Ascitic Fluid and Use of Immunocytochemistry

Mercè Jordà, University of Miami

UHealth
UNIVERSITY OF MIAMI HEALTH SYSTEM
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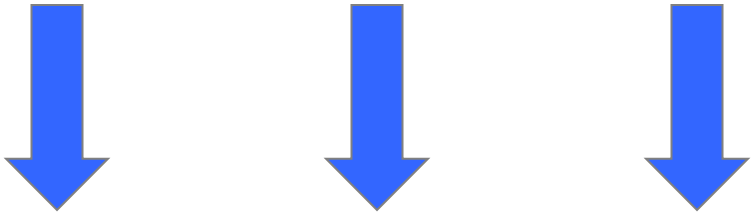
Is It Malignant ?

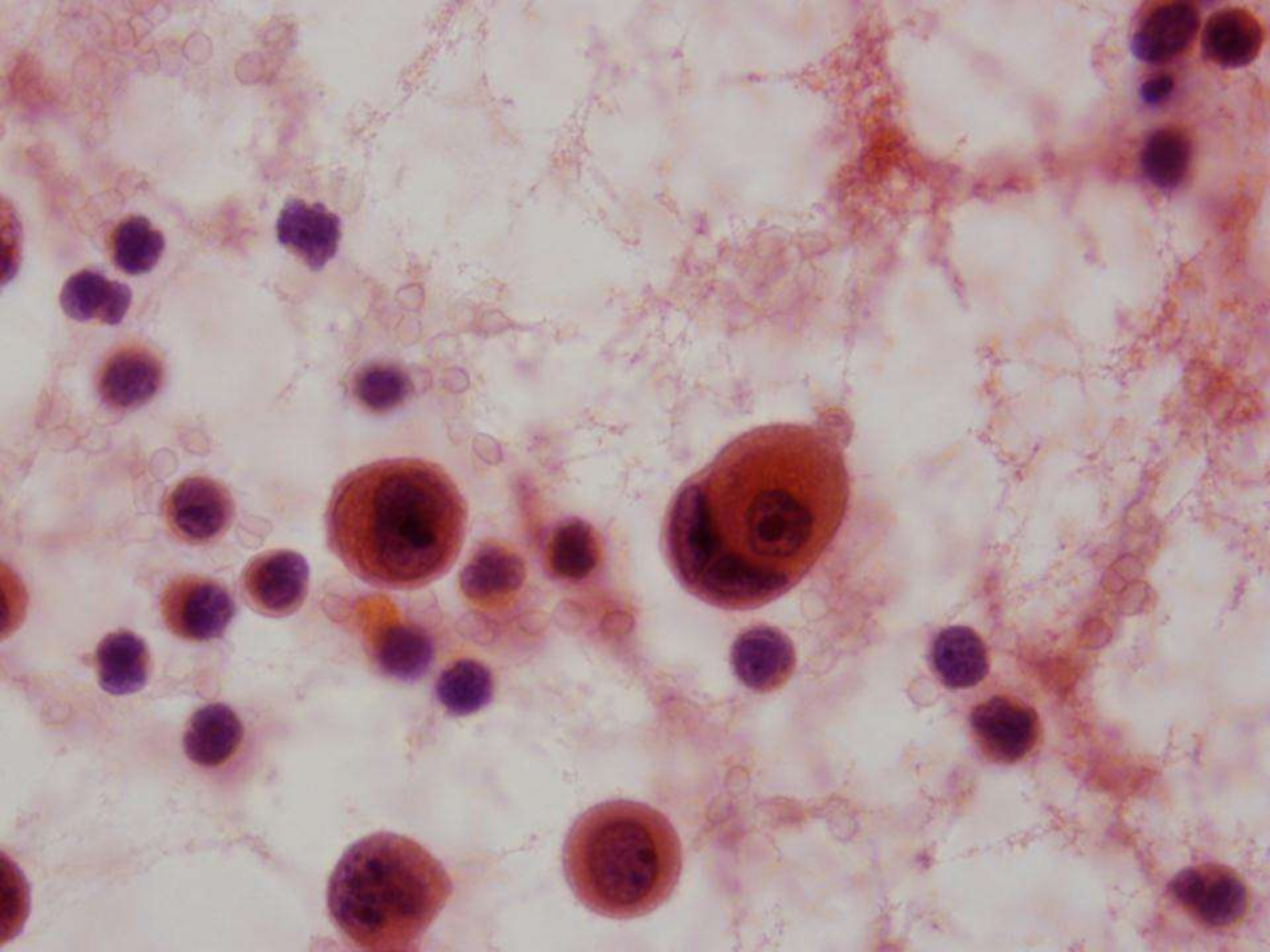


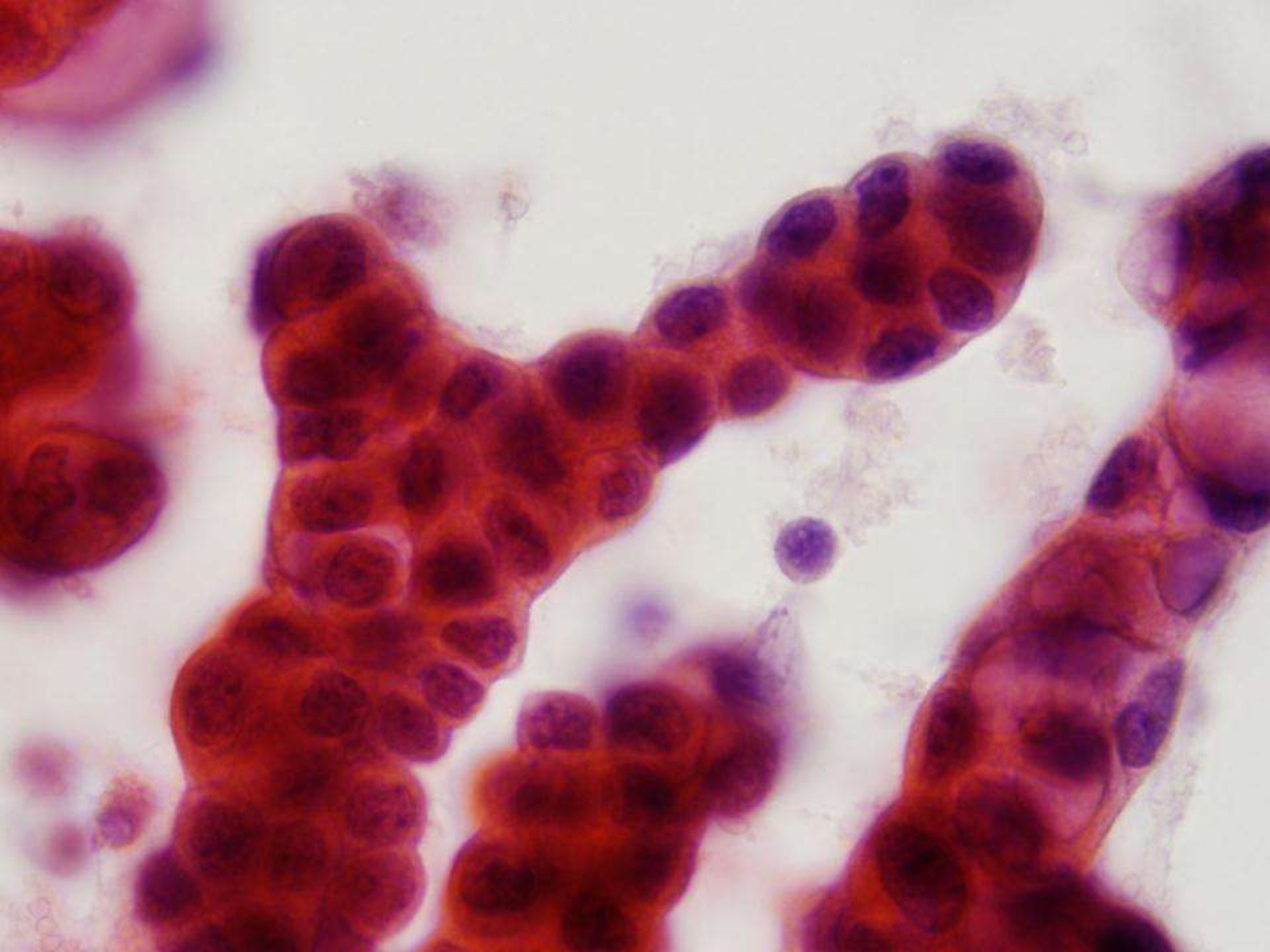
Yes ? No

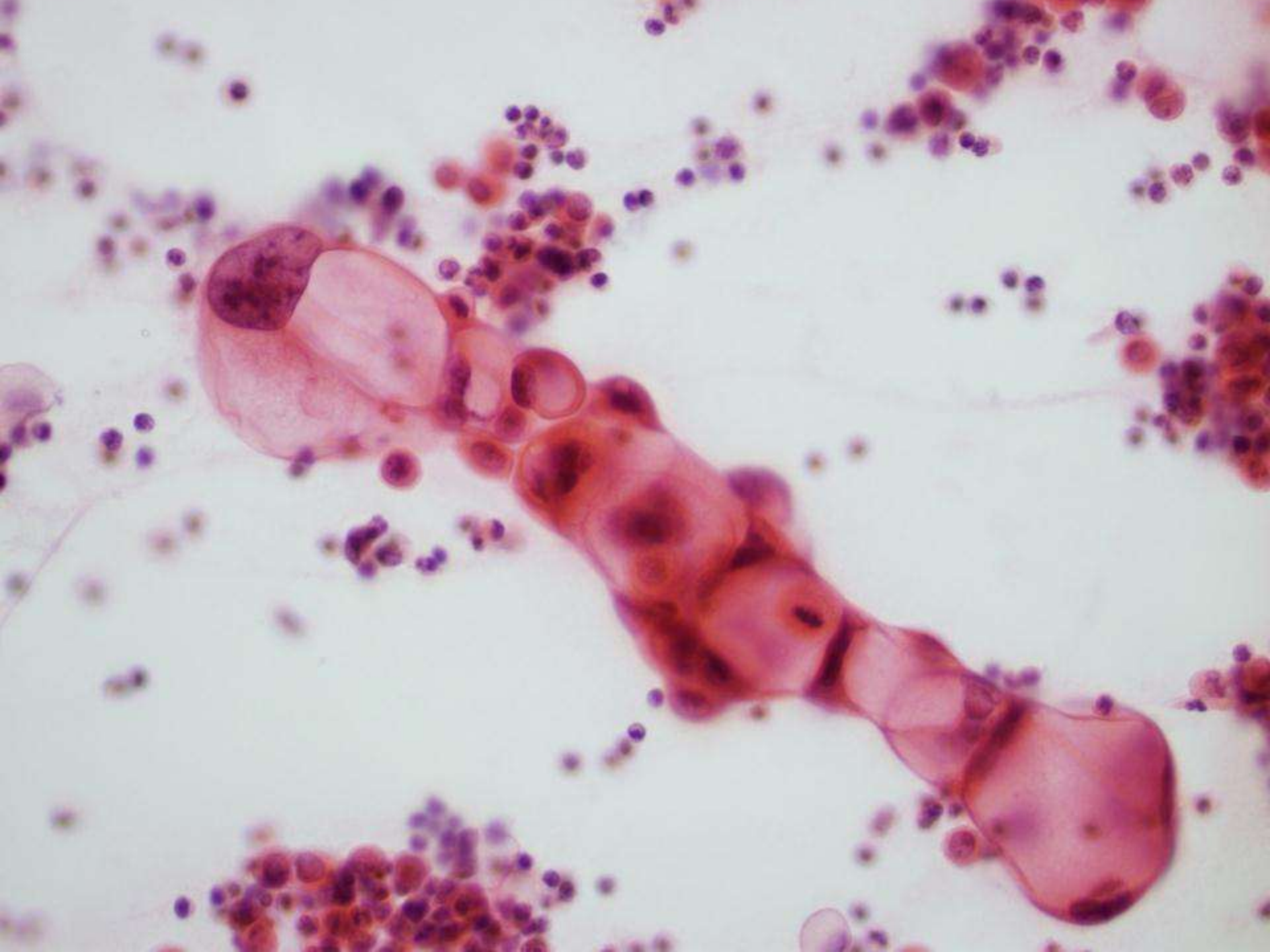
Ascitic Fluid

Cytomorphologic Useful Findings

- Tight clusters with smooth borders
- Cellular and nuclear molding
- Large papillary groups
- Two-cell types
- Signet ring cells in groups
- Abnormal cell morphology







Ascitic Fluid

Cytomorphologic Useless Findings

Cytoplasmic Vacuoles

“Signet Ring Cells” individual

Psammoma Bodies

“Cell within Cell”

Prominent Nucleoli

Mitosis

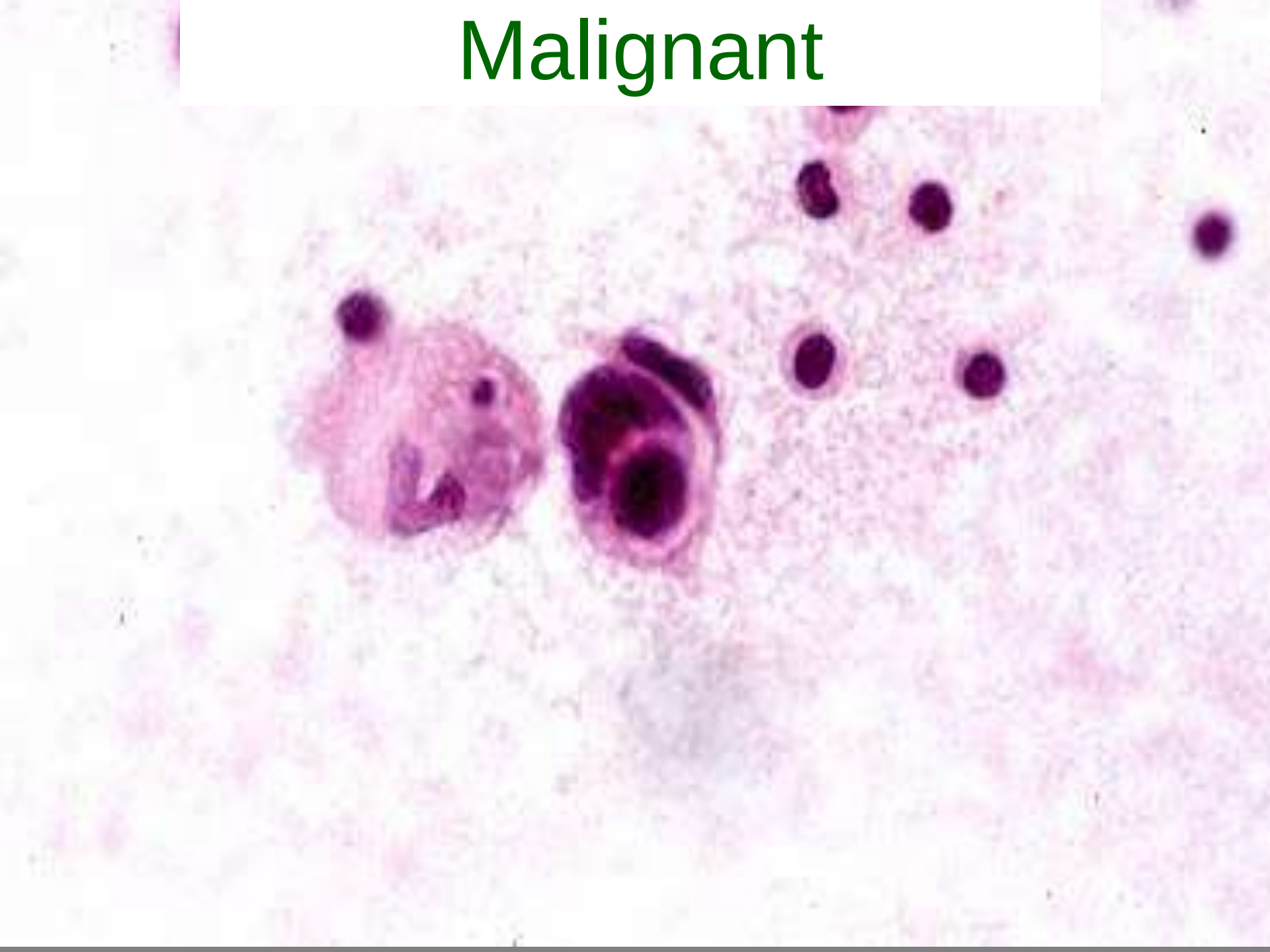
Multinucleation

Reactive Mesothelium

“Signet Ring Cells”

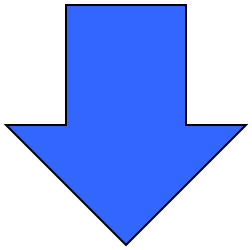
Look for them in Groups!

Malignant



Benign

By cytomorphology



Cellular Pattern

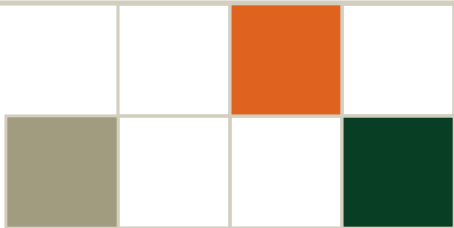
Cellular Morphology

Malignant Ascitic Fluid

Cellular Pattern

Cells in Clusters

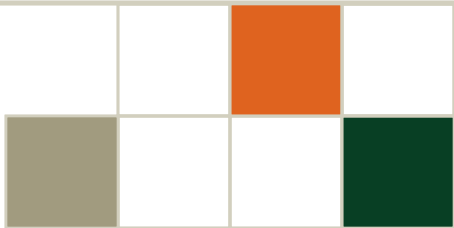
Isolated Cells

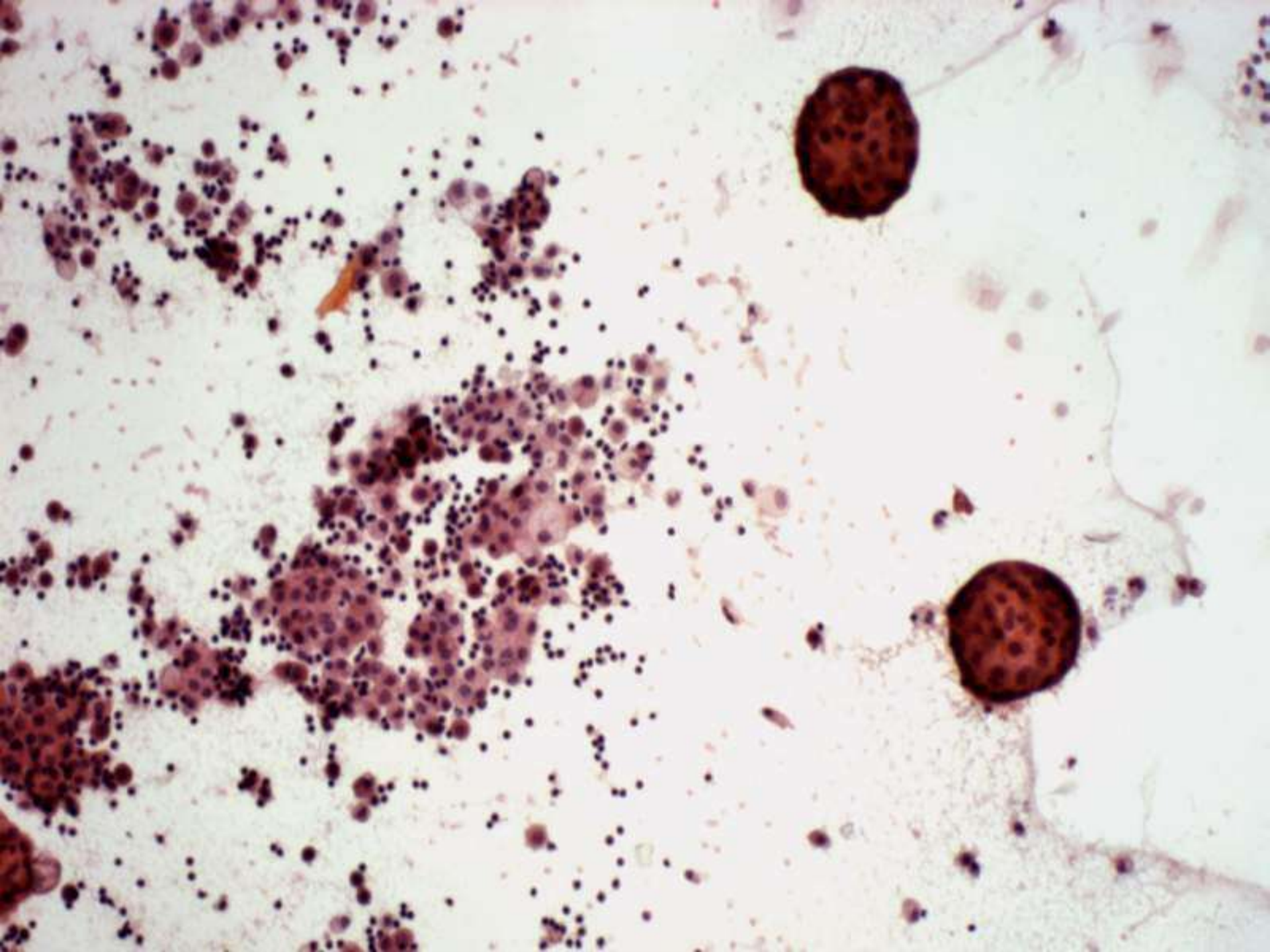


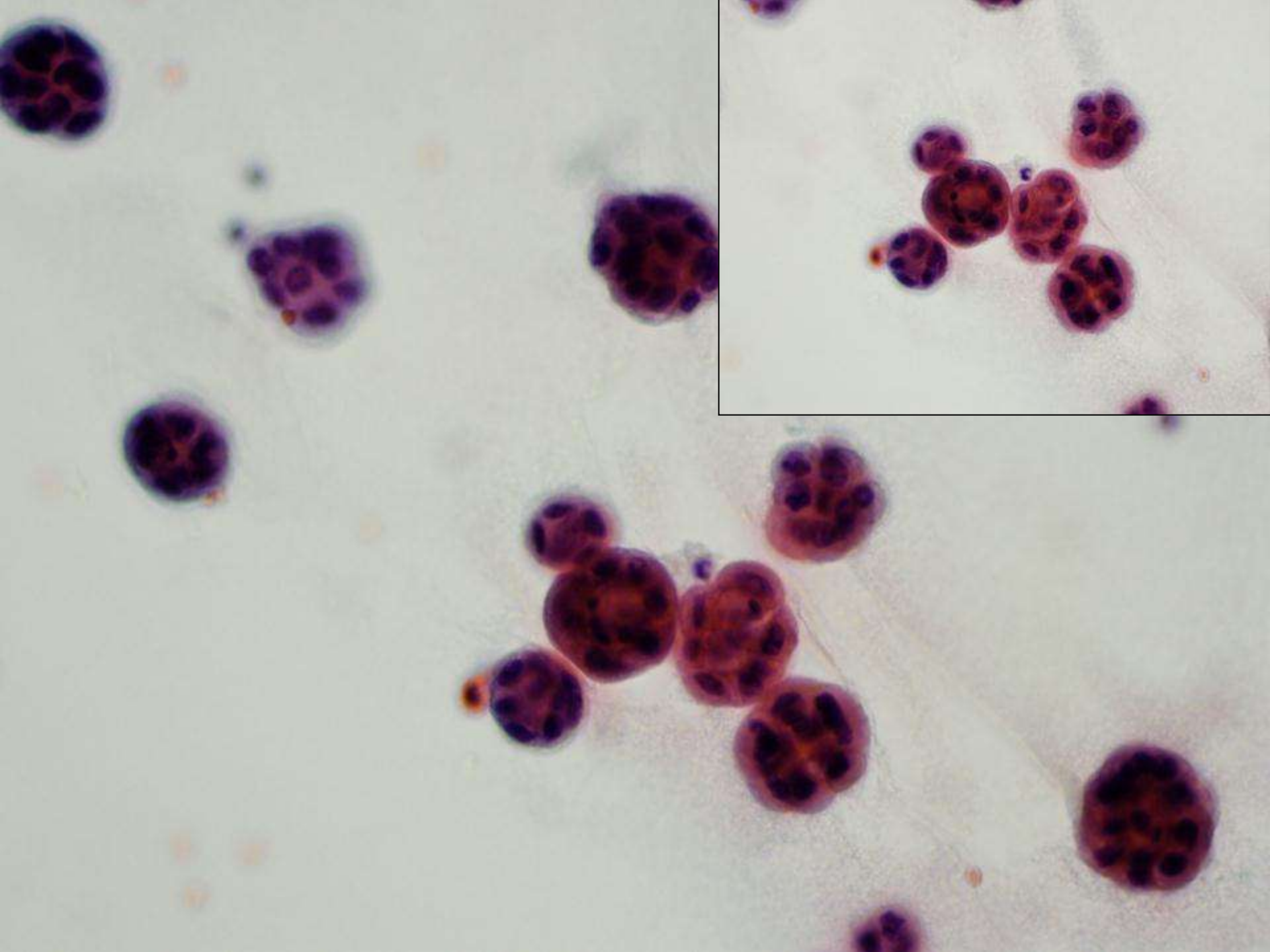
Malignant Ascitic Fluid: Cells in Clusters

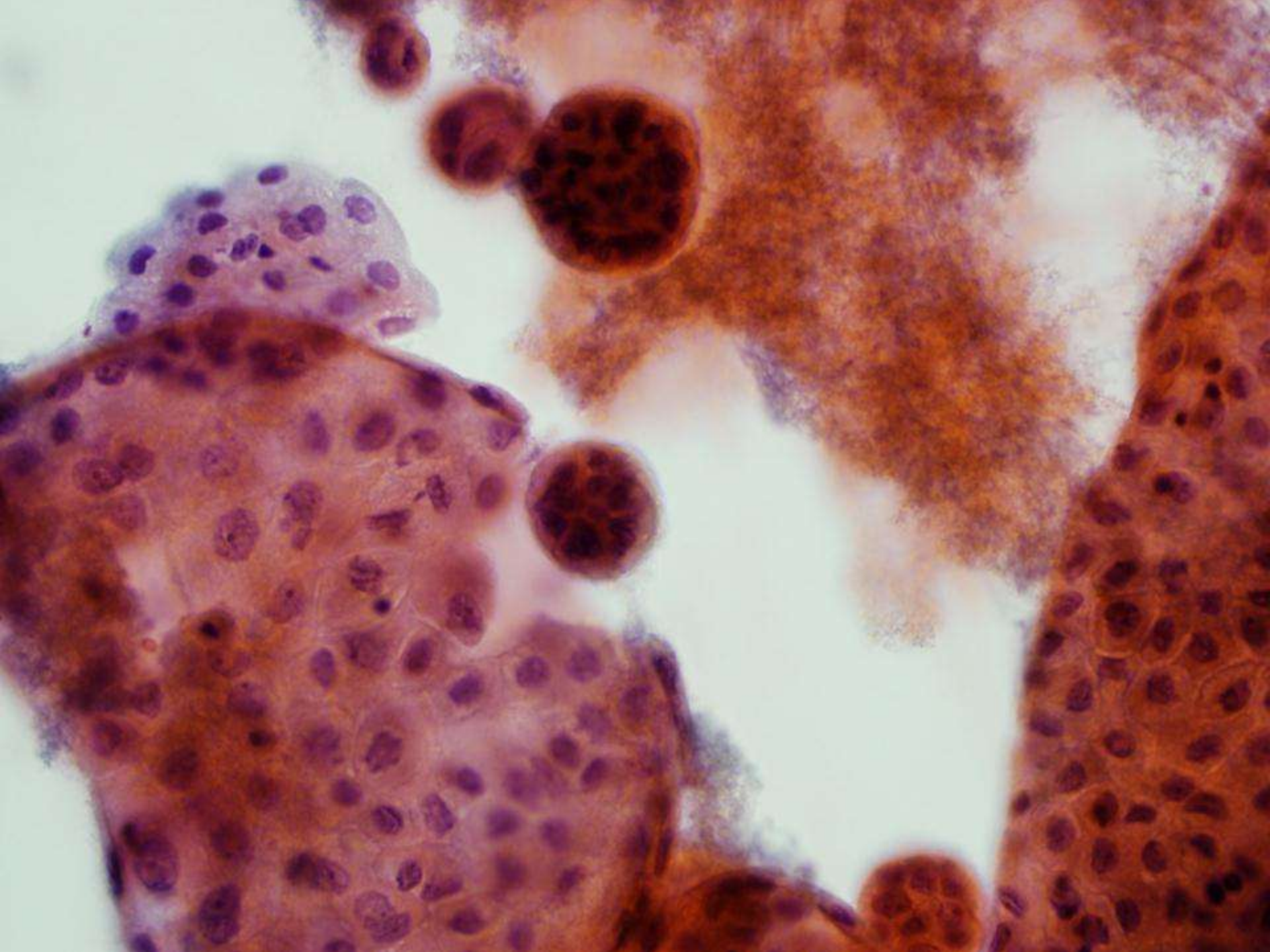
Cells	Background
Tight and compact	No cells
Smooth borders	Reactive mesothelial cells

Diagnosis : Cytomorphology







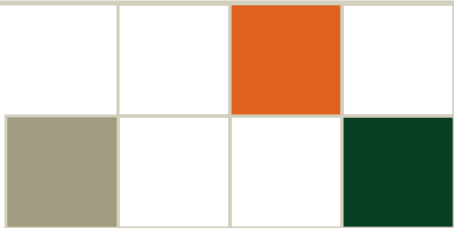


Malignant Effusions: Cells in clusters

Differential Diagnosis

- Carcinoma
- Malignant Mesothelioma

Diagnosis :
Cytomorphology & Immunocytochemistry



Malignant Effusions: Isolated Cells

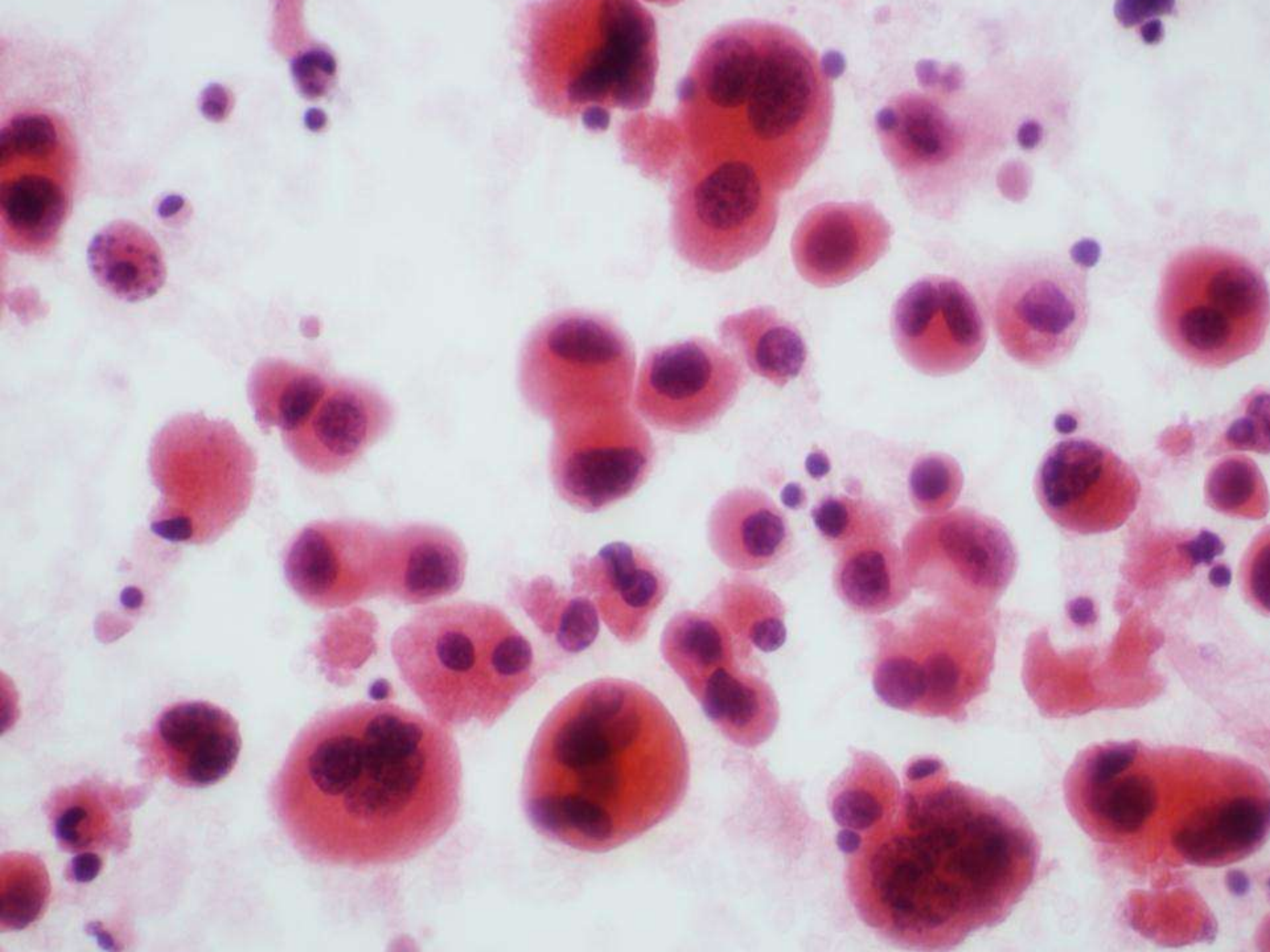
- Abnormal single cells
- May be overlooked in low power
- Look for small clusters

Malignant Effusions: Isolated Cells

Abnormal Cell Morphology

- Pleomorphism
- High N/C ratio
- Hyperchromasia
- Abnormal nucleoli
- Clumped, irregular chromatin
- Intraluminal mucin

