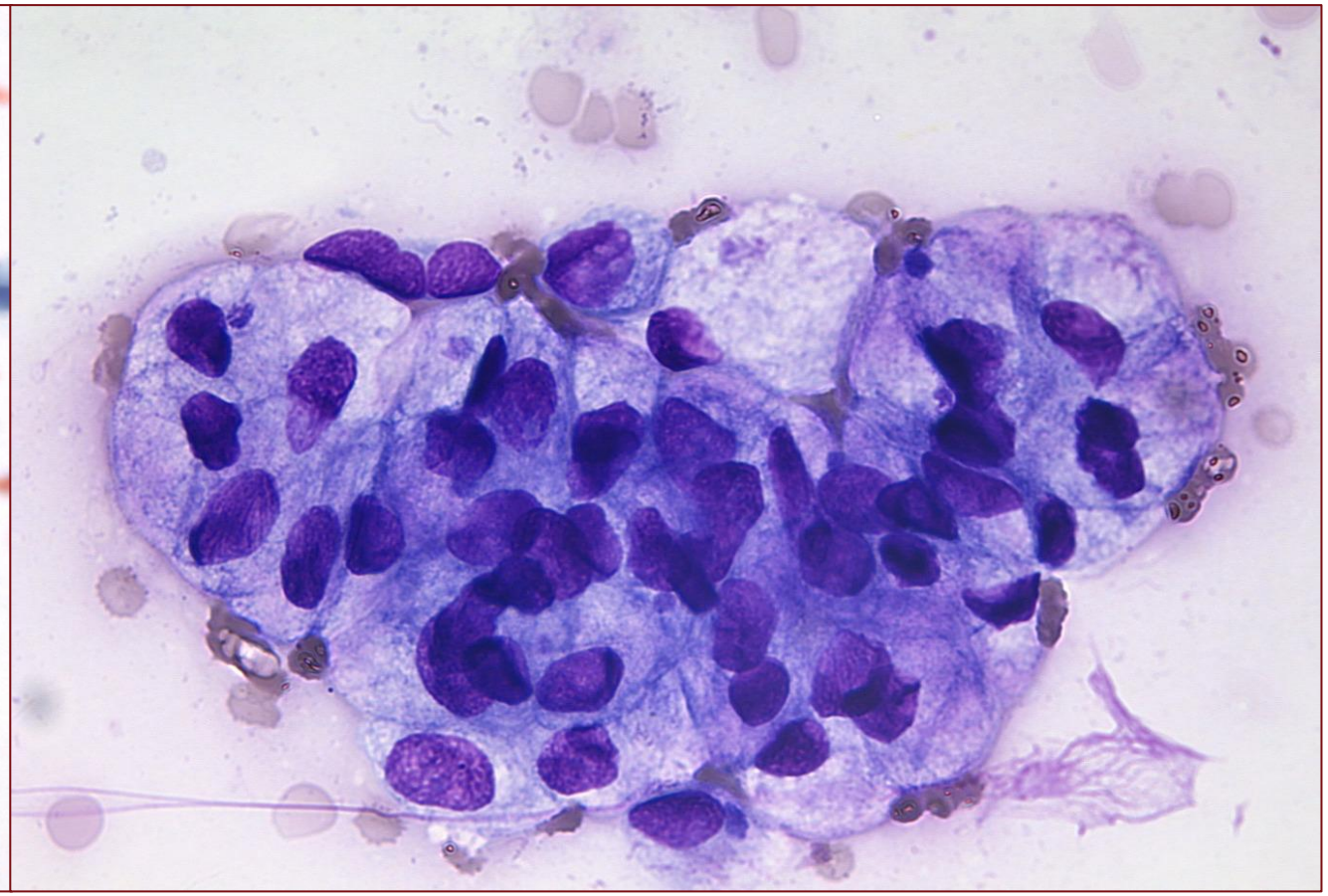


LUNG CANCER
Morphological Aspects

ADENOCARCINOMA	SqCC	SCC
		
		

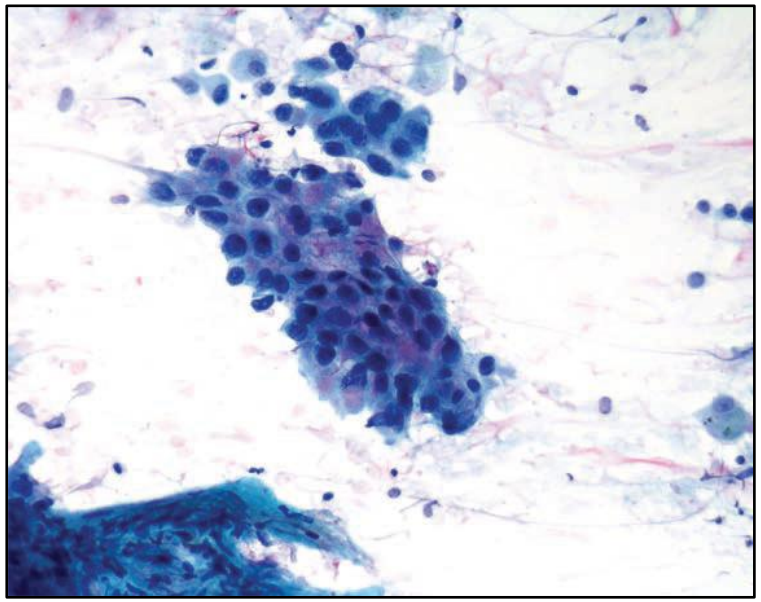
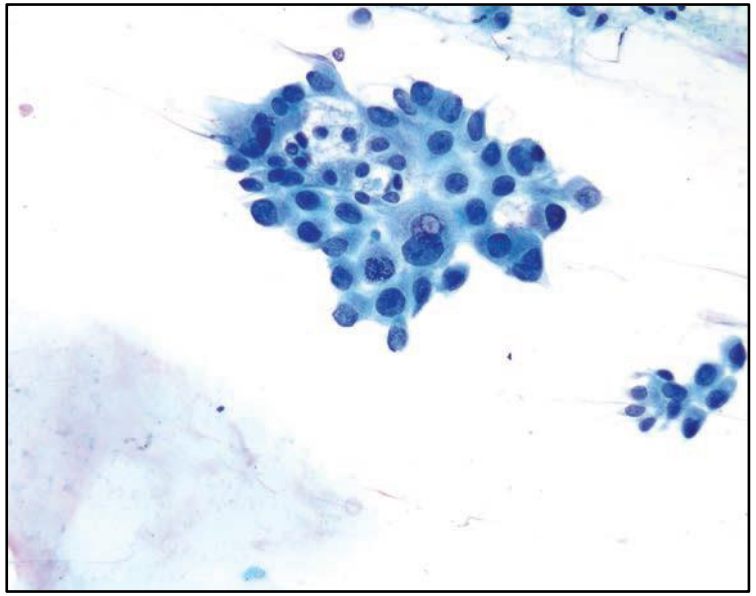
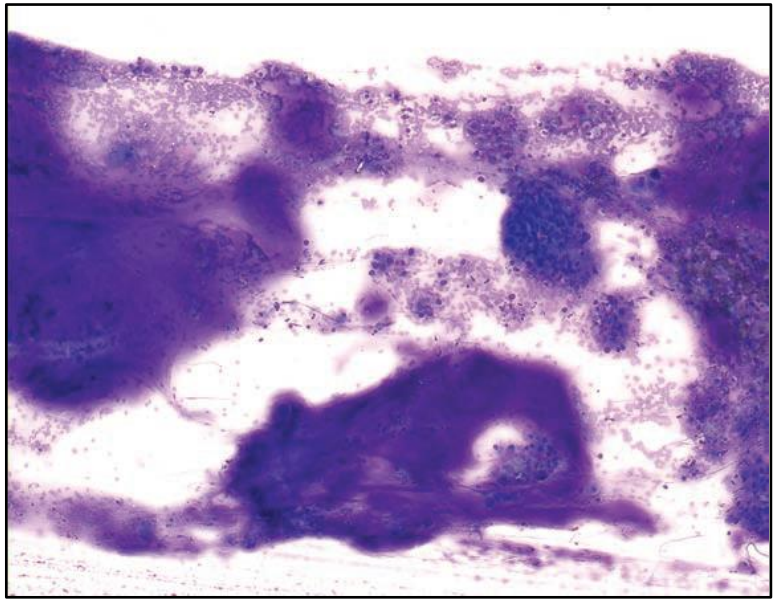
ADENOCARCINOMA





Source: Z Baloch and M Engels, Chapter 8: International System for Reporting Lung Cytopathology, 2022

Malignant - Adenocarcinoma

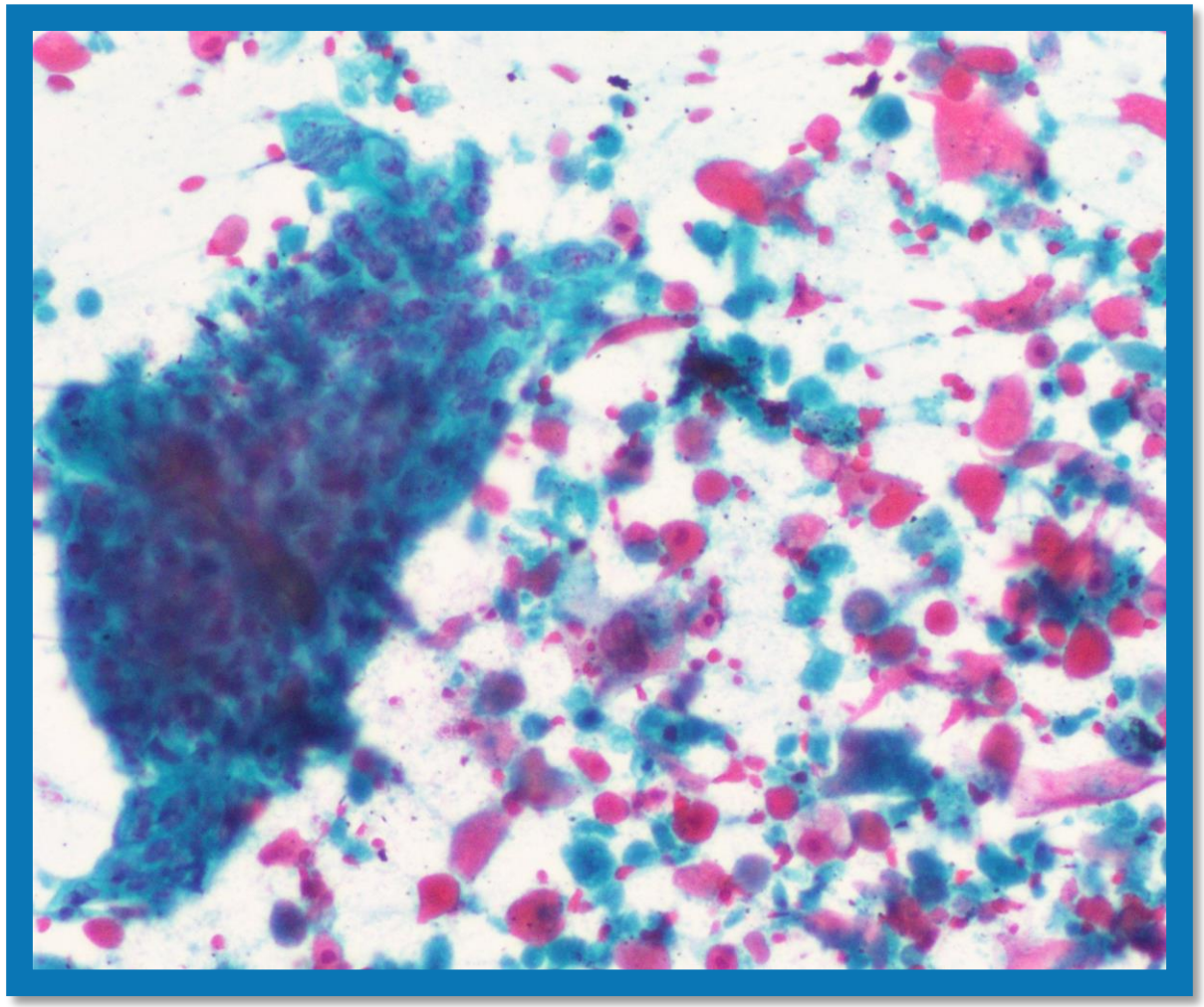
- Mucinous variants of adenocarcinoma can be missed due to its low-grade cytologic features:
- Mitotically inactive malignant cells in a background of mucin;
- Cells can be scattered or arranged in honeycomb-like sheets;
- Can resemble papillary thyroid carcinoma due to optically clear nuclei, inconspicuous nucleoli, grooves, and nuclear pseudoinclusions.

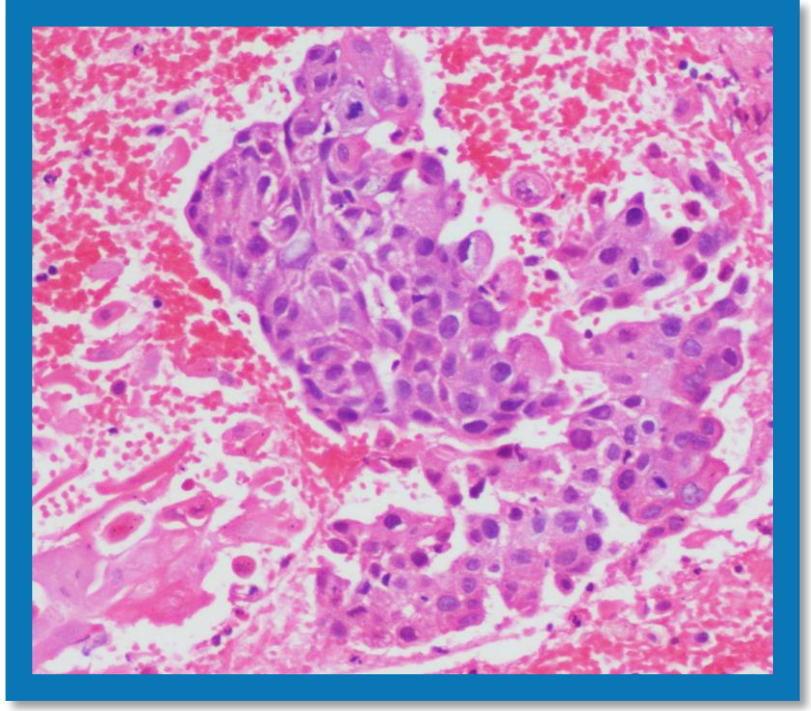
Malignant - Adenocarcinoma

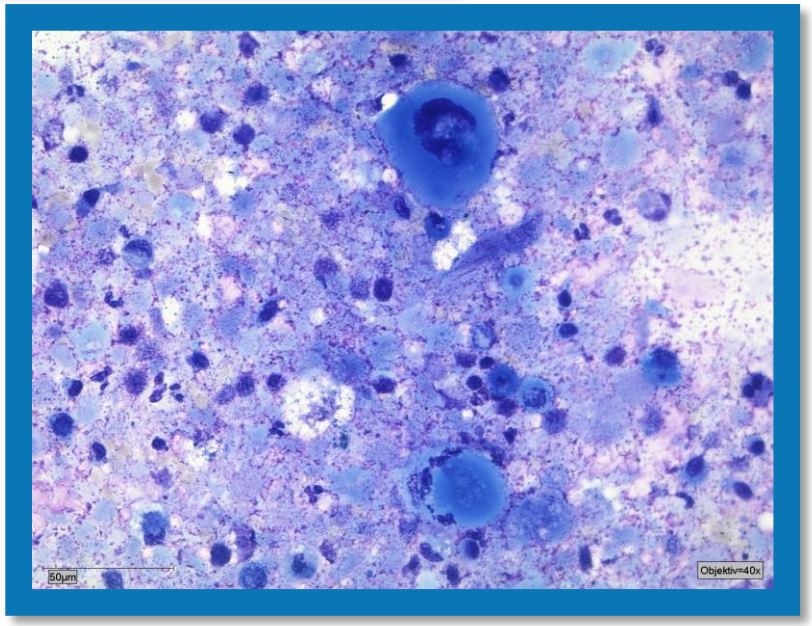
What morfological features distinguish adenocarcinoma from other entities?

Adenocarcinoma vs	Challenging findings	Distinguishing factors
Benign, atypical, and hyperplastic bronchial cells	Reactive atypia within bronchial cells can be misdiagnosed as well- differentiated adenocarcinoma	Presence of cilia favors benign changes continuous spectrum of changes from obviously benign to atypical, i.e., lack of distinct two cell population
Florid type Il pneumocyte hyperplasia	Hyperplastic type Il pneumocytes can yield cellular specimens with worrisome, atypical features and TTF- 1 positivity	Consider pneumocyte hyperplasia if the clinical history and imaging findings are suggestive of lung injury/respiratory distress
Benign mesothelial cells	Can resemble well-differentiated adenocarcinoma and mucinous adenocarcinoma	Mesothelial cells are seen as sheets of uniform cells with round nuclei showing characteristic slit-like windows separating the cells
Pulmonary hamartoma	Prominent glandular component of hamartomas can mimic well/moderately differentiated adenocarcinoma	Presence of chondromyxoid matrix points towards a diagnosis of hamartoma
Squamous cell carcinoma	Both are common primary lung nonsmall cell carcinomas with morphological overlap that can cause considerable diagnostic difficulty	Fine texture of chromatin, thin delicate cytoplasm, intracytoplasmic vacuolation, and abundant intracelular mucin favors adenocarcinoma (note that squamous carcinoma can also show cytoplasmic mucin)

SQUAMOUS CELL CARCINOMA





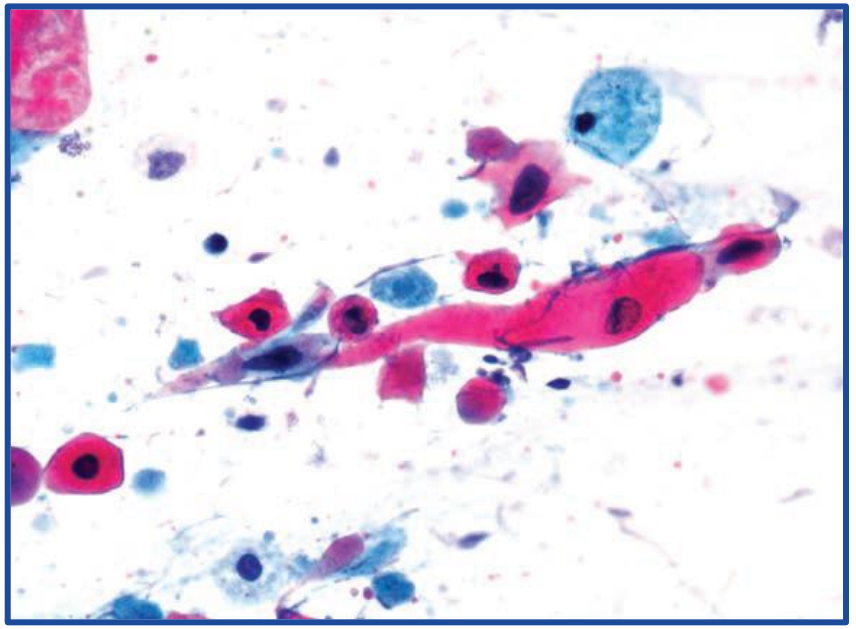


Source: D Jain and M Engels, Chapter 8: International System for Reporting Lung Cytopathology, 2022

