Serous Effusion: Mesothelioma and Mimics

Adebowale Adeniran, MD

Department of Pathology, Yale School of Medicine

Objectives

- Use an algorithmic approach to reach a definitive diagnosis
- Discuss key cytomorphologic features and integrate ancillary tests in precise cytologic diagnosis of mesothelioma and mimics with emphasis on limitations and pitfalls

Effusion Cytology

- Pleural, peritoneal and pericardial cavities are lined by a single layer of flat mesothelial cells
- Normally, these cavities are collapsed and contain only small amounts of fluid
- In disease states, a greater amount of fluid accumulates
- Effusions are classified clinically as transudative or exudative
  - Transudates: Result from imbalance of hydrostatic and oncotic pressures
  - Exudates: Result from injury to the mesothelium
- Distinction important because pleural involvement by a malignancy causes an exudate

Guidelines for interpreting body cavity fluids

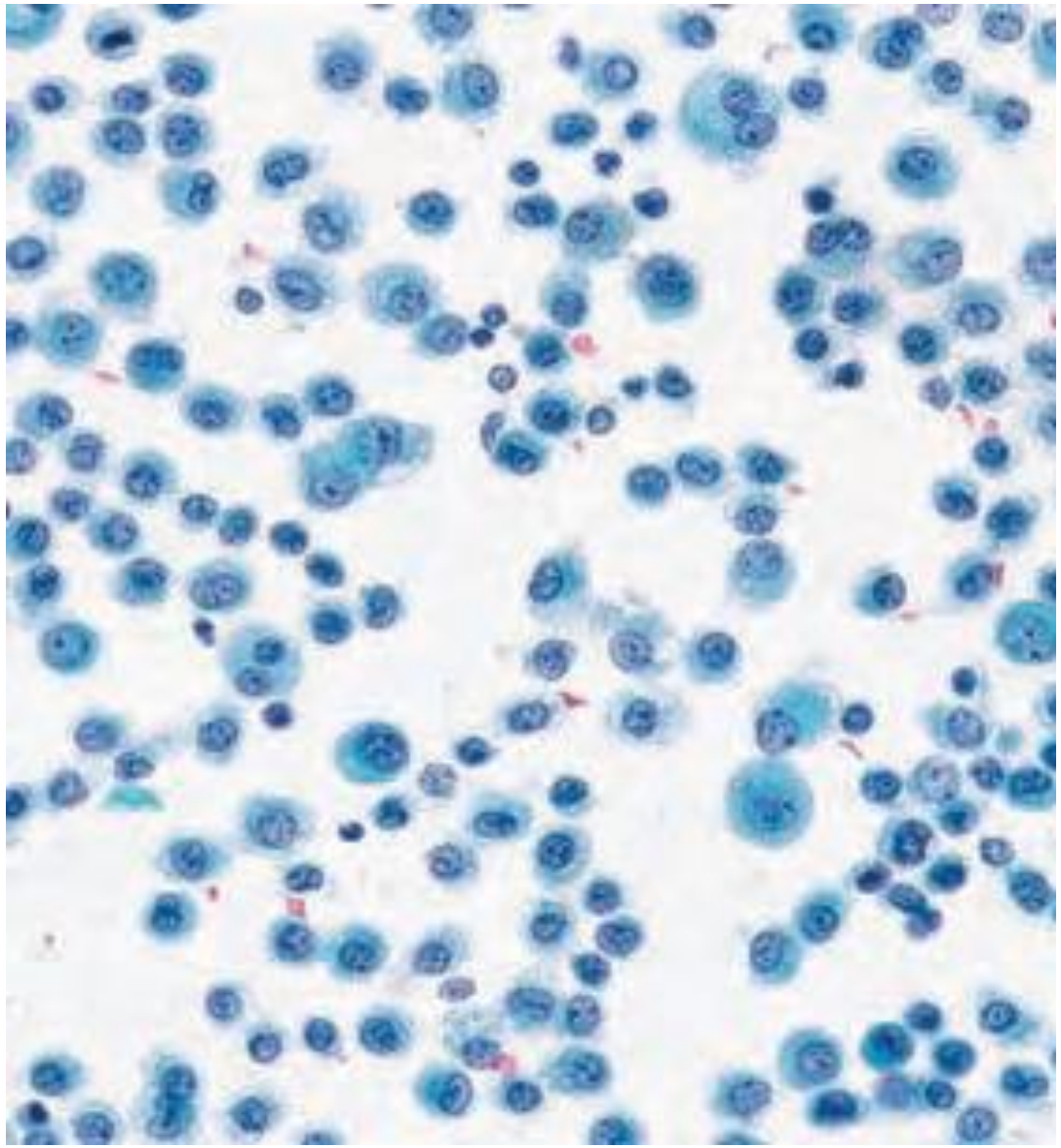
- We need to know:
  1. The significance of a positive diagnosis
  2. The most reliable criteria for a diagnosis of malignancy
  3. The common pitfalls
  4. When to be cautious
  5. When to look really hard for the foreign cells
  6. How to deal with the problematic case

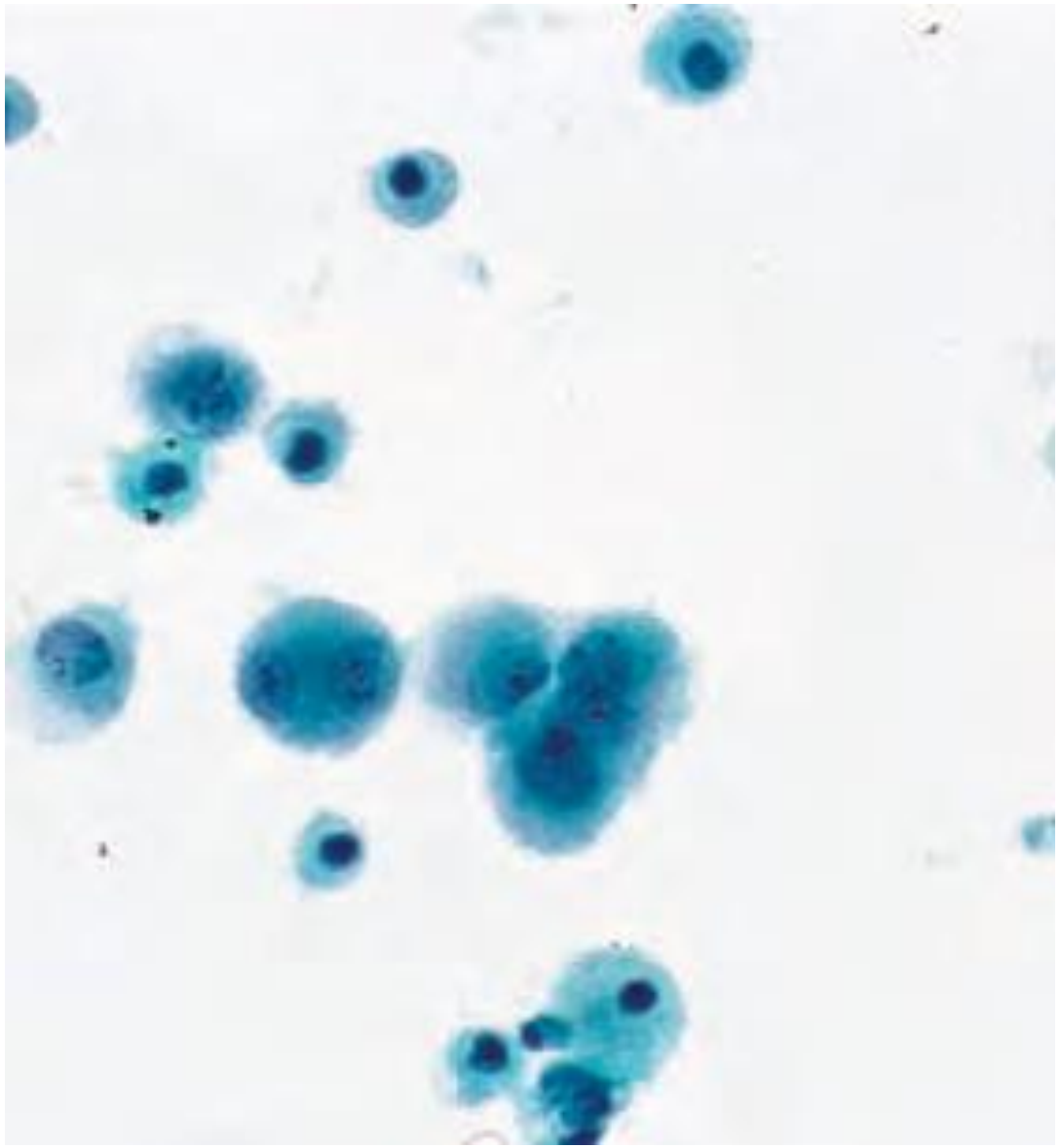
Guidelines for interpreting body cavity fluids

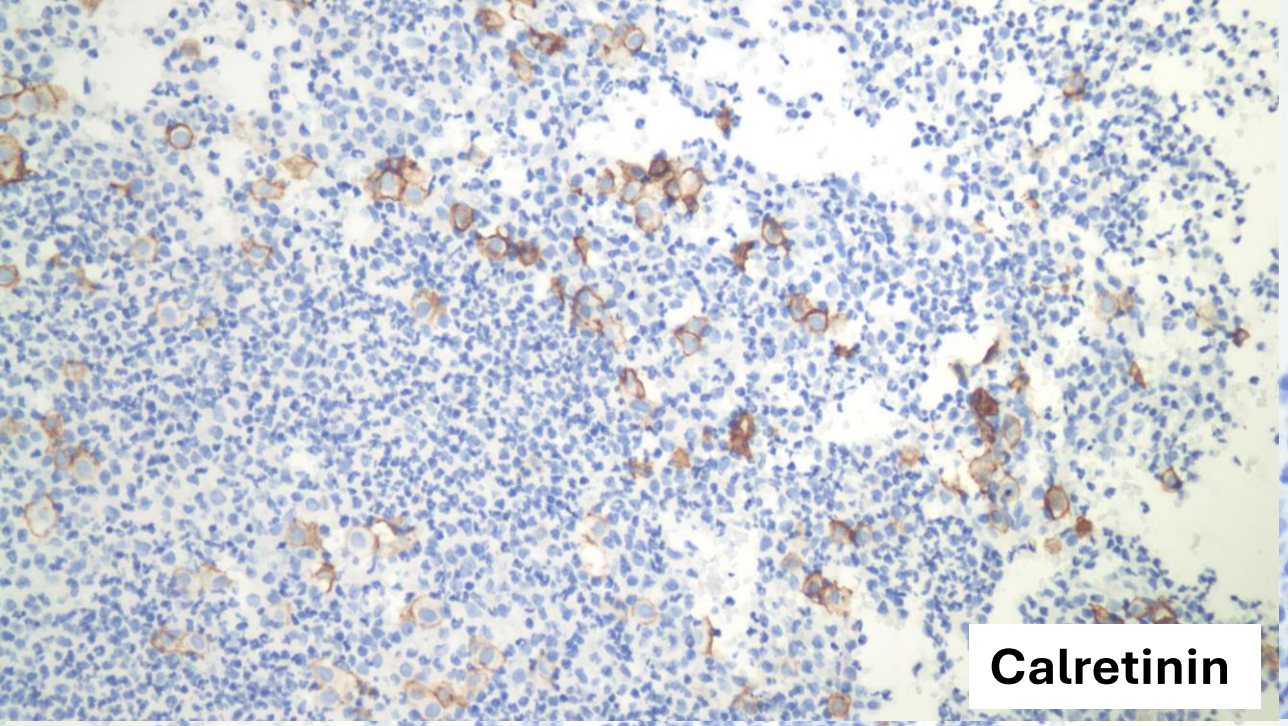
- Cells in 3-D
- Large groups of cells with complex arrangement
- A distinct population of cells distinct from mesothelial cells

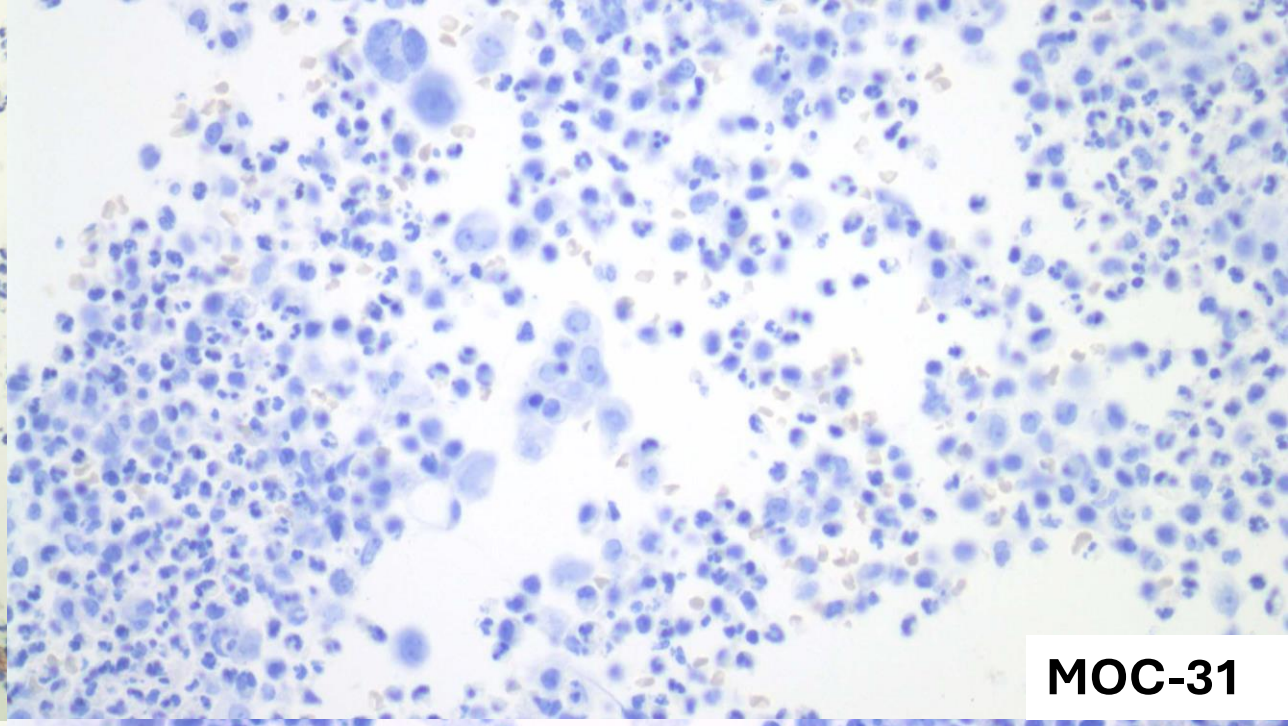
Cytomorphology of mesothelial cells

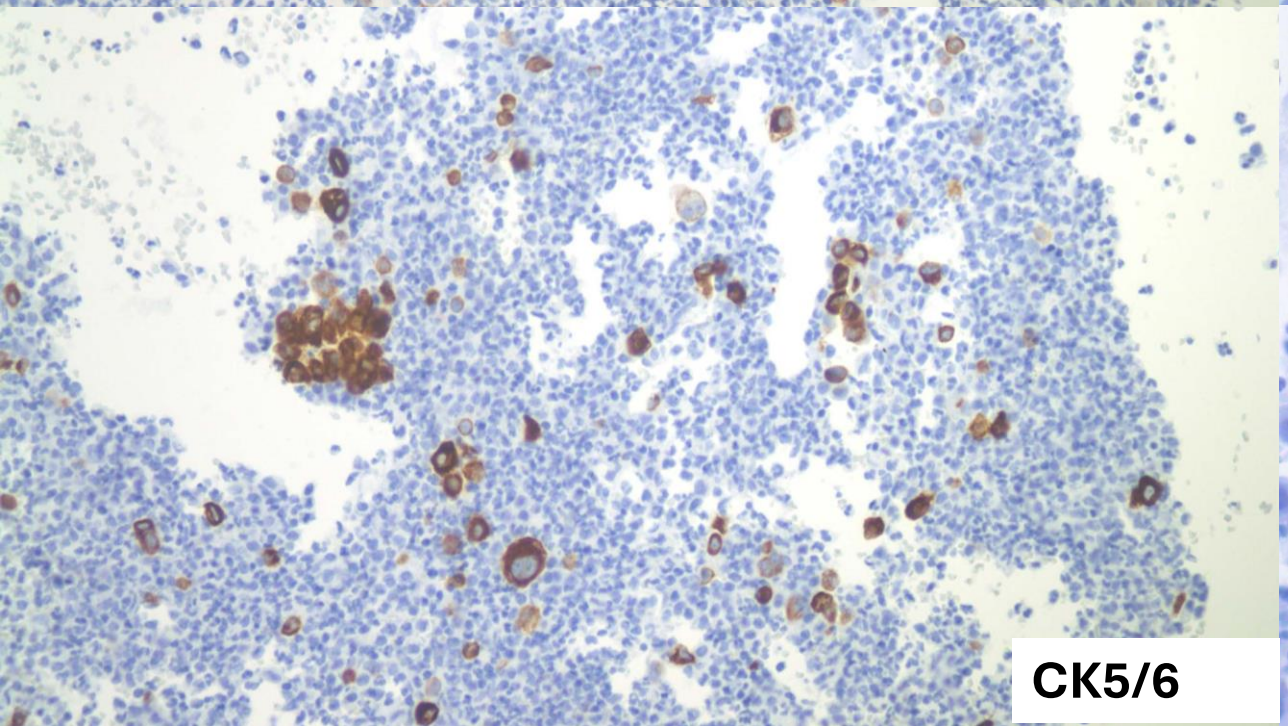
- Often numerous
- Mostly single cell presentation with occasional clusters
- Predominantly mononuclear cells with occasional binucleation
- Round cells with low nuclear/cytoplasmic ratios
- Round, bland nuclei with small, inconspicuous nucleoli
- Dense cytoplasm with clear outer rim (“lacy skirt”)
- Evidence of window-like slits between cells

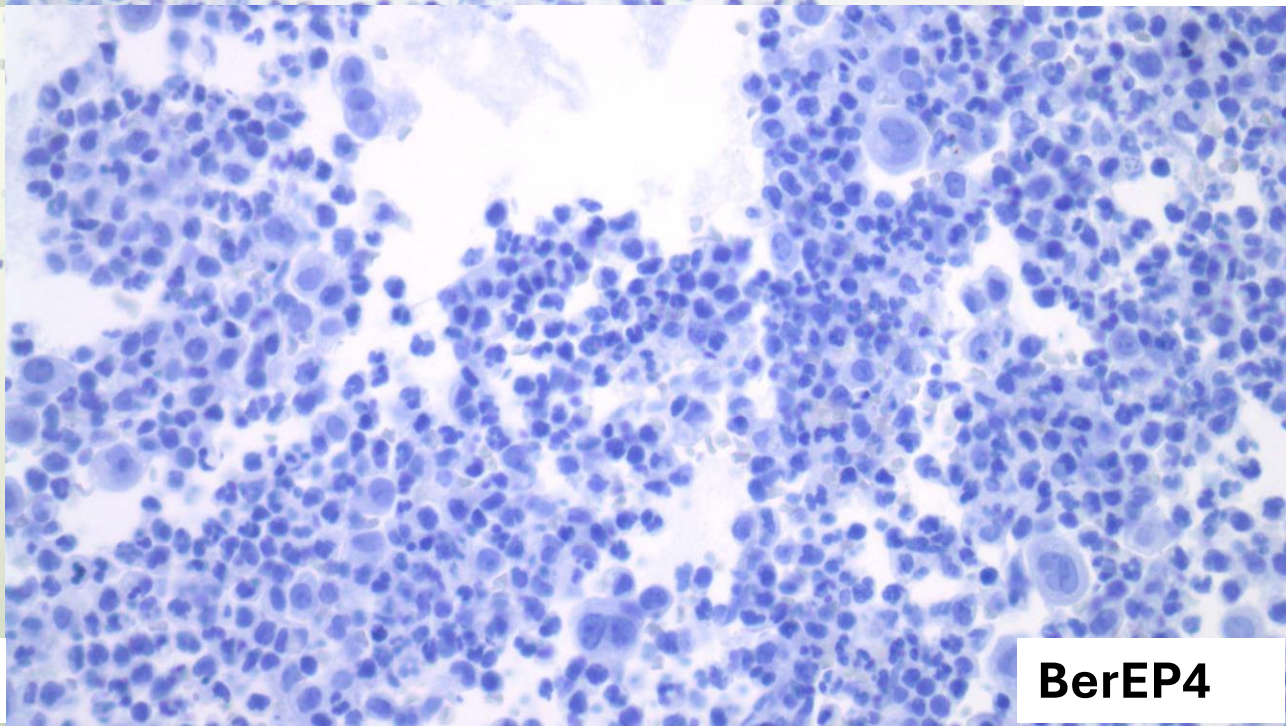





Calretinin


MOC-31


CK5/6


BerEP4

The many faces of mesothelial cells

Cell grouping	Intracellular relationships	Individual cell morphology
Single cells	Pinching	Enlargement
Doublets	Clasping	Elongation
Flat groups	Windows	Hyperchromasia
Rosettes	Side by side	Chromatin clearing
Cells in a row	Molding	Multinucleation
Scalloped cell balls, collagenous cores	Syncytia, cell in cell	Granular inclusions, pseudoacini, blebbing, multiple villi, mitosis