Diagnosis :
Cytomorphology & Immunocytochemistry

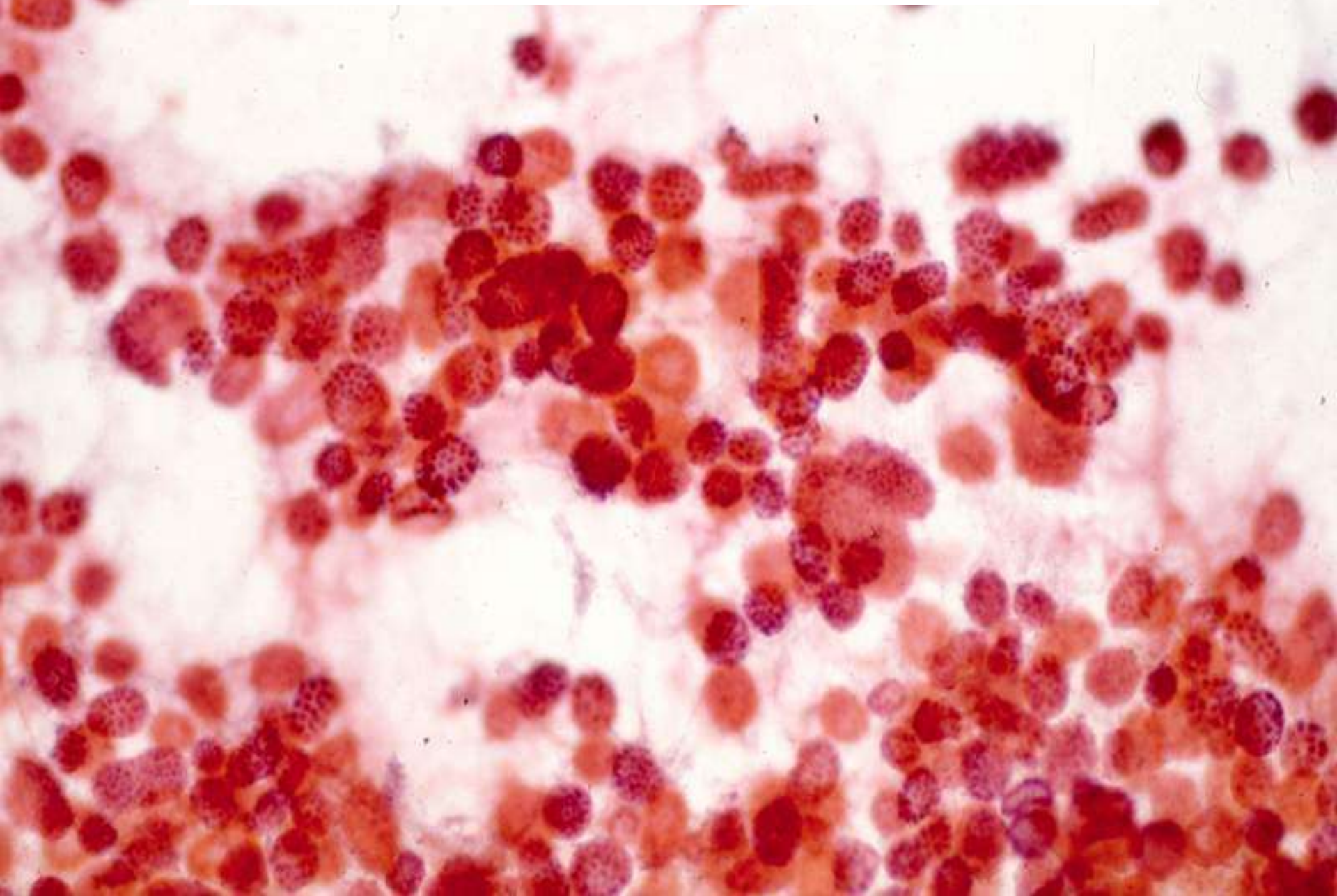


Malignant Ascitic Fluid

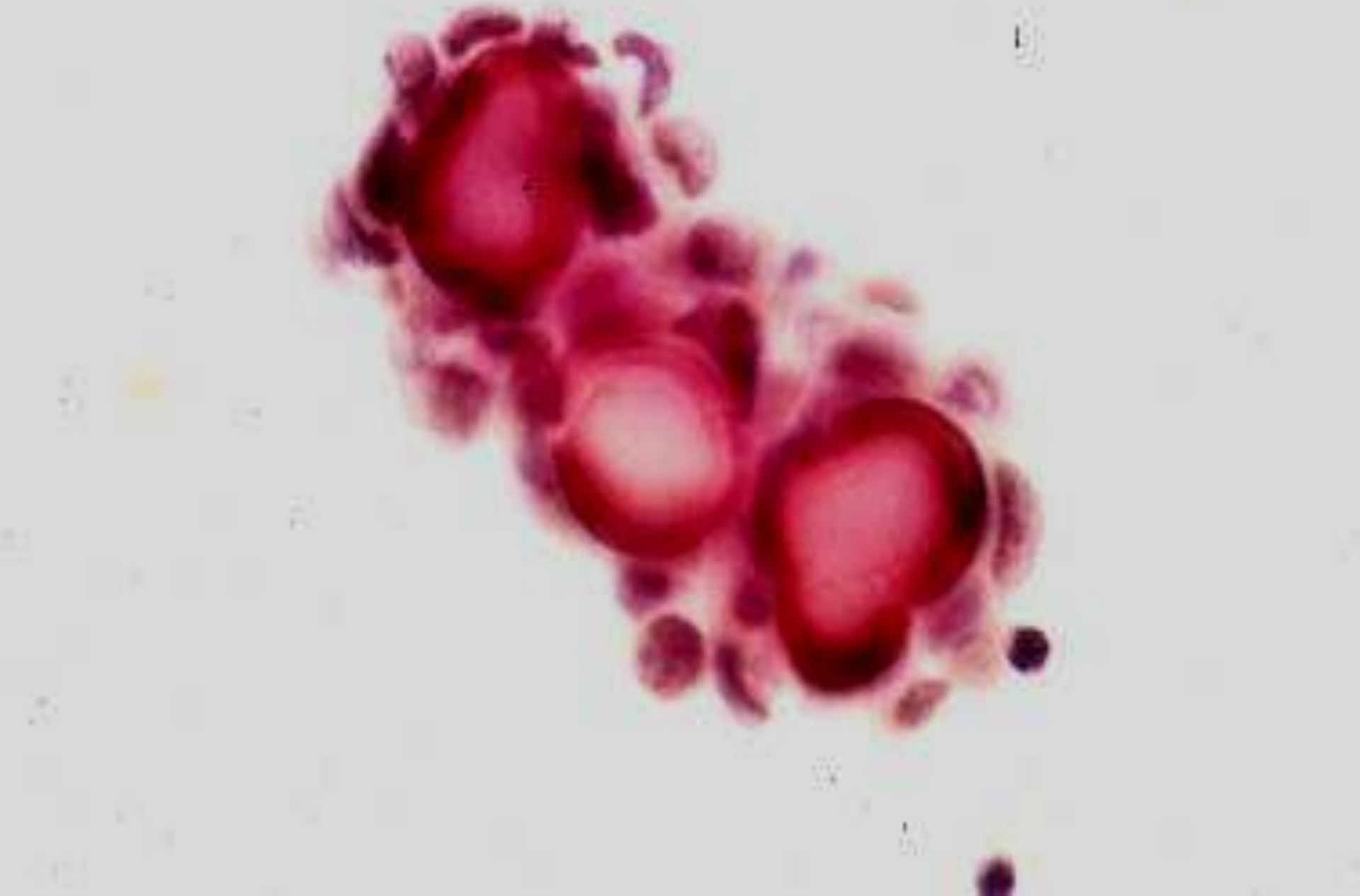
Site of origin by Cytomorphology

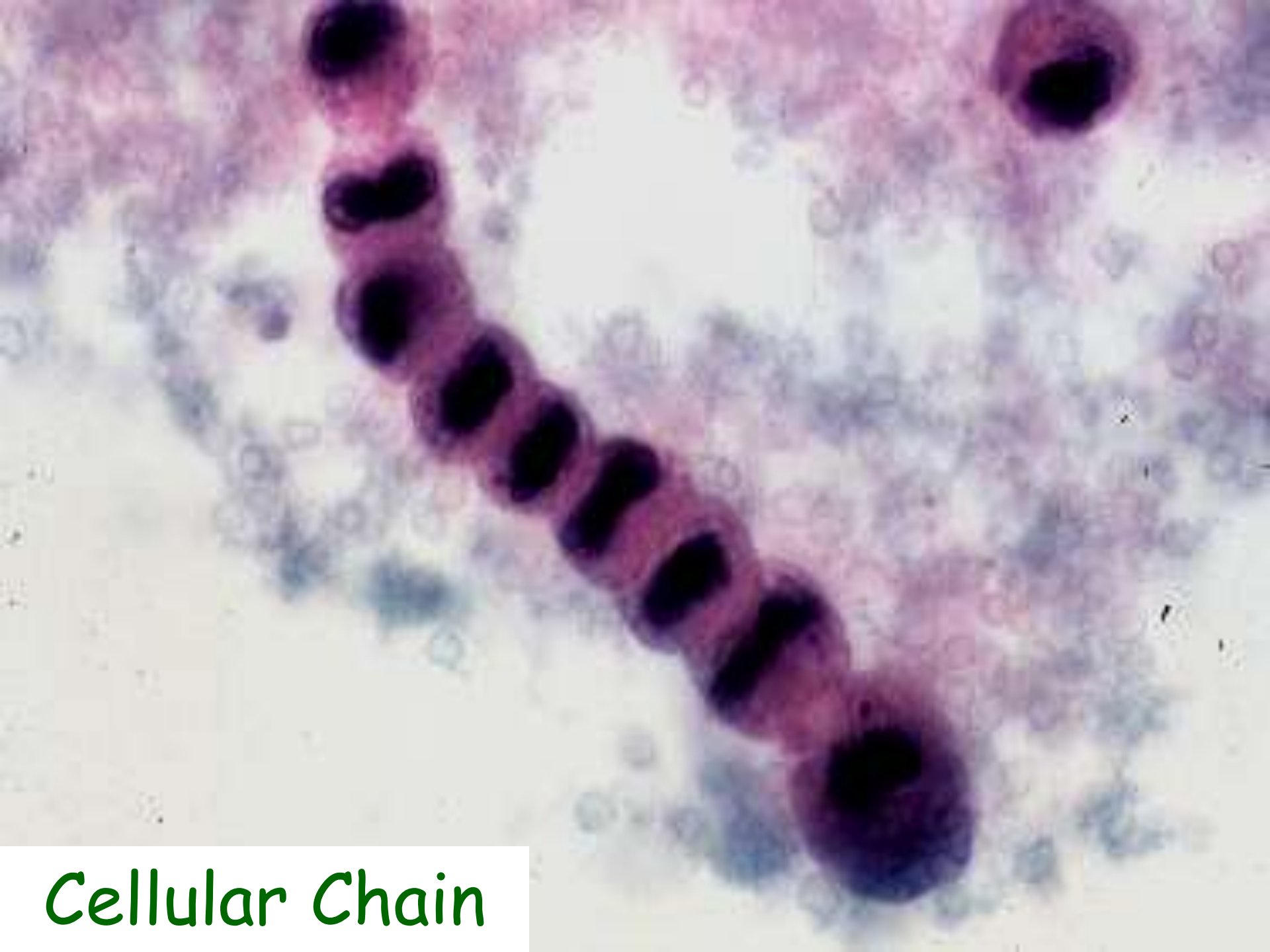
- Tight cell balls in breast Ca.
- Psammoma bodies in serous Papillary Ca.
- “Indian filling” in breast, gastric and pancreatic Ca.
- “Signet ring cells” in breast, gastric and ovarian Ca.
- Keratin pearls in squamous cell Ca.
- Melanin in malignant melanoma.
- Intranuclear inclusions in Adenocarcinoma of lung lipidic, papillary thyroid carcinoma and melanomas
- “Knobby clusters” in mesotheliomas

Cell balls in Breast Ca.



Psammoma Bodies

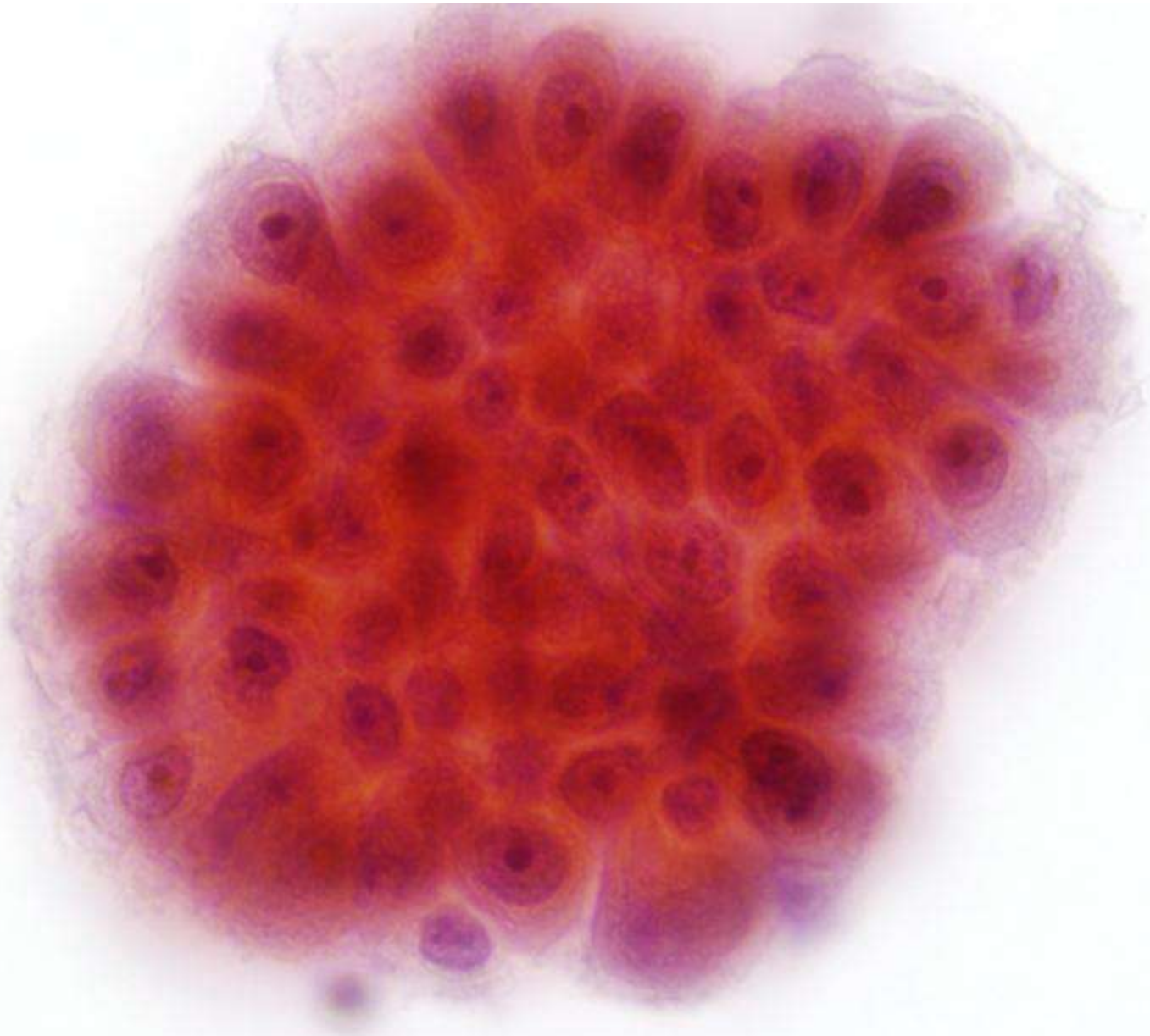




Cellular Chain

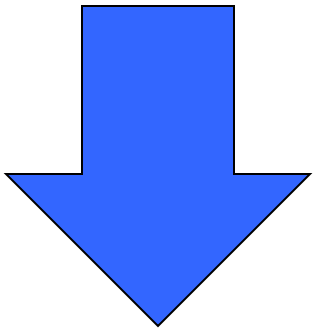
Keratin Pearl

Knobby cluster in Mesothelioma



Malignant Effusions

Specific Type and Site of Origin



Diagnosis :

Cytomorphology & Immunocytochemistry

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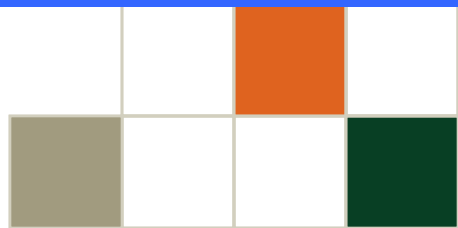
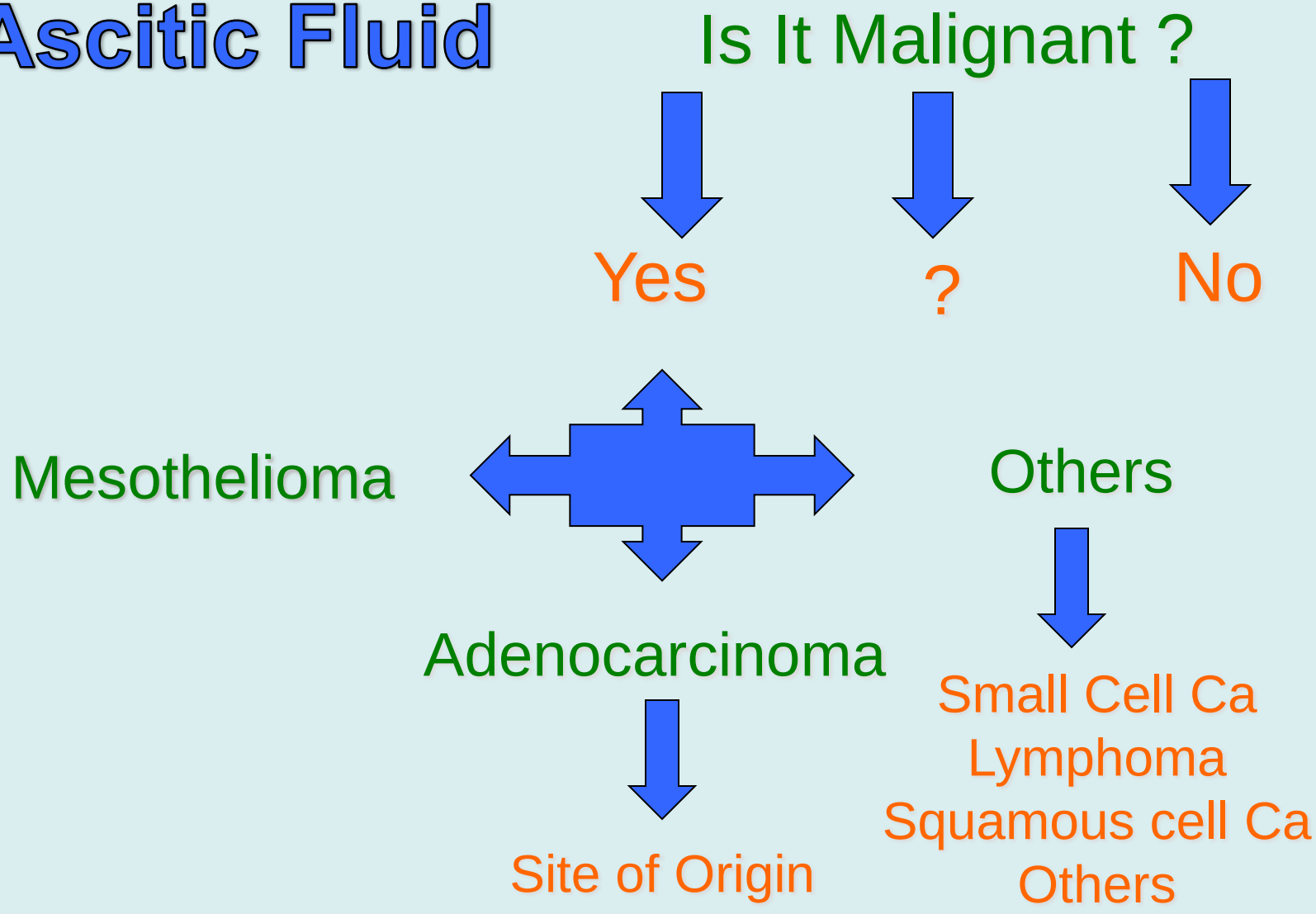


Ascitic Fluid



When to Use Immunocytochemistry in Ascitic fluid Cytology

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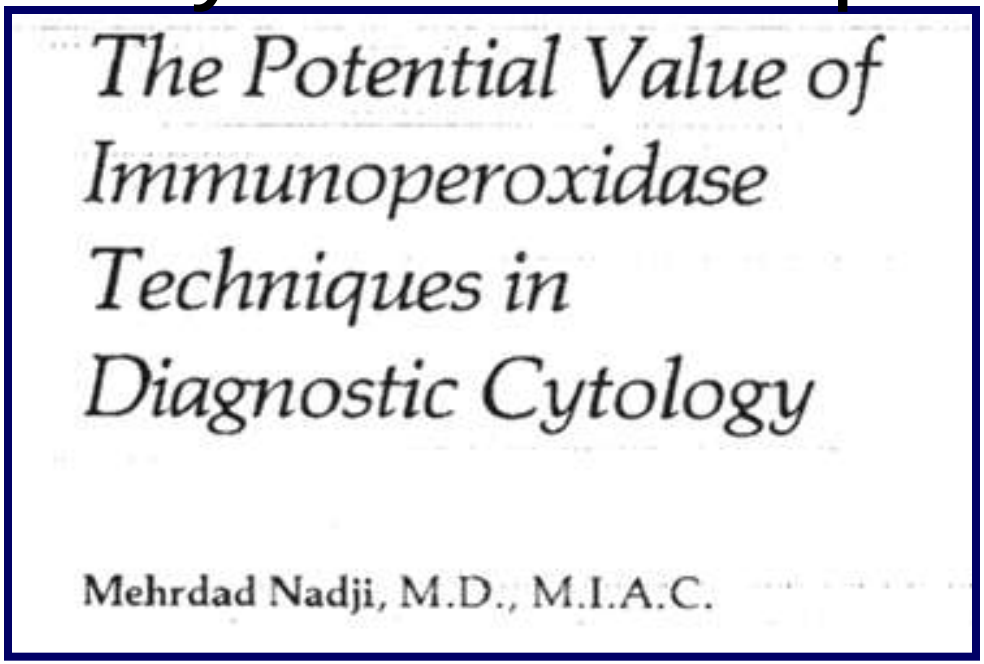
Alamo 1917

Immunocytochemistry in Cytology

University of Miami Experience

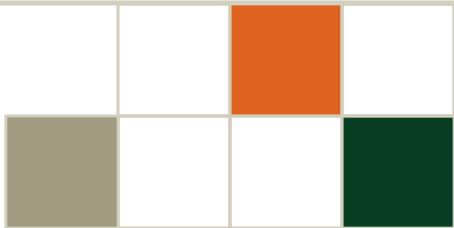


Acta Cytol 1980; 24: 442-447

IHC Applications

University of Miami

- Diagnosis/Classification 65%
- Prognosticators/Predictors 18%
- Target therapy, Others 17%



How Often?

University of Miami

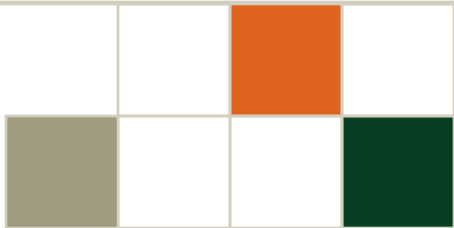
“Percent of our Total Cases”

- Surgical Pathology 5.9%
- Cytopathology 4.9%
- Autopsy Pathology 18%

Type of Specimen

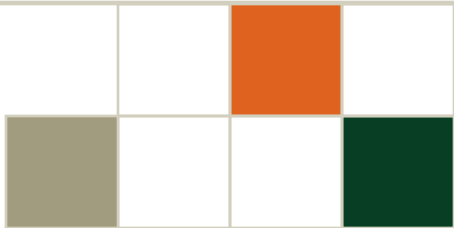
ICC in Cytology

- FNA 55%
- Effusion 41%
- Others 4%



Diagnostic IHC
Facts

- IHC is an important diagnostic tool in tumor pathology
- Traditionally used on histologic material and cytologic cell blocks
- The technique is not widely used in diagnostic cytology



Why IHC is Not Widely Used in Cytology ?

- Limited cytologic material
- Problems in interpretation
- Lack of specific markers to differentiate benign from malignant cells

Technical Considerations

- Use cell block if possible (Cellular)
- Use alcohol fixation (95% isopropyl)
- Alcohol- fixed, Pap- stained archival slides can be used
- No de-staining is necessary
- Most cytology samples can be used

Immunocytochemistry

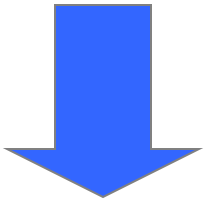
Not good in:

- Air-dried slides
- Diff-Quick-stained slides
- De-stained slides (cellular antigens maybe removed)
- Slides with plastic coverslip

Immunocytochemistry

Not good in:

- Filter preparation
- Serous fluid specimens with excess blood and proteins



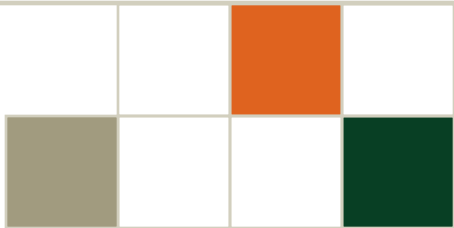
Wash specimen or use Saccomanno solution

Immunocytochemistry
Fixation

- 95% isopropyl alcohol
- Buffered formalin
- Formol-acetone
- Mixture of ethanol & formalin

Immunocytochemistry
Fixation

- Prolonged fixation (wks/months) in *formalin* may result in antigenic loss
- Prolonged fixation in *alcohol-based* fixatives is not a major problem



Easy 3-Step Procedure

1. Use a diamond pen to mark the cells on the back of the slide
2. Remove the coverslip
3. Start your routine IHC/ICC procedure

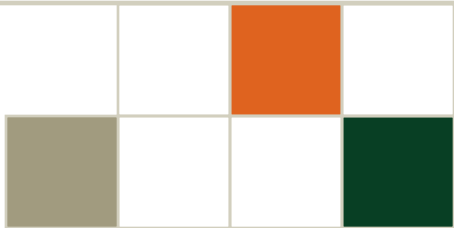
Immunocytochemistry
Using Archival Slides

- Removal of coverslip may be difficult
- When diagnostic slides are limited, ICC can be performed on a previously negative slide

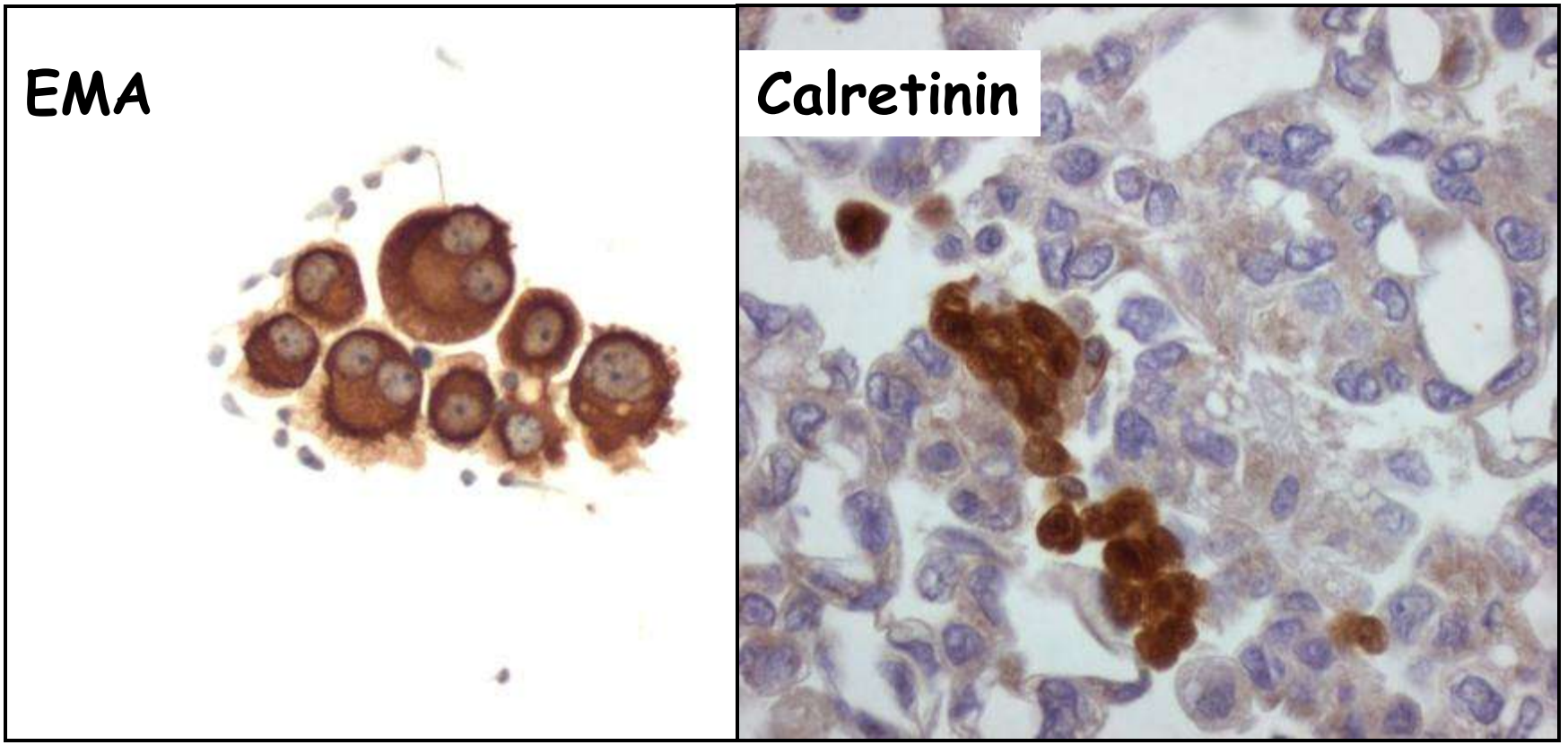
IHC=ICC

Technique

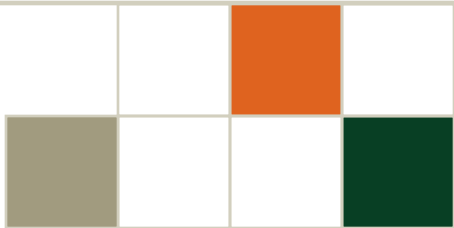
No technical alterations needed for cytologic specimens



True Positive



You Should Know your Antibody



ICC in Diagnostic Cytology
Applications

- Tumor Diagnosis/Classification
- Prognostic/Predictor Markers
- Target Therapy

ICC in Diagnostic Cytology
Applications

- Tumor Diagnosis/Classification
- Prognostic/Predictor Markers
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Selection of Markers

Cytomorphology

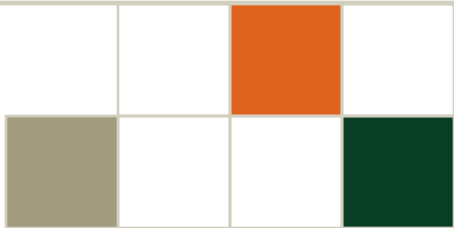
Clinical Information

Working Diagnosis

Differential Diagnosis

Selection of ICC Markers

Final Interpretation



ICC in Diagnostic Cytology
Selection of Markers
"tailor-made" Approach

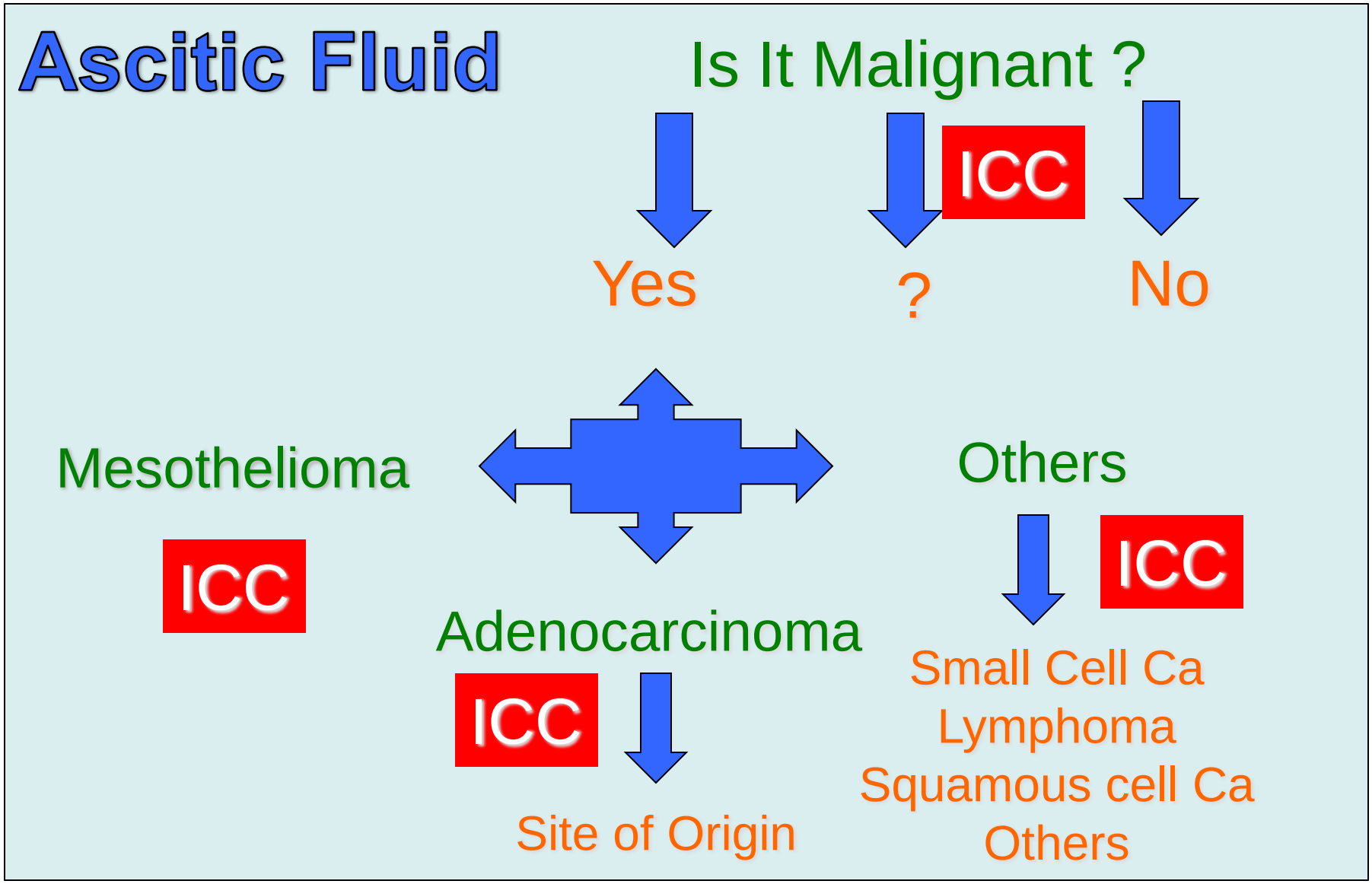
- When the differential diagnosis is narrowed down, usually not more than 2-3 markers are needed ("tailor-made")
- In many occasions only one marker is used to confirm the working diagnosis

