# Simple guide for triaging lymphocyte-rich effusions for ancillary studies

台灣臨床細胞學會
細胞診斷繼續教育
劉之怡 醫師

*2021.8.15*

# Outline

- ***Morphological assessment of effusion specimens***
- ***Lymphocyte-rich effusions***
- ***Triage algorithm proposed for lymphocyte-rich effusions***

  *Cellular atypia in small lymphoid cells*

  *Cellular atypia in large lymphoid cells*

  *Ancillary tests*

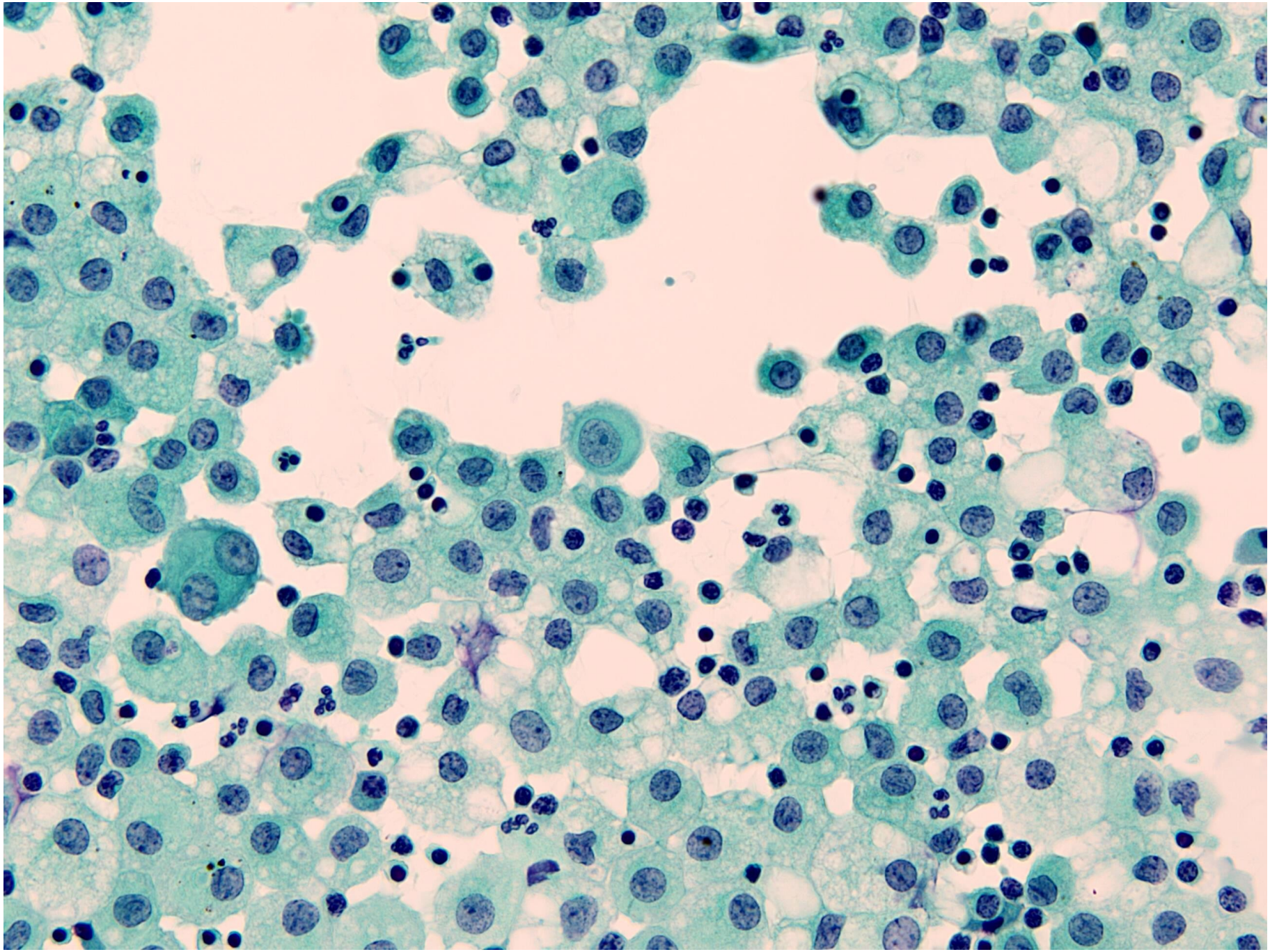
- ***Non-cohesive atypical cells: Differential Diagnosis***

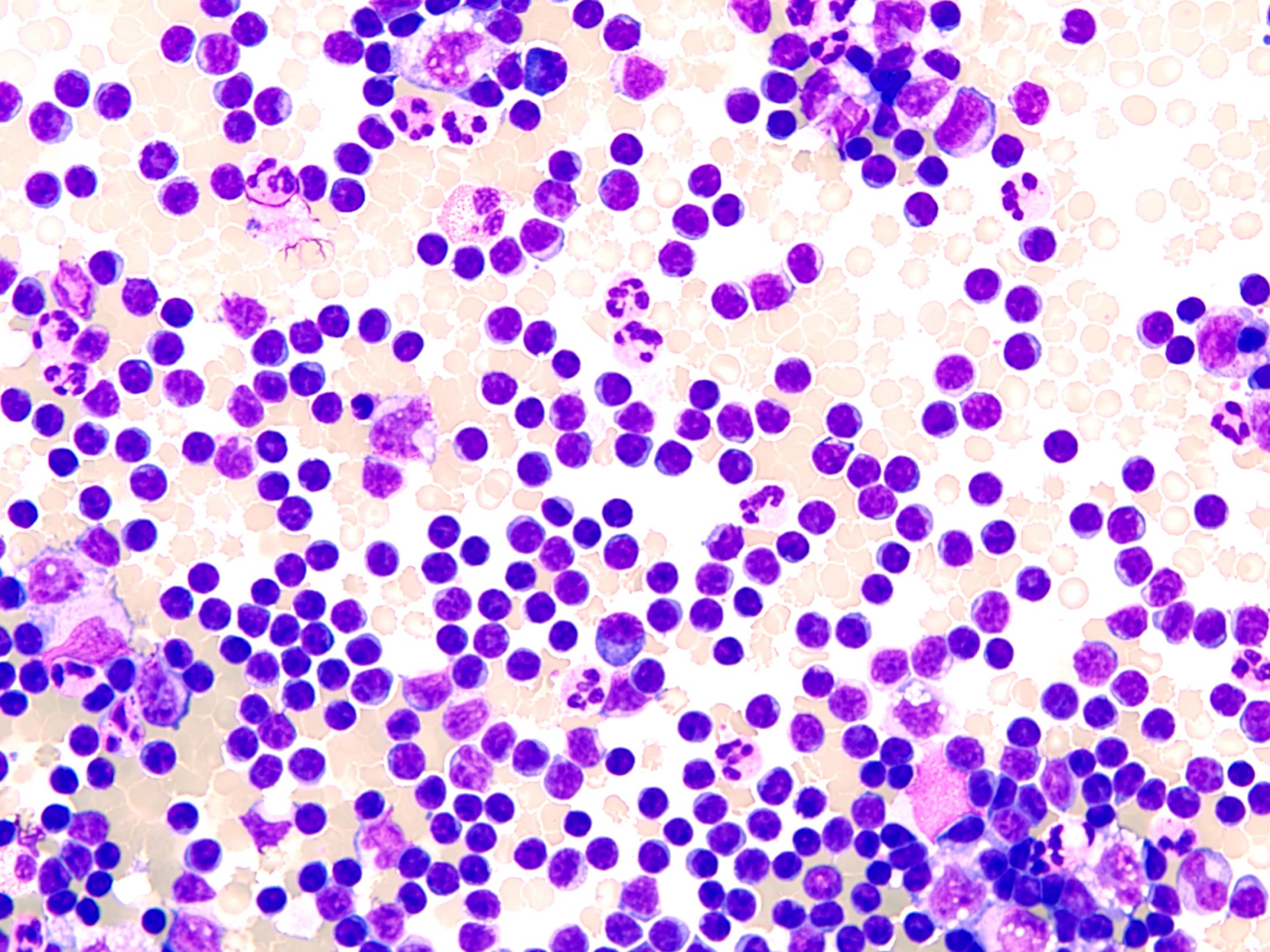
# Non-neoplastic cellular components in effusion specimens