Malignant – Squamous cell carcinoma

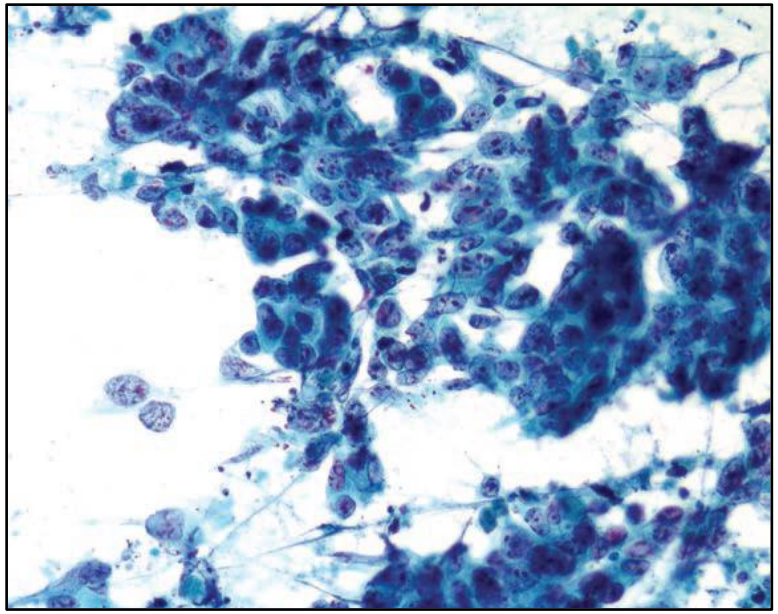
Cytomorphologic features **vary** depending on the degree of **squamous differentiation** within the tumor:

Distinguishing features	Well-differentiated Scc
Cytology	• Cell clusters are discohesive and show polygonal, round, spindle, and tadpole-shaped cells • Frequent anucleate cells are seen
Cytoplasm	• Abundant, smooth, dense 'squamoid' cytoplasm filled with keratin • Stains green, yellow, or densely orange with pap stains and blue with Romanowski stains
Nuclei	• Small, hyperchromatic, smudgy pyknotic-looking nuclei
Nucleoli	• inconspicuous



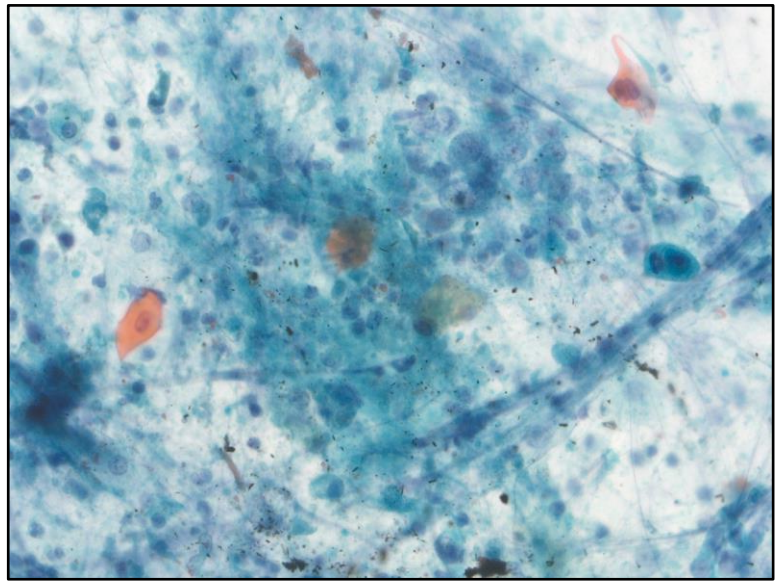
Malignant – Squamous cell carcinoma

•**Cytomorphologic** features **vary** depending on the degree of **squamous differentiation** within the tumor:

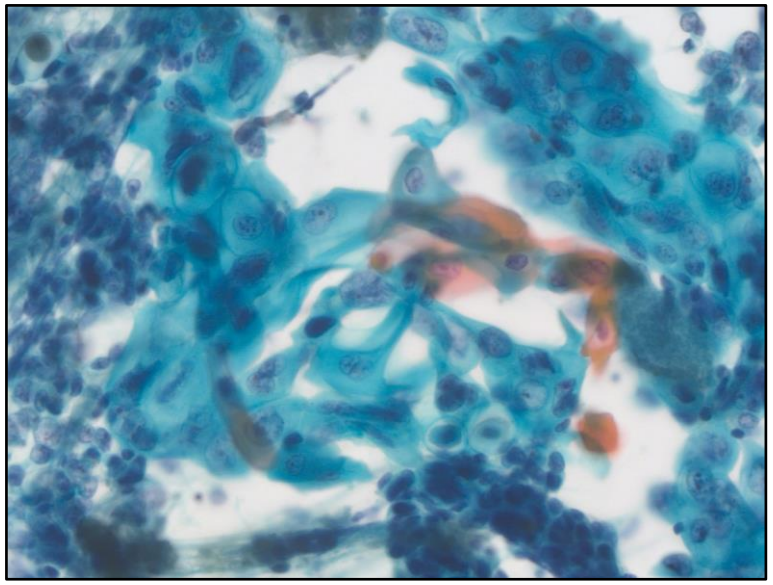


Distinguishing features	Moderately/poorly-differentiated Scc
Cytology	• Larger, denser, and more cohesive clusters of spindle cells that exhibit 'school of fish' appearance (i.e. tumor cell nuclei streaming in parallel along their long axes)
Cytoplasm	• Can range from smooth and dense (similar to well-differentiated squamous cell carcinoma) to granular • Keratinization is much less prominent and can be difficult to appreciate
Nuclei	• Larger nuclei with coarsely textured chromatin
Nucleoli	• Prominent

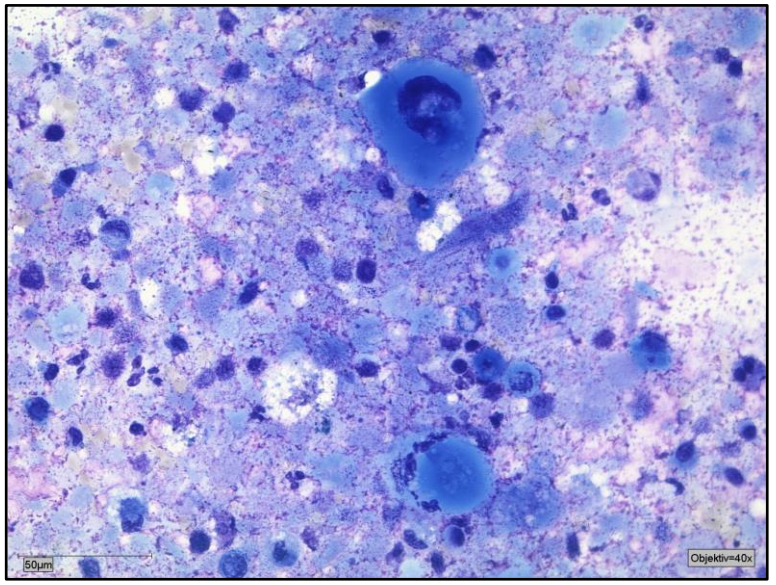
Malignant – Squamous cell carcinoma



Necrotic debris



Squamous morules



Anucleate keratinized ghost cell

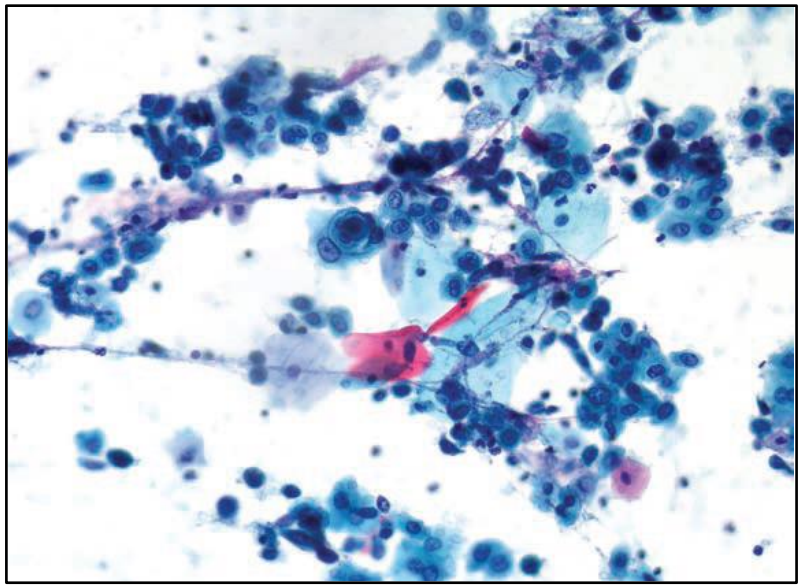
Malignant – Squamous cell carcinoma

What morfological features distinguish squamous cell carcinoma from other entities?

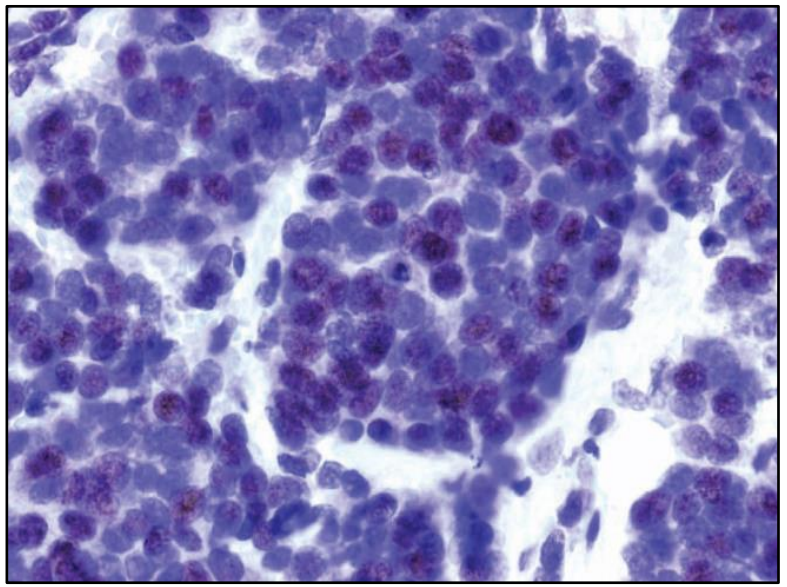
Squamous cell carcinoma vs	Distinguishing factors
Squamous metaplasia	Metaplastic cells have small, round nuclei without hyperchromasia.
Degenerative changes	Even though degenerative cells have dark pyknotic nuclei, they are generally of smaller size and show much less anisonucleosis than scc.
Reactive squamous atypia	Consider reactive squamous atypia in the differential when sampling is adjacent to a site of lung injury (e.g., stoma, cavitary fungal infection) or in the setting of sepsis, chemo/radiation therapy, alveolar damage, or any lung injury).
Adenocarcinoma	Fine texture of chromatin, thin delicate cytoplasm, intracytoplasmic vacuolation, and abundant intracelular mucin favors adenocarcinoma (note that squamous carcinoma can also show cytoplasmic mucin).
NUT midline carcinoma	Cytology of NUT midline carcinoma shows dispersed primitive-appearing, monomorphic, non-cohesive cells with vesicular chromatin, scant cytoplasm +/- prominent nucleoli.

Malignant – Squamous cell carcinoma

What morfological features distinguish squamous cell carcinoma from other entities?



Squamous cell metaplasia



NUT carcinoma

Differential Diagnosis Adeno vs SqCC

Adenocarcinoma

- ✓ Sheets, papillary structures
- ✓ Acini
- ✓ Eccentric, round/oval nuclei
- ✓ Granular chromatin
- ✓ Large nucleoli
- ✓ Pale cytoplasm
- ✓ Indistinct cytoplasmic borders
- ✓ Mucin secretion

Squamous Cell Carcinoma

- ✓ Clusters, streaming, whorling
- ✓ Cell in cell pattern
- ✓ Central, oval/ elongated nuclei
- ✓ Course-dense chromatin
- ✓ Small nucleoli
- ✓ Dense cytoplasm
- ✓ Distinct cytoplasmic borders
- ✓ Keratinized cells

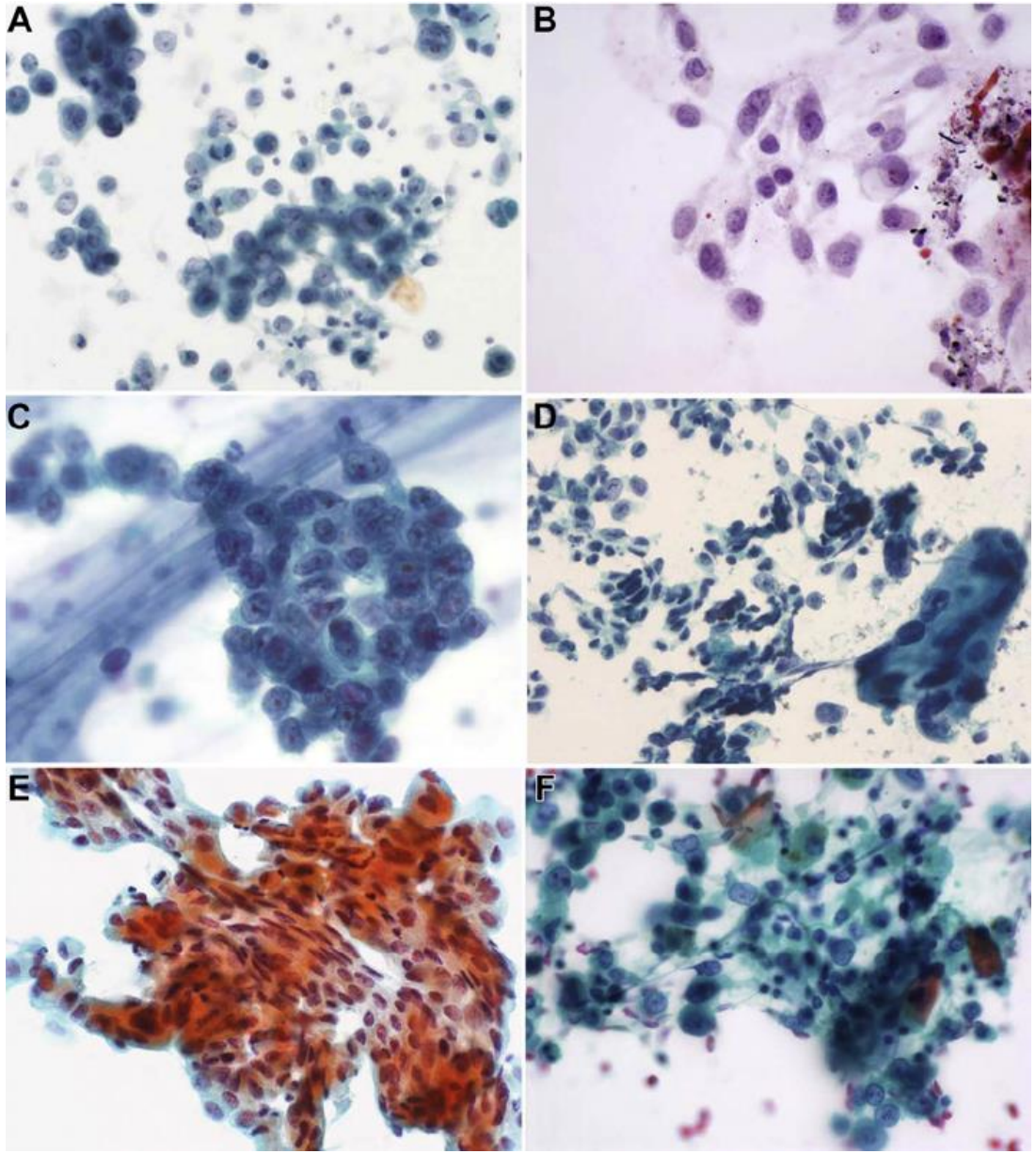
NSCLC Subtyping in Conventional Cytology: Results of the International Association for the Study of Lung Cancer Cytology Working Group Survey to Determine Specific Cytomorphologic Criteria for Adenocarcinoma and Squamous Cell Carcinoma

Deepali Jain, MD, FIAC,[a,*] Aruna Nambirajan, MD,[a] Gang Chen, MD,[b] Kim Geisinger, MD,[c] Kenzo Hiroshima, MD, PhD,[d] Lester Layfield, MD,[e] Yuko Minami, MD, PhD,[f] Andre L. Moreira, MD,[g] Noriko Motoi, MD, PhD,[h,i] Mauro Papotti, MD,[j] Natasha Rekhtman, MD, PhD,[k] Prudence A. Russell, FRCPA Spasenija Savic Prince, MD,[m] Fernando Schmitt, MD, PhD, FIAC,[n] Yasushi Yatabe, MD, PhD,[h] Serenella Eppenberger-Castori, PhD,[m] Lukas Bubendorf, MD,[m] for the IASLC Pathology Committee

Common Cytologic Features Observed in Concordant, Misclassified and Failure to Classify Responses in Gold Standard LUAD and LUSC by 13 Expert Cytopathologists

Jain D et al. JTO doi.org/10.1016/j.jtho.2022.02.013

Categorisation of responses (n=1487)	All	Gold standard LUAD		Gold standard LUSC	
		Category	Common cytological features	Category	Common cytological features
Concordant	53% (792/1487)	58% (580/992)	Vesicular nuclei with open chromatin (58%) Prominent nucleoli (56%) Delicate/translucent cytoplasm (50%)	43% (212/495)	Hyperchromatic nuclei (79%) Dense opaque cytoplasm (75%) Keratinisation (70%)
Misclassification	10% (152/1487)	5.5% (55/992)	Dense (opaque) cytoplasm (80%) Hyperchromatic nuclei (71%) Tadpole or spindle cell morphology without keratinisation (56%)	20% (97/495)	Vesicular nuclei with open chromatin (48%) Rounded 3D cell clusters (43%) Prominent nucleoli (42%) Acinus formation (42%)
Failure to classify	37% (543/1487)	36% (357/992)	Prominent nucleoli (47%) Translucent cytoplasm (31%) Hyperchromatic nuclei (31%)	37.5% (186/495)	Hyperchromatic nuclei (43%) Dense cytoplasm (40%) Prominent nuclei (34%)



Cytologic features of frequently misclassified NSCLC:

- LUSC misclassified as LUAD (A-C)
- LUAD misclassified as LUSC (D-F)

