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# Respiratory Cytology and Ancillary diagnostic techniques

Dr Alex Rice
Royal Brompton Hospital

# Overview

- Specialist Cardiothoracic centre
  - BAL specimens and cell differential counts
  - EBUS
    - Diagnostic pitfalls
    - Subtyping lung cancer
    - Molecular techniques
  - Recent advances pleural fluids

# Bronchiolo-alveolar Lavage (BAL) specimens

# BAL Cytology

- Indications (Acute or Chronic LD)
  - Interstitial and alveolar lung disease
  - Infections
    - E.g Pneumocystis, fungi, viral
  - Drug reactions
  - Malignancy

- Techniques
  - Cell differential count
  - Fat laden macrophage
  - Immunofluorescence

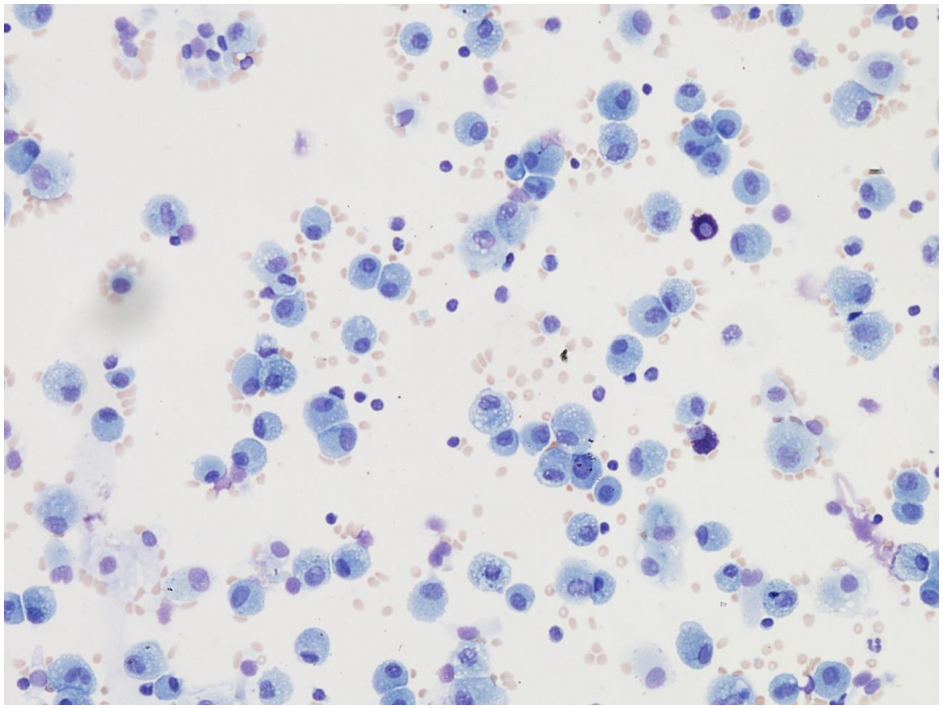
**CLINICAL DETAILS**
?IPF

| **Volume in:** | -ml | **Volume out:** | -ml |
|---|---|---|---|
| Volume Received | 40ml | Total Cells counted | 300 |
| | **Cells Counted** | **%** | **Normal Range** |
| Macrophages | 184 | 61.3 | >80% |
| Lymphocytes | 18 | 6 | ≤14% |
| Neutrophils | 75 | 25 | ≤ 4% |
| Eosinophils | 21 | 7 | ≤ 3% |
| Mast Cells | 2 | 0.7 | ≤ 0.5% |
| Ciliated Epithelial Cells | 0 | 0 | |
| Squamous Epithelial Cells | 0 | 0 | |
| Others | 0 | 0 | |
| | | | |
| Debris/Mucus | ++ | | |
| RBC | ++ | | |
| Inclusion Bodies | - | | |
| Pigmented Macrophages | - | | |

**Comments:** Neutrophilia. Mild Eosinophilia. Raised Mast Cells.

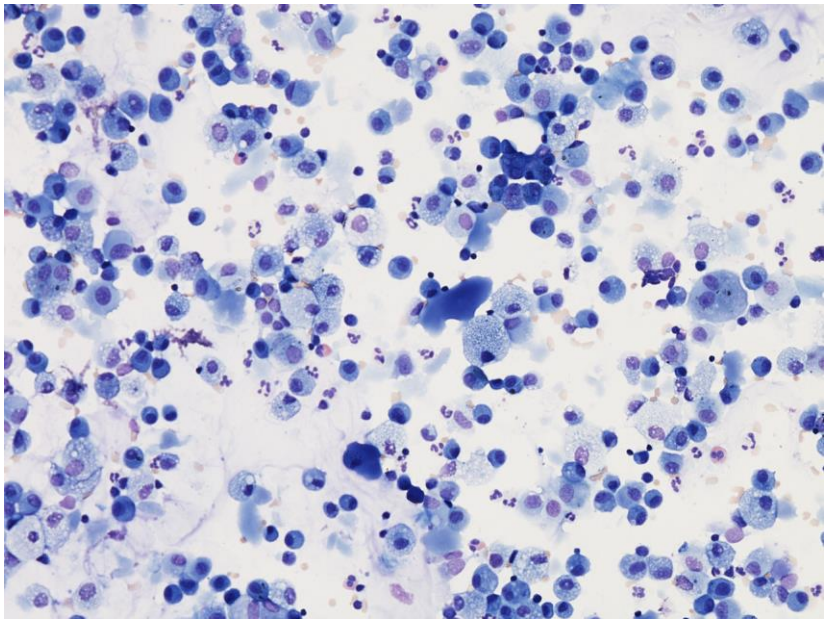
# Lymphocytosis

- ILDs associated with lymphocytosis include
  - Sarcoidosis
  - Hypersensitivity pneumonitis
  - NSIP/CTD
  - OP
- Drug related
- Infection: TB, viral pneumonia
- Lymphoma



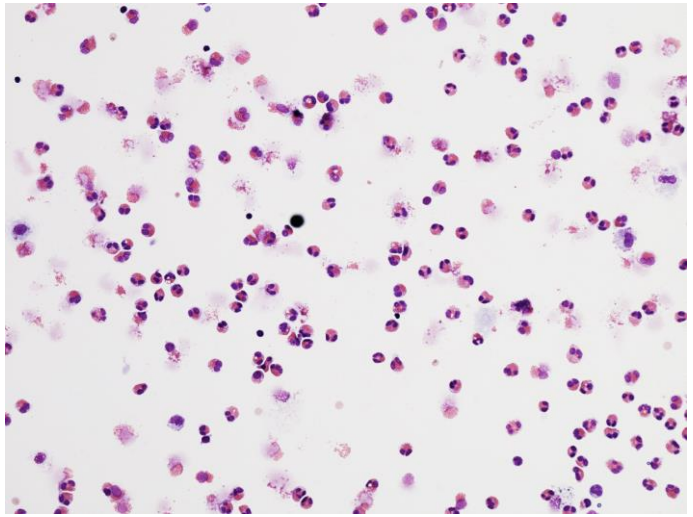
# Neutrophilia

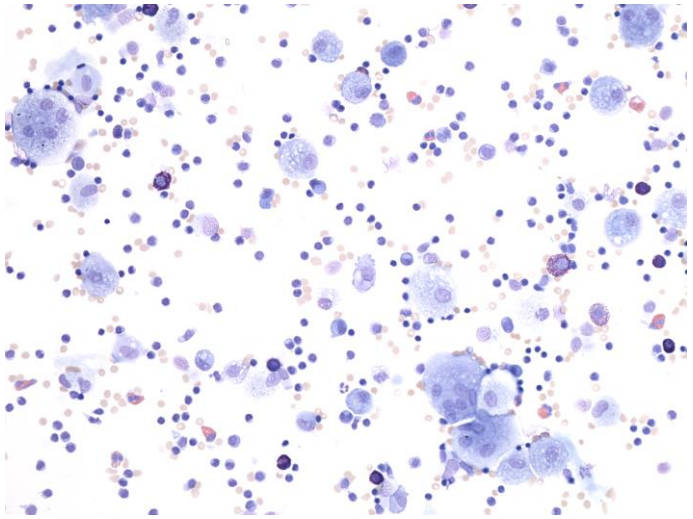
- ILDs associated with neutrophilia
  - IPF/UIP
- Infection
  - Fungal stains
  - Correlate with microbiology
- Vasculitis
  - Leukocytoclasis
  - haemosiderin laden macrophages



# Eosinophilia

- Eosinophilic pneumonia
- Asthma
- ABPA
- Drug reaction
- Parasitic infection
- Vasculitis (Churg Strauss)
- Langerhans cell histiocytosis

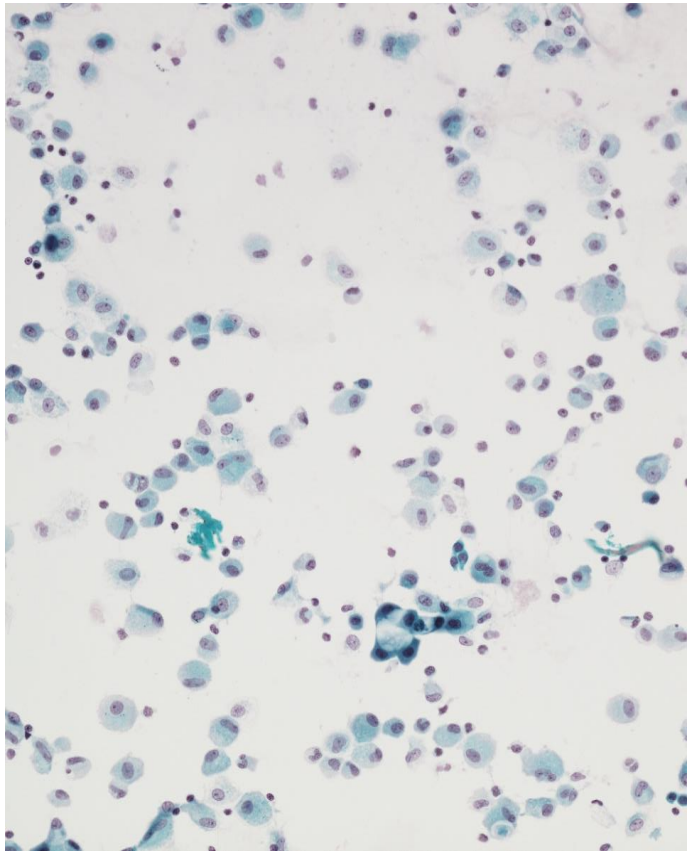




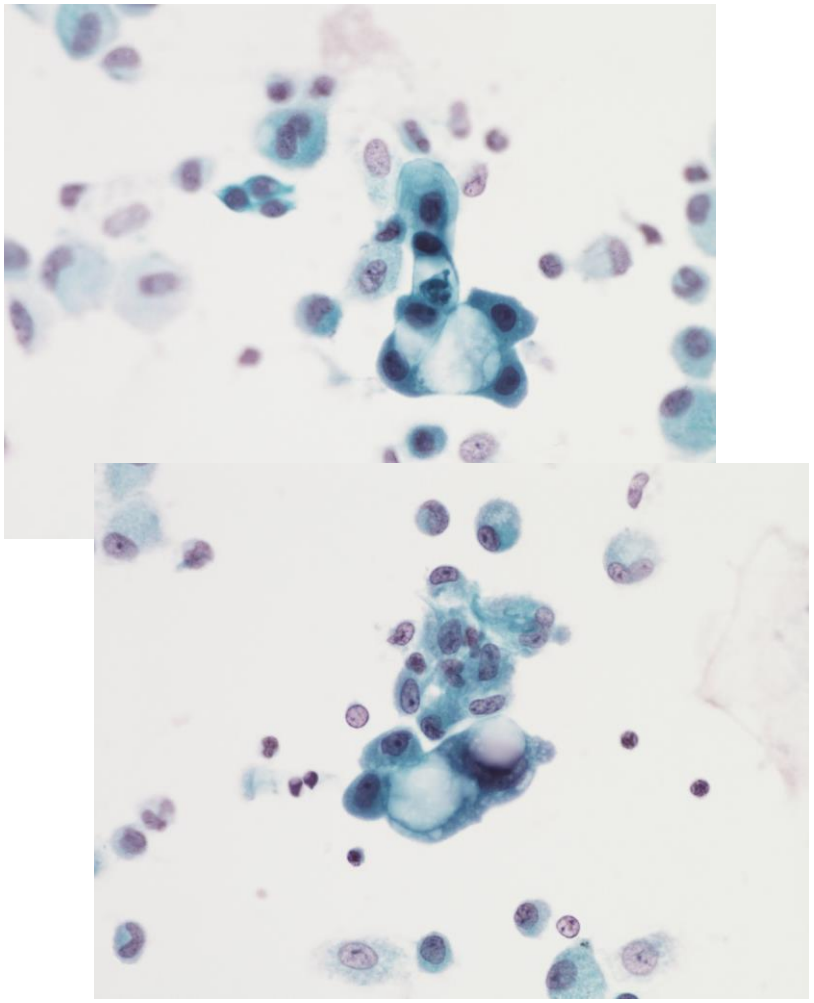
# ITU – Acutely sick patient

- Neutrophilia
  - +/- intracytoplasmic organisms (special stains)
  - Leukocytoclasis ->capillaritis?
- Eosinophilia
  - Eosinophilic pneumonia/drug reaction/infection
- Infectious agents
  - PCJ, fungi, CMV, HSV, TB
  - Gram, grocott, IHC, IF stains
- Iron
  - Pulmonary haemorrhage syndrome/vasculitis
- Reactive type 2 pneumocytes, debris, fibrin

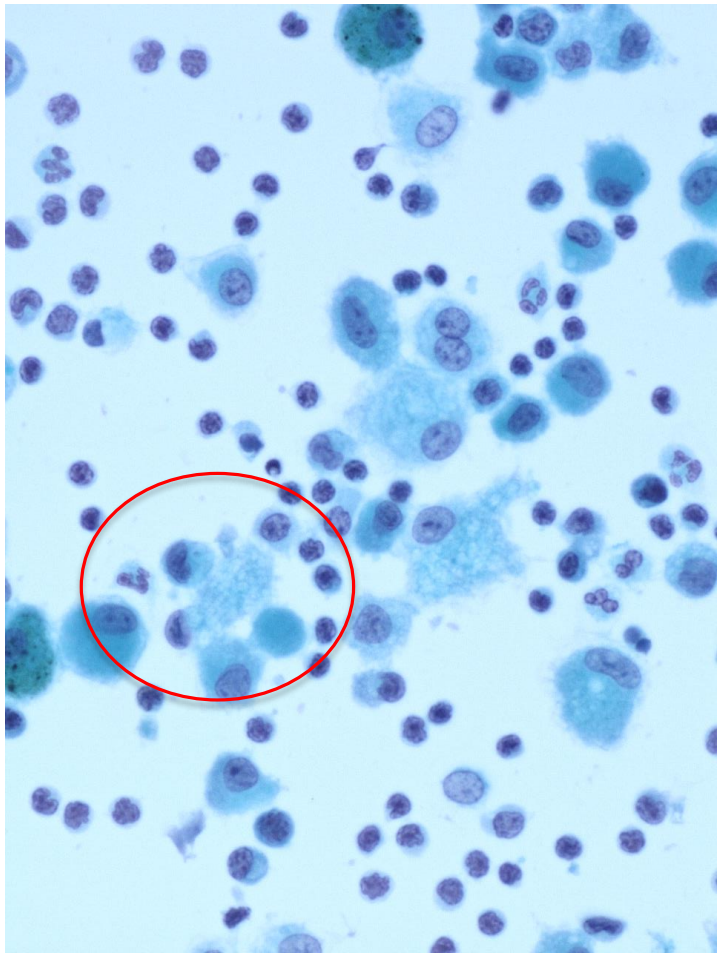
# Reactive cytological atypia

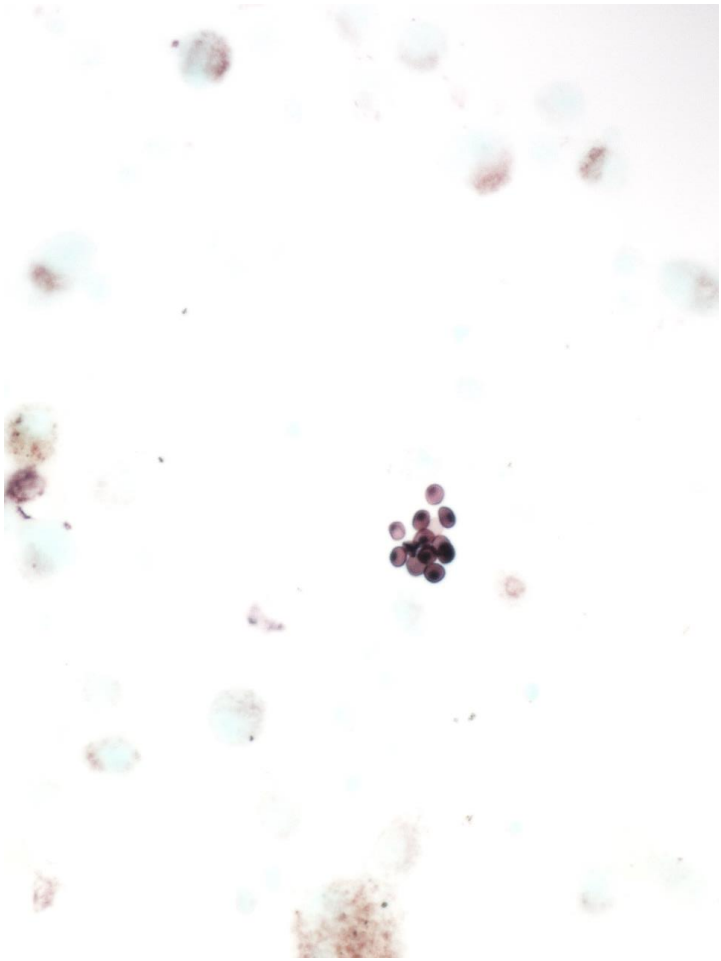


Post radiotherapy interstitial infiltrates



# Infection





Pneumocystis

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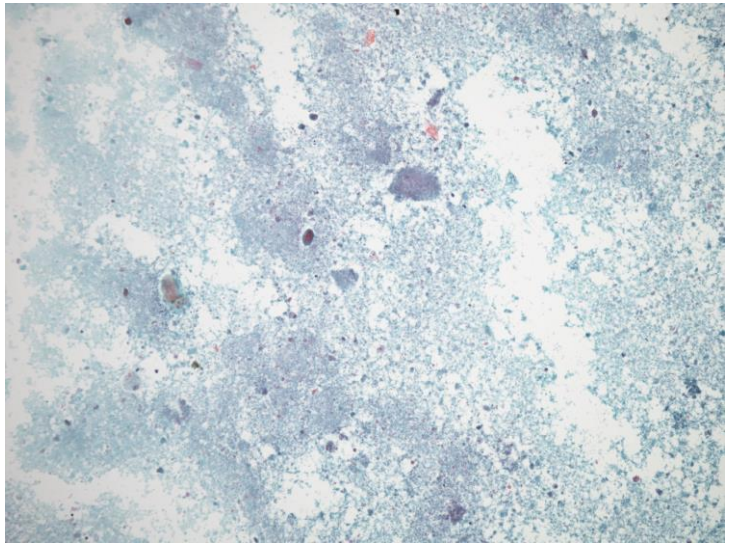
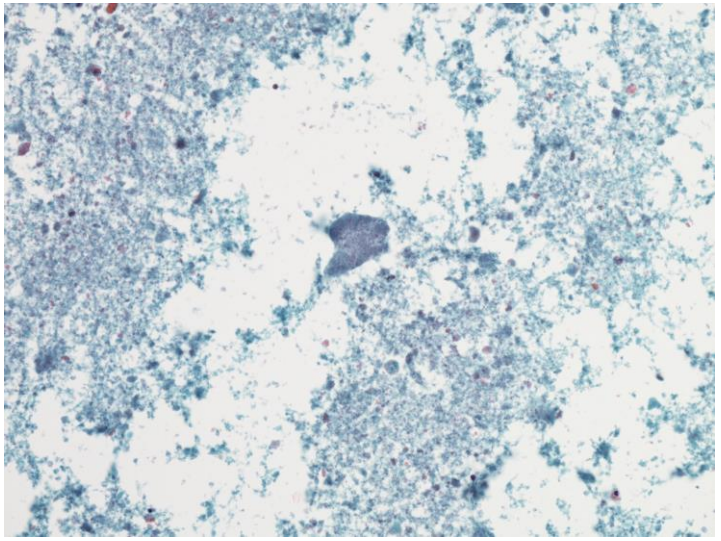
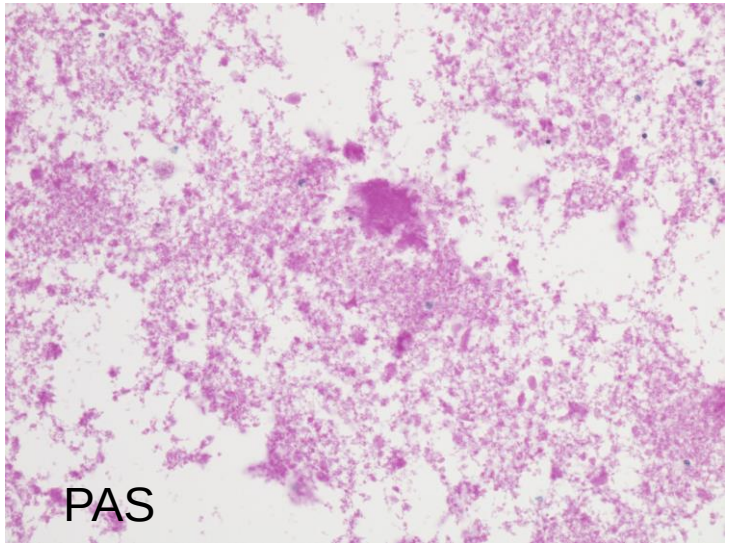
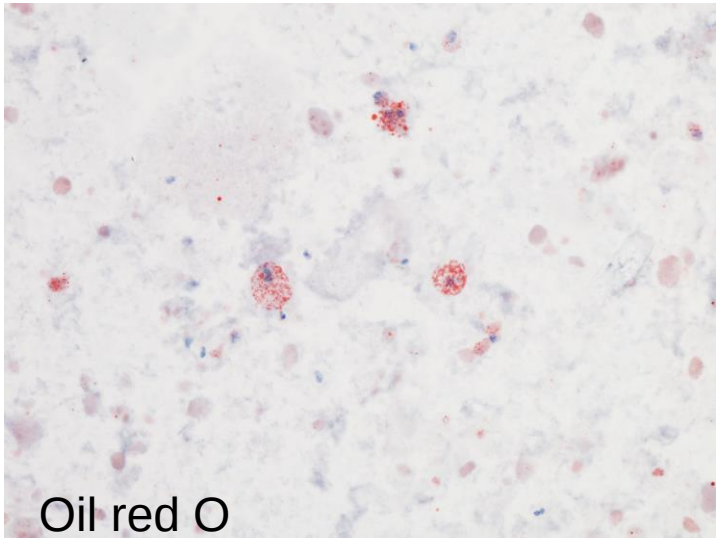
# Pulmonary Alveolar Proteinosis