**Cellular components** usually seen in a serous cavity specimen (histiocytes, mesothelial cells, and lymphocytes)

**Cytomorphology of histiocytes:** Smaller nucleus than that of mesothelial cells, Nucleus often folded, Cytoplasm granular or vacuolated, No "windows" between adjacent cells

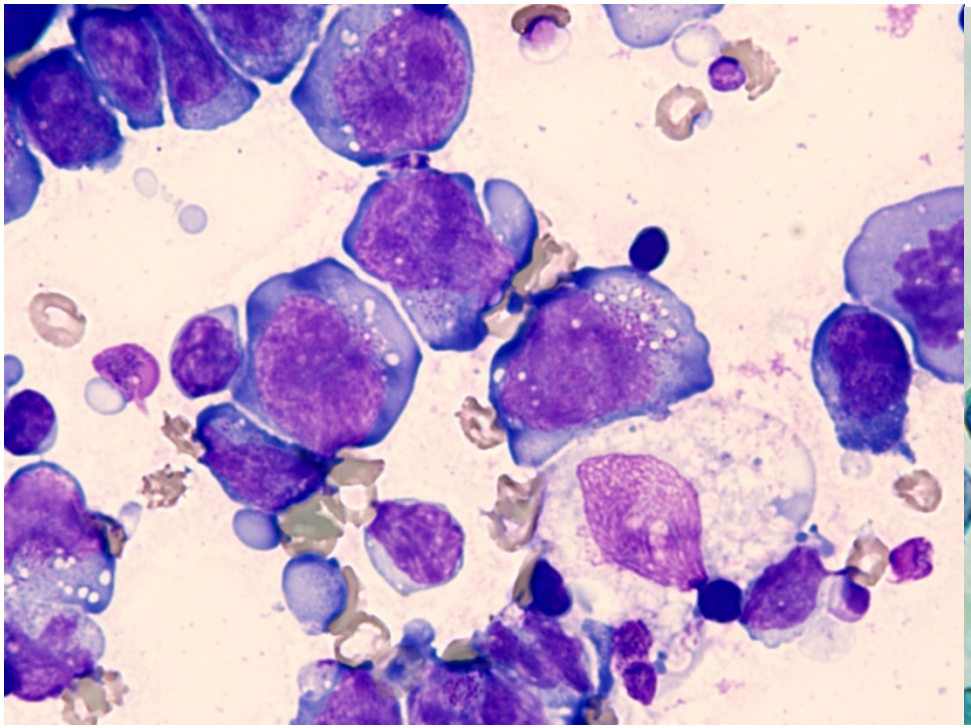
**Non-neoplastic (in reactive processes by approximately 80%):** Lymphocytosis may indicate an infectious process or reactive process.

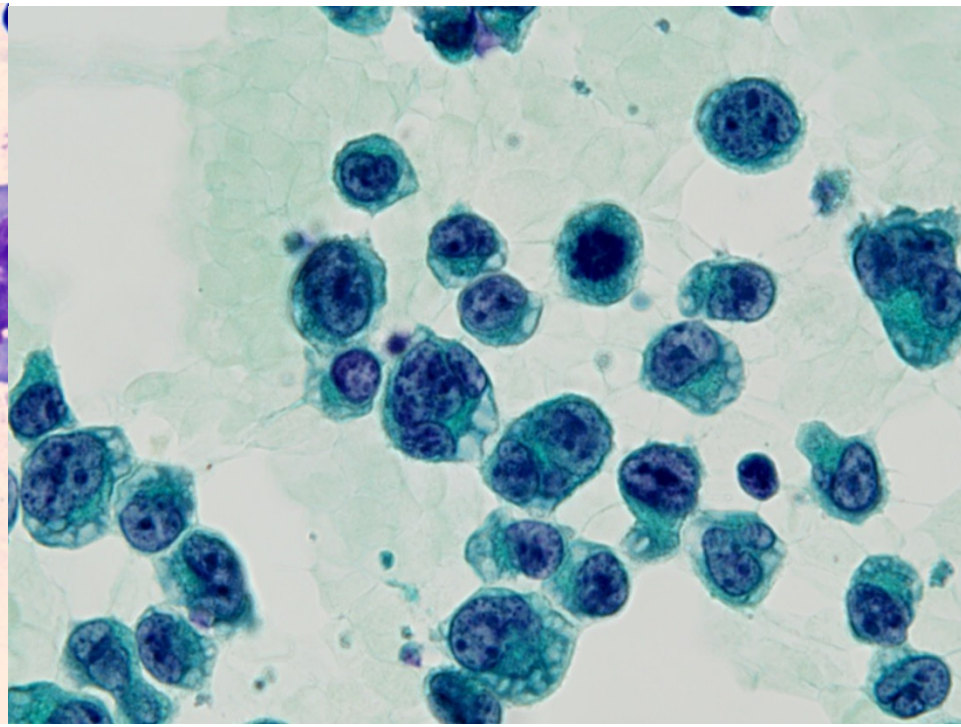




# Cytology preparation

- **Romanowsky-type stains**: Giemsa, Diff-Quik, Wright, Wright-Giemsa, or Liu stain
  - Prototypical stains used in **hematology** laboratories and effective in assessing the cytomorphology of hematopoietic cells, particularly cytoplasmic features
  - Heterogeneity and polymorphism of the cellular populations
- **Papanicolaou stain**:
  - Emphasizes the nuclear details
- **Cell block**:
  - H&E stained samples allow for "microhistology" of the cellular elements in effusion cytology
  - Preservation of cellular components and provision of materials for subsequent ancillary tests





# Lymphocytic or lymphocyte-rich effusions

More than 50% lymphocytes

The lymphocytes in such situations are small and may either be monotonous or polymorphous.

The presence of lymphocytosis may indicate an **infectious process** or **metastatic malignancy** that is not otherwise seen in the fluid specimen.

**Lymphocytosis indefinite for a lymphoproliferative disorder**:
Low-grade lymphoma is difficult to exclude without flow cytometric analysis. For this reason, lymphocytosis should be reported as an atypical finding and requires further workup by the clinical team.

# Lymphocytic or lymphocyte-rich effusions

D/D: Reactive lymphocytosis, lymphoma, and round cell malignancies

Reactive lymphocytosis: mainly of small mature lymphocytes. Lymphocytes may range from small and mature to reactive forms that can include occasional cells with plasmacytoid and/or immunoblastic features

**Lymphomatous effusion**: A predominance of specific lymphocytic populations (small vs. intermediate vs. large cells), and/or lack of maturation