The International System for Reporting Serous Fluid Cytopathology

- To enhance consensus on the meanings assigned to diagnostic terminology
- To prevent clinical misunderstanding that might undermine therapeutic decisions
- Template for improving communication of serous fluid cytology results that strives to reduce reporting variability
- Promotes comparison of research results
- Improves efficiency of electronic record data capture
- Promotes a common language for teaching
- Provides meaningful correlation with follow-up cytology and surgical specimens
- Ultimately improve patient management and quality of clinical care

The International System for Reporting Serous Fluid Cytopathology

- Consists of 5 diagnostic categories
  - Nondiagnostic
  - Negative for malignancy
  - Atypia of undetermined significance
  - Suspicious for malignancy
  - Malignant

Most common tumors that cause malignant pleural effusions by sex

Men	Women
Lung	Breast
Lymphoma/leukemia	Lung
Gastrointestinal tract	Lymphoma/leukemia
Sarcoma	Ovary
Mesothelioma	Gastrointestinal tract
Genitourinary (kidney, bladder, prostate)	Endometrium
Melanoma	**Mesothelioma**

Malignant-Primary (Mesothelioma) Definitive criteria

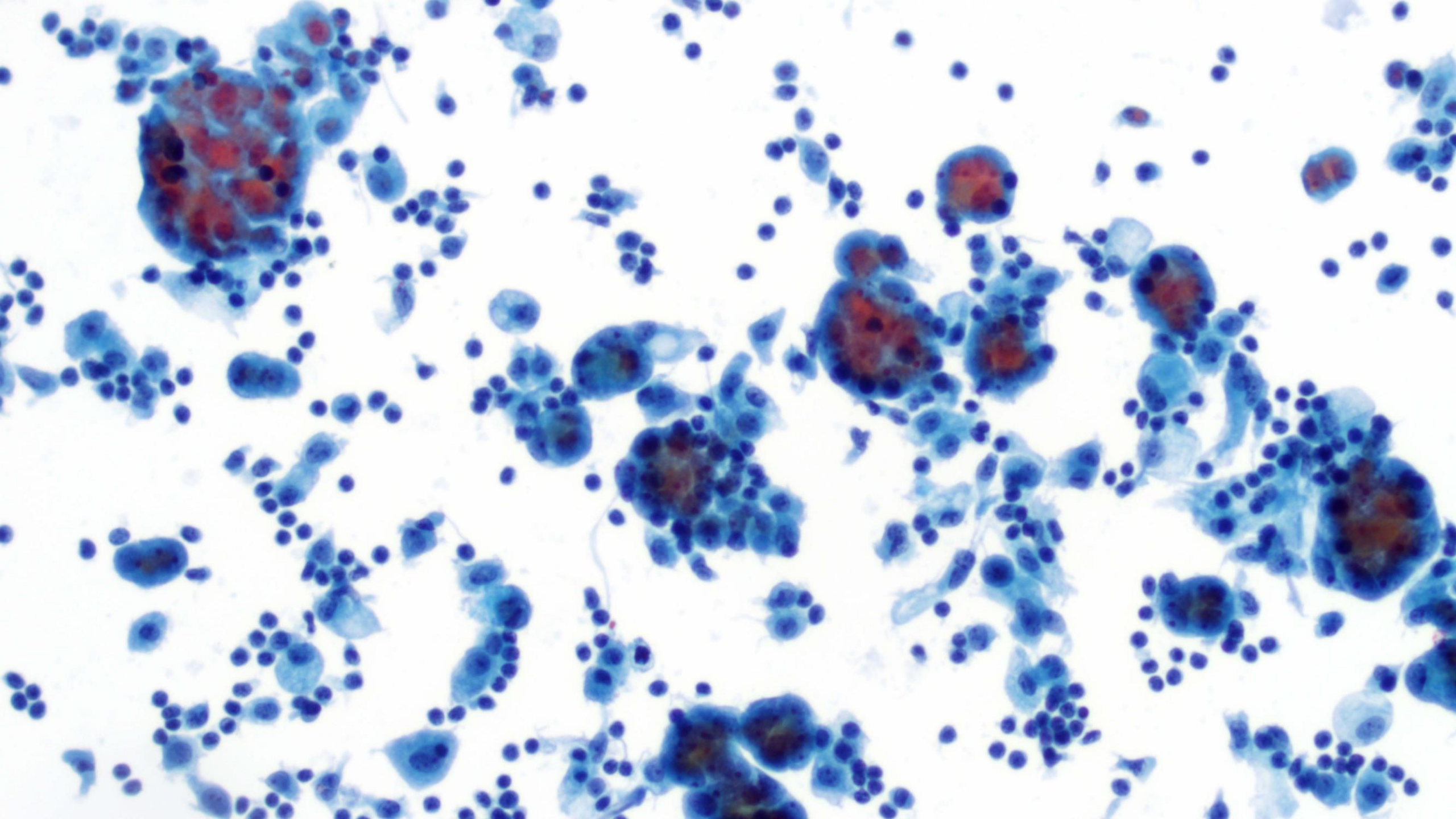
- Hypercellularity
- Numerous cellular spheres, papillary tissue fragments, berry-like morules, single cells or a mixture
- Malignant features identified by either:
  - Overt nuclear abnormalities diagnostic of malignancy (nuclear enlargement, irregular nuclear membranes, macronucleoli, frequent binucleation, and multinucleation, cellular pleomorphism, atypical mitoses)
  - Numerous large tissue fragments and cellular clusters

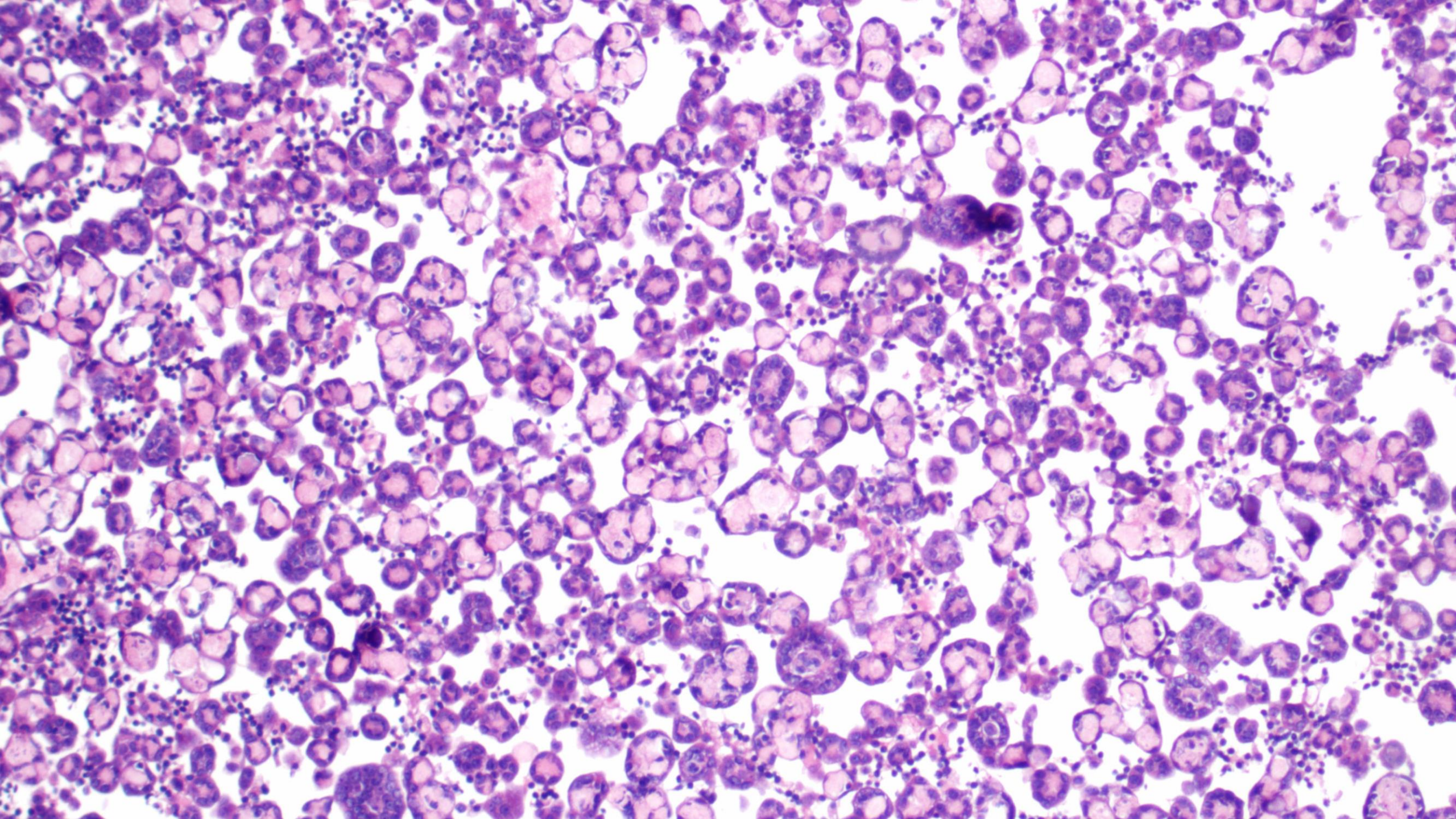
Malignant-Primary (Mesothelioma) Supportive criteria

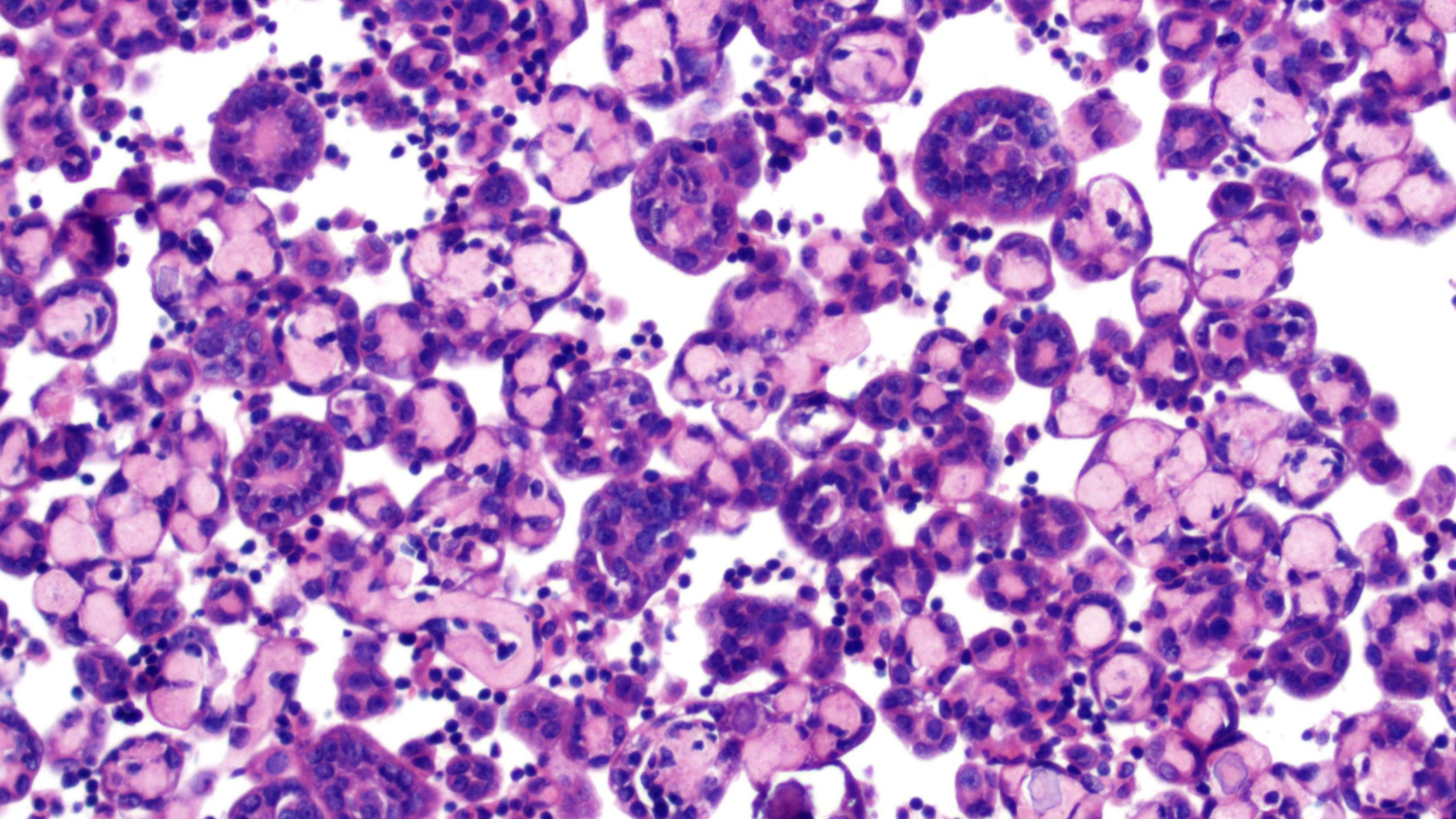
- Significantly enlarged mesothelial cells with abundant cytoplasm
- Large nuclei with subtle atypia
- Prominent nucleoli, often variable in size and number
- Wide variation in cellular size
- Numerous multinucleated cells
- Tissue fragments or papillary groups with collagen or basement membrane cores
- Pseudokeratotic cells
- Large clusters with scalloped (“knobby”) edges
- Giant mesothelial cells, including binucleated and multinucleated forms
- Cellular clasping and “cell within cell” appearance

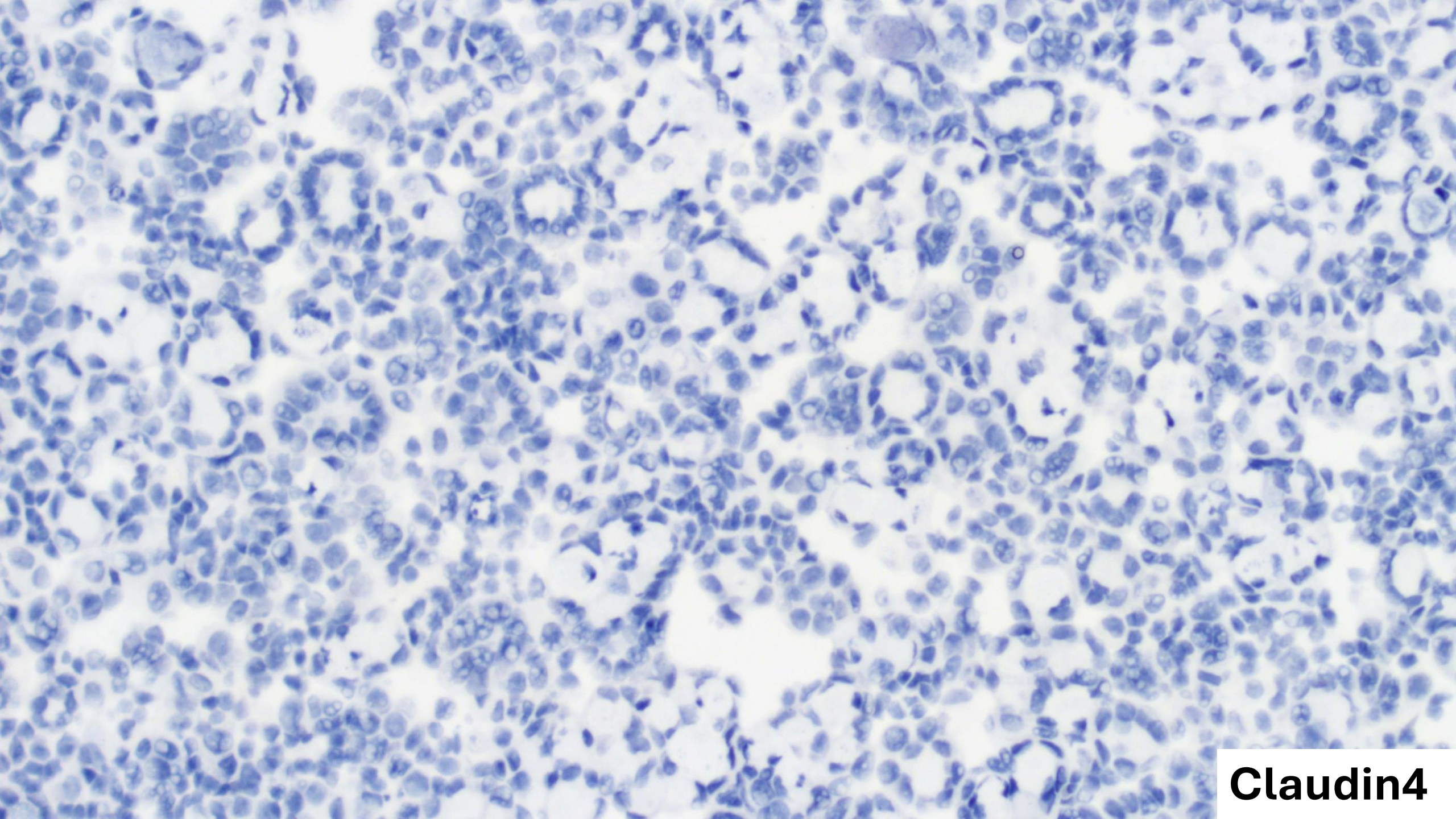
Malignant-Primary (Mesothelioma)

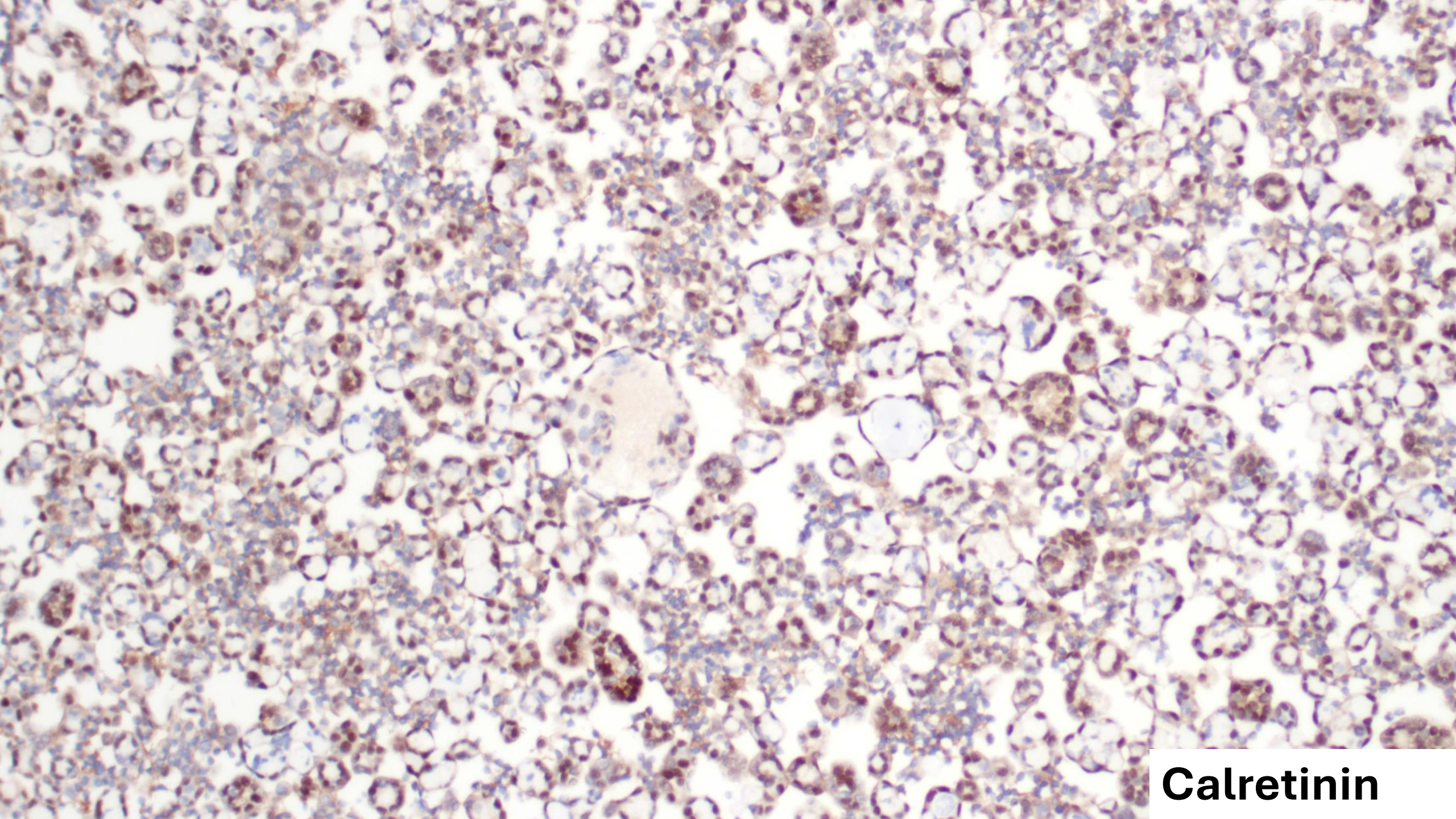
- Risk of malignancy: ~99% (in cases with strong supporting ancillary testing)
- Diagnosis sufficient to initiate treatment without further delay or invasive tissue sampling