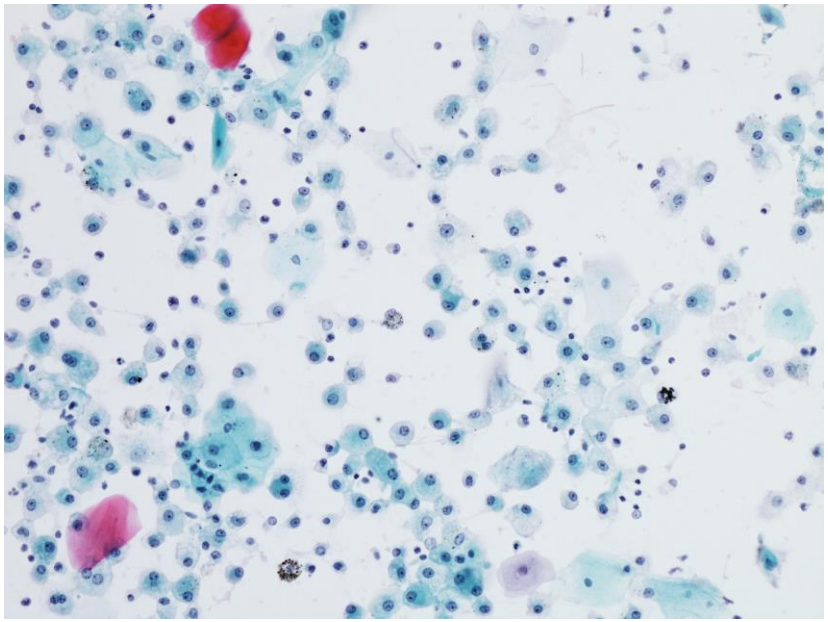


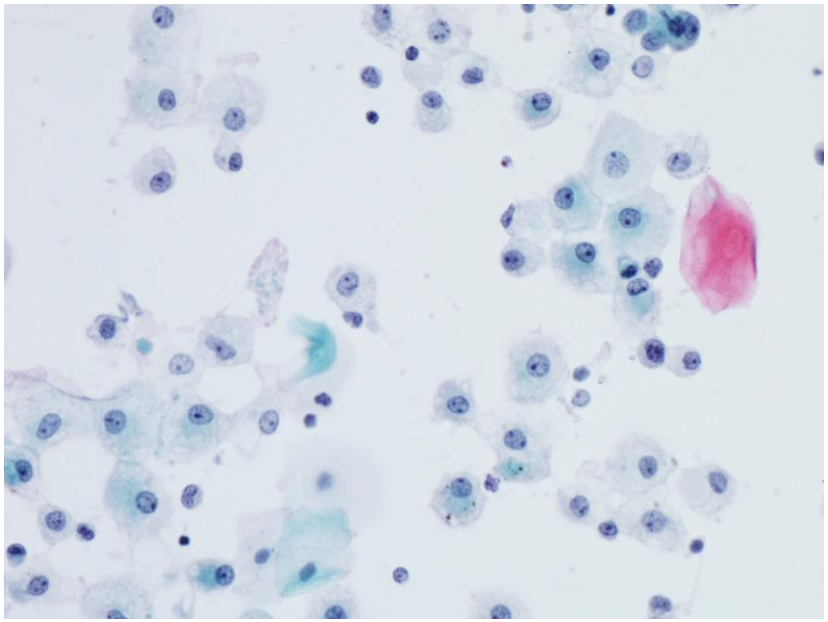




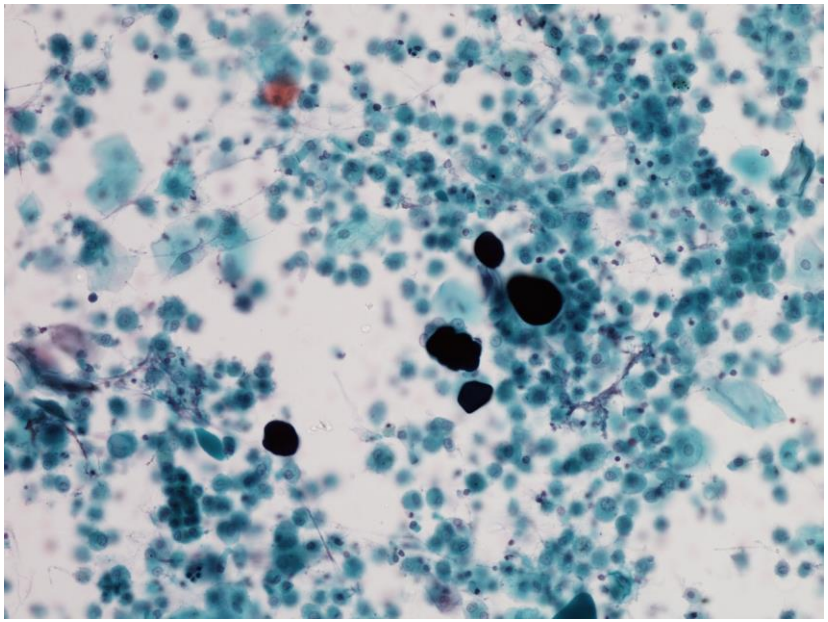



# Mucinous adenocarcinoma ('BAC')

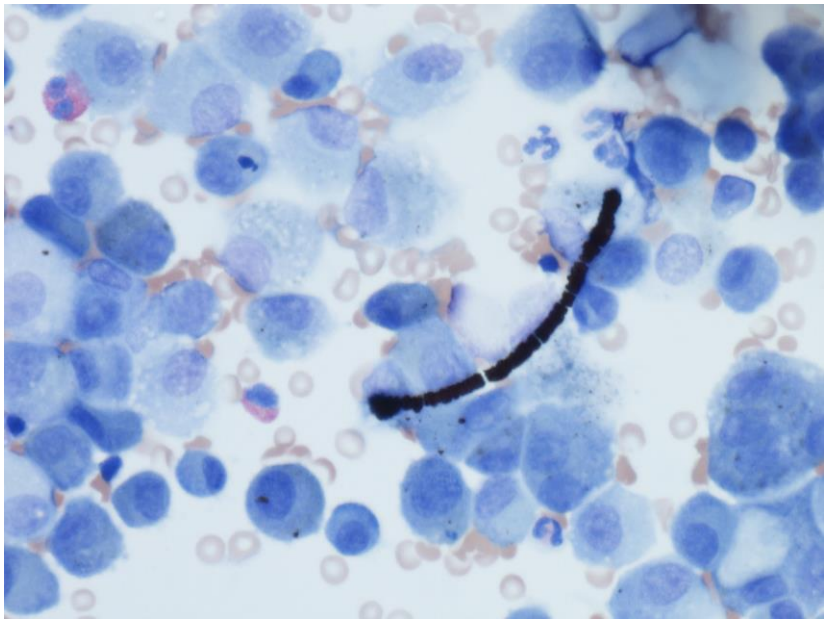




# Other features



Microlithiasis



Asbestos bodies

# EBUS TBNA

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# EBUS TBNA

**Endoscope**

**Real time imaging needle sample**

# ROSE – Rapid on-site evaluation

- Advantages
  - Assessment of adequacy (esp for new operators)
  - Triage specimens (micro, flow cytometry)
  - Rapid diagnosis and progression to treatment

- Disadvantages
  - False -/+ material
  - Use some material making air dried slide
  - Pathologist time and resources
  - Some studies show no significant improvement in samples

# Criteria for Adequacy - LN

**Lymph node sample**

- Abundant lymphocytes or lymphohistiocytic aggregates
- More lymphocytes than expect from blood alone (TBNA samples quite bloody)

**But NO agreed gold standard**

- Presence lymphoid cells, TBM or anthracotic macrophages
- >40L/hpf or >5lpf with >100L

# Criteria for Adequacy - Lung

**Lung sample**

- Assessment of adequacy much harder
- “negative” vs “non-diagnostic”
- Sparsely cellular pneumocytes, alveolar macrophages may not be representative. Don’t forget peritumoural lymphoid or granulomatous reactions.

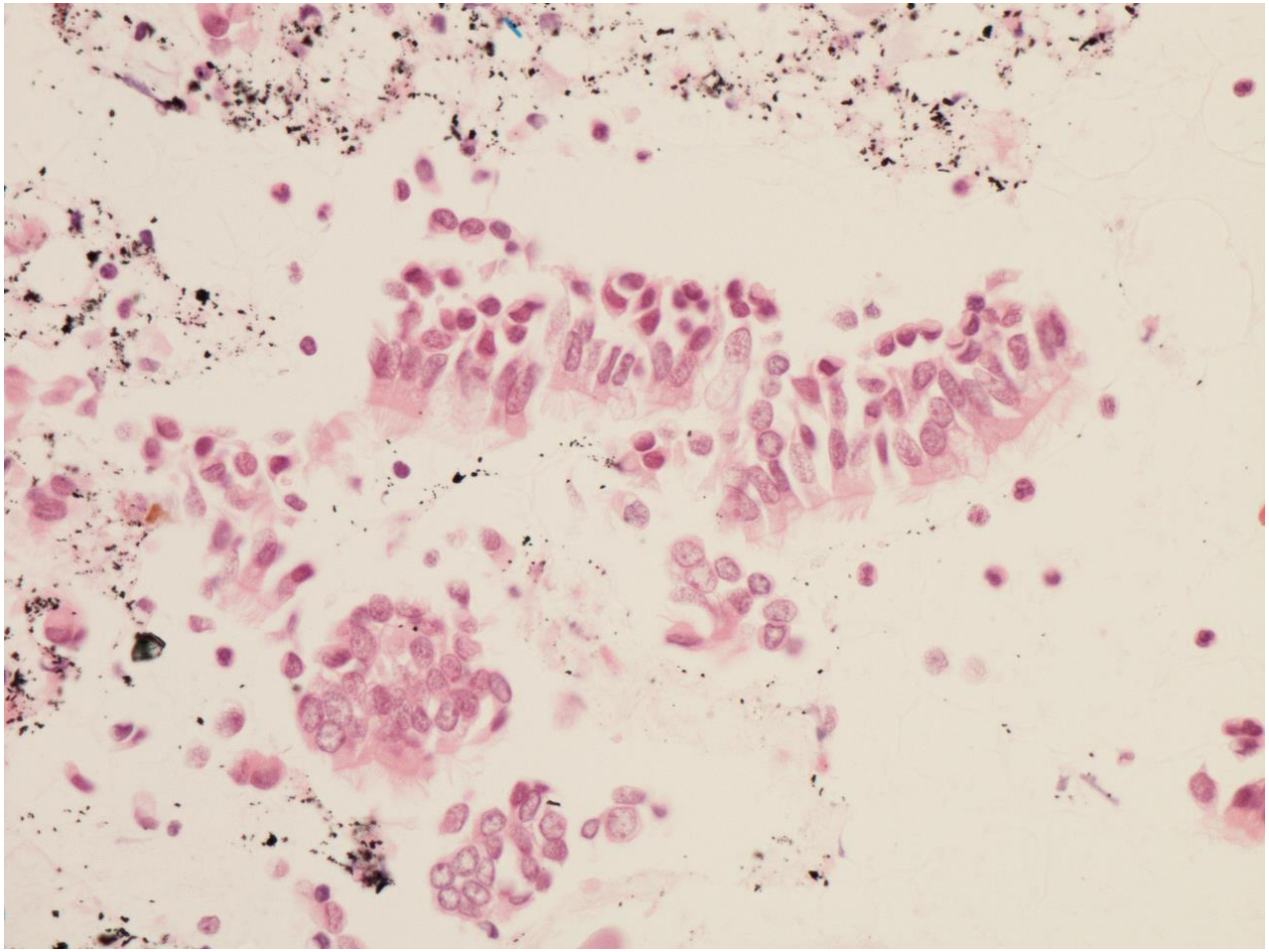
- ***Qu: Does the cellular or acellular material present explain the radiographic and clinical characteristics of the targeted lesion?***

- If not: Non-diagnostic

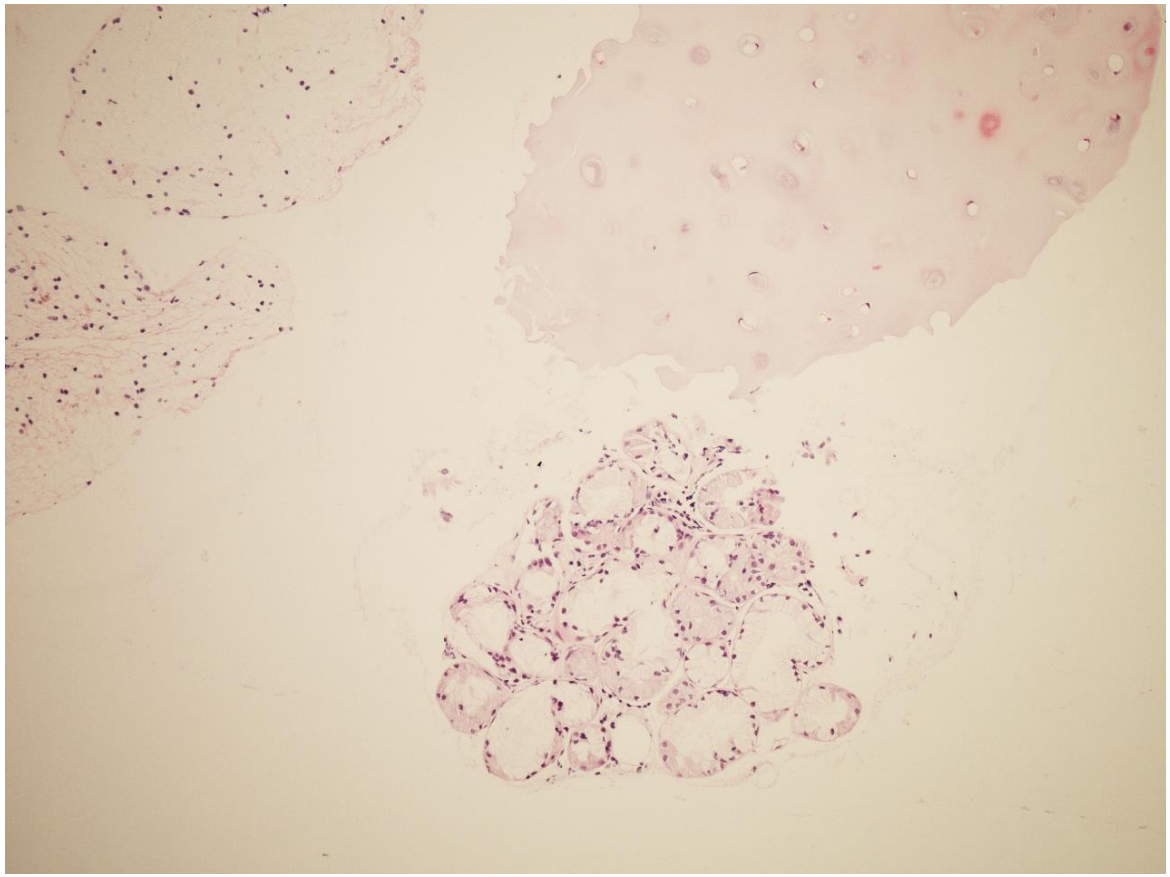
# Cellular contents

- Lymph node sample: Lymphocytes, follicle fragments, TBM, anthracotic macrophages
- Bronchial wall: Ciliated respiratory epithelial cells, goblet cells, metaplastic cells, seromucinous glands, cartilage and mesothelial cells
- Lung: Pneumocytes, alveolar macrophages

# Clumps of reactive bronchial epithelial cells with reserve cell hyperplasia



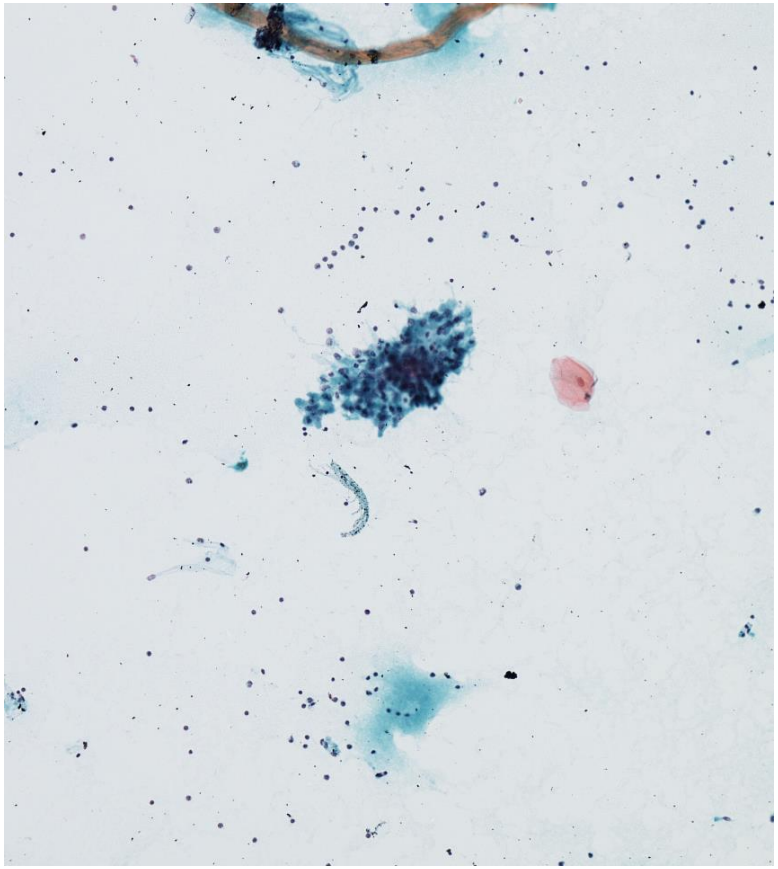
# Cartilage and seromucinous glands

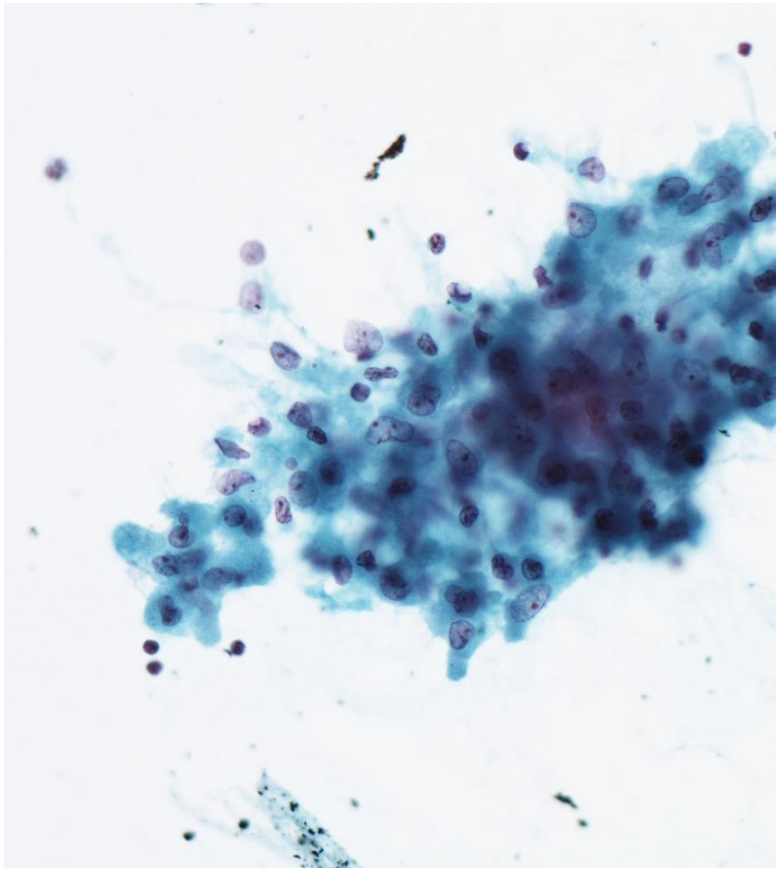


# EBUS – Pathology Non-neoplastic disease

- Granulomatous disease
  - Sarcoid, TB, other infections
    - Granulomas
      - Necrosis, suppuration
    - Cell block > cytospin preparations
    - Special stains; ZN, grocott

# Granuloma





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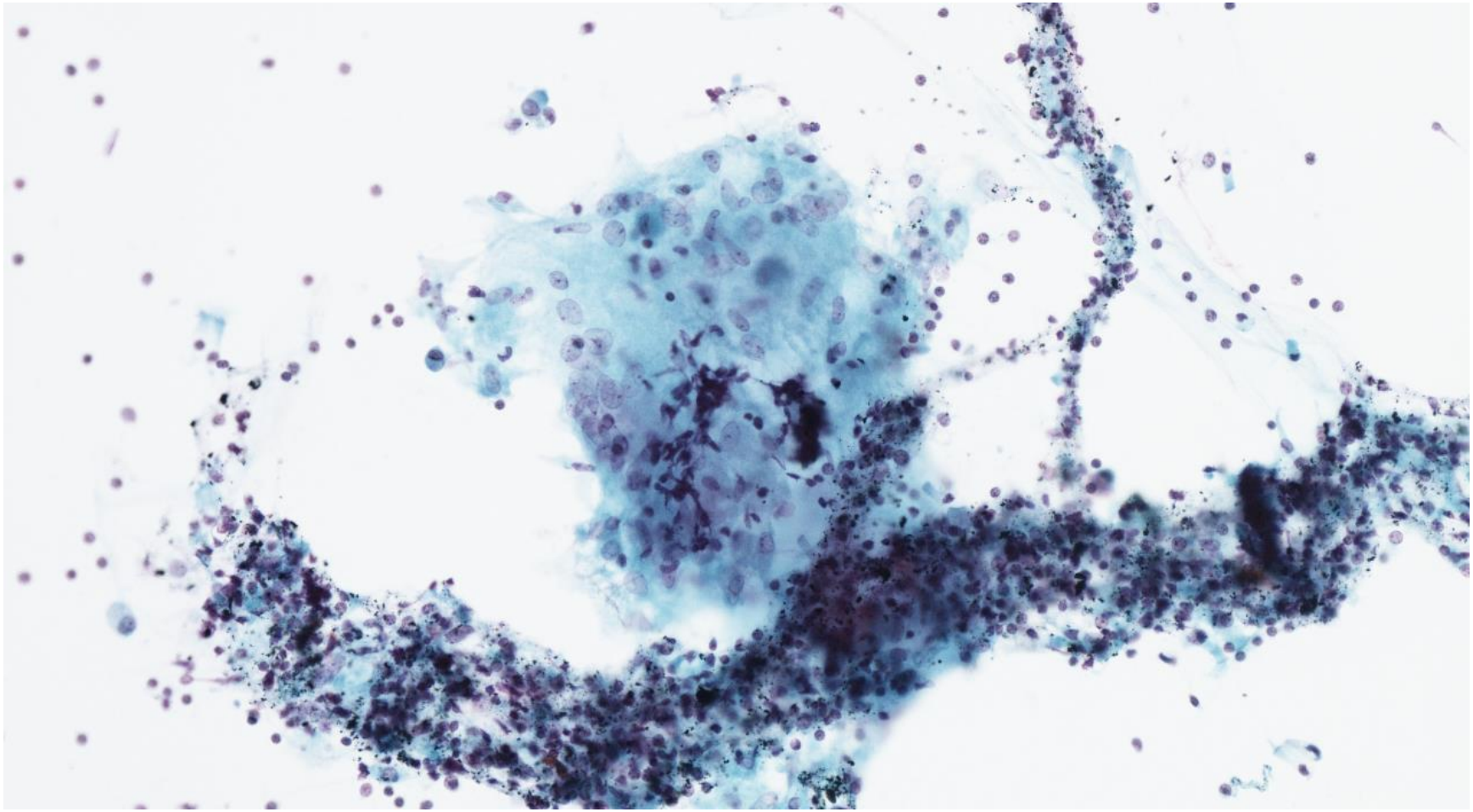
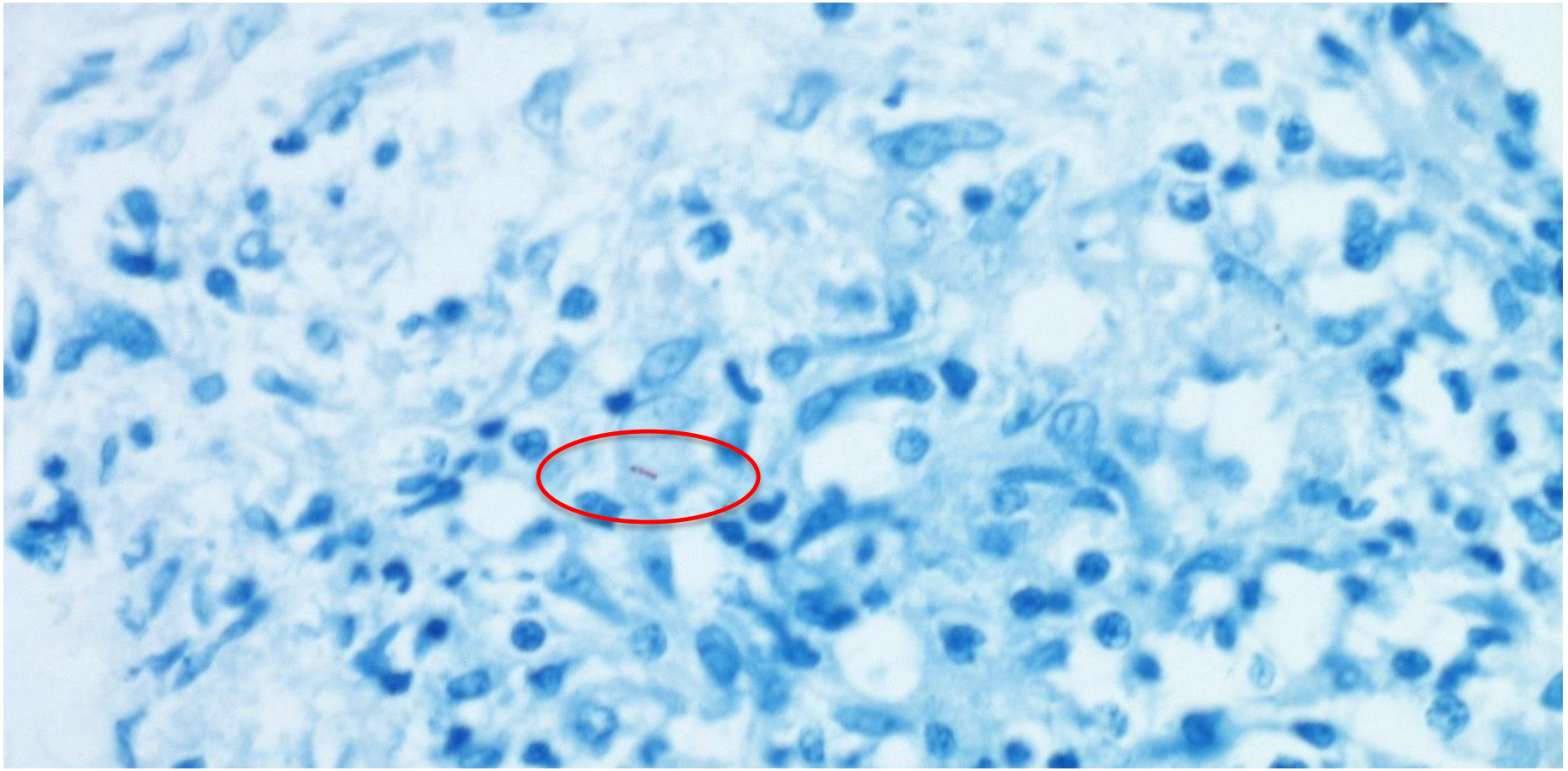