Jain D et al. JTO doi.org/10.1016/j.jtho.2022.02.013

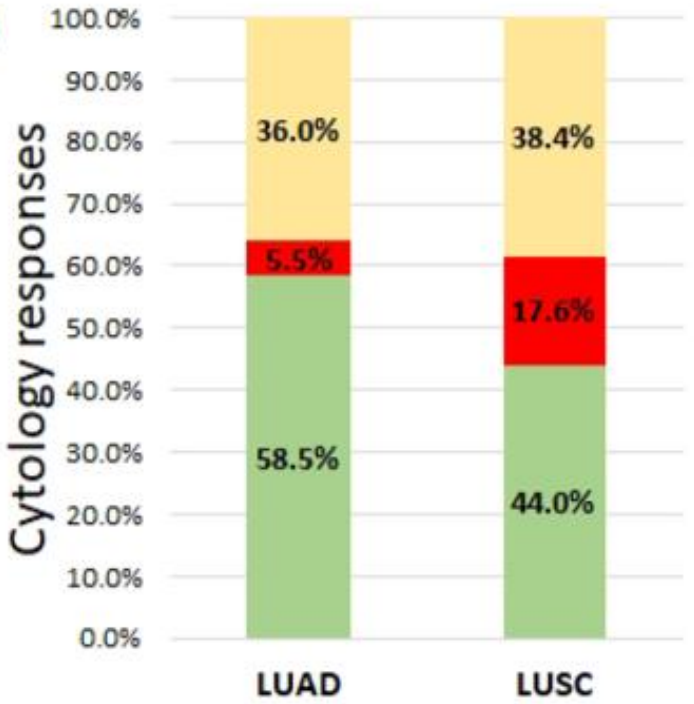
IASLC Cytology Working Group survey to determine specific cytomorphological criteria for adenocarcinoma and squamous cell carcinoma

A

Gold standard subtype	Cytology subtype			Total responses
	LUAD	LUSC	NSSC-NOS	
LUAD (n=80)	**580**	**55**	**357**	992
LUSC (n=39)	**85**	**212**	**185**	482
Total responses	665	267	542	1474

Concordant Misclassification Failure to classify

B



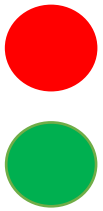
Jain D et al. JTO doi.org/10.1016/j.jtho.2022.02.013

Markers for subtyping

Table 2
Common immunocytochemistry markers used in lung cytology

	Markers					
Lung Cancer Subtype	TTF-1	Napsin A	P63	P40	CK5/6	Neuroendocrine Markers
ADC	+/–[a]	+	–/+	–	–	–
SqCC	–	–	+	+	+	–
SCLC	+	–	–	–	–	+
NSCLC-NOS	+/–	–	–	–	–	–
Metastatic carcinoma	–/+	–	–/+	–/+	–/+	–/+

[a] Especially mucinous ADC.



nuclei

cytoplasm

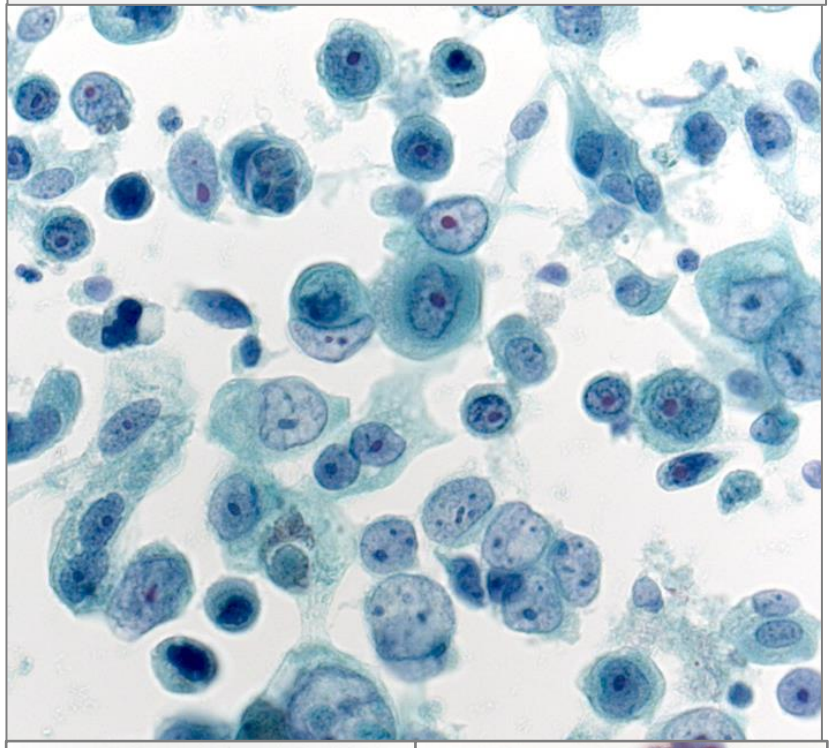
TTF1 clone: 8G7G3/1
Specific but less sensitive

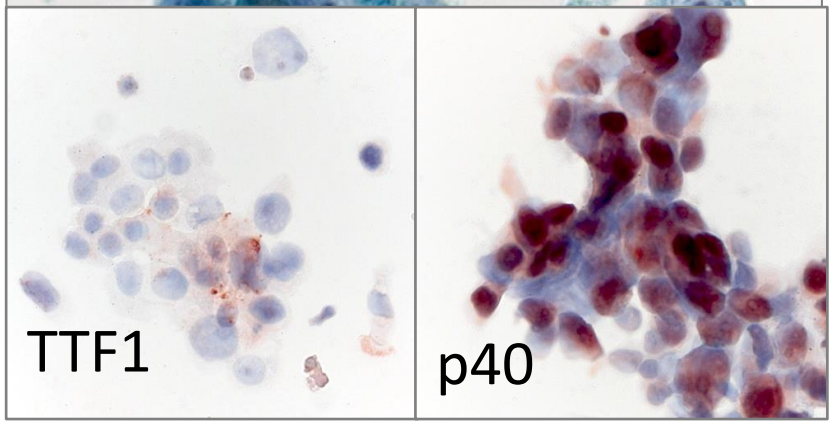
Ancillary Studies, Including Immunohistochemistry and Molecular Studies, in Lung Cytology

Fernando Schmitt, MD, PhD, FIAC[a,b,*], José Carlos Machado, PhD[b]

SqCC

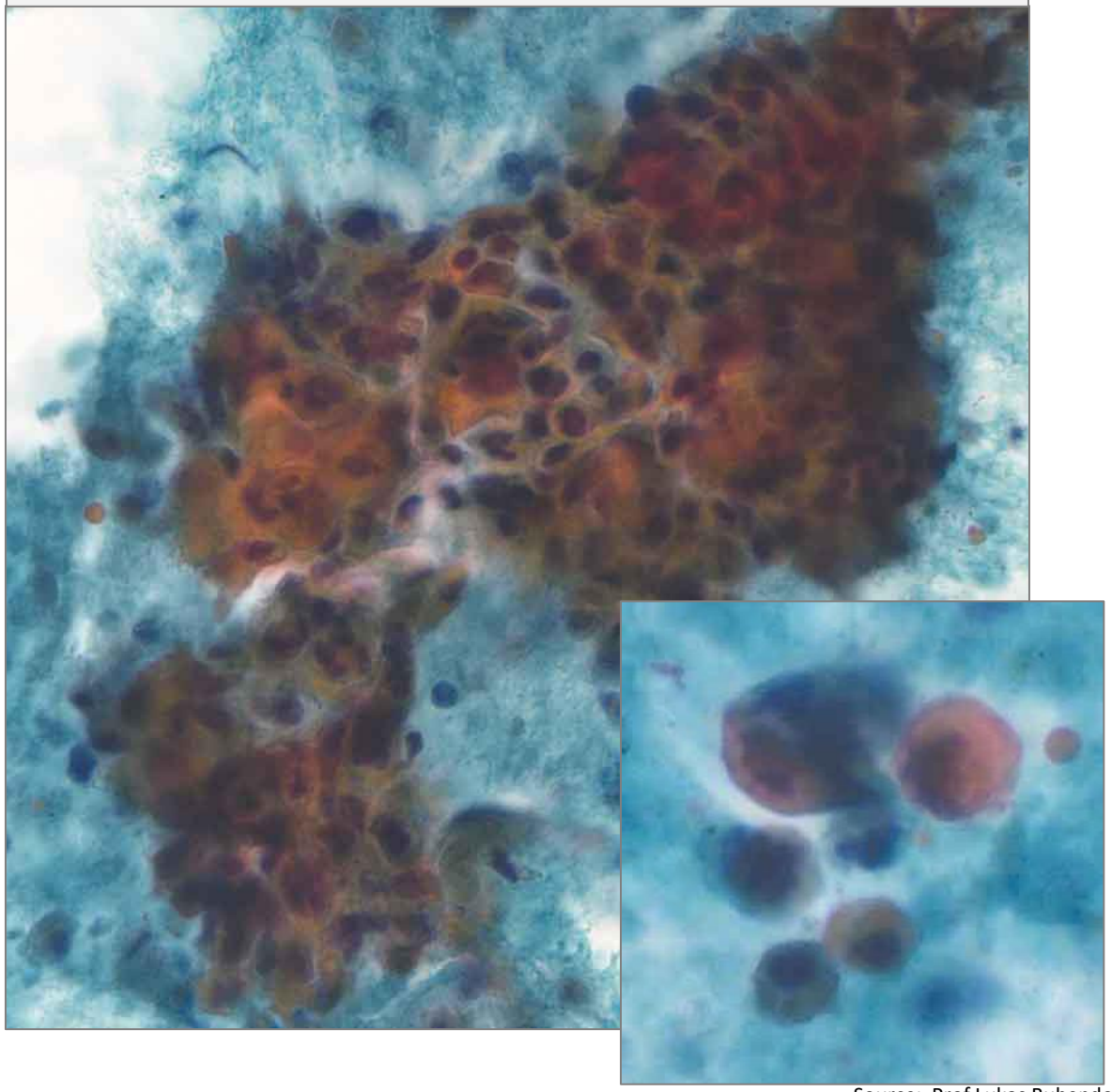
(with cytological AC features)



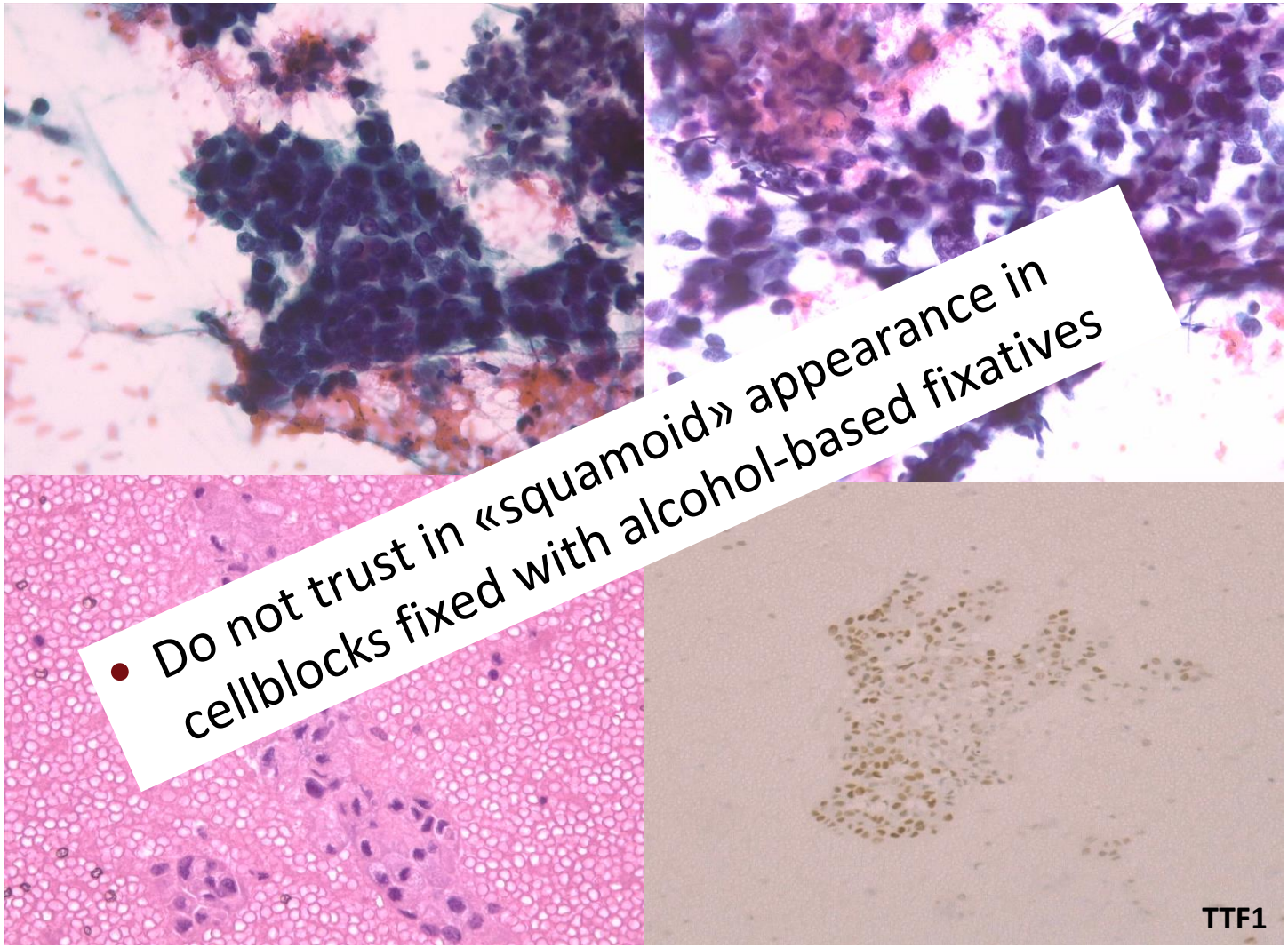


Solid AC (TTF1+)

(with necrosis/degen. eosinophilia)

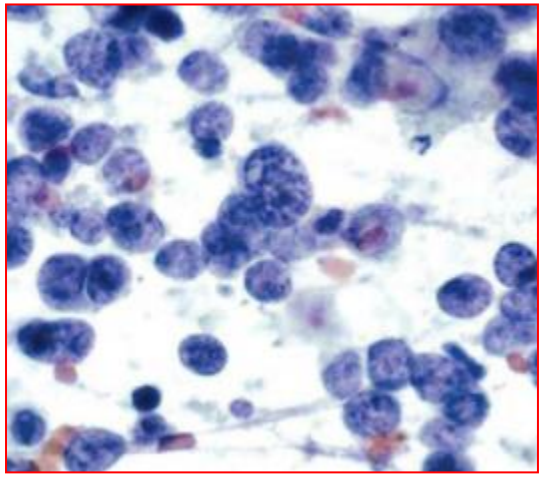


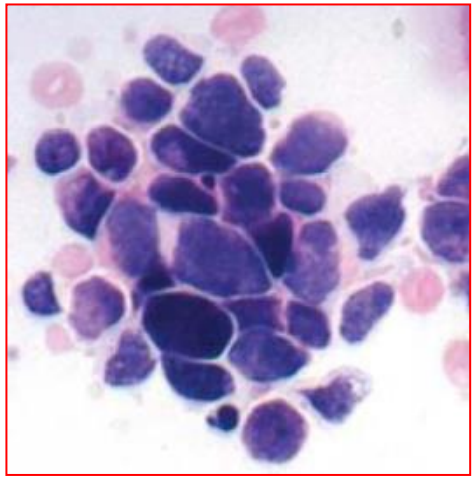
Source: Prof Lukas Bubendorf

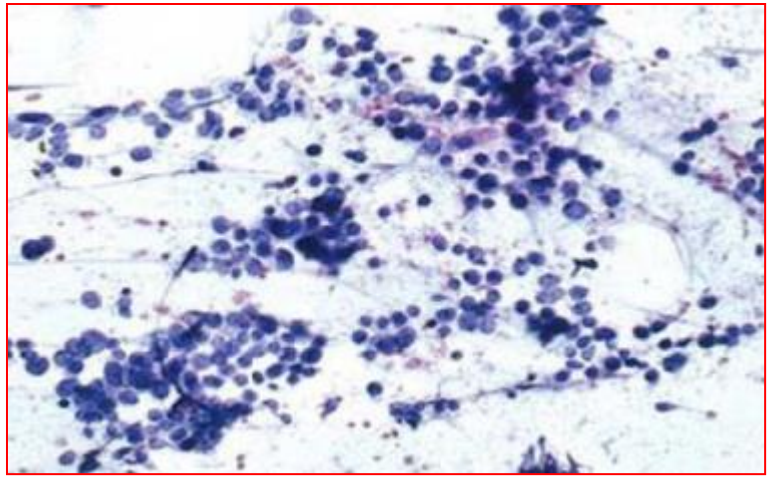

• Do not trust in «squamoid» appearance in cellblocks fixed with alcohol-based fixatives
TTF1

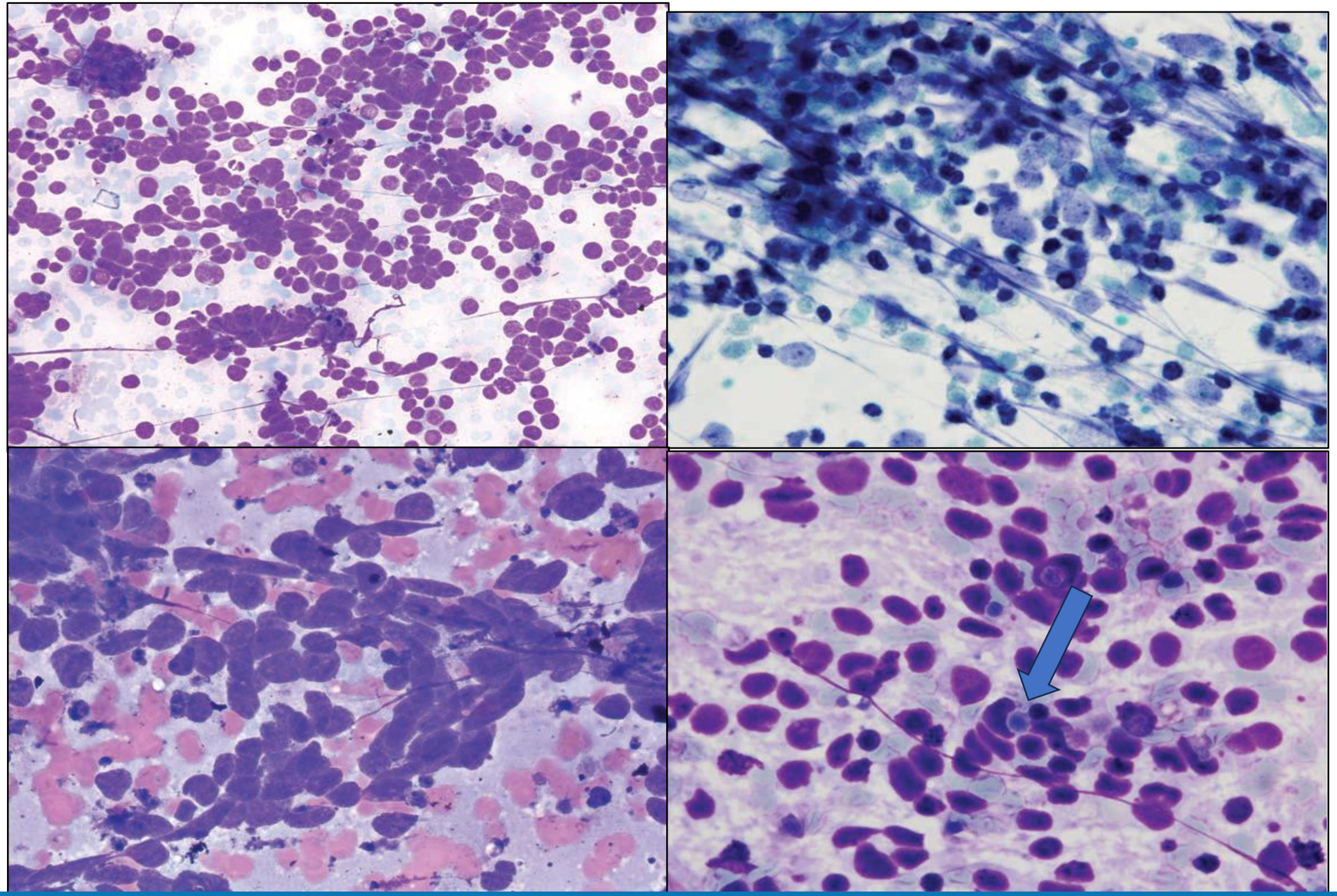
LUNG CYTOLOGY
Morphological Aspects

- **SMALL CELL CARCINOMA**
  - Small cells in loose clusters with single cells
  - Molding
  - No or small nucleoli
  - Salt and pepper chromatin
  - Mitosis
  - Necrosis