Diagnosis/Classification

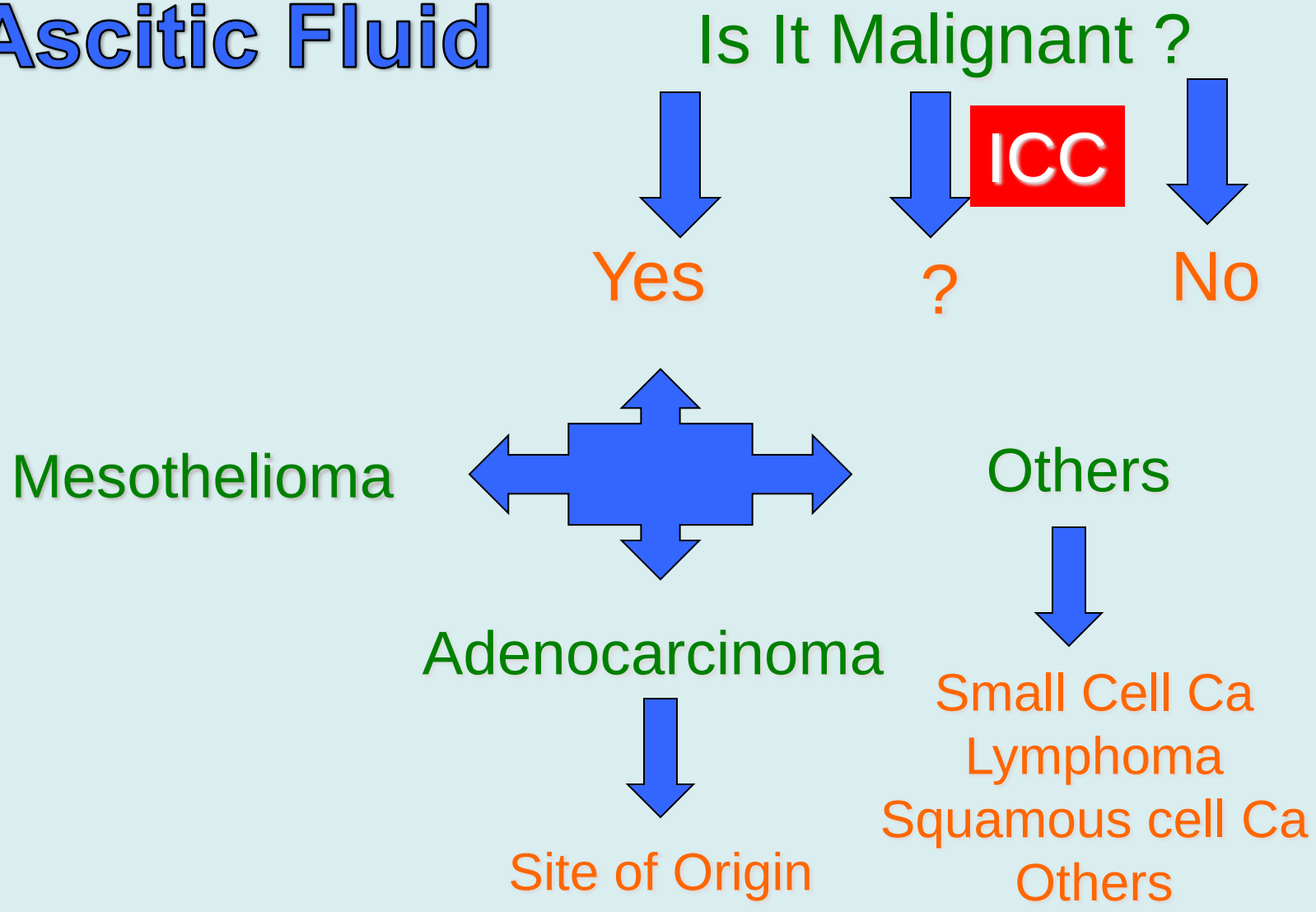
Our 3-Step Approach

1. Define a specific differential Dx
2. Select a small panel of ICC markers
3. Combine cytomorphology and ICC

Ascitic Fluid



Ascitic Fluid



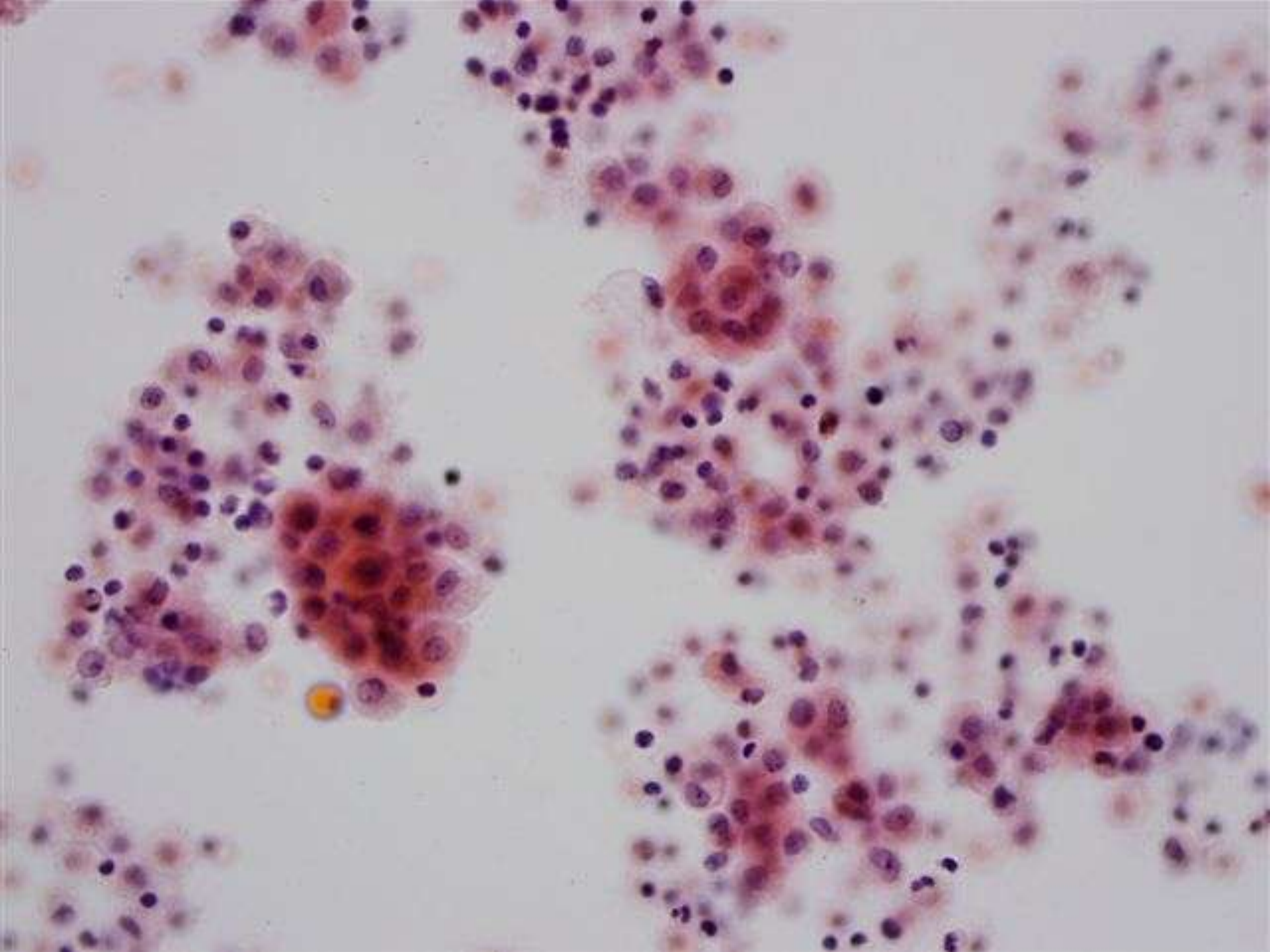
First Step…..

Reactive Mesothelial cells

versus

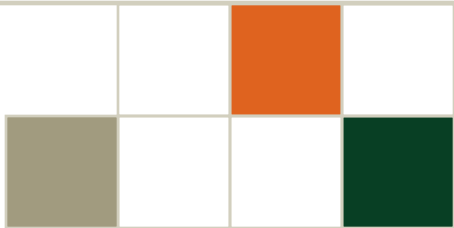
Malignant Process

- The reactive mesothelial cells may group.
- If so, the grouping usually presents as loose clusters, without nuclear overlapping.



Differential Diagnosis of Atypical cells in Ascitic Fluid

	Malignant Morphology	Resemble Mesothelial Cells
Reactive Mesothelial Cells	NO	YES
Malignant Mesothelioma	YES	YES
Adenocarcinoma	YES	NO



Commonly Used Markers In Effusions

EMA: Malignant: adenocarcinoma,
malignant mesothelioma

CEA: Malignant: adenocarcinoma

Ber-EP4: Malignant: adenocarcinoma

LeuM1: Malignant: adenocarcinoma

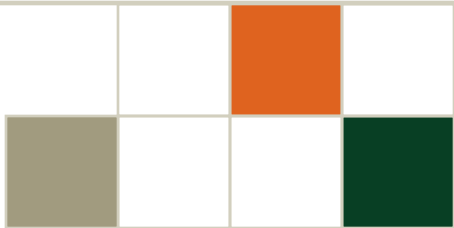
Desmin: Benign: reactive mesothelium

In our laboratory, EMA (clone E29) is the most frequently used antibody in defining “atypical cells” in effusions.

Reactive Mesothelium vs. Adenocarcinoma and Mesothelioma

EMA

Reactive	Negative
Adenocarcinoma	Positive (Cytoplasm)
Mesothelioma	Positive (membrane)

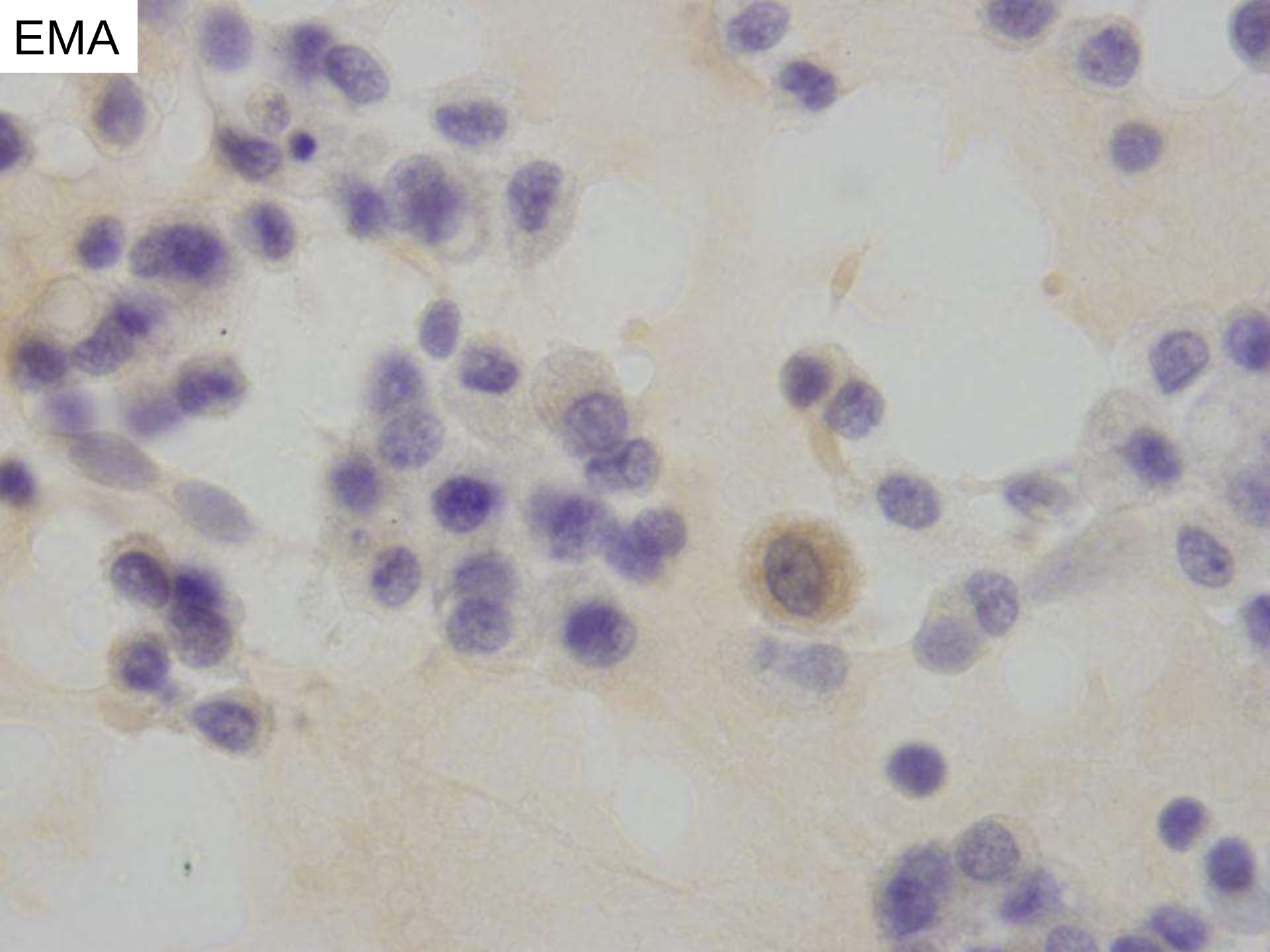


- In our experience, the most useful limited panel of ICC includes:
  - EMA
  - Calretinin
- Nuclear and intracytoplasmic positivity for calretinin and negativity for EMA confirms a reactive mesothelial proliferation.

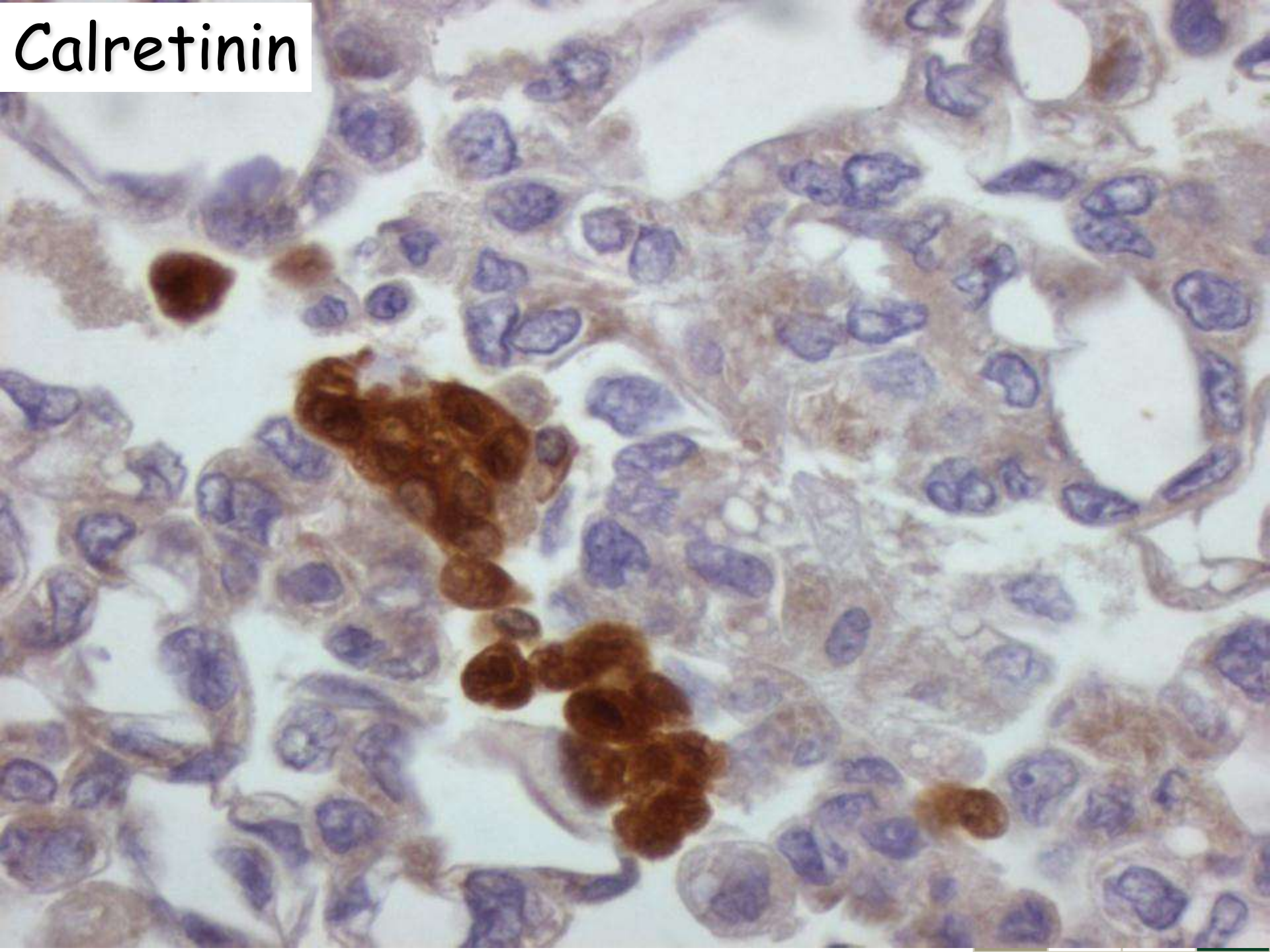
Acta Cytol 2000; 44 : 854

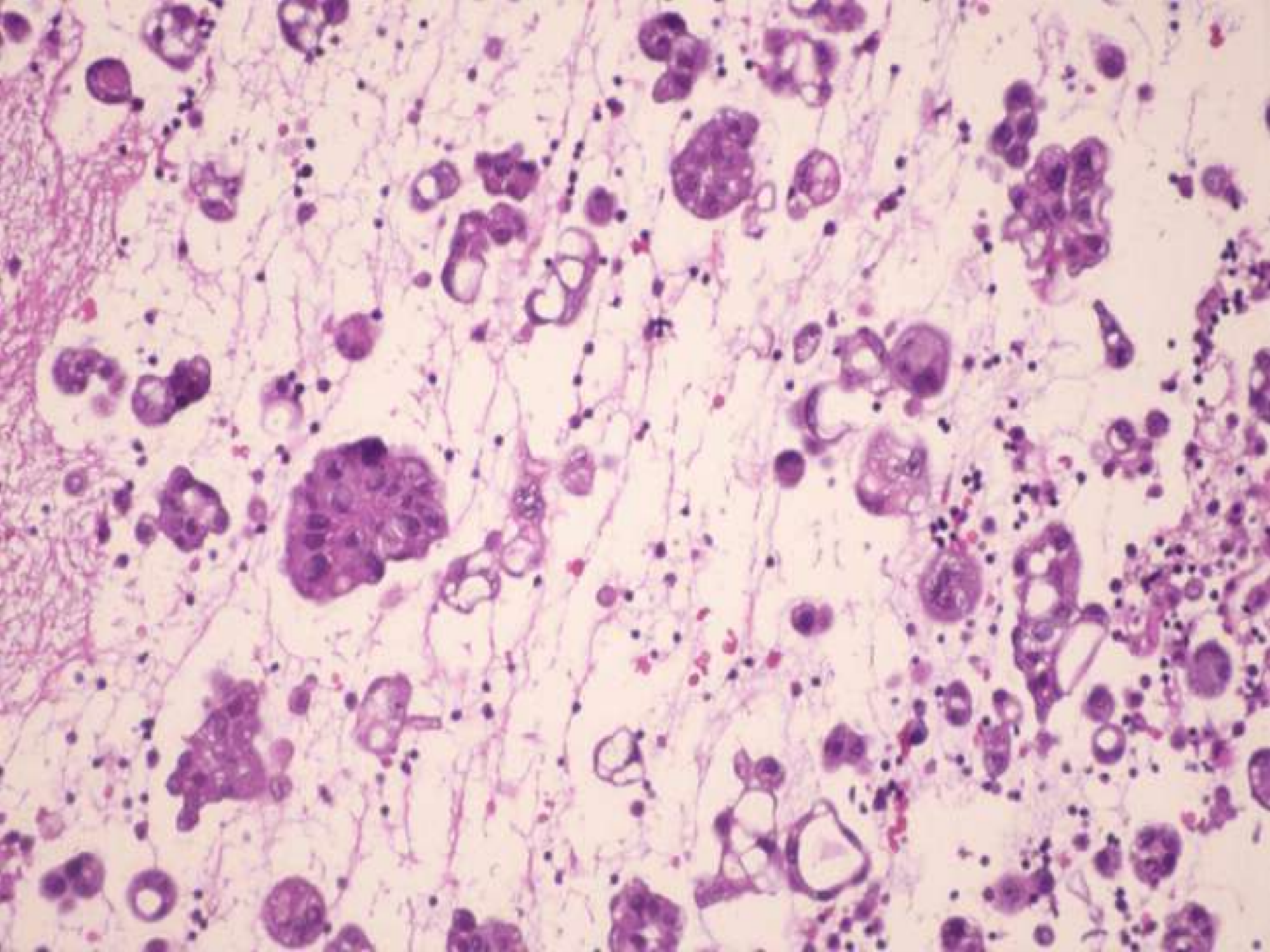
Diag Cytopathol 2008, 34:

Reactive Mesothelium


EMA

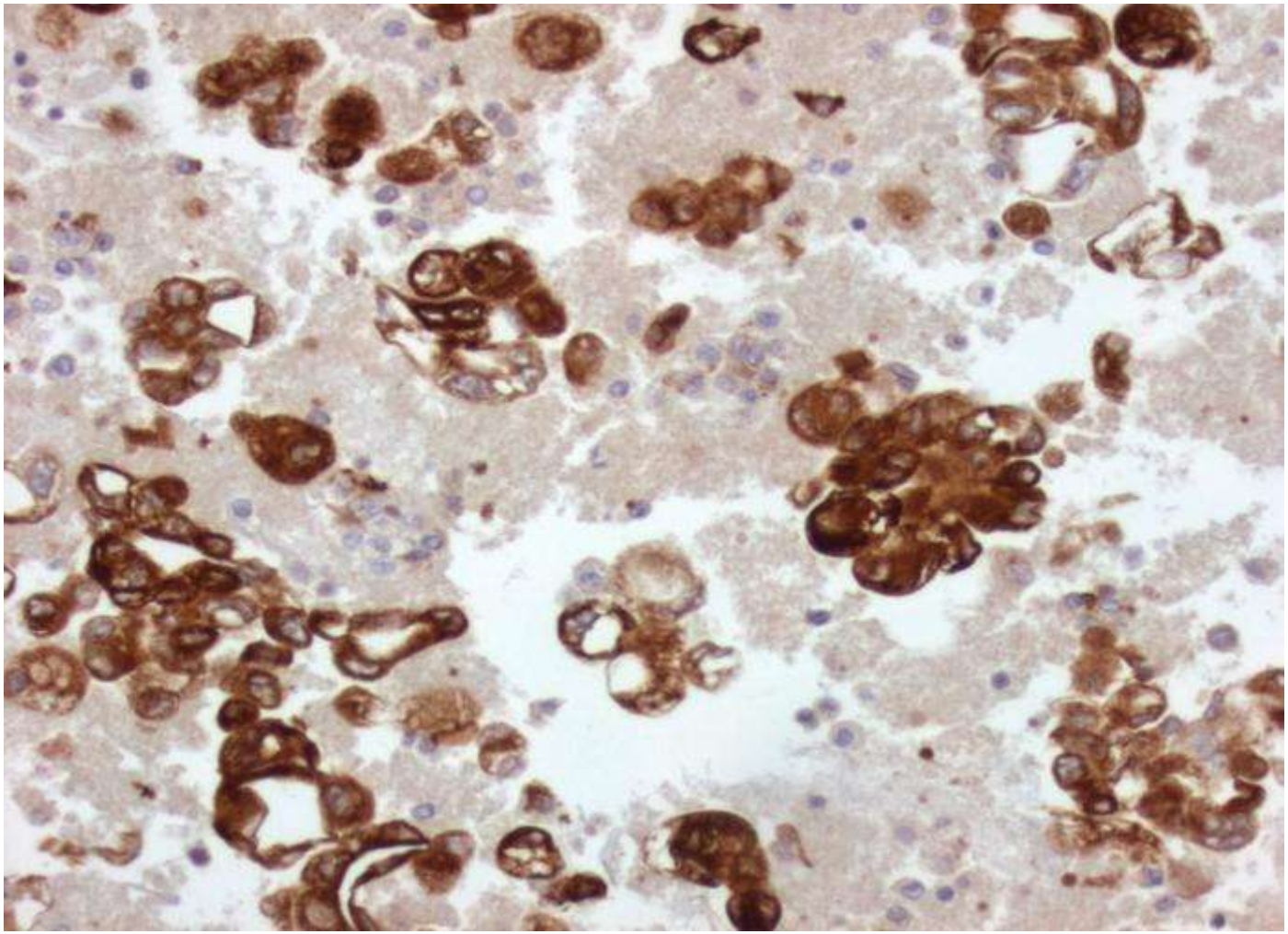
Calretinin

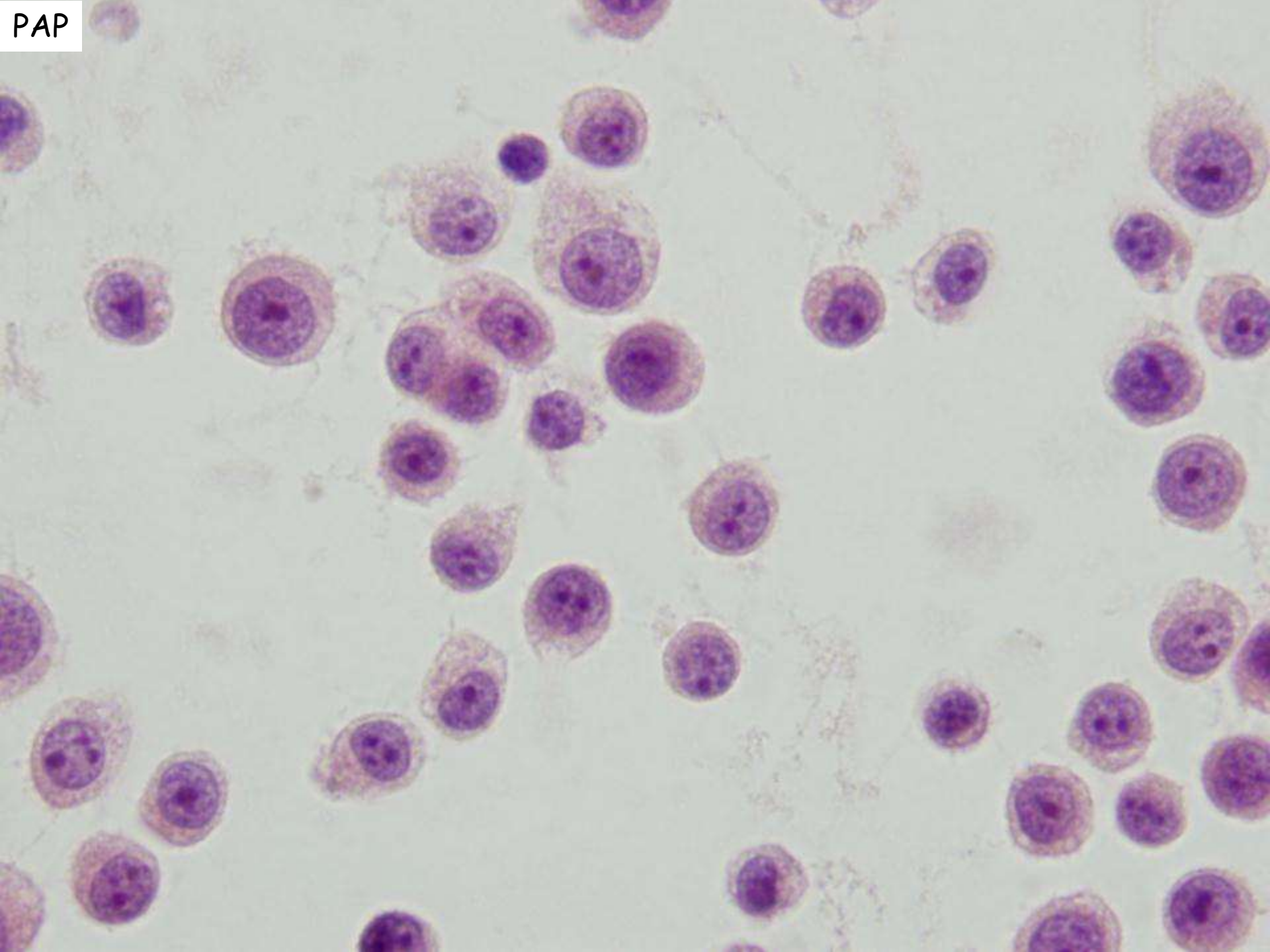


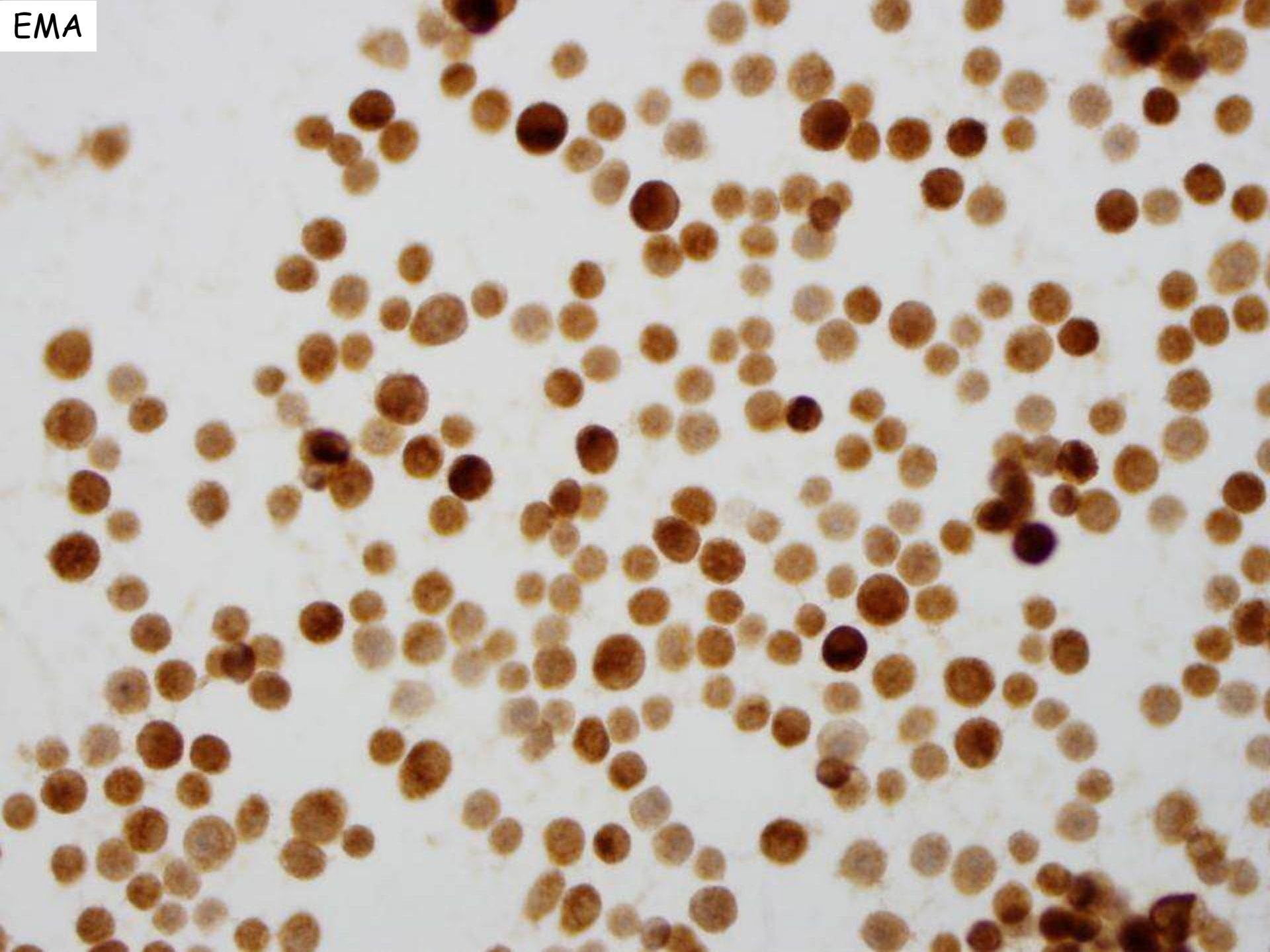


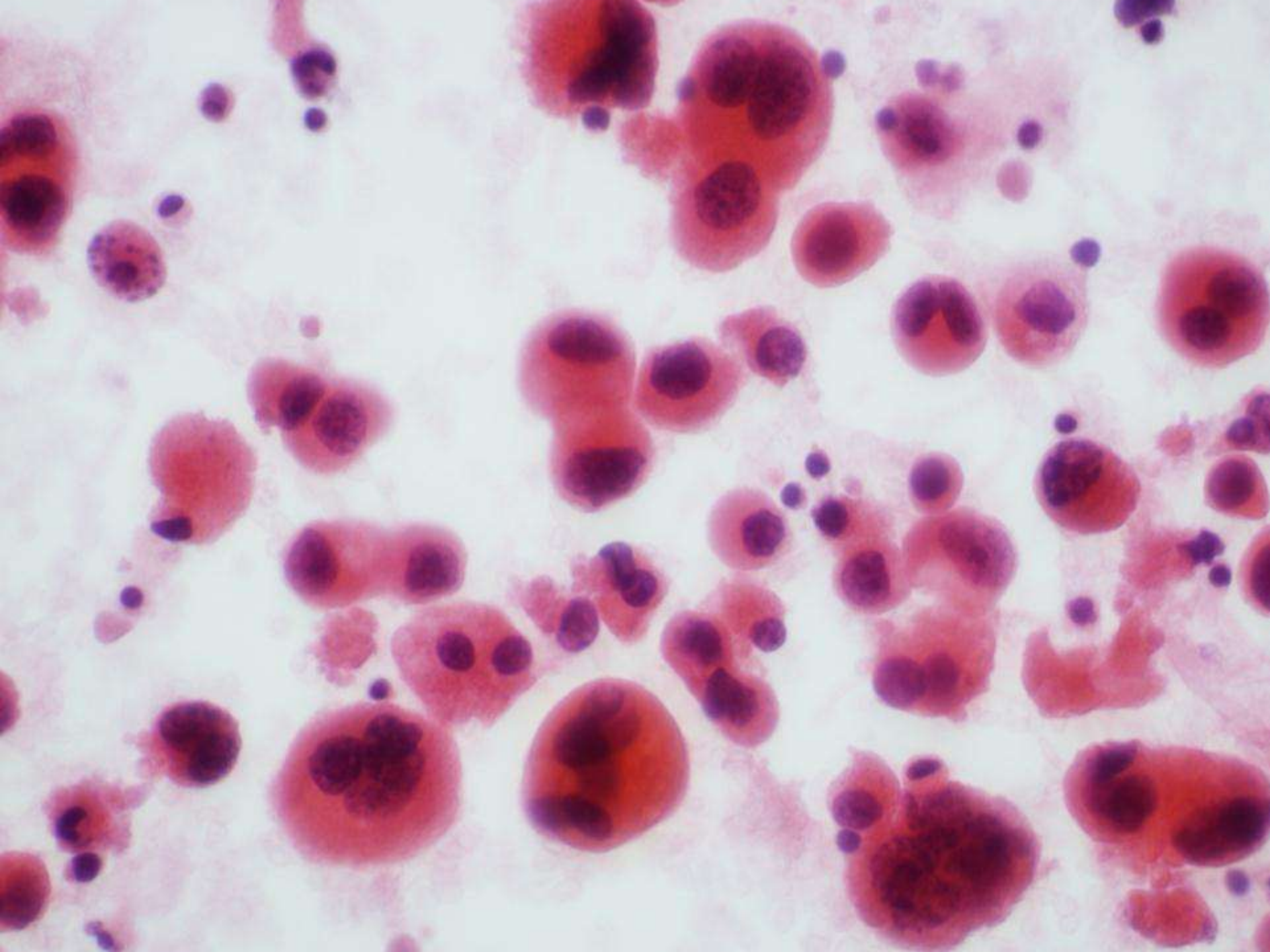
EMA Positivity

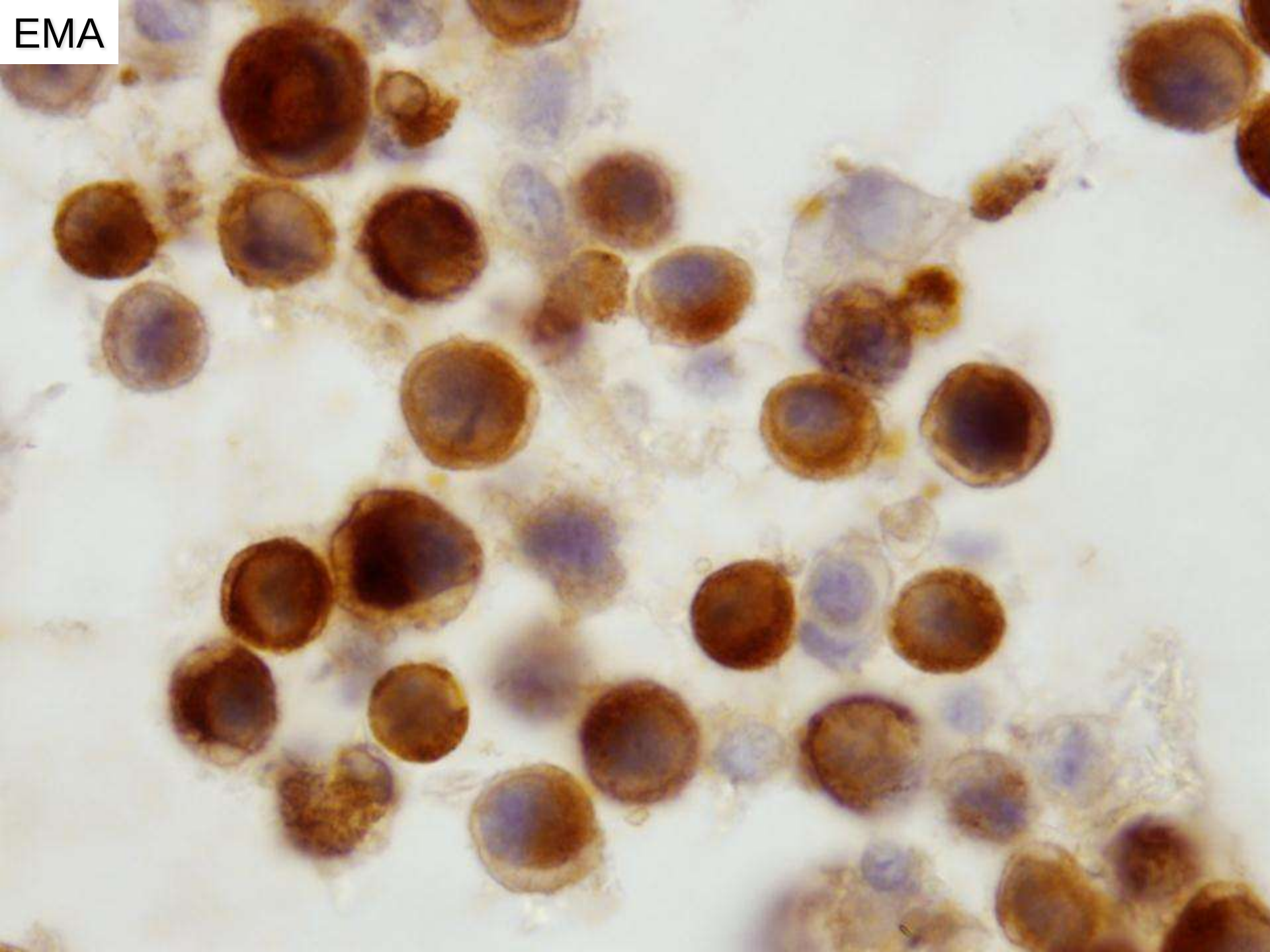
Strong, Intracytoplasmic & Easily seen on Low Power



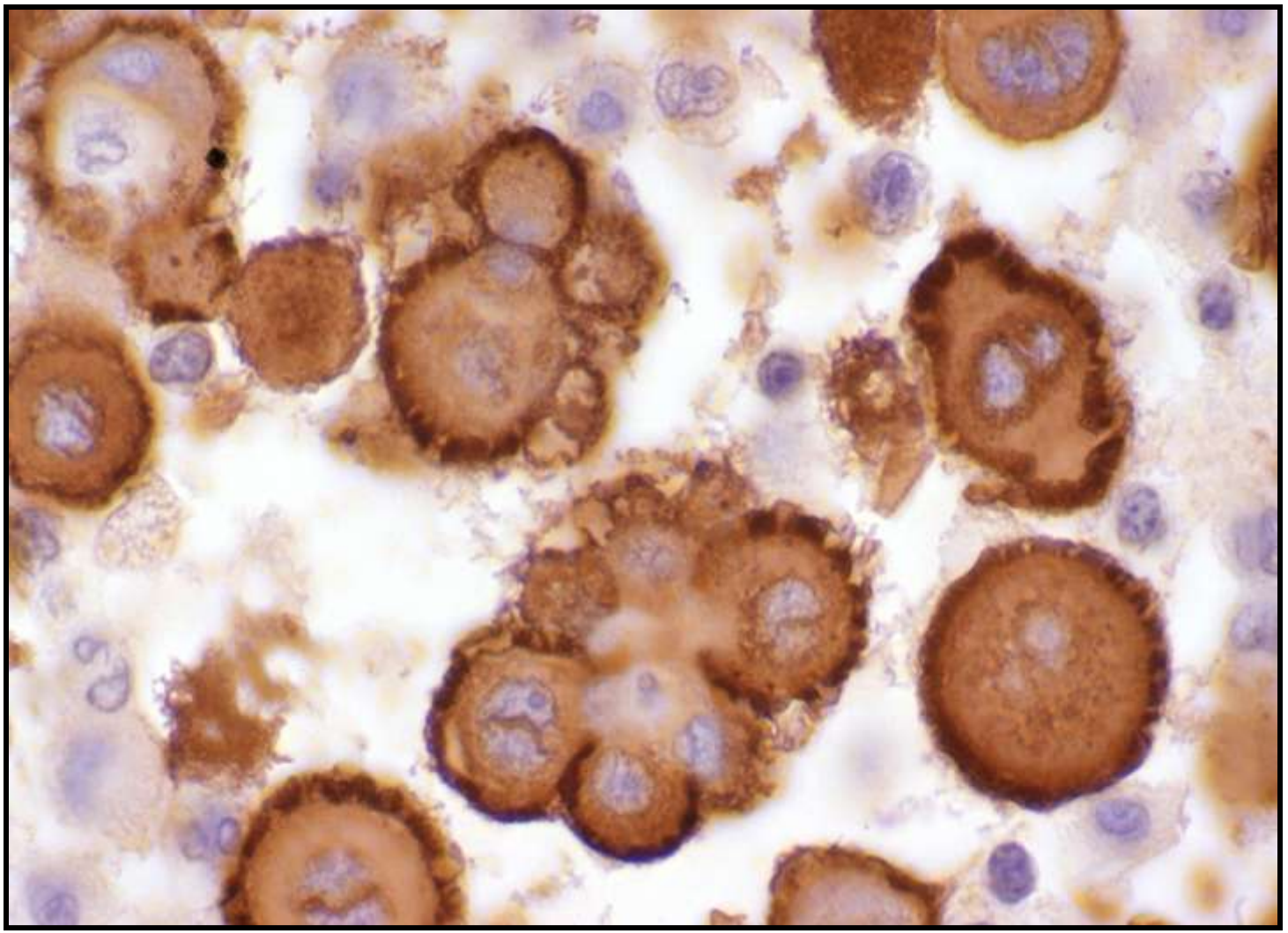

PAP


EMA

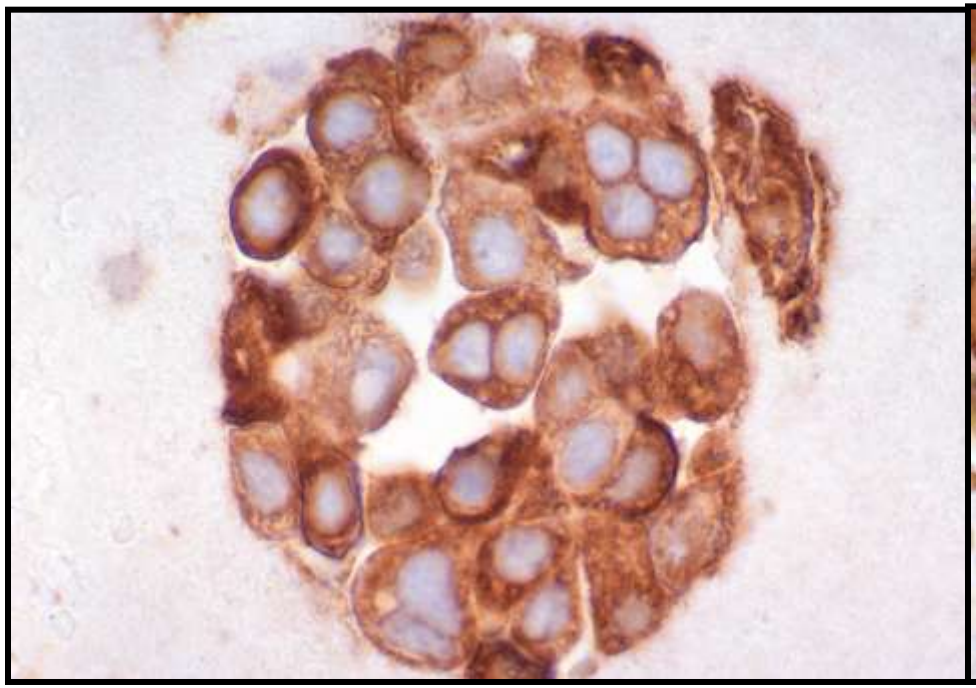



EMA

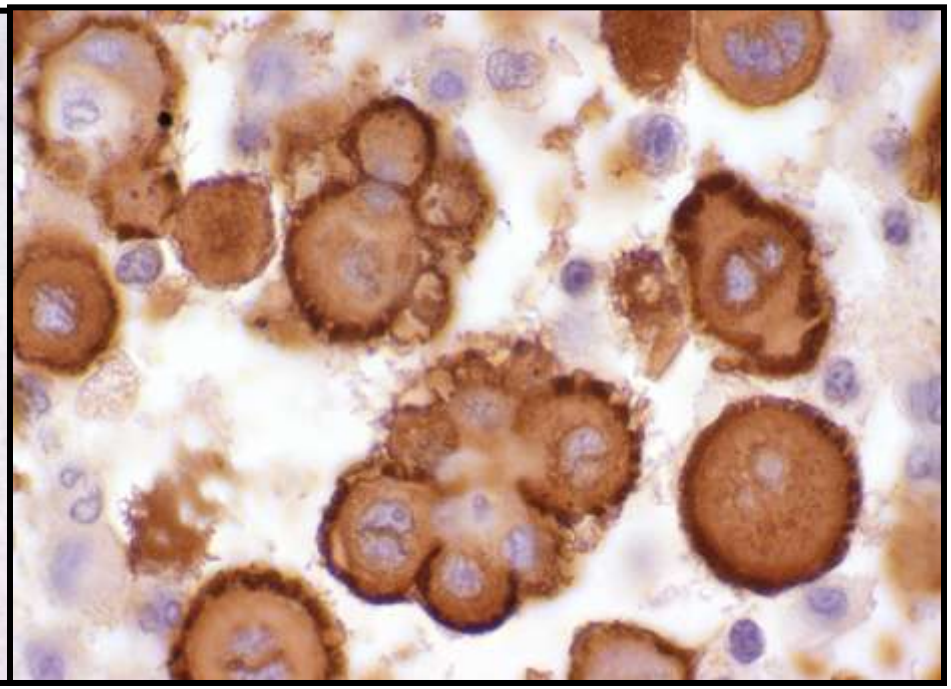
EMA Positive in MM: Strong Membrane/Cytoplasmic



EMA Positive in MM



Strong
Cytoplasmic

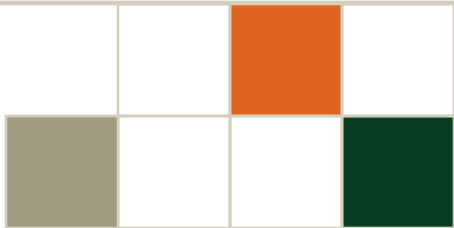


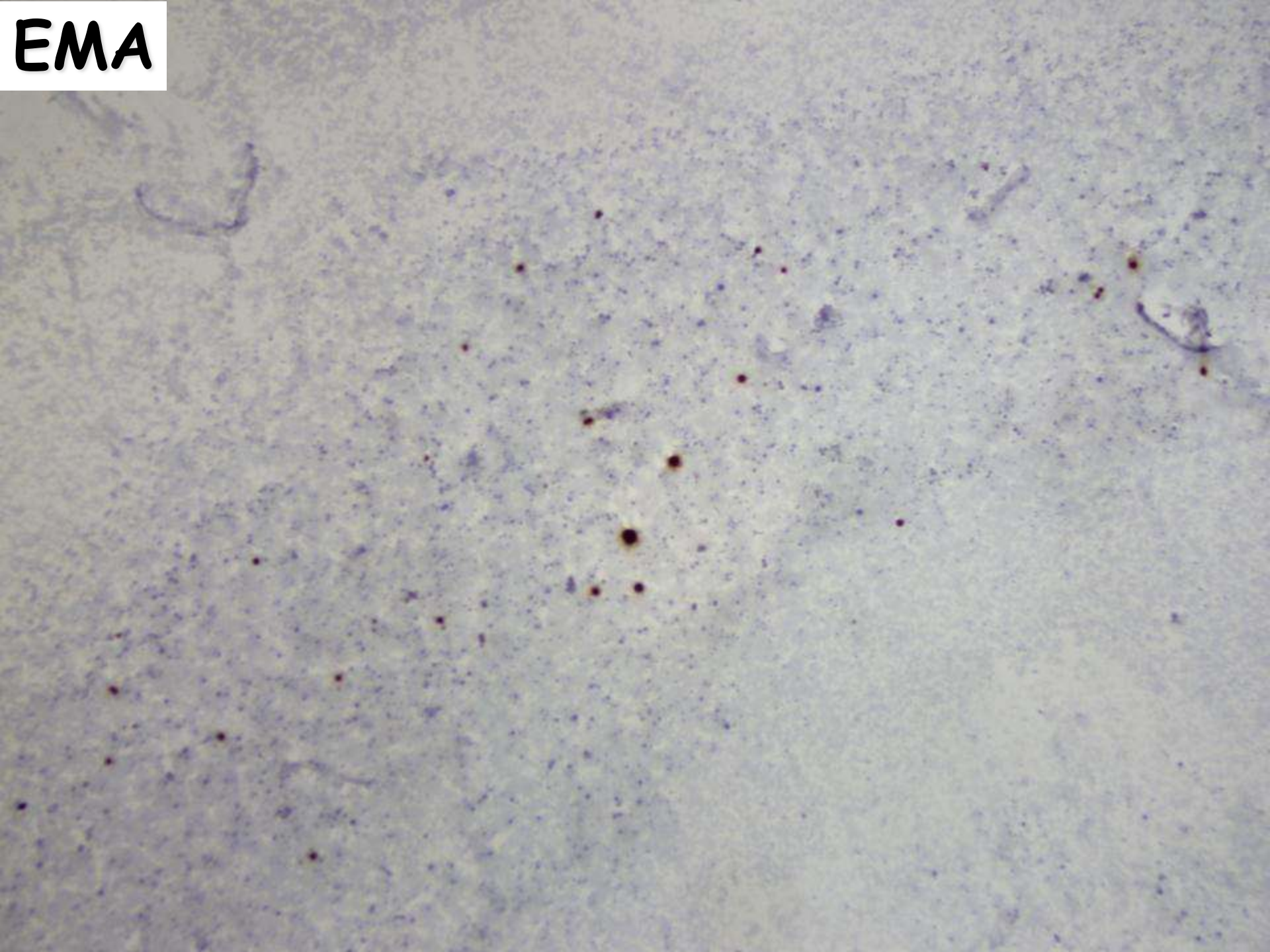
Strong
Membrane/Cytoplasmic

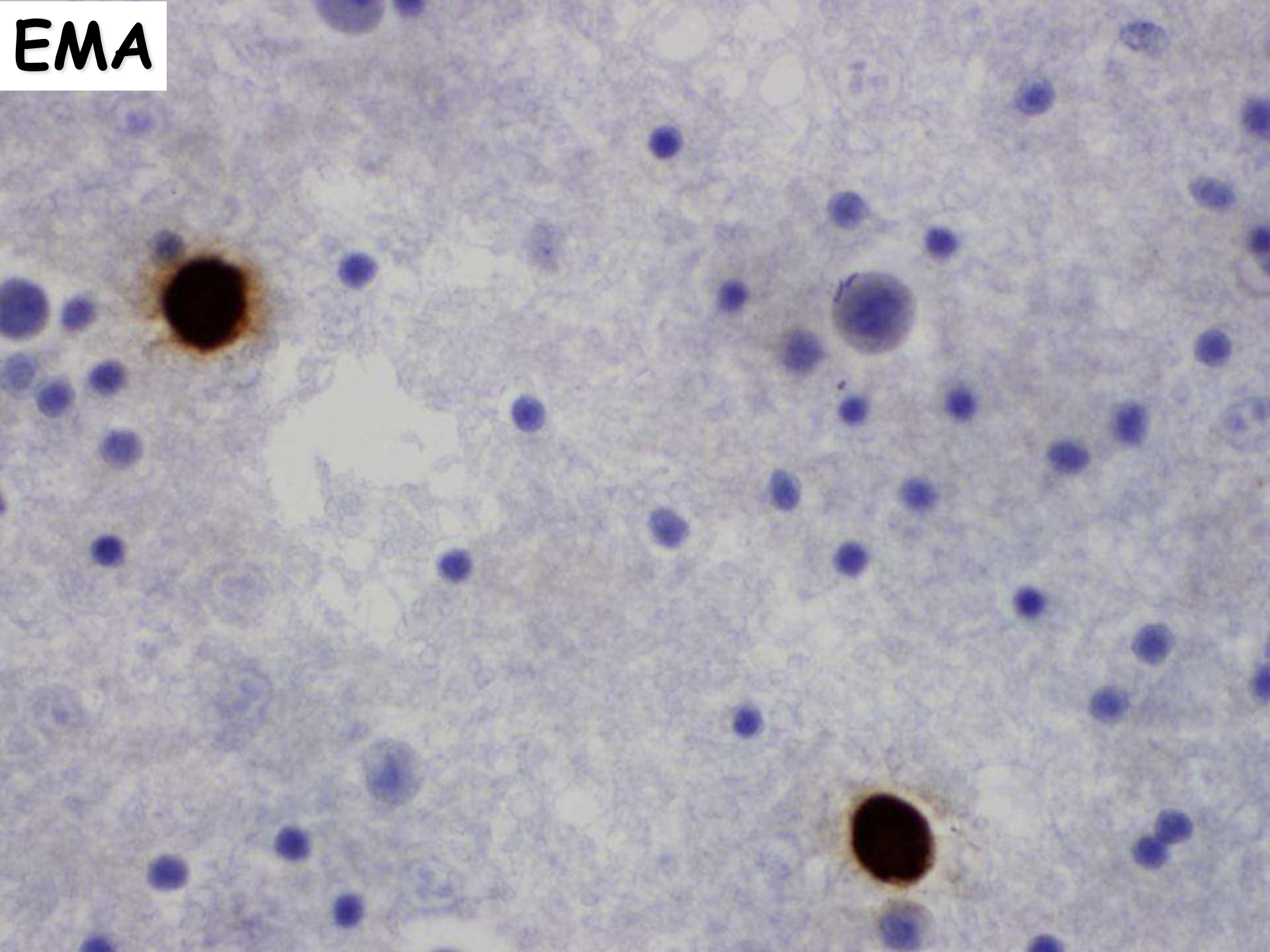
Positive EMA in Serous Effusions

Represents adenocarcinoma, if:

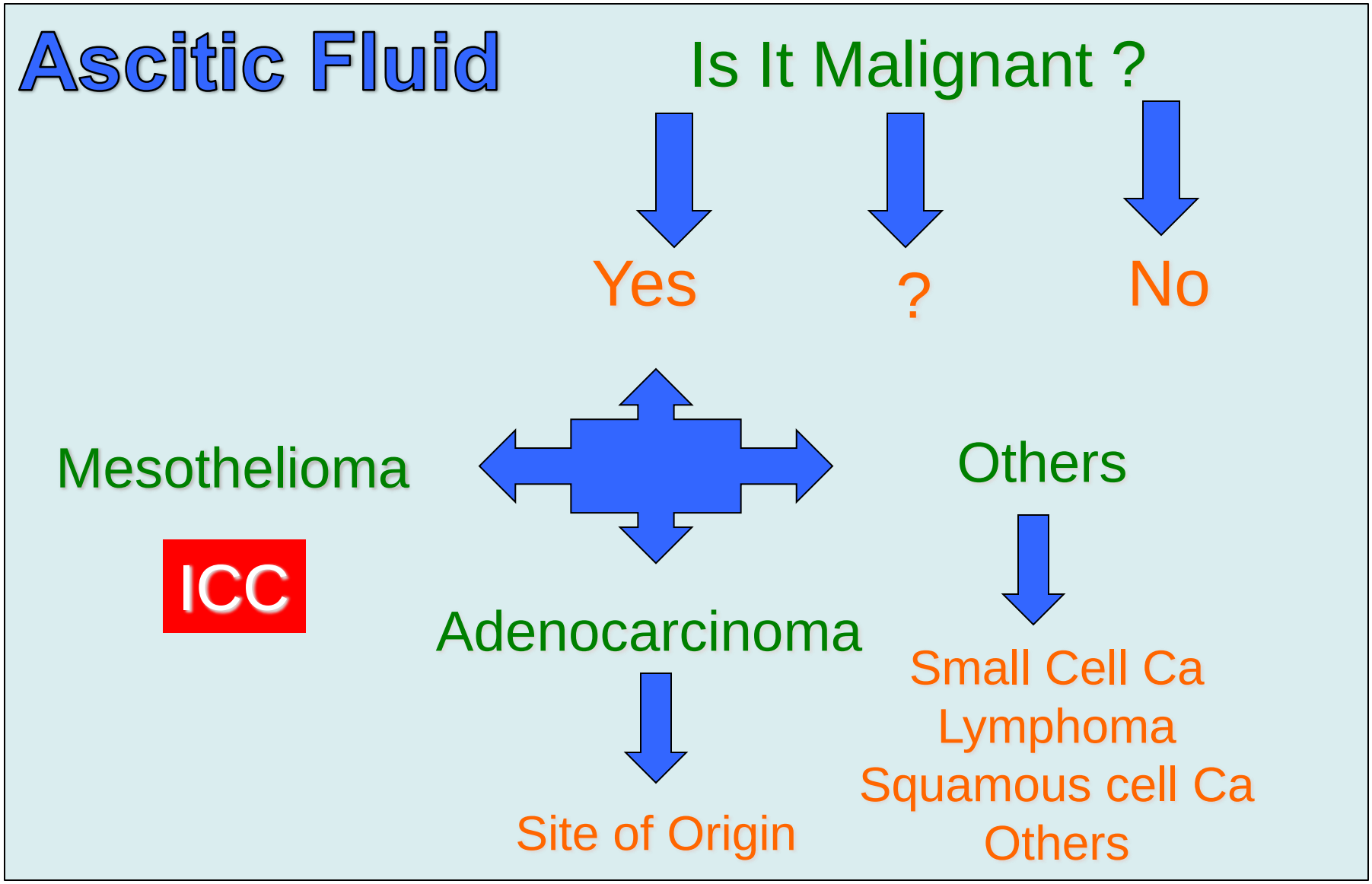
- Easily seen on low power
- Is strong and intracytoplasmic




EMA


EMA

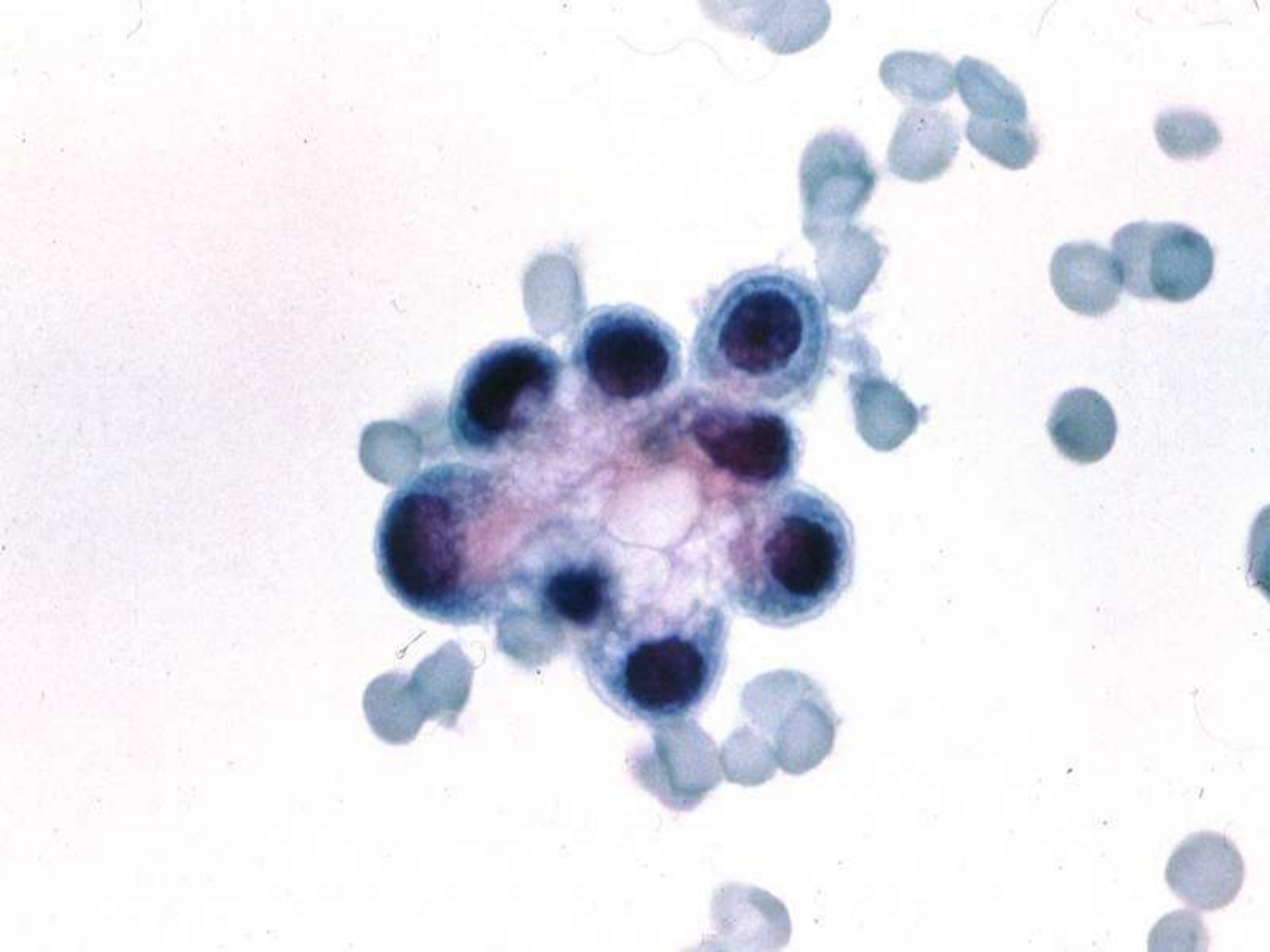
Ascitic Fluid



Malignant Mesotheliomas in Effusions

Low Power

- Small or large 3D groups
- “Knobby clusters”



Resemblance to Mesothelial Cells

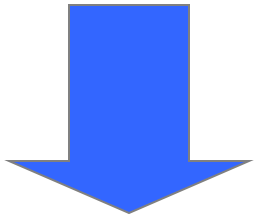
Malignant Mesothelioma in Effusions

Differential Diagnosis of Mesothelioma

- Cytomorphology
- Electron microscopy
- Cytochemistry
- Immunocytochemistry (ICC)

When

Malignant Mesothelioma Mimics Adenocarcinomas

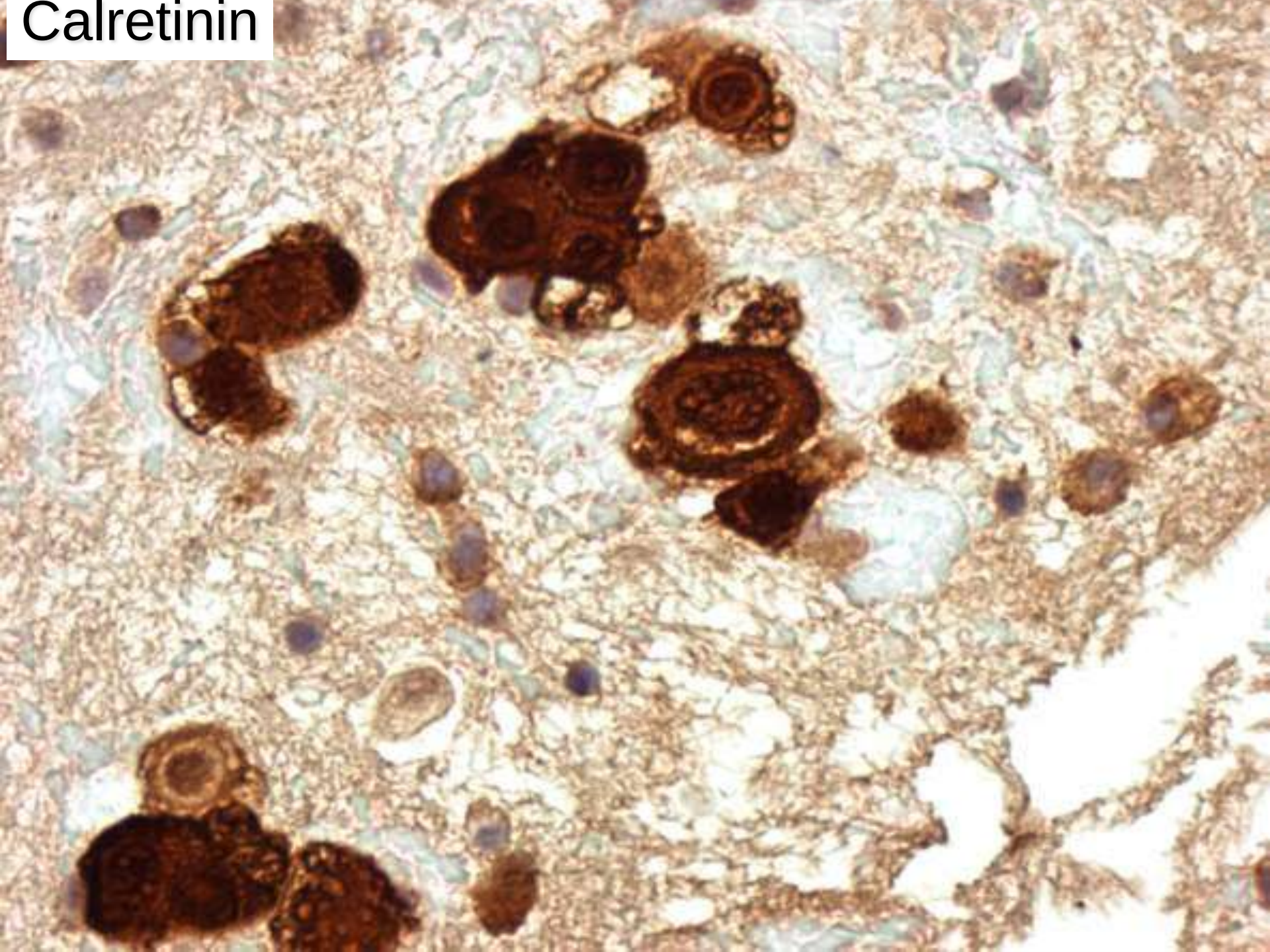


Use ICC

- In our experience, the most useful limited panel of ICC includes:
  - EMA
  - Calretinin
- Nuclear and intracytoplasmic positivity for calretinin and Positivity for EMA confirms a Malignant Mesothelioma

Acta Cytol 2000; 44 : 854

Diag Cytopathol 2008, 34:

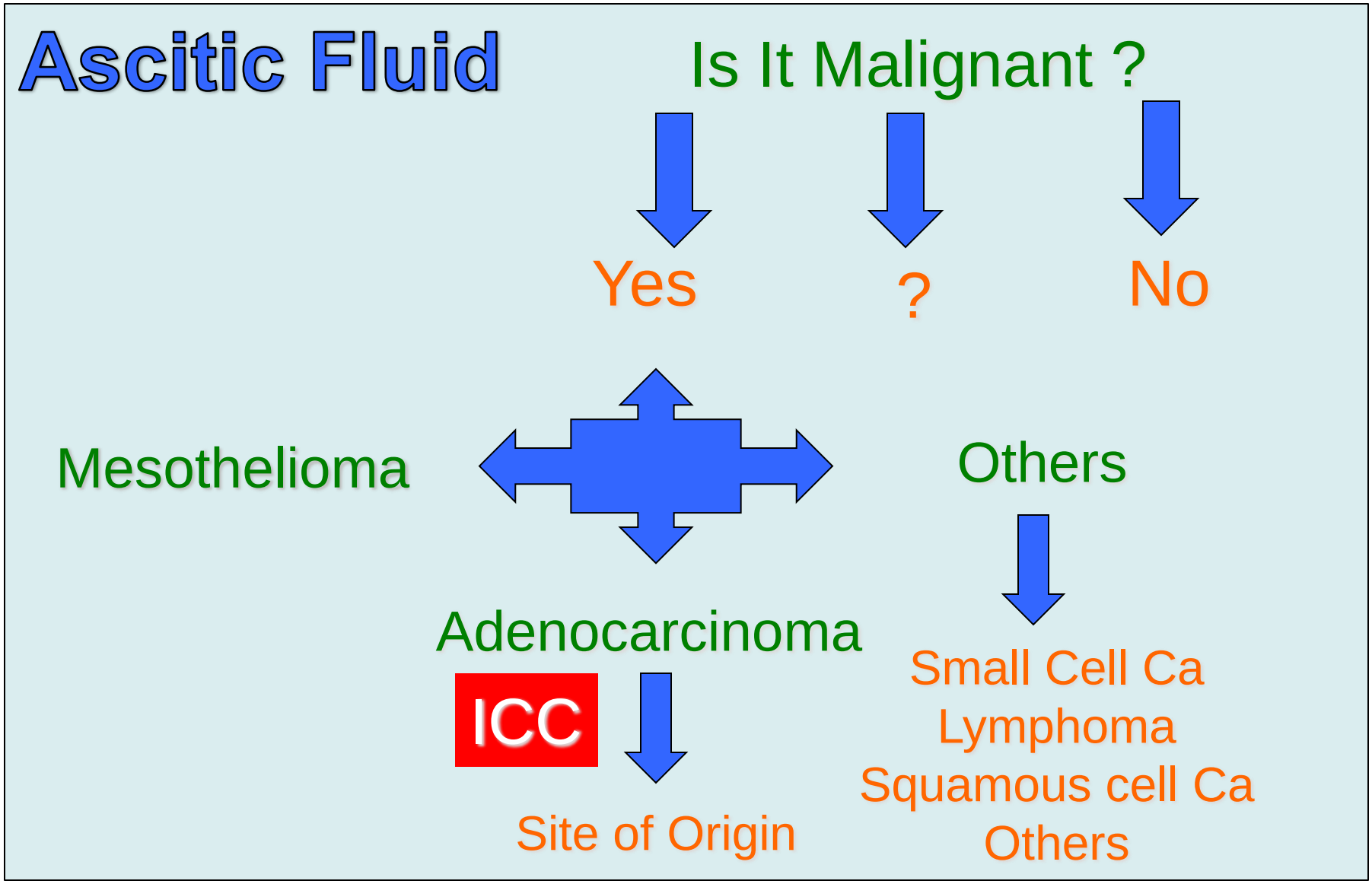

Calretinin

Ascitic Fluid

Malignant Mesothelioma Vs Lung Adenocarcinoma

	Calret	TTF-1	CEA	D2-40
MM	Pos	Neg	Neg	Pos
LA	Neg	Pos	Pos	Neg

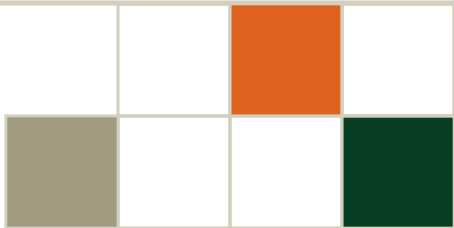
Ascitic Fluid



Adenocarcinoma in Ascitic Fluid

Primary Sites in Adult Male

- Adenocarcinoma
  - –GI tract-
    Pancreas
  - –GU
  - –Lung

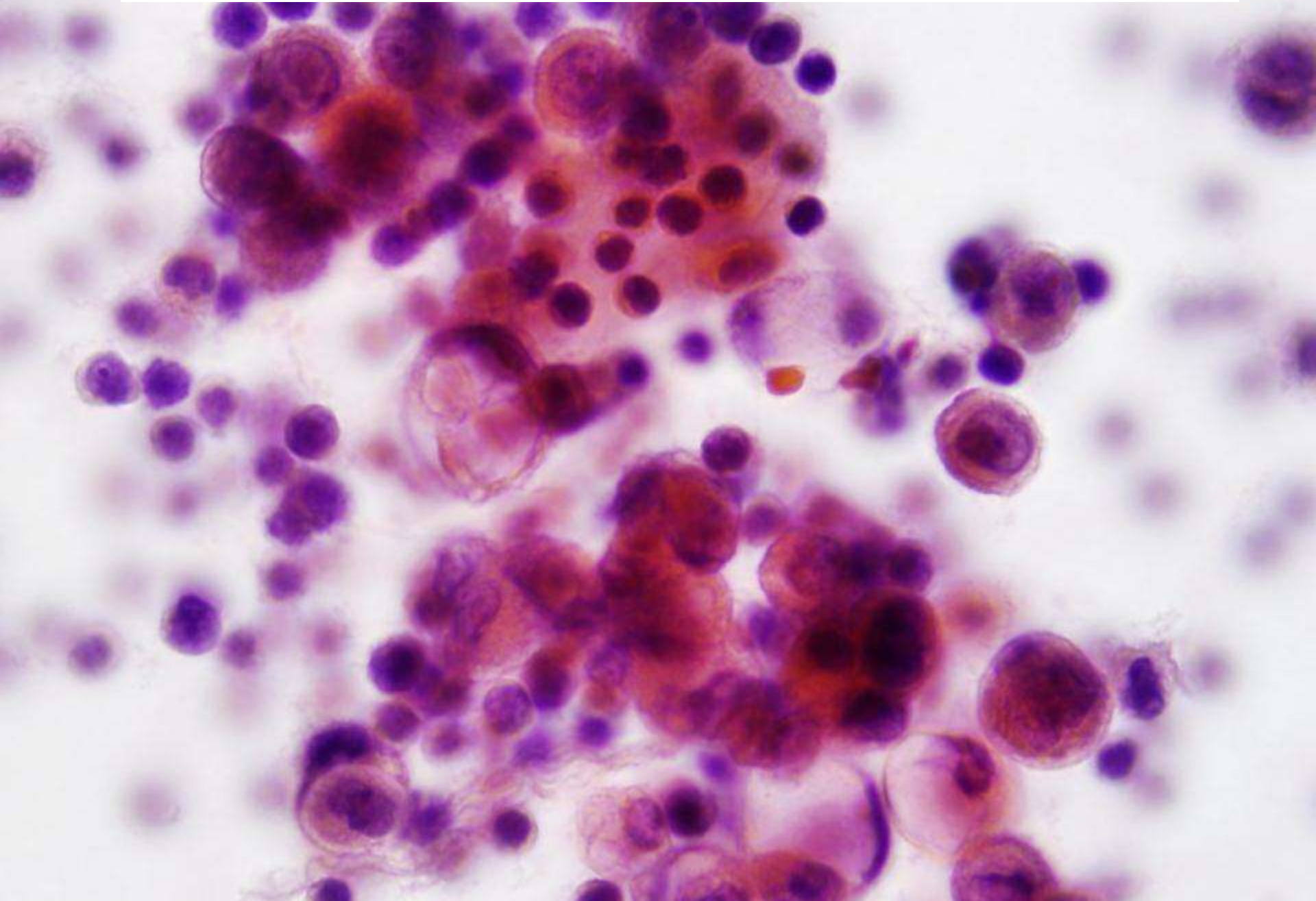


Adenocarcinoma in Ascitic Fluid

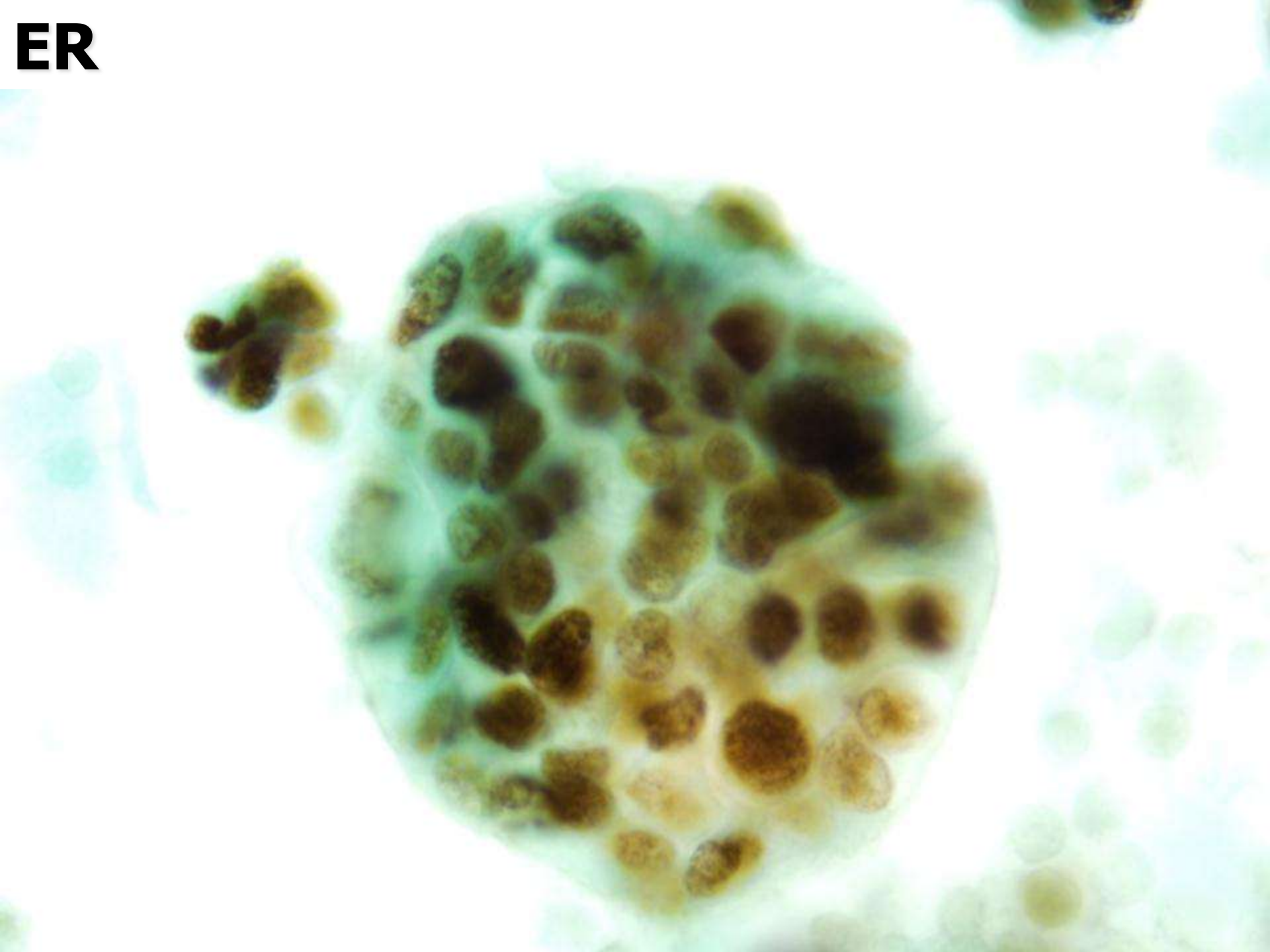
Primary Sites in Adult Female

- Adenocarcinoma
  - Ovary
  - Breast
  - GI Tract-Pancreas
  - Lung

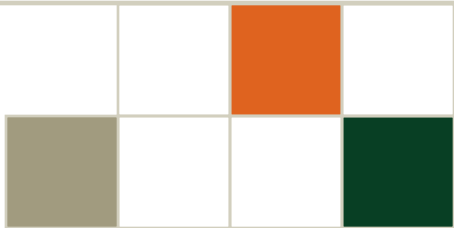
Breast/GYN adenocarcinoma



ER



ER-1D5
In Fluids



Remember !

- Be careful with the use of ER in peritoneal effusions of female patients
- Benign epithelial inclusions may cause false positive results
- First establish the malignant nature of the cells by cytomorphology

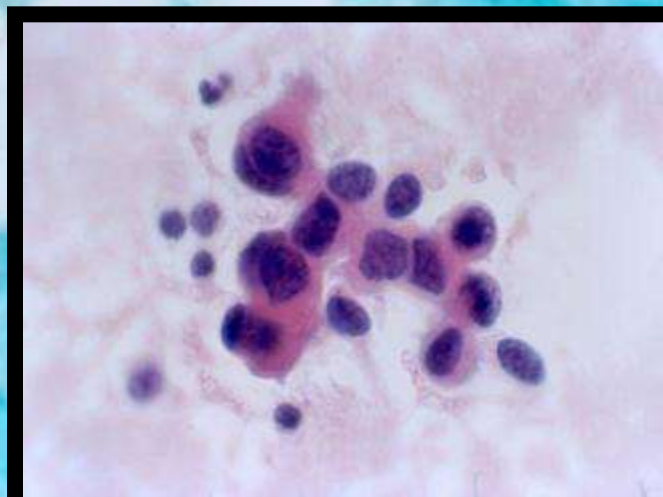
Adenocarcinoma of Lung Vs. Colonic Carcinoma

	TTF-1	CK20	CK7
Adenoca of Lung	+	-	+
Colonic Carcinoma	-	+	-

ICC Markers for Colon Cancer

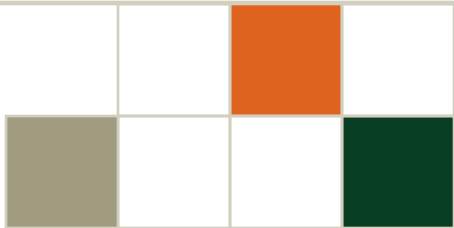
- CK 7 Negative
- CK 20 Positive
- CDX-2 Positive
- CEA Positive

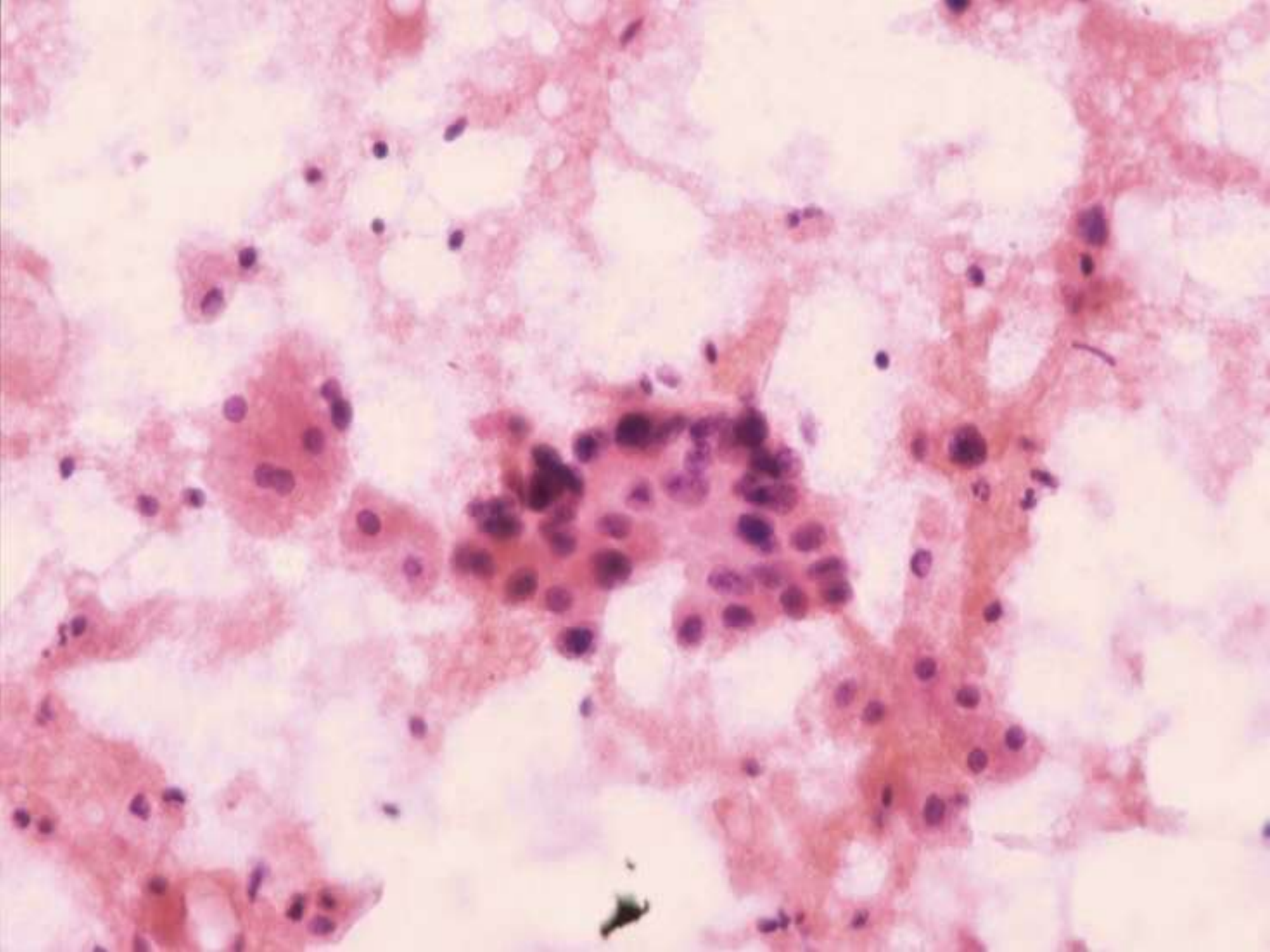
CK20

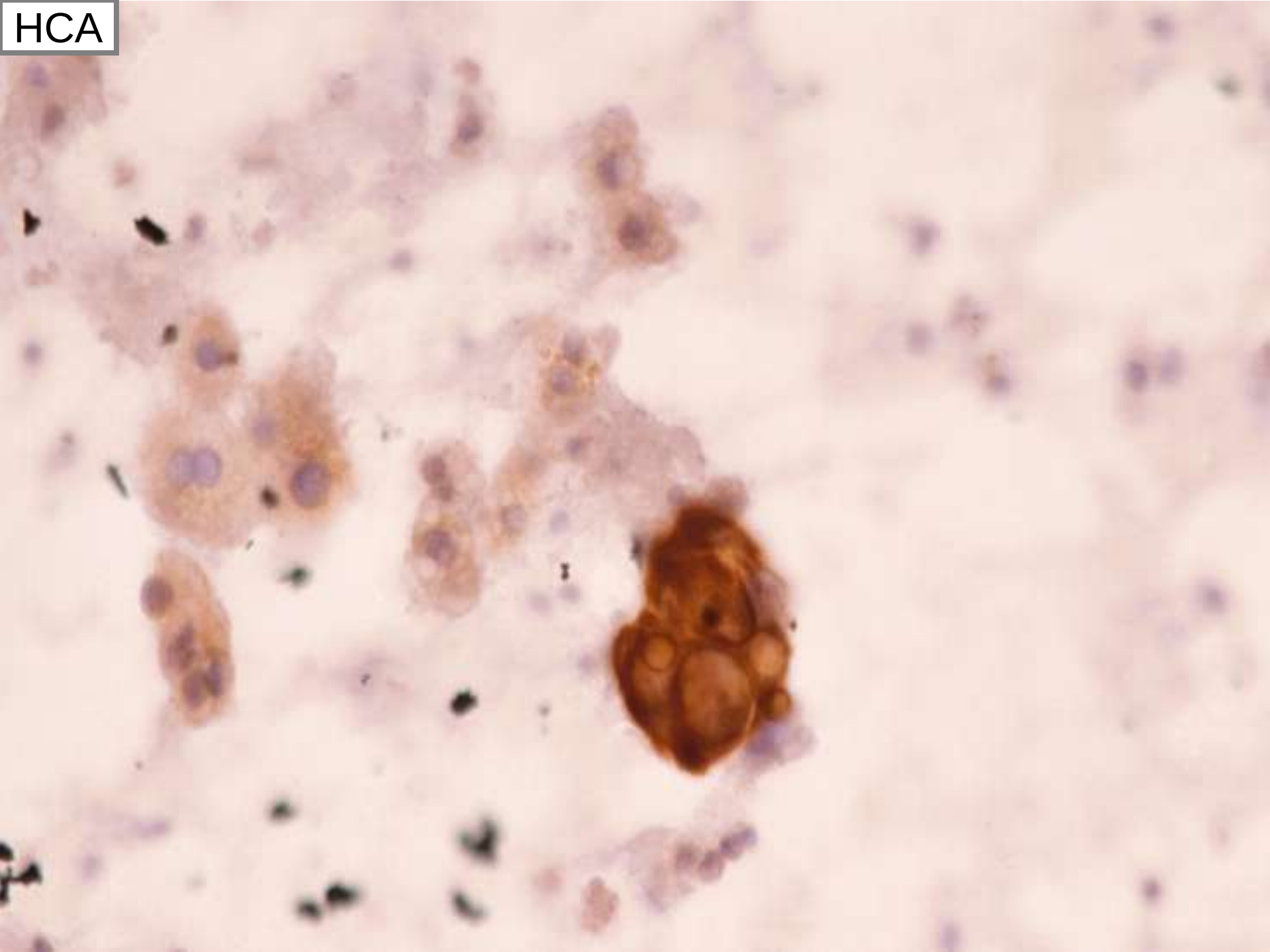


Hepatocellular Carcinoma vs Metastatic Adenocarcinoma

	CK7	HCA
Hepatocellular Ca	-	+
Adenocarcinoma	+	-

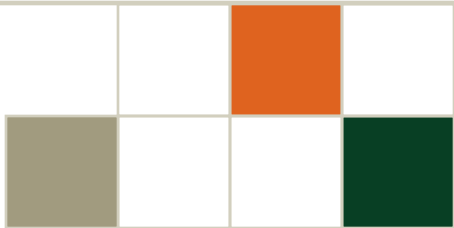


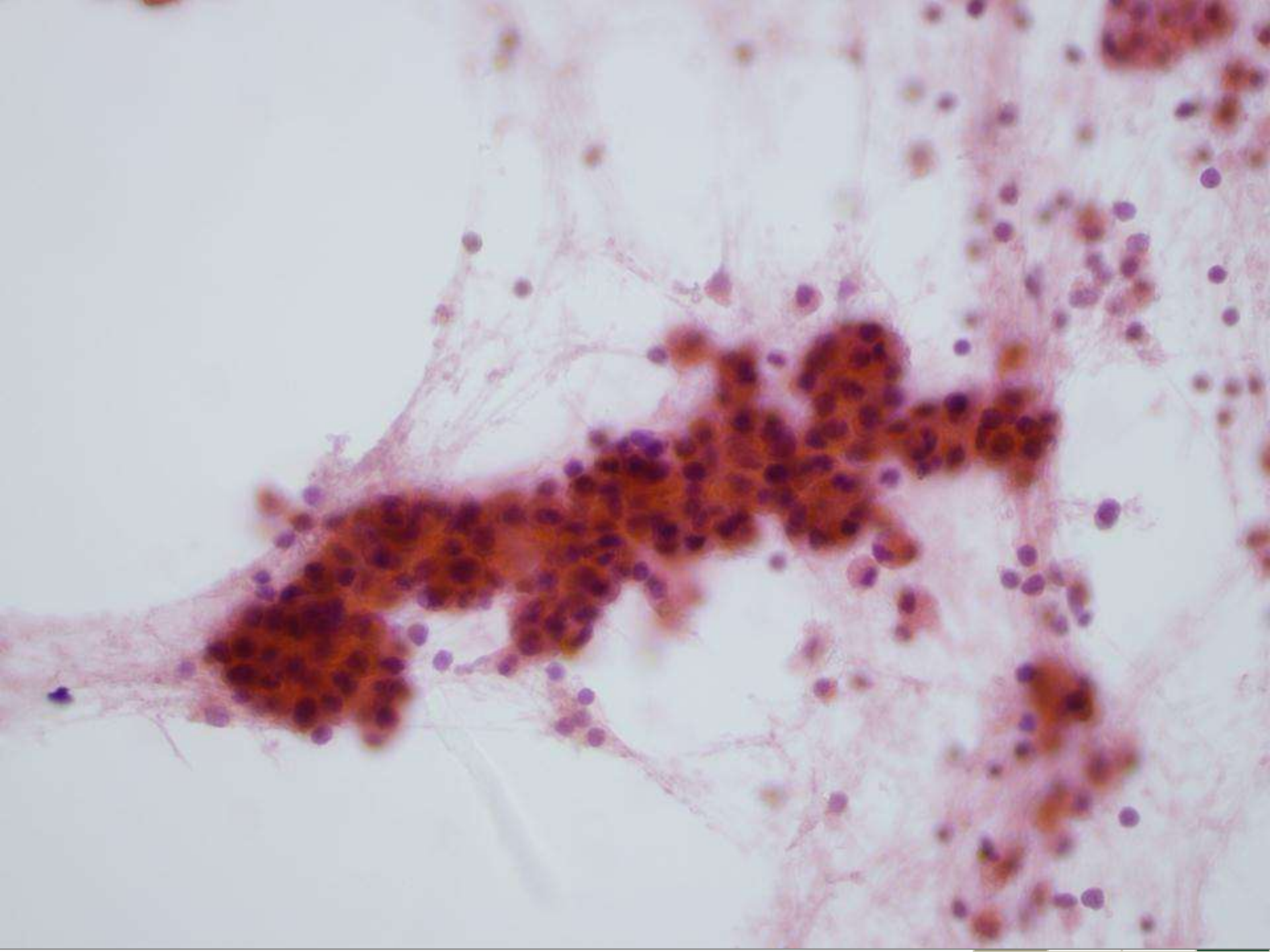



HCA

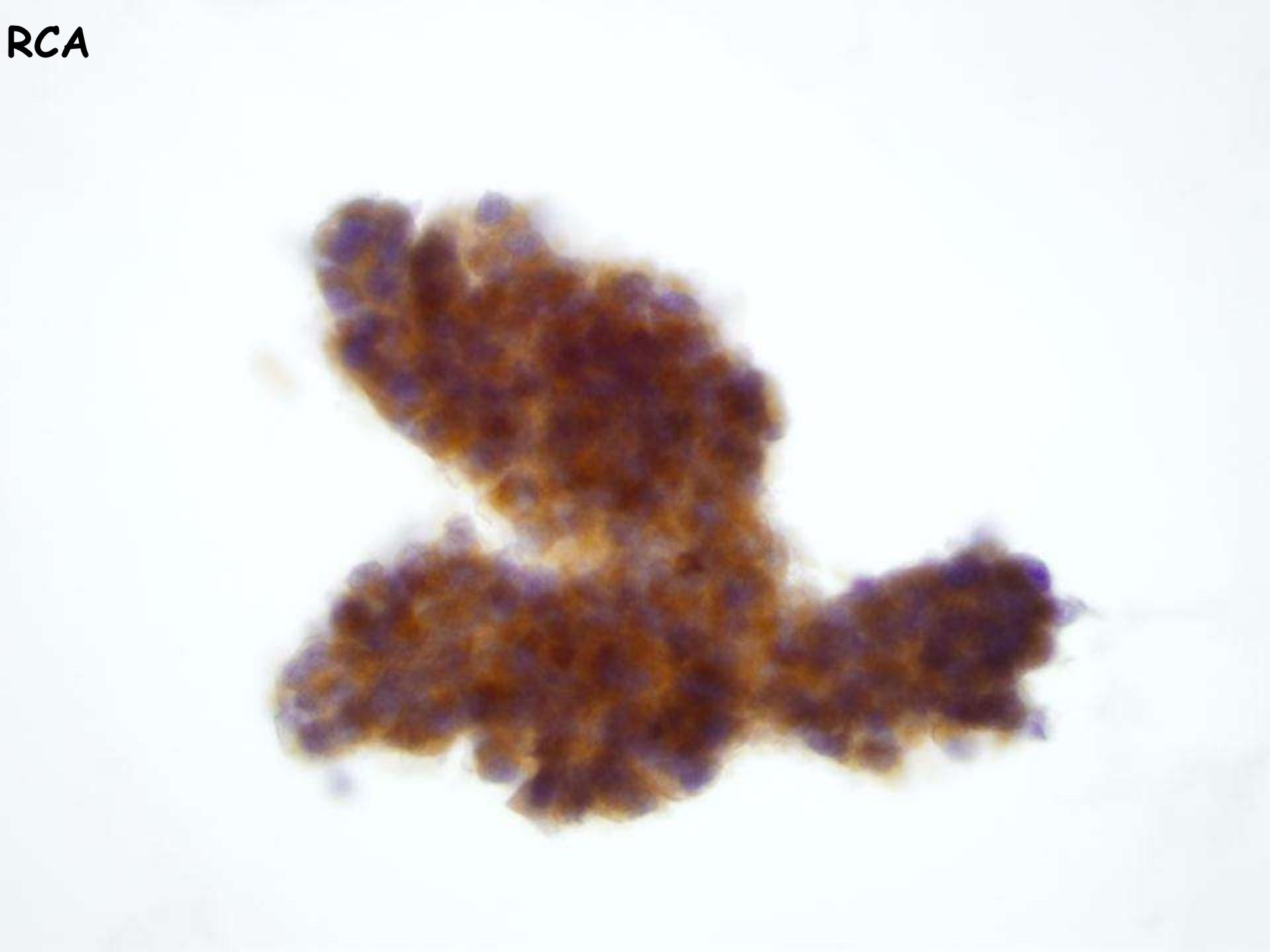
Hepatocellular Carcinoma Vs . Renal Cell Carcinoma