Cell pellet

# EBUS - Pathology
# Neoplastic lung disease

Sub-typing non-small cell lung cancer

Molecular techniques

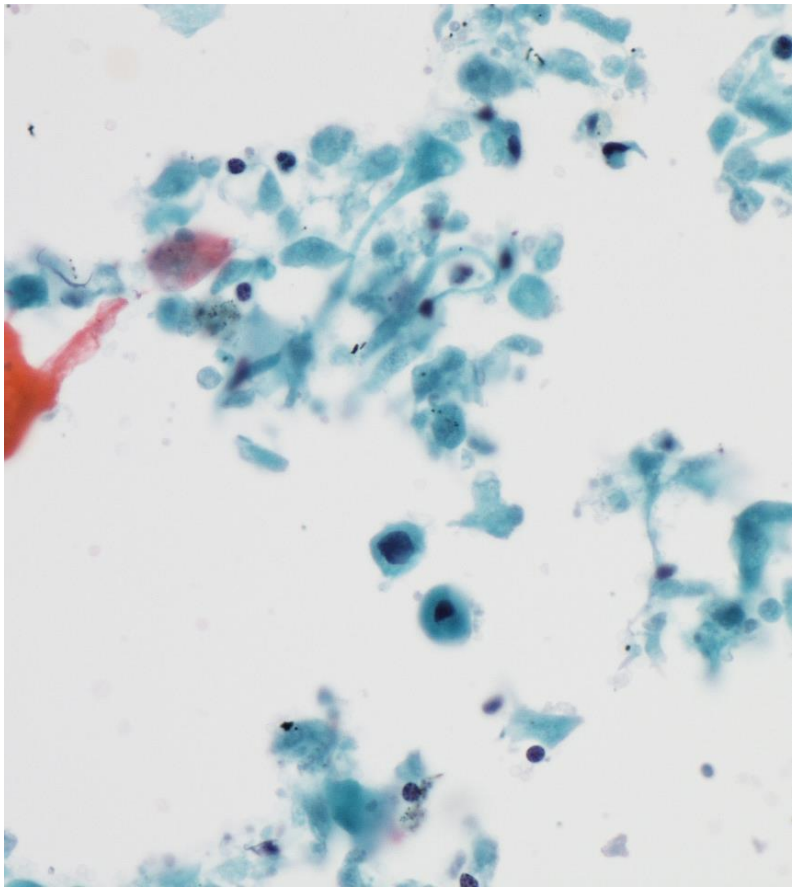
Diagnostic pitfalls

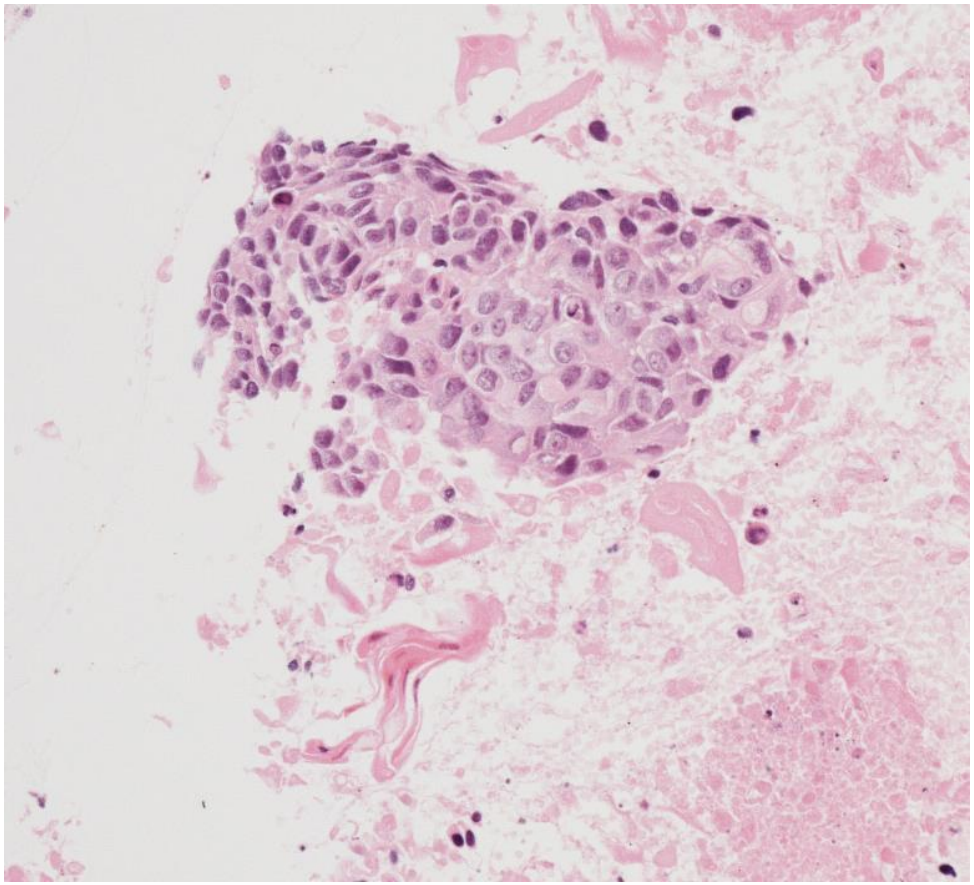
# Sub-typing NSCLC

- Some studies 85% of cases classified as adeno or squamous using smears, cell pellet and immunohistochemistry

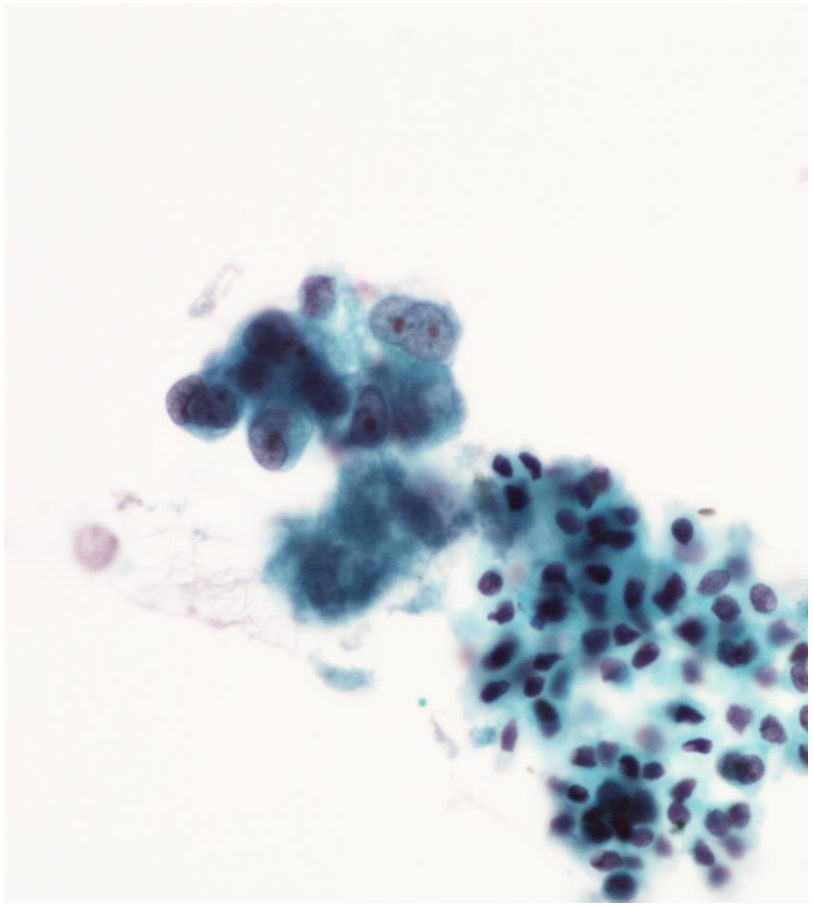
- NSCLC NOS
  - Immuno: TTF-1, CK5/6, p63, p40

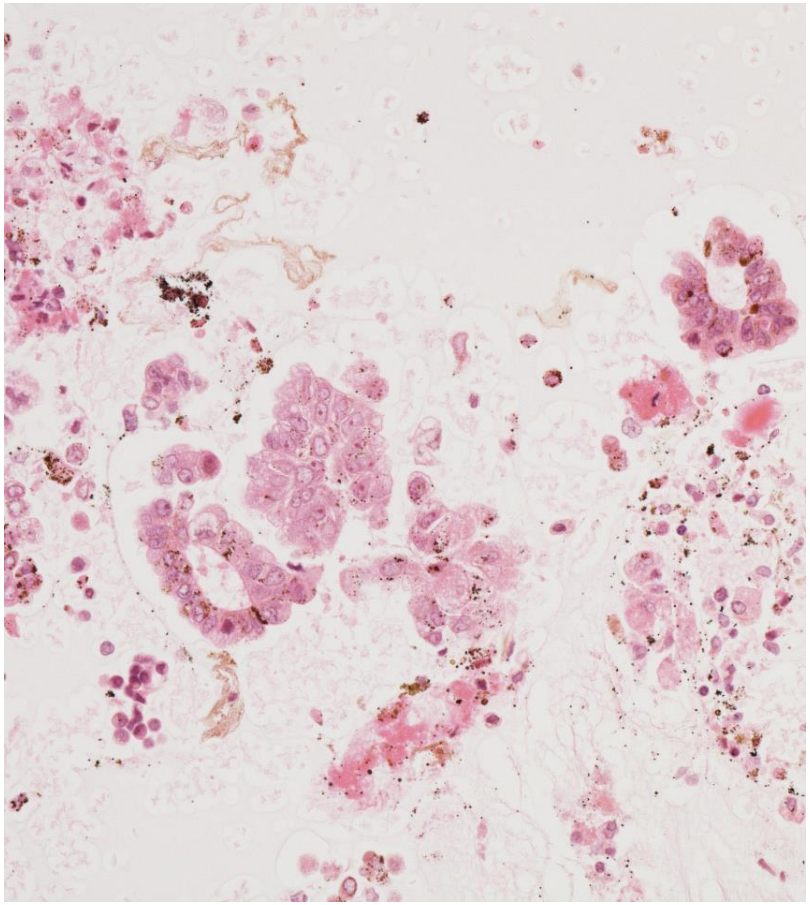
# Metastatic Squamous cell ca





# Metastatic adenocarcinoma





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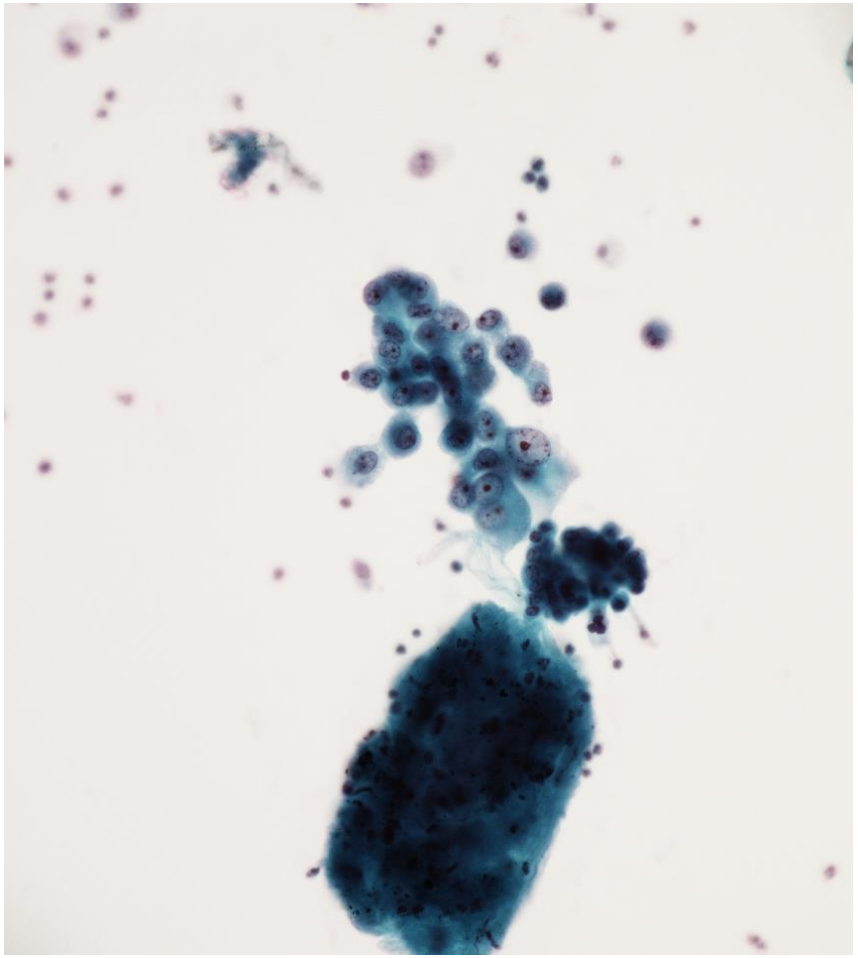
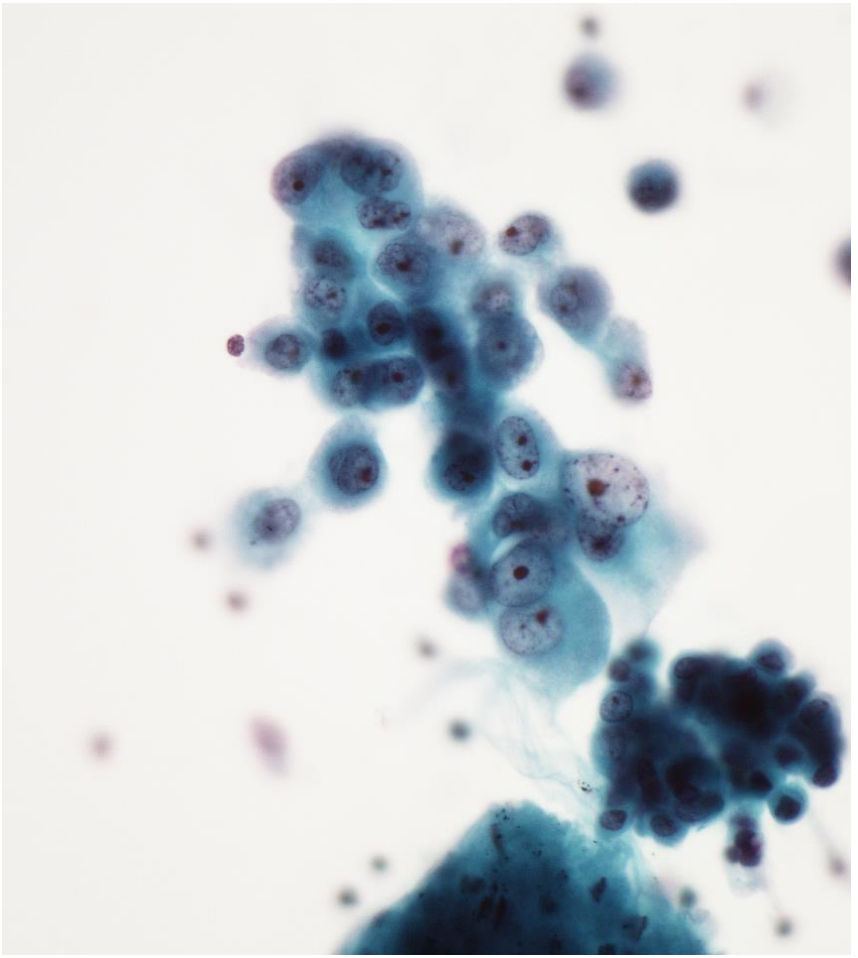
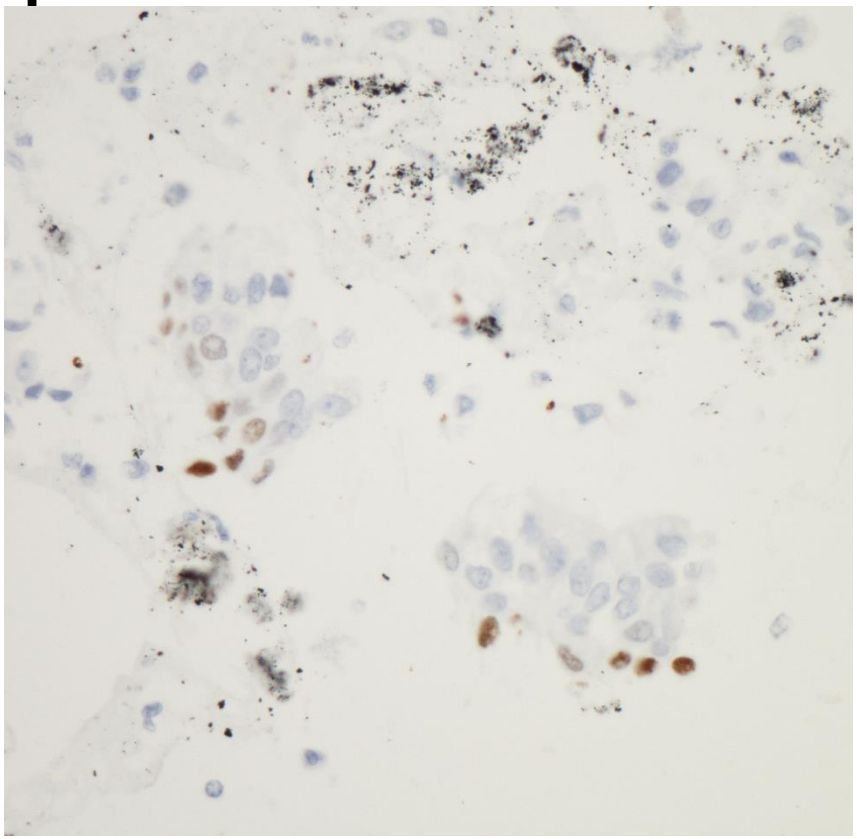
# EBUS TBNA LN - NSCLC



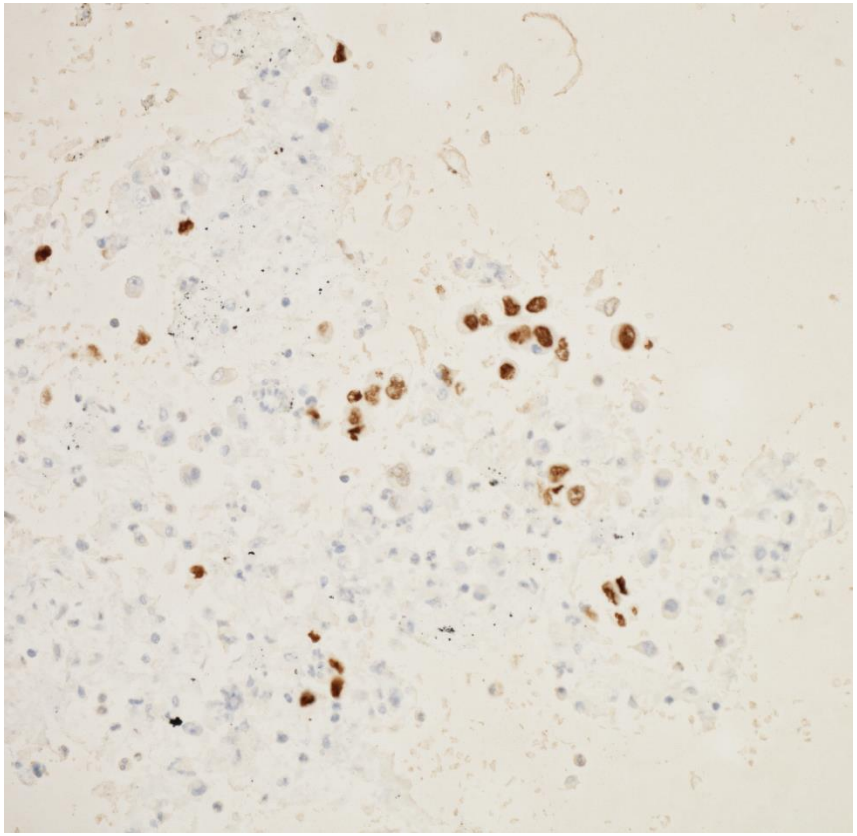


# Immunohistochemistry

**p40**



**TTF-1**



Adenocarcinoma

# Molecular Techniques in Respiratory Cytology

# Molecular analysis

- Suitable for analysis of molecular changes including EGFR, KRAS and ALK
- Block sent to molecular diagnostics laboratory – 5 day turnaround
- Studies in literature show successful testing of at least 1 gene target in 72-98% of cases using both FFPE cell block and air dried slides.

# Do More With Less

## Tips and Techniques for Maximizing Small Biopsy and Cytology Specimens for Molecular and Ancillary Testing: The University of Colorado Experience

*Dara L. Aisner, MD, PhD; Mathew D. Rumery, MD; Daniel T. Merrick, MD; Kimi L. Kondo, DO; Hala Nijmeh, PhD; Derek J. Linderman, MD; Robert C. Doebele, MD, PhD; Natalie Thomas, MPhil; Patrick C. Chesnut, BA; Marileila Varella-Garcia, PhD; Wilbur A. Franklin, MD; D. Ross Camidge, MD, PhD*

**Table 3. Success Rate of Molecular Testing Based on Cumulative Tumor Cell Count**

| No. Cumulative Tumor Cells[a] | No. of Cases | Molecular Success Rate,[b] % |
|---|---|---|
| >1000 | 11 | 100 |
| >500–1000 | 6 | 100 |
| >100–500 | 25 | 100 |
| >50–100 | 6 | 100 |
| 10–50 | 3 | 66 |
| <10 | 3 | 33 |
| ND[c] | 13 | 79 |

Abbreviations: H&E, hematoxylin-eosin; ND, not determined.

[a] As determined by evaluation of 1 H&E slide per block for all blocks in the case.

[b] Molecular success determined by successful completion of at least 1 molecular test.

[c] All representative H&Es slides were required to be available for evaluation of tumor cell content.

*Arch Pathol Lab Med.* doi: 10.5858/arpa.2016-0156-RA

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# Quality and Quantity Tissue Handling

- Problem
  - Increasing number of tests
    - Multiple genes: mutations, deletions, amplifications chromosomal rearrangements
    - Companion IHC diagnostics
  - Smaller samples

- Workflows for tissue optimisation (quantity material and quality DNA)

## Clinicians!

- Multiple passes – separate sample containers
- ROSE
- Indicate “Molecular Priority Sample”

## Processing

- Cytology preparation techniques
  Dual sample preparation: cell block and centrifuged specimen for storage
- Minimise cell block trimming & save trimmings

## Examination

- Minimal diagnostic IHC panel: p40 TTF-1

## Post examination

- Microdissection, laser capture

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# Preanalytic Variables in Cytology

## Lessons Learned From Next-Generation Sequencing—The MD Anderson Experience

*Sinchita Roy-Chowdhuri, MD, PhD; John Stewart, MD, PhD*

**Table 2. Advantages and Disadvantages of the Cytologic Substrates Commonly Used for Molecular Testing**

| Cytologic Substrate | Advantages | Disadvantages |
|---|---|---|
| Direct smear[a] | • Immediate assessment for adequacy<br>• High-quality nucleic acid<br>• Whole cells with whole nuclei<br>• Superior tumor mapping in samples with low tumor fraction | • Sacrificing slide from archival material (potential medicolegal issues)<br>• Additional validation |
| Liquid-based cytology[b] | • Standardized processing with optimal preservation of cells<br>• Whole cells with whole nuclei<br>• High-quality nucleic acid | • Lack of immediate assessment<br>• Additional validation<br>• Nucleic acid retrieval may be variable based on preservative/fixative |
| Cell block[c] | • Ease of acquisition<br>• Multiple serial sections<br>• Standardized validation in most molecular laboratories | • Lack of immediate assessment<br>• Frequently suboptimal cellularity<br>• Nucleic acid may be suboptimal because of formalin fixation<br>• Partial nuclei on standard 4- to 5-micron sections |

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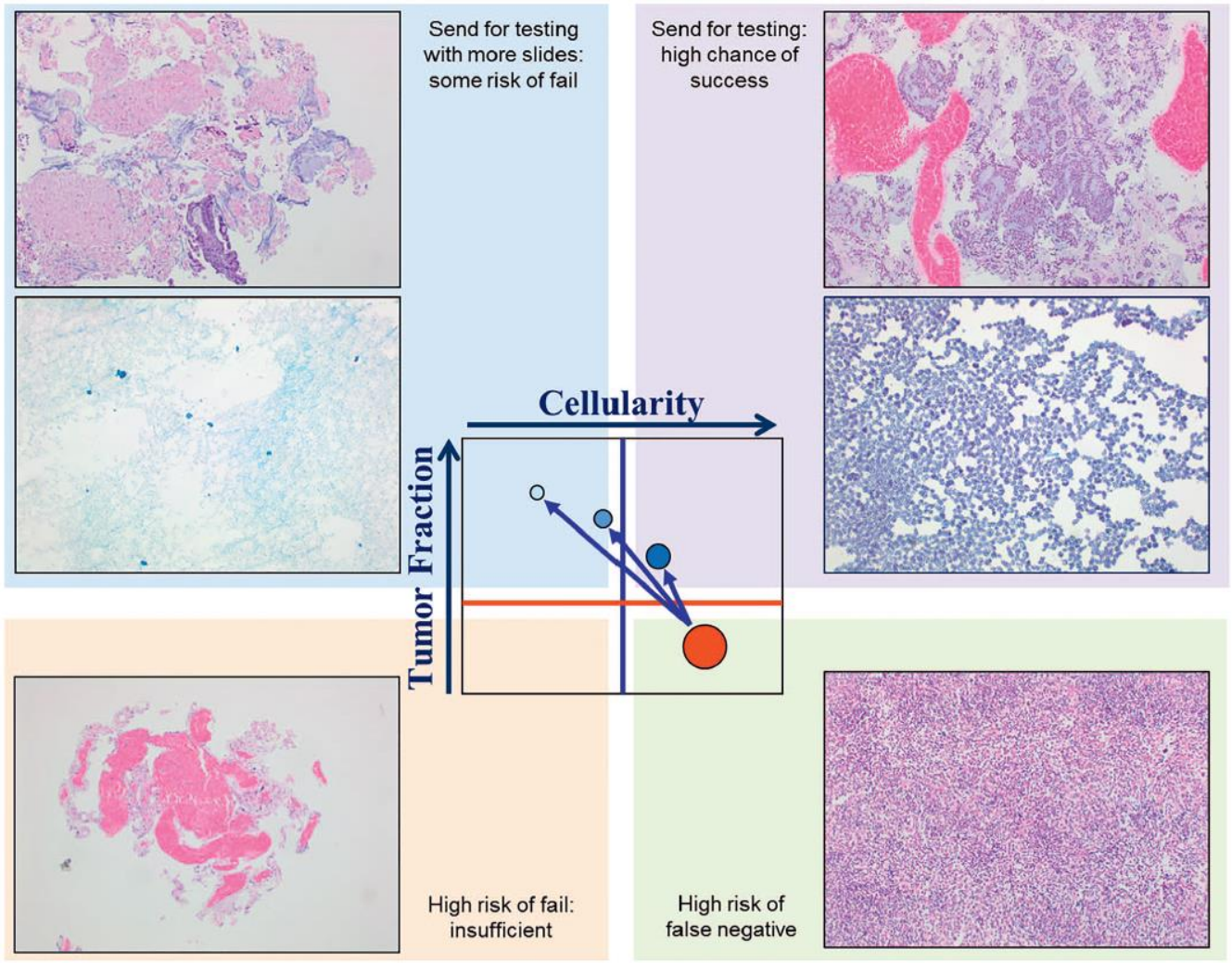
# Cellularity and tumour fraction