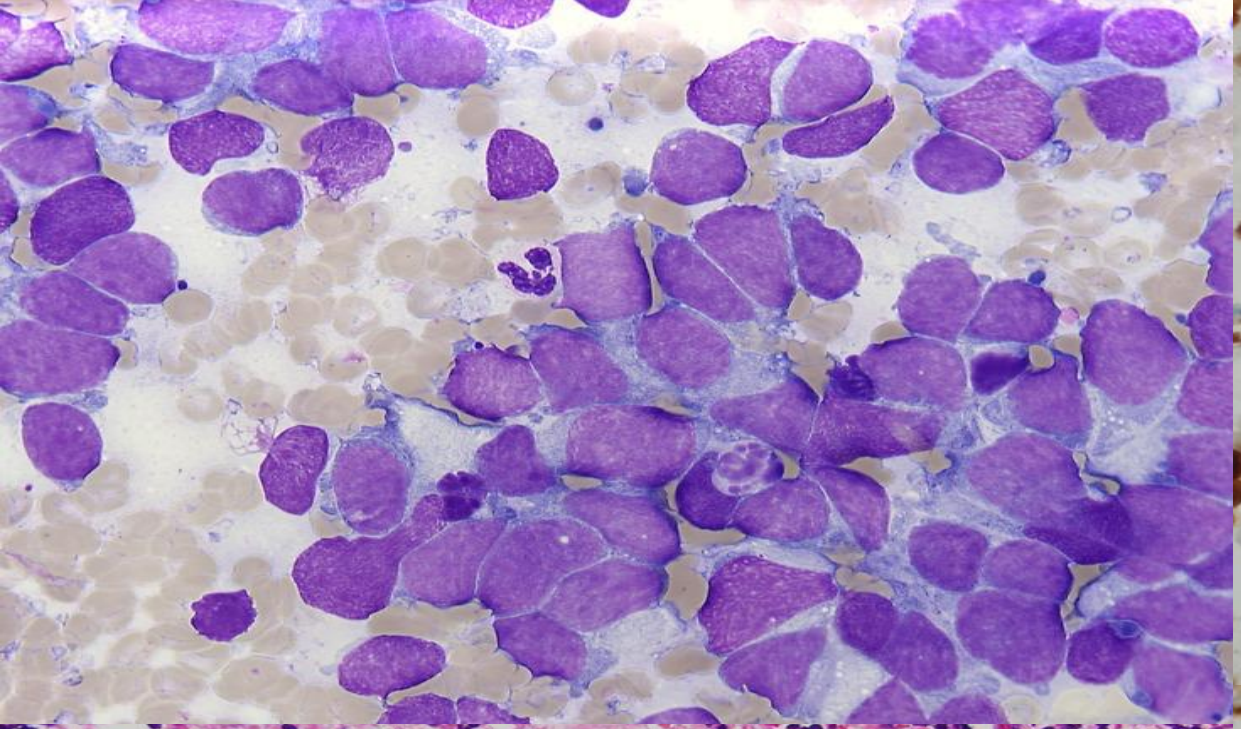


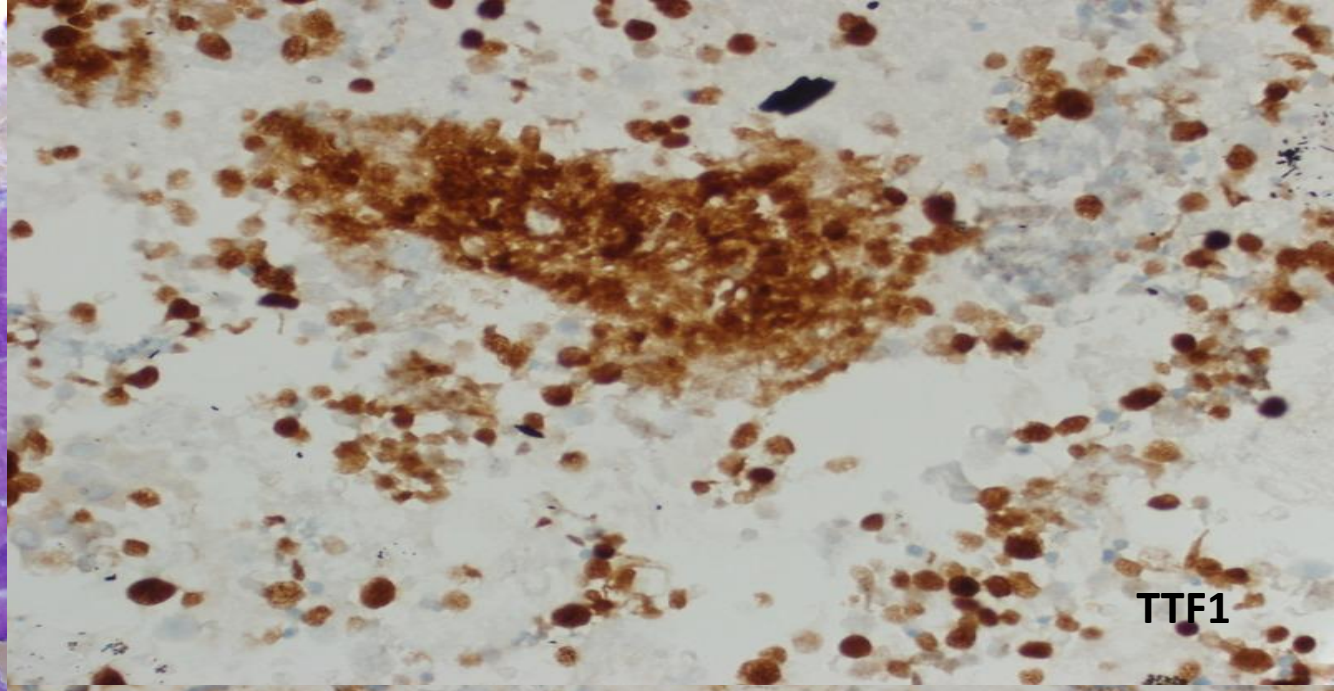


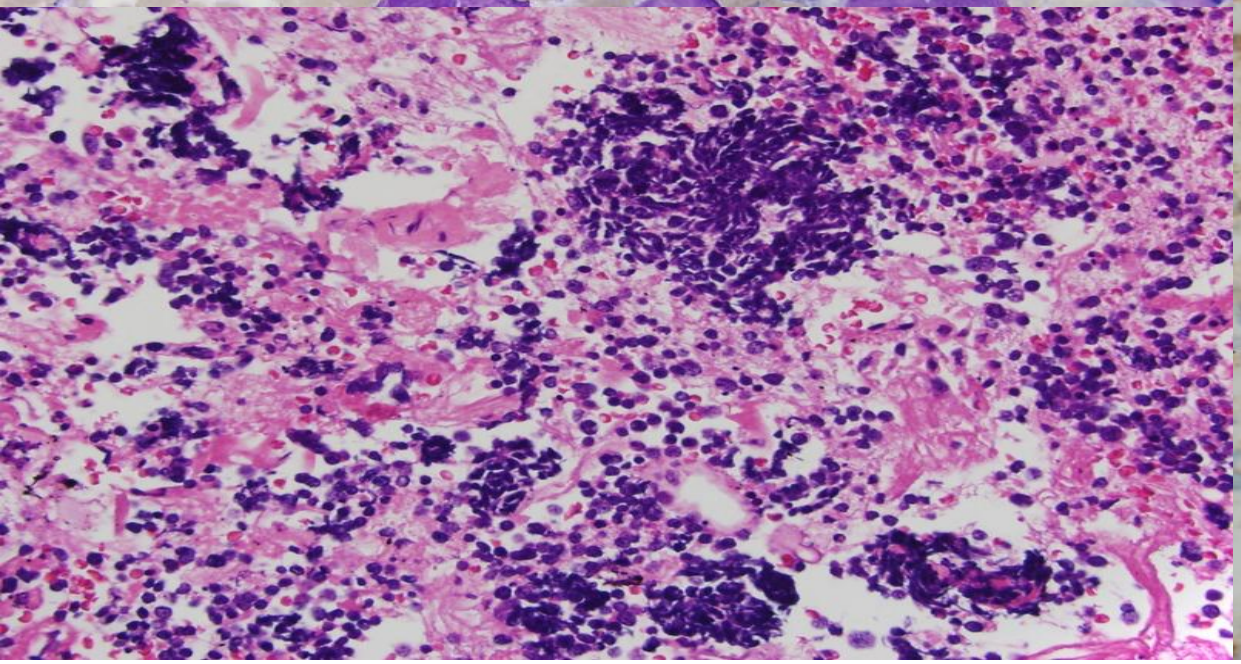


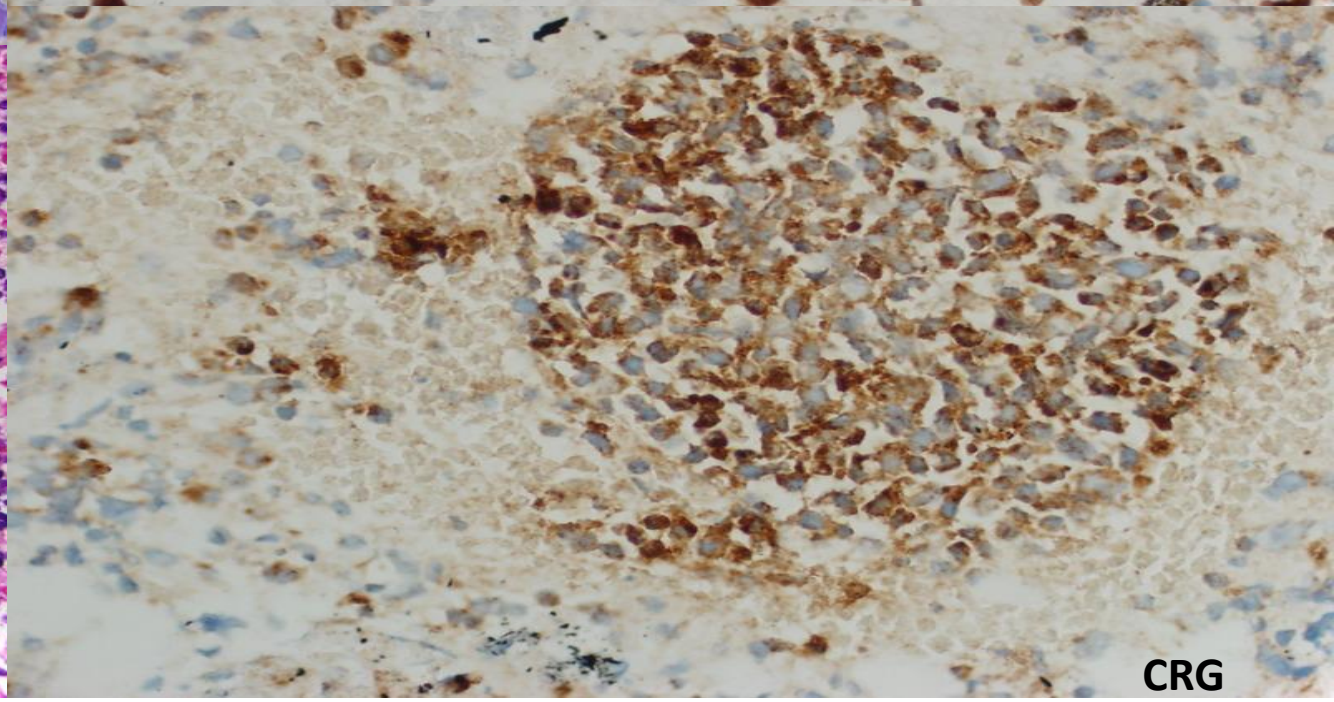
Malignant – Small cell carcinoma

Small cell carcinoma vs	Similarities	Differences
Reserve cell hyperplasia	• Small cells that show molding	• RCH cells are smaller and tend to cluster • RCH does not show necrosis or mitoses • RCH cells have smudged, featureless chromatin whereas small cell carcinoma cells have finely textured chromatin
Lymphoid cells	• Discohesive cells with scant cytoplasm	• Lymphoid cells are smaller (one half to one third the size of small cell carcinoma cells) • Lymphoid cells are evenly spaced rather than molded
Typical carcinoid tumor	• Some typical carcinoid tumors can bear slight resemblance to small cell carcinoma (e.g., spindle cell variant)	• Typical carcinoids lack molding, necrosis, high • mitotic activity
Atypical carcinoid tumor	• Medium sized cells with scant cytoplasm • Both tumors can display nuclear molding, necrosis and nuclear pleomorphism	• Small cell carcinoma cells tend to be dispersed, with finely granular chromatin unlike atypical carcinoids which form clusters/rosettes and have coarsely granular chromatin • Small cell carcinomas have a greater degree of necrosis, nuclear crush artifact, and nuclear molding • Mitotic rate much higher in small cell carcinoma





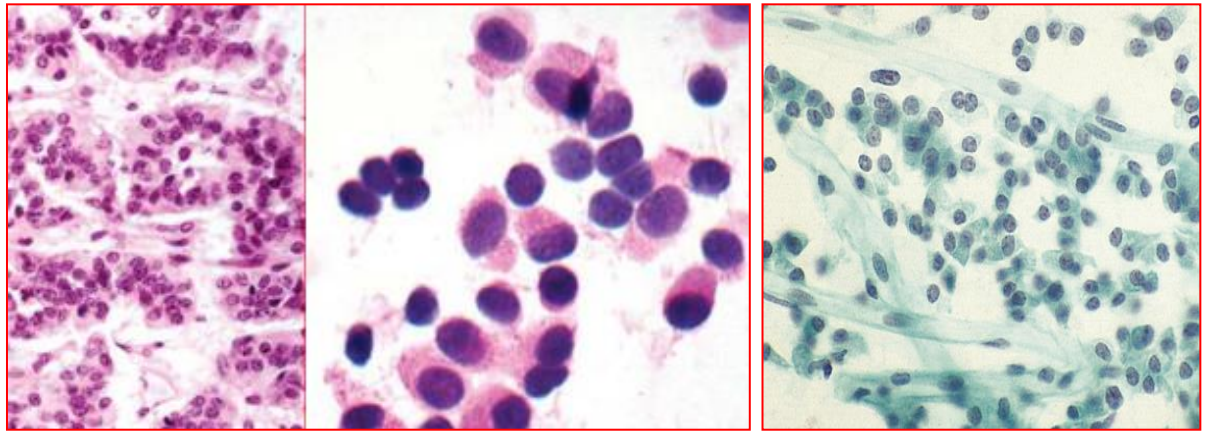


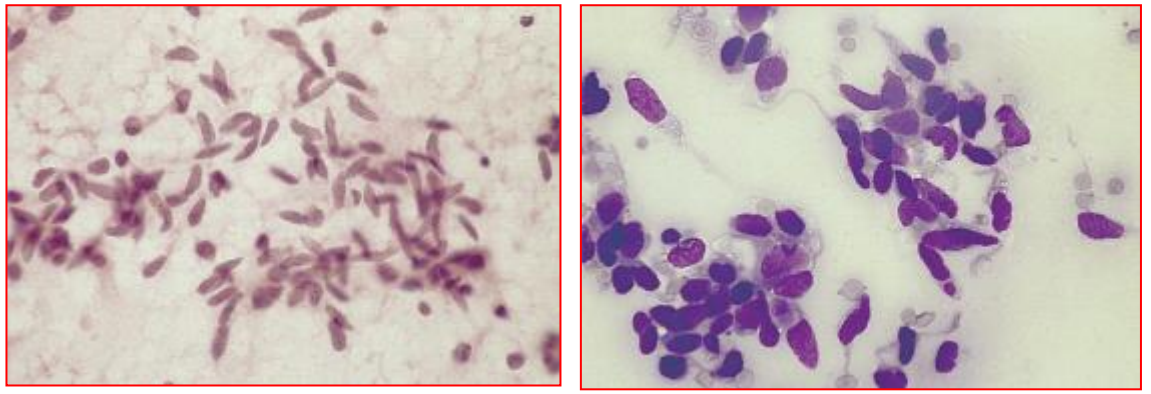


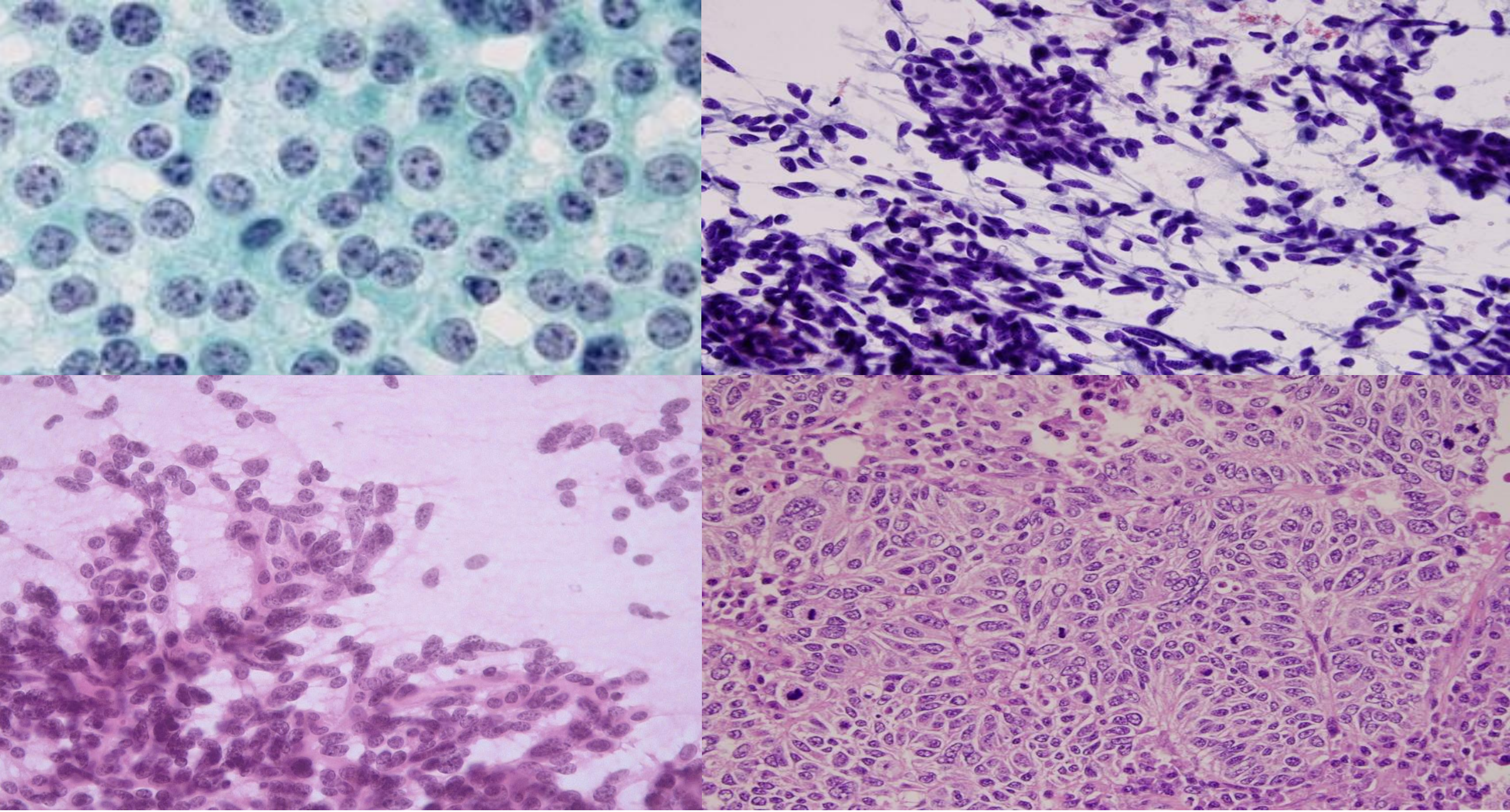
Source: C Michael and M Engels, Chapter 8: International System for Reporting Lung Cytopathology, 2022

LUNG CYTOLOGY
Morphological Aspects

- **CARCINOID**
  - Single cells
  - Round cells
  - Salt and pepper chromatin
  - No necrosis, molding, or mitosis
  - Spindle-cell type







Source: C Michael and B Osamura, Chapter 8: International System for Reporting Lung Cytopathology, 2022; F Schmitt

Neuroendocrine tumours – Typical and atypical carcinoids

What about some clues between typical and atypical carcinoids?