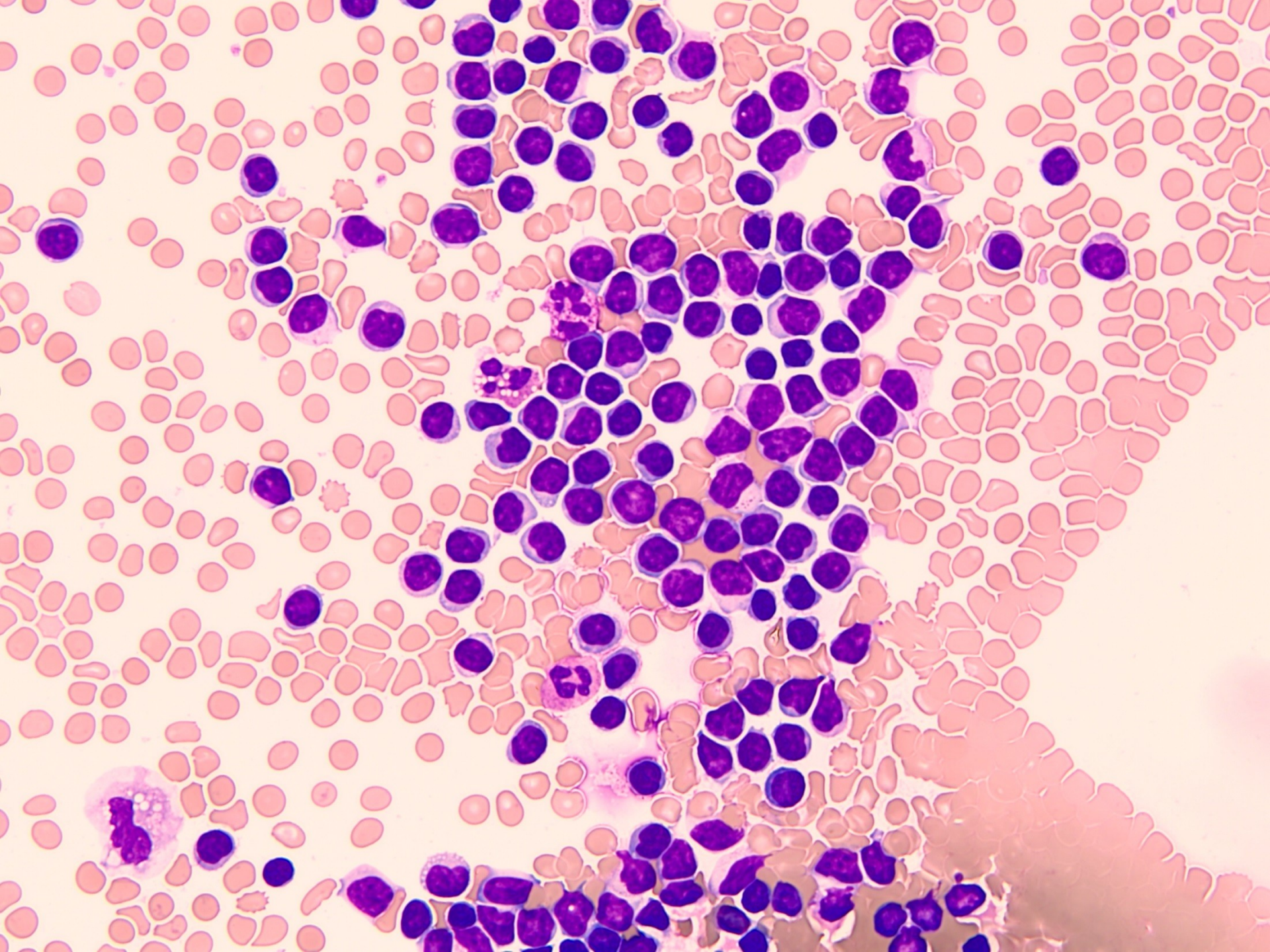
# General diagnostic principles

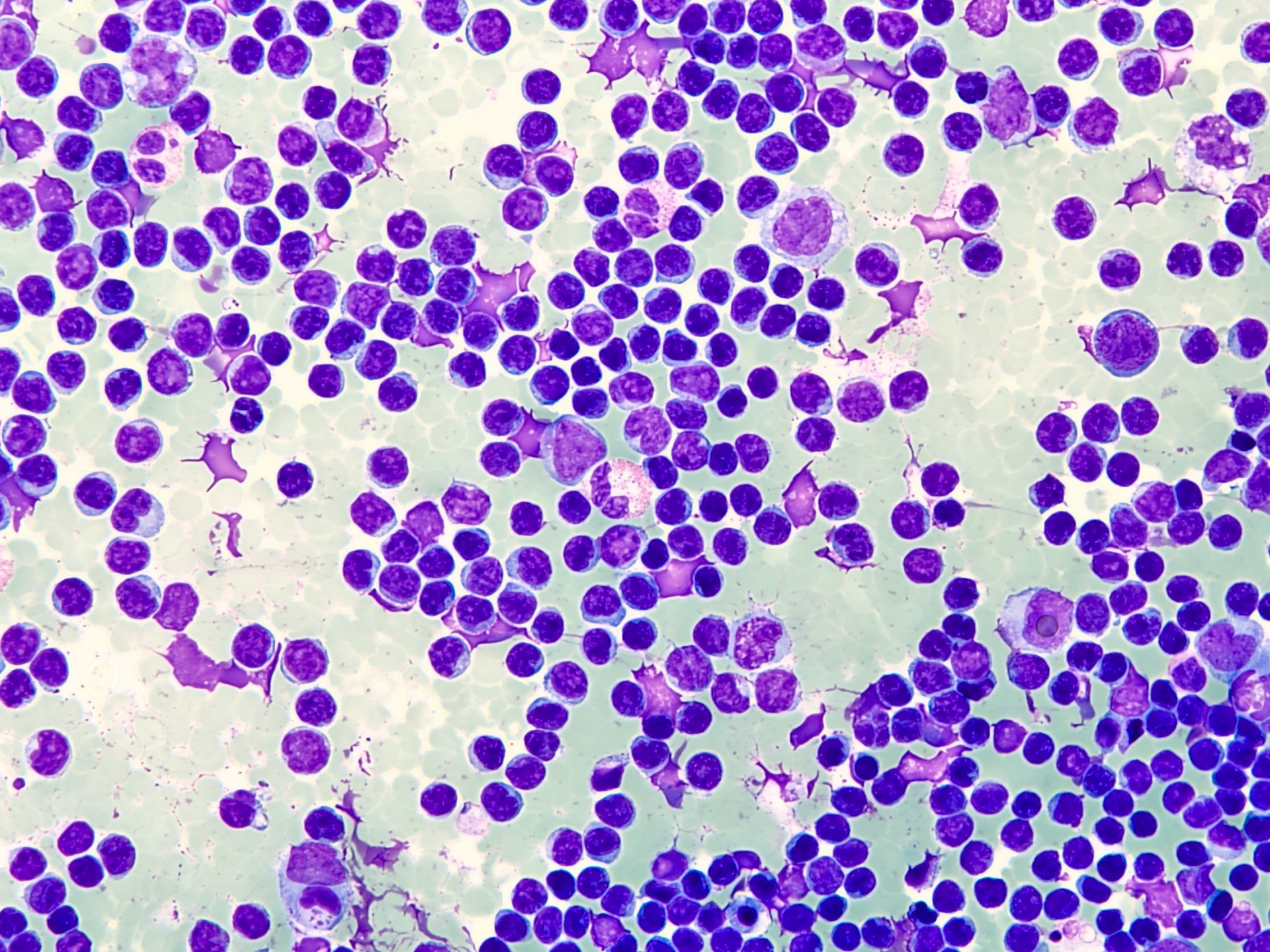
## Low/medium-power examination of the cellular pattern

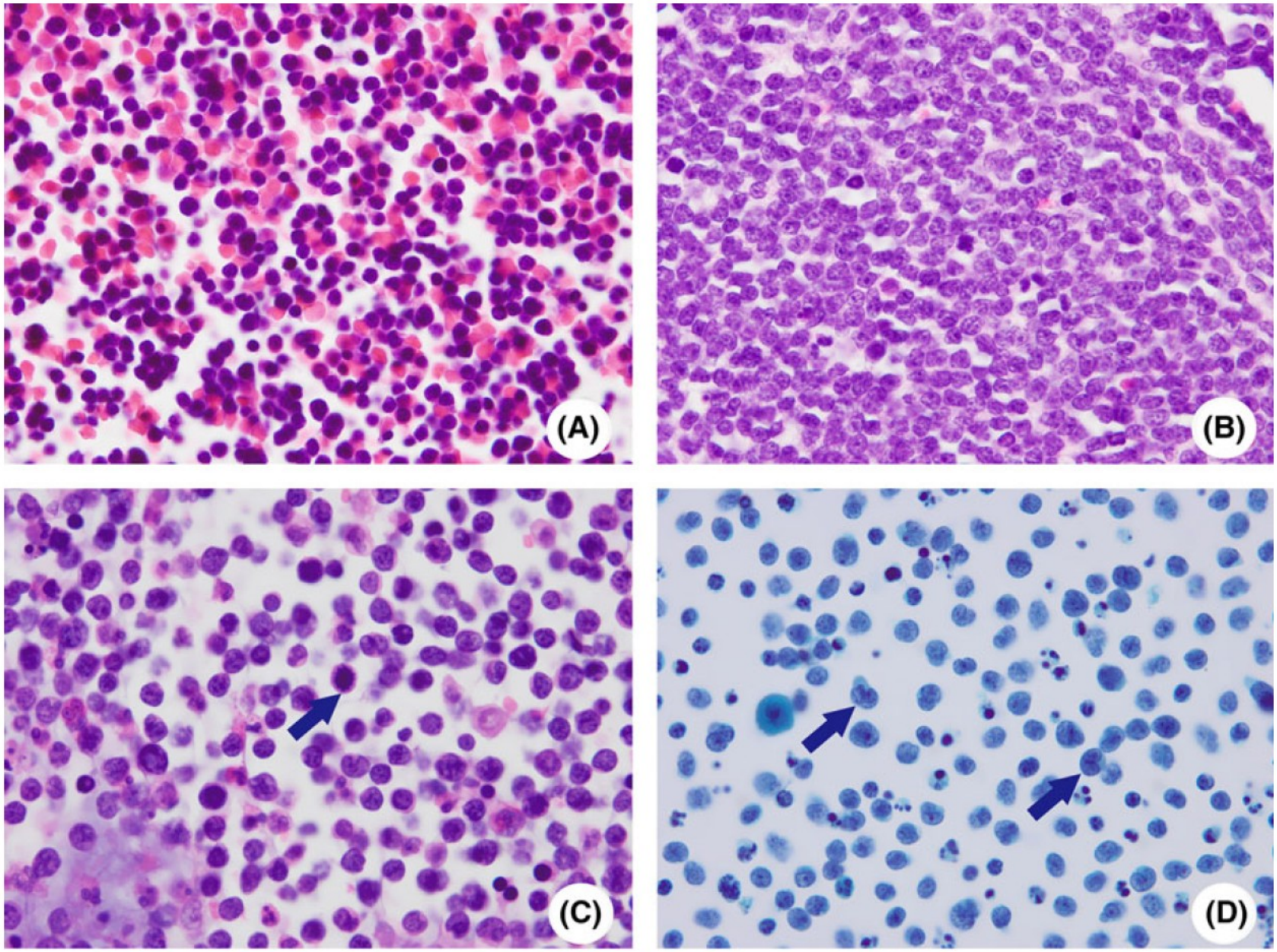
- Monomorphic or Polymorphic
- Index of suspicion: Patients with a history or a clinical suspicion of lymphoma