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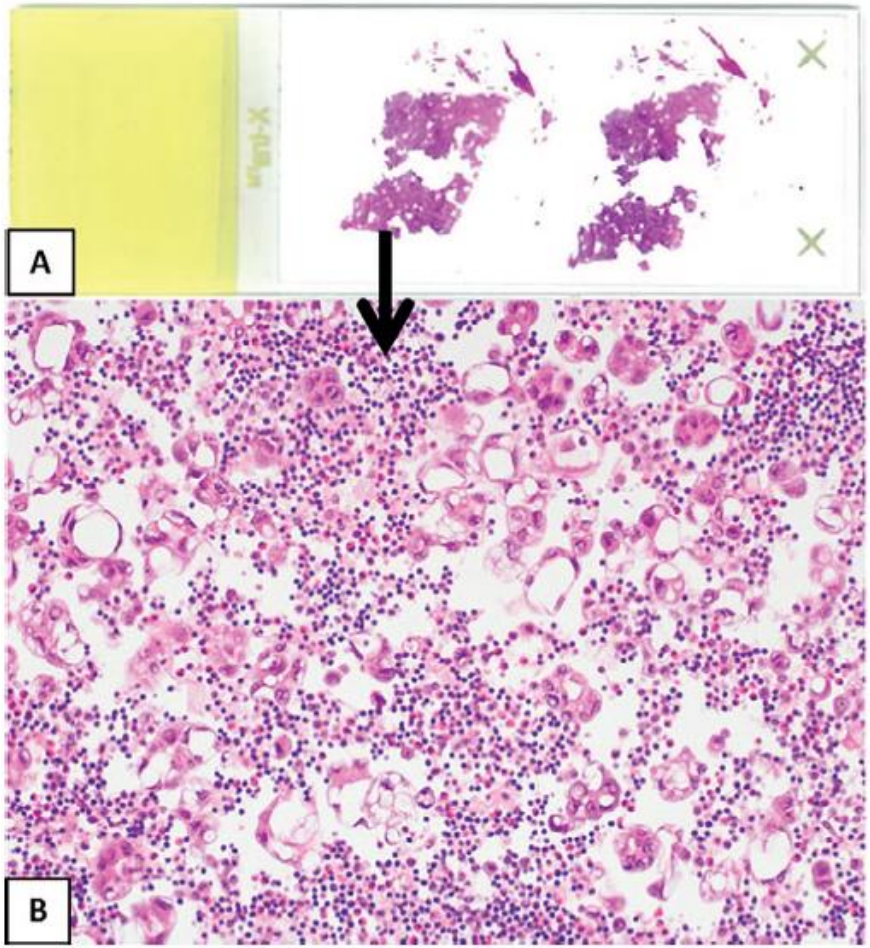
**Preanalytic Variables in Cytology**

**Lessons Learned From Next-Generation Sequencing—The MD Anderson Experience**

*Sinchita Roy-Chowdhuri, MD, PhD; John Stewart, MD, PhD*

# Tumour fraction assessment



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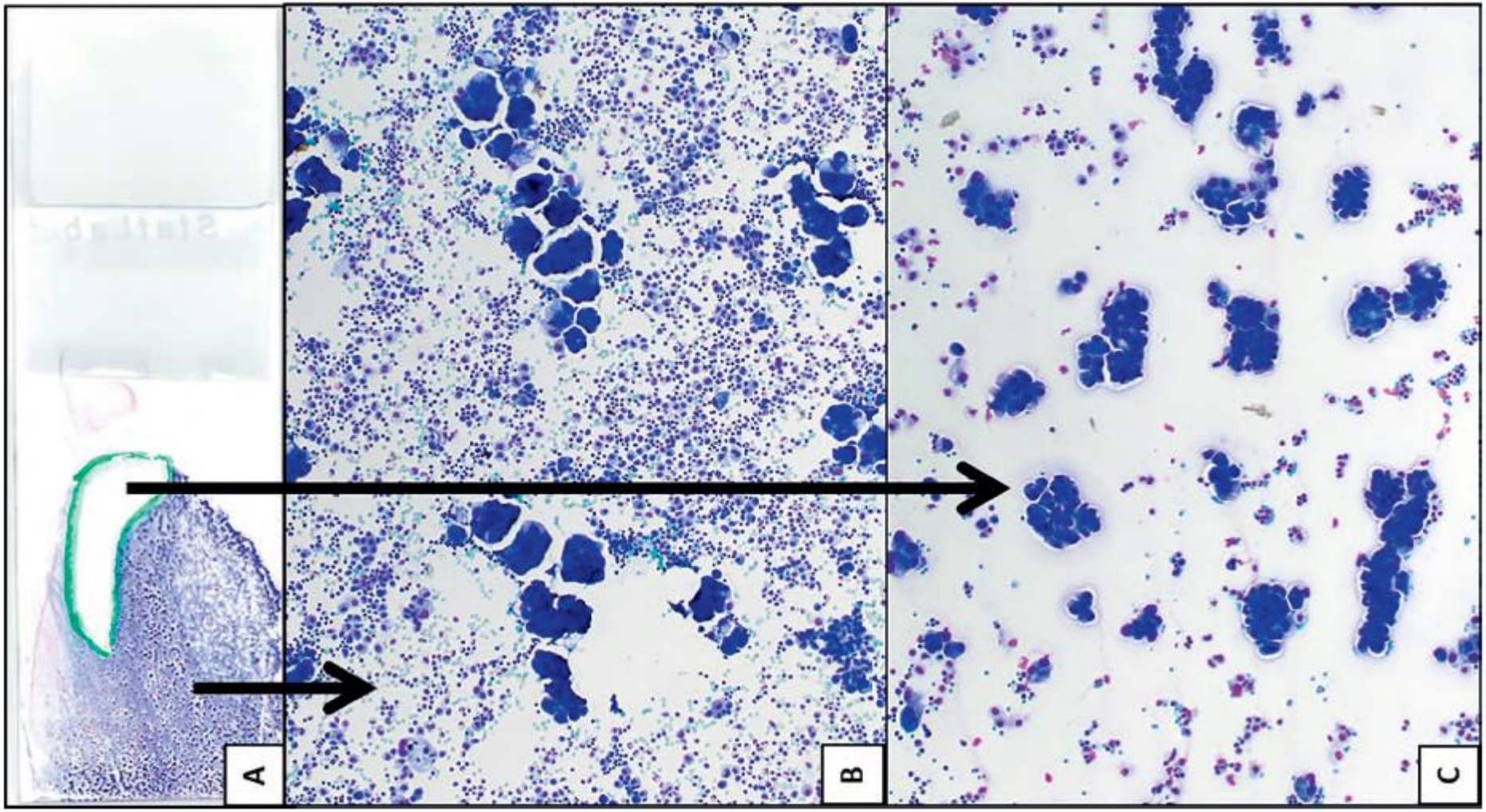

# Companion Diagnostics

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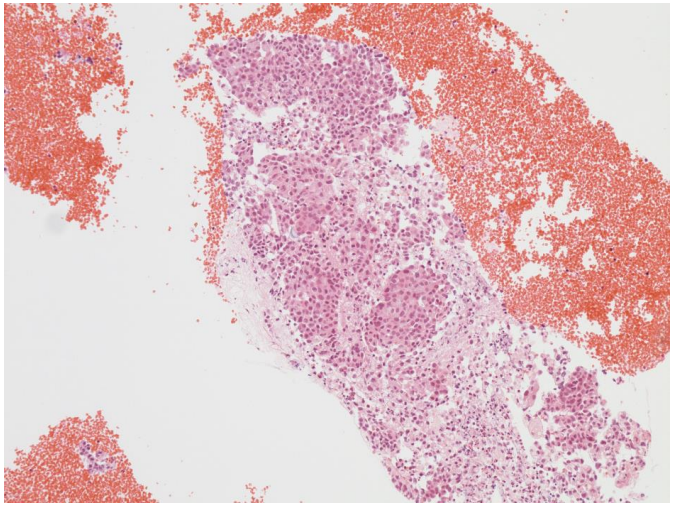
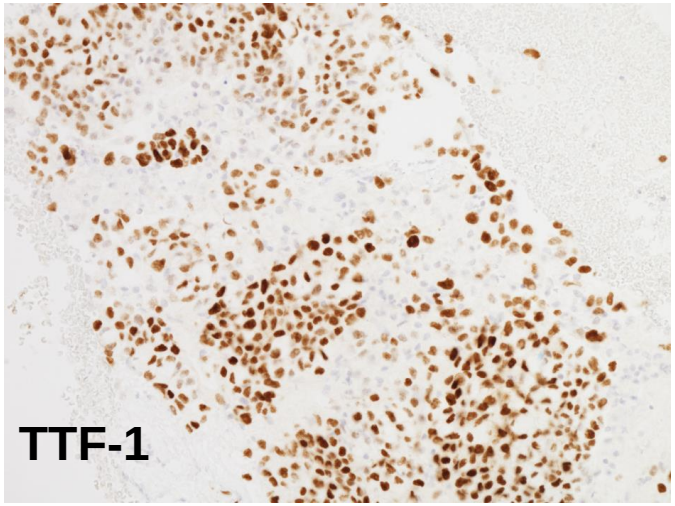
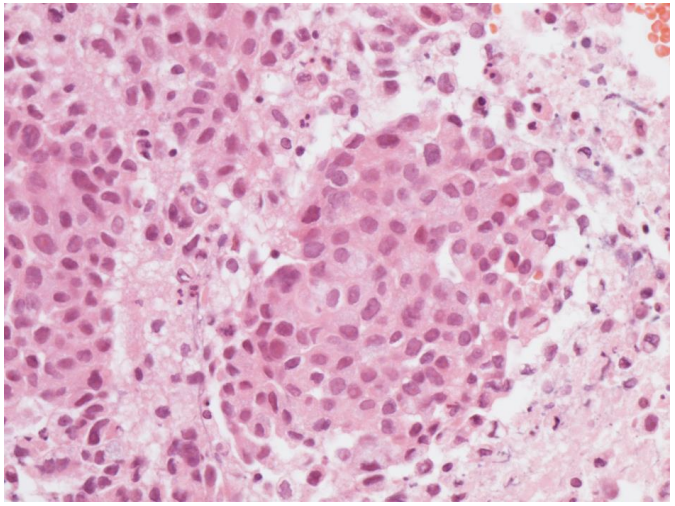
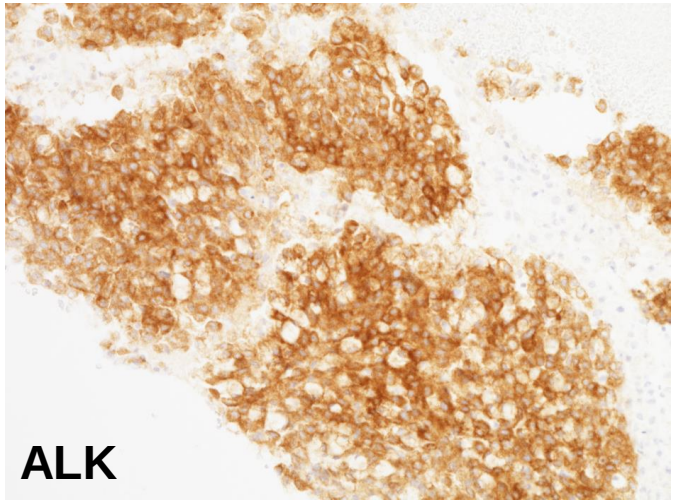
# ALK immunohistochemistry







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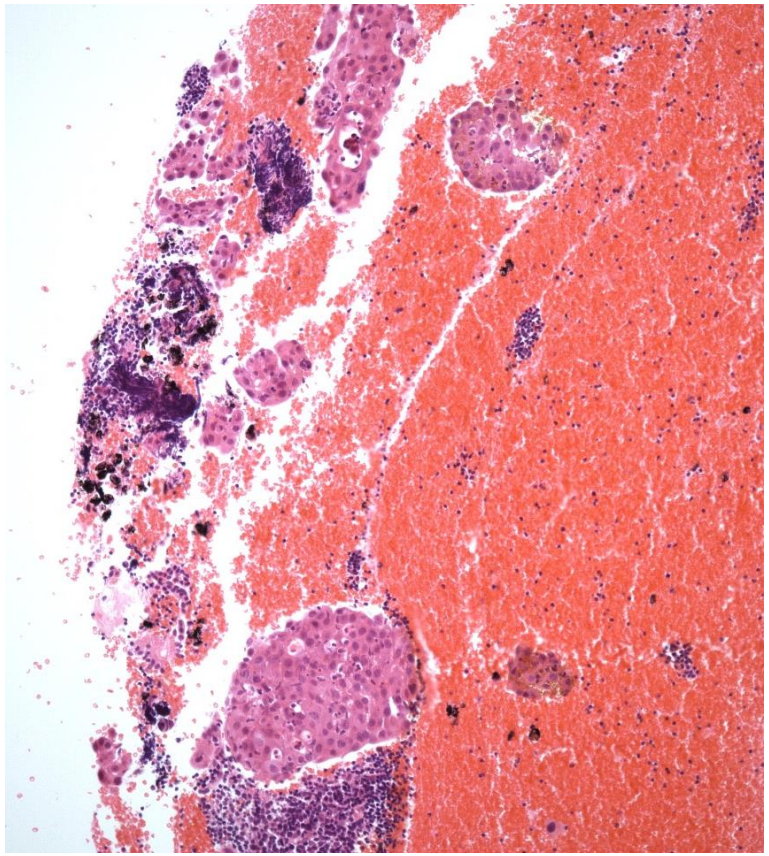
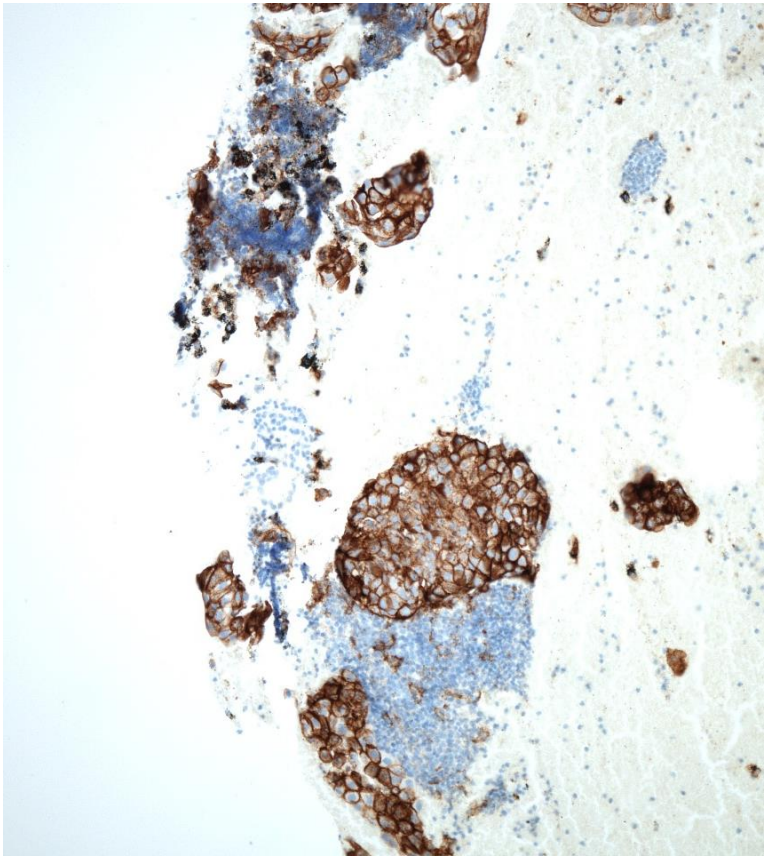
# PD-L1 Immunohistochemistry





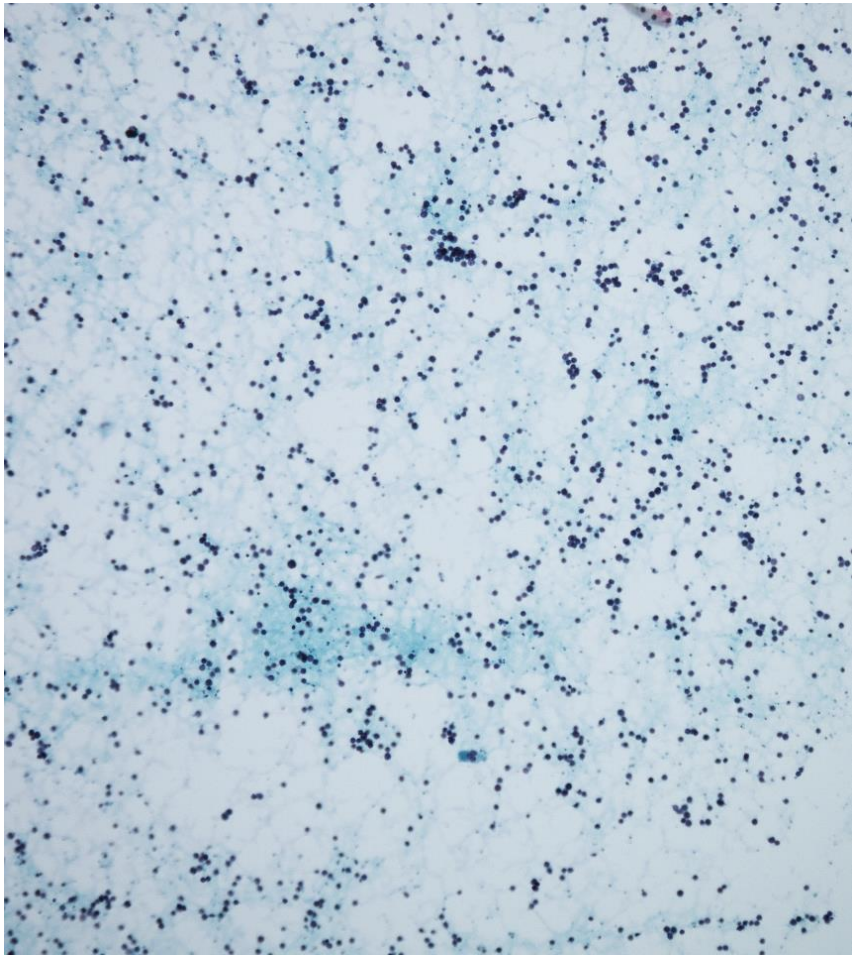
# Diagnostic Pitfalls

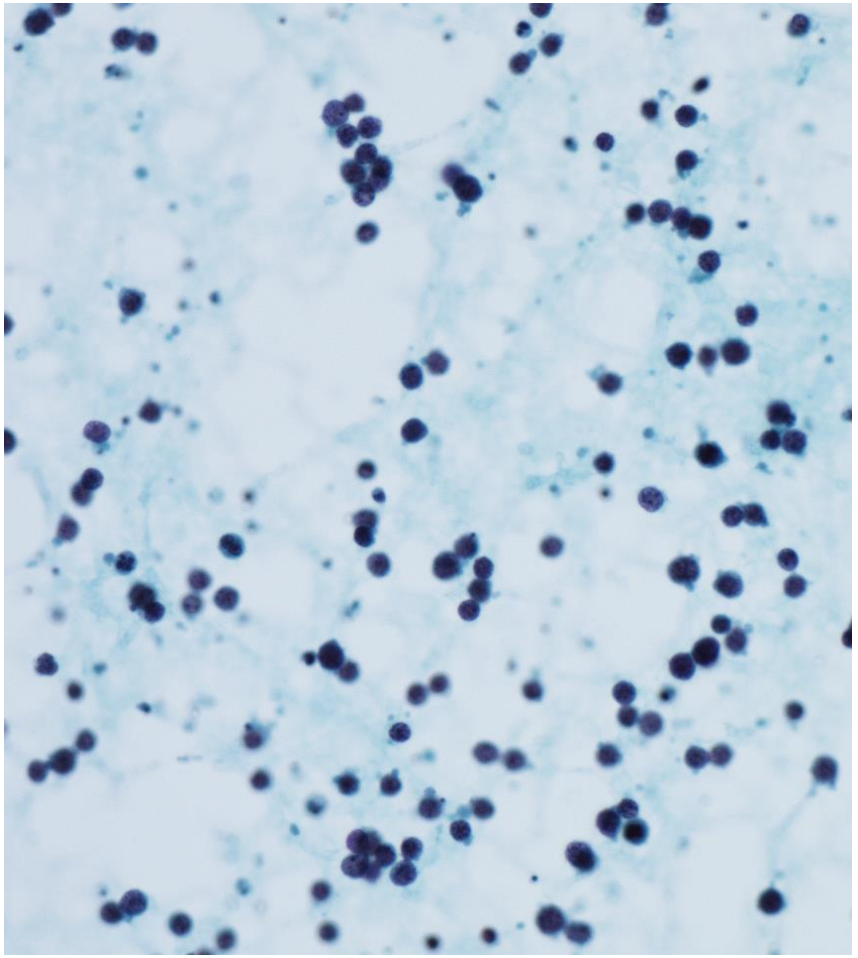
# EBUS Lymph nodes and lung masses

- Dispersed population tumour cells
- Metastases from extrathoracic malignancies
- Rare tumours
- Low cellularity & necrosis
  - Review at high power, beware cellular necrosis
- Reactive bronchial epithelial cells, goblet cells, seromucinous glands, mesothelial cells

# Dispersed tumour cells

# Originally reported as negative, lymphocytes





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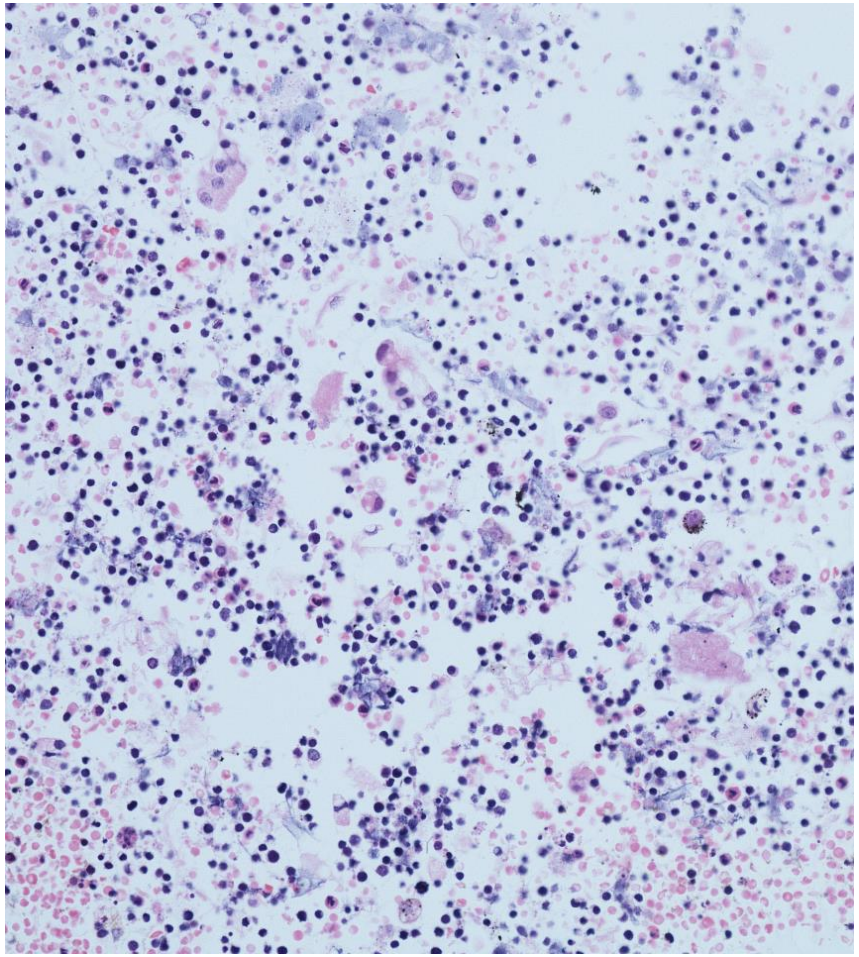
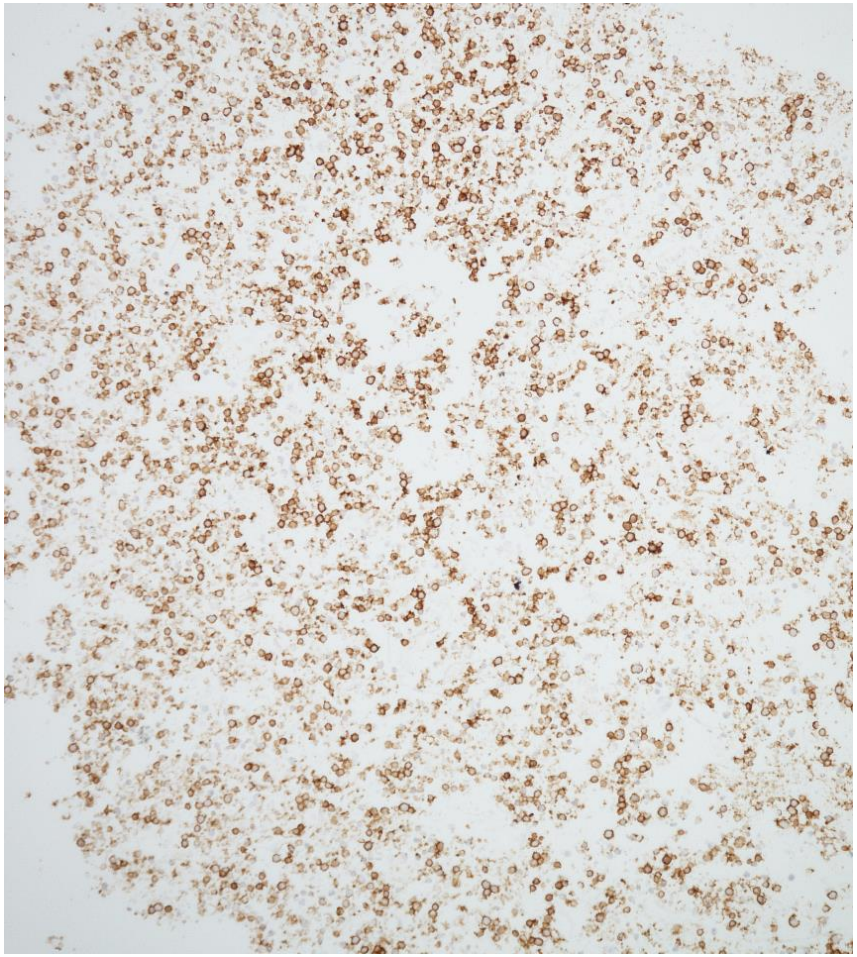
# Metastatic small cell carcinoma