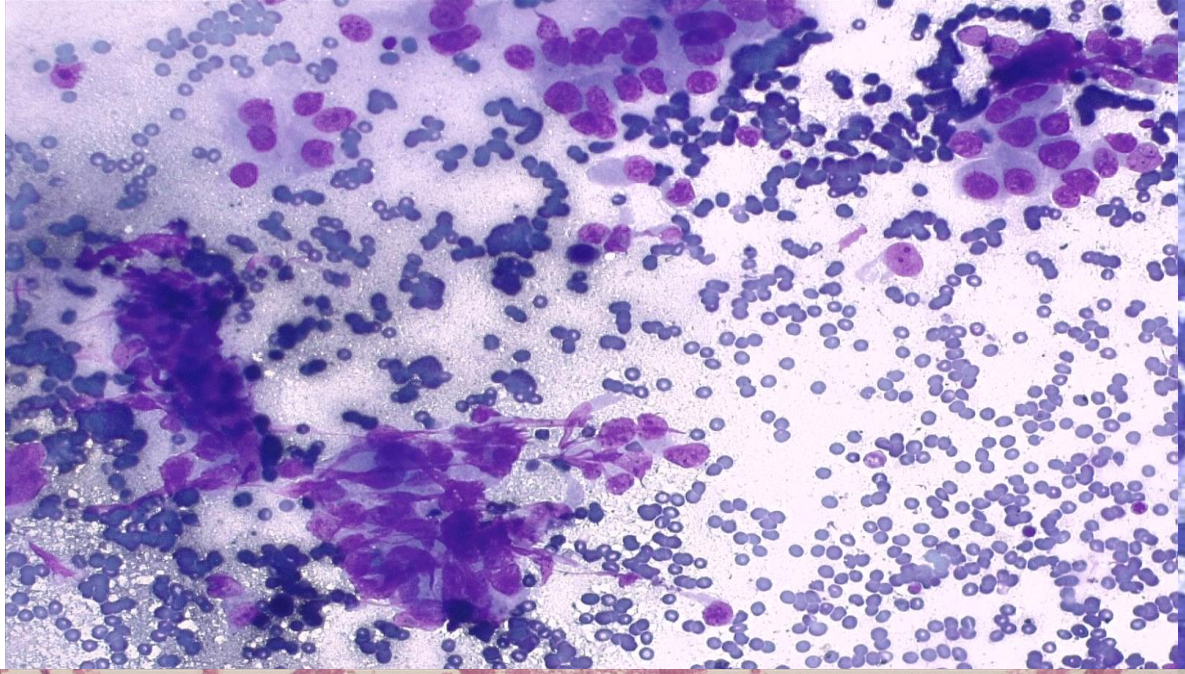
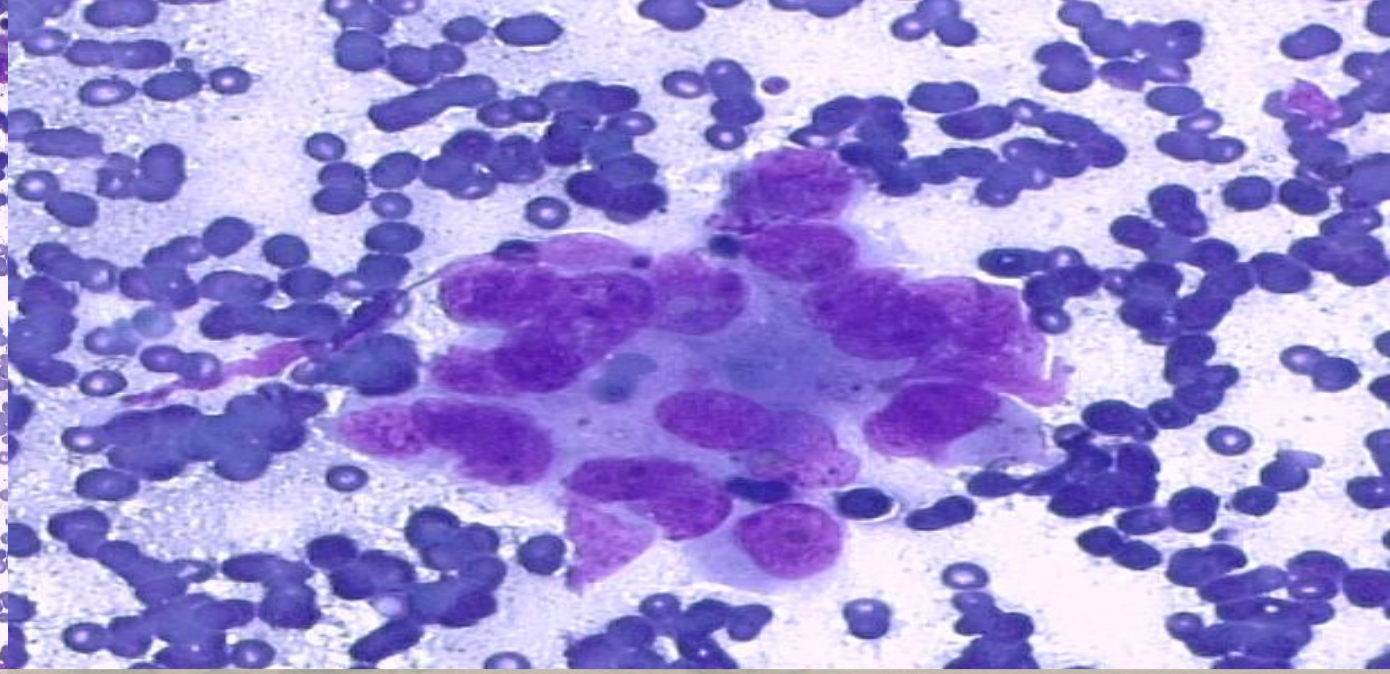
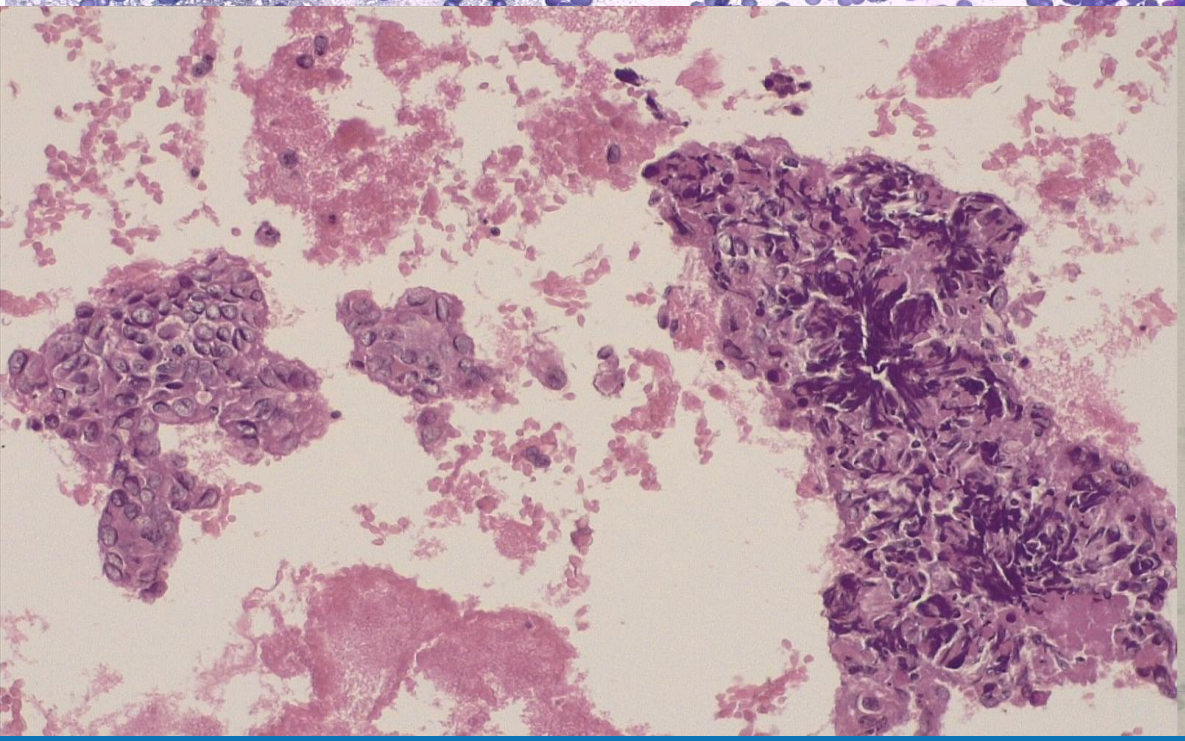
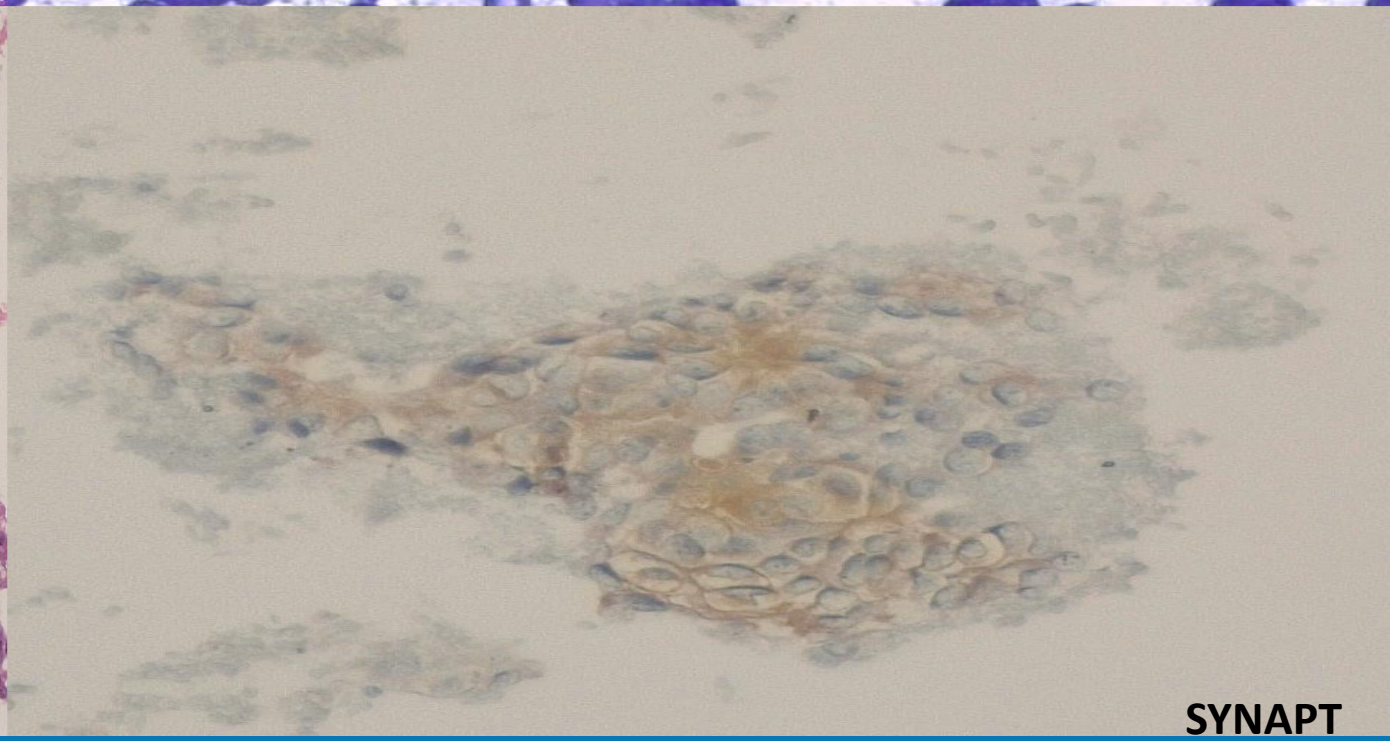
Distinguishing features	Typical carcinoid	Atypical carcinoid
Cell size	Cell size tends to be smaller	Medium sized cells
Nucleoli	Small, inconspicuous nucleoli	Nucleoli can be prominent
Mitosis	Mitoses rare (<2/10HPF)	Mitoses between (2-10/10 HPF)
Pleomorphism	No/mild nuclear pleomorphism	Moderate pleomorphism
Necrosis	Necrosis absent by definition	Necrosis can be present

Accurate classification requires resected specimen!

LUNG CYTOLOGY
Large Cell Neuroendocrine Carcinoma
LNEC

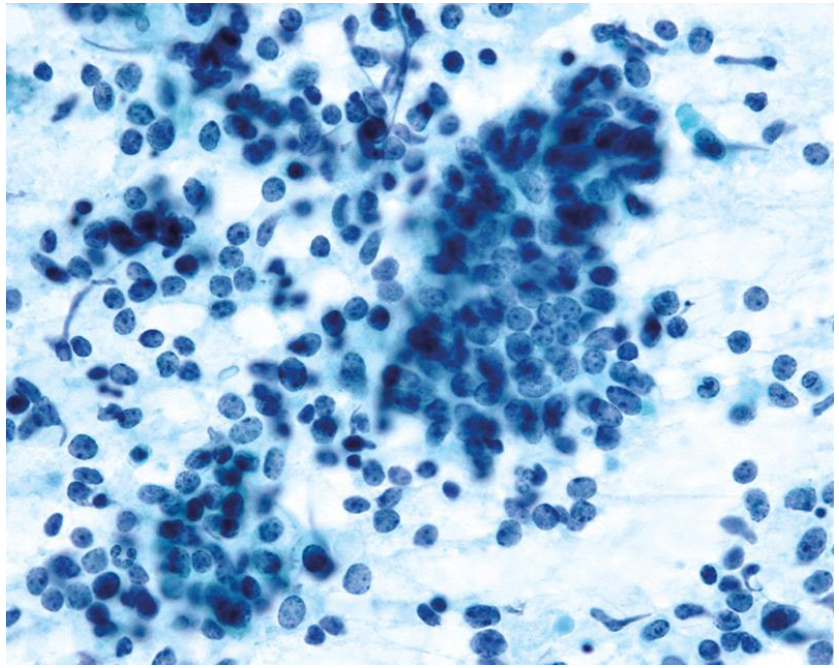
- Large cell neuroendocrine carcinoma (LCNEC) is a high-grade NSCLC with neuroendocrine morphology and neuroendocrine marker expression

- Key Diagnostic Cytopathological Features
  - Moderately to highly cellular
  - Small cohesive or loosely cohesive tissue fragments in a background of single cells and necrosis.
  - Cells are intermediate to large (usually >3 lymphocyte diameters) with moderate to abundant cytoplasm
  - Nuclear molding may be present but is subtler than SCLC and the chromatin is typically granular and fine but can be coarse or show perinucleolar clearing (in Pap stain)
  - Nucleoli range from inconspicuous to large
  - Chromatinic smearing, necrosis and mitosis are common