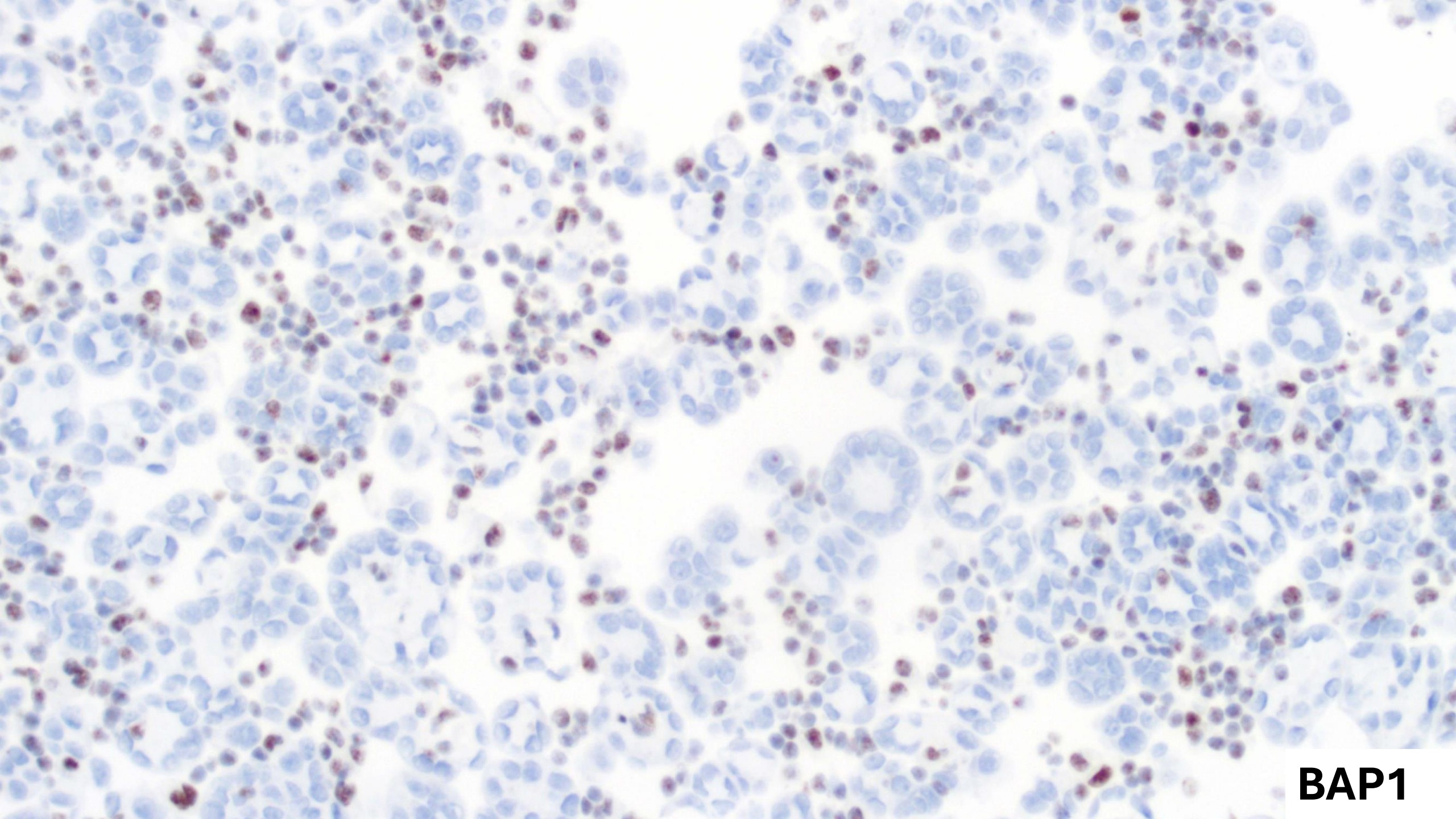


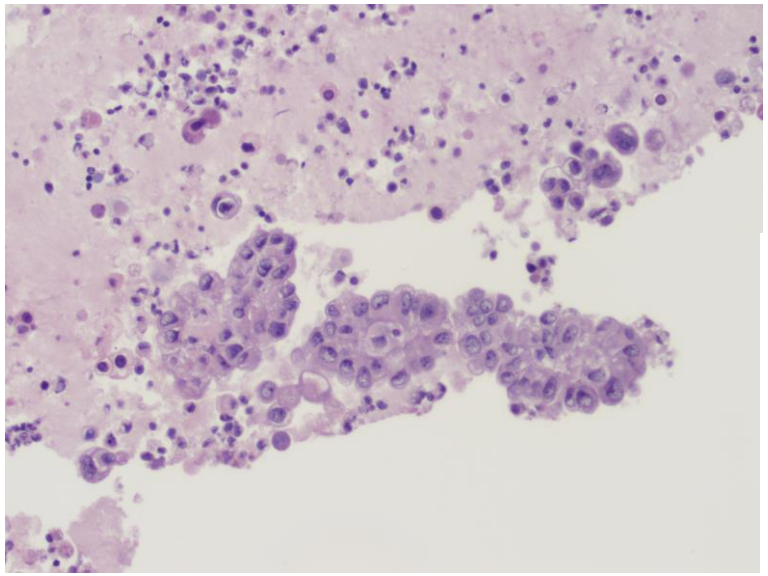


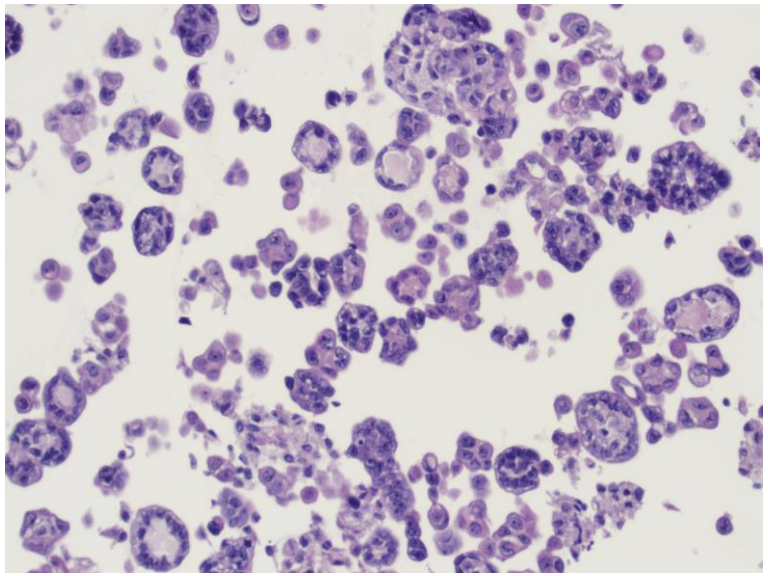


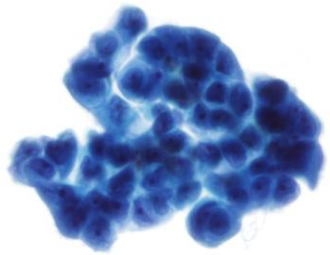

Claudin4

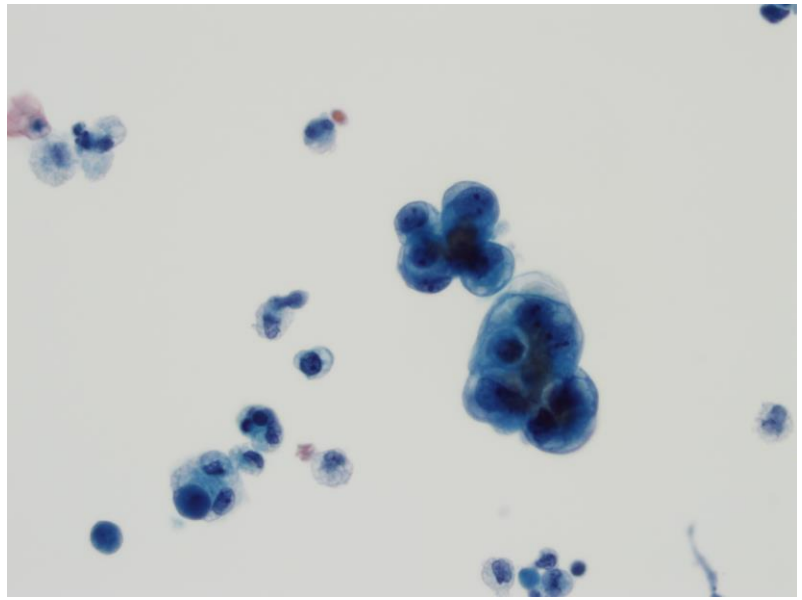

Calretinin

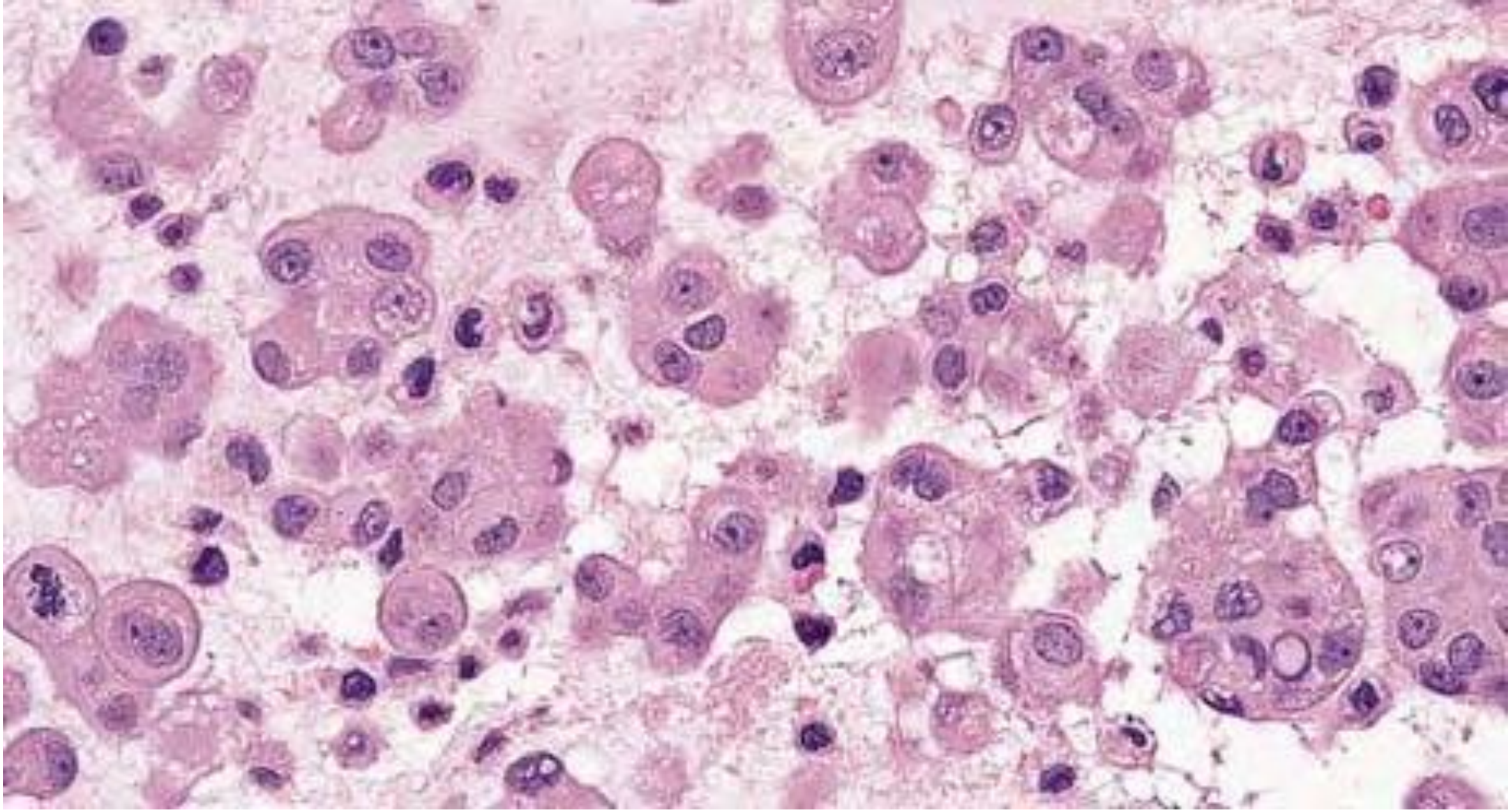

BAP1

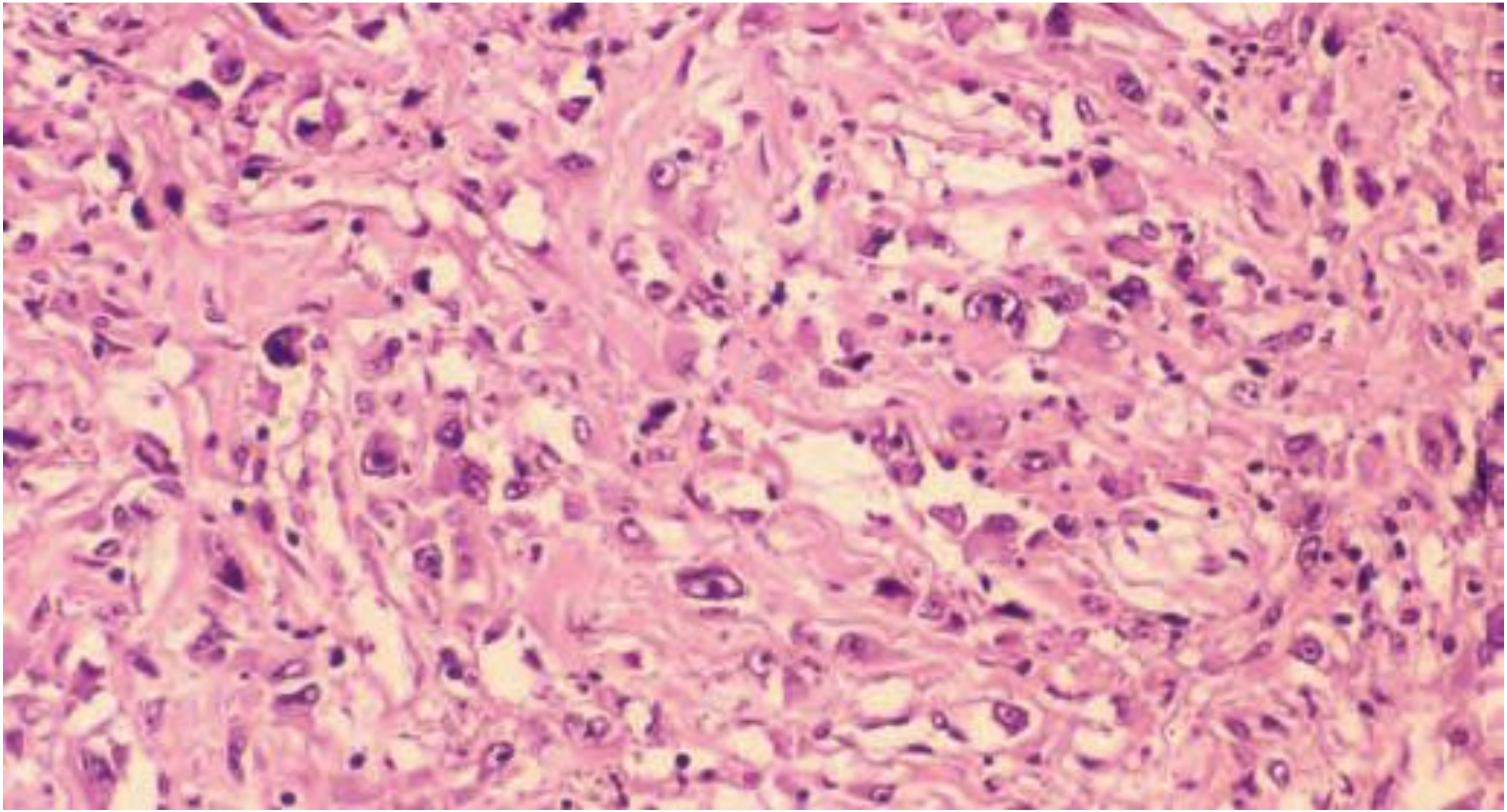


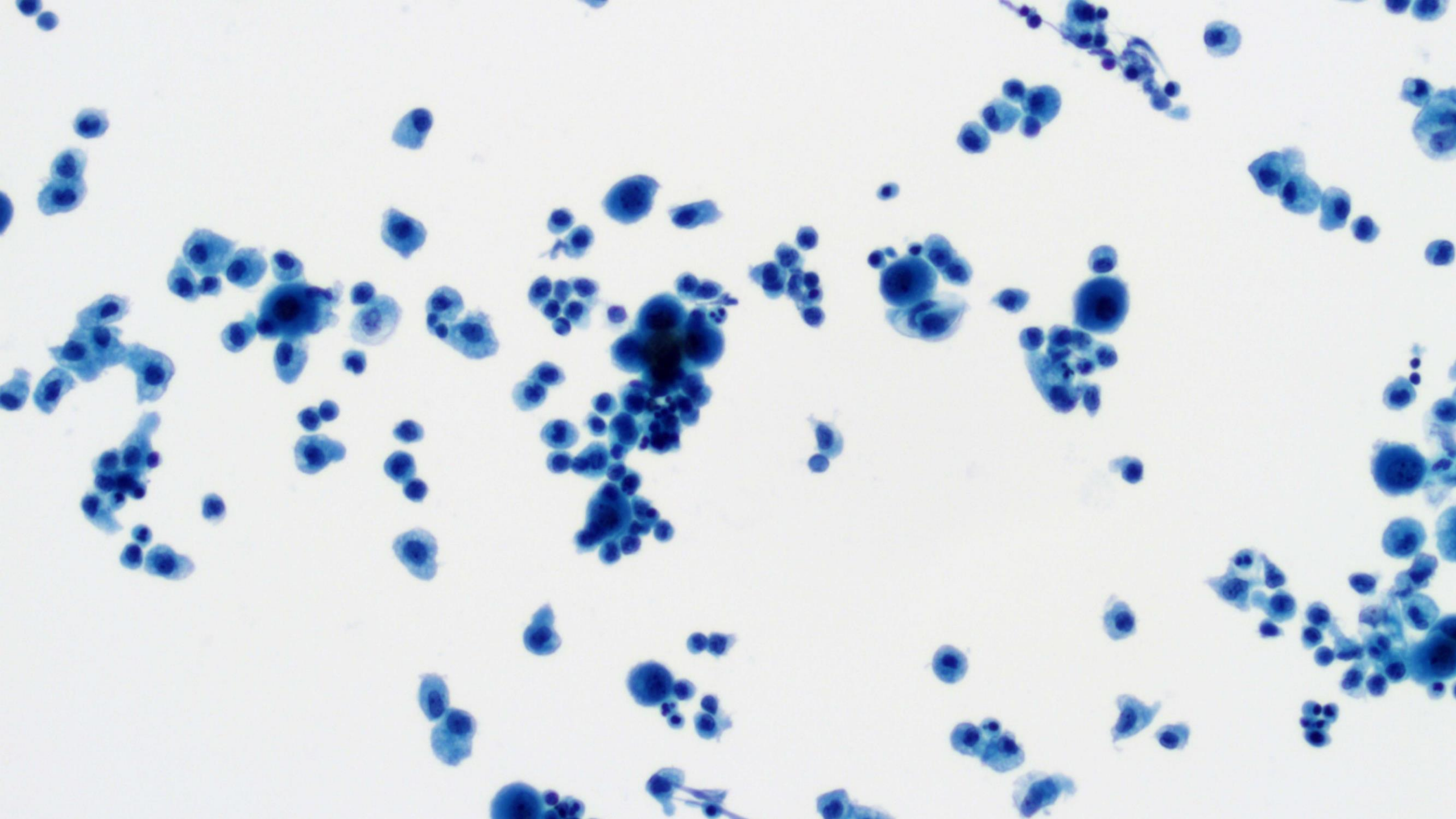


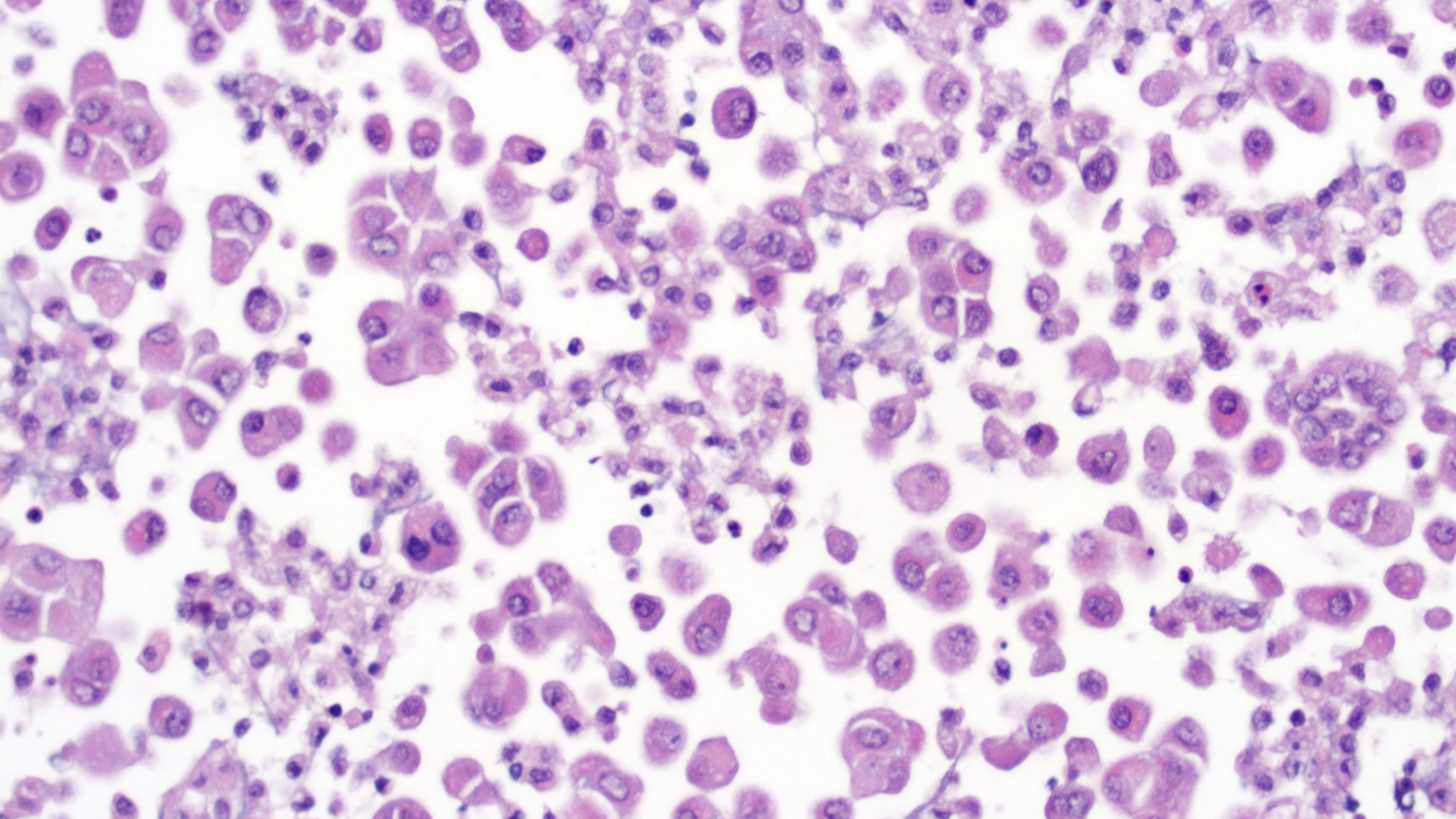


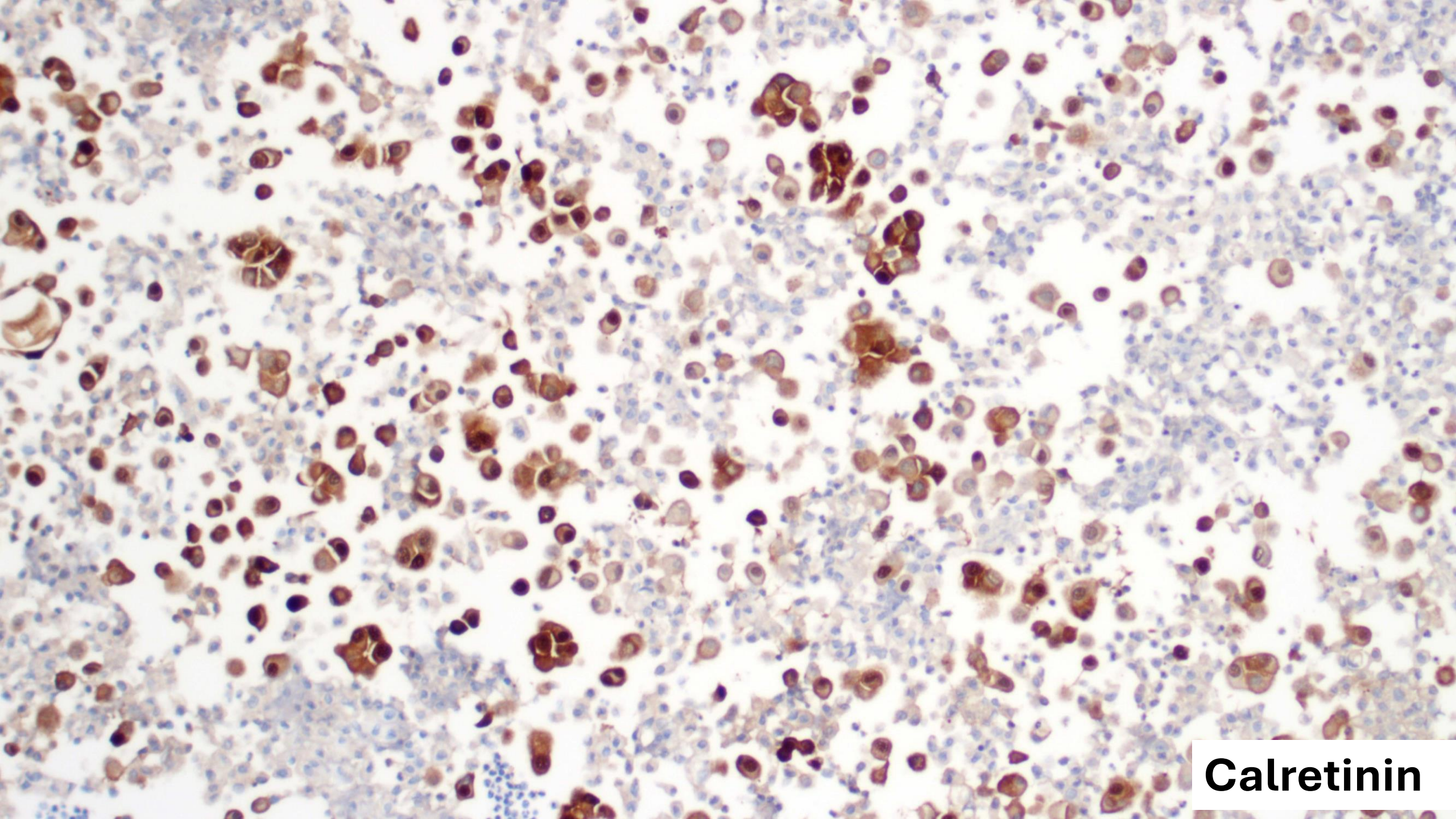


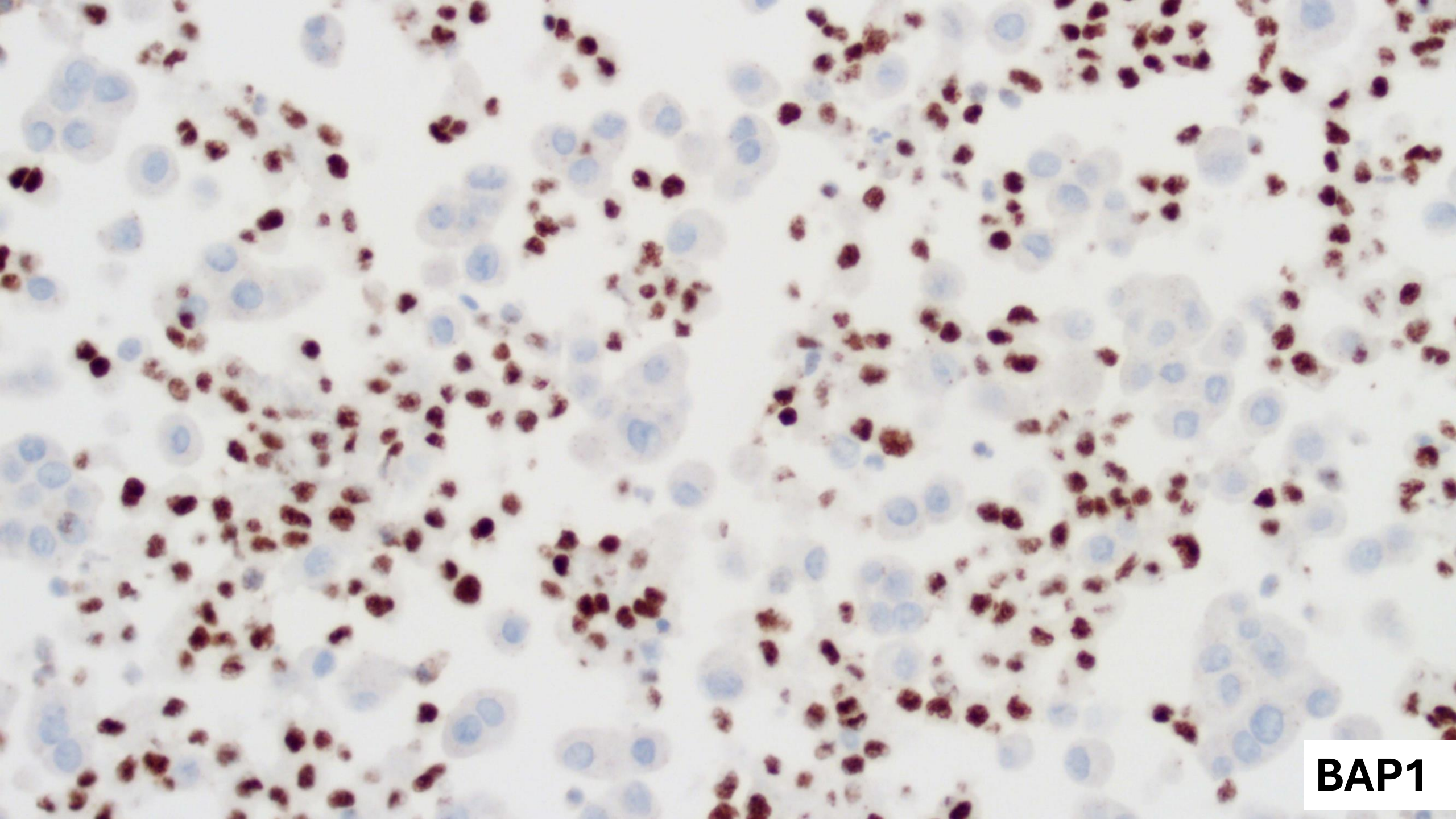









Calretinin


BAP1

Common Differential diagnoses of mesothelioma

- Reactive mesothelial cells
- Metastatic tumor
  - Adenocarcinoma
  - Squamous cell carcinoma
  - Epithelioid hemangioendothelioma

Mesothelioma versus reactive mesothelial cells

- Mesothelioma cells are markedly larger in size
- Chromatin in mesothelioma stains variably darker and may be irregular in distribution
- Nucleoli are usually present and may be enlarged and multiple
- Macronucleoli are associated with malignancy and may be the sole criterion of malignancy
- Reactive mesothelial proliferations may show high cellularity, cytologic atypia, papillary excrescences, and entrapment
- Malignant mesotheliomas may appear bland

Mesothelioma versus reactive mesothelial hyperplasia

	Reactive mesothelial hyperplasia	Mesothelioma
Cellularity	May be prominent within the mesothelial space, but not within the stroma	Increased cellularity can be seen within stroma
Cell arrangement	Single or small number of cell layers limited to the surface; papillae without fibrovascular cores	Complex, branching papillae with fibrovascular cores; tubules with irregular slit-like spaces; solid growth pattern