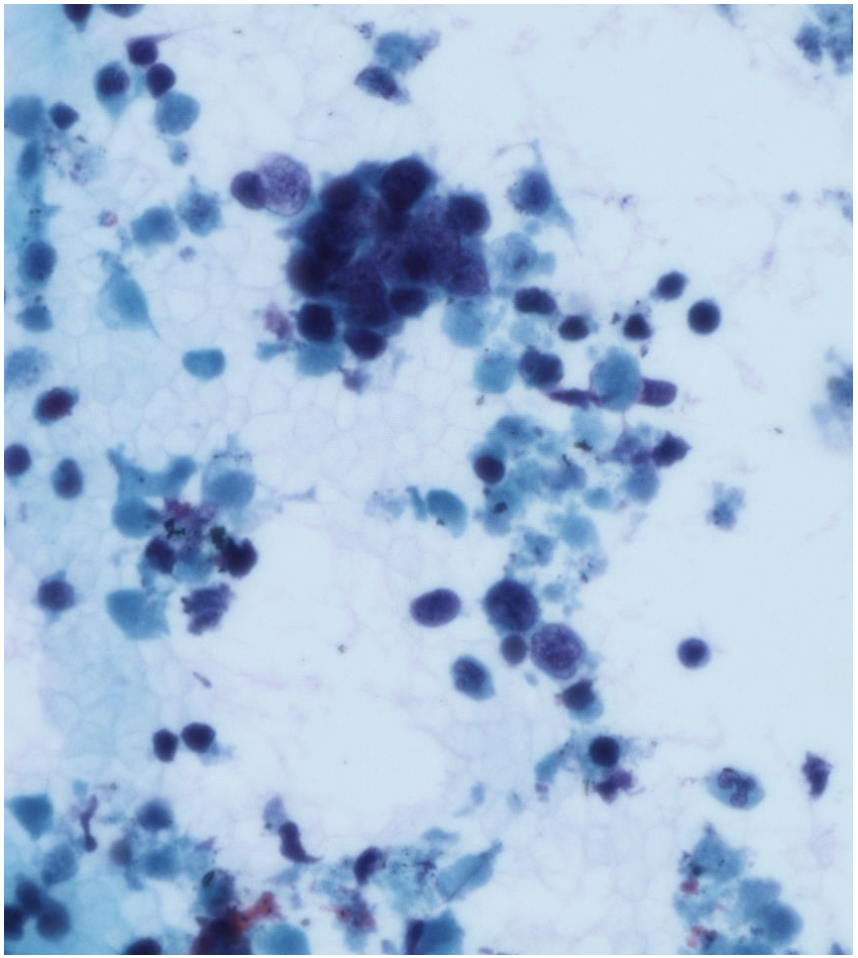


Cell pellet

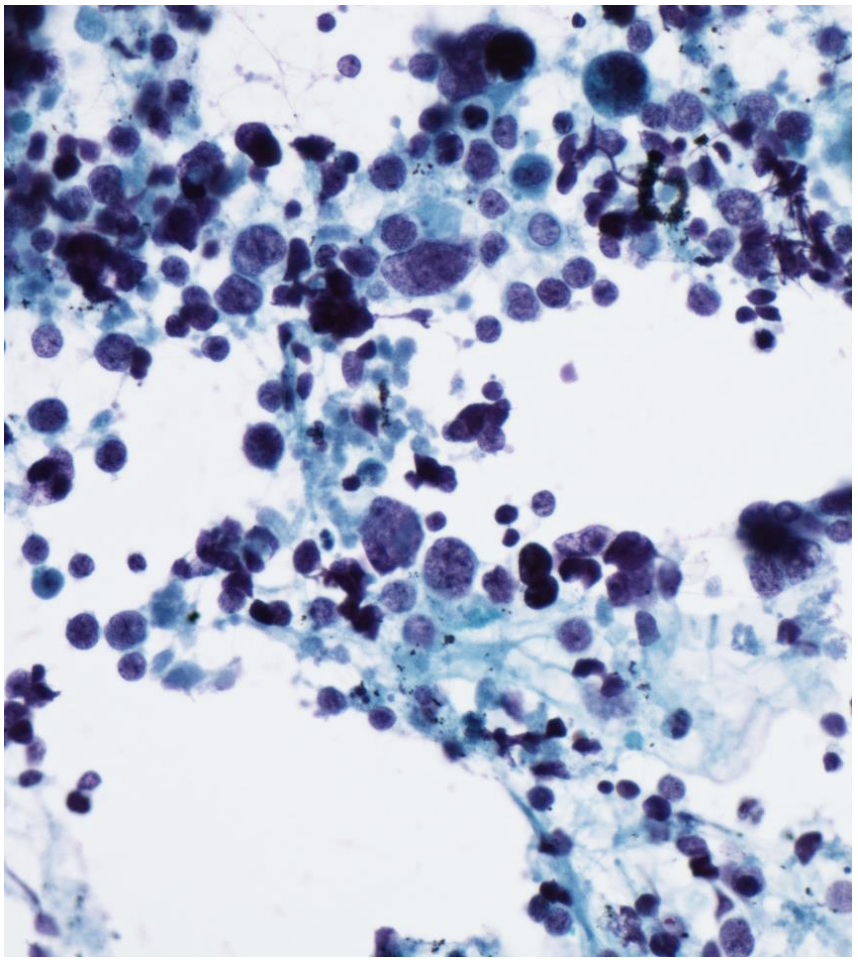


CD56

# More typical appearance Small cell carcinoma

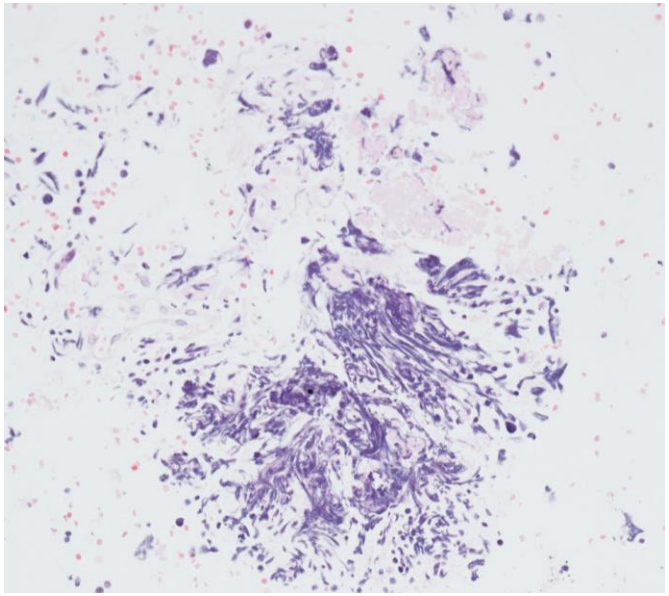


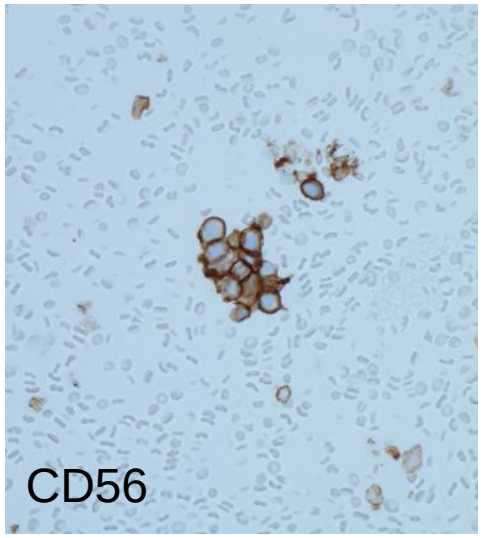
Necrosis

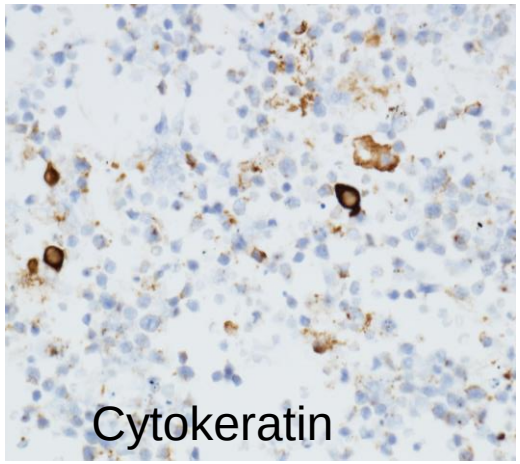


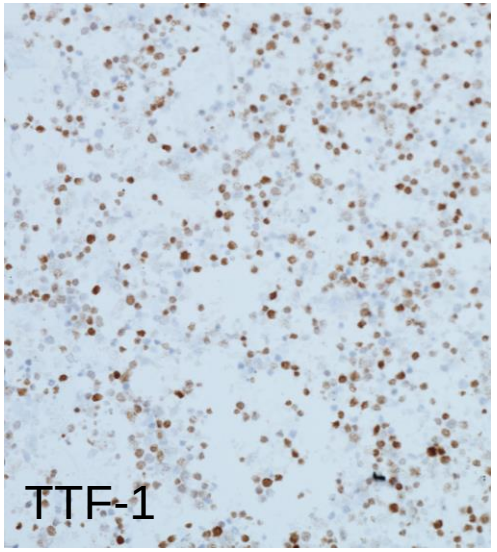
Scattered larger cells

# Small cell carcinoma

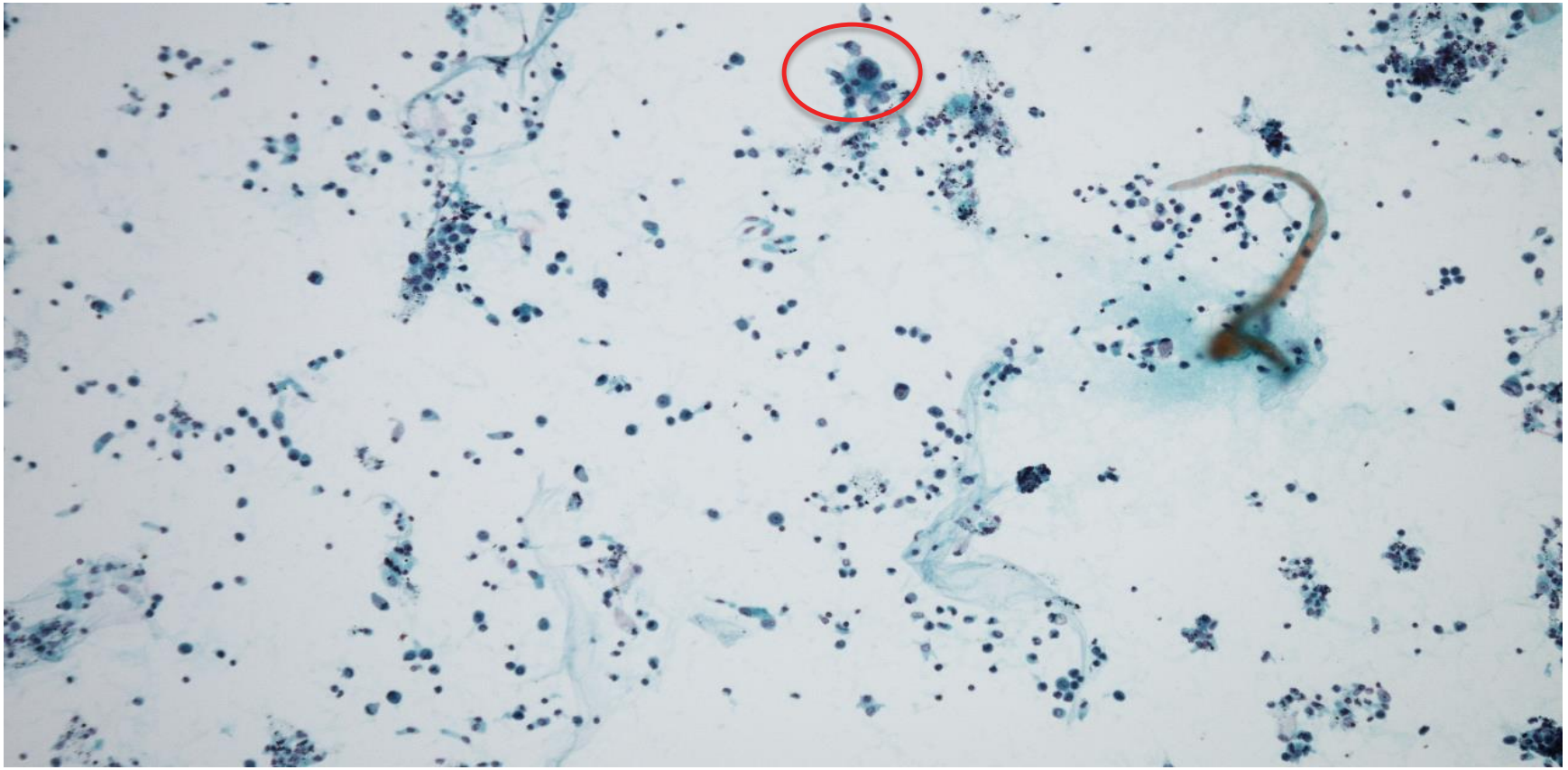




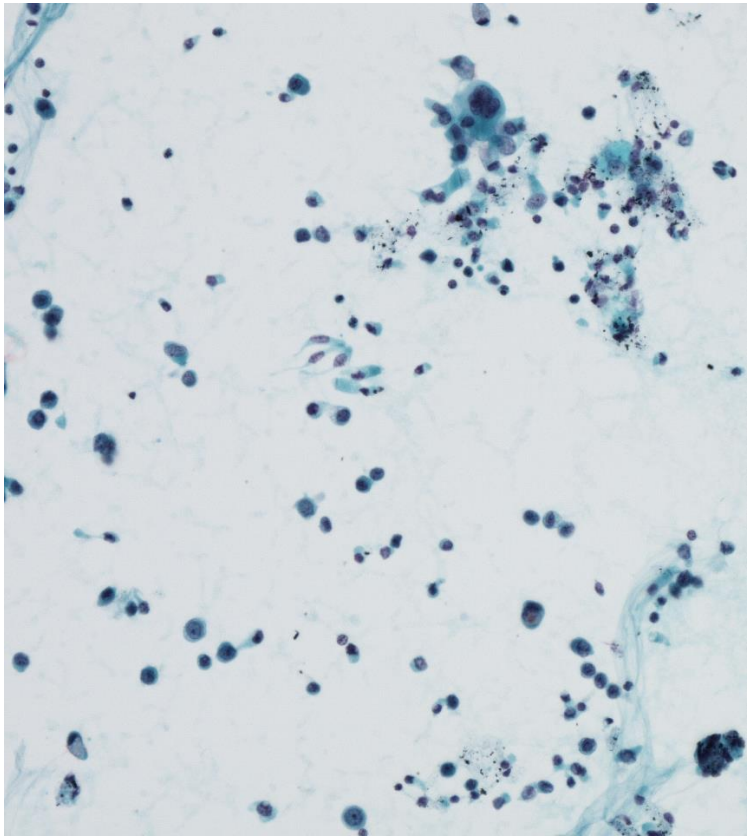



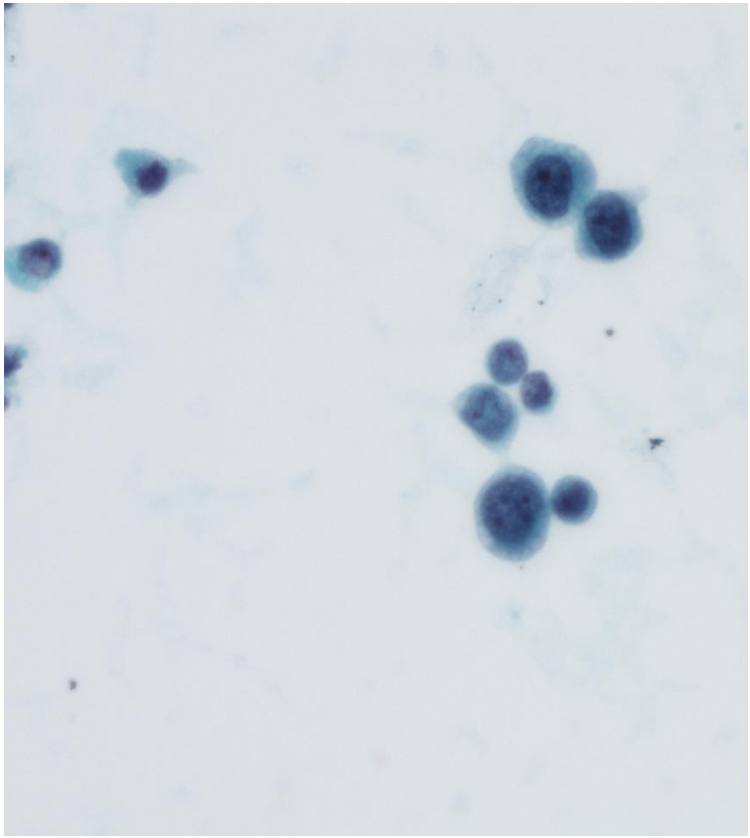



# ? Reactive lymph node



# High power review





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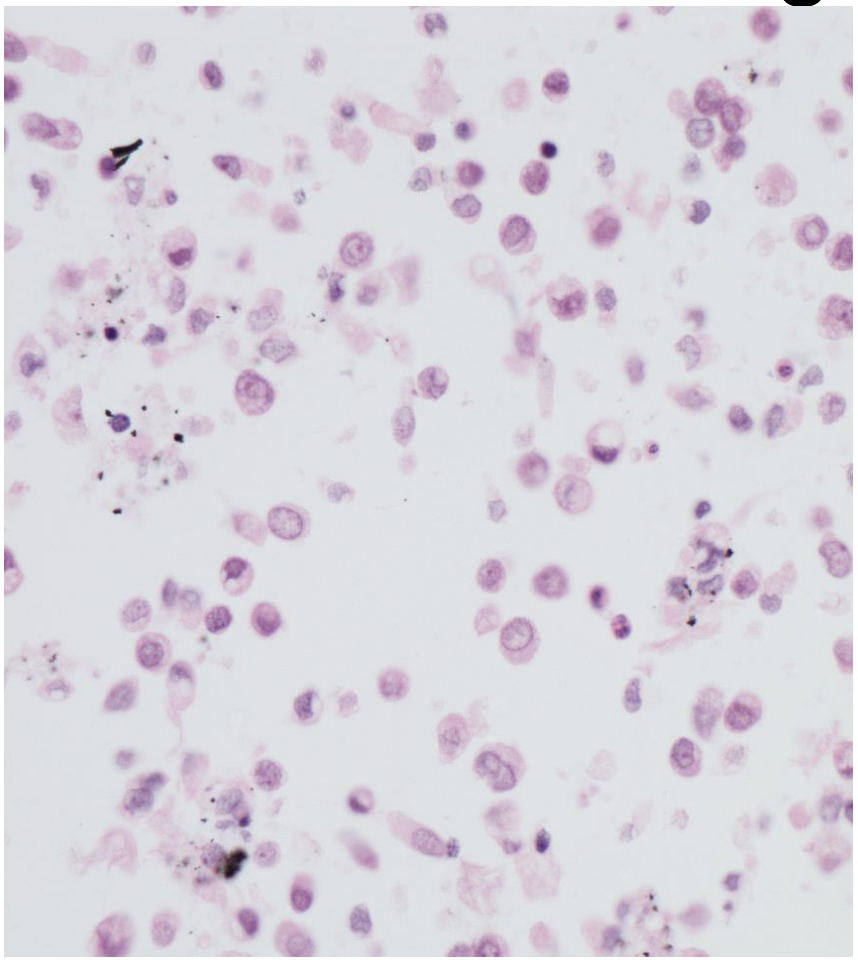
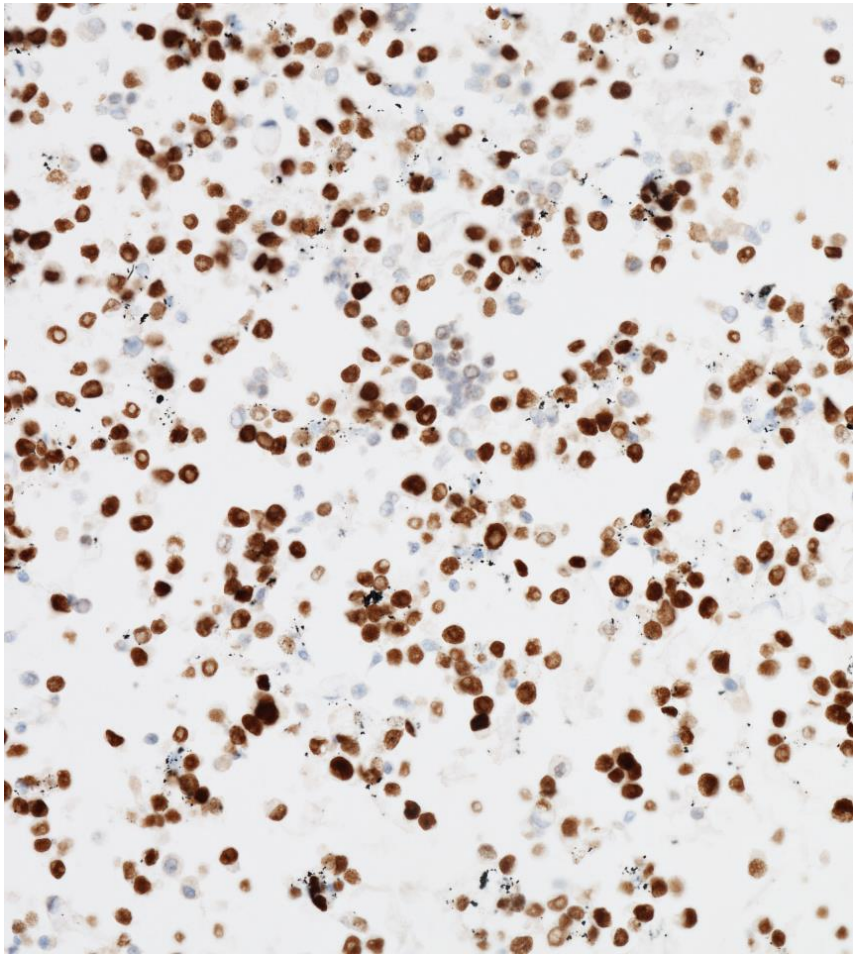
# Metastatic lung adenocarcinoma