SYNAPT

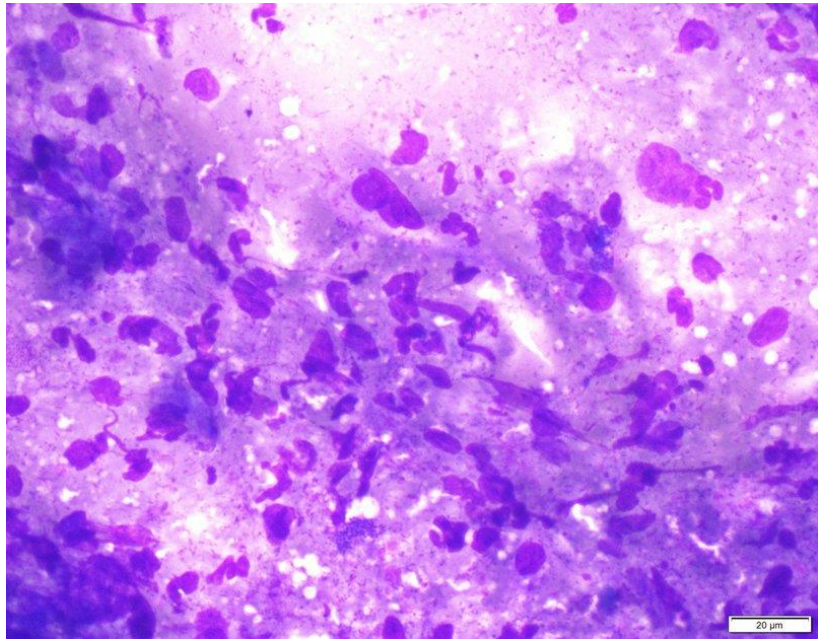
LUNG - MALIGNANT

PULMONARY BLASTOMA



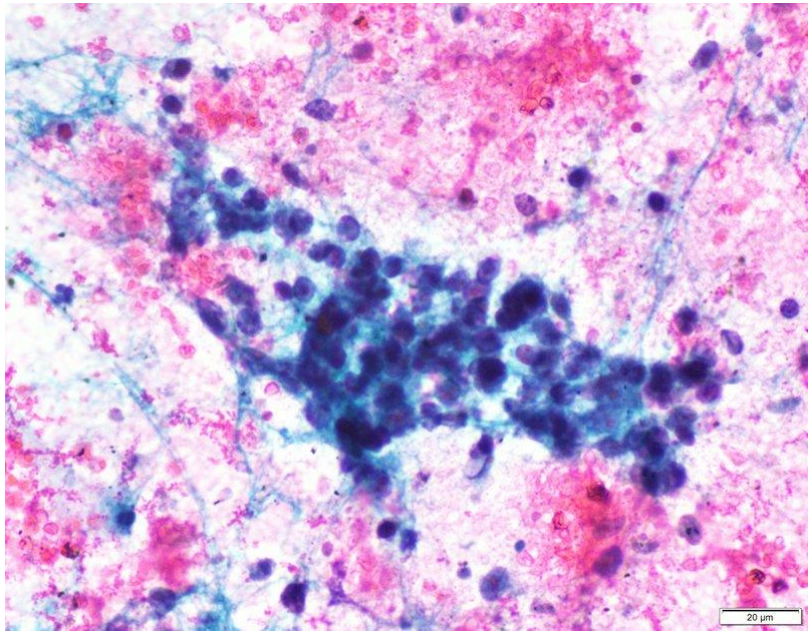
Source: Nakatani Yukio

CARCINOSARCOMA



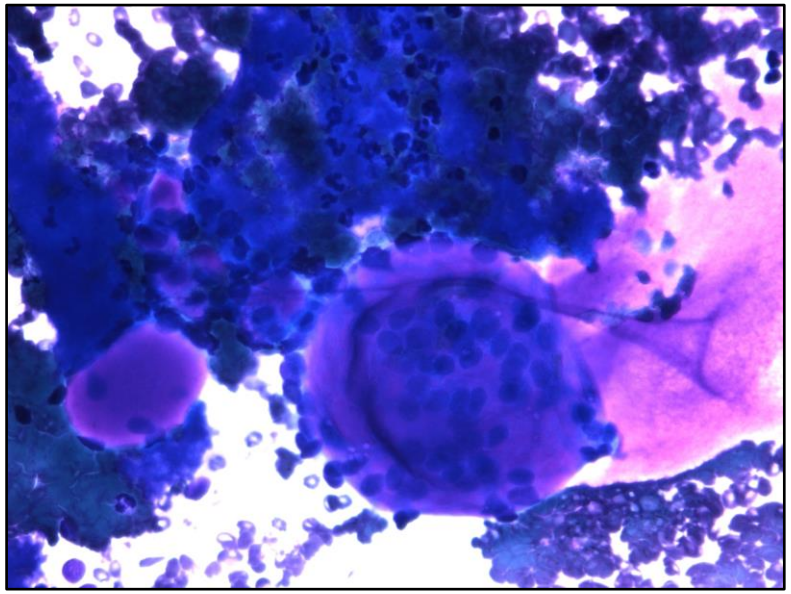
Source: Deepali Jain

NUT CARCINOMA



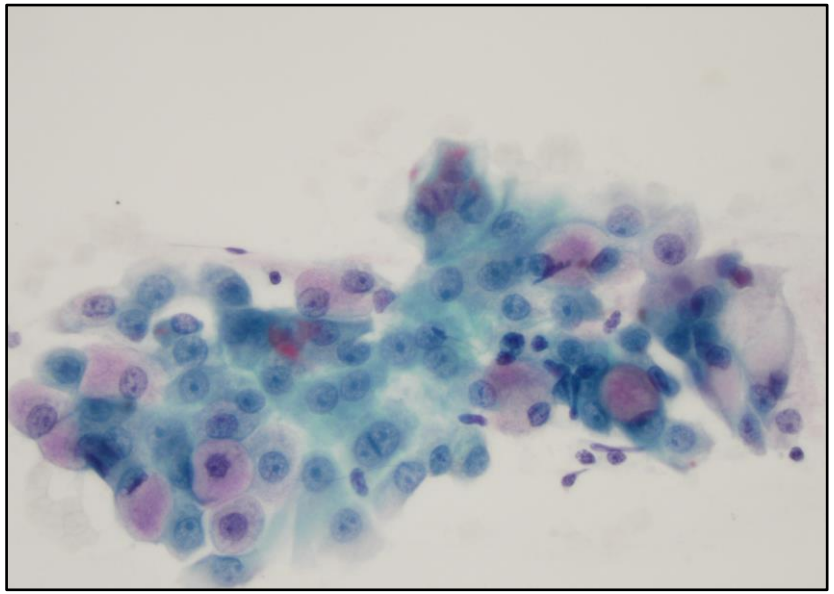
Source: Deepali Jain

Malignant – Salivary glad-type tumours of the lung



Adenoid cystic carcinoma:

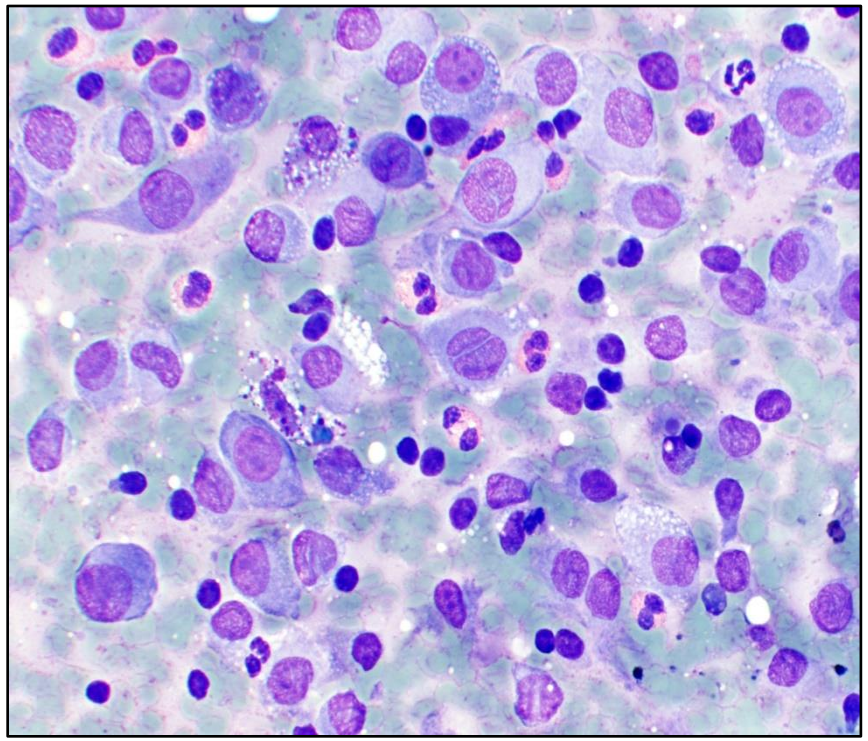
- Samples show small, bland uniform epithelial cells that surround cylinders of the myxochondroid matrix.



Mucoepidermoid carcinoma:

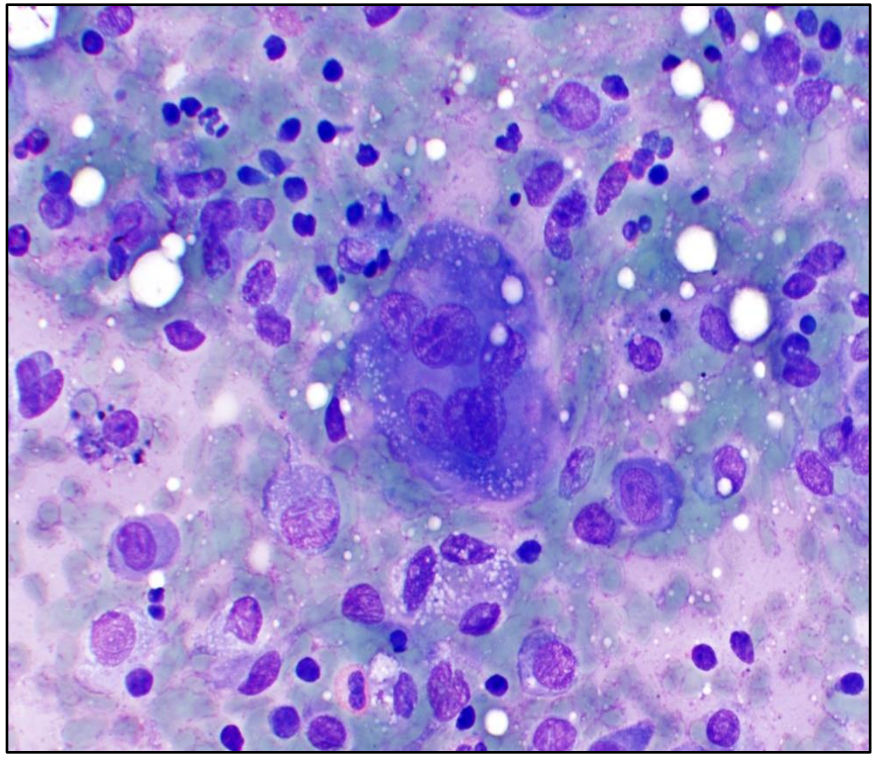
- Can be low or high grade and shows varying proportions of squamous, glandular, and intermediate cells.

Malignant – Langerhans cell histiocytosis

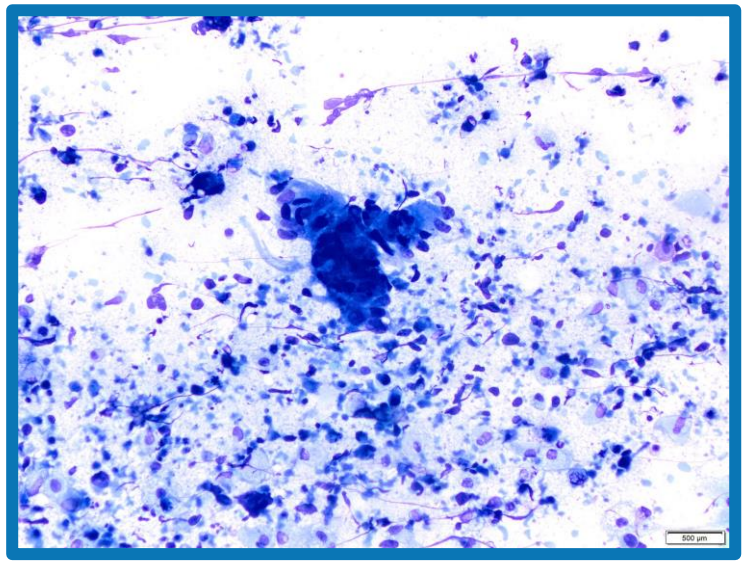


Langerhans cell histiocytosis

- Shows a combination of Langerhans cells, pigmented macrophages, and a mixed inflammatory infiltrate.
- Lesional cells show histiocytoid morphology characterized by abundant granular, mildly eosinophilic cytoplasm, elongated or convoluted nuclei with prominent nuclear grooves.
- Mixed inflammatory infiltrate often shows abundant eosinophils, an important diagnostic clue.



Malignant – Metastasis to the lung

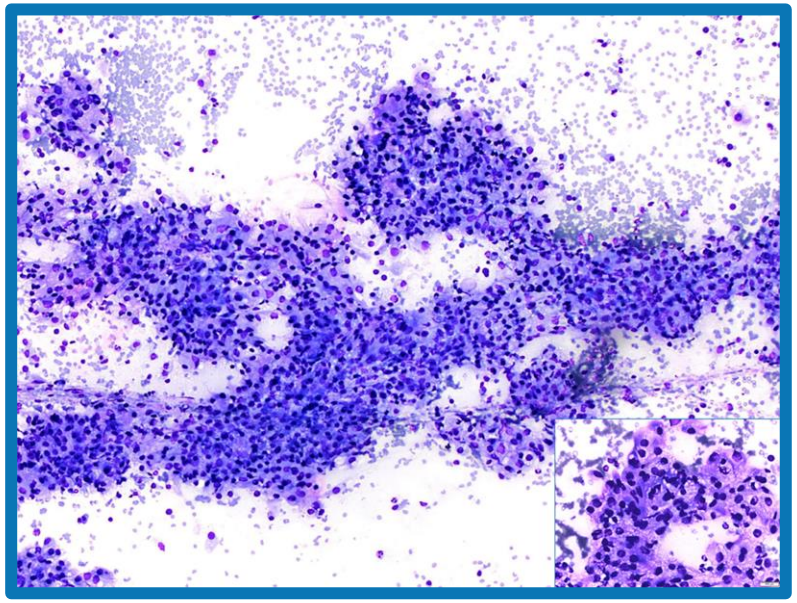


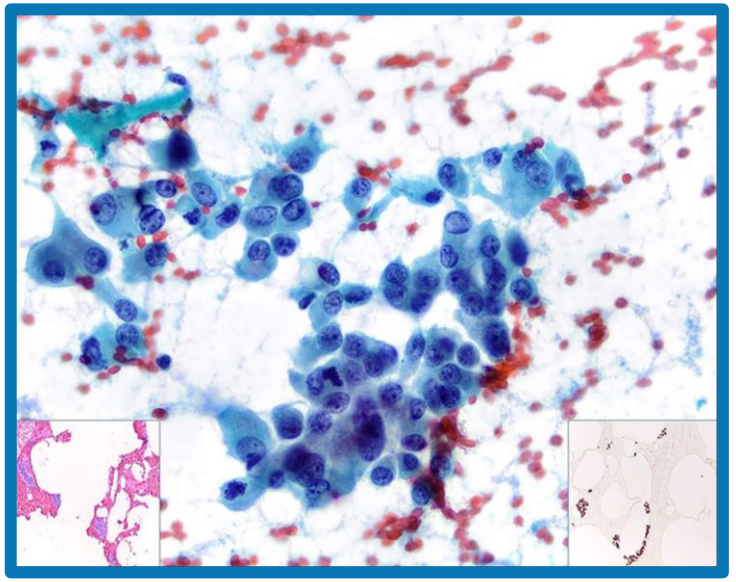
Metastasis of colorectal adenocarcinoma:

- Tall, columnar glandular cells with a picket fence appearance;
- Dirty necrosis, tumour cavitation.

Metastasis of renal carcinoma (clear cell):

- Large, polygonal cells with abundant clear or vacuolated cytoplasm;
- Enmeshed capillaries can be present.





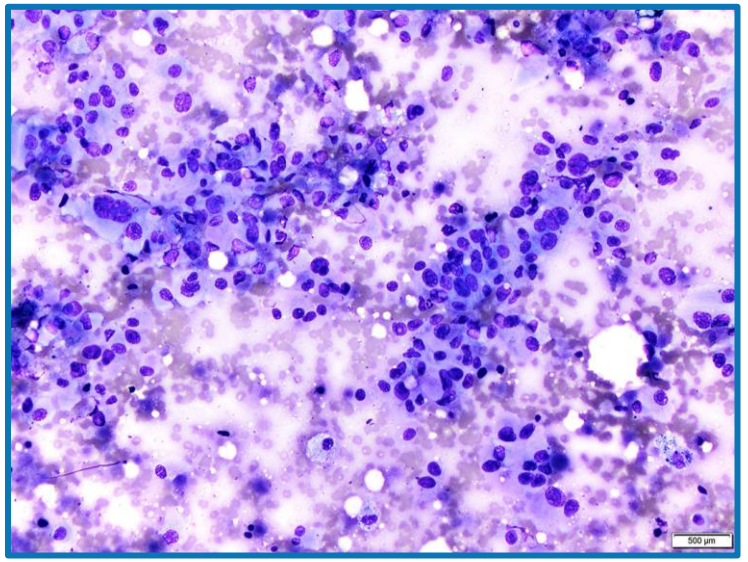
Metastasis of breast carcinoma:

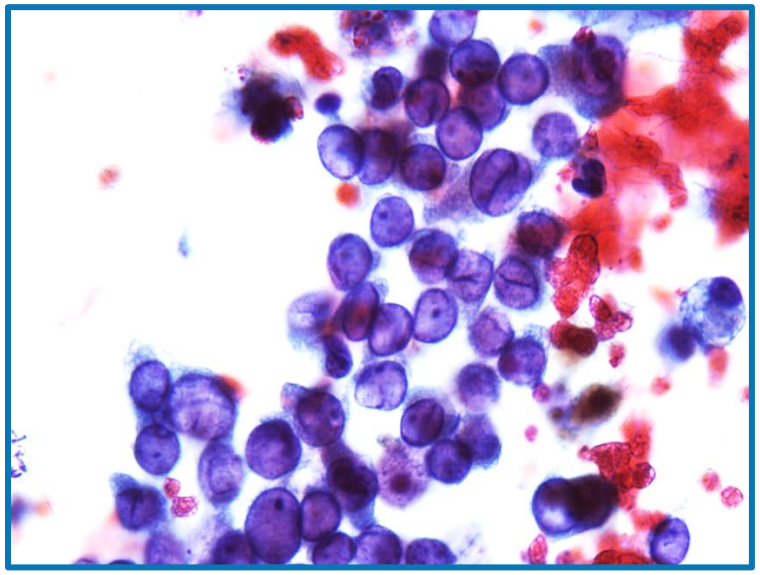
- Tends to replicate the morphology of the primary tumour;
- Single file appearance, lumen formation, and intracytoplasmic mucin may be present.

Malignant – Metastasis to the lung

Metastasis of melanoma:

- ✓ Highly variable in appearance; classical cytology is of discohesive, epithelioid or spindle cells with large, atypical nuclei and characteristically prominent red nucleoli;
- ✓ Intracytoplasmic melanin pigment and melanophages might be present.