Stromal invasion	Absent (beware of entrapment)	Present (highlight with pan-cytokeratin or calretinin staining)
Necrosis	Absent usually	Present occasionally
Ancillary techniques	Negative for EMA, p53; positive for desmin	Positive for EMA, P53; negative for desmin

Mesothelioma versus reactive mesothelial cells

- Ancillary studies were of little value in this scenario
  - Desmin
  - EMA
  - GLUT-1
  - p53
  - Ki67
  - Oncofetal Protein IMP3
- Distinction remained a clinicopathologic one
- Pathologic parameter was morphologic assessment by standard H&E light microscopy

Mesothelioma versus reactive mesothelial cells

Markers	Sensitivity	Specificity
Loss of expression of nuclear BAP1		
All mesothelioma	27 - 67	100
Epithelioid mesothelioma	56 – 81	
Sarcomatoid mesothelioma	0 - 63	
Loss of expression of cytoplasmic MTAP		
Epithelioid mesothelioma	37	100
Sarcomatoid mesothelioma	80	
Homozygous deletion of CDKN2A (p16) by FISH		
All mesothelioma	58 - 62	100
Epithelioid mesothelioma	48 - 78	
Sarcomatoid mesothelioma	67	

Mod Pathol 2015;28:1043, Am J Surg Pathol 2015;39:977, Am J Surg Pathol 2016;40:714, Arch Pathol Lab Med 2018;142:1549, Hum Pathol 2017;60:86, Lung Cancer 2017;104:98, J Thorac Oncol 2015;10:565, Lung Cancer 2018;125:198, Mod Pathol 2020;33:245, Ann Diagn Pathol 2017;26:31

Types of epithelioid mesothelioma

- Small cell
- Deciduoid
- Adenomatoid
- Mucin-positive
- Signet-ring cell
- Mesothelioma with rhabdoid features