## High-power examination

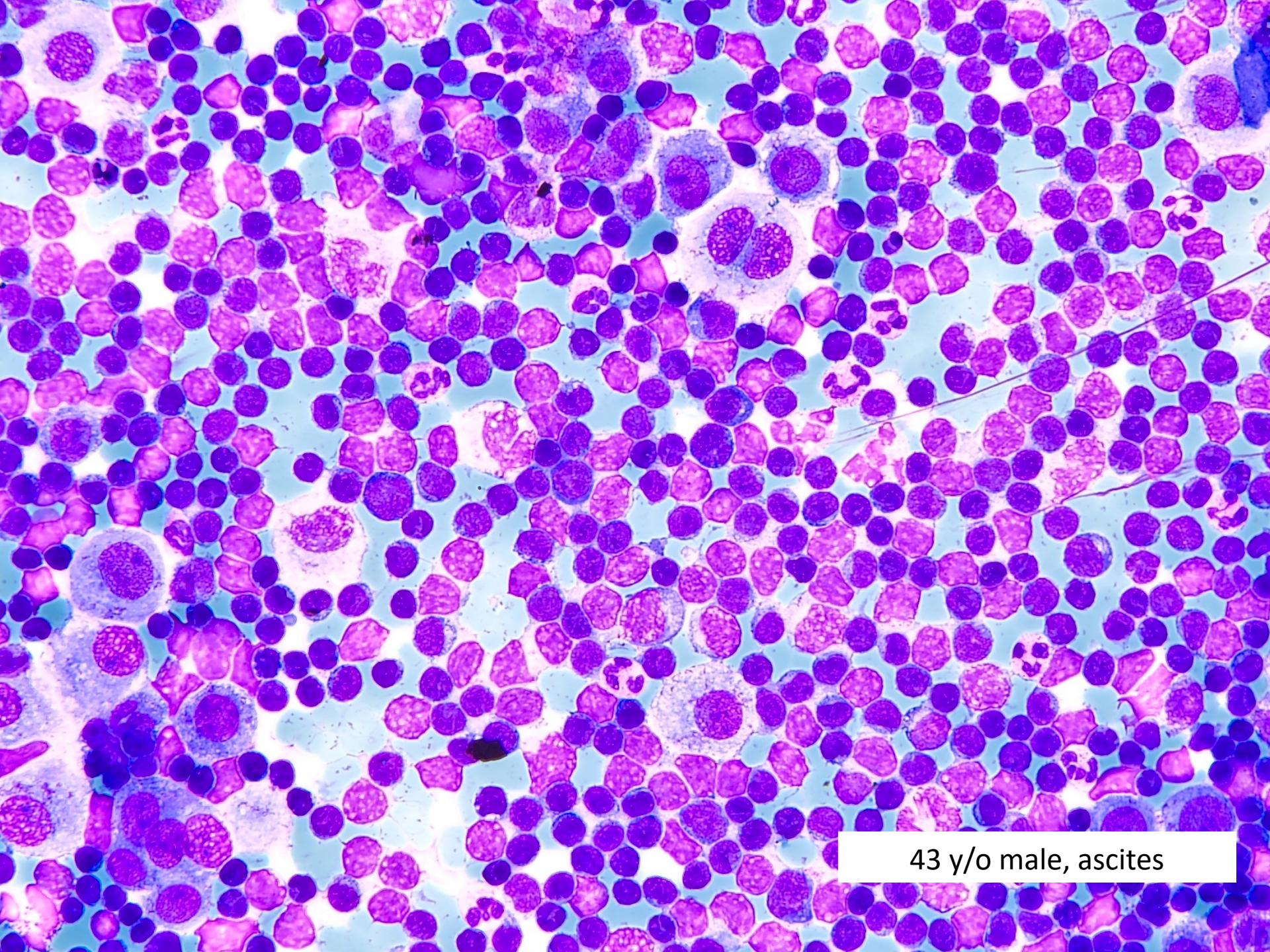
- Cellular size: small/medium-sized or large cell predominant pattern
- Nuclear atypia in small/medium-sized cells: Cleaved or folded nuclei, irregular nuclear contours, coarsely textured chromatin, and clotted or soccer-ball-like chromatin pattern
- Cytoplasmic features