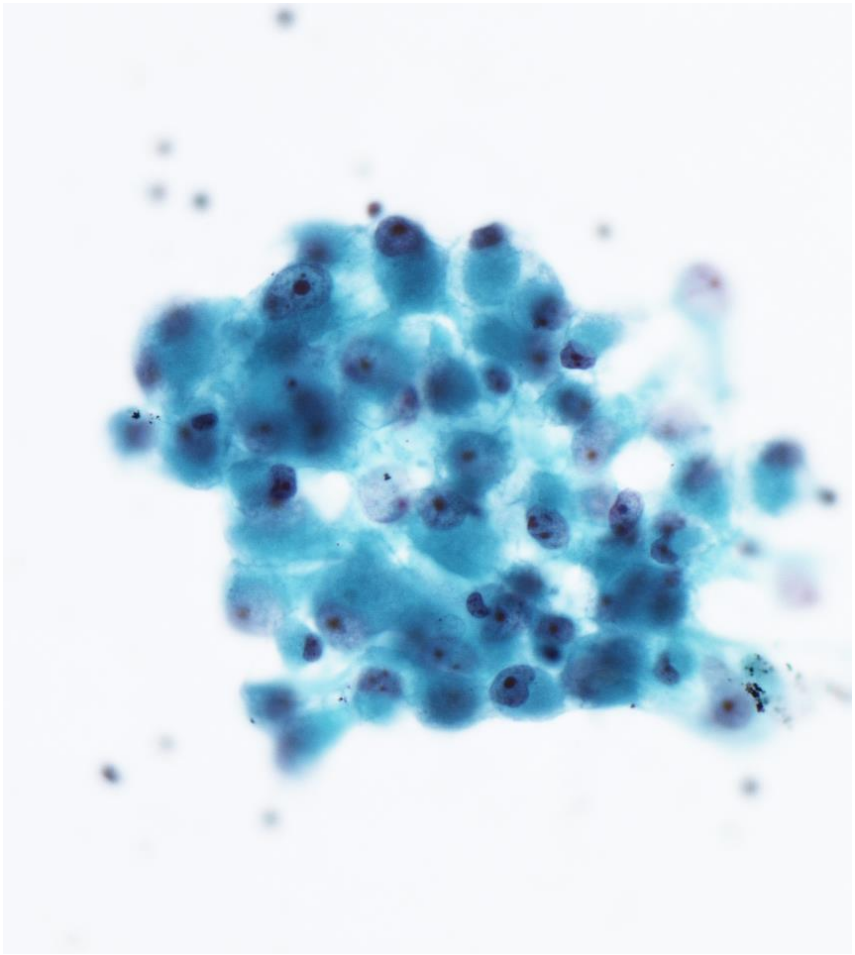


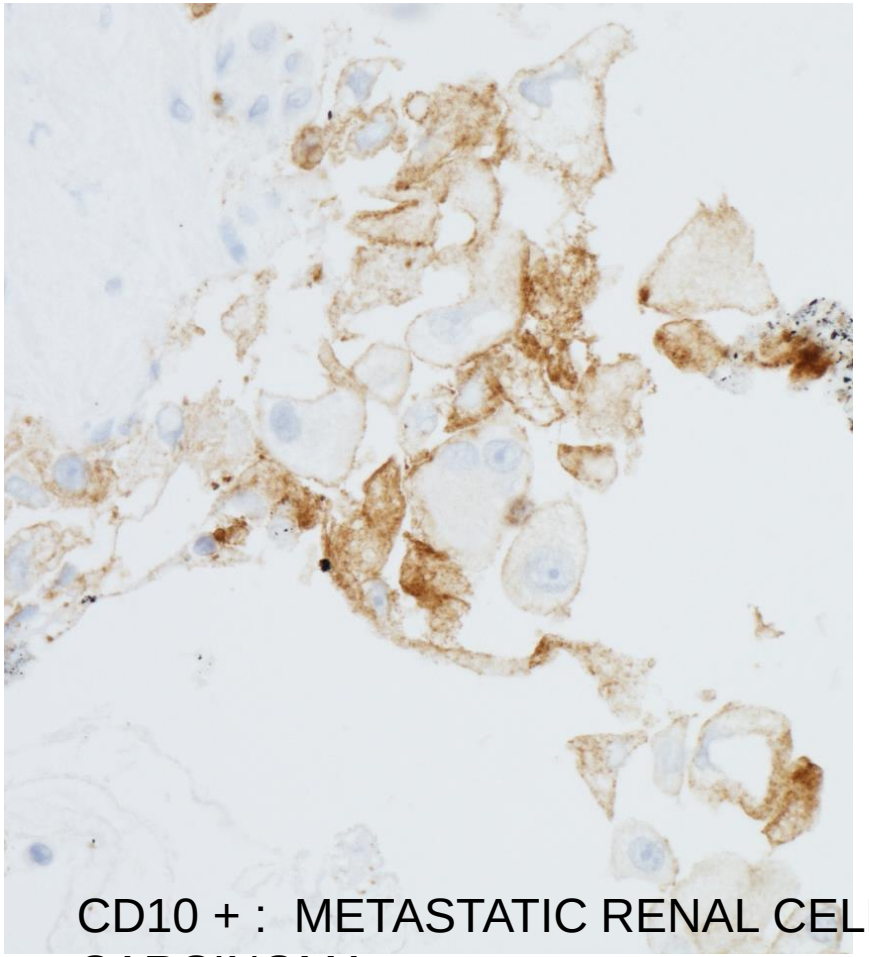


TTF-1

# Metastatic extrathoracic malignancies

# “Metastatic non-small cell carcinoma”





CD10 + : METASTATIC RENAL CELL CARCINOMA

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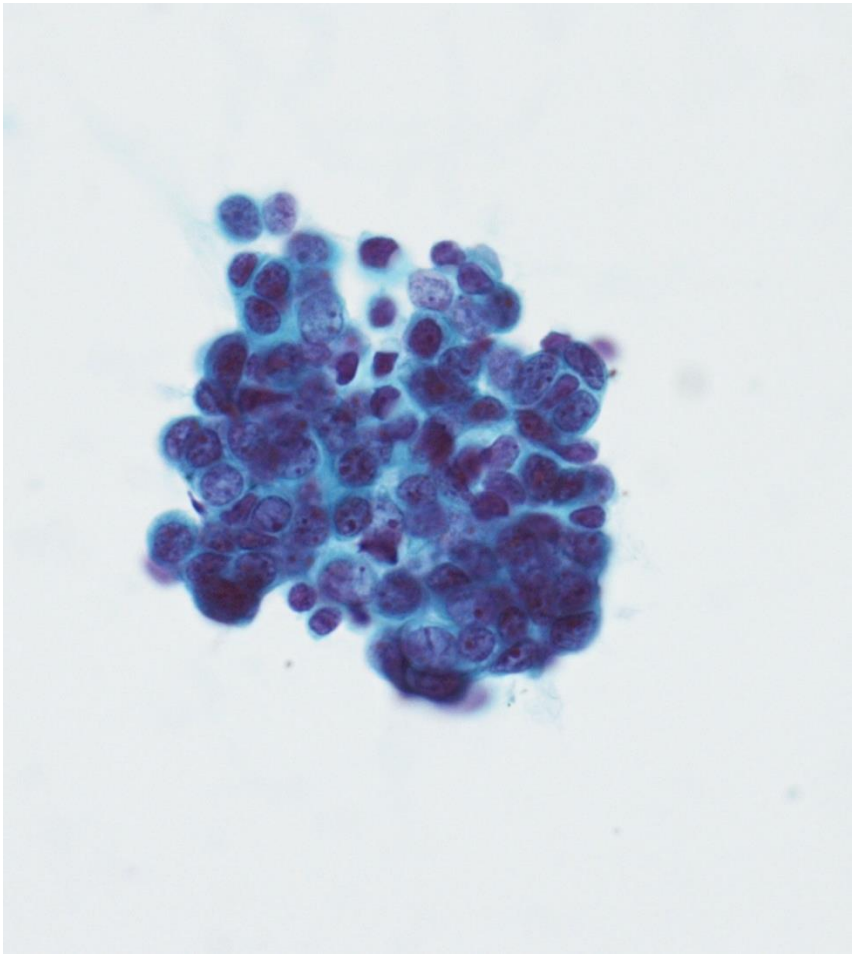
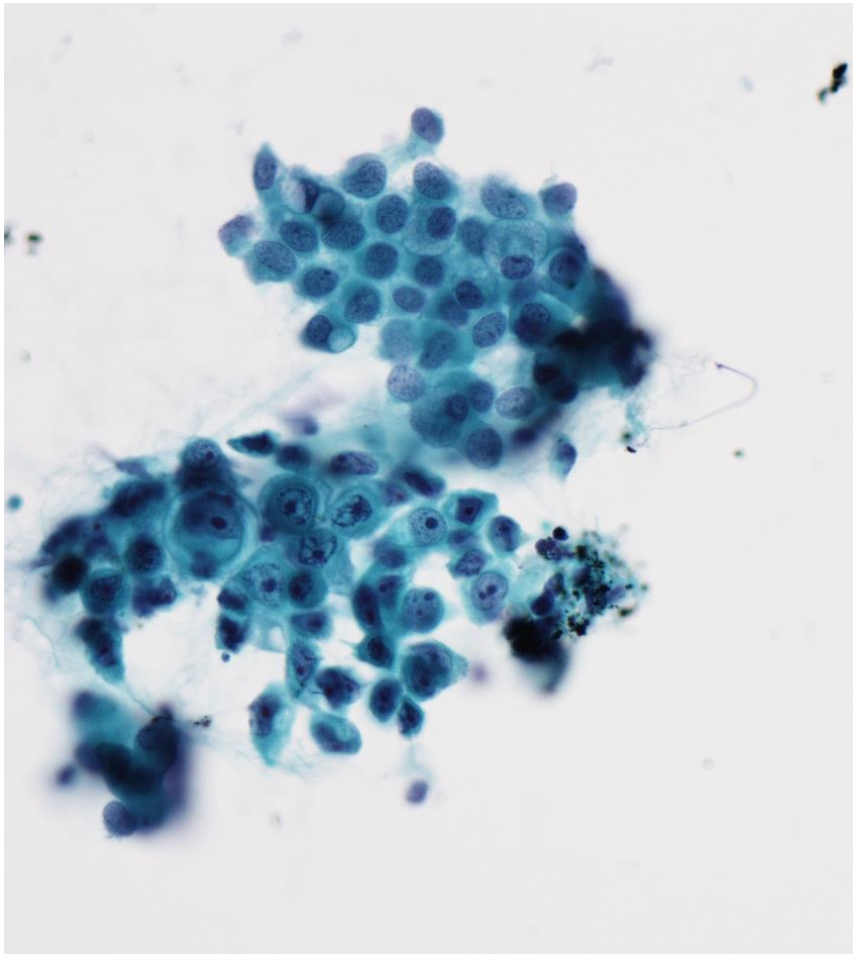
# “Metastatic carcinoma”



Metastatic breast carcinoma



Metastatic TCC

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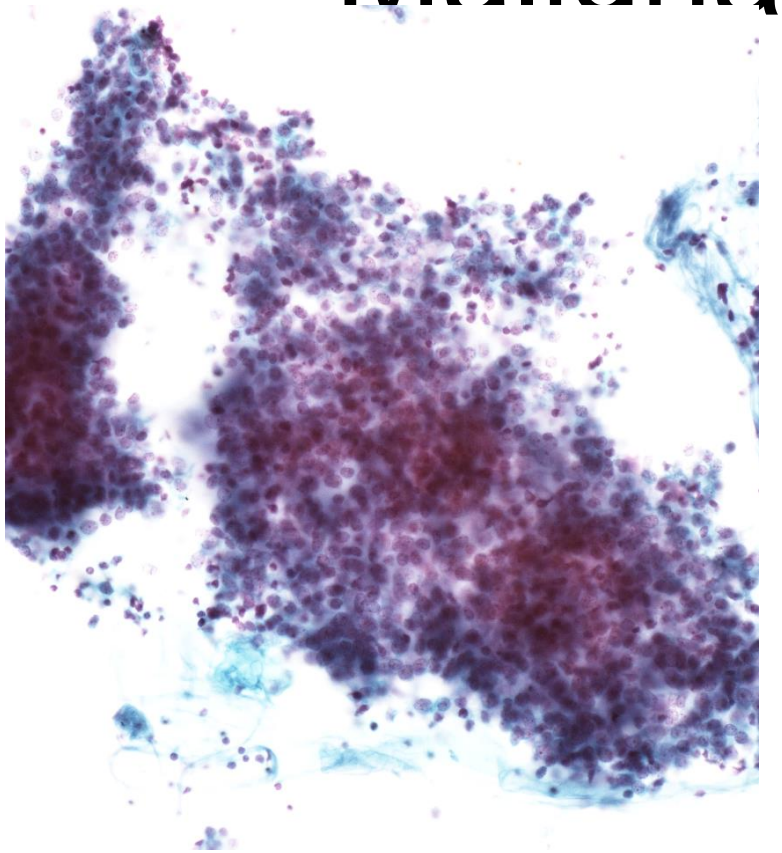
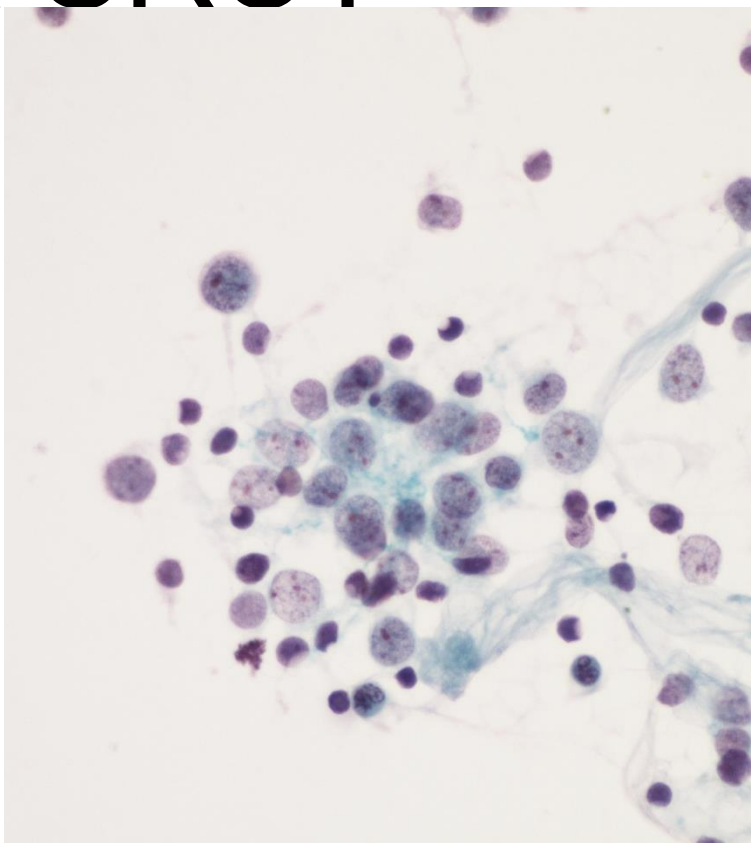
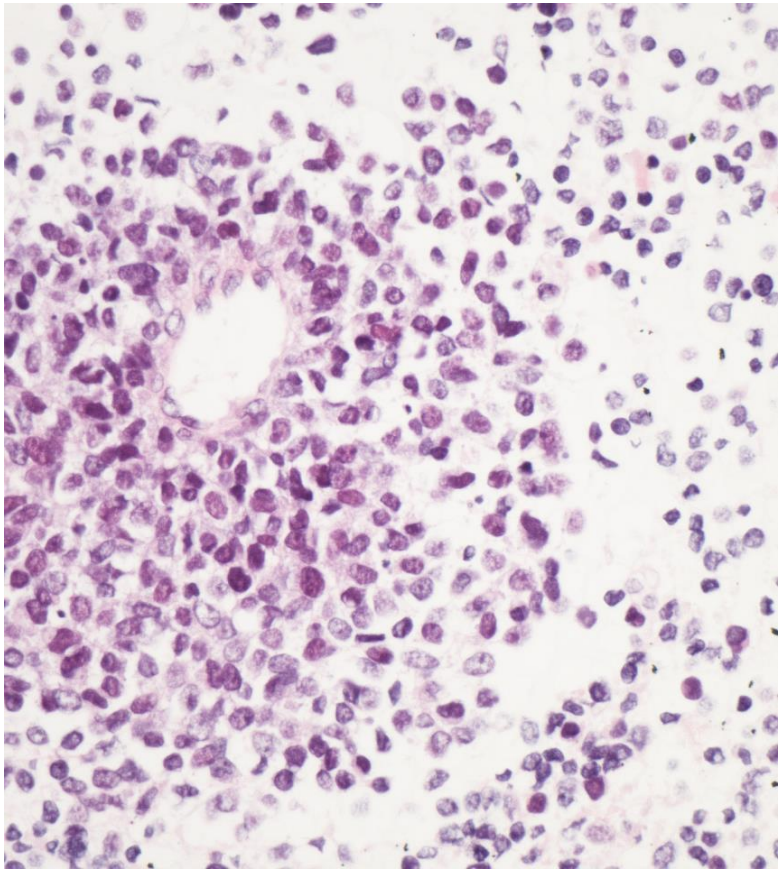
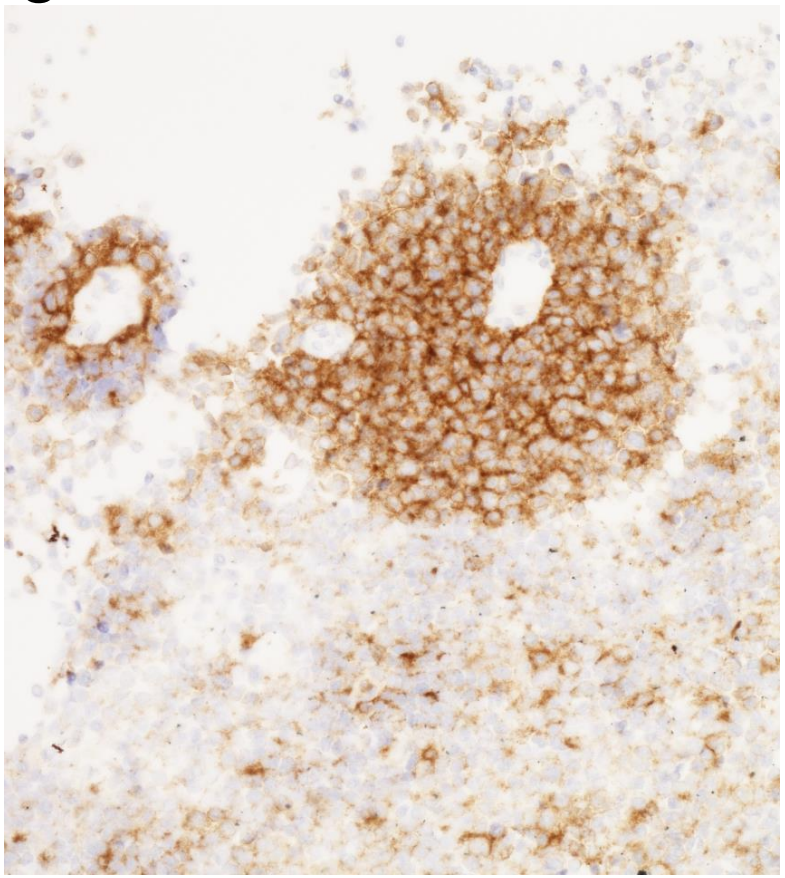
# “Lung mass and lymph nodes - Malignant SRCT”





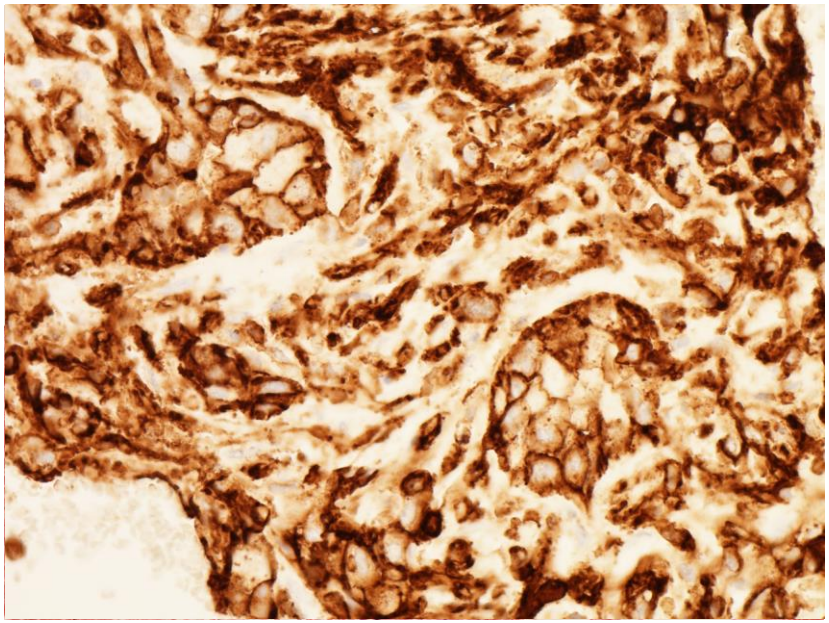
# History previous Ewings sarcoma
# - Metastatic Ewings sarcoma

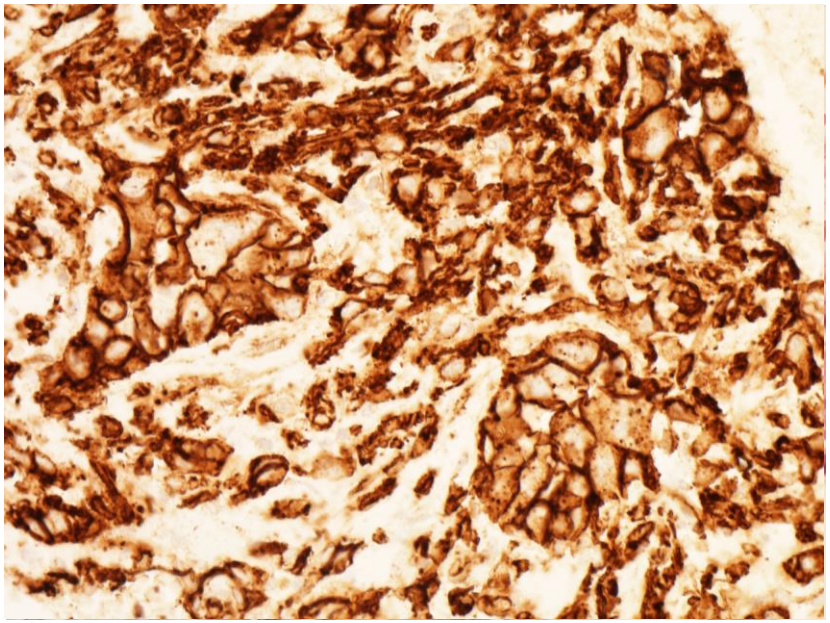




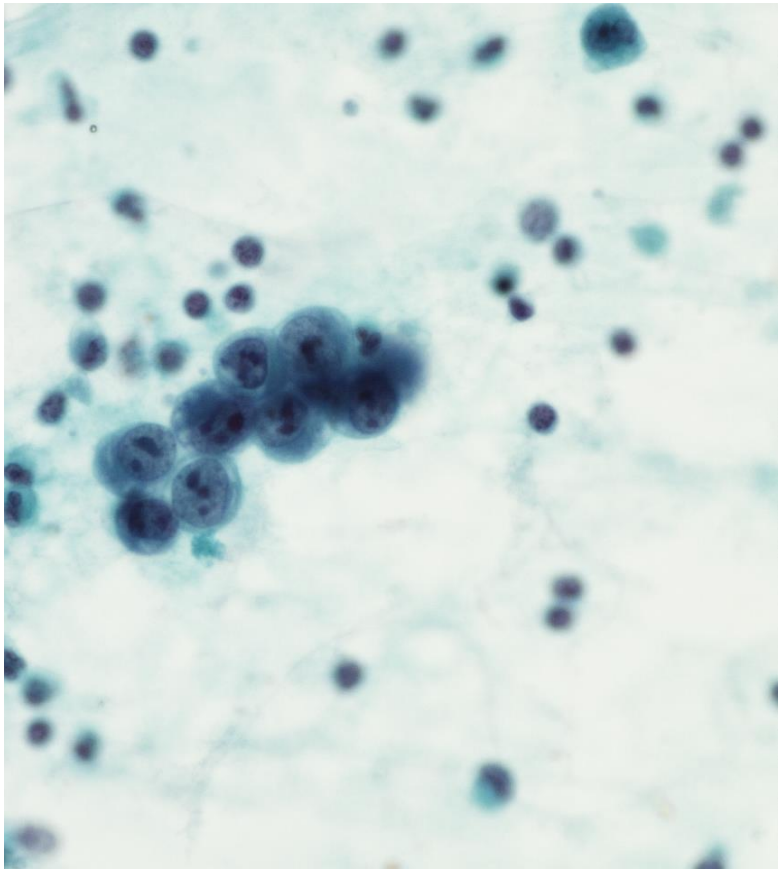
CD99

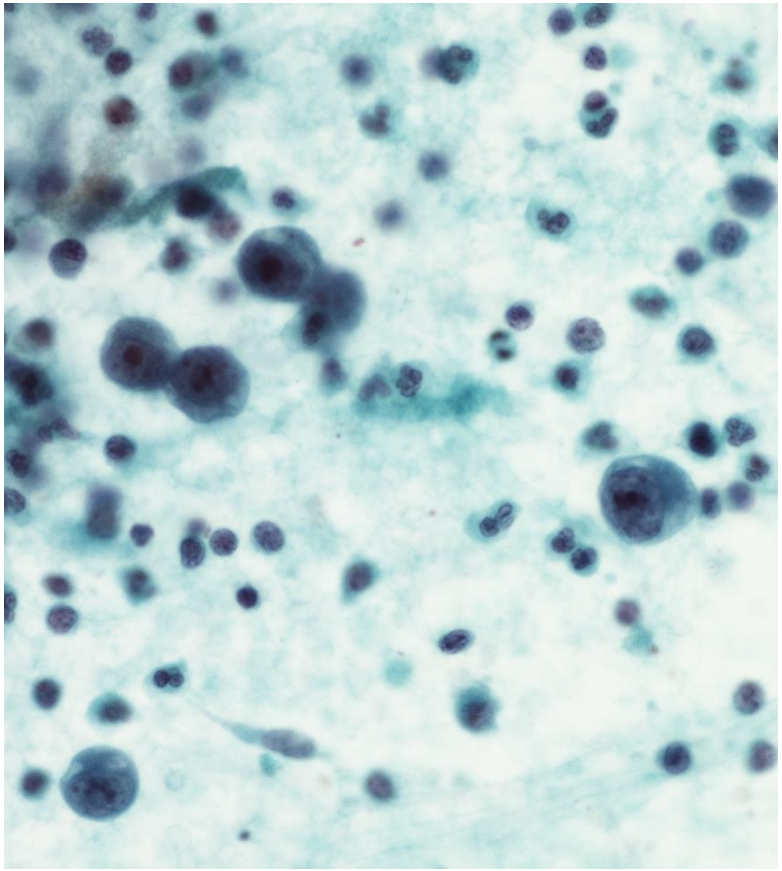
Mediastinal lymph nodes – also has skin lesion