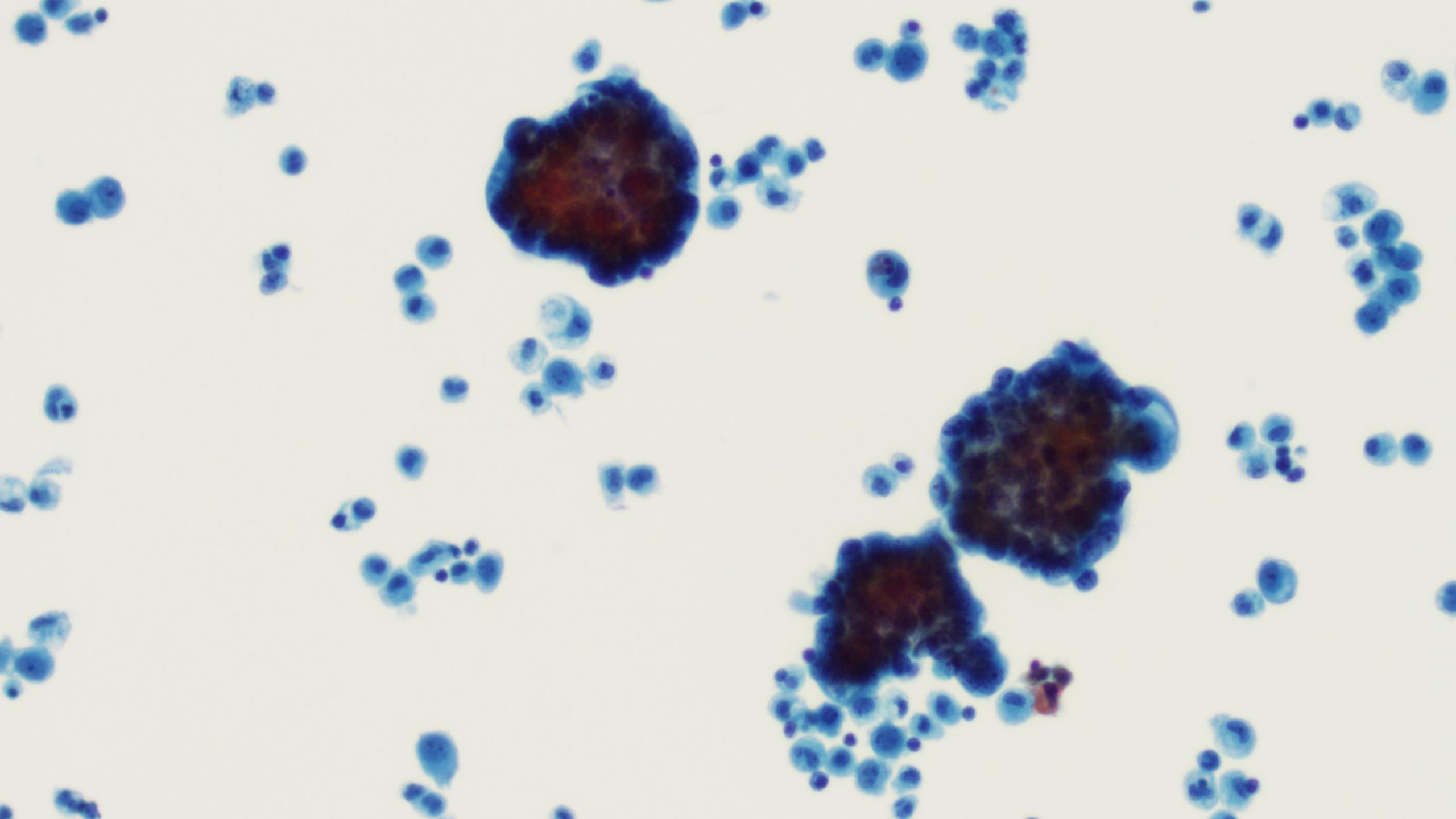
Mimics of epithelioid mesothelioma

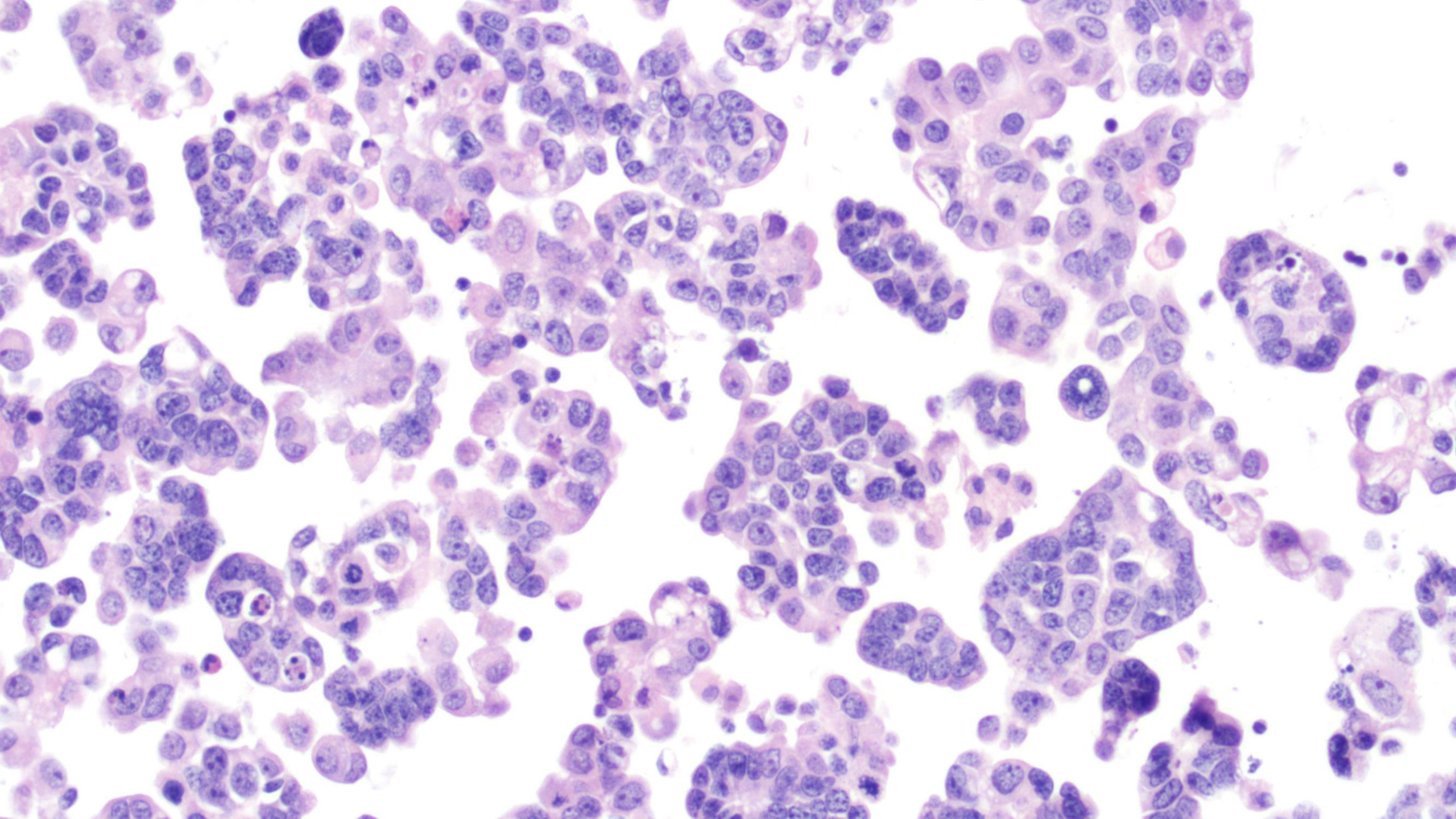
- NSCLC – adenocarcinoma
- NSCLC – squamous cell carcinoma
- Metastatic carcinoma e.g. breast, GI, ovarian, urothelial
- Melanoma

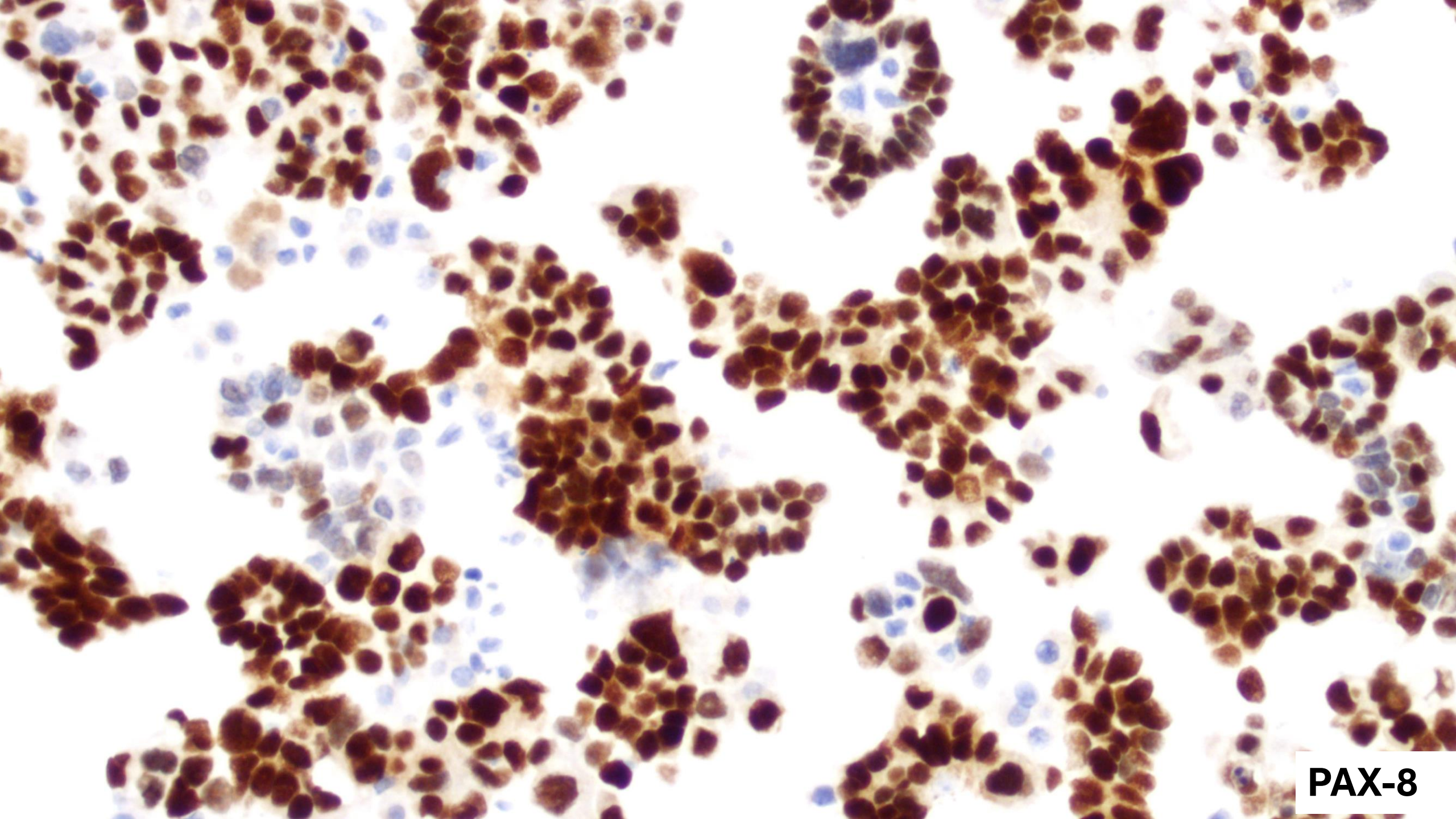
Epithelioid mesothelioma versus metastatic carcinoma

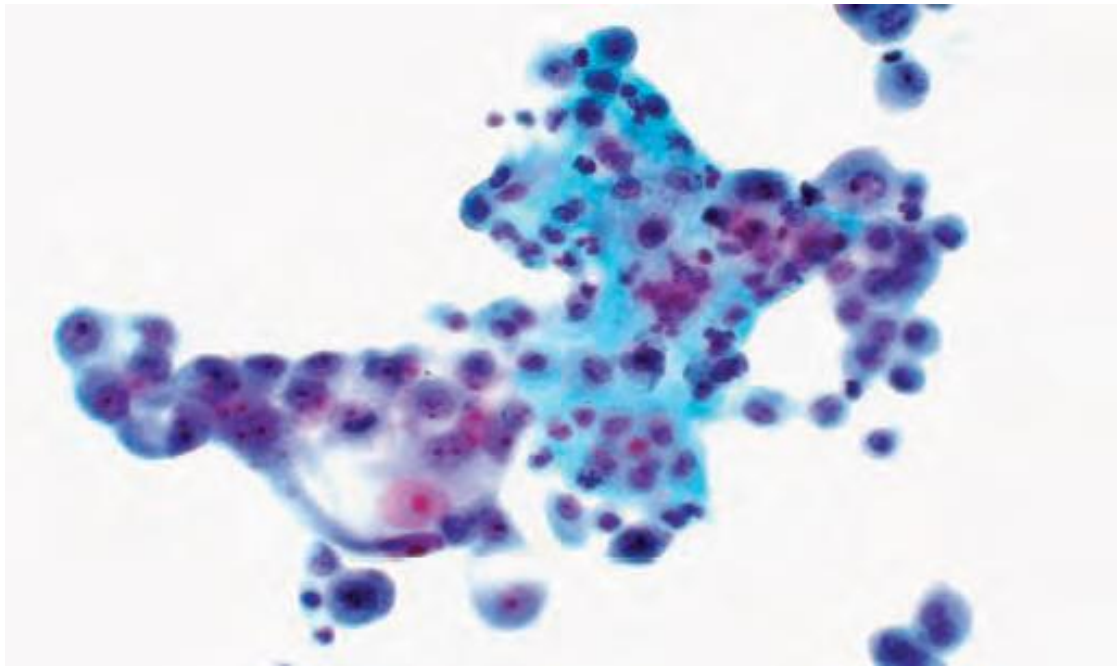
- Complex tubules and papillae lined by uniform cells with vesicular nuclei, single central nucleoli and a moderate amount of pale-staining cytoplasm in epithelioid mesothelioma may mimic metastatic adenocarcinoma.
- Intracellular vacuoles may be present in epithelioid mesothelioma
- Tumor giant cells or anaplastic cells may be present in poorly differentiated epithelioid mesothelioma
- Mesothelioma may demonstrate diffuse clear cell changes which may be mistaken as metastatic clear cell carcinoma
- Diagnosis relies heavily on Immunohistochemistry