	HCA	RCA	EMA
Hepatocellular Ca.	+	-	-
Renal Cell Ca.	-	+	+





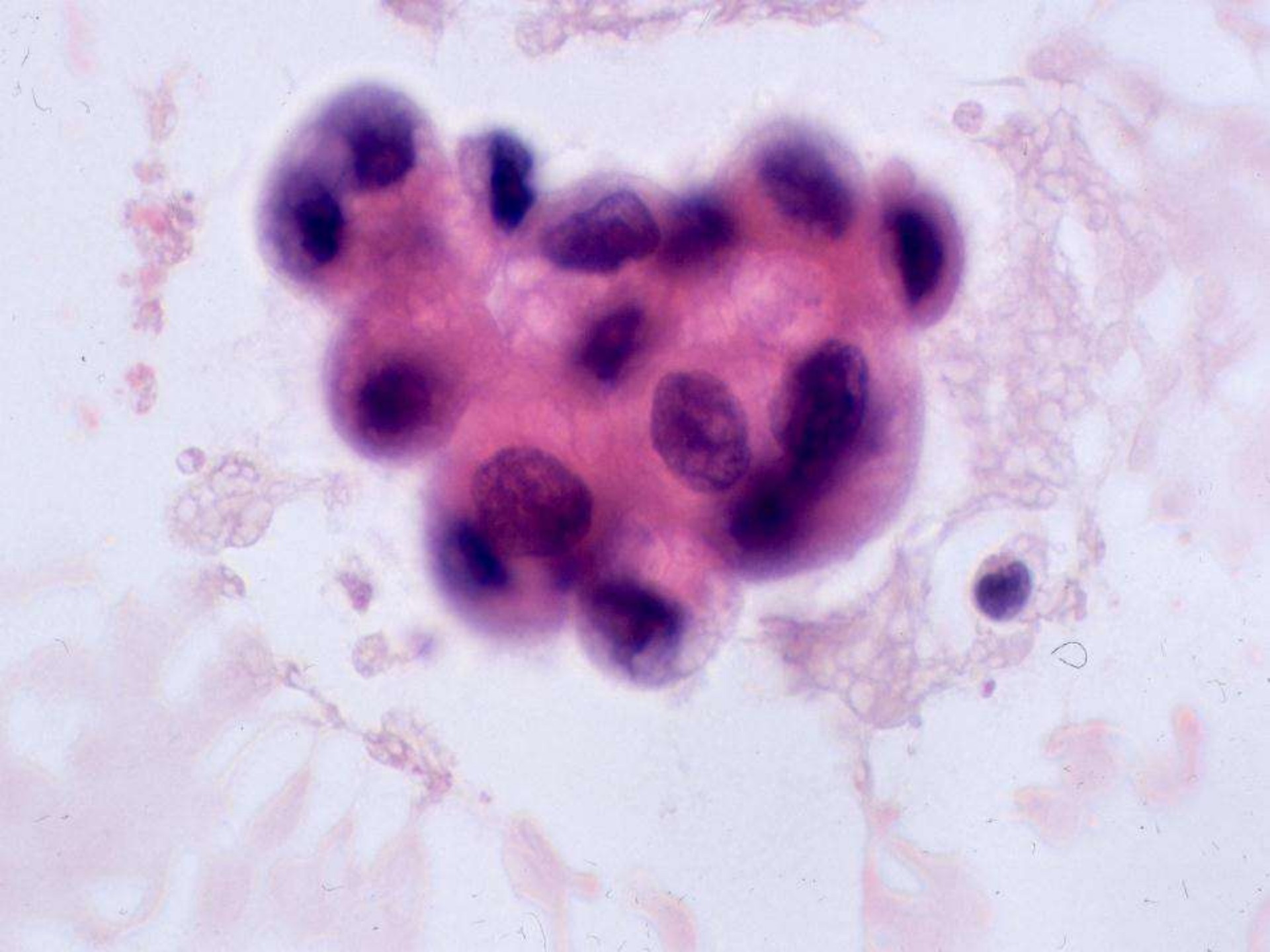
RCA

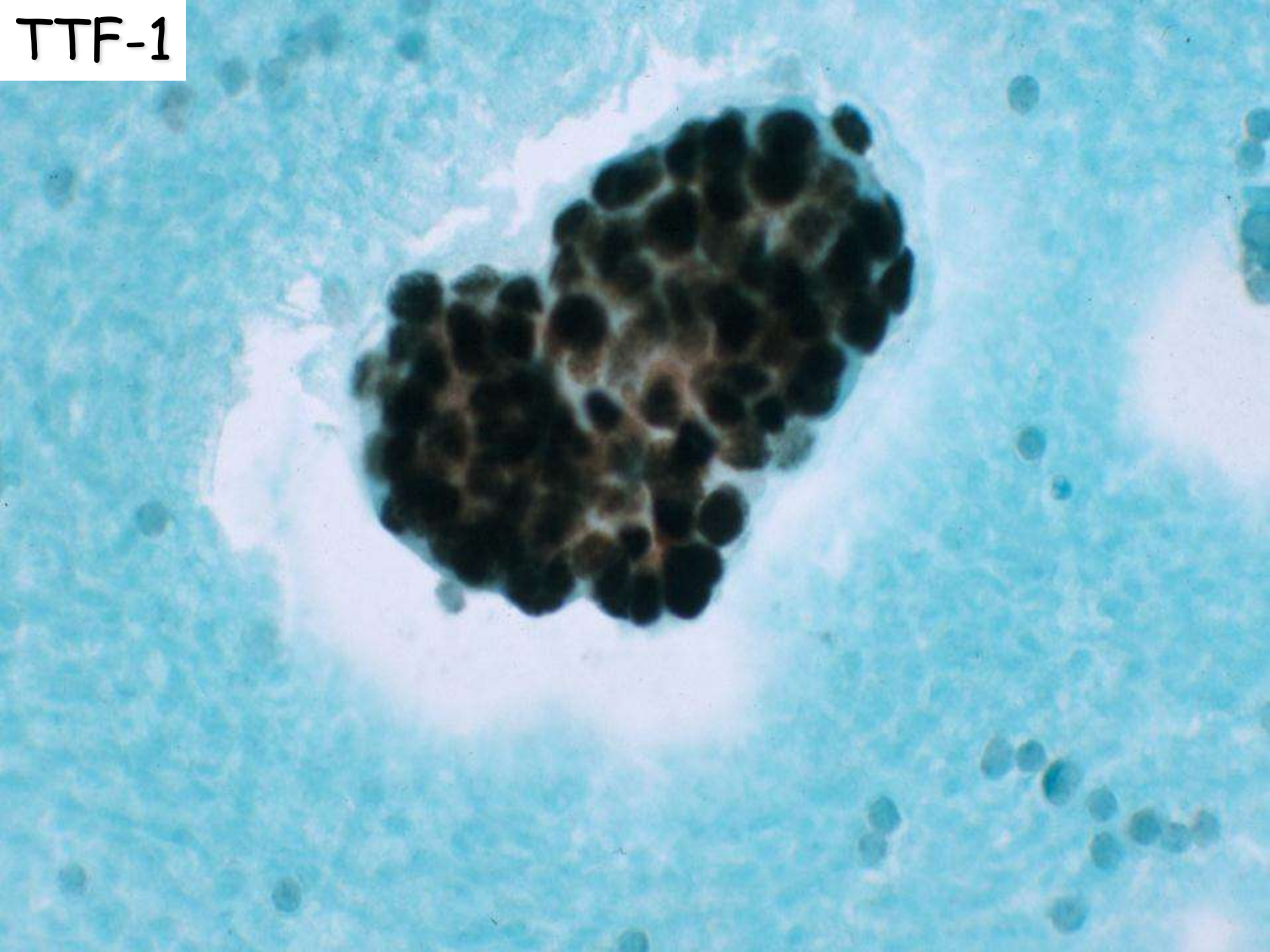


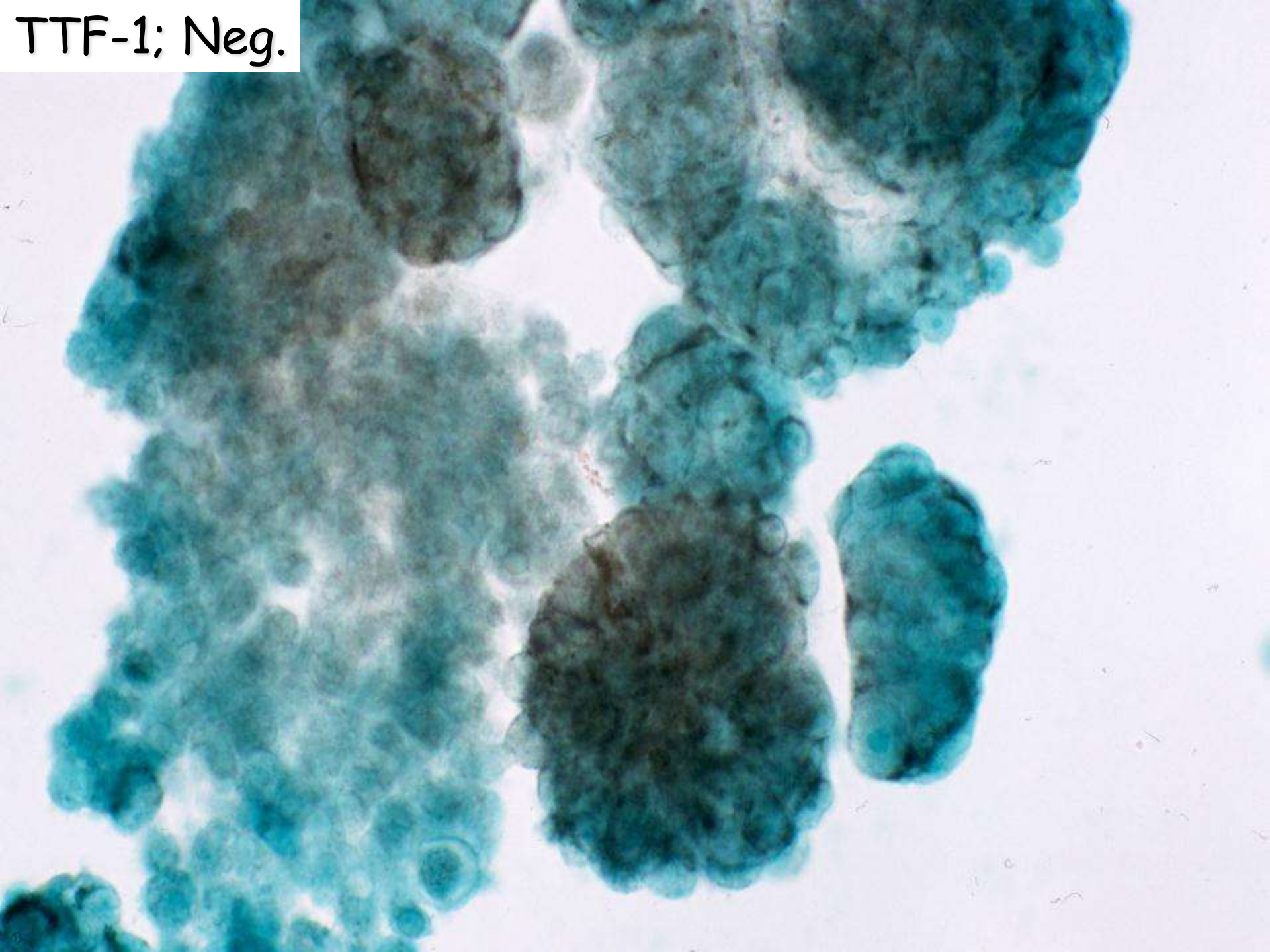
TTF-1 in Lung Adenoca.

- TTF-1 is useful for diagnosis of lung adenocarcinomas in effusions
- Only nuclear staining must be considered positive

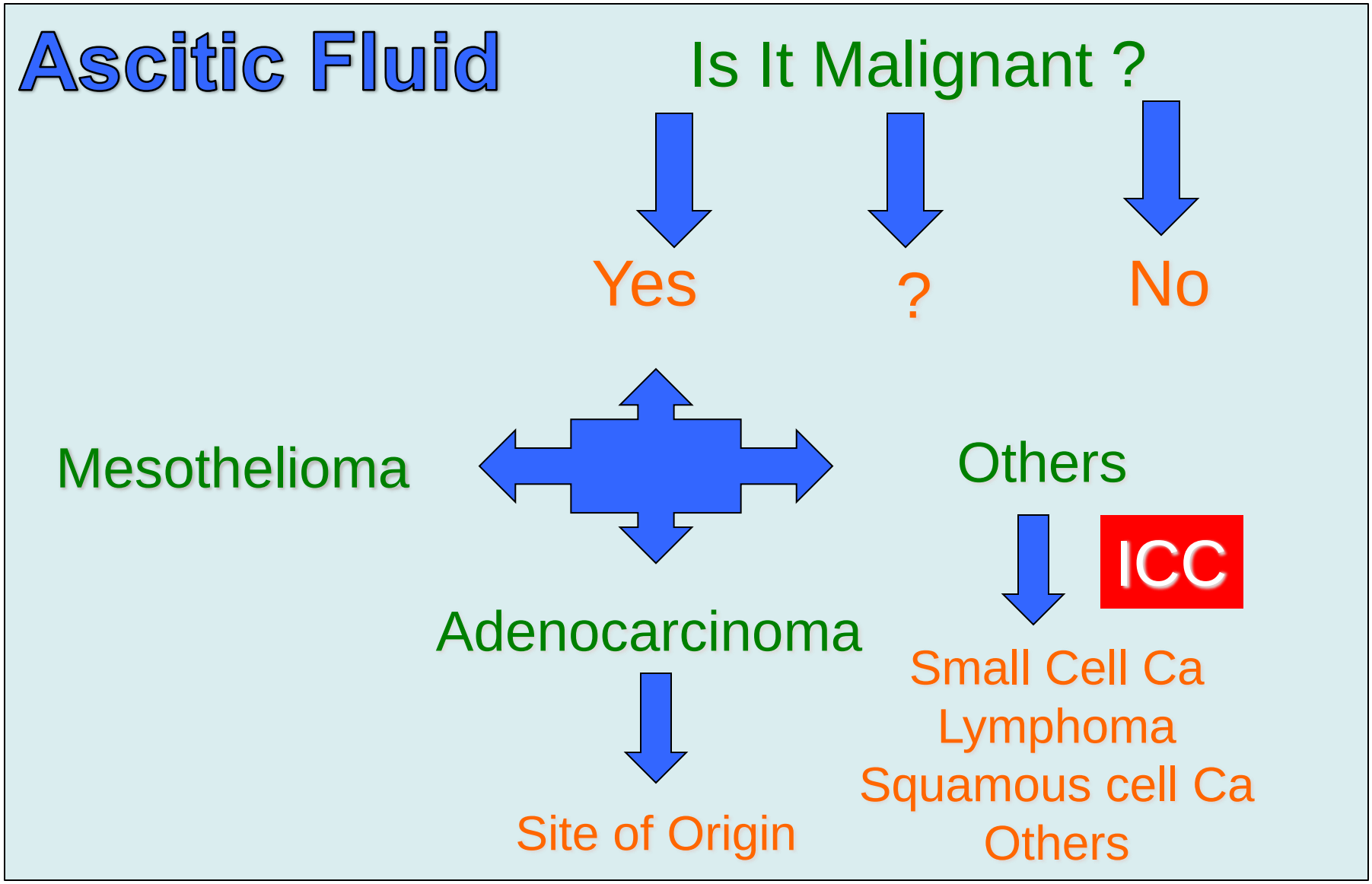
Cancer Cytopathol 96: 289-93, 2002




TTF-1


TTF-1; Neg.

Ascitic Fluid



Small Cell Carcinoma in Ascitic Fluid

Low Power

- Tight cell balls
- Indian file/chain
- Isolated cells may be overlooked

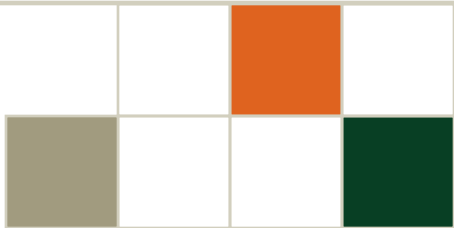
High Power

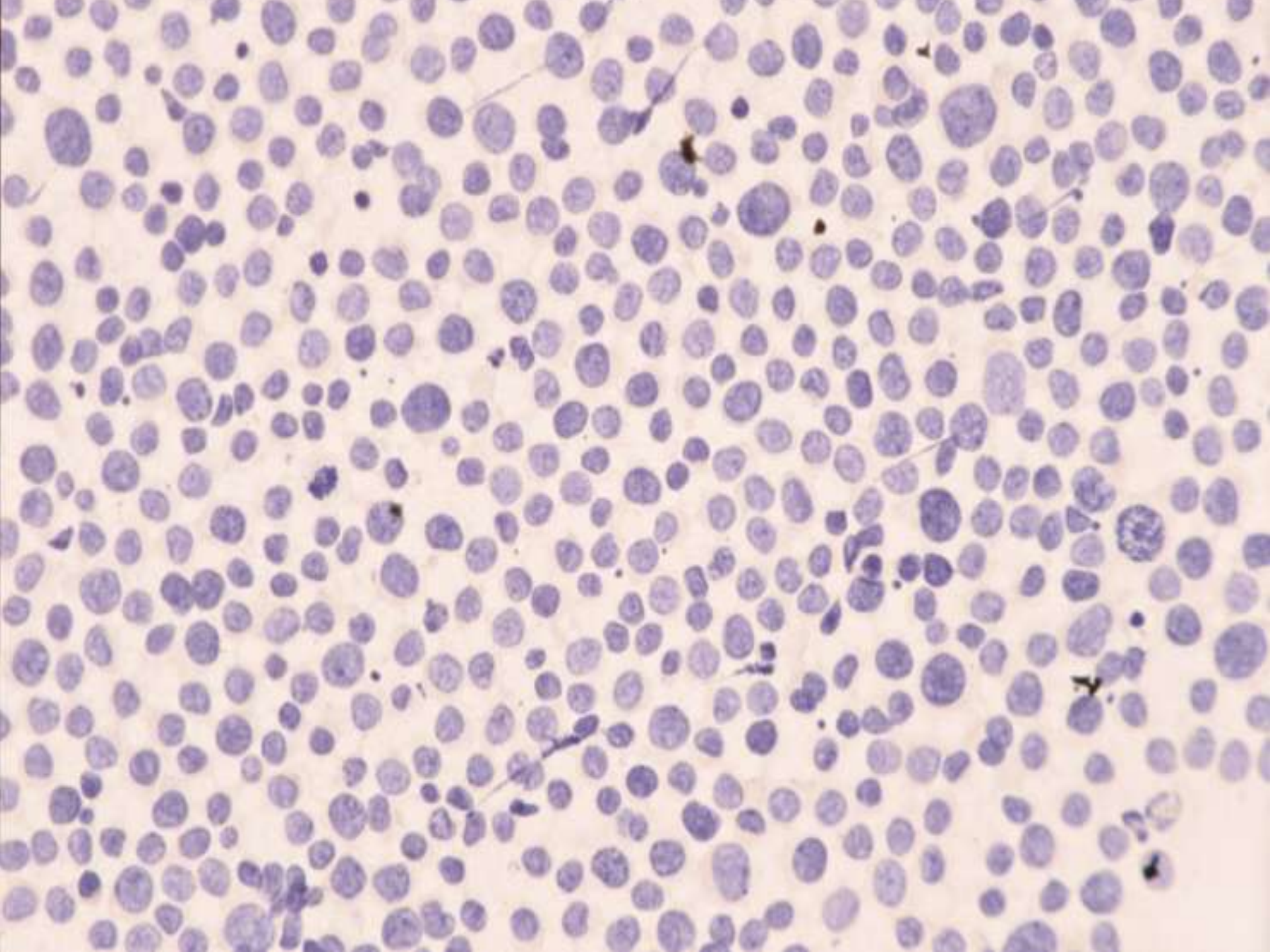
- Nuclear molding
- Coarse chromatin
- Wrinkled nuclear membrane
- Occasional cells with nucleoli

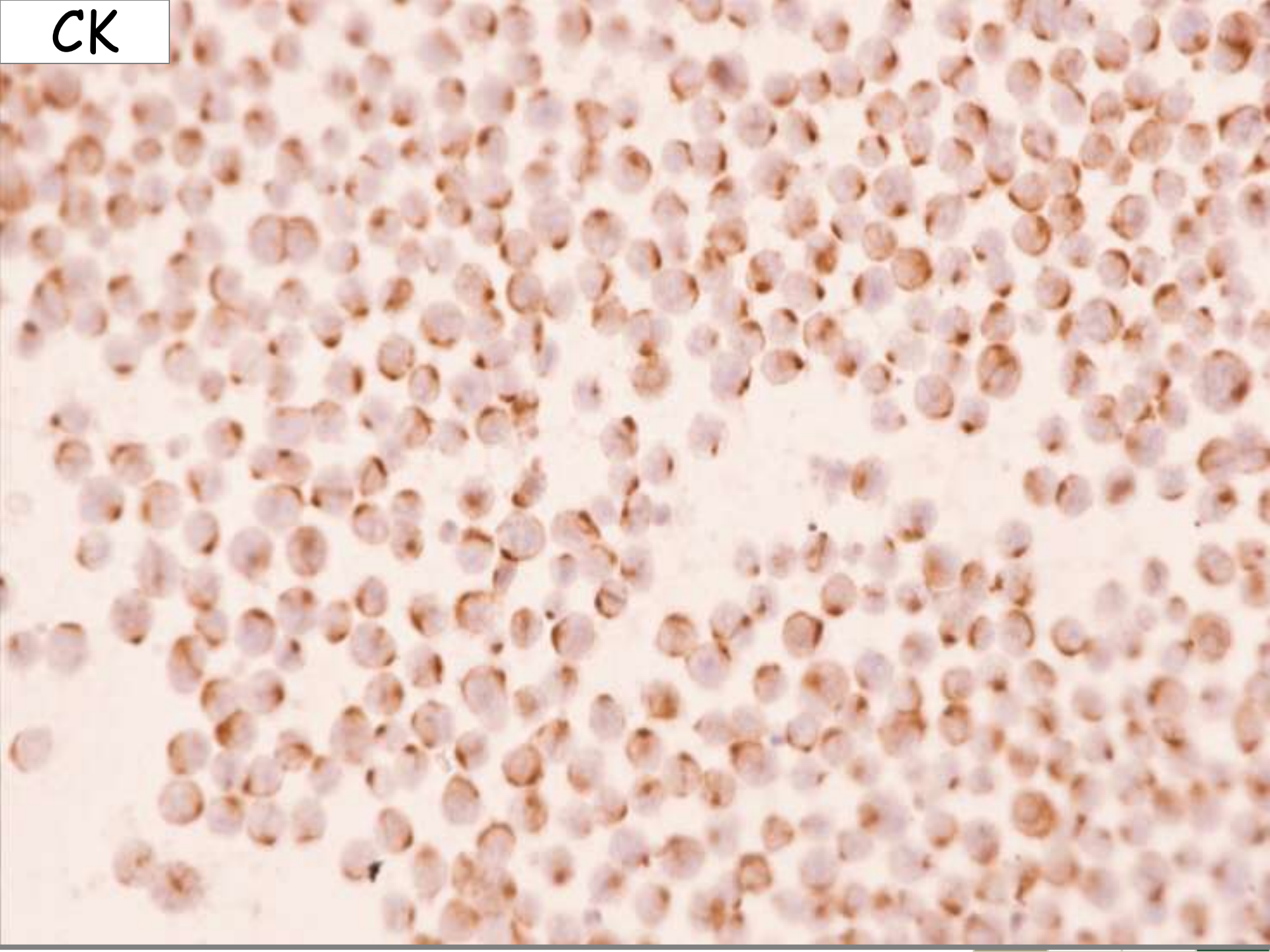
Lung Carcinoma

Non- Small vs Small Cell

	CK	SYN	CHR	TTF-1
Non-Small	+	-/+	-	+/-
Small Cell	+(dot)	+	+/-	+






CK

Small Cell Carcinoma in Ascitic Fluid

Differential Diagnosis

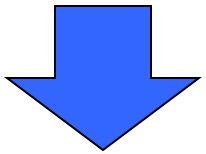
- Malignant lymphoma
- “Small blue cell tumors”

ICC in Differential Diagnosis of Small Cell Malignancies

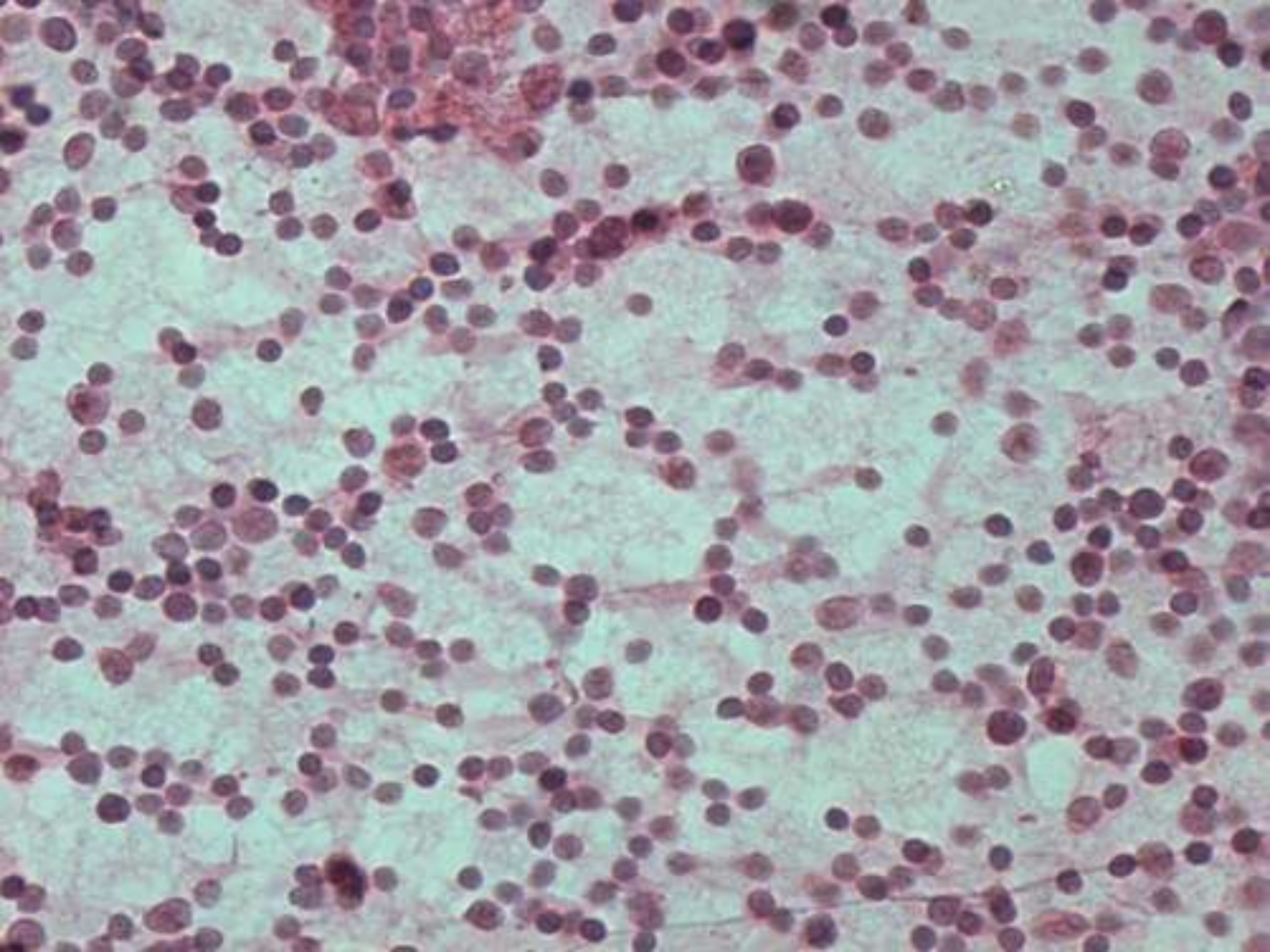
	LCA	KER	CHR	DES	NB
Small Cell Ca	-	+	+/-	-	-
Lymphoma	+	-	-	-	-
Rhabdomyosarcoma	-	-	-	+	-
Neuroblastoma	-	-	-	-	+

Malignant Lymphoma in Ascitic Fluid

Low Power



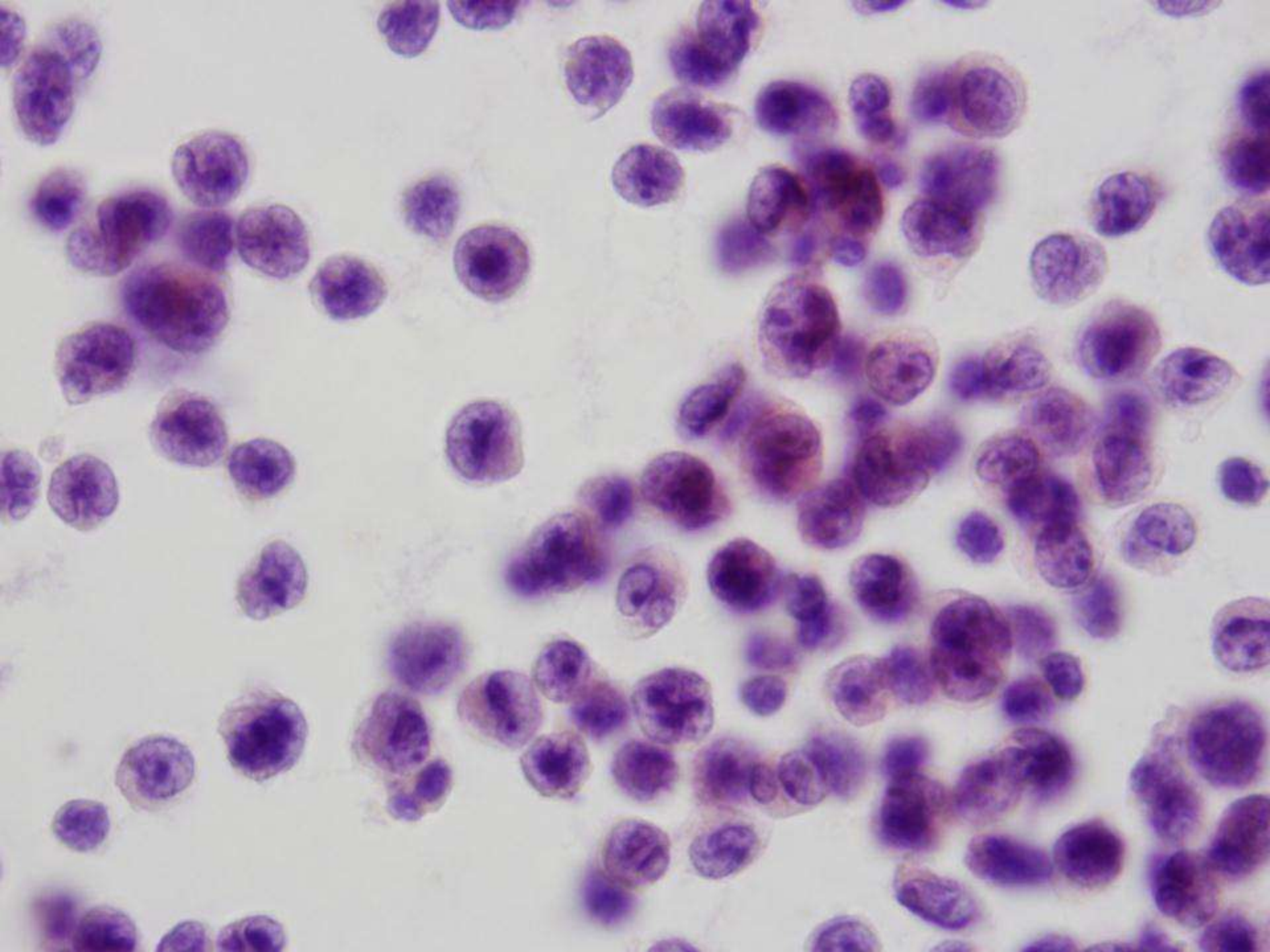
Isolated Cells

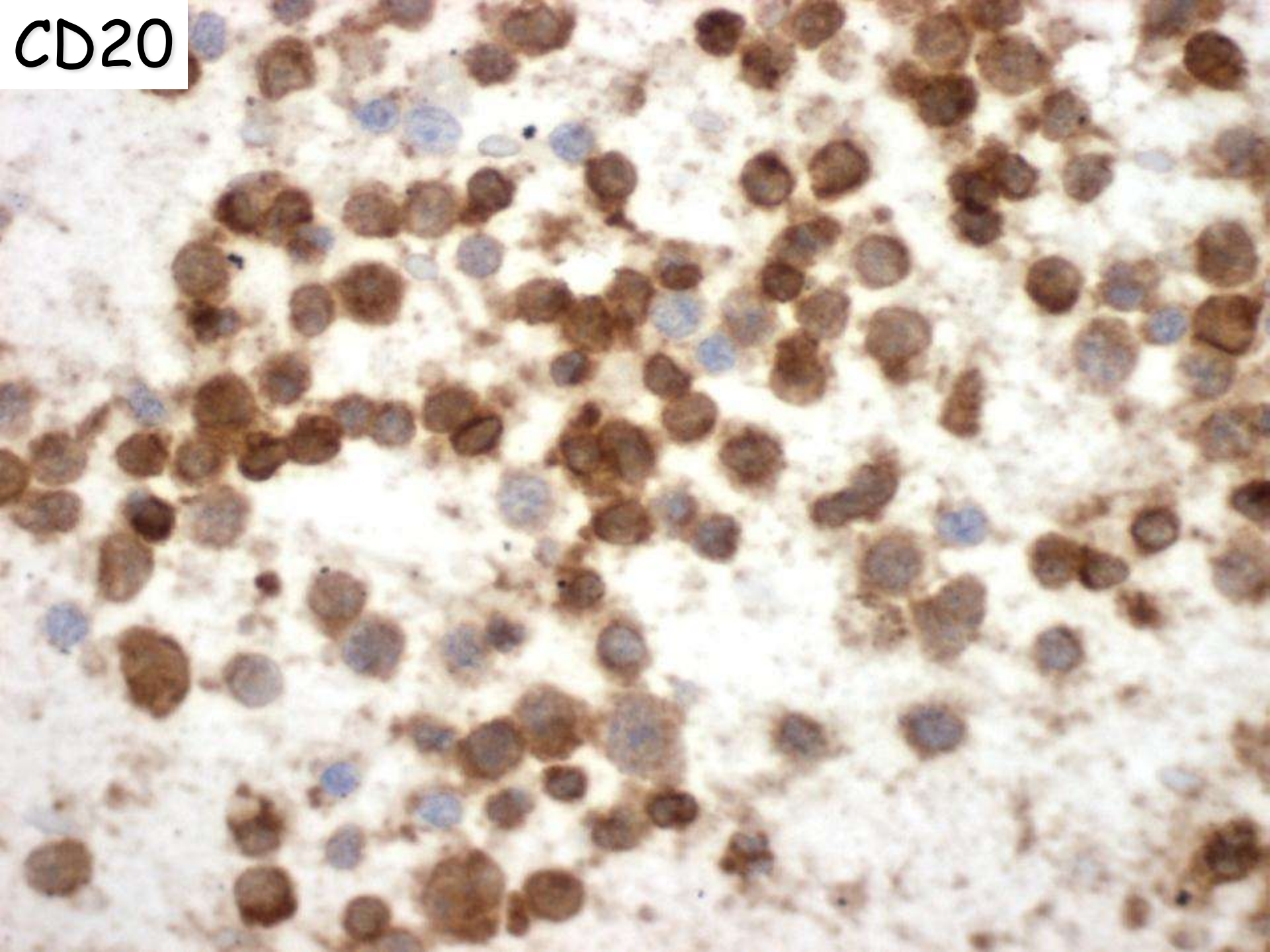


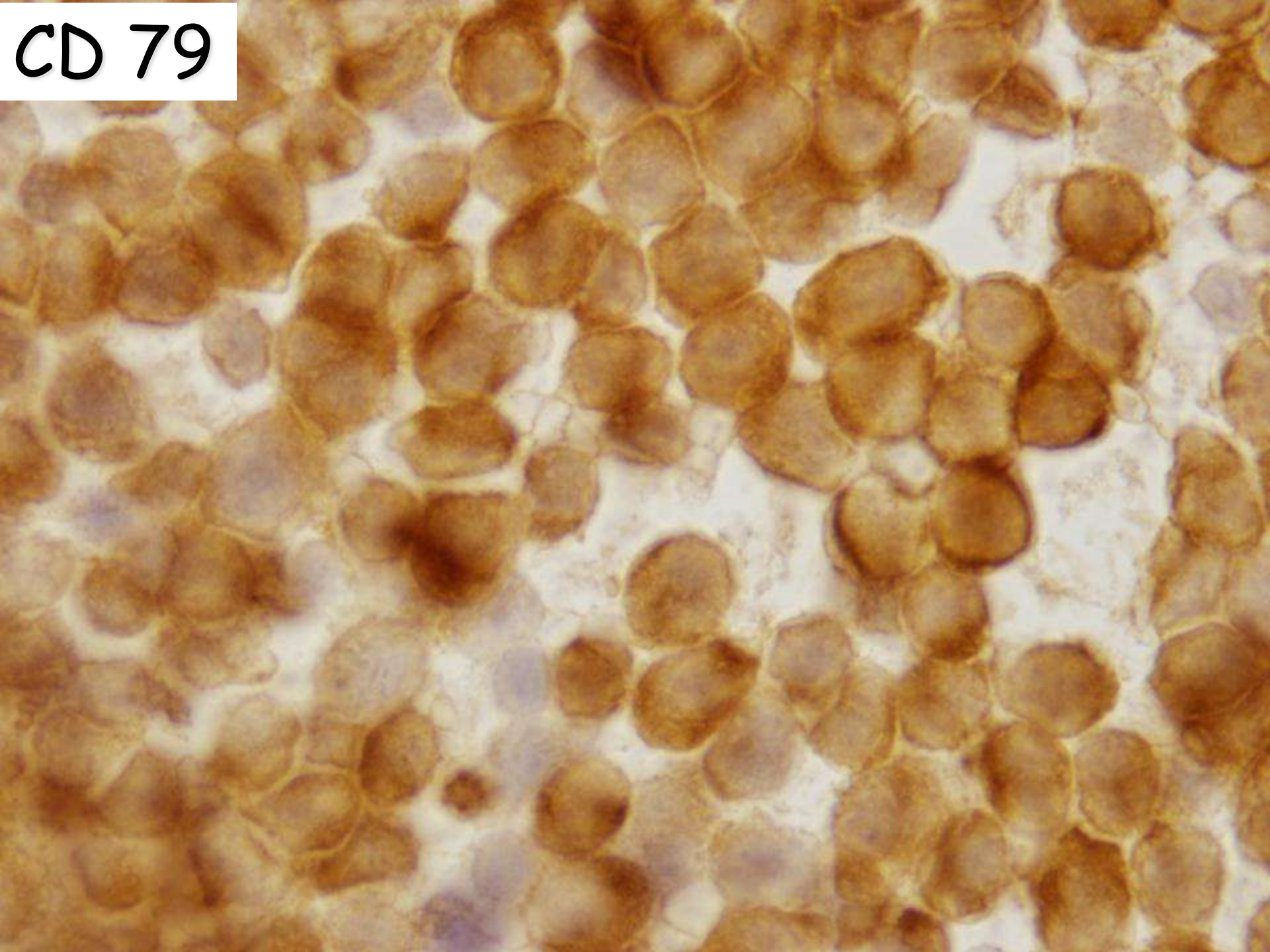
Malignant Lymphoma in Ascitic Fluid

High Power

- Nuclear variation in size and shape
- Nuclear indentation/convolution
- Vesicular nuclei with prominent nucleoli
- Individual cell necrosis (apoptosis)
- Scant, basophilic cytoplasm, rarely well preserved