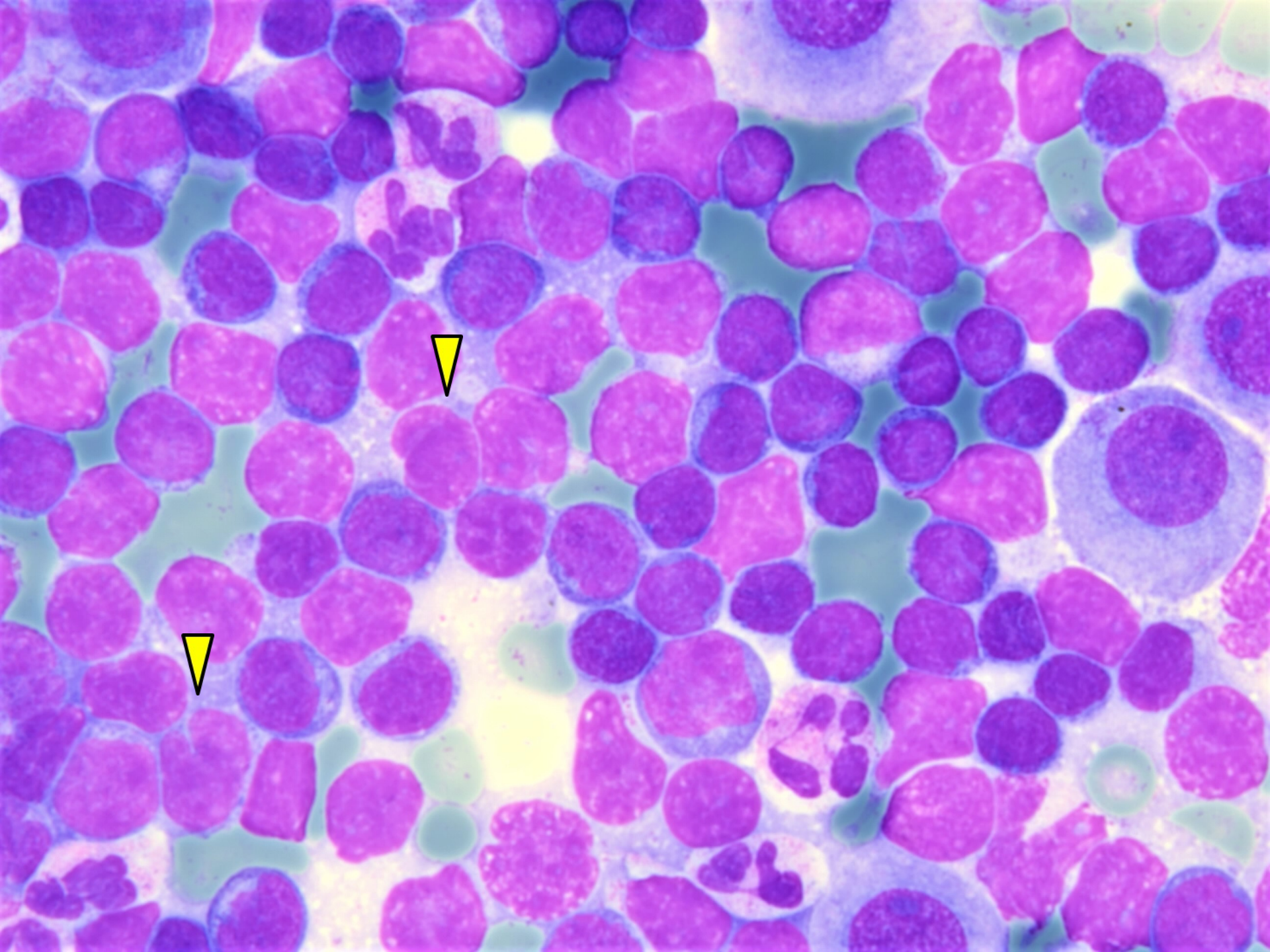


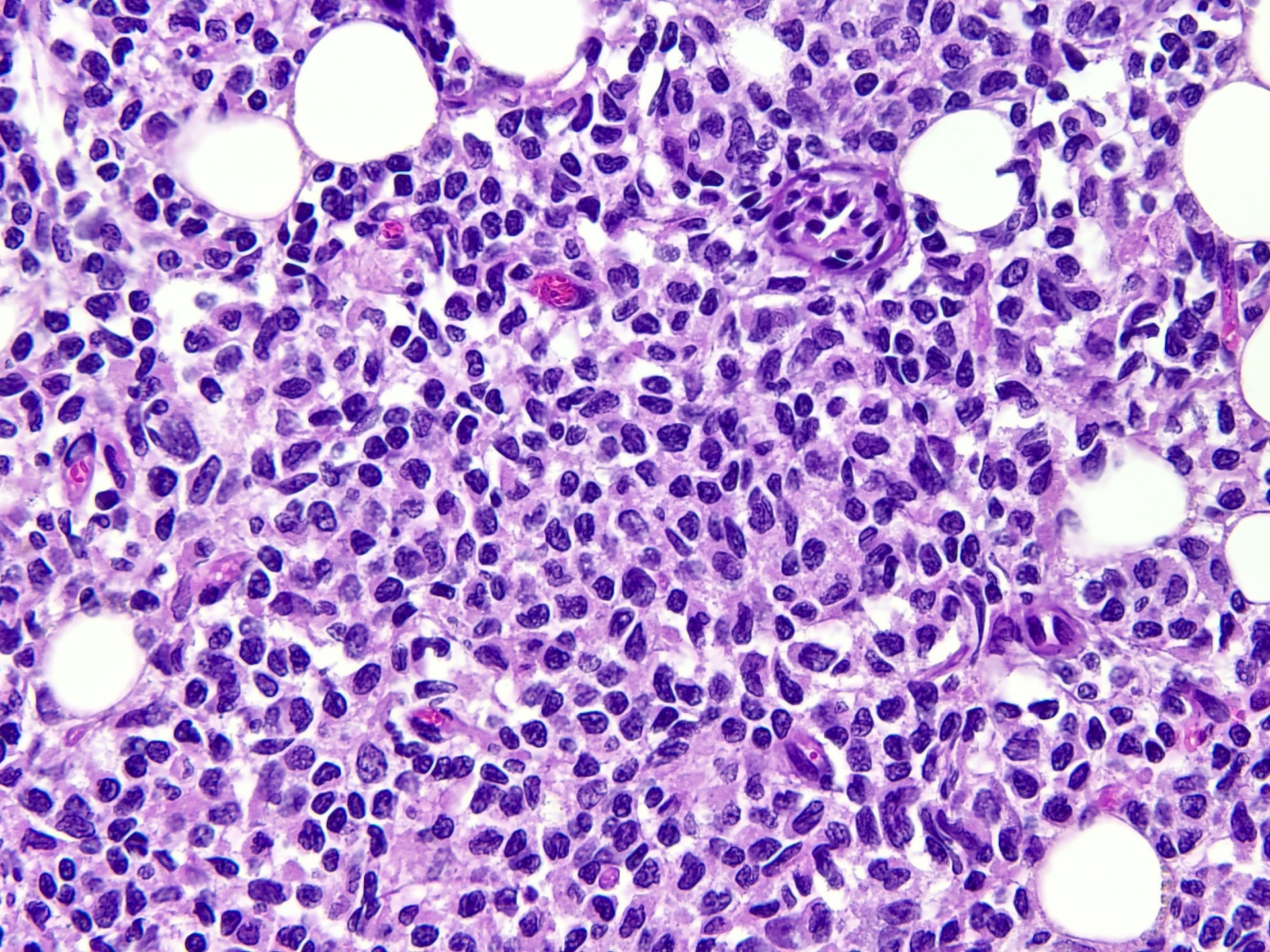





Li J, Zhang W, Wang W, Jiang Y, Zhao S, Liu W, Li G, Liu N, Li Q, Su X. Forty-nine cases of acute lymphoblastic leukaemia/lymphoma in pleural and pericardial effusions: A cytological-histological correlation. Cytopathology. 2018 Apr;29(2):172-178.


43 y/o male, ascites





# Follicular lymphoma

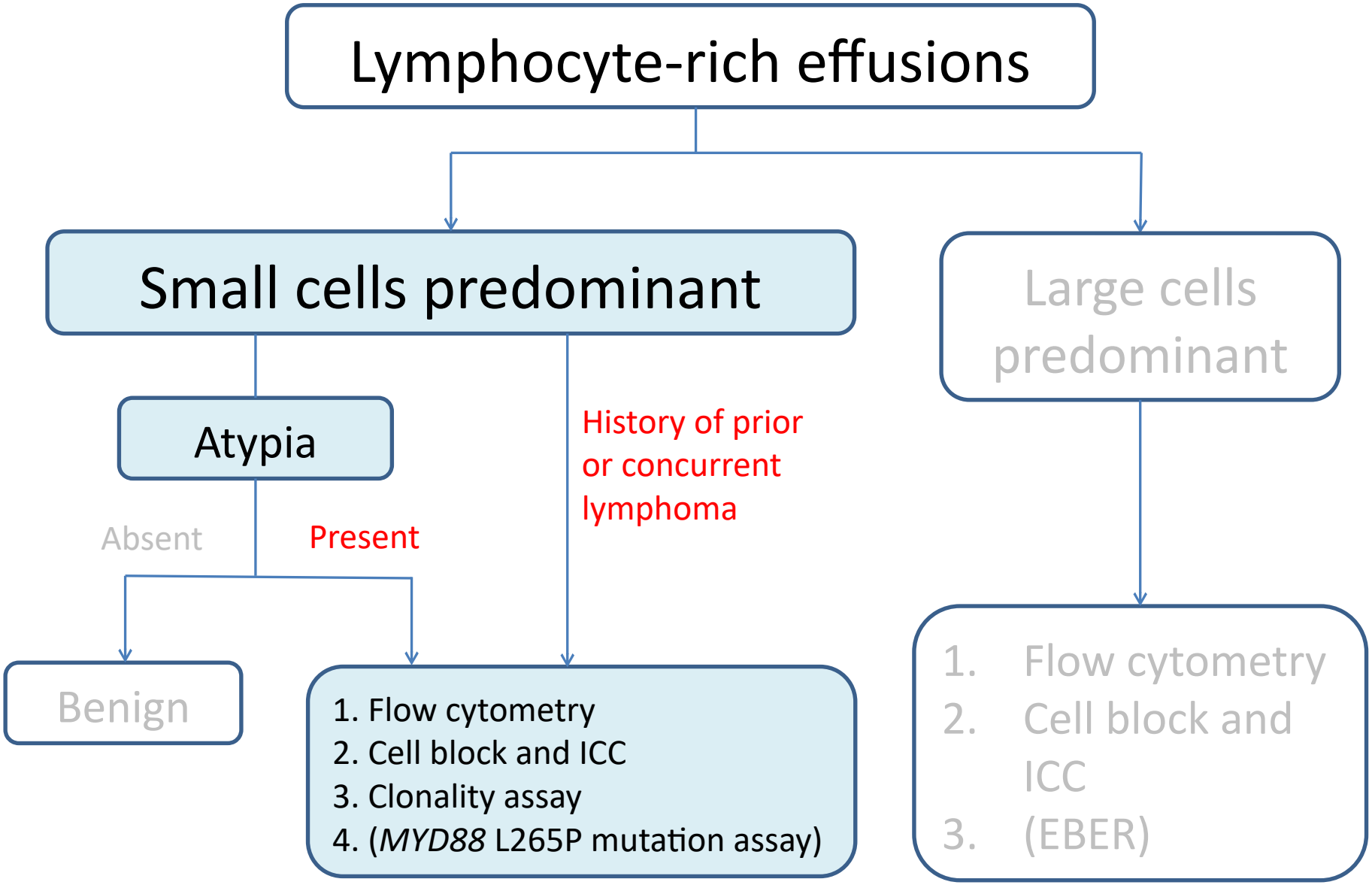
## Low/medium-power examination of the cellular pattern:

- Monomorphic or Polymorphic
- Index of suspicion: Patients with a history or a clinical suspicion of lymphoma

## High-power examination

- Cellular size: small/medium-sized or large cell predominant pattern
- Cellular features as atypia: Cleaved or folded nuclei, irregular nuclear contours, coarsely textured chromatin, and clotted or soccer-ball-like chromatin pattern

Dual population: Foreign-appearing cells in a background of small lymphocytes or reactive mesothelial cells
Ancillary studies and clinical correlation




*Liu CY, Chuang SS. A Simple and Practical Guide for Triaging Lymphocyte-rich Effusions for Ancillary Studies. Adv Anat Pathol. 2021 Mar 1;28(2):94-104.*

# Lymphomatous effusions

10% to 15% of malignant effusions are caused by lymphoma

Lymphomatous effusions might either be primary or secondary

Most represent secondary involvement of serosal surfaces; very few are primary effusion lymphomas.

Lymphomatous effusion more commonly represents a complication of a widely disseminated disease.

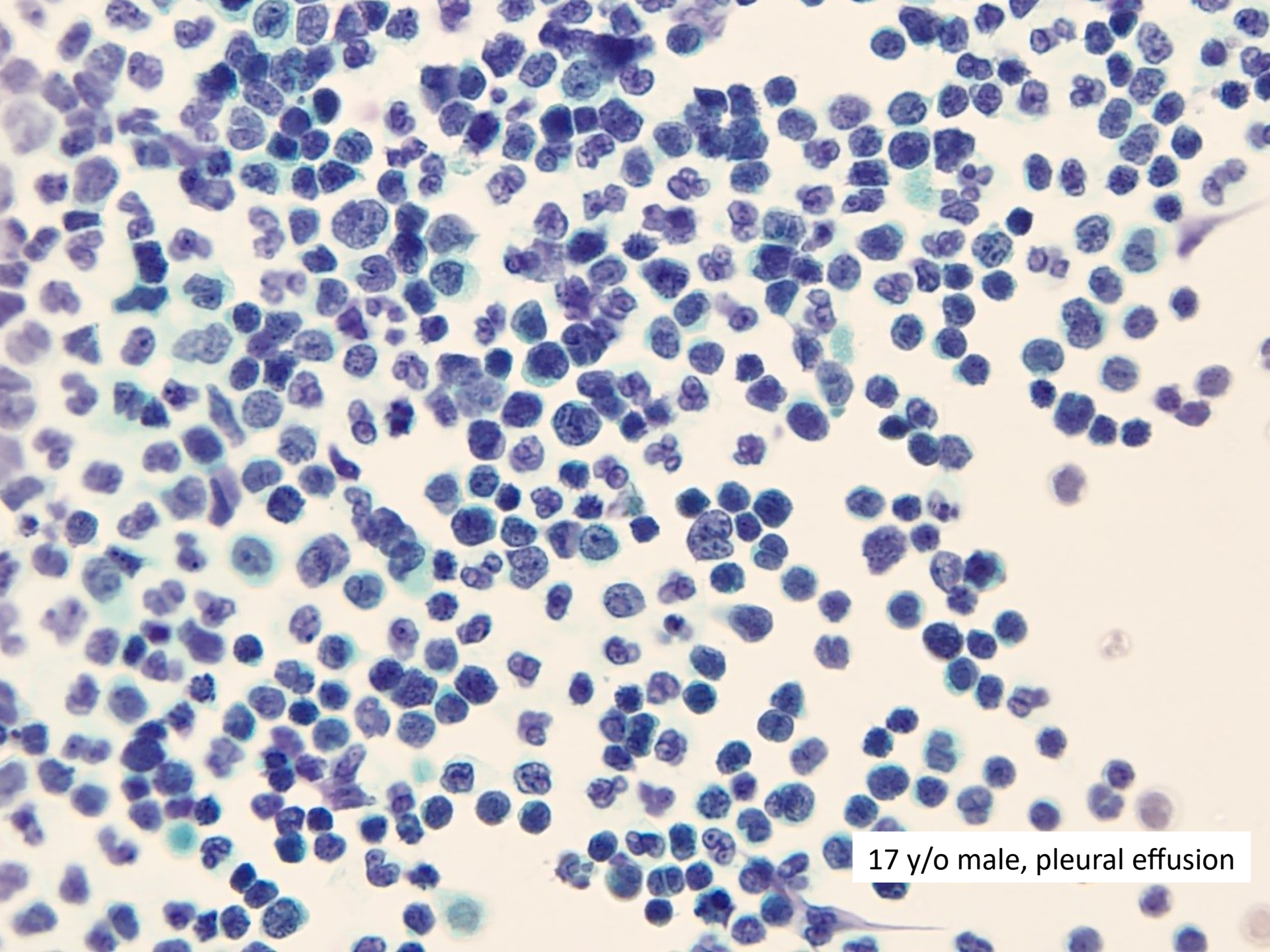
# Lymphomatous effusions

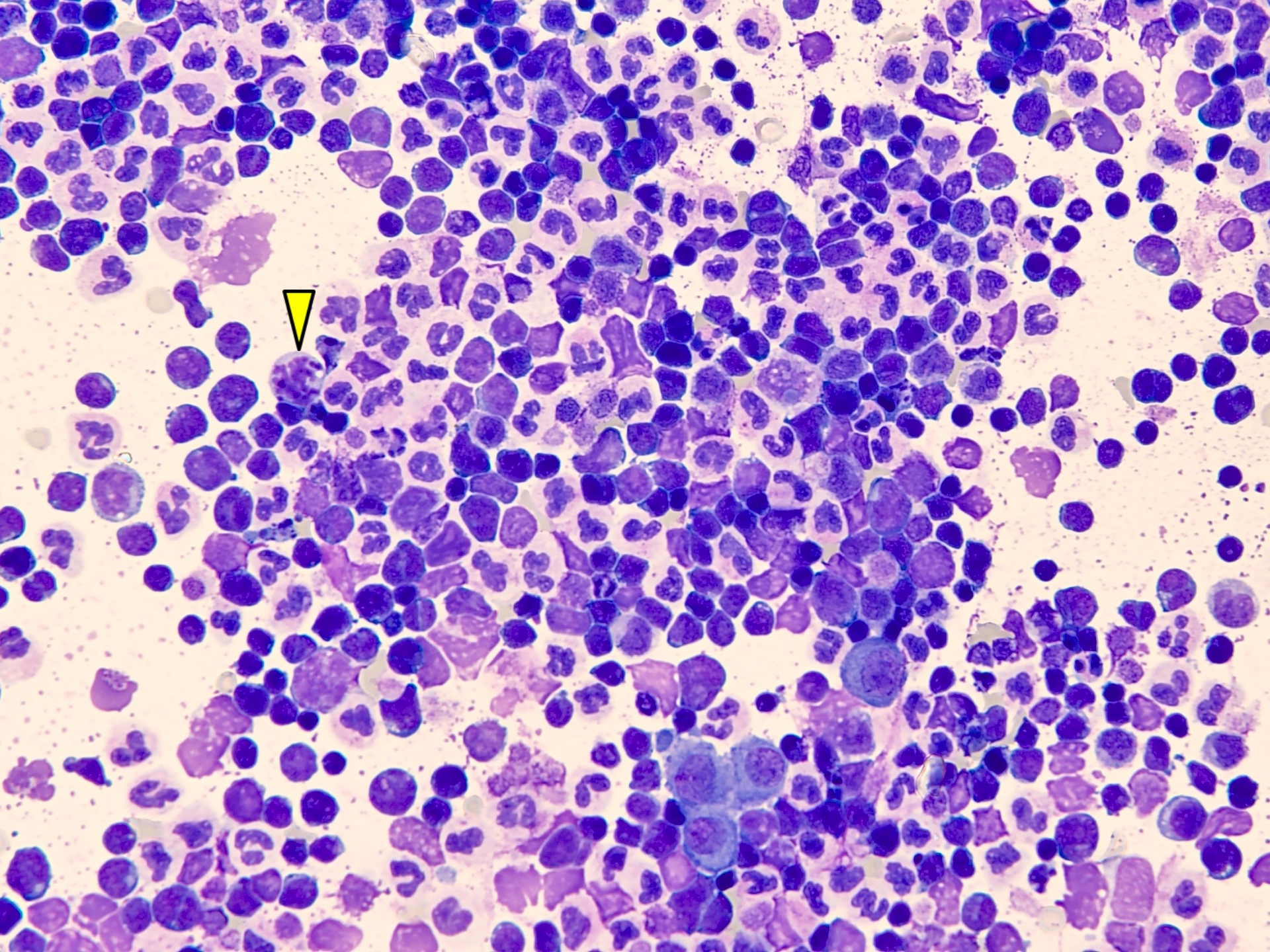
| | |
|---|---|
| **Classical Hodgkin lymphoma** | **1 (3%)** |
| B cell lymphoma | 27 (75%) |
| DLBCL and high grade B cell neoplasm[a] | 16 |
| Mantle cell lymphoma | 5 |
| LPL | 1 |
| NMZL | 1 |
| CLL/SLL | 2 |
| Unspecified B cell neoplasm | 2 |
| T cell lymphoma | 8 (22%) |
| T-LBL | 2 |
| PTCL | 6 |
| ALK+ ALCL | 2 |
| ALK- ALCL | 1 |
| AITL | 1 |
| MEITL | 1 |
| PTCL, NOS | 1 |

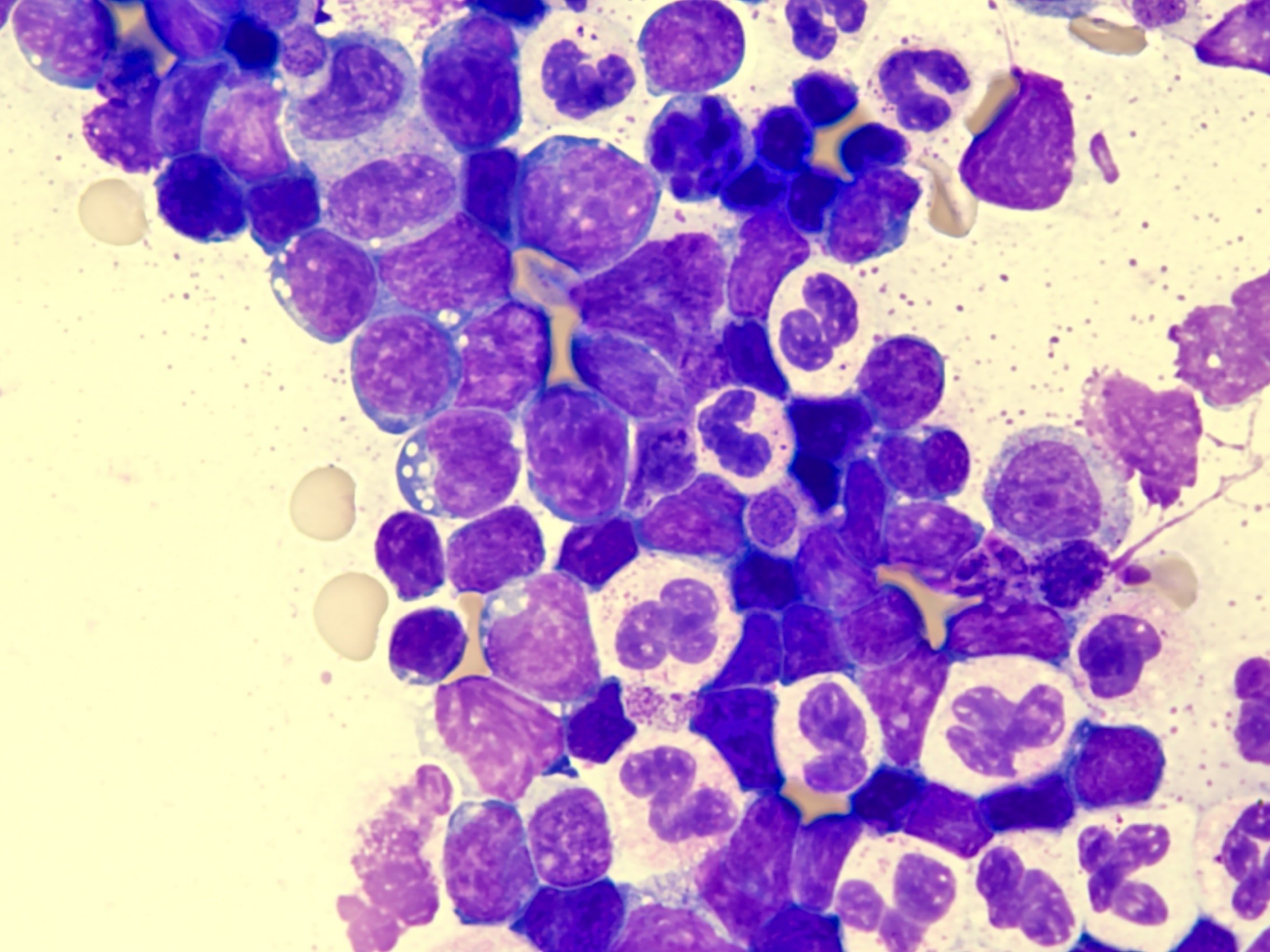
Abbreviations: ALK, anaplastic lymphoma kinase; ALCL, anaplastic large cell lymphoma; AITL, angioimmunoblastic T cell lymphoma; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B cell lymphoma; LAP, lymphadenopathy; LBL, lymphoblastic lymphoma; LPL, lymphoplasmacytic lymphoma; MEITL, monomorphic epitheliotropic intestinal T cell lymphoma; NMZL, nodal marginal zone lymphoma; NOS, not otherwise specified; PTCL, Peripheral T-cell lymphoma; SLL, small lymphocytic lymphoma.