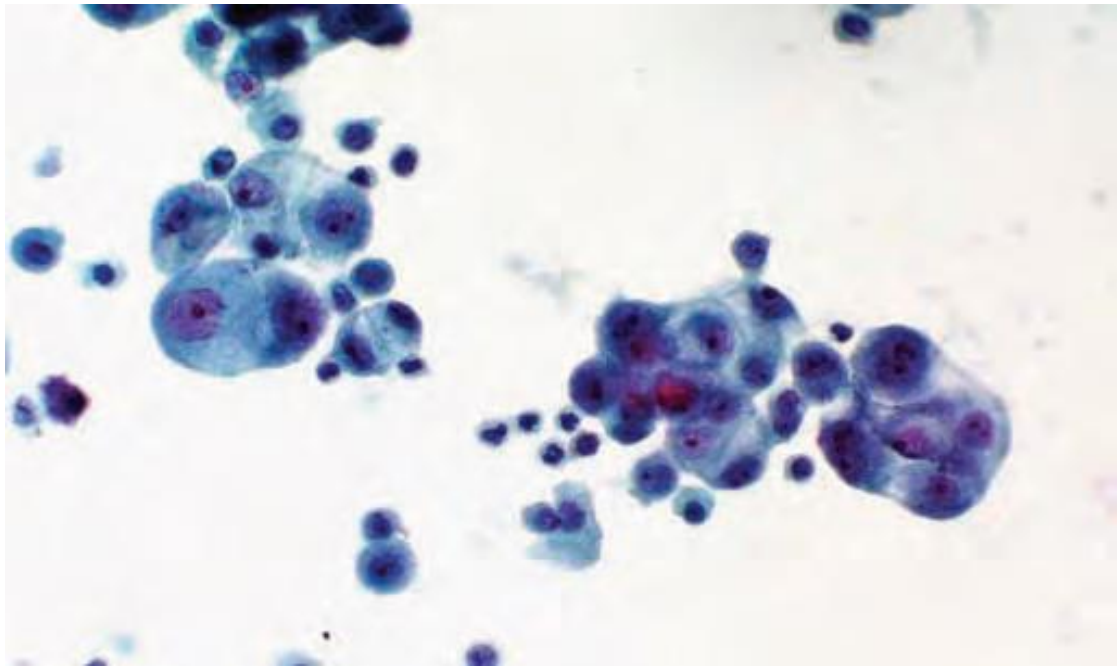
Mesothelioma versus Adenocarcinoma

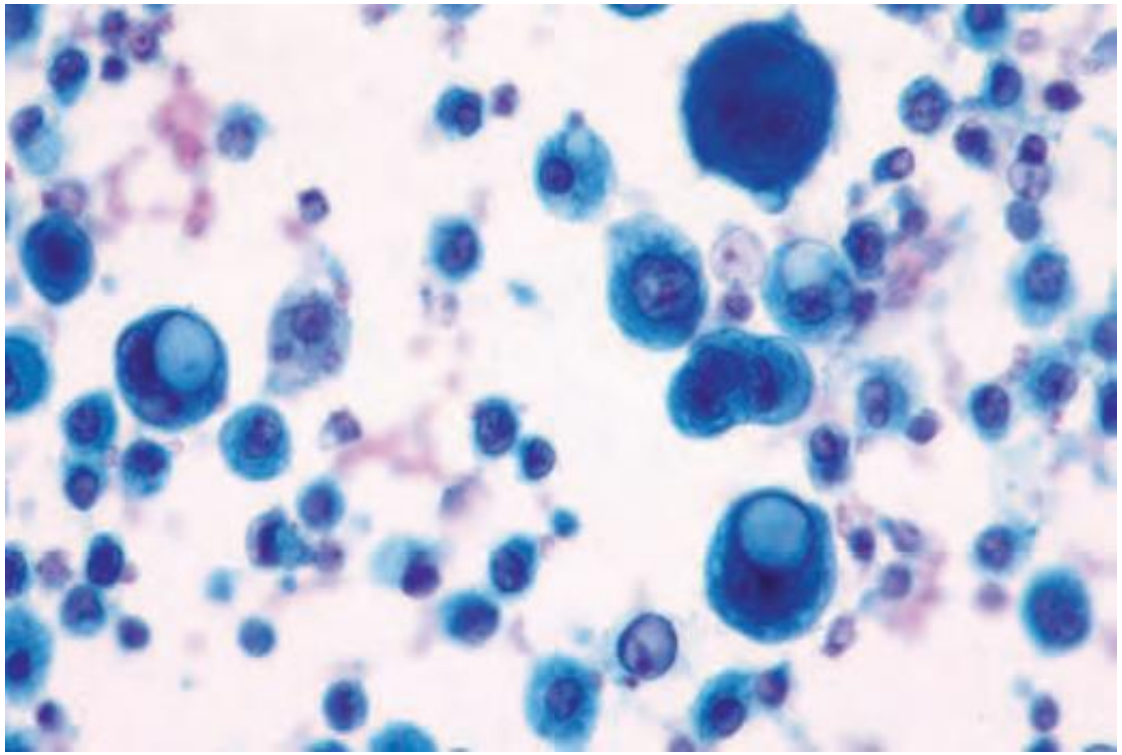


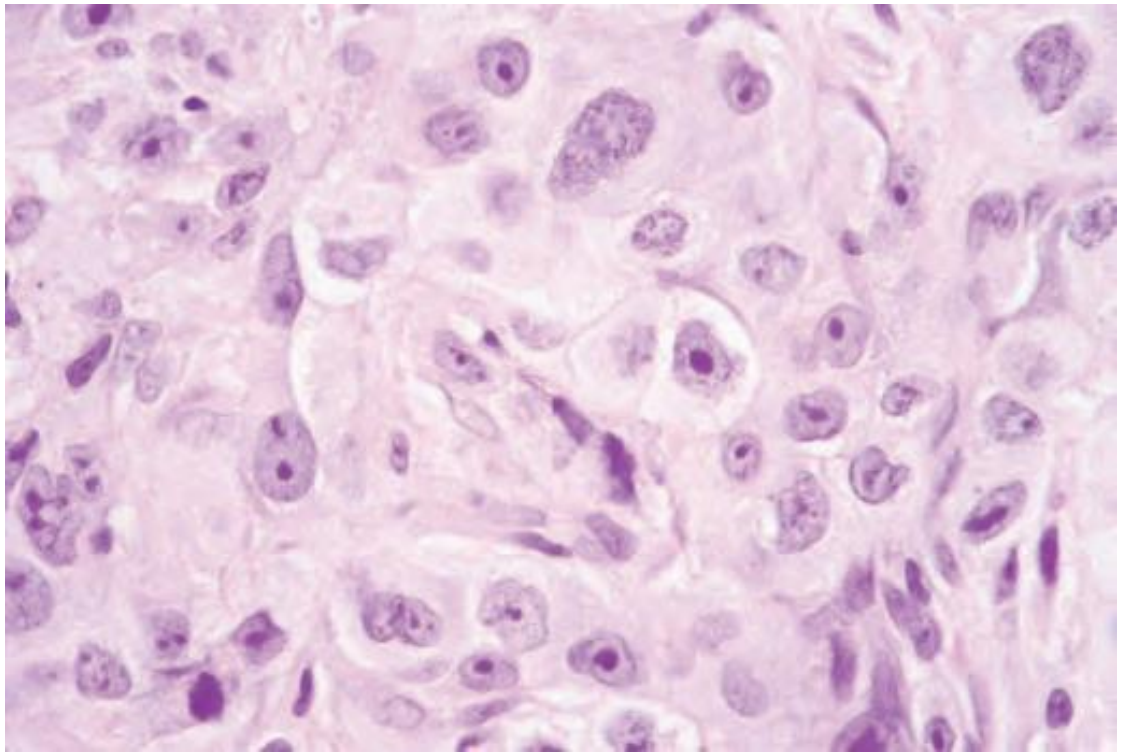


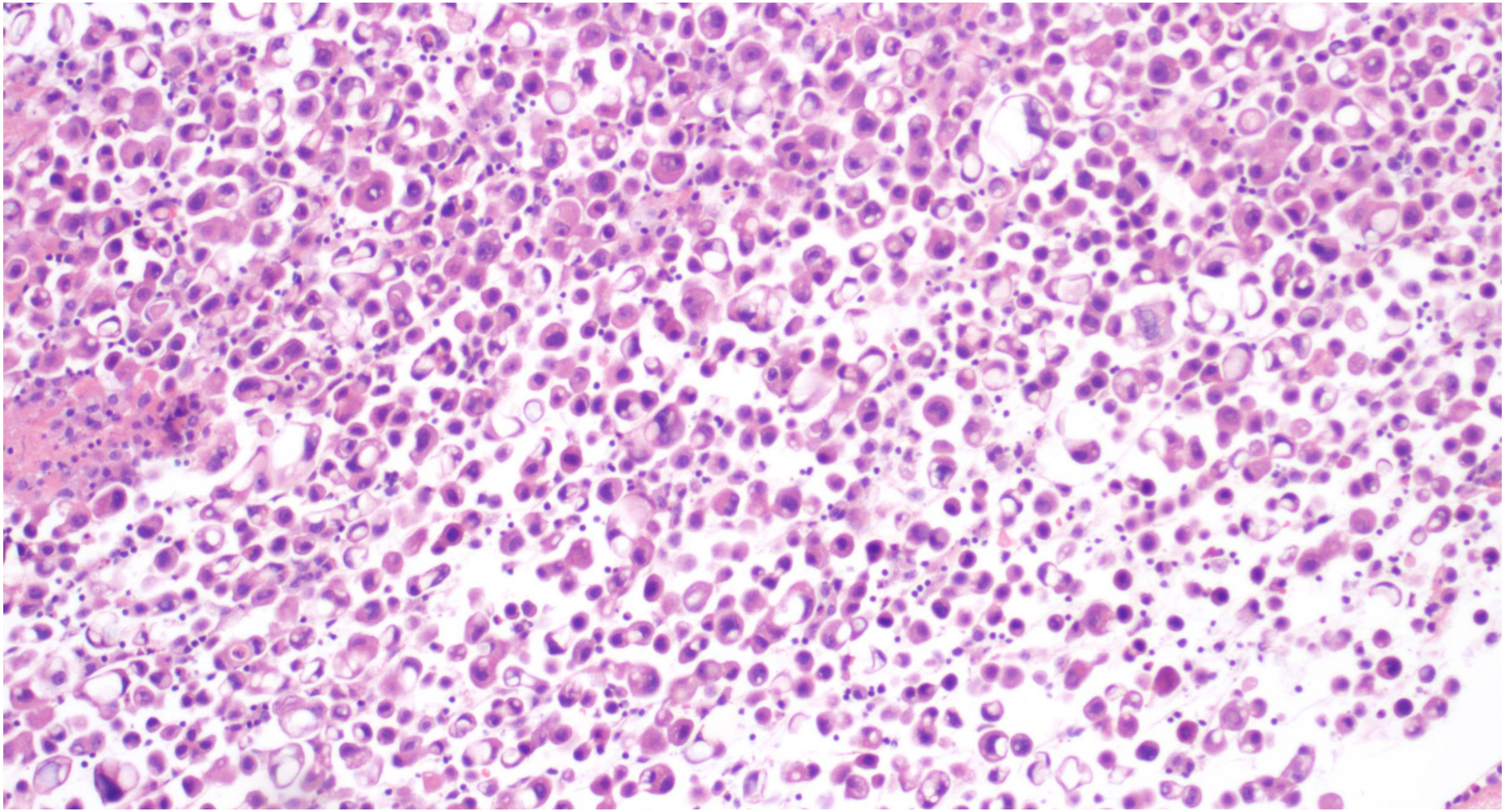

PAX-8

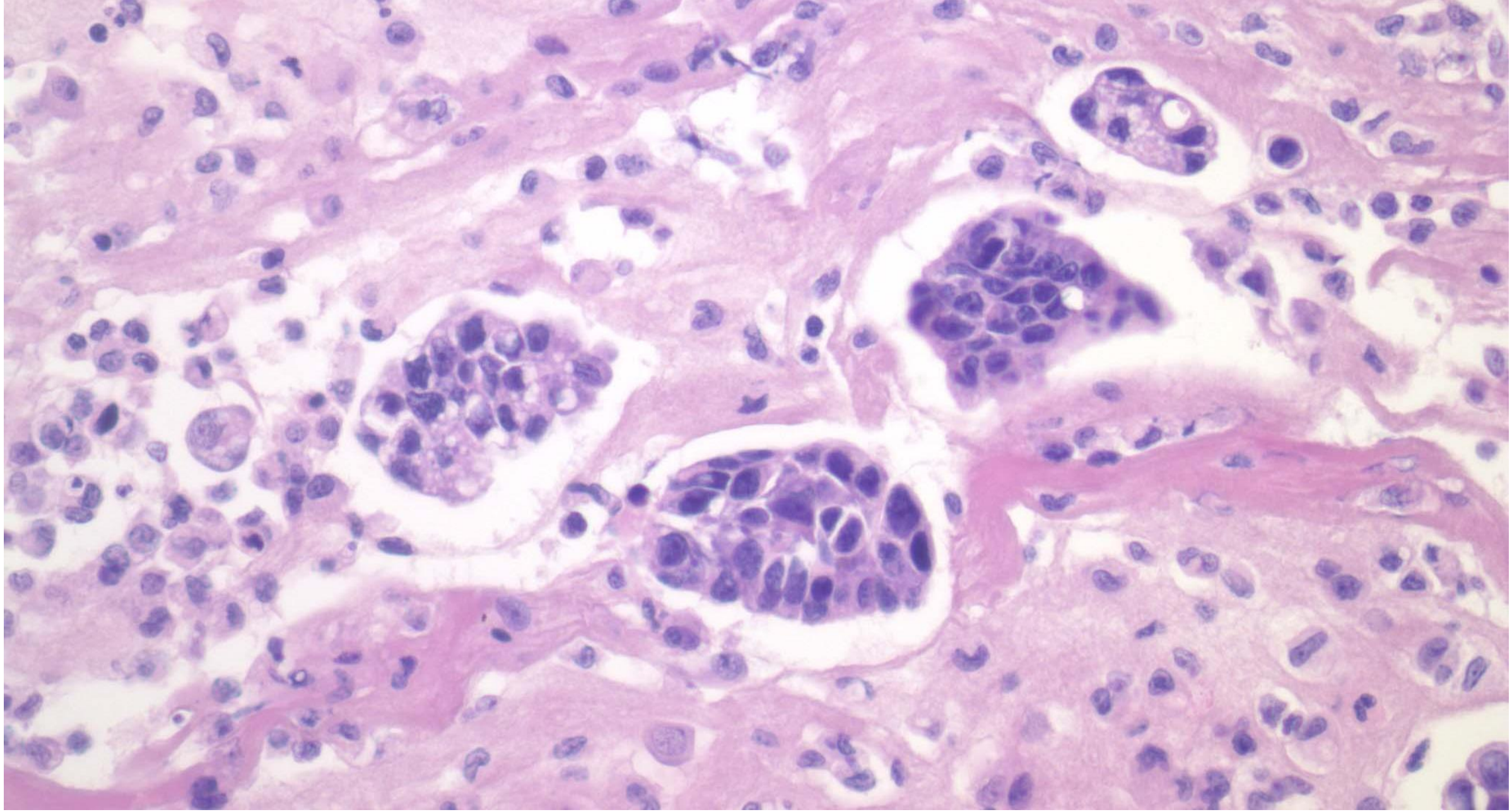












Cytologic differences between Malignant Mesothelioma and Adenocarcinoma

Feature	Malignant Mesothelioma	Adenocarcinoma
Cell population	Monotonous mesothelial cells	2-cell population
Cellular groups	3-D, tight spheres and loose clusters with knobby borders Windows commonly seen	Syntitial 3-D cluster or papillae with community borders Windows unusual
Psammoma bodies	Few in number when present	Numerous when present
Nucleus	Usually central or paracentral Mild hyperchromasia Small or very prominent nucleoli	Usually eccentric Marked hyperchromasia Frequently prominent irregular nucleoli
Cytoplasm	Dense center with fuzzy edges 2-tone staining	Delicate, homogeneous Uniform stain
Vacuoles	Perinuclear and submembranous	Secretory, large Randomly distributed
Multinucleated giant cells	Common	Rare

Typical immunostaining patterns of Malignant Mesothelioma and Adenocarcinoma

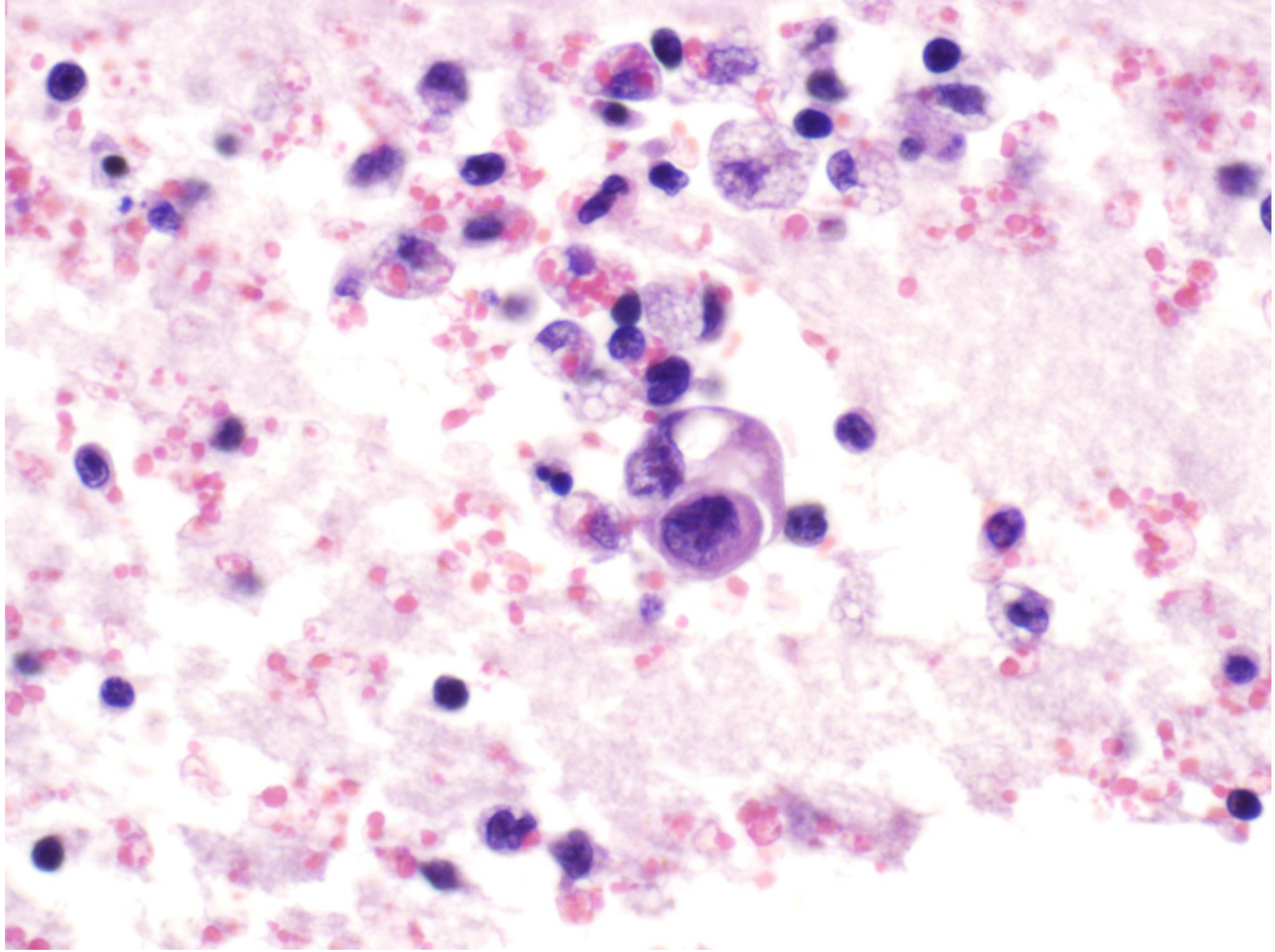
Tumor	Malignant mesothelioma	Adenocarcinoma
AE1/AE3	+	+
CK5/6	+	-
Calretinin	+	-
HBME-1	+	-/+
WT-1	+	-
D2-40	+	-
Claudin-4	-	+
CEA	-	+
Ber-EP4	-	+
B72.3	-	+
CD15	-	+
MOC-31	-	+
Mucicarmine	-	+
TTF-1	-	+

Suspicious for malignancy

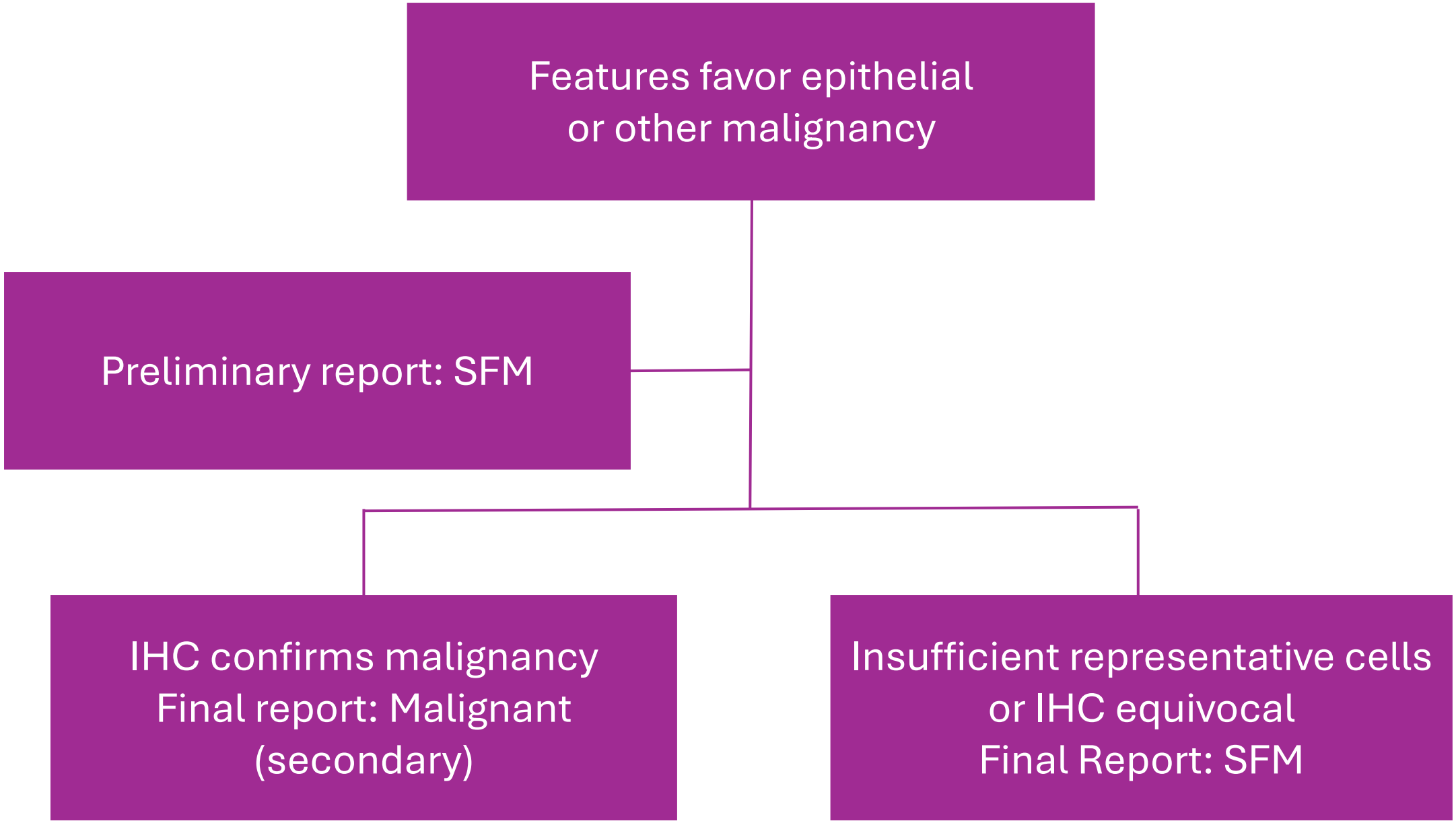
- Evidence falls short of confirming malignancy based on cytomorphology and results of ancillary tests
- Cells occurring in small or occasionally in large numbers but limited by artifact and raising the suspicion of malignancy
- Monomorphous lymphoid population or atypical lymphoid cells in varying numbers
- Presence of mucinous material alone or with small numbers of bland epithelial cells
- Mesothelial proliferation suspicious for mesothelioma
- Presence of epithelial cells in peritoneal washings

Suspicious for malignancy

- Risk of malignancy: ~80%
- Patients managed same way as proven malignant effusions
- Use should be reserved for highly probable malignant cases



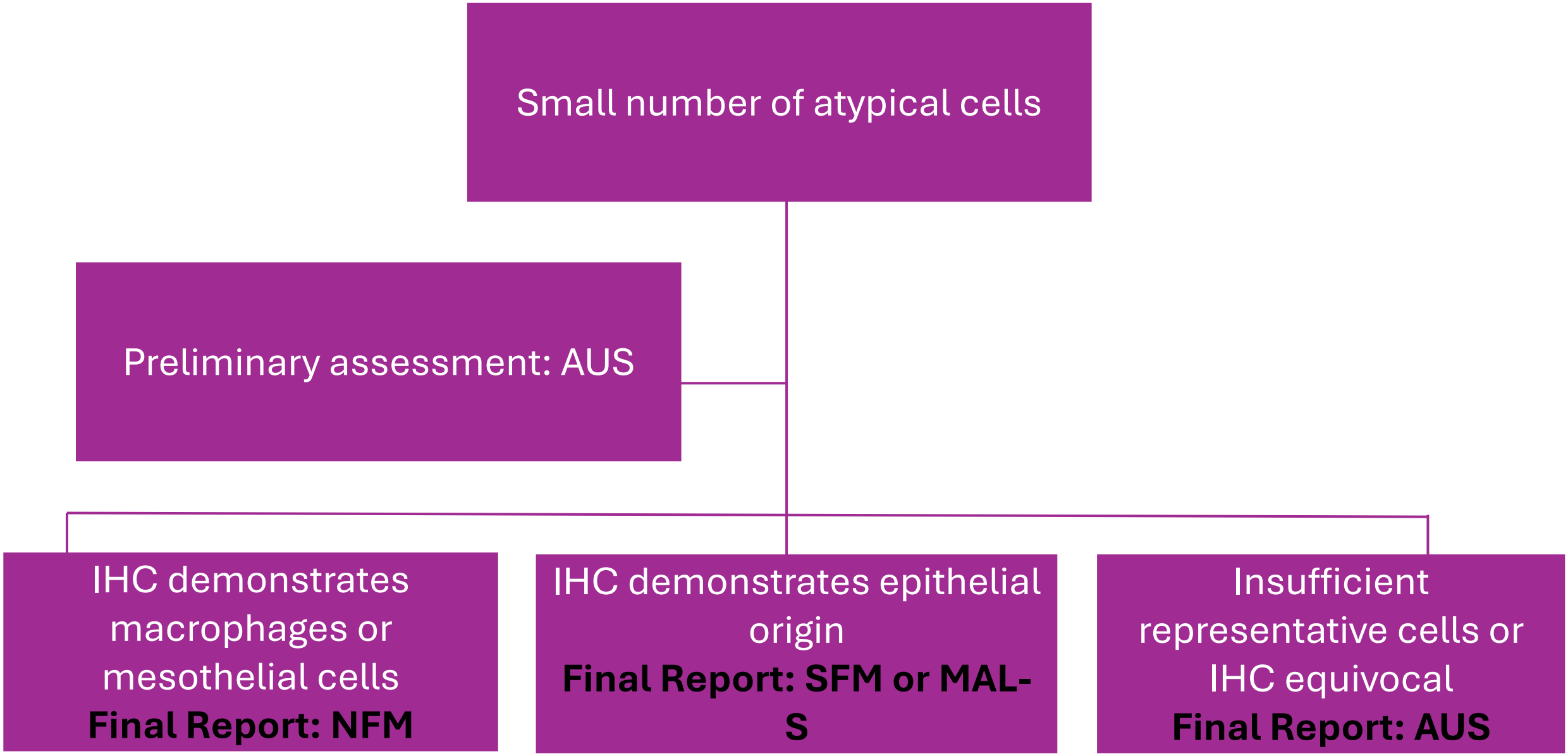
Algorithm for SFM



Atypia of undetermined significance

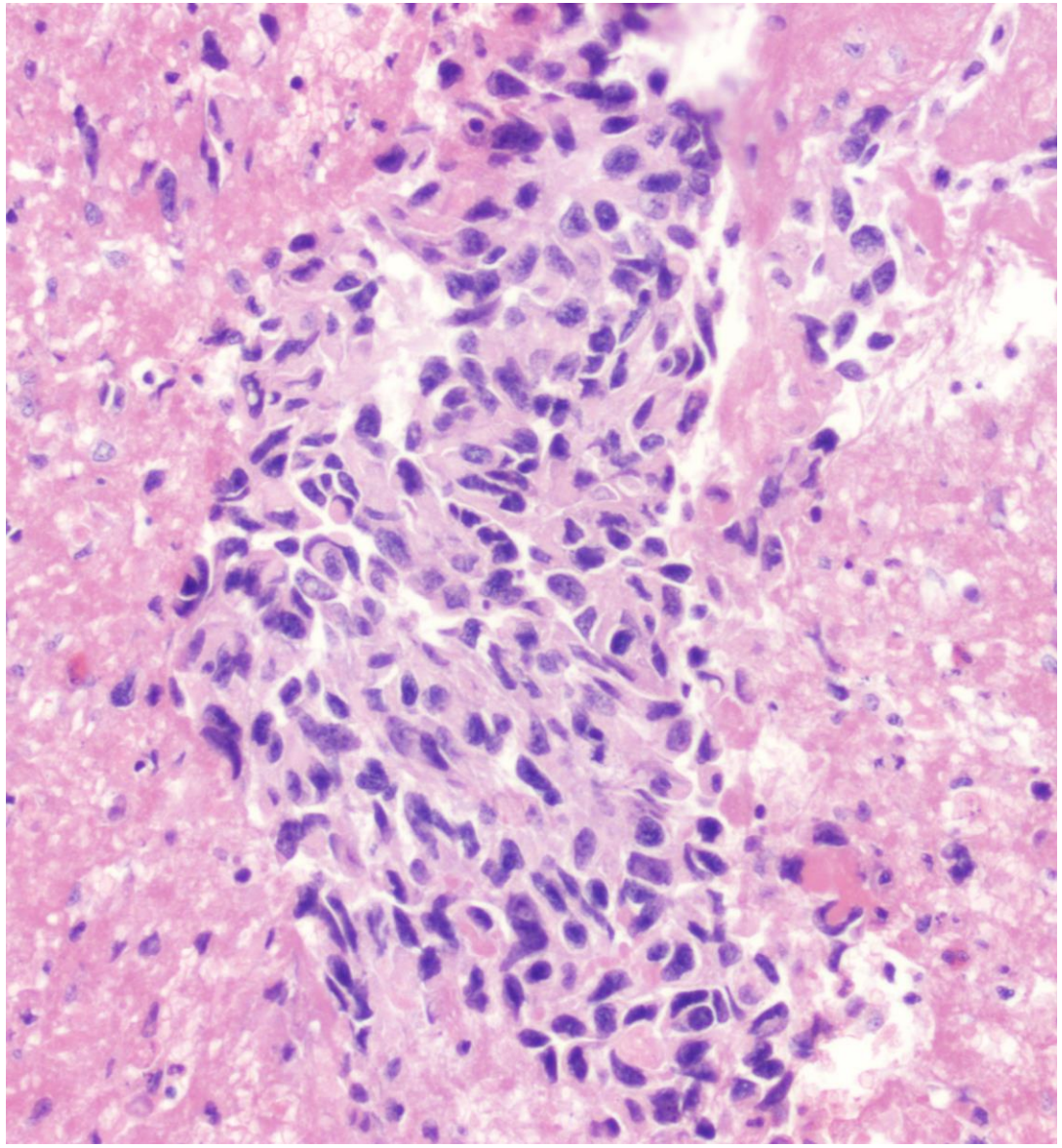
- Specimen indeterminate for mesothelial or non-mesothelial malignancy
- Represents a true gray zone in effusion cytology
- Includes cases showing extremes of reactive atypia or specimens containing few or degenerated tumor cells
- Mild to moderate nuclear enlargement, slightly increased N/C ratio, prominent or variable nucleoli, slight nuclear membrane irregularities, altered chromatin
- Lymphocytosis indefinite for lymphoproliferative disorder
- Epithelial cells of unknown or indeterminate origin with bland features
- Risk of malignancy: ~66%*