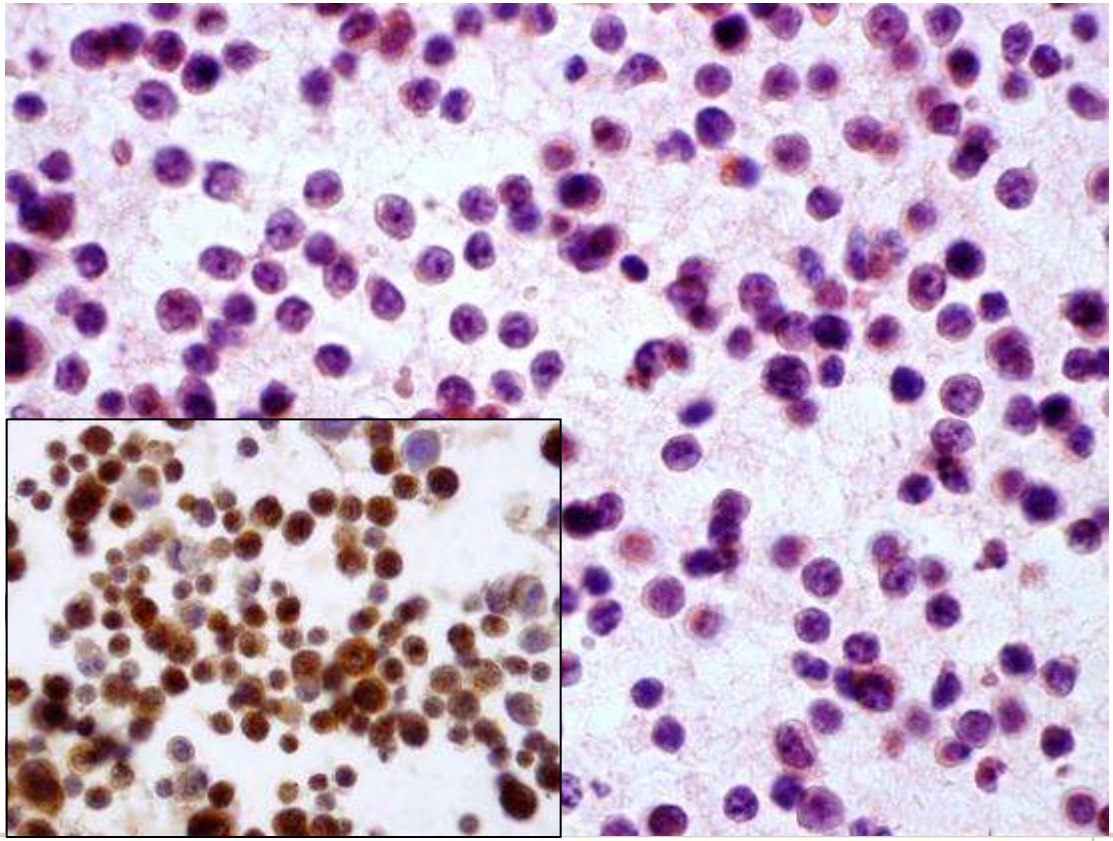



CD20


CD 79

Malignant Lymphoma in Ascitic Fluid

HHV8 associated lymphoma



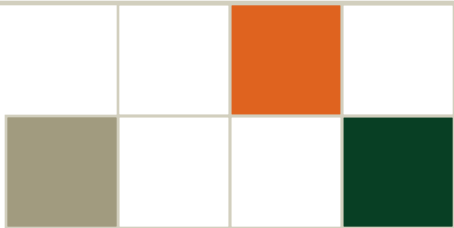
Lymphoma vs. Carcinoma vs. Germinoma vs. Melanoma

Favor Lymphoma

- Only isolated cells
- Nuclear clefts
- Apoptotic cells

Immunocytochemistry

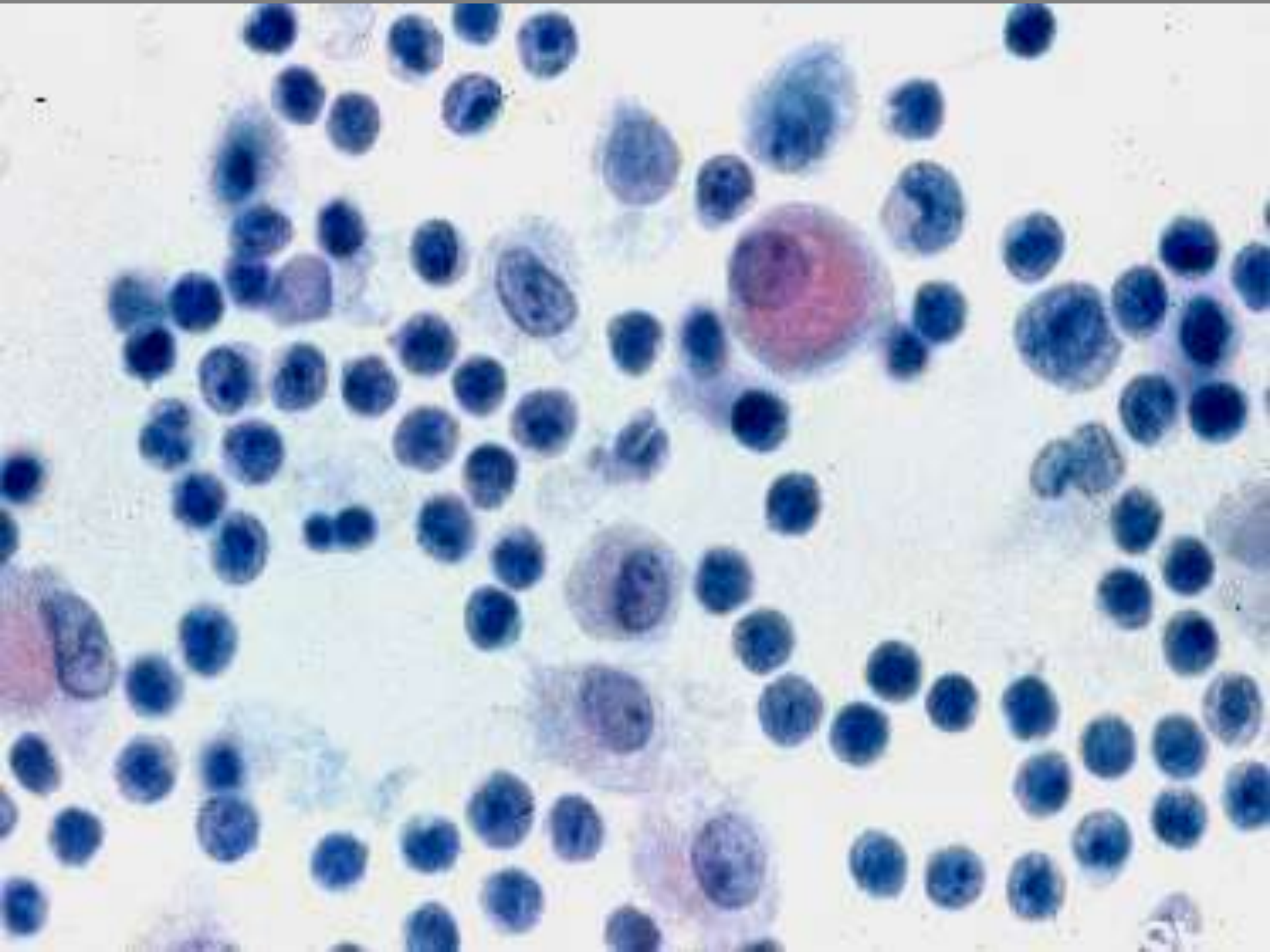
- LCA (+)
- Keratin (-)
- PLAP (-)
- S100 (-)



Small “Mature-Looking” Lymphocytes in Effusions

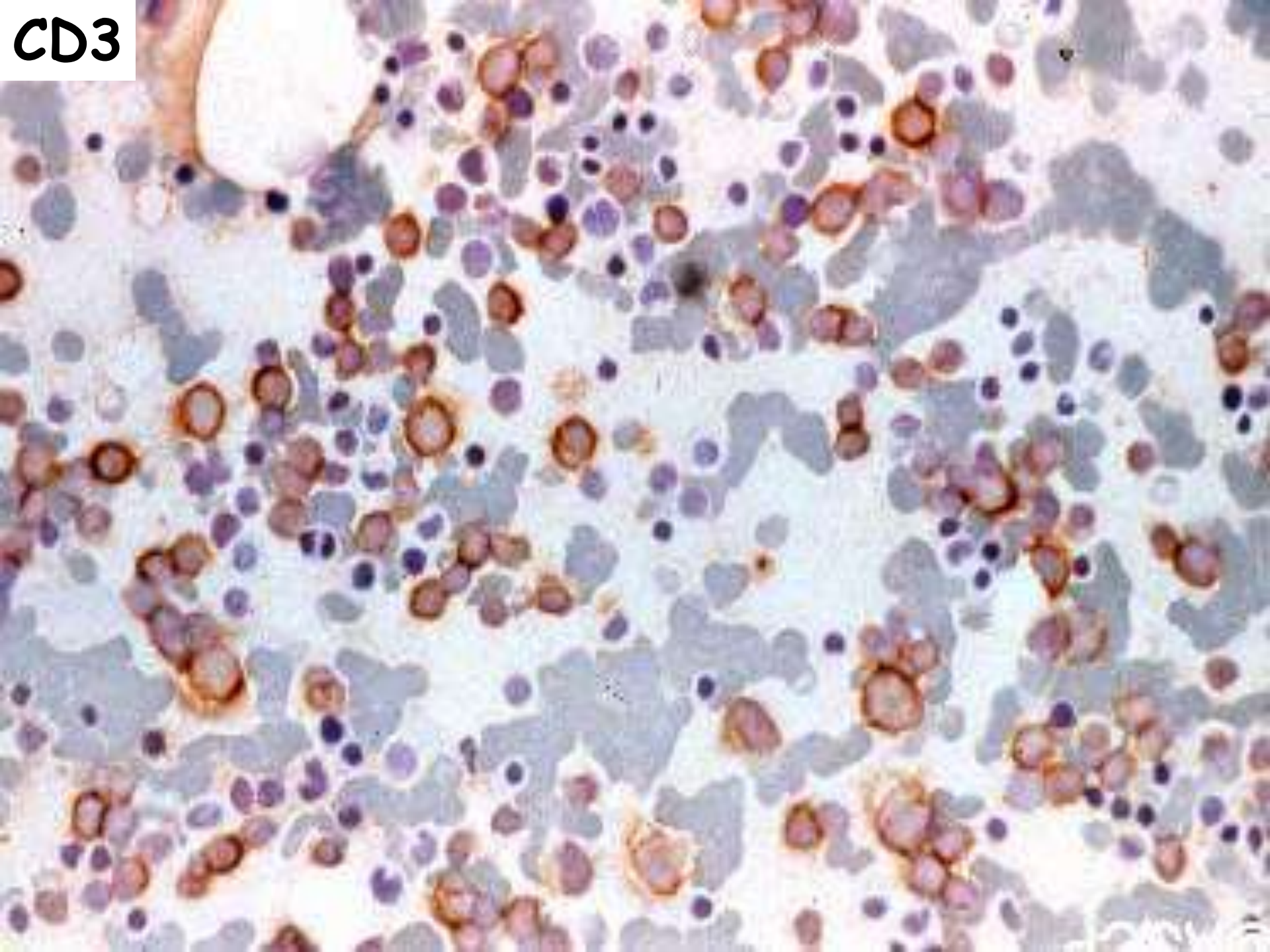
Differential Diagnosis

- Chronic pleuritis (TB)
- Small cell lymphomas
- Chronic lymphocytic leukemia
- Waldenstrom’s macroglobulinemia



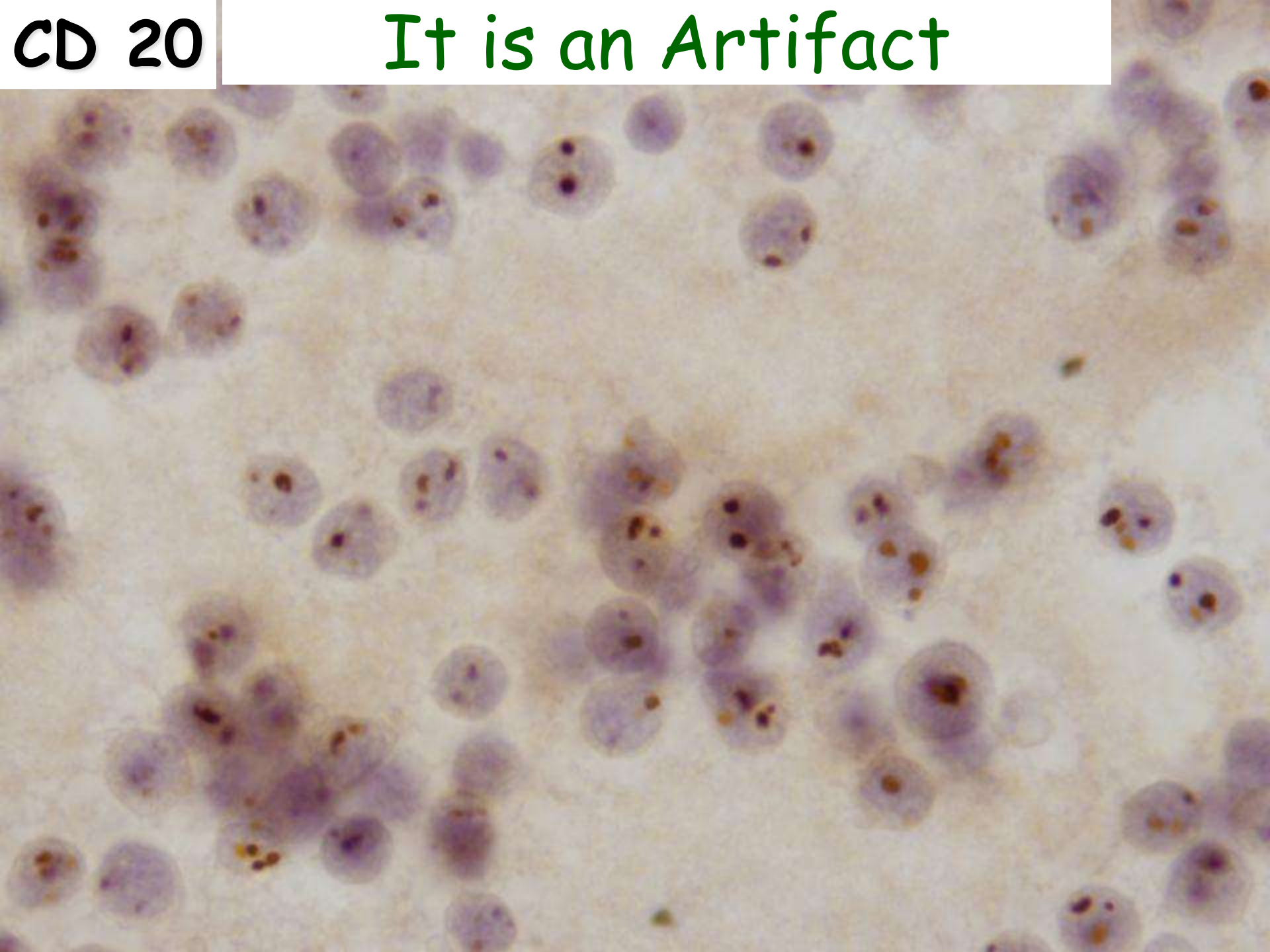
Lymphocytes in Effusions

Effusion Type	CD45 (LCA)	CD20 (B-cell)	CD3 (T-cell)
Benign	+	-	+
Malignant	+	+	-


CD3

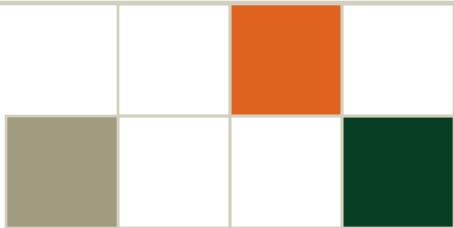
CD 20

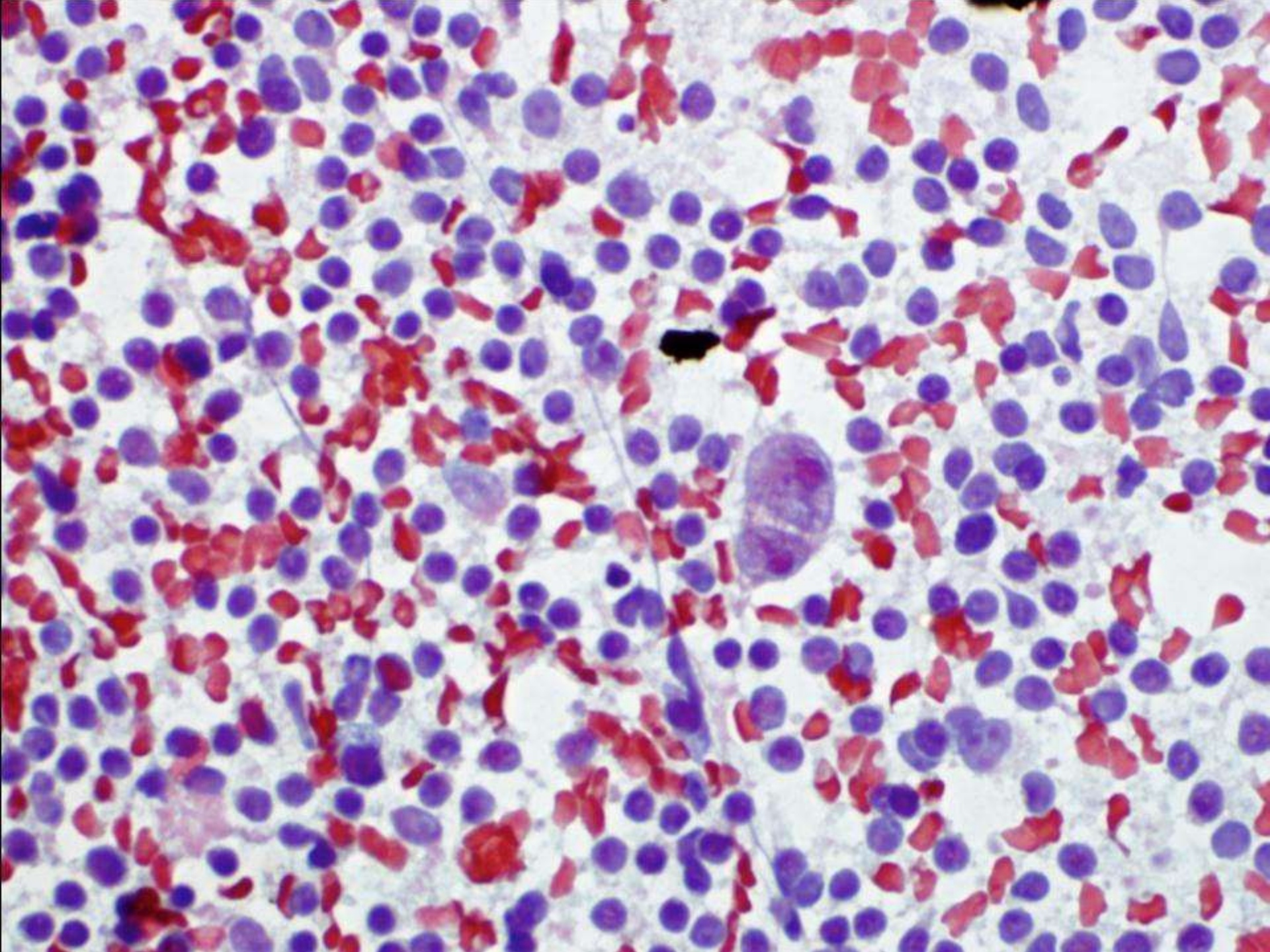
It is an Artifact



Ancillary Techniques to Rule Out Malignant Lymphoma

- Flow cytometry
- Gene rearrangement





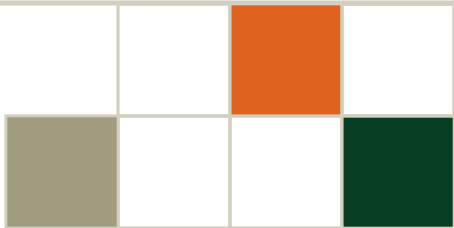
Squamous Cell Carcinomas are Rare in Effusions

Site of Origin

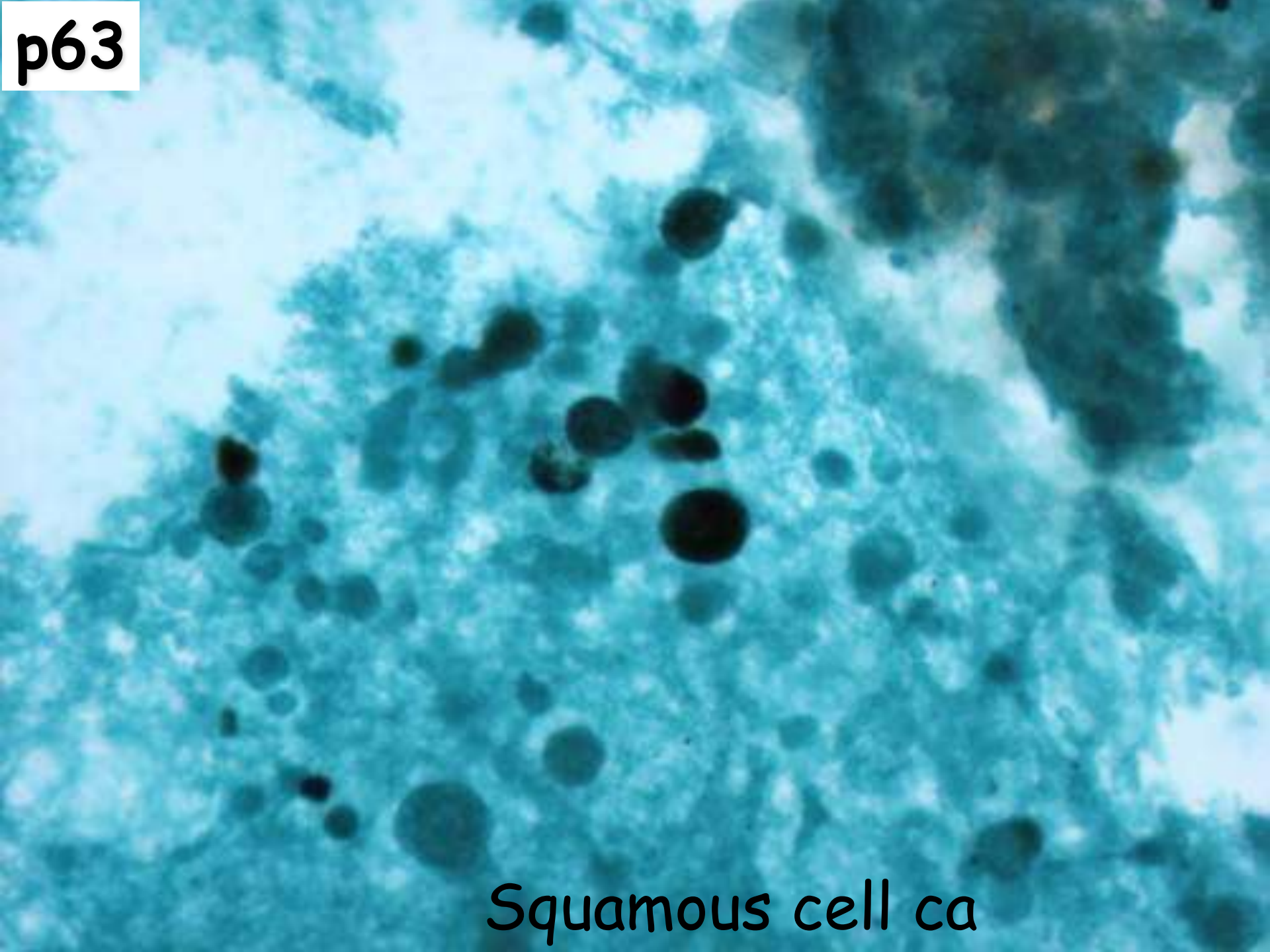
- Lung
- Cervix
- Skin
- Esophagus

Diagnostic Difficulties

- Tumor cells do not shed
- May be mistaken for poorly differentiated adenocarcinomas or mesotheliomas




Squamous cell ca


p63
Squamous cell ca

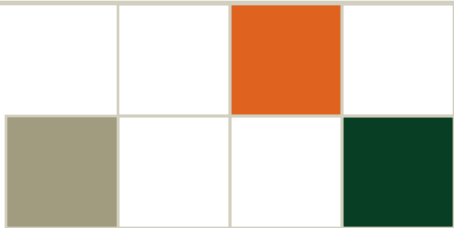
Remember !

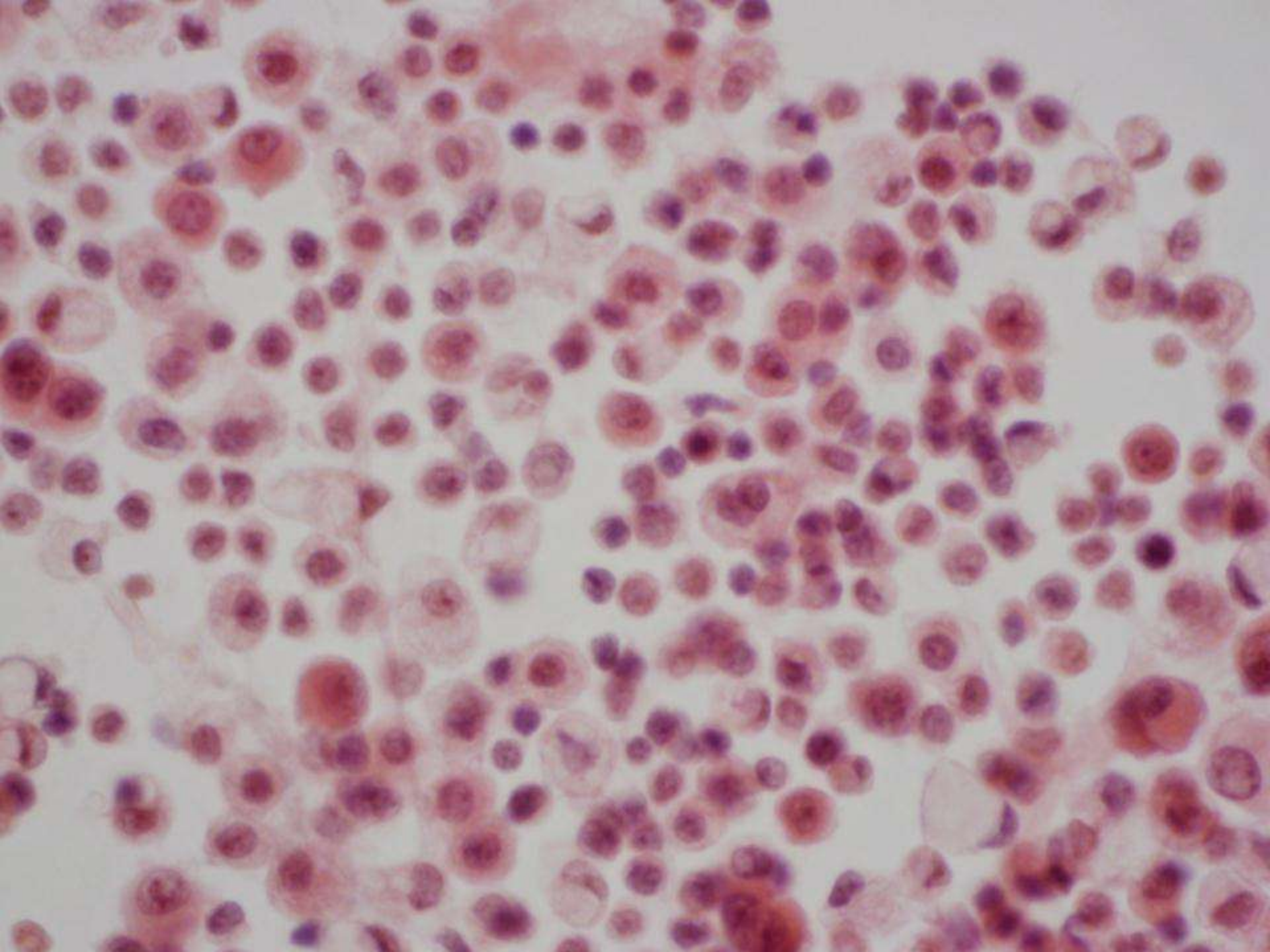
- Squamous carcinoma cells are usually overlooked in body cavity fluid cytology - Only few cells shed
- They might be confused with necrotic /degenerative mesothelial cells
- p63 and p40 are very helpful to detect squamous cells

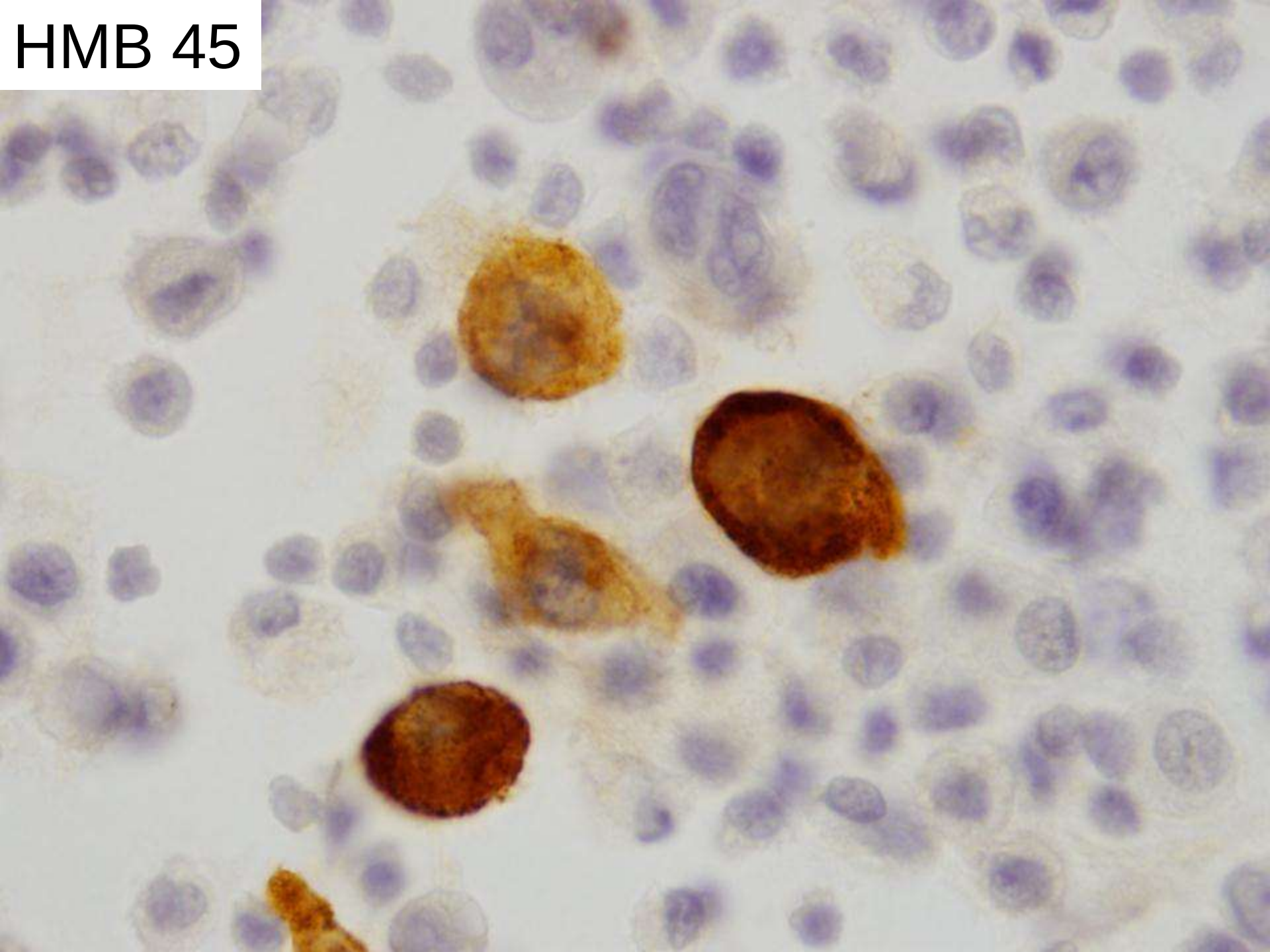
Cancer Cytopathol 2009; 117: 46-50

Carcinoma vs Melanoma

	CK	S100	HMB45
Carcinoma	+	-/+	-
Melanoma	-	+	+






HMB 45

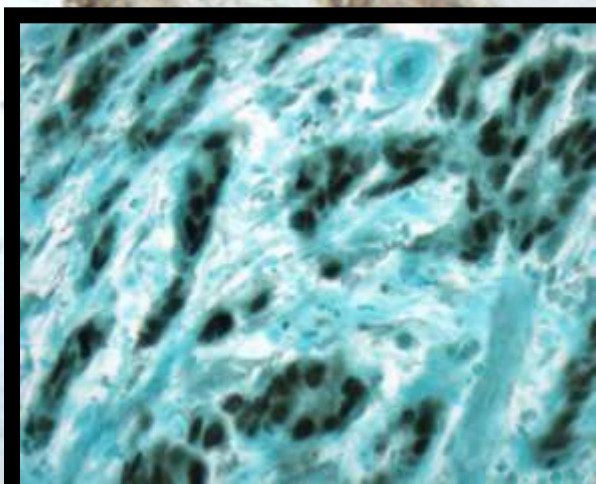
Melanoma Markers

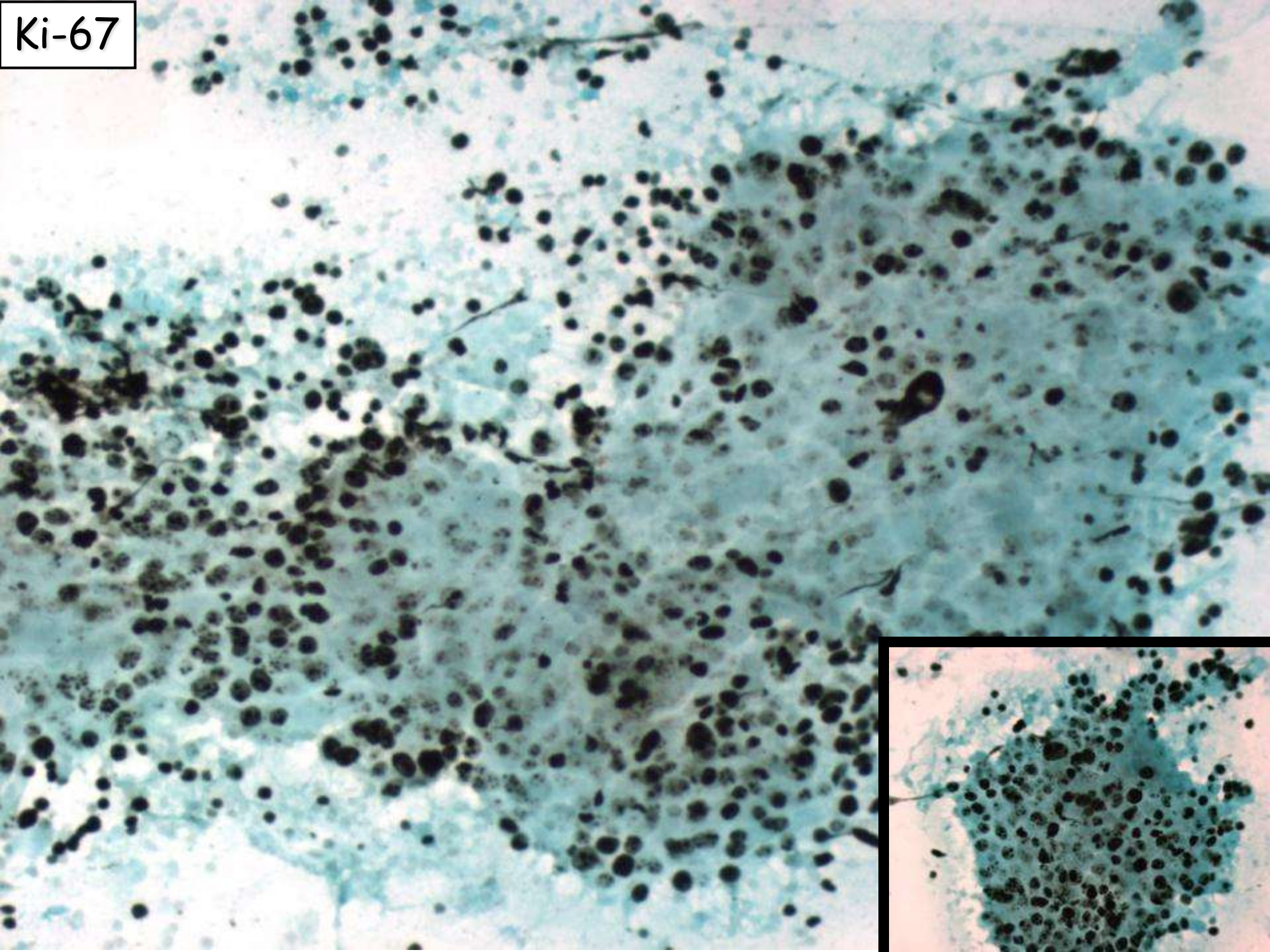
- S100 Protein +++
- HMB-45 +++
- Melan-A ++
- Tyrosinase ++