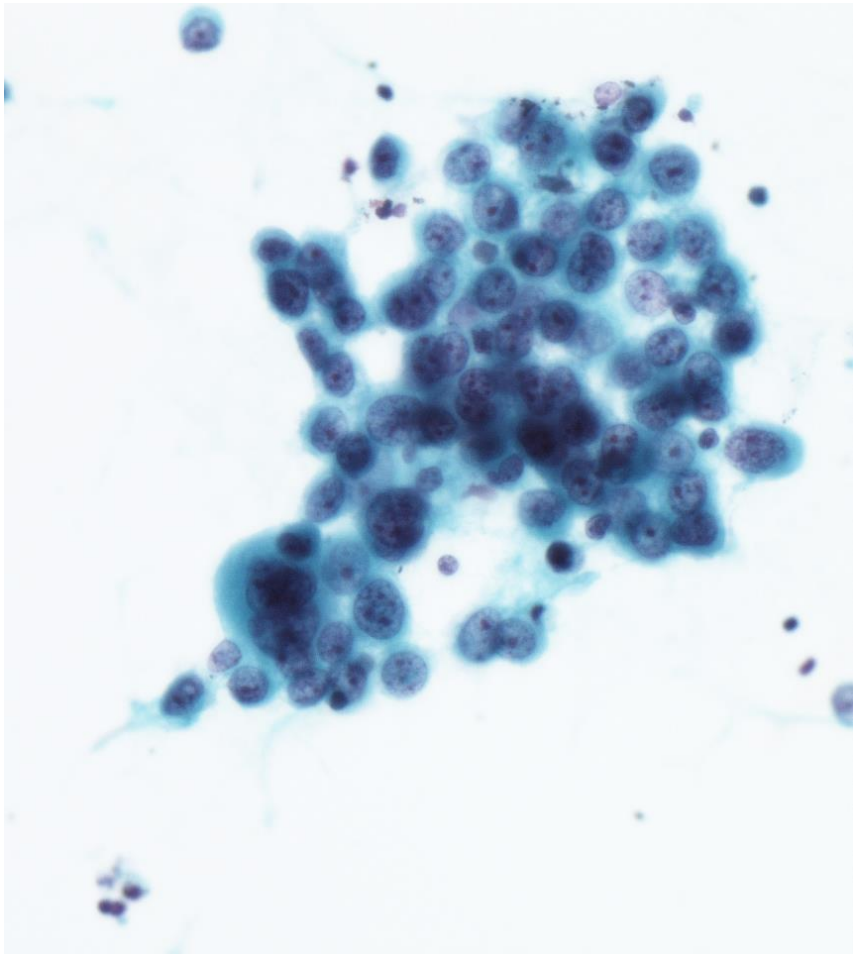
# Metastatic Angiosarcoma

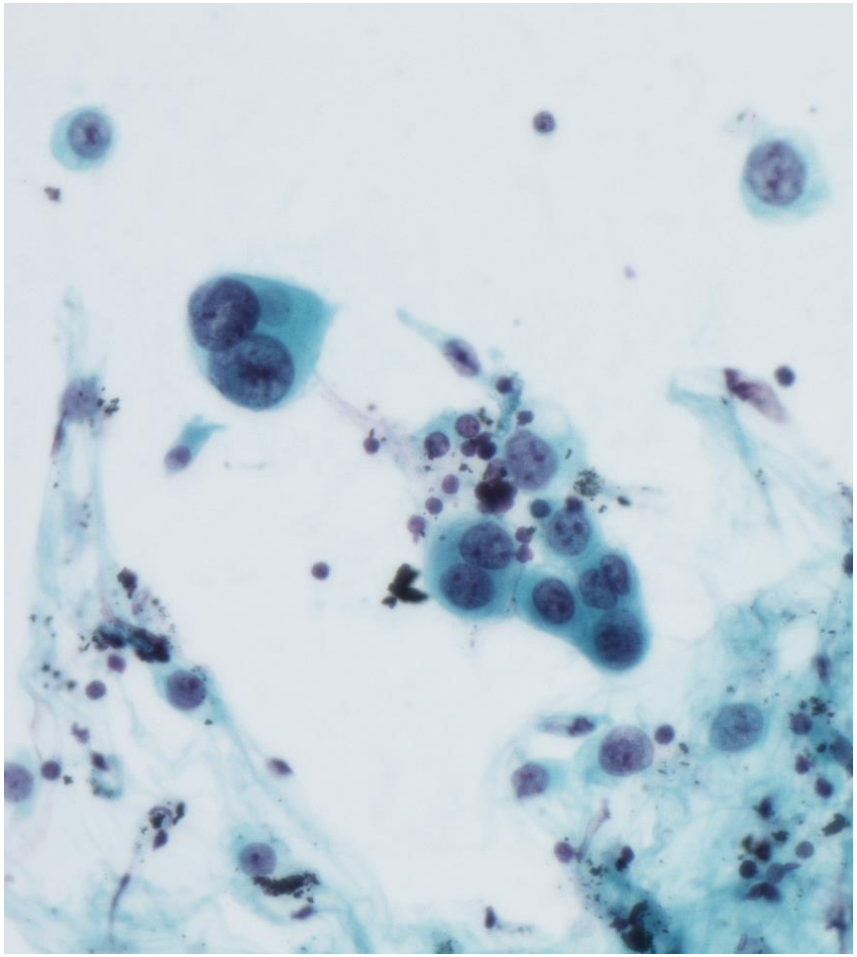




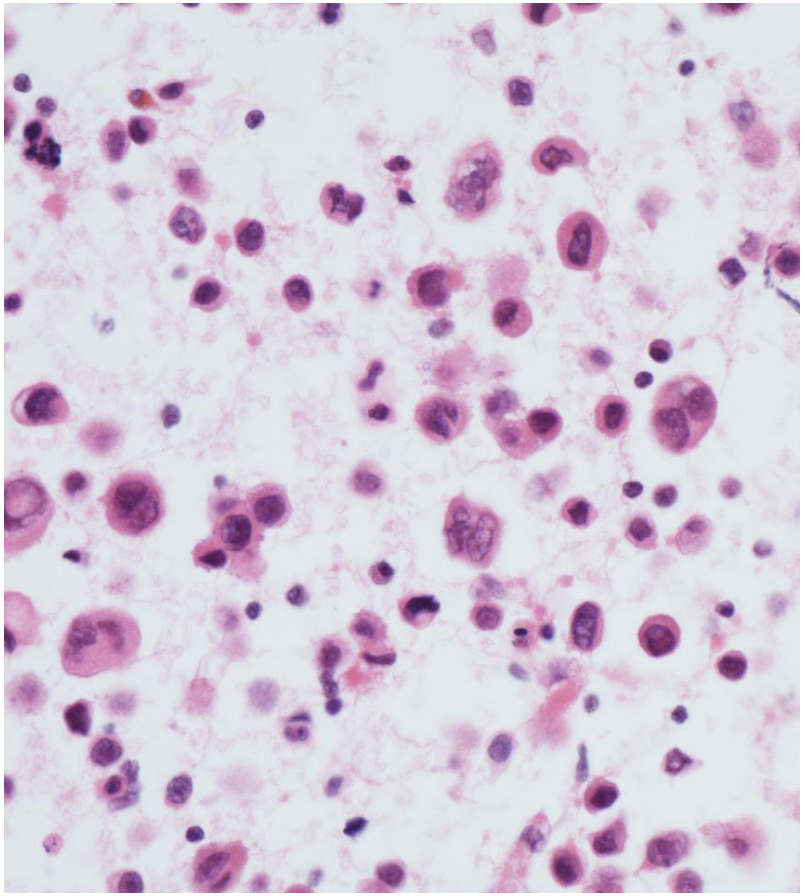
# Metastatic seminoma

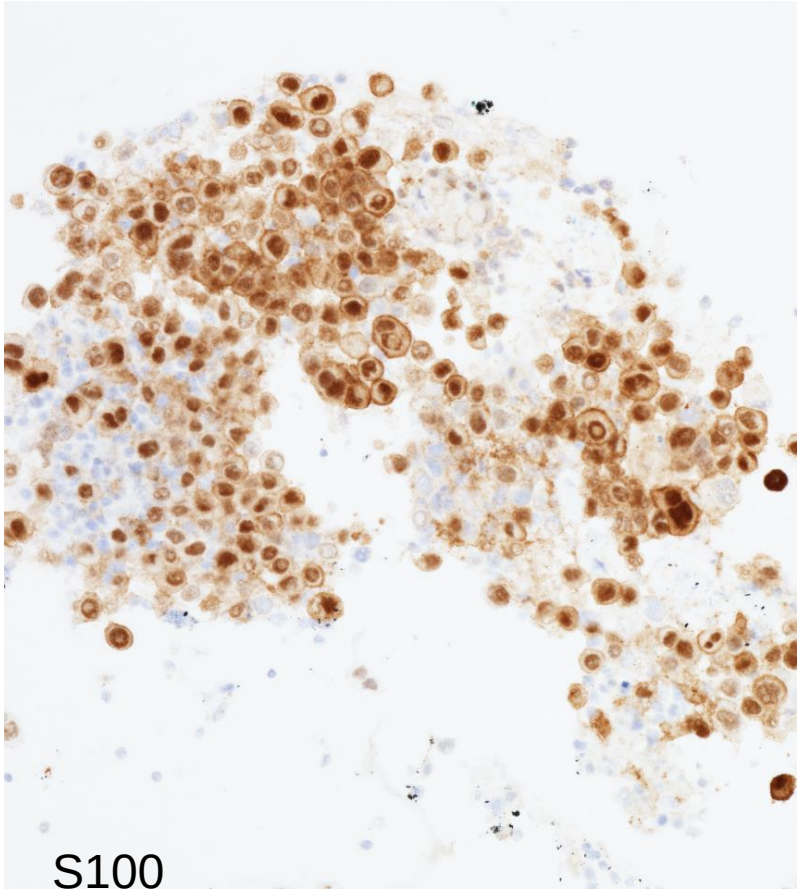
# “Metastatic non-small cell carcinoma/epithelioid tumour”





# Metastatic melanoma





S100

# CLINICAL HISTORY IS ESSENTIAL

# EBUS Lung lesions

- Non-neoplastic
  - granulomatous, inflammatory, cystic
- Benign
  - Chondroid hamartoma, chondroma
- Malignant
  - Common lung cancers
  - Carcinoid
  - Salivary gland tumours
  - Sarcomas

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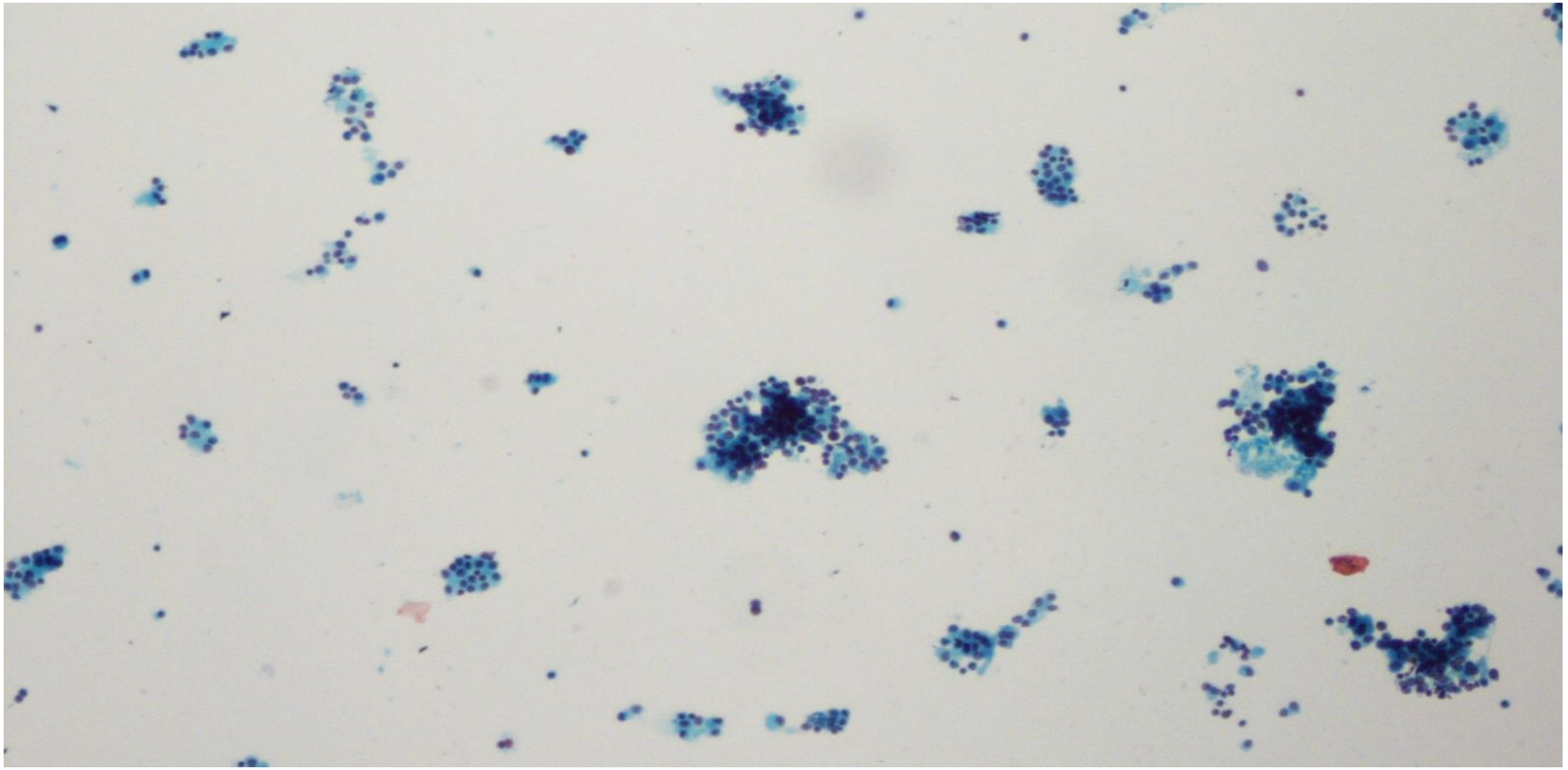
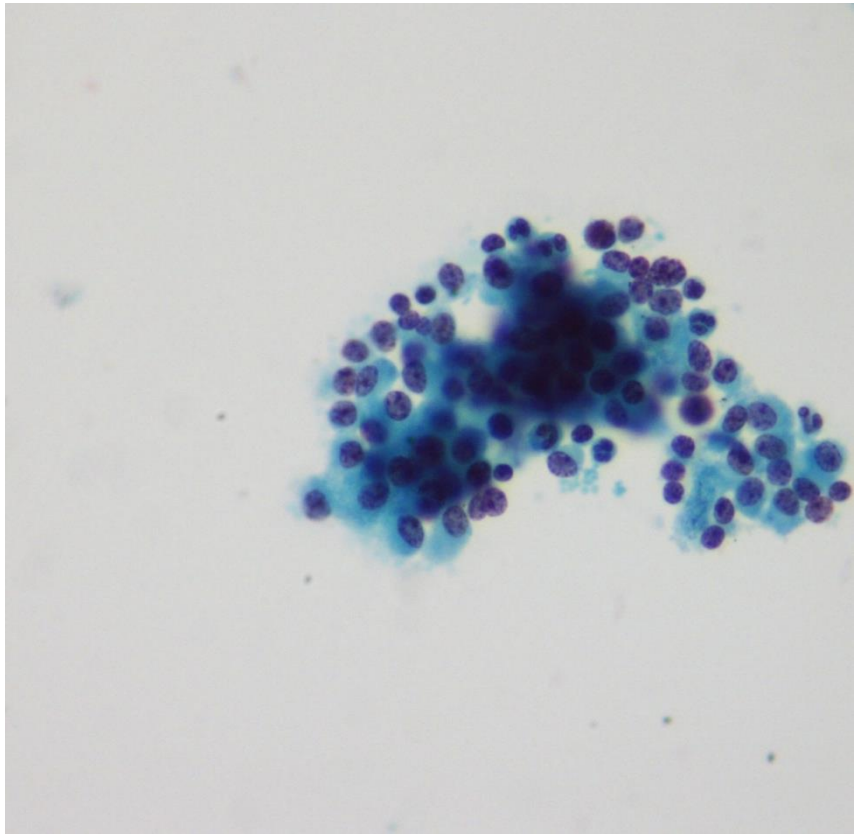
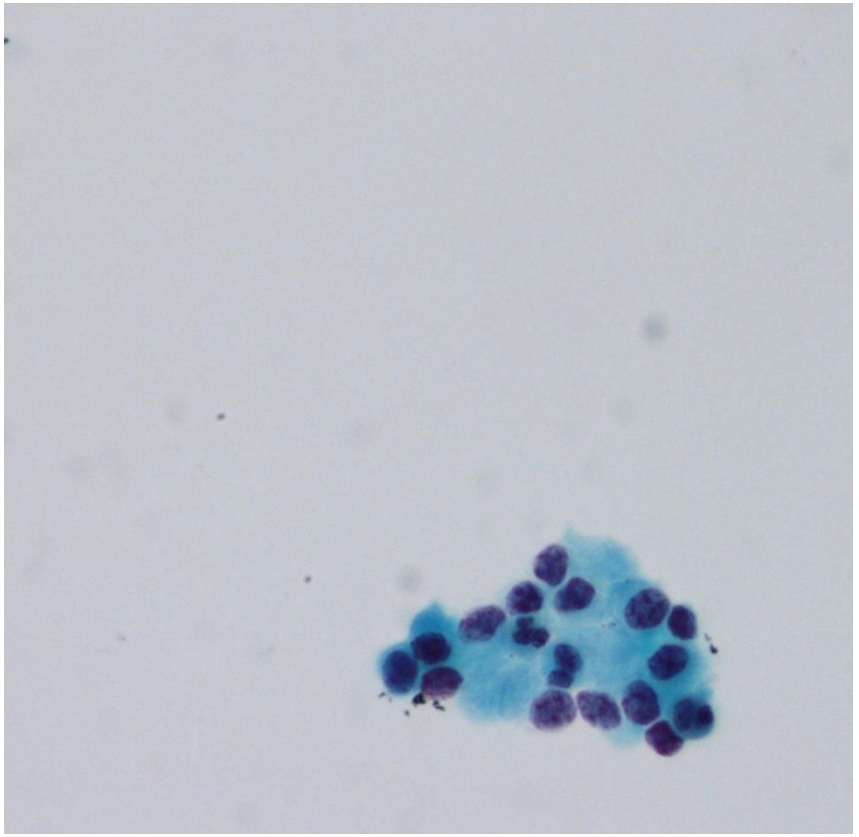
# EBUS TBNA parabronchial mass "low grade epithelioid tumour"