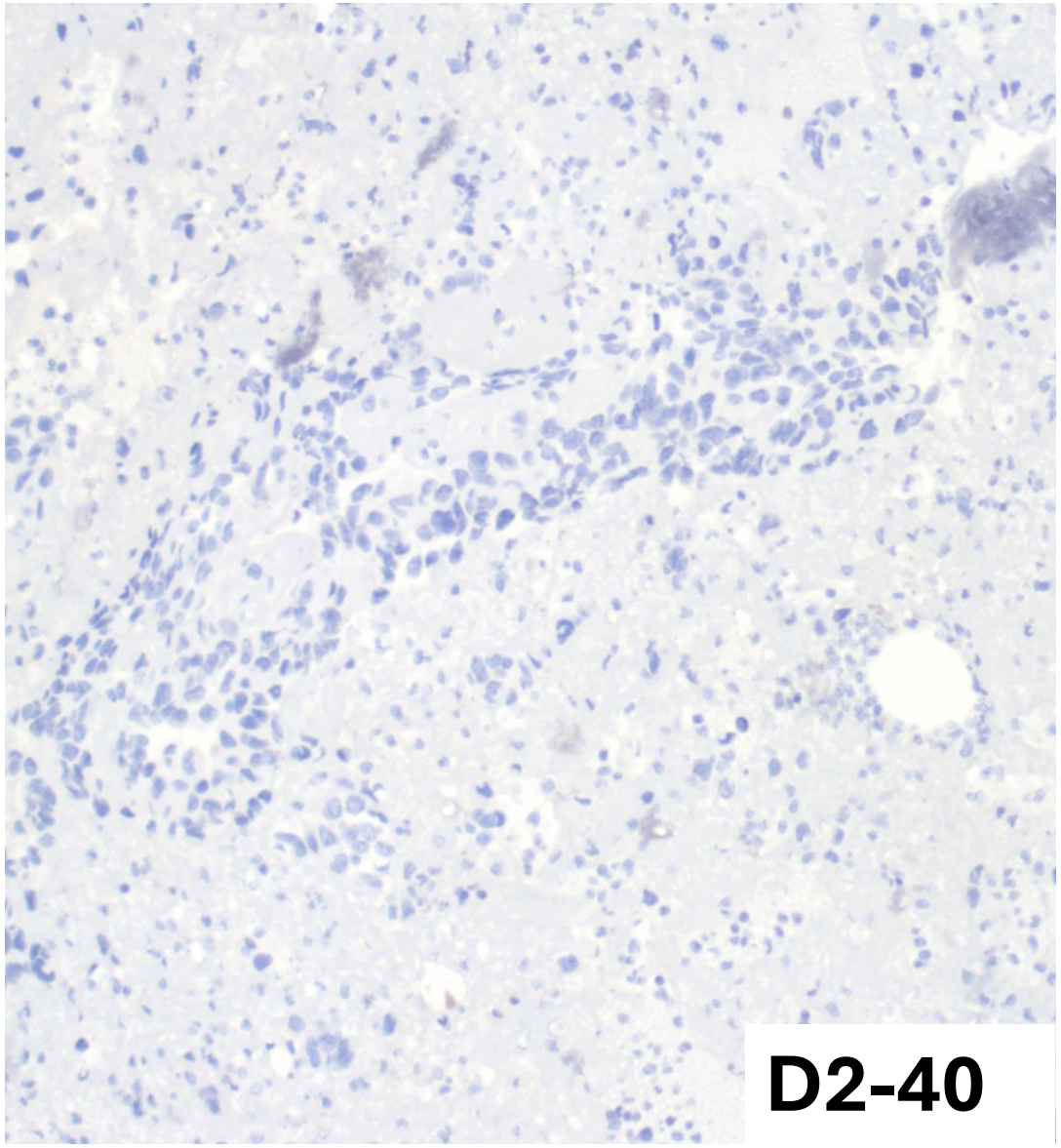
Approach to reporting of atypia of undetermined significance



Mesothelioma versus vascular tumors

- Angiosarcomas and hemangioendotheliomas
- Arise in the lung but spread to involve the pleural surface in a diffuse pattern
- They have a pseudomesotheliomatous growth pattern that mimics mesothelioma
- Immunohistochemistry is key
  - Mesothelial markers: calretinin, WT-1, D2-40
  - Vascular markers: CD31, CD34, ERG
  - Nuclear expression of CAMTA1 characteristic of epithelioid hemangioendothelioma versus other endothelial neoplasms




D2-40

Key diagnostic features of keratinizing Squamous cell carcinoma

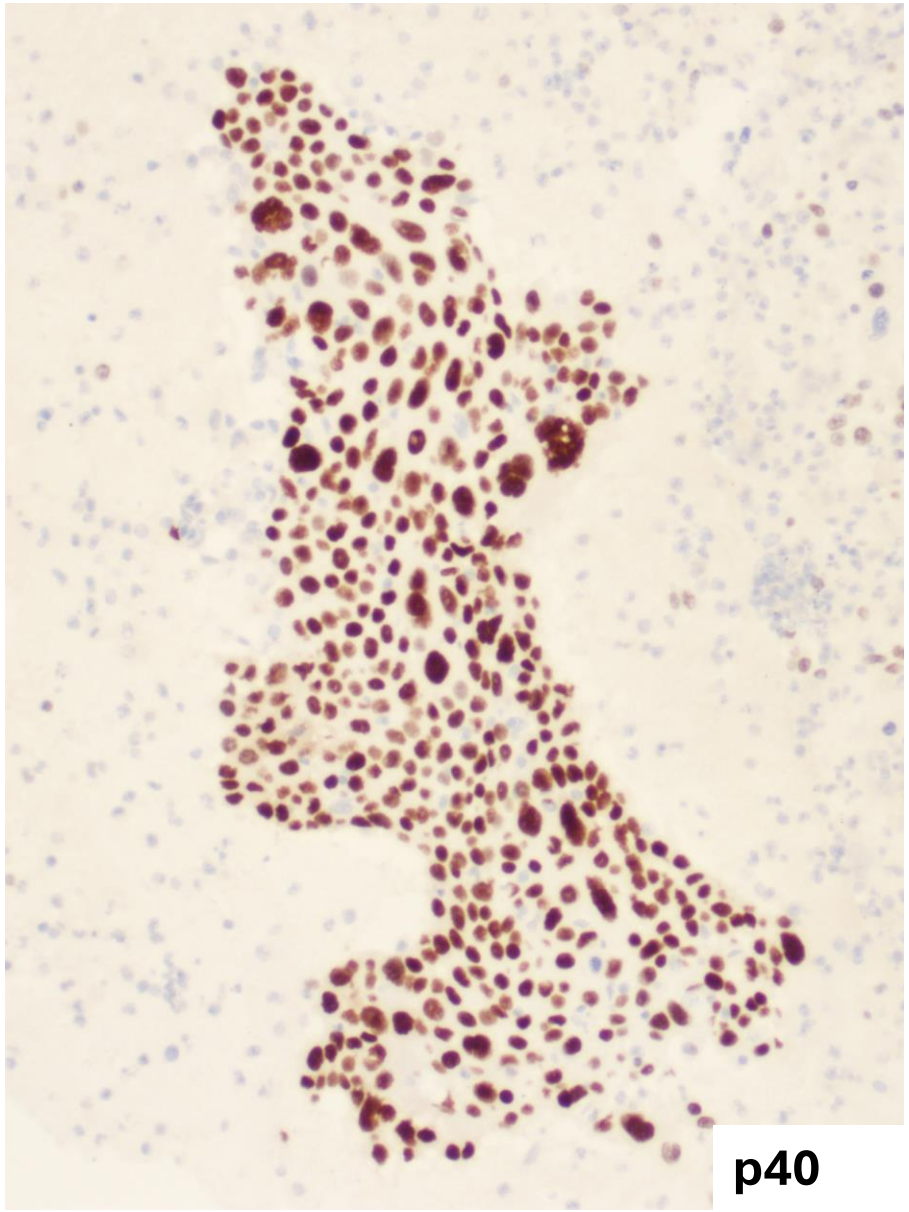
- Predominantly single-cell pattern or small clusters
- Enlarged, irregular hyperchromatic nuclei
- Dense, cyanophilic to eosinophilic to dyskeratotic/orangiophilic cytoplasm (on Pap stain)
- Sharply defined cell borders
- Polygonal cells, tadpole cells.
- Fiber cells and anucleated cells
- Keratin debris, squamous pearls

Key diagnostic features of non-keratinizing Squamous cell carcinoma

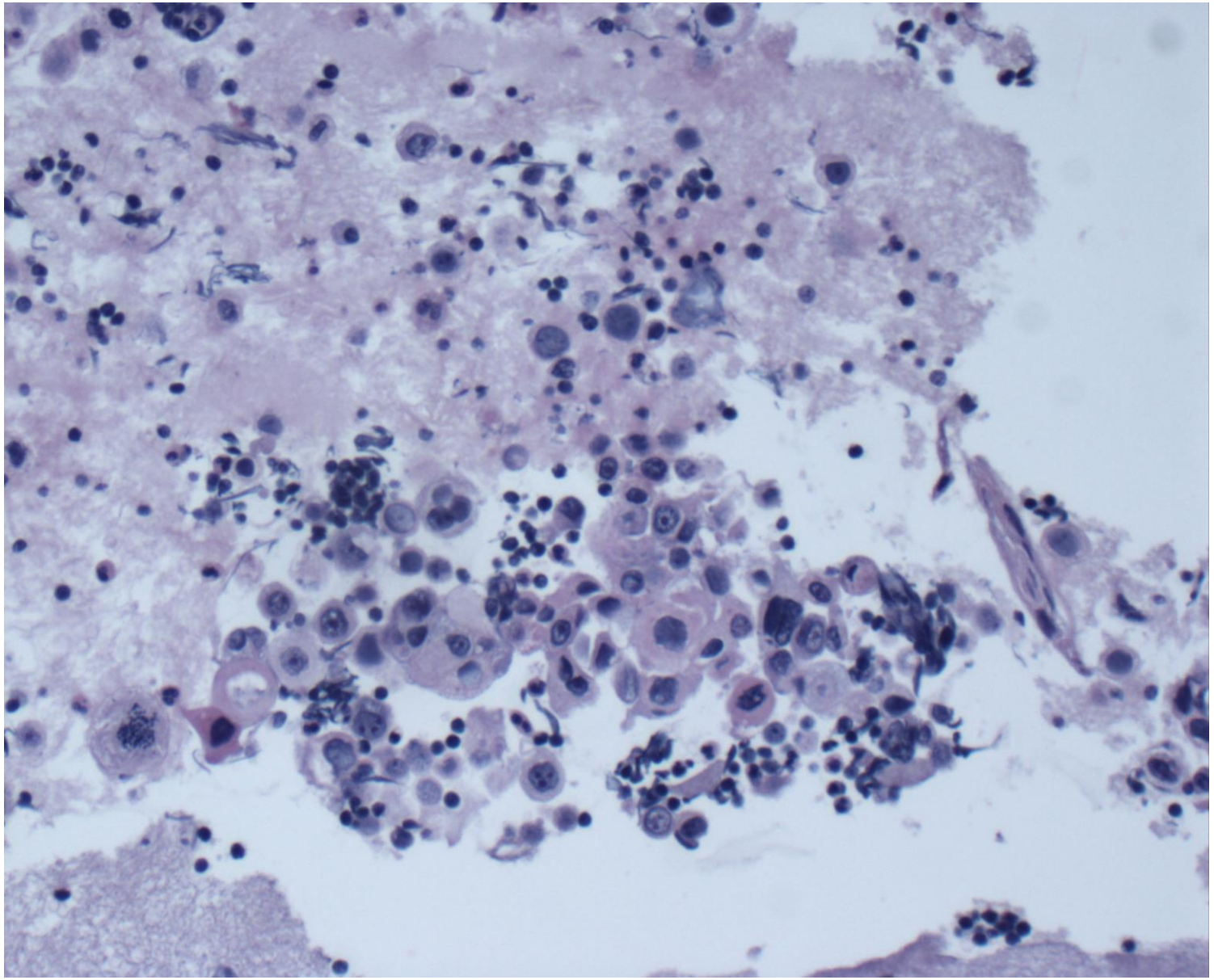
- Clusters of syncytial groups of cells
- High nuclear-to-cytoplasmic ratio
- Enlarged, irregular, hyperchromatic nuclei
- Coarse chromatin
- Thin rim of ill-defined, delicate, granular cytoplasm to focally dense, cyanophilic cytoplasm

Squamoid mesothelioma versus squamous cell carcinoma

- Both typically have dense cytoplasm
- Immunohistochemistry is very helpful
  - Mesothelial markers: calretinin, WT-1, D2-40
  - Squamous carcinoma markers: p40, p63, MOC-31
  - CK5/6 unhelpful in this scenario



p40



Types of Sarcomatoid Mesothelioma

- Desmoplastic
- Lymphohistiocytoid
- Heterologous elements

Mimics of Sarcomatoid Mesothelioma

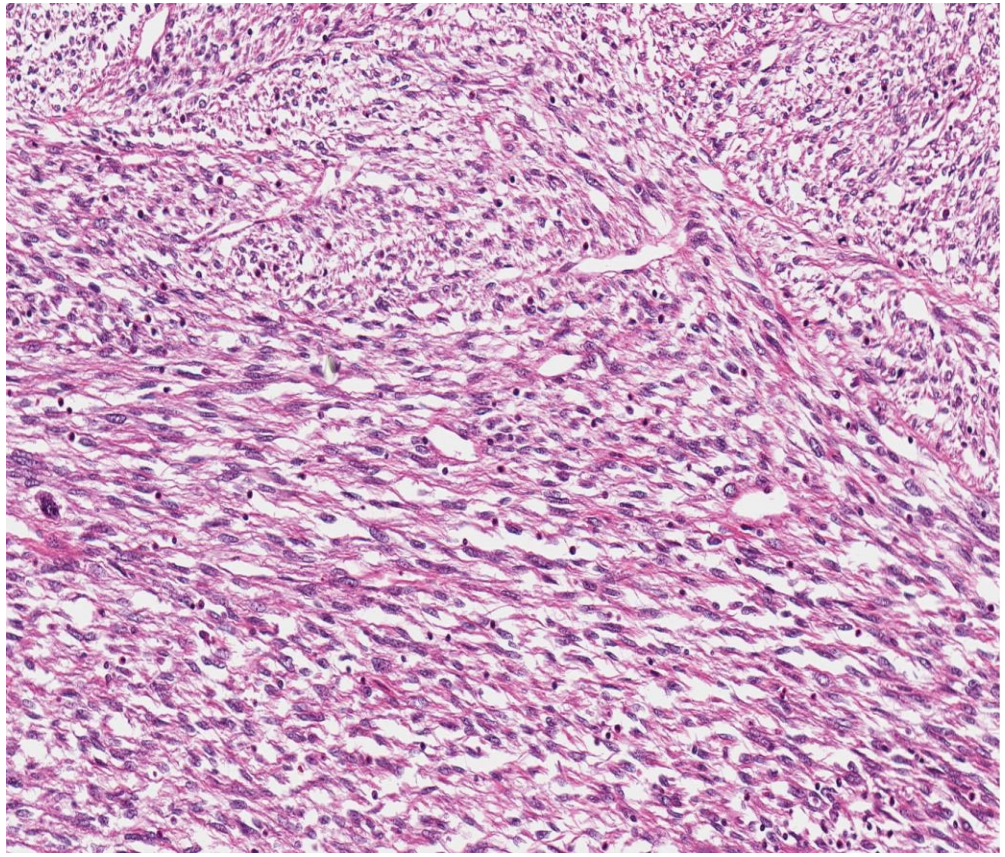
- Benign fibrous pleurisy
- Sarcomatoid carcinoma
- Synovial sarcoma
- Extraintestinal GIST
- Malignant solitary fibrous tumor
- Desmoid tumor
- Biphasic epithelioid hemangioendothelioma

Benign fibrous pleurisy versus desmoplastic mesothelioma

Feature	Benign fibrous pleurisy	Desmoplastic mesothelioma
Storiform growth pattern	Not prominent	Can be prominent
Vasculature	Perpendicularly oriented	Haphazardly oriented
Stromal invasion	Absent	Present
Necrosis	Rare, associated with acute inflammation	Bland, infarct-like necrosis common
Zonation	Hypercellular at the surface with decreased cellularity and maturation	Lack of maturation and zonation

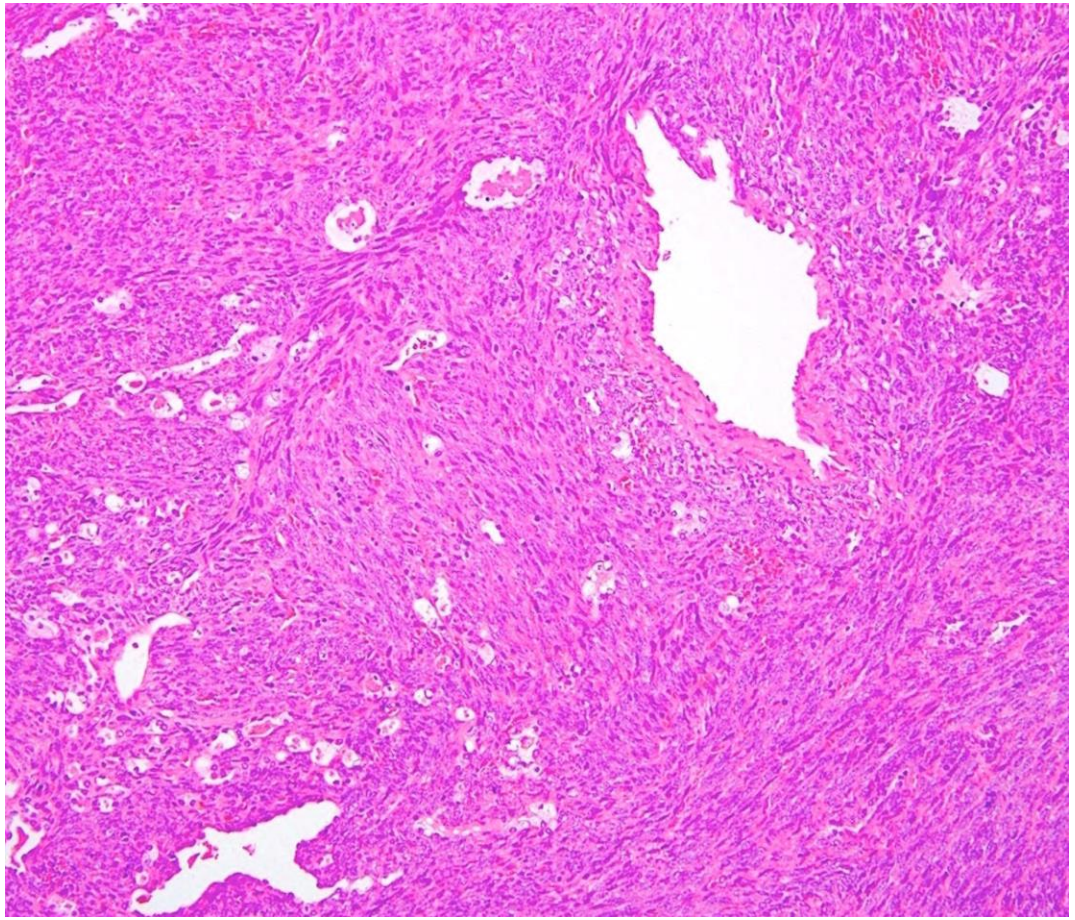
Sarcomatoid carcinoma

- Malignant cells showing epithelial and mesenchymal features
- Often include spindle cells, pleomorphic cells and giant cells
- Pan-cytokeratin, CK7, TTF-1 and EMA expression