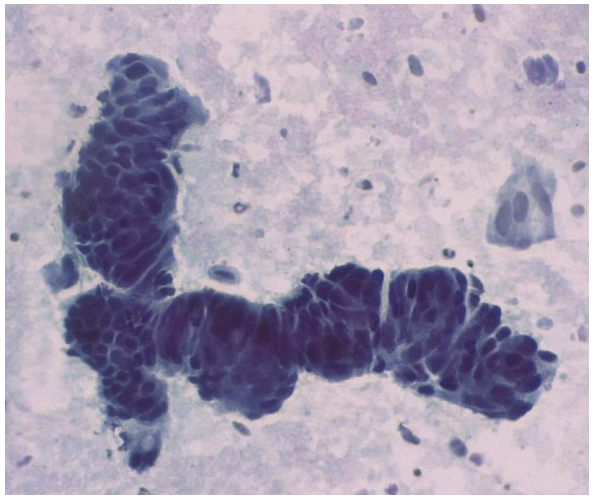


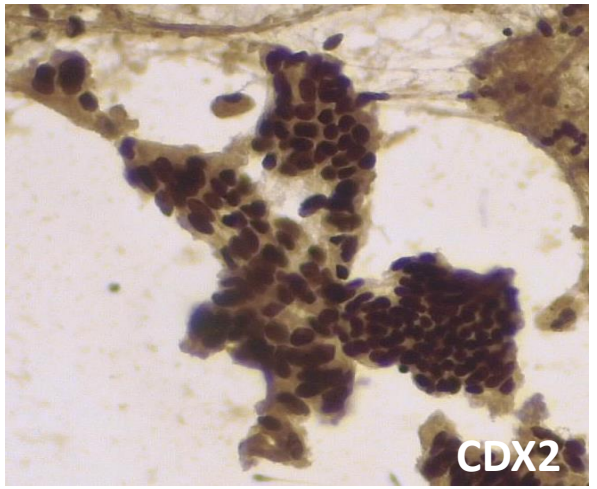
Metastasis of papillary thyroid carcinoma:

- ✓ Characteristic cytology shows elongated overlapping tumour cells with nuclear grooves, clearing, and intranuclear pseudoinclusions; papillary architecture can occasionally be represented on cytology;
- ✓ Psammoma bodies can be seen.

Immunoprofile of Pulmonary Secondary Neoplasms

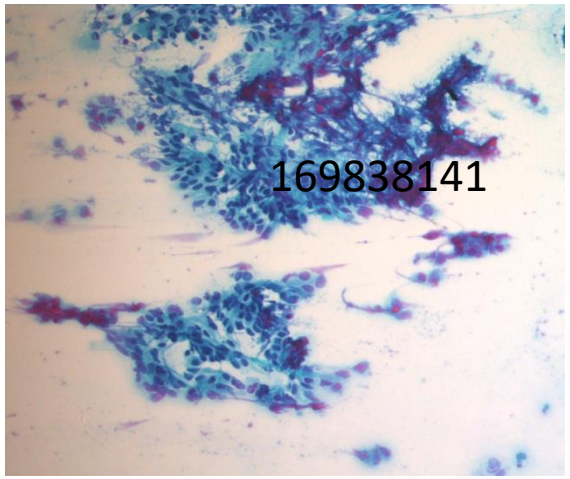
Site of Origin	Immunostaining Profile*
Gastrointestinal Tract	
1. Esophagus	CK7+, CK20-, TTF-1 -, CDX2 +/-, CEA+, MUC1-/+, MUC5AC -/+, SATB2-
2. Stomach	CK7+, CK20+, TTF-1 -, CEA+, CDX2-/=, MUC1 -/+, MUC5AC-/+
3. Colorectal	CK7-, CK20+, CDX2+, SATB2+, MOC31+
Breast	CK7+, CK20-, GATA3+, Mammoglobin+/-, GCDFP15-/+, ER+, PR+, TTF-1
Melanoma	SOX10+, Melan-A+, S100+, HMB45+, CK7-, CK20-
Pancreas	CK7+, CK20+, DPC4 -/+, CK17 +/-, pVHL -, Maspin +, S100p+, MOC31+, MUC5AC+
Liver	Ck7-, CK20-, HepPar1+, AFP+, Glypican+, Arginase 1+, CD10+, pCEA+, mCEA-,
Genitourinary Tract	
1. Urinary Bladder	CK7+, CK20+/-, GATA3+, p63+, p40+, CK5/6+, S100+, CK903+, Uroplakin +, Thrombomodulin +
2. Prostate	CK7-, CK20-, PSA+, NKX3.1+, PSAP+, P504S+
3. Ovary (serous)	CK7+, CK20-, PAX8 +, ER+, WT1+, TTF-1, TFF3 -, GATA3-
4. Ovary (clear cell)	CK7+, CK20 -, pVHL+, Napsin A+, WT1-, ER-, AFP-
5. Ovary (mucinous)	CK7+, CK20-, DPC4+, CA-125.5+, CDX2 +/-
6. Endometrium	CK7+, CK20-, ER+, PR+, PAX8+, CEA+ (foci of squamous metaplasia)
7. Uterine - Cervix	CK7+, CK20-, p16+, CEA+, PR-, PAX8+/-
Kidney	
1. Clear cell	CK7-, PAX8+, PAX2+, CAIX+, CD10+, RCC+, AE1/AE3+, CAM5.2+, EMA+, AMACR+/-, GATA3 -, TTF-1-
2. Clear cell papillary	CK7+, PAX8+, CAIX +, CD10-, RCC+/-, AMACR-, GATA3 -/+ (rare cases)
3. Papillary renal cell	CK7+, PAX8+, CA1X+/-, CD10+, RCC+, AMACR +, GATA3 -
Thyroid	
1. Papillary or Follicular	CK7+, CK20-, TTF1+, PAX8+, Thyroglobulin +
2. Medullary	CK7+, TTF-1+, PAX8-, Calcitonin +, CEA+, Synaptophysin +, Chromogranin+, Thyroglobulin -
Germ Cell Tumors	CD117+, OCT4+, CD30+, Glypican-3+, PLAP+, SALL4+

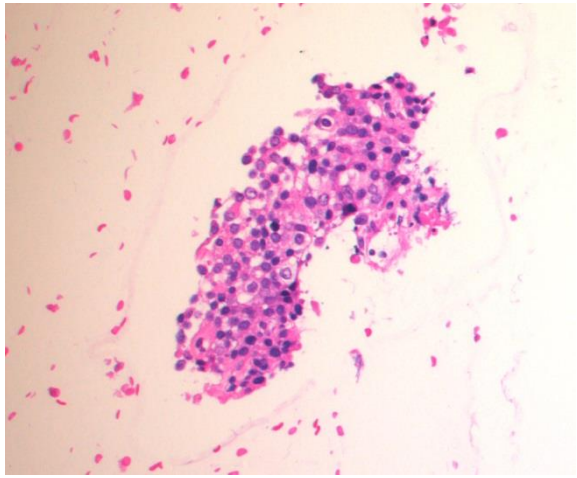


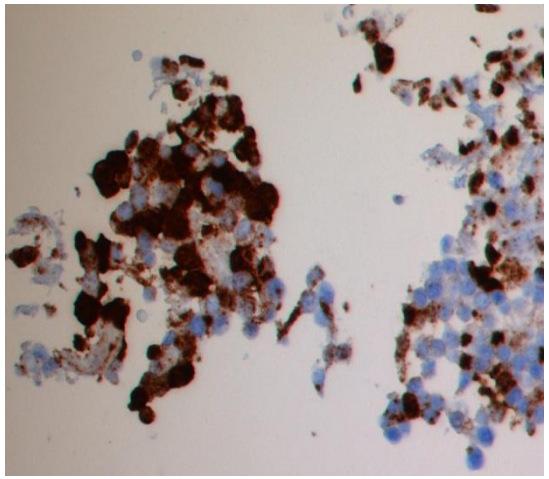


Other use of P16…..

- 39 y-old lady with lung nodule. History of a SCC of cervix operated three years ago. A lung FNA CT-guided with rapid on site assessment was performed.







	p16 Immunoreactivity				
	0	1+	2+	3+	Total Positive Cases
Cervical SCC (n = 48)	1 (2)	0 (0)	0 (0)	47 (98)	47 (98)
Pulmonary SCC (n = 33)	26 (79)	3 (9)	1 (3)	3 (9)	7 (21)

Am J Clin Pathol 2009;131:715-722

The Standardized Cytopathology Report

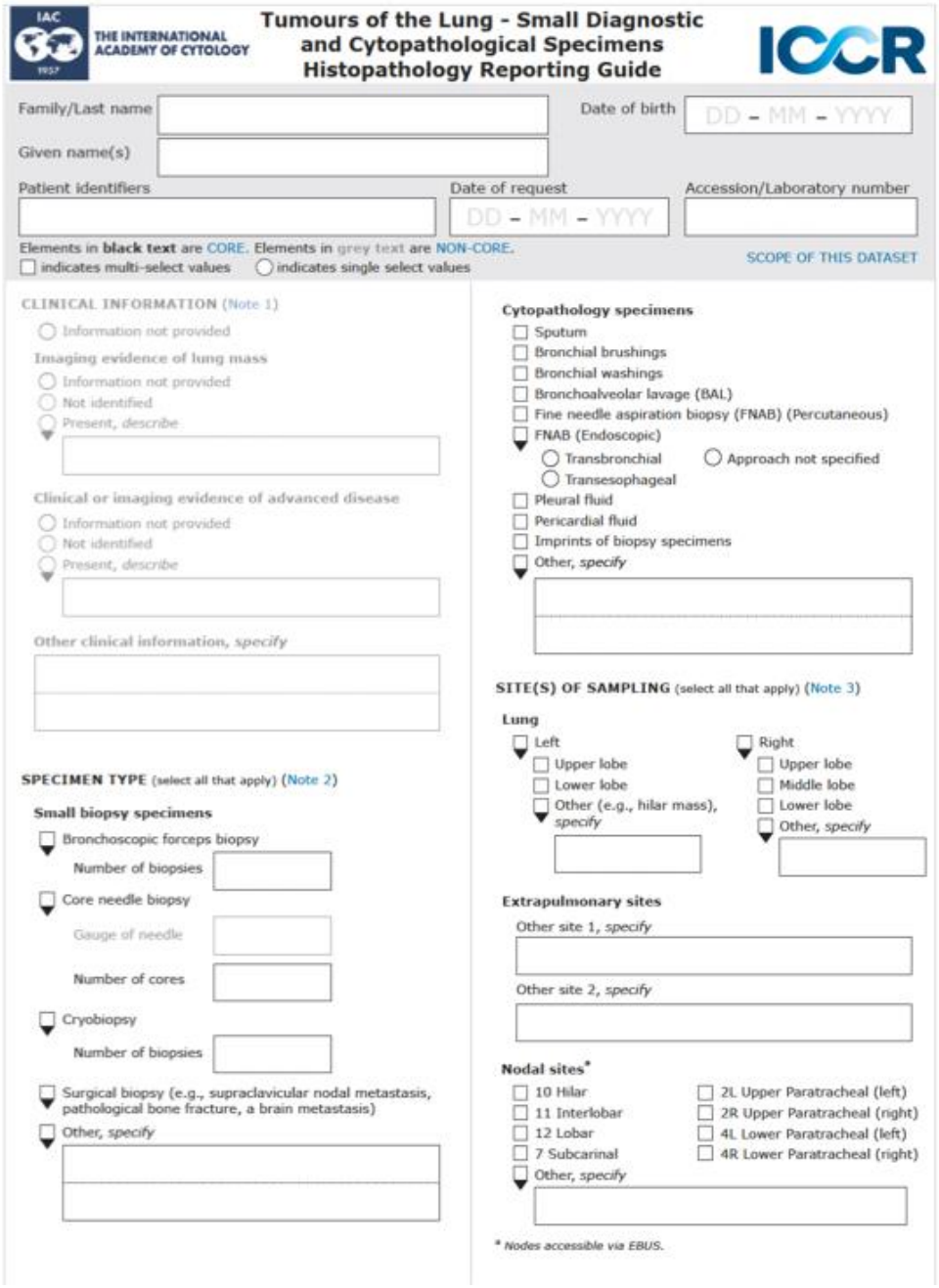
- **Demographic information:**
  - -patient's name, date of birth, address, patient identifiers, date of request, and laboratory accession number
  - -referring doctor and contact details

- **Type of Specimen:**
  - -sputum, bronchial wash, bronchial lavage, bronchial brush, FNAB (EBUS, transthoracic)

- **Clinical & Imaging information:**
  - -site, size (mm), imaging (ultrasound, CXR, tomogram, CT, MRI) features
  - -previous cytopathology procedures and results and previous other biopsy results when available

- **Category:** (example: Malignant)

- **Diagnosis:** (example: cytological findings of small cell carcinoma)
  - *-reporting system Category: using terminology, not a number*
  - *-specific diagnosis or differential diagnosis*



Tumours of the Lung - Small Diagnostic and Cytopathological Specimens Histopathology Reporting Guide

THE INTERNATIONAL ACADEMY OF CYTOLOGY

ICCR

Family/Last name

Given name(s)

Date of birth DD – MM – YYYY

Patient identifiers

Date of request DD – MM – YYYY

Accession/Laboratory number

Elements in **black text** are CORE. Elements in grey text are NON-CORE.

☐ indicates multi-select values ◯ indicates single select values

SCOPE OF THIS DATASET

CLINICAL INFORMATION (Note 1)

◯ Information not provided

Imaging evidence of lung mass

◯ Information not provided
◯ Not identified
◯ Present, describe

Clinical or imaging evidence of advanced disease

◯ Information not provided
◯ Not identified
◯ Present, describe

Other clinical information, *specify*

SPECIMEN TYPE (select all that apply) (Note 2)

Small biopsy specimens

☐ Bronchoscopic forceps biopsy
Number of biopsies
☐ Core needle biopsy
Gauge of needle
Number of cores
☐ Cryobiopsy
Number of biopsies
☐ Surgical biopsy (e.g., supraclavicular nodal metastasis, pathological bone fracture, a brain metastasis)
☐ Other, *specify*

Cytopathology specimens

☐ Sputum
☐ Bronchial brushings
☐ Bronchial washings
☐ Bronchoalveolar lavage (BAL)
☐ Fine needle aspiration biopsy (FNAB) (Percutaneous)
☐ FNAB (Endoscopic)
◯ Transbronchial ◯ Approach not specified
◯ Transesophageal
☐ Pleural fluid
☐ Pericardial fluid
☐ Imprints of biopsy specimens
☐ Other, *specify*

SITE(S) OF SAMPLING (select all that apply) (Note 3)

Lung

☐ Left
☐ Upper lobe
☐ Lower lobe
☐ Other (e.g., hilar mass), *specify*

☐ Right
☐ Upper lobe
☐ Middle lobe
☐ Lower lobe
☐ Other, *specify*

Extrapulmonary sites

Other site 1, *specify*

Other site 2, *specify*

Nodal sites*

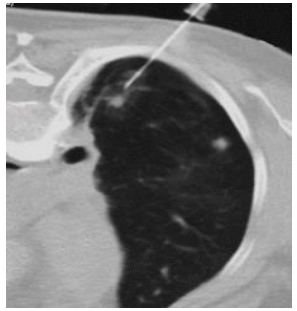
☐ 10 Hilar
☐ 11 Interlobar
☐ 12 Lobar
☐ 7 Subcarinal
☐ 2L Upper Paratracheal (left)
☐ 2R Upper Paratracheal (right)
☐ 4L Lower Paratracheal (left)
☐ 4R Lower Paratracheal (right)
☐ Other, *specify*

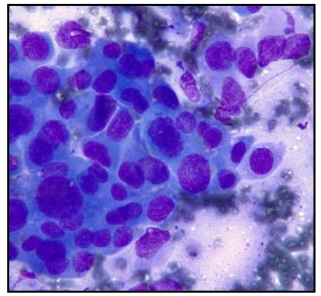
* *Nodes accessible via EBUS.*

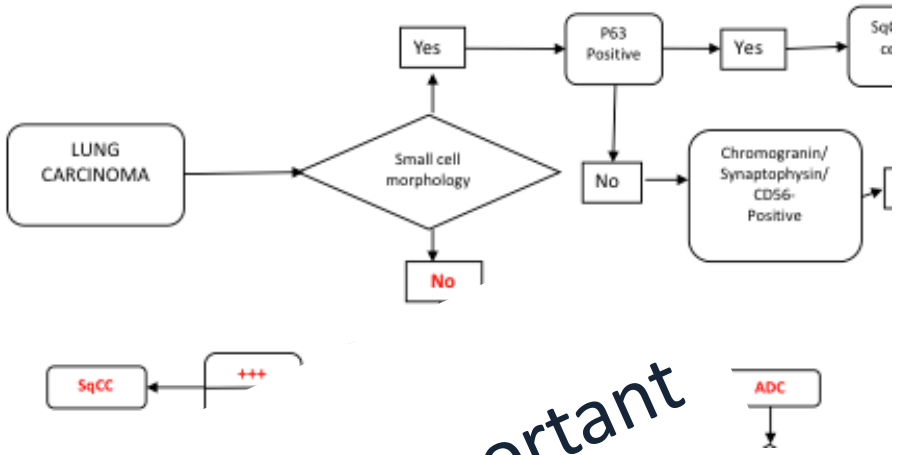
Reporting dataset IAC-ICCR

https://www.iccr-cancer.org/datasets/datasets-under-consultation/.