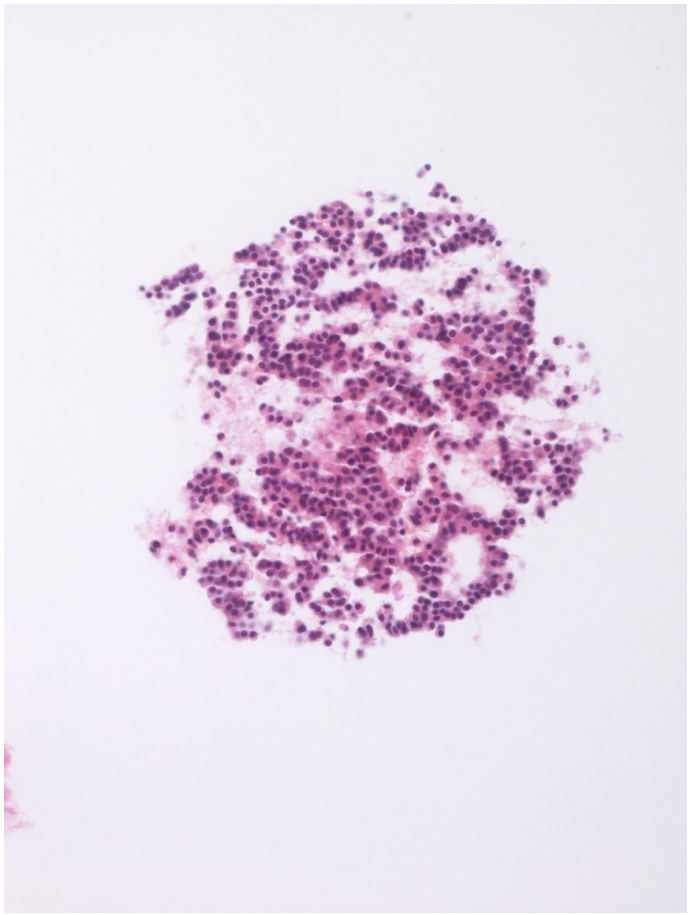
## Bland plasmacytoid cells

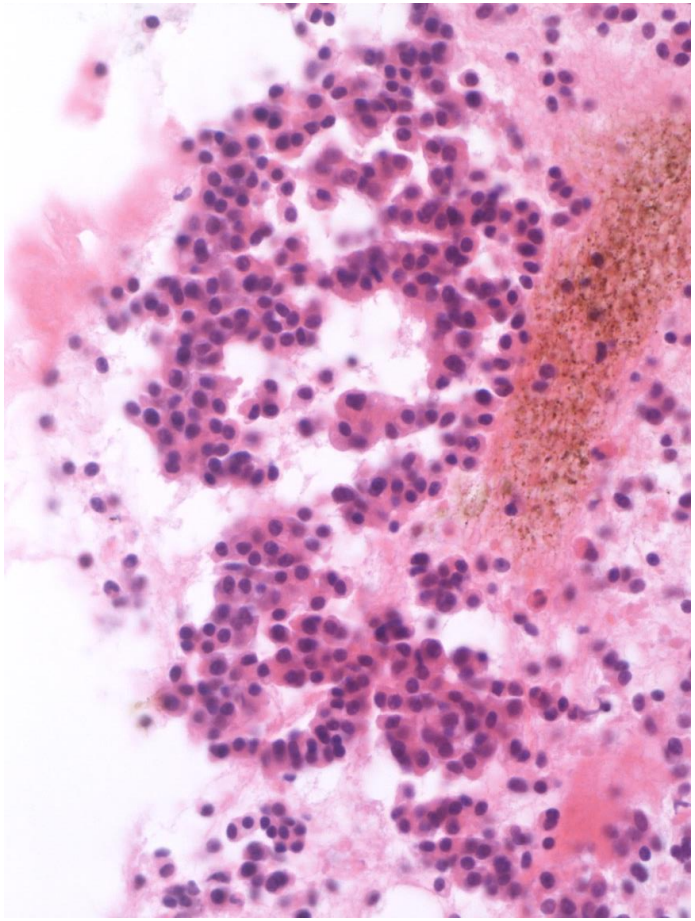


## Rosettes

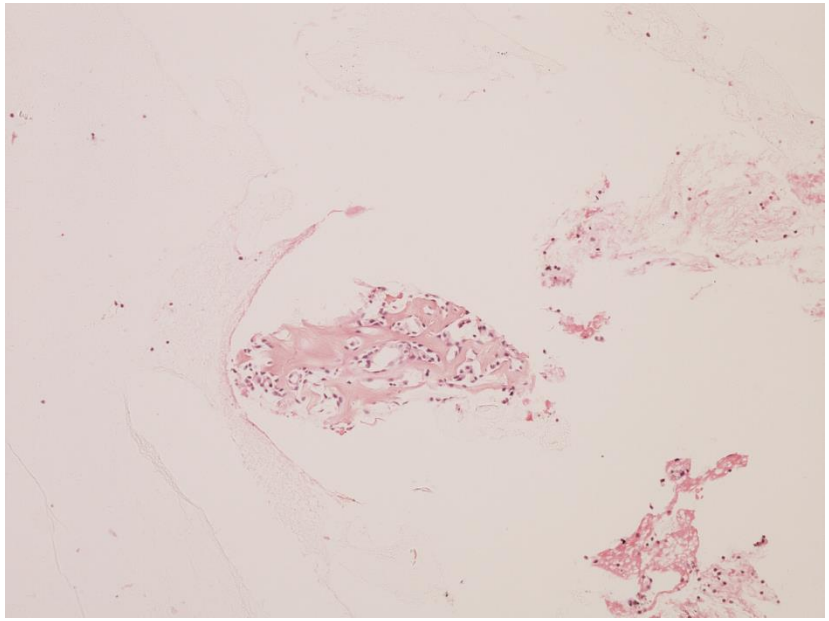


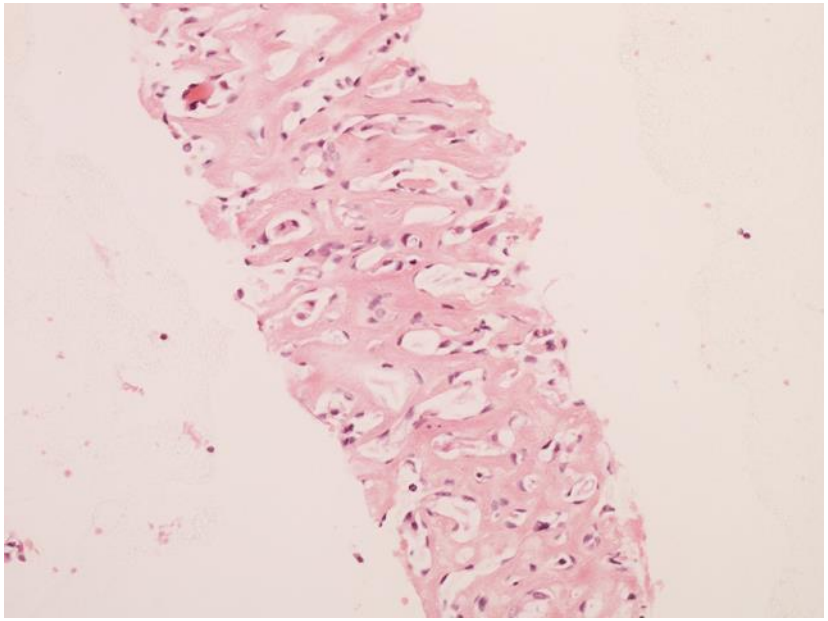
# Carcinoid tumour – cell block





# EBUS TBNA parabronchial mass





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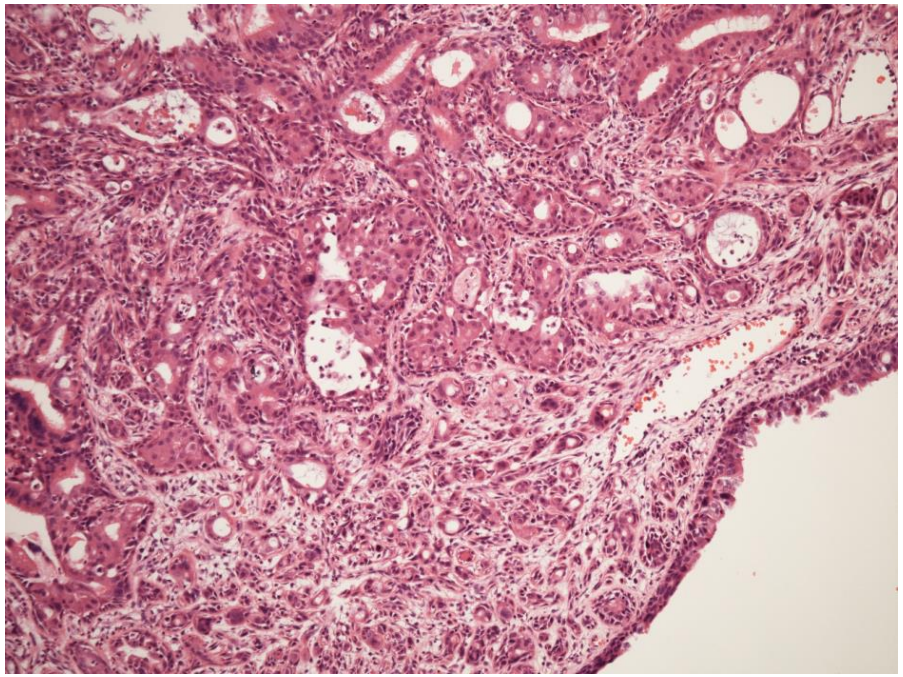
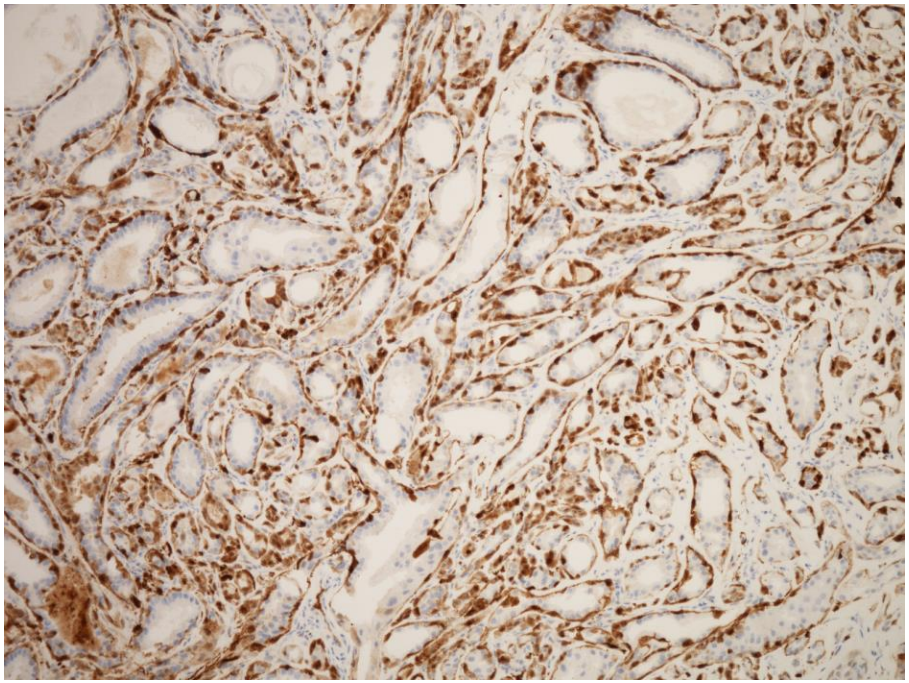
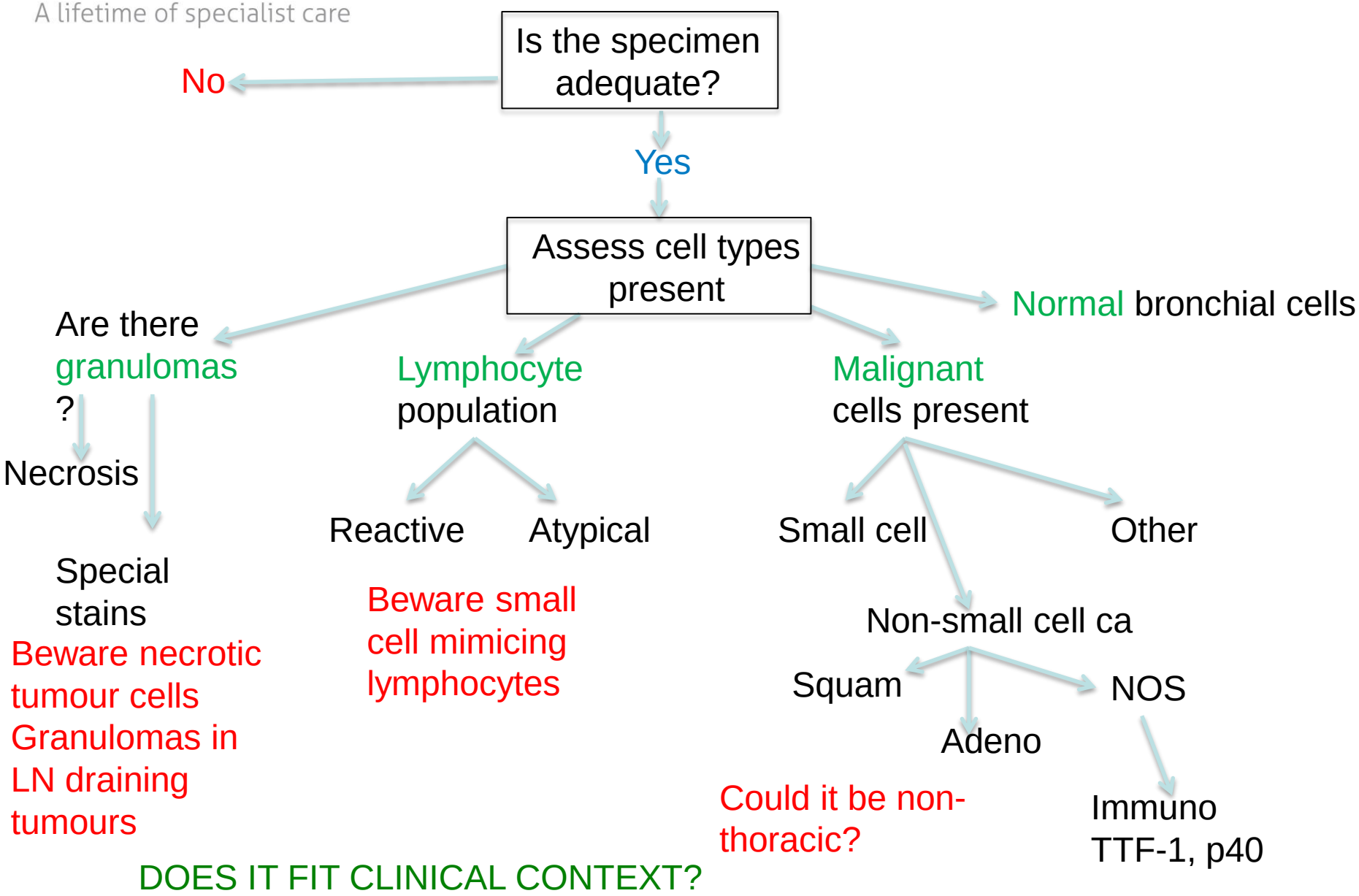
# Epithelial-myoepithelial carcinoma






Is the specimen adequate?
No
Yes
Assess cell types present
Are there granulomas ?
Necrosis
Special stains
Beware necrotic tumour cells
Granulomas in LN draining tumours
Lymphocyte population
Reactive
Atypical
Beware small cell mimicing lymphocytes
Normal bronchial cells
Malignant cells present
Small cell
Other
Non-small cell ca
Squam
Adeno
NOS
Immuno
TTF-1, p40
Could it be non-thoracic?
DOES IT FIT CLINICAL CONTEXT?

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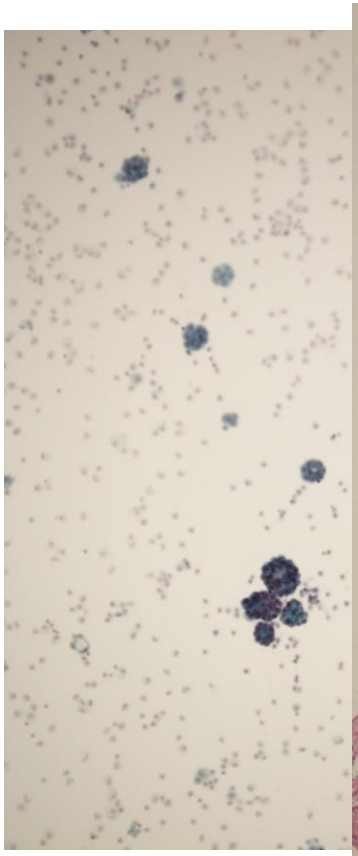
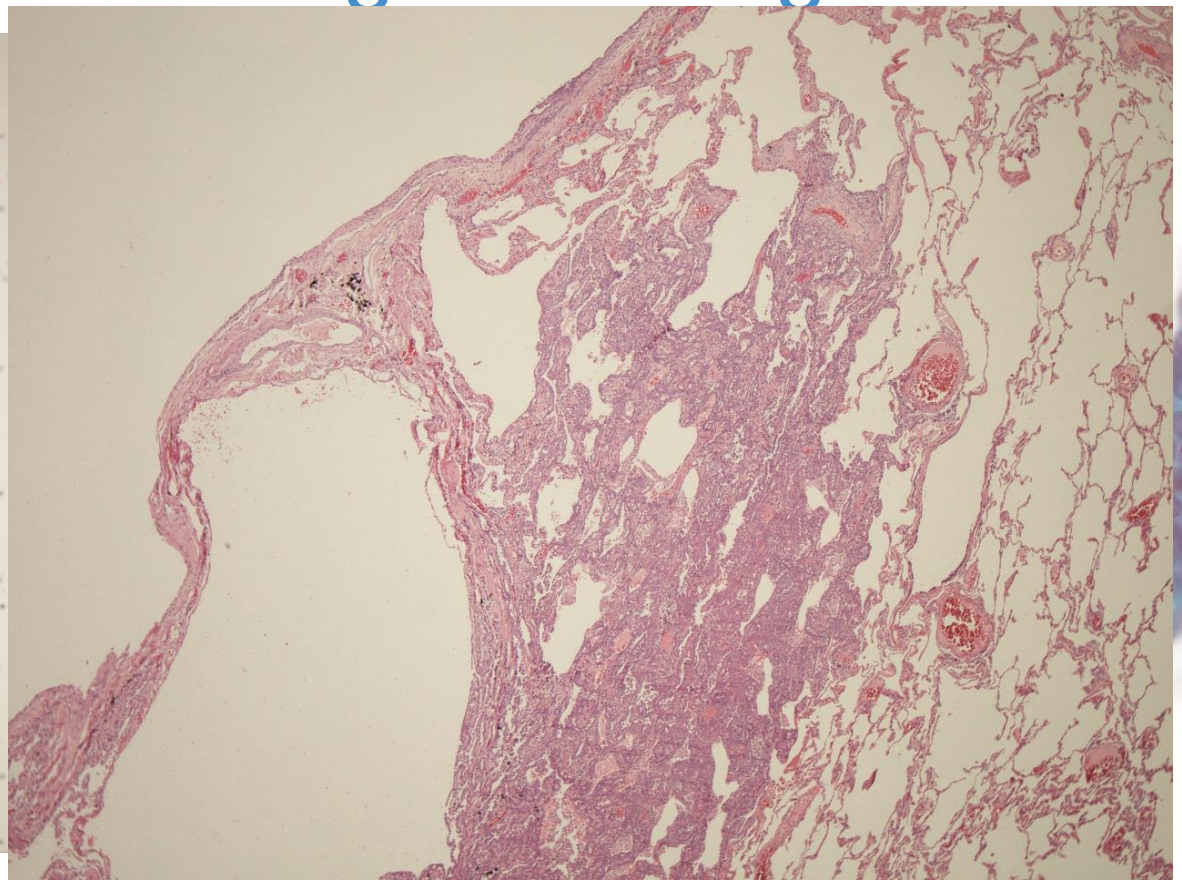
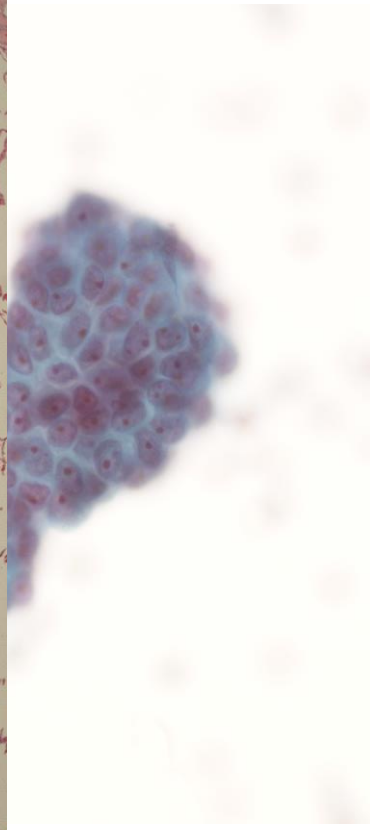
# Pleural Effusions: Mesothelioma
# Benign vs malignant







# Mesothelioma Molecular Pathogenesis

- Homozygous deletions 9p21: p16
  - Only seen in MM
  - % of cases
  - Detected by FISH
- Loss of BRCA Associated Protein 1: BAP1
  - Only seen in MM
  - Germline mutation : renal cell and uveal malignancies
  - % cases
  - IHC good correlate with mutation/deletion

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# Loss BAP1 IHC

Arch Pathol Lab Med—Vol 140, April 2016

**Table 2. Frequency of BAP1 Loss by Immunohistochemistry in Mesotheliomas**

| Source, y | Epithelial, No. (%) | Mixed, No. (%) | Sarcomatous, No. (%) |
|---|---|---|---|
| Nasu et al,[31] 2015[a] | 50/63 (79) | 9/16 (56) | 5/8 (63) |
| Farzin et al,[36] 2015 | 75/120 (63) | 19/42 (45) | 12/67 (18) |
| Yoshikawa et al,[37] 2012 | 10/12 (81) | 1/5 (20) | |
| Sheffield et al,[39] 2015[b] | 5/9 (56) | 2/12 (15) | |
| Cigognetti et al,[38] 2015 | 128/184 (70) | 9/15 (60) | 2/13 (15) |

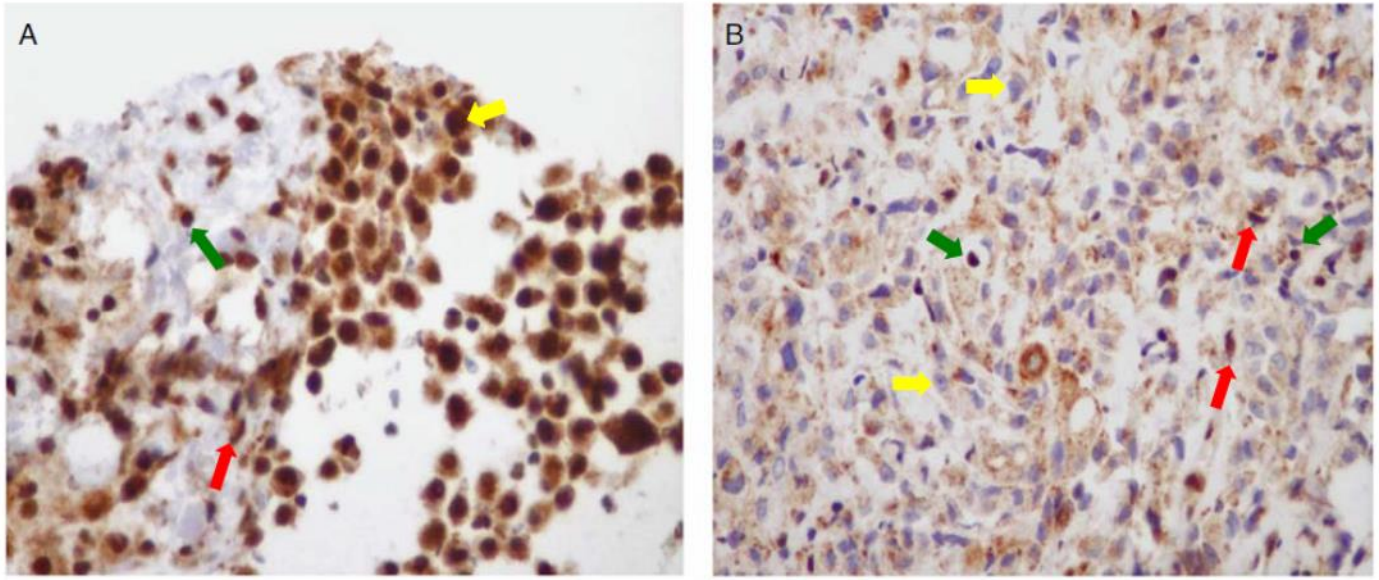
**Table 3. Frequency of BAP1 Loss by Immunohistochemistry in Benign Reactions**

| Source, y | BAP1 Loss in Benign Reactions, No. (%) |
|---|---|
| Sheffield et al,[39] 2015 and Churg (unpublished data, June 2015) | 0/53 (0) |
| Galateau-Salle (unpublished data, June 2015) | 0/23 (0) |
| Cigognetti et al,[38] 2015 | 0/25 (0) |

Abbreviation: BAP1, BRCA1-associated protein 1.

# BAP1 Immunohistochemistry and p16 FISH to Separate Benign From Malignant Mesothelial Proliferations

*Brandon S. Sheffield, MD,* Harry C. Hwang, MD,† Anna F. Lee, MD,‡ Kim Thompson, HT, ASCP, QIHC,† Stephanie Rodriguez, HT, MB, ASCP,† Christopher H. Tse, MBBS,† Allen M. Gown, MD,† and Andrew Churg, MD**




| Marker | BAP1 IHC | p16 FISH |
|---|---|---|
| n | 75 | 67 |
| Benign | 0/49 | 0/40 |
| Malignant | 7/26 | 14/27 |
| Sensitivity (95% CI) (%) | 27 (17-37) | 52 (40-64) |
| Specificity (95% CI) (%) | 100 (100-100) | 100 (100-100) |

*Am J Surg Pathol* 2015;39:977–982

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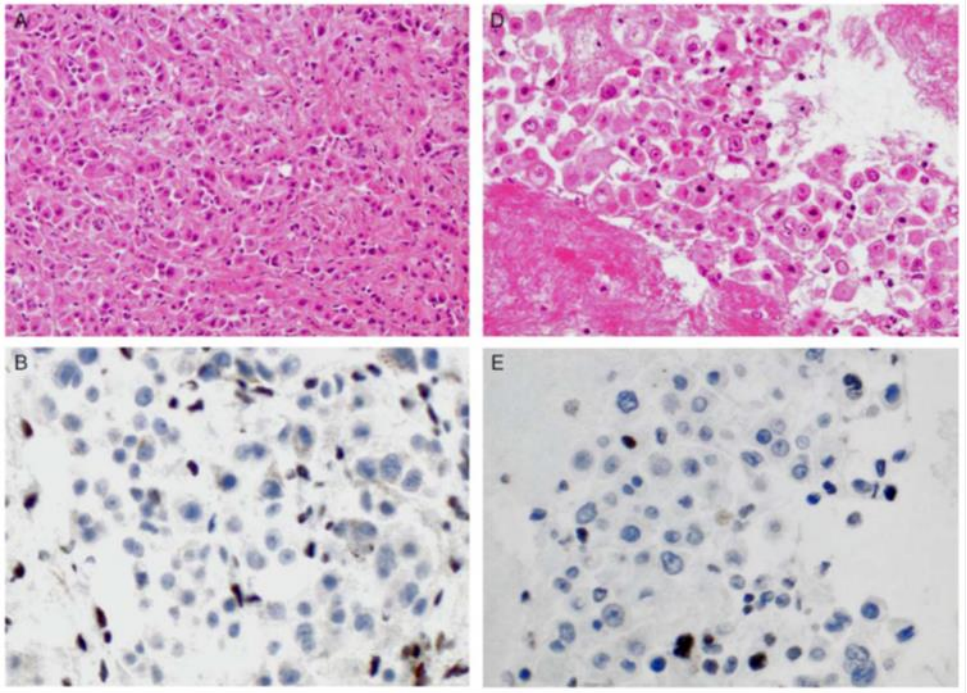
# Utility of BAP1 Immunohistochemistry and p16 (CDKN2A) FISH in the Diagnosis of Malignant Mesothelioma in Effusion Cytology Specimens

*Harry C. Hwang, MD,* Brandon S. Sheffield, MD,†‡ Stephanie Rodriguez, HT, MB, ASCP,* Kim Thompson, ASCP, QIHC,* Christopher H. Tse, MBBS,* Allen M. Gown, MD,* and Andrew Churg, MD†‡*




Loss of BAP1 expression in malignant mesothelioma cells (note positive internal control inflammatory cells)

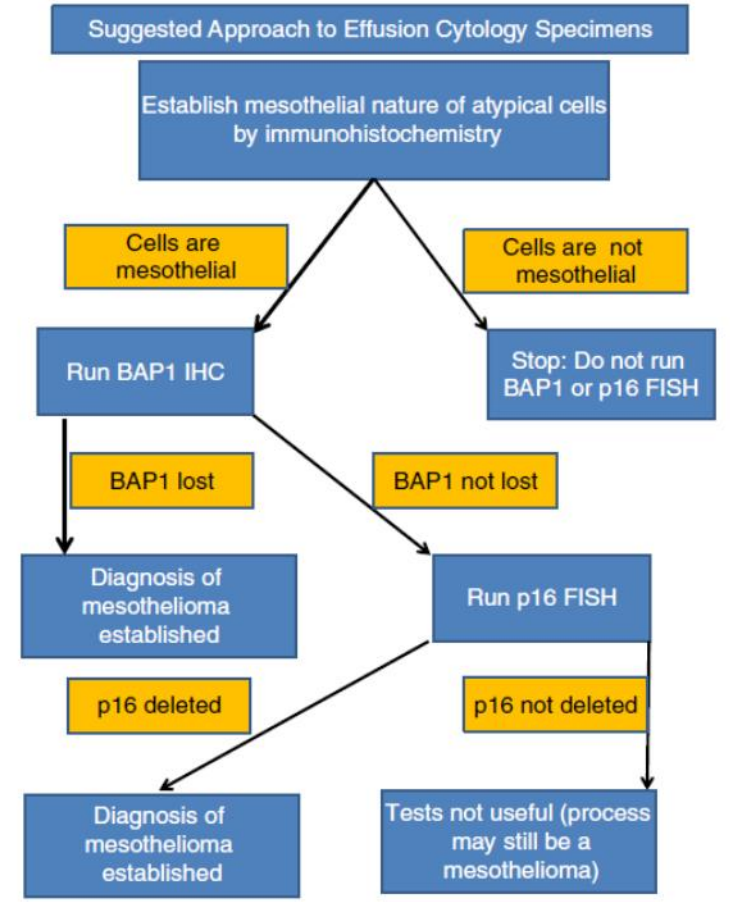
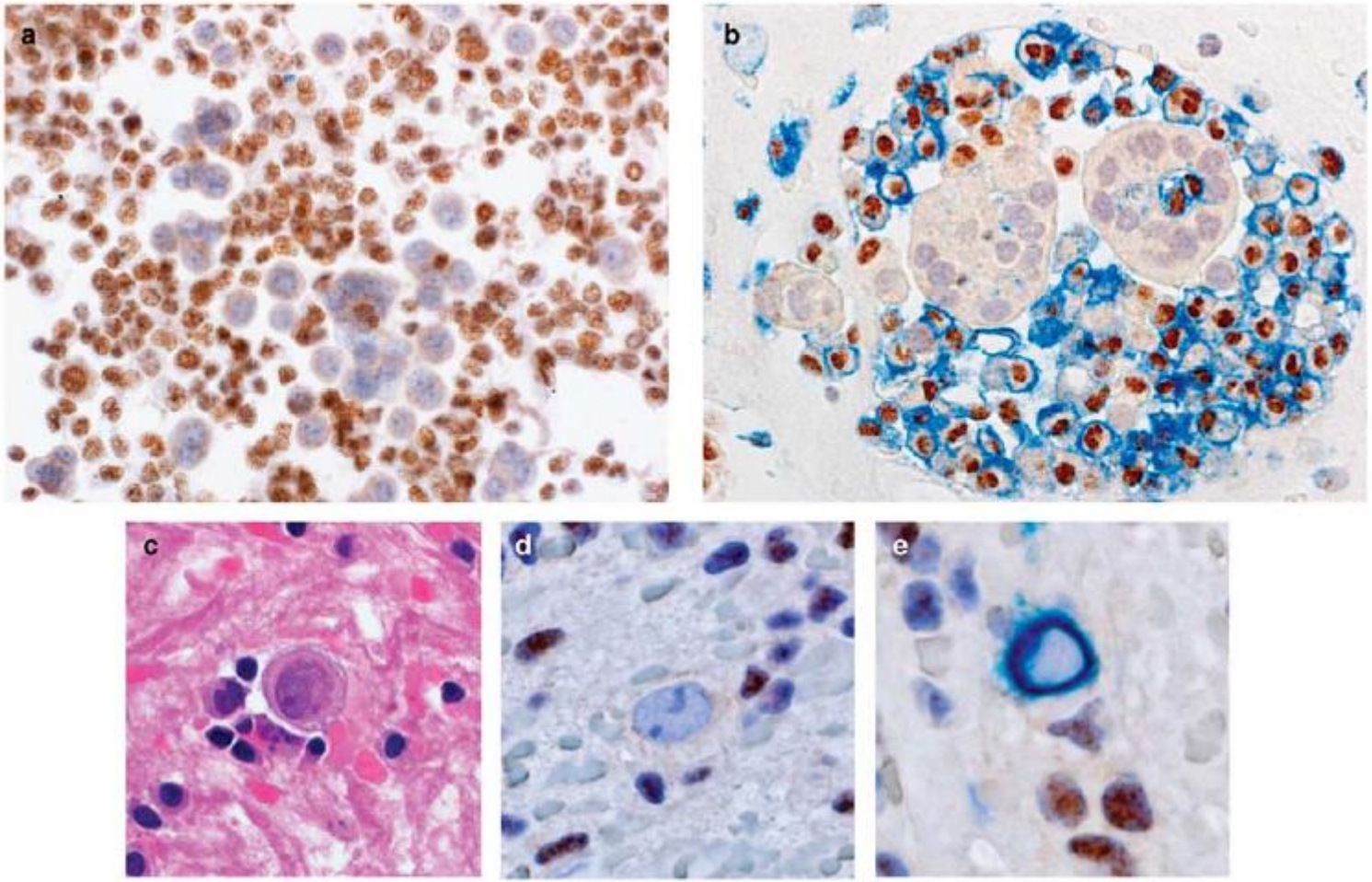



**FIGURE 3.** Recommended scheme for using BAP1 IHC and p16 FISH in effusion cytology specimens.

*Am J Surg Pathol* 2016;40:120–126




*Modern Pathology* (2015) **28,** 1043–1057

# But Note:

- 40% of mesotheliomas will still express BAP1 and retain p16
- TISSUE INVASION still best marker of malignancy
- BAP1 and p16 analysis may be useful where not possible to assess invasion or equivocal or in cytology specimens

# Conclusion

- Minimally invasive diagnostic techniques increasing
- Expanding role molecular and companion diagnostics
- Pathologists major role in optimising tissue pathways
- New diagnostic markers
- Still role for humble pathologist and microscope

# Thank you

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