ICC in Diagnostic Cytology
Applications

- Tumor Diagnosis/Classification
- Prognostic/Predictor Markers
- Target Therapy

ER-1D5




Ki-67

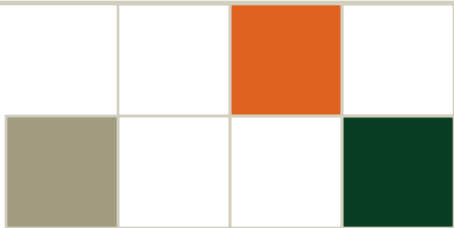
Detection of HER2 in cytology

ICC, FISH, CISH

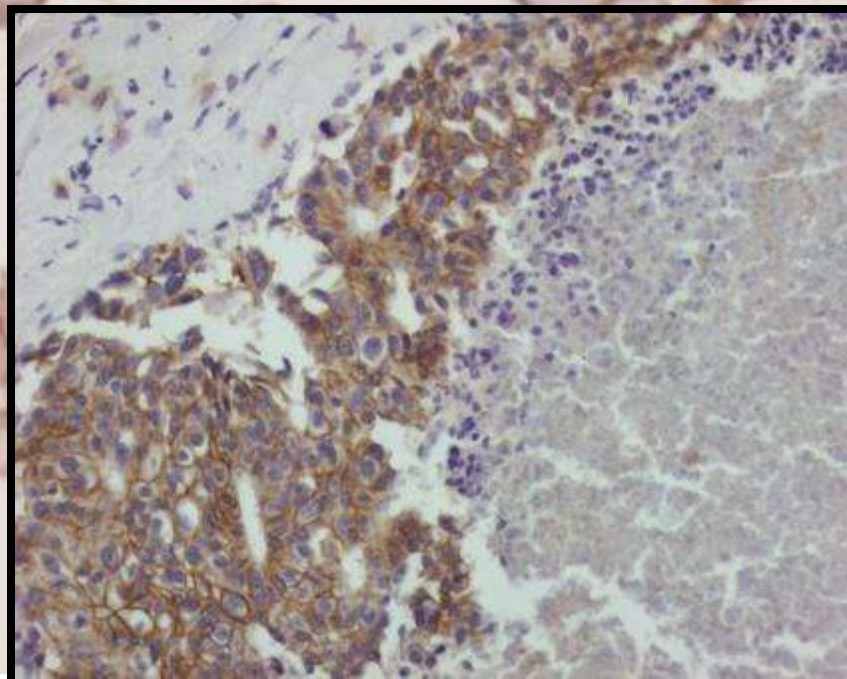
Predictive Value

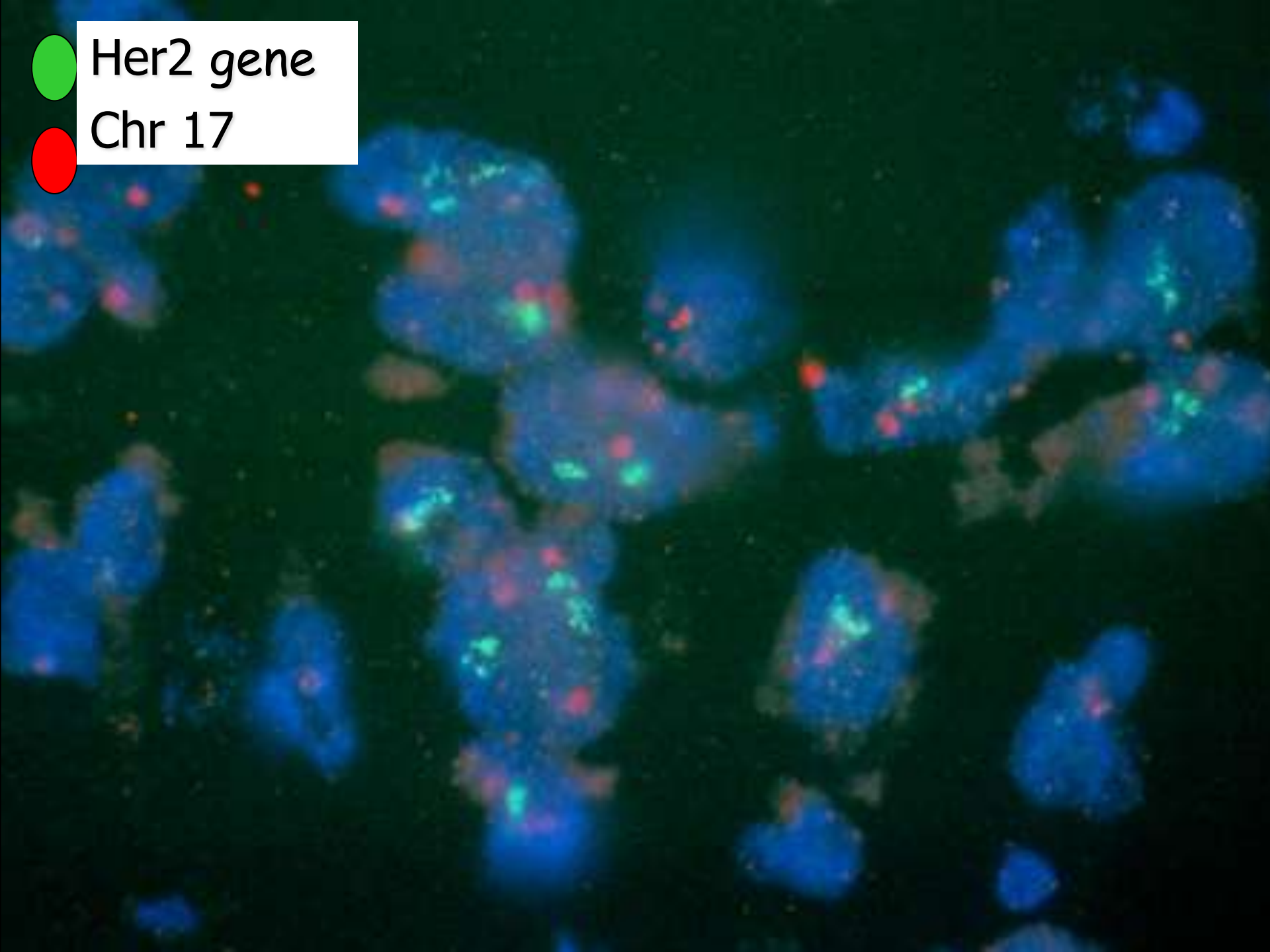
NOT standard of Care for Breast CA

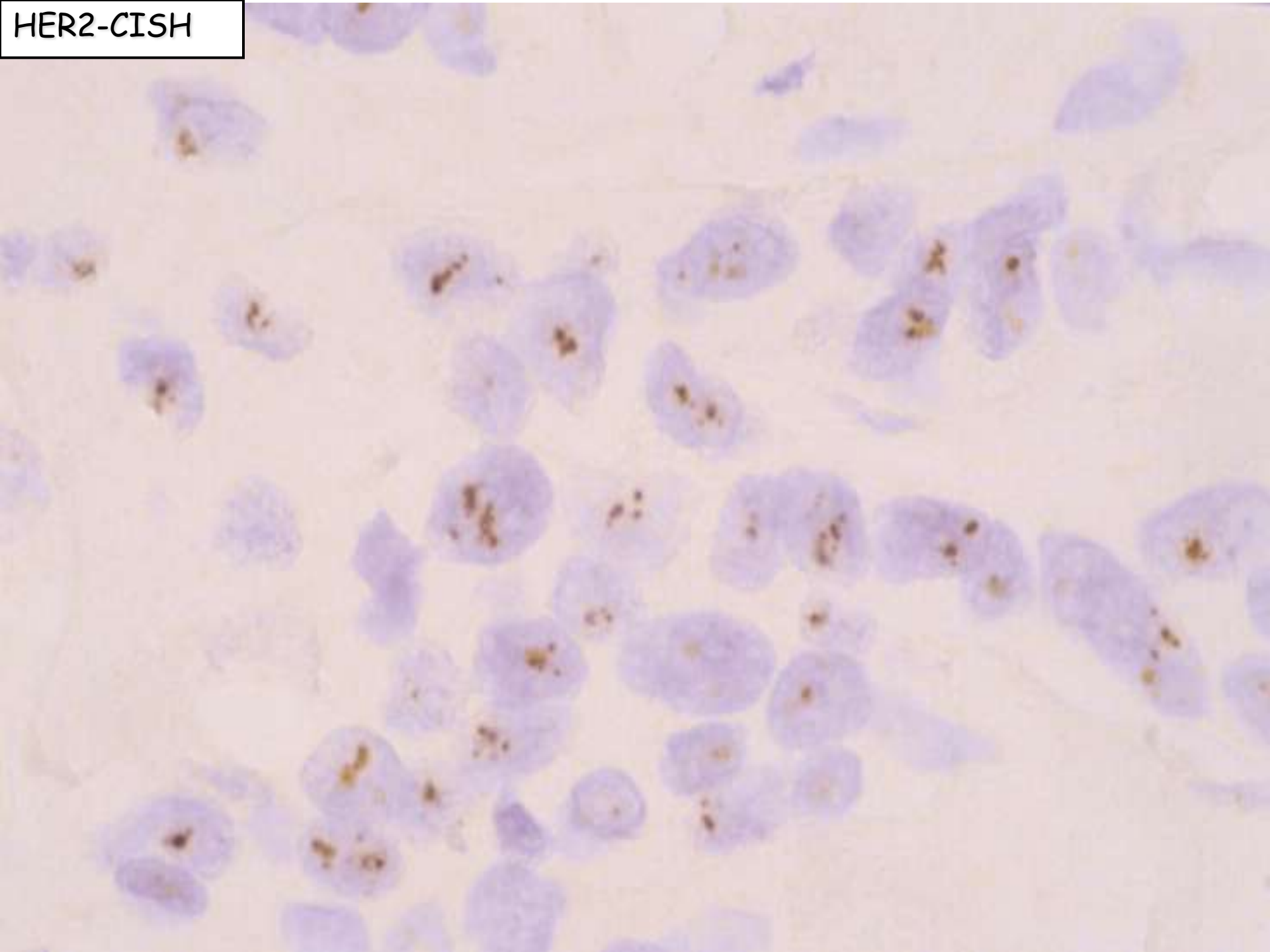
Diagn Cytopathol 1994; 11:262-265

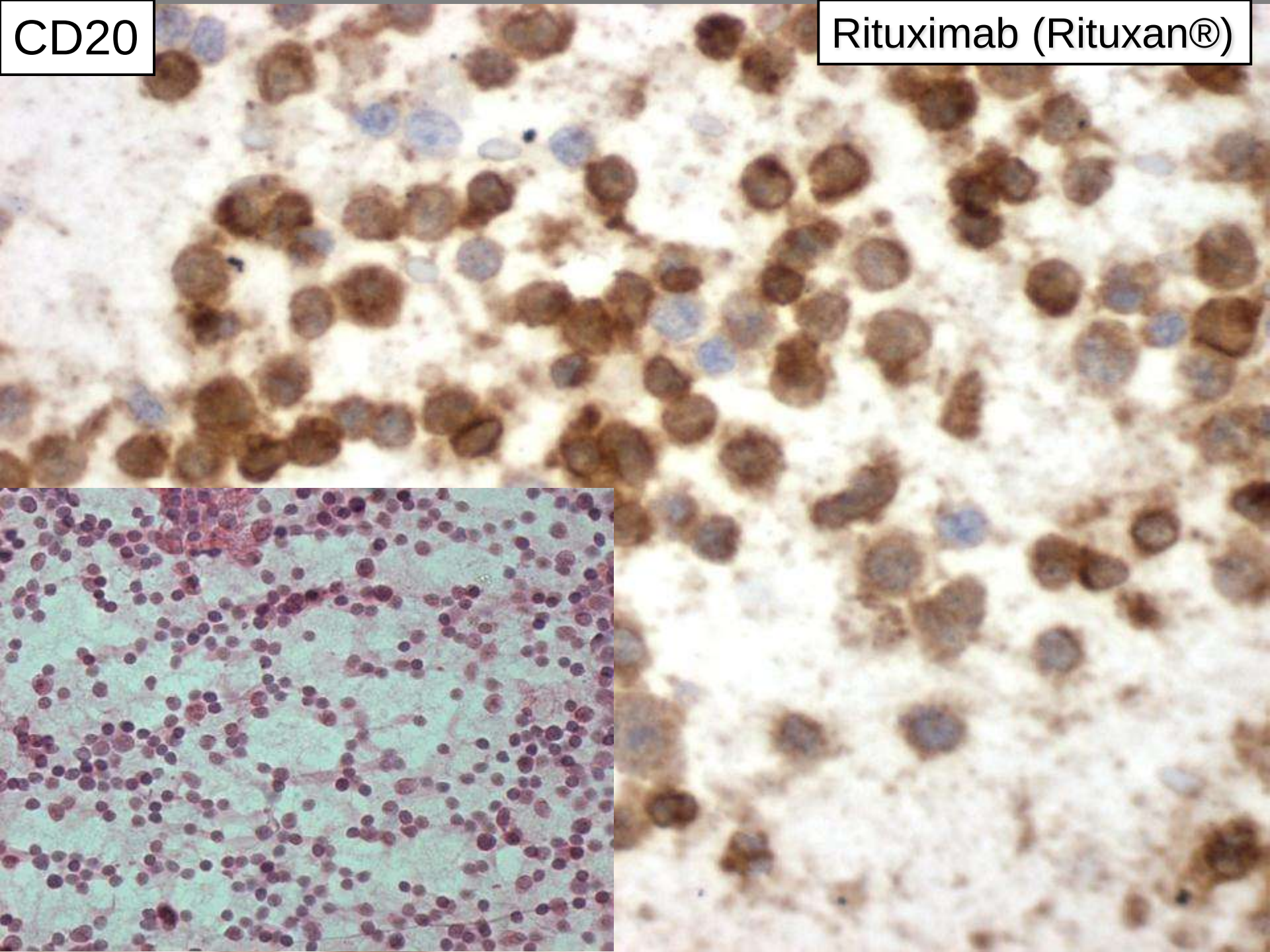


HER2-ICC




Her2 gene
Chr 17


HER2-CISH


CD20
Rituximab (Rituxan®)

ICC in Diagnostic Cytology
Applications

- Tumor Diagnosis/Classification
- Prognostic/Predictor Markers
- Target Therapy

NSCLC: Target Therapy

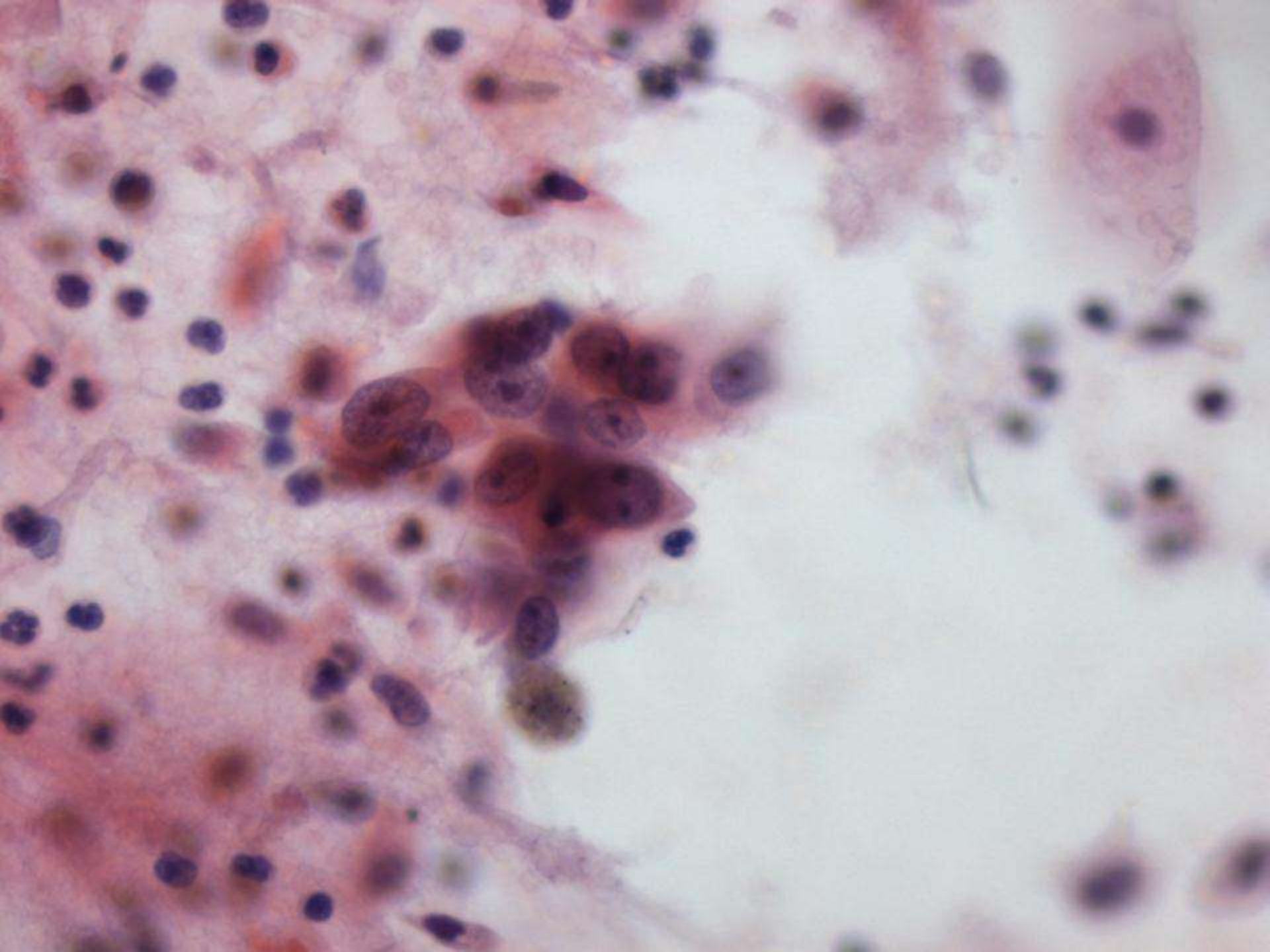
- tyrosine kinase inhibitors (TKI) first-line therapy in patients with advanced lung adenocarcinoma with **EGFR mutations**
- adenocarcinomas with **ALK rearrangements** are responsive to crizotinib (AlK inhibitor).
- Patients with **KRAS or BRAF mutation** do not respond to TKI, ALKI

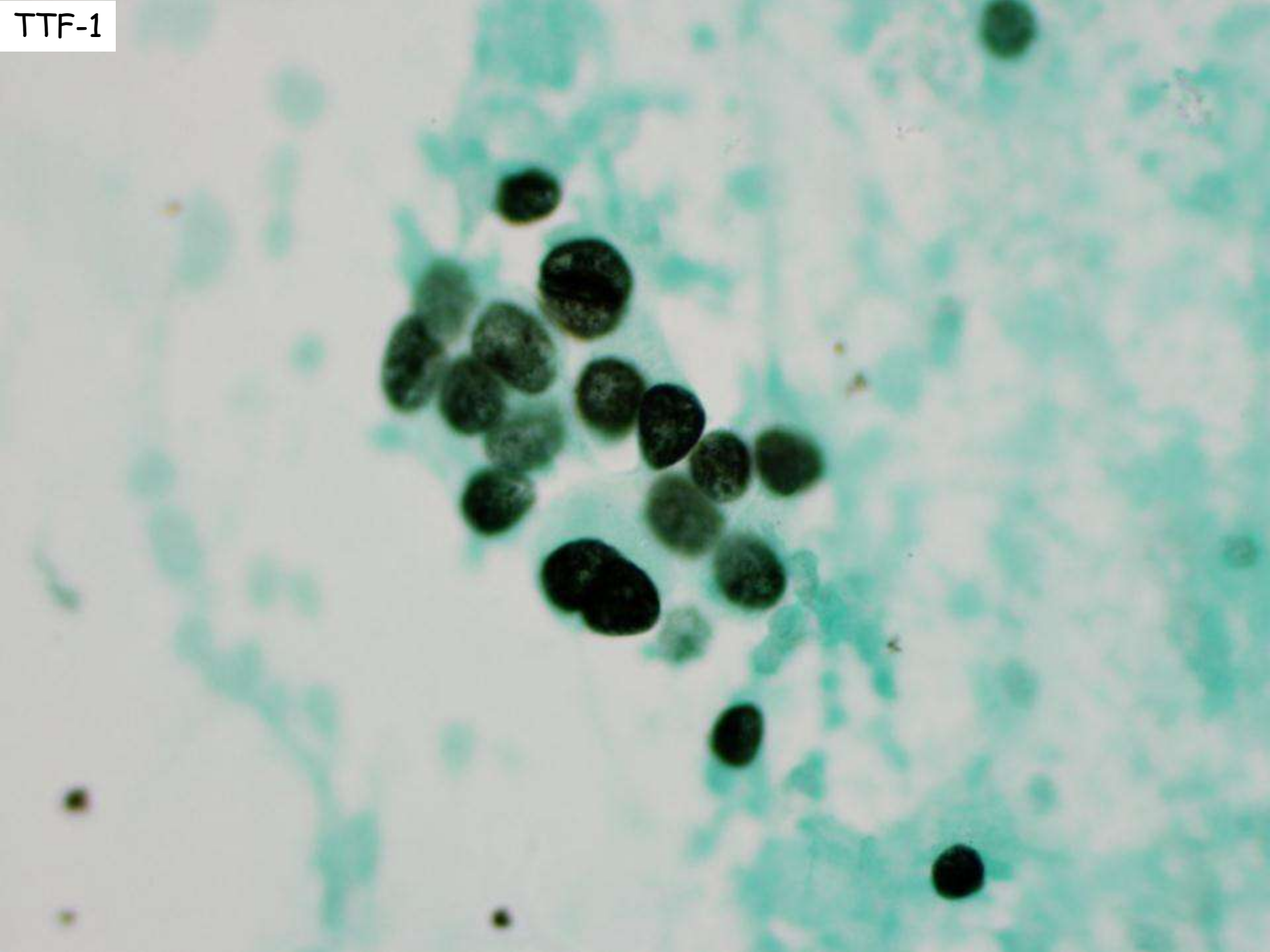
Arch Pathol Lab Med 2013, 137:668-684

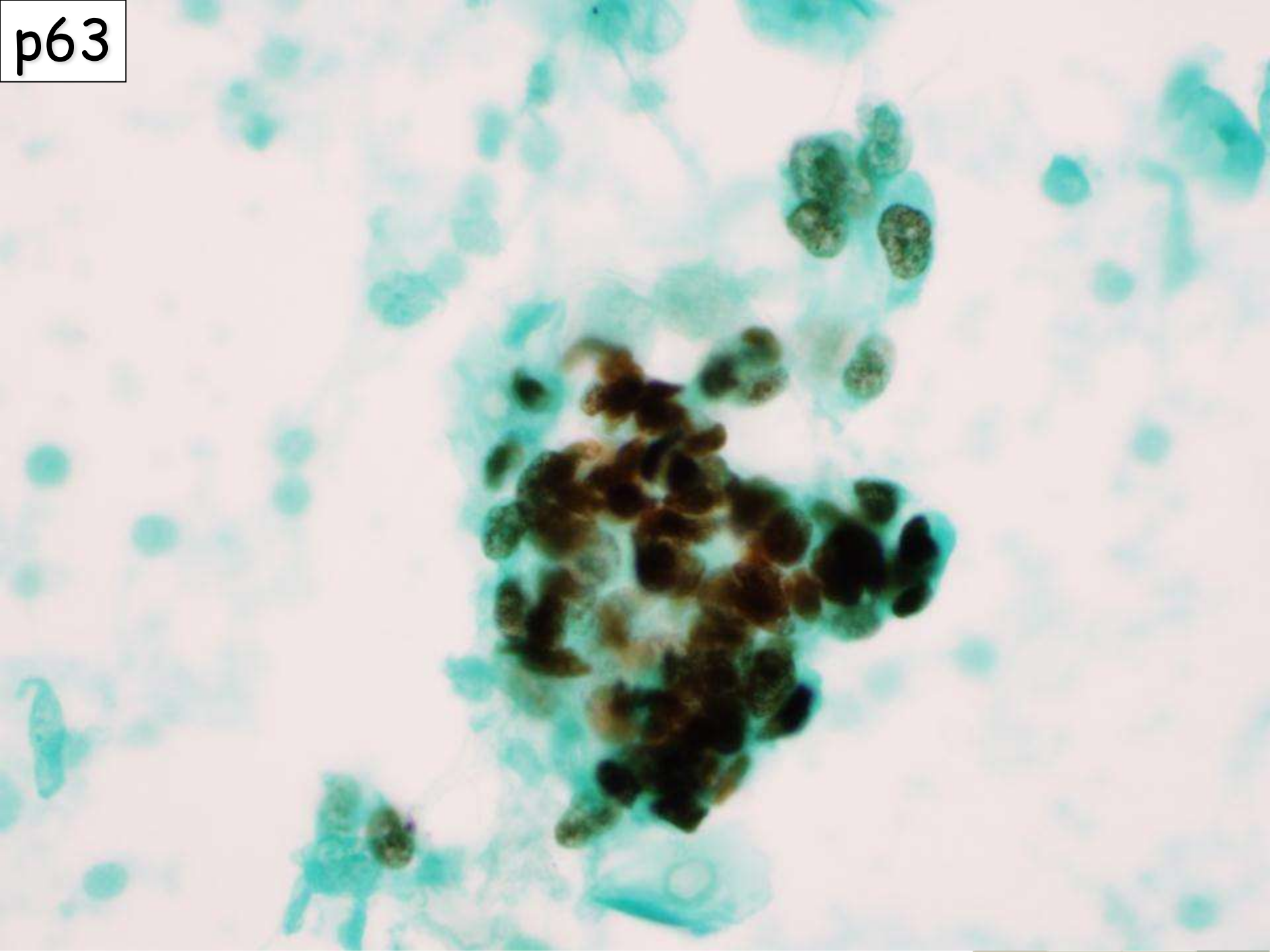
NSCLC: Target Therapy

- patients with adenocarcinoma or NSCLC, not otherwise specified (NSCLC-NOS),are more responsive to pemetrexed than those squamous cell carcinoma
- squamous cell carcinoma is associated with life-threatening hemorrhage in patients treated with bevacizumab; therefore, it is contraindicated in lung cancer patients with this histology.

Arch Pathol Lab Med 2013, 137:668-684




TTF-1


p63

ICC Limitations

- Large 3D cellular clusters in cytospin samples
- Histiocytes, macrophages, cells in mitosis , tumor giant cells

Look for single cells or smaller 2D groups

AM J Clin Pathol 1990; 94:470-475.

ICC Limitations

- Lack of internal control
- Negative results in ICC are not as meaningful as positive reactions

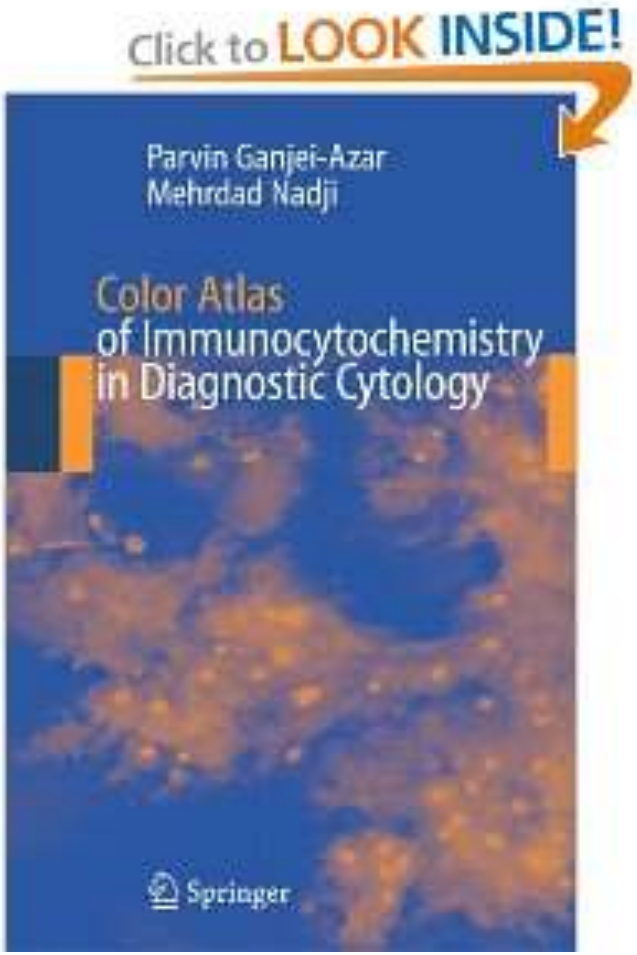
Diag Cytopathol 1986; 81-2, 1986

Final Words….

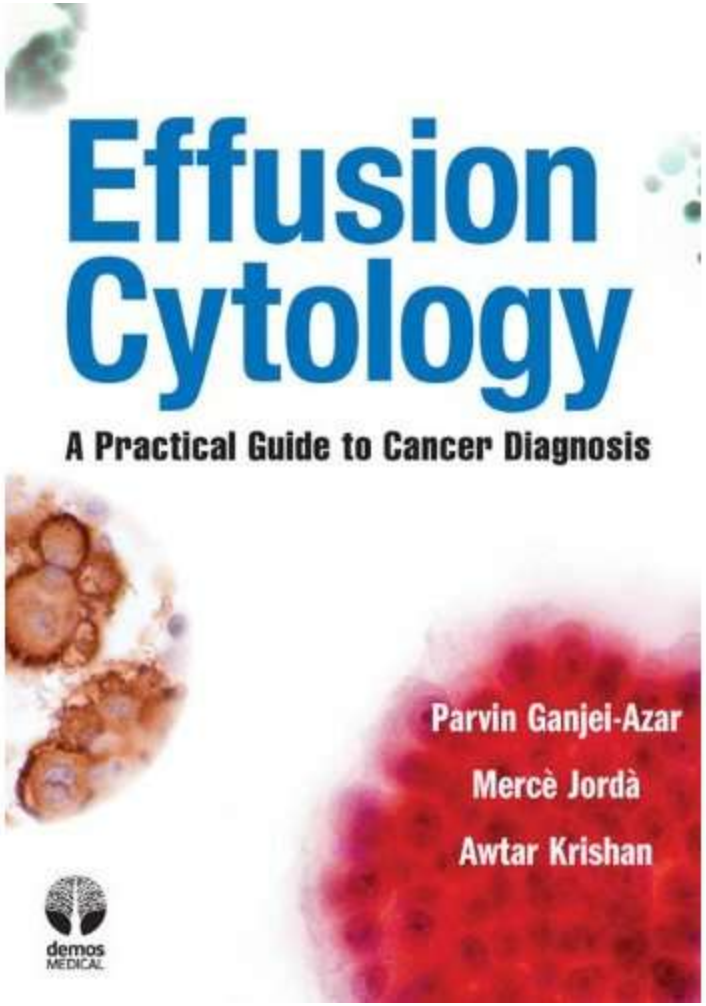
- Use our 3-step approach:
  - Define a specific differential Dx
  - Select a small panel of ICC markers
  - Combine Cytomorphology and ICC

Final Words….

- ICC can be used on previously alcohol-fixed Pap-stained slides without de-staining
- The technique does not require any modification of the routine ICC staining protocol



Springer, 2007



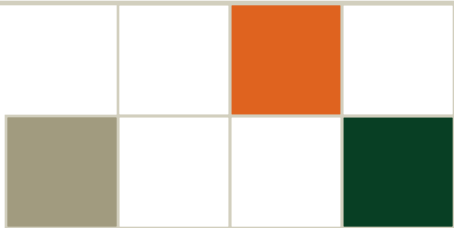
Demos, 2011

ASCP Workshops

Diagnostic problems in body cavity fluid cytology; a practical approach.

Immunocytochemistry in Diagnostic Cytology:
Values and Limitations

Parvin Ganjei-Azar MD, FASCP
Mercè Jordà MD, PhD, FASCP



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