Approach to small biopsies and cytologic samples

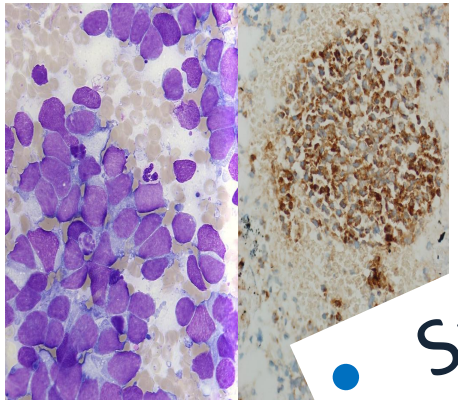




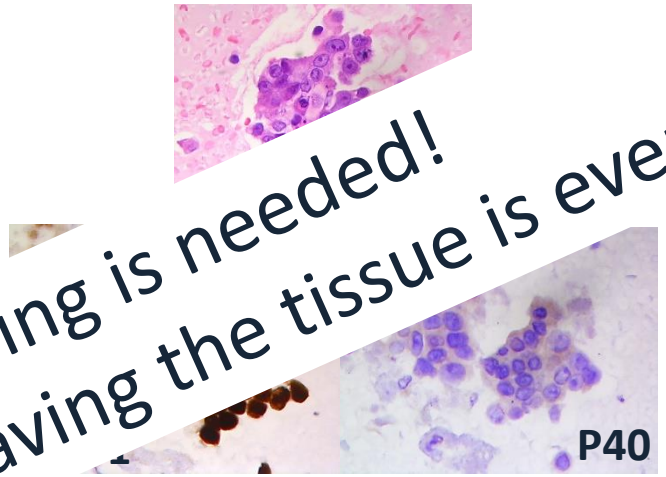


Barroca H & Schmitt f, Cancer Cytopathol 2011

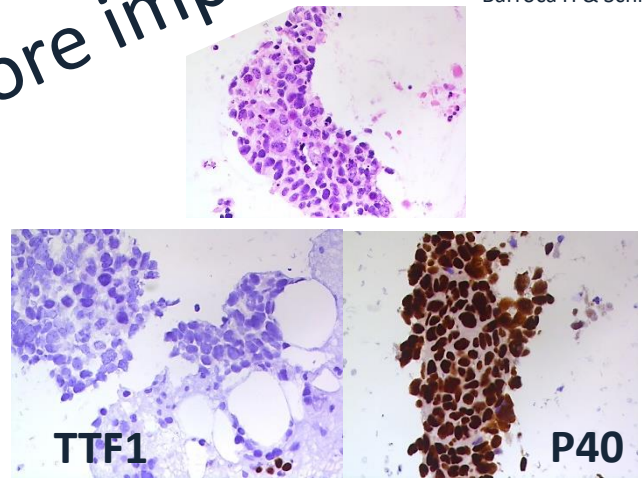
- Subtyping is needed!
- But saving the tissue is even more important



SMALL CELL CARC



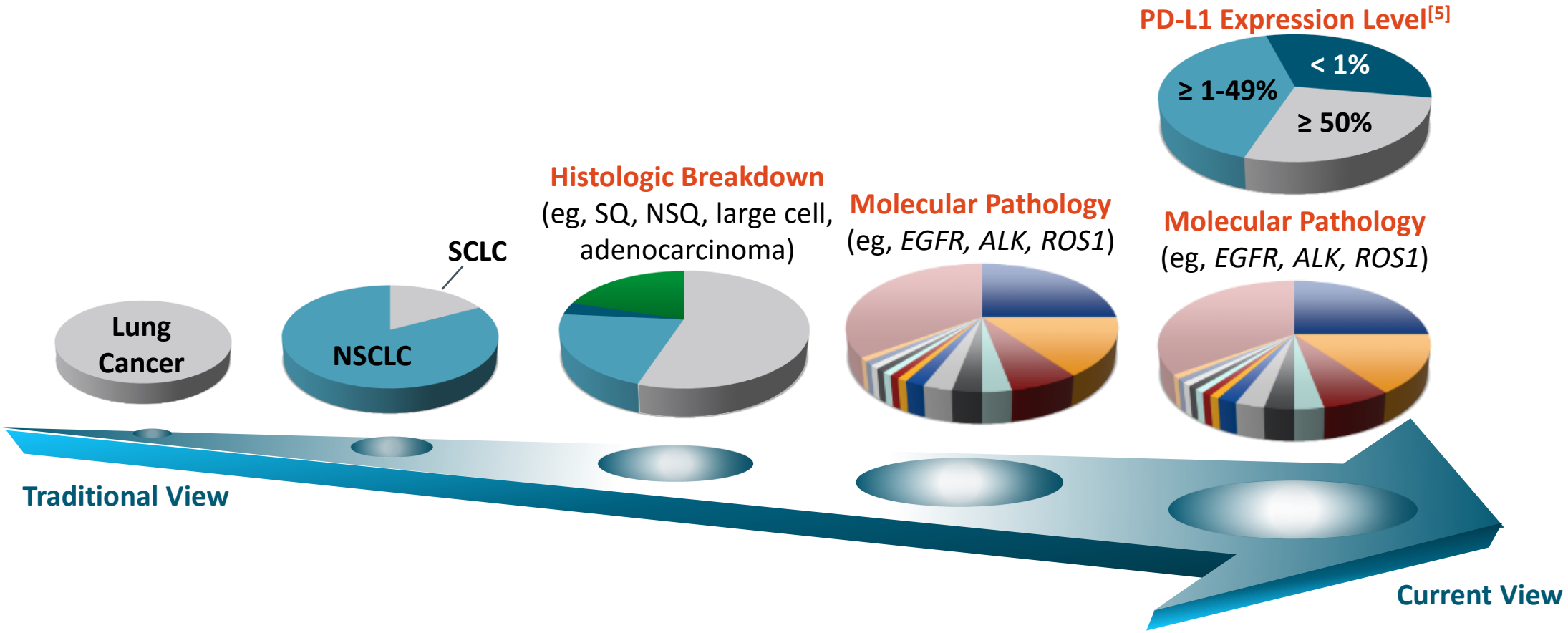
ADENOCARCINOMA



SQUAMOUS CELL CARCINOMA

LUNG CANCER AND PERSONALIZED THERAPY

- NSCLC was considered a single disease, until distinct subtypes, and characteristics were identified[1-4]
- NSCLC subtype characteristics are clinically relevant for treatment planning from diagnosis[1]

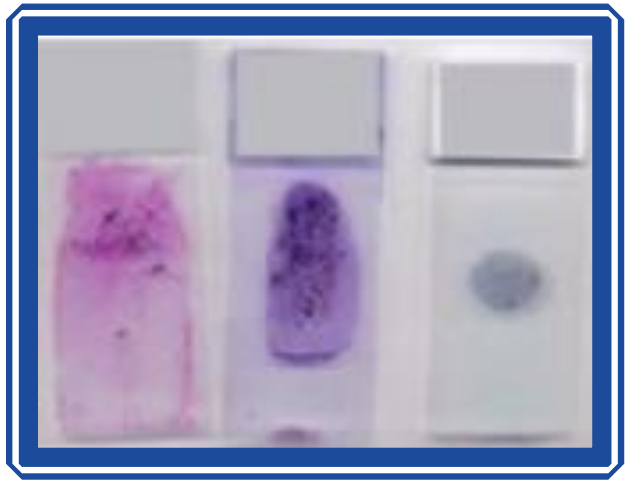


1. Cooper. Pathology. 2011;43:103. 2. Langer. J Clin Oncol. 2010;28:5311. 3. Galon. Immunity. 2013;39:11. 4. Pao. Lancet Oncol. 2011;12:175. 5. Krigsfeld. AACR 2017. Abstr CT143. 6. Hellmann. NEJM. 2018;378:2093. Slide Credit: clinicaloptions.com

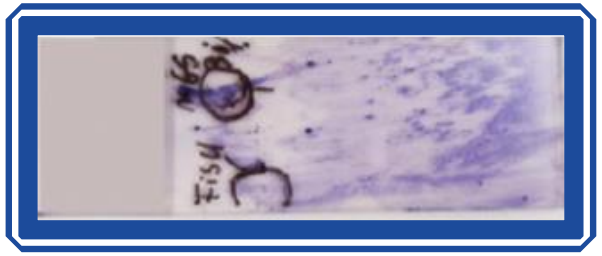
Working with cytological specimen for Molecular Tests

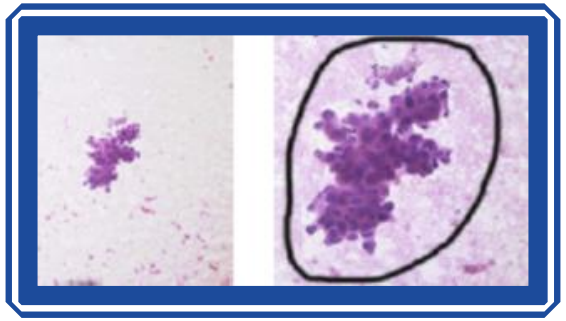
- Different cytological specimen:
  - ✓ Brush/washings
  - ✓ TNBA
  - ✓ Pleural effusions

- Different dyes:
  - ✓ H&E
  - ✓ MGG
  - ✓ PAP



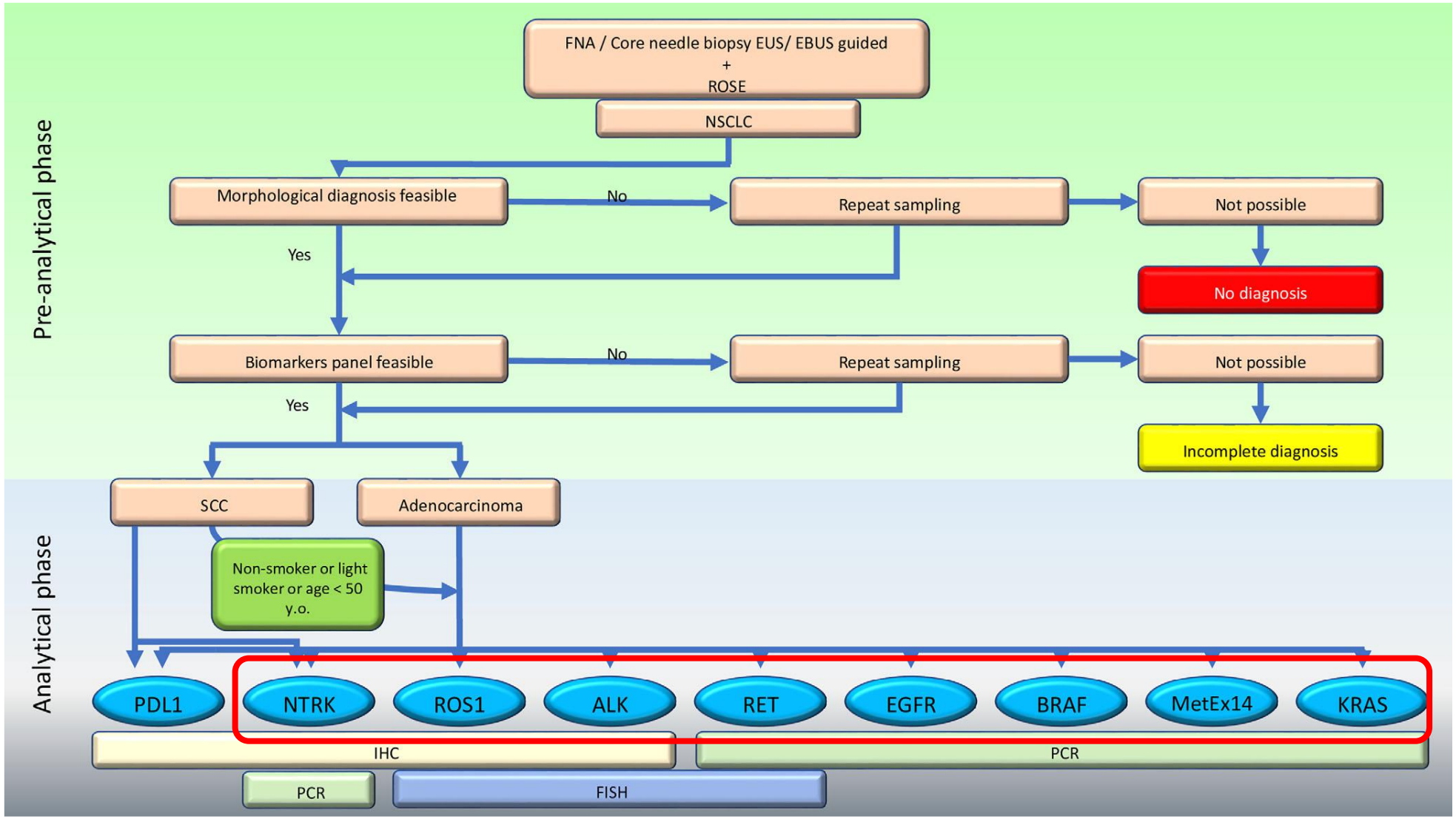
- requirements: a sufficient number of malignant cells (850-1700 cells/ 10-20%)
- all slides should be stained, and looked through, areas with malignant cells should be highlighted
- all materials, all standard staining can be used for NGS analysis





Molecular/biomarker testing in lung cytology: A practical approach

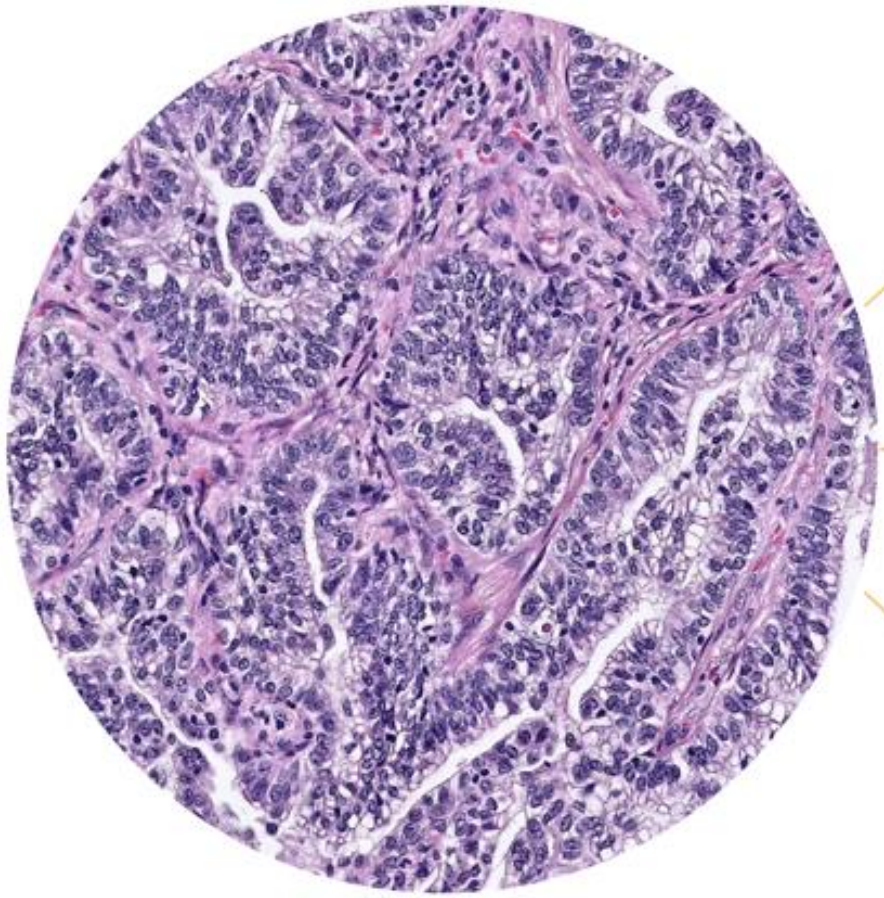
Fernando Schmitt MD, PhD, FIAC[1,2,3] | Maria D. Lozano MD, PhD, MIAC[4,5,6]



NGS

Virchows Archiv
https://doi.org/10.1007/s00428-022-03344-1

NSCLC: options for first-line therapy



Targeted Therapy

- EGFR
- ALK
- ROS1
- BRAF V600E
- MET ex 14 skipping
- RET
- NTRK

ICI monotherapy

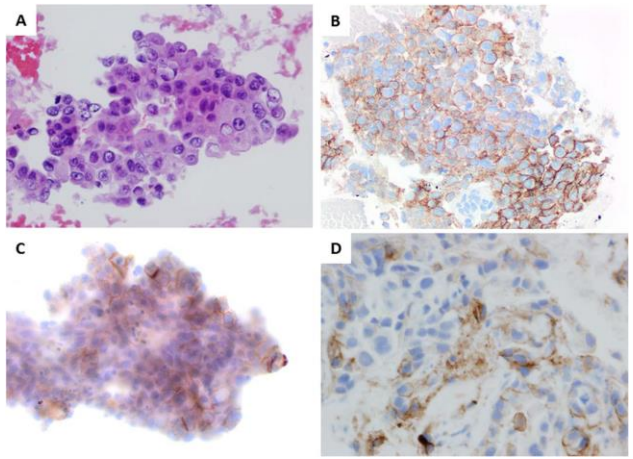
- If no driver mutation *and*
- PD-L1 IHC TPS ≥50%

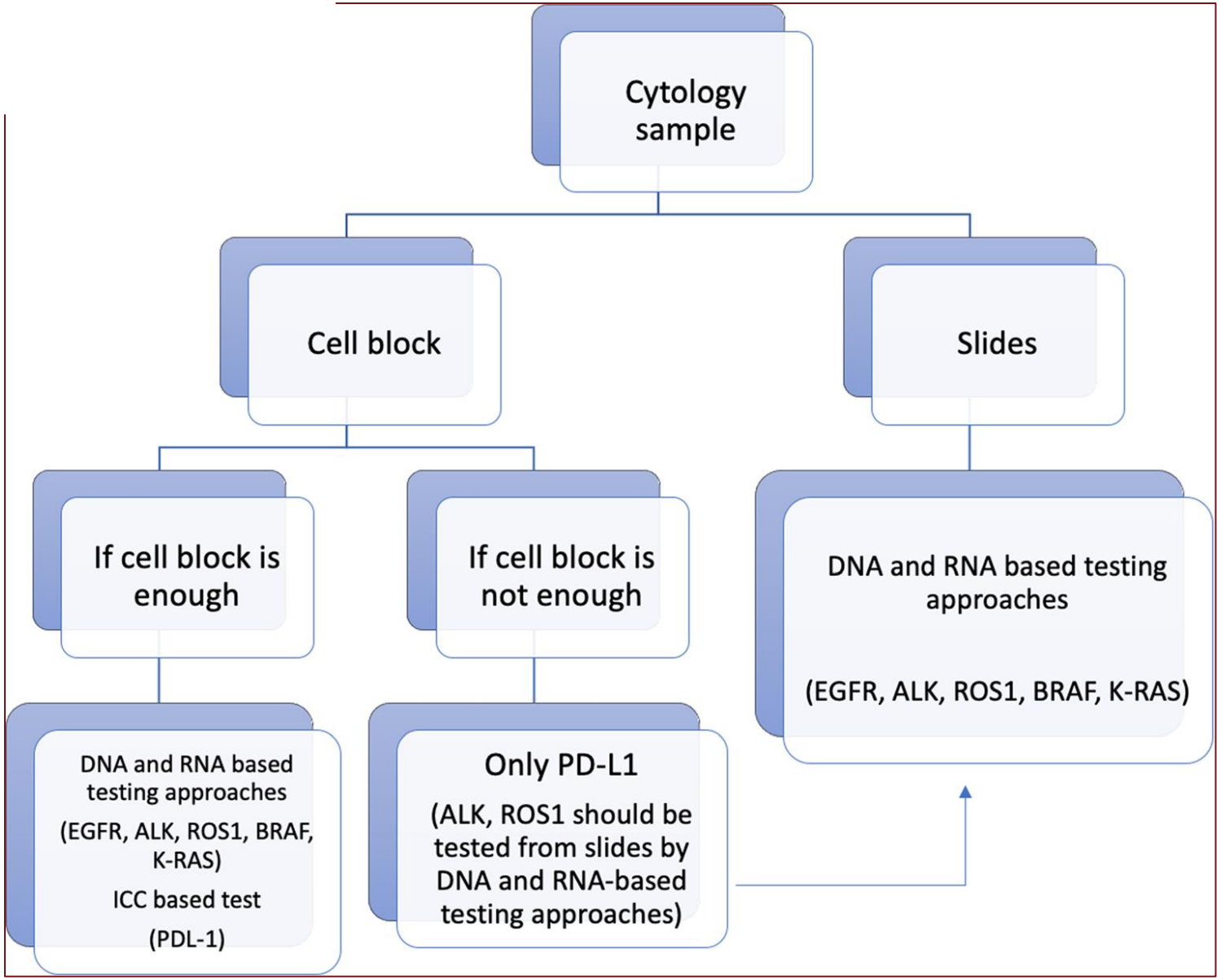
Chemo + ICI

- If no driver mutation *and*
- PD-L1 IHC TPS <50%

Cytology samples and molecular biomarker testing in lung cancer—advantages and challenges

Sule Canberk [1,2,3] • Marianne Engels [4]





CONCLUSIONS

- For 70% of lung cancer patients who present with advanced-stage, diagnosis and classification must be based primarily on small biopsy and/or cytology specimens.
- A solid knowledge of the wide range of morphological findings in lung cytopathology is key for the correct diagnosis.
- The WHO System is an international consensus about the key diagnostic cytopathologic criteria for each lesion or lung tumour, which contributes to improve the quality of diagnostic assessment and reporting of lung cytopathology.
- The System is designed to improve communication between clinicians and cytopathologists. Each specimen type and its category have a specific initial ROM and this will directly influence clinical diagnostic management algorithms.


SAVE
THE DATE
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