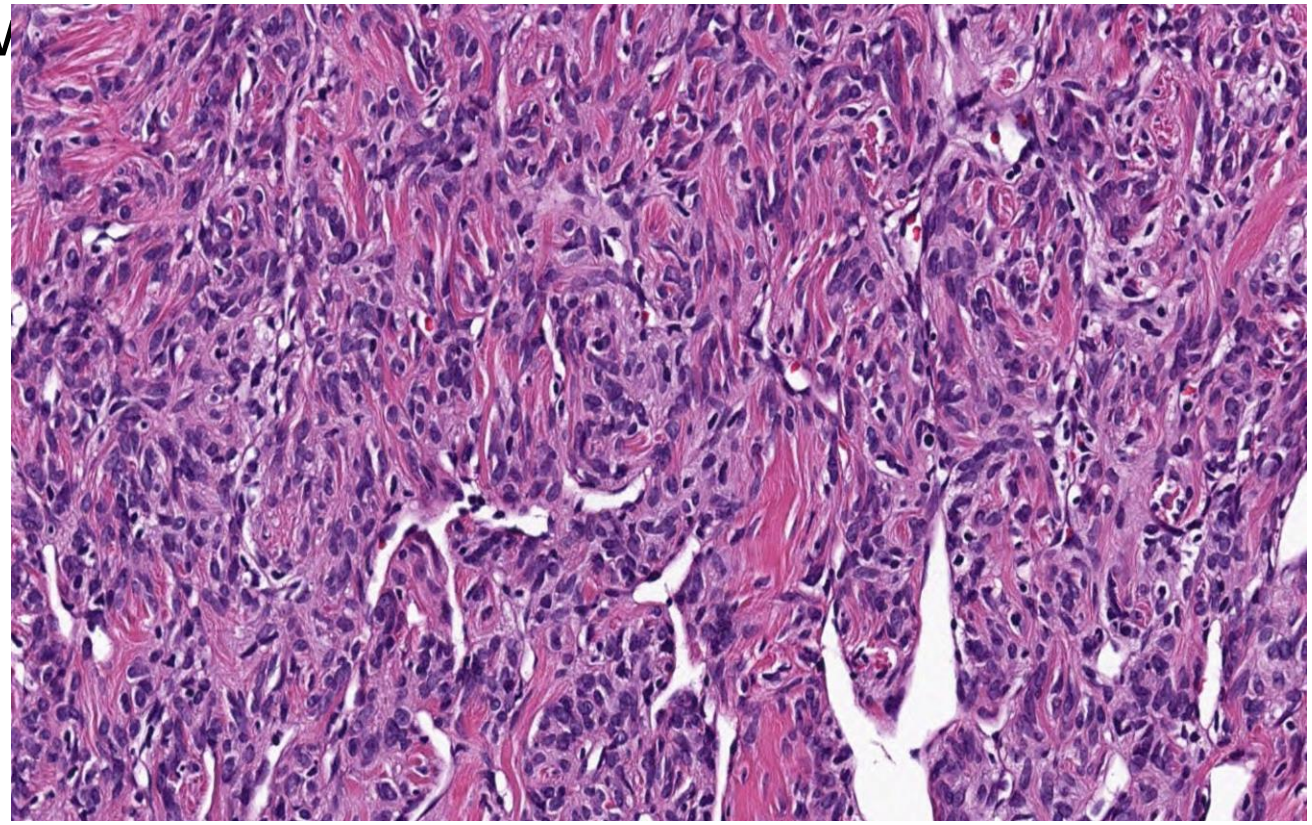
Synovial sarcoma

- Hypercellular spindle cell neoplasm with cells often arranged in branching clusters with thin-walled capillaries
- Cytokeratin (focal),EMA, CD99, Bcl-2 expression
- t(X;18) translocation by FISH



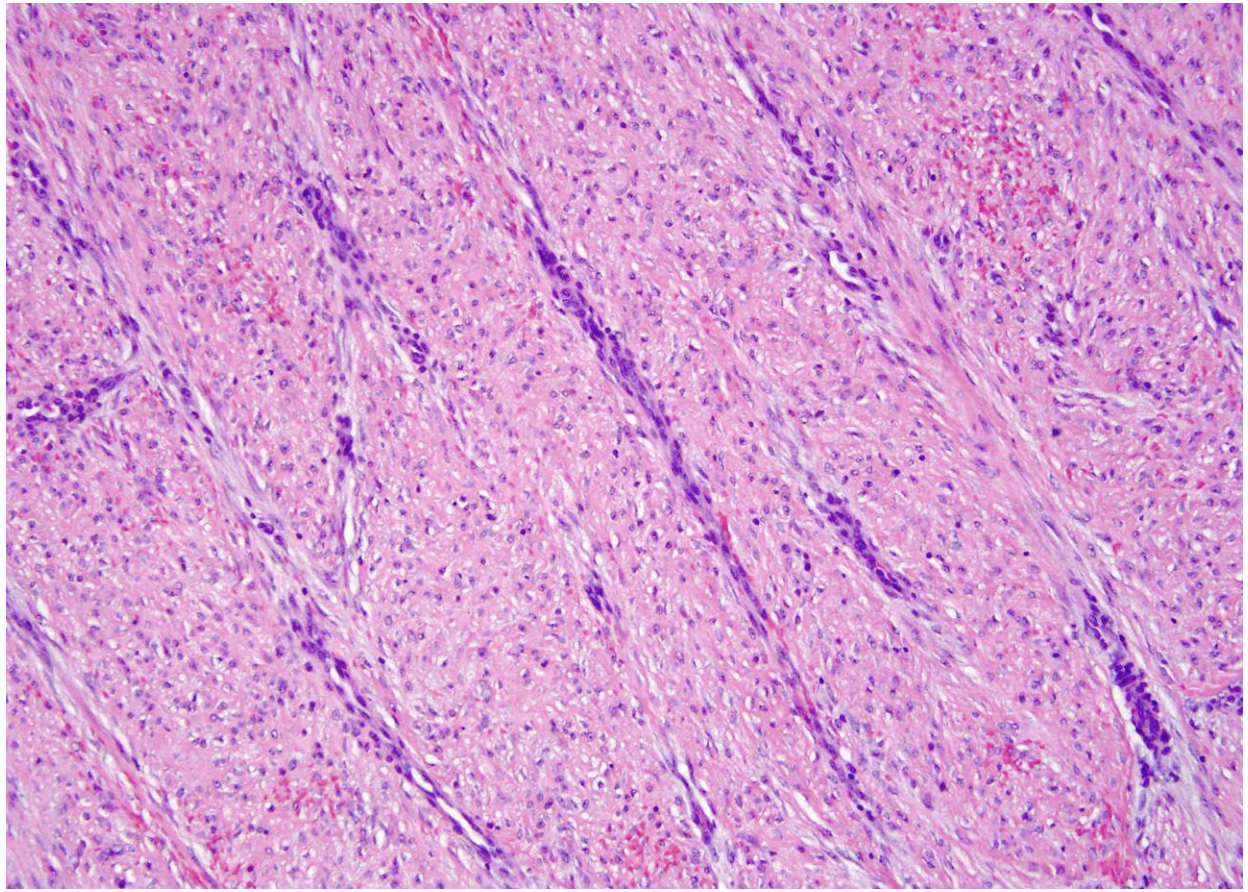
Solitary fibrous tumor

- Spindle cell neoplasm with low to moderate cellularity
- Background of collagenous stroma and blood vessels
- CD34, CD99, Bcl-2, STAT-6 expression

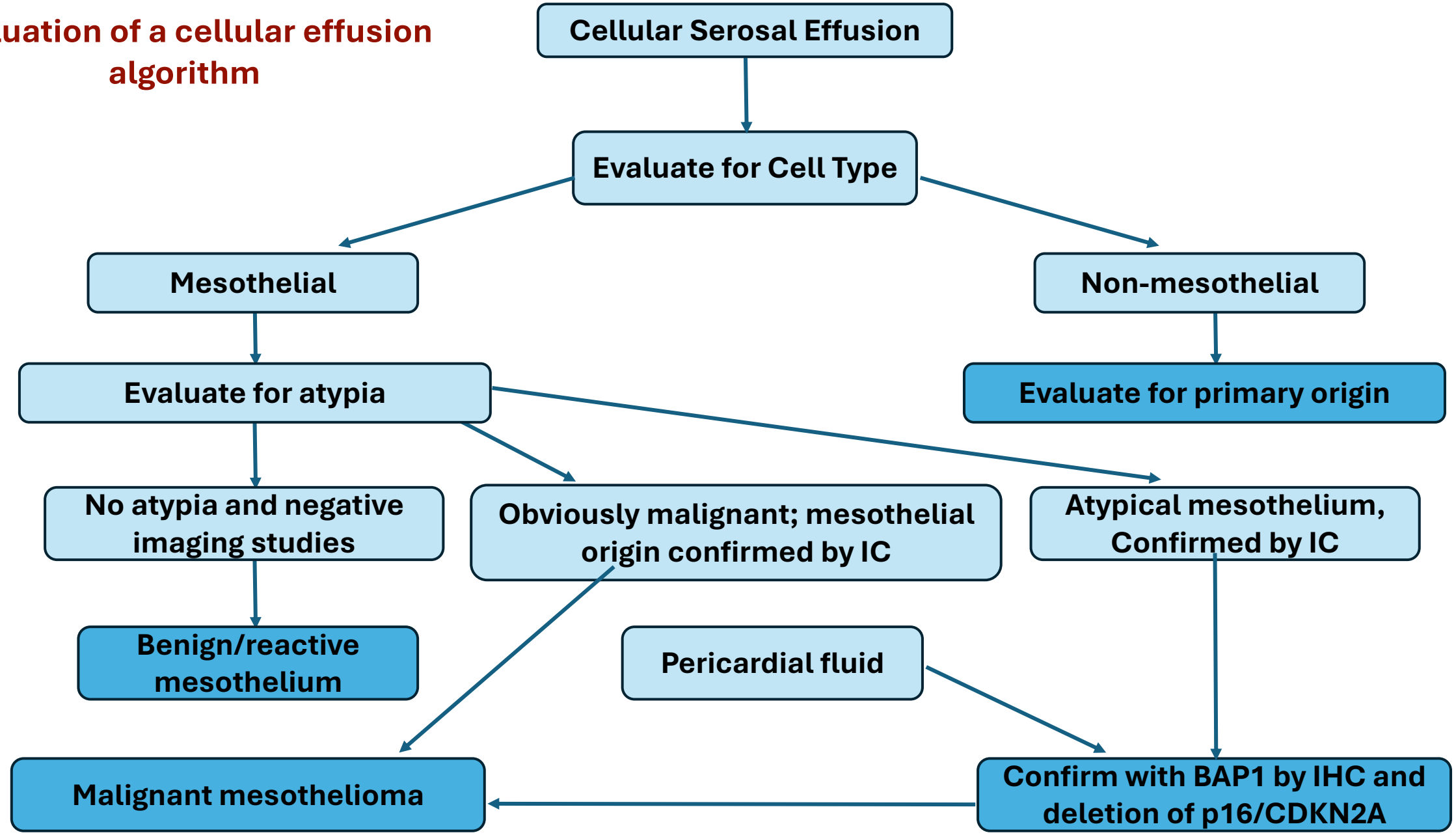


Desmoid tumor

- Proliferation of spindle cells with bland nuclei and abundant cytoplasm
- Often arranged in fascicles within a collagenous background
- Generally, lacks significant nuclear atypia
- SMA, desmin, beta-catenin expression



Evaluation of a cellular effusion algorithm



When to use Immunohistochemistry for effusions

- Confirming malignancy when morphology alone is equivocal
- Distinguishing adenocarcinoma from mesothelioma
- Screening an effusion for lobular breast cancer
- Establishing the primary site of a malignant effusion
  - Occult primary
  - Multiple primaries
- Establishing vulnerability of advanced lung and other cancers to targeted therapy and immunotherapy
- Assessing receptor status (e.g., HER2) for patients with breast and gastric cancers

Key Take Away Points

- **Approach to a difficult effusion**
  - History
  - Cell arrangement
  - Comparison with obvious mesothelial cells
  - Cytomorphology
  - Prepare additional smears
  - Prepare cell block
  - Ancillary studies
  - Consultation
  - Communicate limitations
- **Therapeutic implications**
  - Positive effusion = stage 4 metastatic disease
  - Mesothelioma = radical surgery + radiation and chemotherapy

References

Michael C, Hiroshima K, Hjerpe A, Michelow P, Onal B, Segal A. Malignant-Primary (MAL-P) (Mesothelioma). In: Chandra A, Crothers B, Kurtycz, Schmitt F, editors. The International System for Serous Fluid Cytopathology. Switzerland; 2020. P. 63-98.

Cigognetti M, Lonardi S, Fisogni S, et al. BAP1 (BRCA1-associated protein 1) is a highly specific marker for differentiating mesothelioma from reactive mesothelial proliferations. Mod Pathol. 2015;28:1043-57.

Roh MH, Michael CW. Common Metastatic Epithelial Neoplasms. In: Michael CW, Chhieng DC, Bedrossian CWM, eds. Cytohistology of the Serous Membranes Cambridge: Cambridge University Press; 2015:125-168.

Miki Y, Tabatabai ZL, Davidson B. Malignant-Secondary (MAL-S). In: Chandra A, Crothers B, Kurtycz, Schmitt F, editors. The International System for Serous Fluid Cytopathology. Switzerland; 2020. P. 99-128.

Mikou P, Engels M, Roy Chowdhuri S, Santis G. Atypia of Undetermined Significance. In: Chandra A, Crothers B, Kurtycz, Schmitt F, editors. The International System for Serous Fluid Cytopathology. Switzerland; 2020. P. 53-62.

Eccher A, Girolami I, Lucenteforte E, Troncone G, Scarpa A, Pantanowitz L. Diagnostic mesothelioma biomarkers in effusion cytology. Cancer Cytopathol. 2021 Jul;129(7):506-516.

Sheffield BS, Hwang HC, Lee AF, Thompson K, Rodriguez S, Tse CH, Gown AM, Churg A. BAP1 immunohistochemistry and p16 FISH to separate benign from malignant mesothelial proliferations. Am J Surg Pathol. 2015 Jul;39(7):977-82.

Hwang HC, Pyott S, Rodriguez S, Cindric A, Carr A, Michelsen C, Thompson K, Tse CH, Gown AM, Churg A. BAP1 Immunohistochemistry and p16 FISH in the Diagnosis of Sarcomatous and Desmoplastic Mesotheliomas. Am J Surg Pathol. 2016 May;40(5):714-8.

Berg KB, Dacic S, Miller C, Cheung S, Churg A. Utility of Methylthioadenosine Phosphorylase Compared With BAP1 Immunohistochemistry, and CDKN2A and NF2 Fluorescence In Situ Hybridization in Separating Reactive Mesothelial Proliferations From Epithelioid Malignant Mesotheliomas. Arch Pathol Lab Med. 2018 Dec;142(12):1549-1553.

McGregor SM, McElherne J, Minor A, Keller-Ramey J, Dunning R, Husain AN, Vigneswaran W, Fitzpatrick C, Krausz T. BAP1 immunohistochemistry has limited prognostic utility as a complement of CDKN2A (p16) fluorescence in situ hybridization in malignant pleural mesothelioma. Hum Pathol. 2017 Feb;60:86-94.

Hida T, Hamasaki M, Matsumoto S, Sato A, Tsujimura T, Kawahara K, Iwasaki A, Okamoto T, Oda Y, Honda H, Nabeshima K. Immunohistochemical detection of MTAP and BAP1 protein loss for mesothelioma diagnosis: Comparison with 9p21 FISH and BAP1 immunohistochemistry. Lung Cancer. 2017 Feb;104:98-105.

Nasu M, Emi M, Pastorino S, Tanji M, Powers A, Luk H, Baumann F, Zhang YA, Gazdar A, Kanodia S, Tiirikainen M, Flores E, Gaudino G, Becich MJ, Pass HI, Yang H, Carbone M. High Incidence of Somatic BAP1 alterations in sporadic malignant mesothelioma. J Thorac Oncol. 2015 Apr;10(4):565-76.

Chapel DB, Schulte JJ, Berg K, Churg A, Dacic S, Fitzpatrick C, Galateau-Salle F, Hiroshima K, Krausz T, Le Stang N, McGregor S, Nabeshima K, Husain AN. MTAP immunohistochemistry is an accurate and reproducible surrogate for CDKN2A fluorescence in situ hybridization in diagnosis of malignant pleural mesothelioma. Mod Pathol. 2020 Feb;33(2):245-254.

Wu D, Hiroshima K, Yusa T, Ozaki D, Koh E, Sekine Y, Matsumoto S, Nabeshima K, Sato A, Tsujimura T, Yamakawa H, Tada Y, Shimada H, Tagawa M. Usefulness of p16/CDKN2A fluorescence in situ hybridization and BAP1 immunohistochemistry for the diagnosis of biphasic mesothelioma. Ann Diagn Pathol. 2017 Feb;26:31-37.

Kinoshita Y, Hamasaki M, Yoshimura M, Matsumoto S, Sato A, Tsujimura T, Ueda H, Makihata S, Kato F, Iwasaki A, Nabeshima K. A combination of MTAP and BAP1 immunohistochemistry is effective for distinguishing sarcomatoid mesothelioma from fibrous pleuritis. Lung Cancer. 2018 Nov;125:198-204.

Wojcik EM, Jing XS, Alshaikh S, Lobo C. Negative for Malignancy. In: Chandra A, Crothers B, Kurtycz, Schmitt F, editors. The International System for Serous Fluid Cytopathology. Switzerland; 2020. P. 17-40.

Cibas ES. Pleural, pericardial and peritoneal fluids. In: Cibas ES, Ducatman BS, eds. Cytology: Diagnostic Principles and Clinical Correlates. Elsevier; 2021:141-170.

Adeniran AJ, Chhieng DC. Primary and Metastatic Hematopoietic Diseases. In: Michael CW, Chhieng DC, Bedrossian CWM, eds. Cytohistology of the Serous Membranes Cambridge: Cambridge University Press; 2015:214-26.

Shaw JA, Diacon AH, Koegelenberg CFN. Tuberculous pleural effusion. Respirology. 2019;24:962-971.

Depascale R, Del Frate G, Gasparotto M, et al. Diagnosis and management of lung involvement in systemic lupus erythematosus and Sjögren's syndrome: a literature review. Ther Adv Musculoskelet Dis. 2021;13:1759720X211040696.

Wang DY. Diagnosis and management of lupus pleuritis. Curr Opin Pulm Med. 2002;8:312-6.

Balbir-Gurman A, Yigla M, Nahir AM, Braun-Moscovici Y. Rheumatoid pleural effusion. Semin Arthritis Rheum. 2006 Jun;35(6):368-78.

Komarla A, Yu GH, Shahane A. Pleural effusion, pneumothorax, and lung entrapment in rheumatoid arthritis. J Clin Rheumatol. 2015 Jun;21(4):211-5.

THANK YOU!