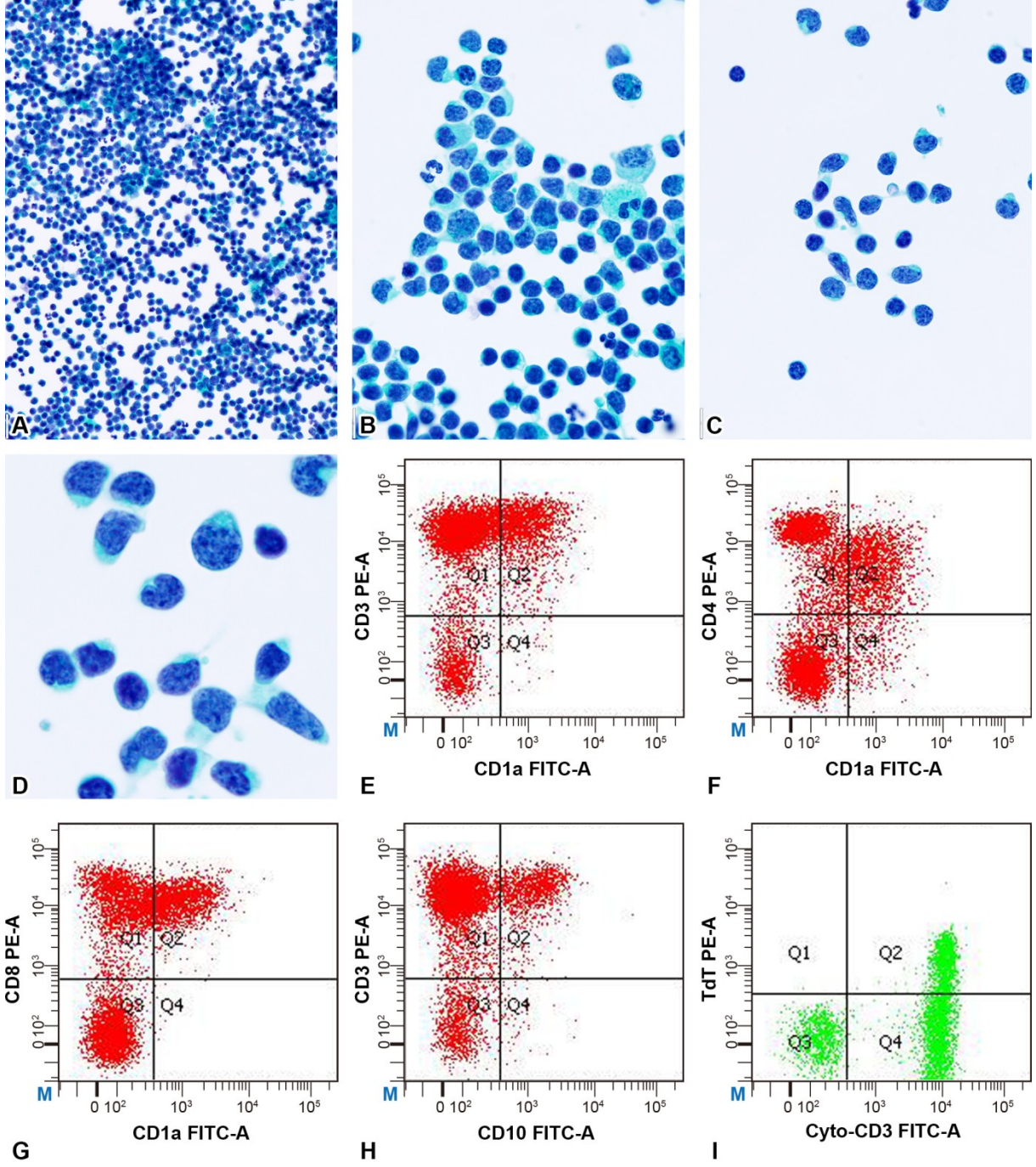
[a]Including one case of plasmablastic lymphoma in the colon.

Wang RC, Chen YH, Chen BJ, Chuang SS. The cytopathological spectrum of lymphomas in effusions in a tertiary center in Taiwan. Diagn Cytopathol. 2021 Feb;49(2):232-240.


17 y/o male, pleural effusion








29-year-old male presented with a mediastinal mass and massive pleural effusion

*Liu CY, Chuang SS. A Simple and Practical Guide for Triaging Lymphocyte-rich Effusions for Ancillary Studies. Adv Anat Pathol. 2021 Mar 1;28(2):94-104.*

# T-lymphoblastic lymphoma

## Low/medium-power examination of the cellular pattern:

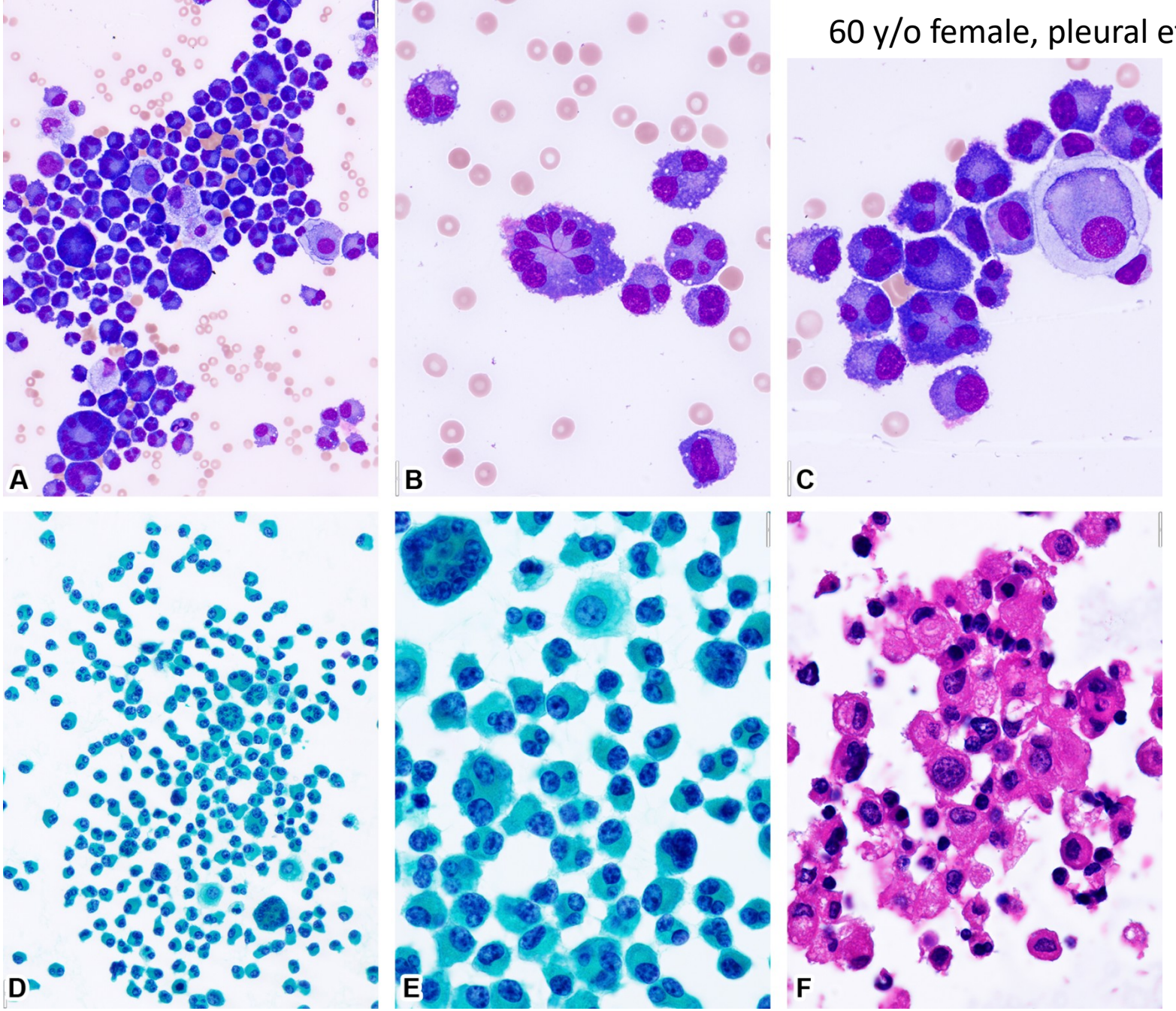
- Monomorphic or Polymorphic
- Index of suspicion: Patients with a history or a clinical suspicion of lymphoma

## High-power examination

- Cellular size: Small/medium-sized or large cell predominant pattern
- Cellular features as atypia: Round or convoluted nuclei, irregular nuclear contours; Mitotic figures are variable

Young or middle-aged male patients with a mediastinal mass
Detailed immunophenotypic study

60 y/o female, pleural effusion




*Liu CY, Chuang SS. A Simple and Practical Guide for Triaging Lymphocyte-rich Effusions for Ancillary Studies. Adv Anat Pathol. 2021 Mar 1;28(2):94-104.*

# Plasma cell myeloma

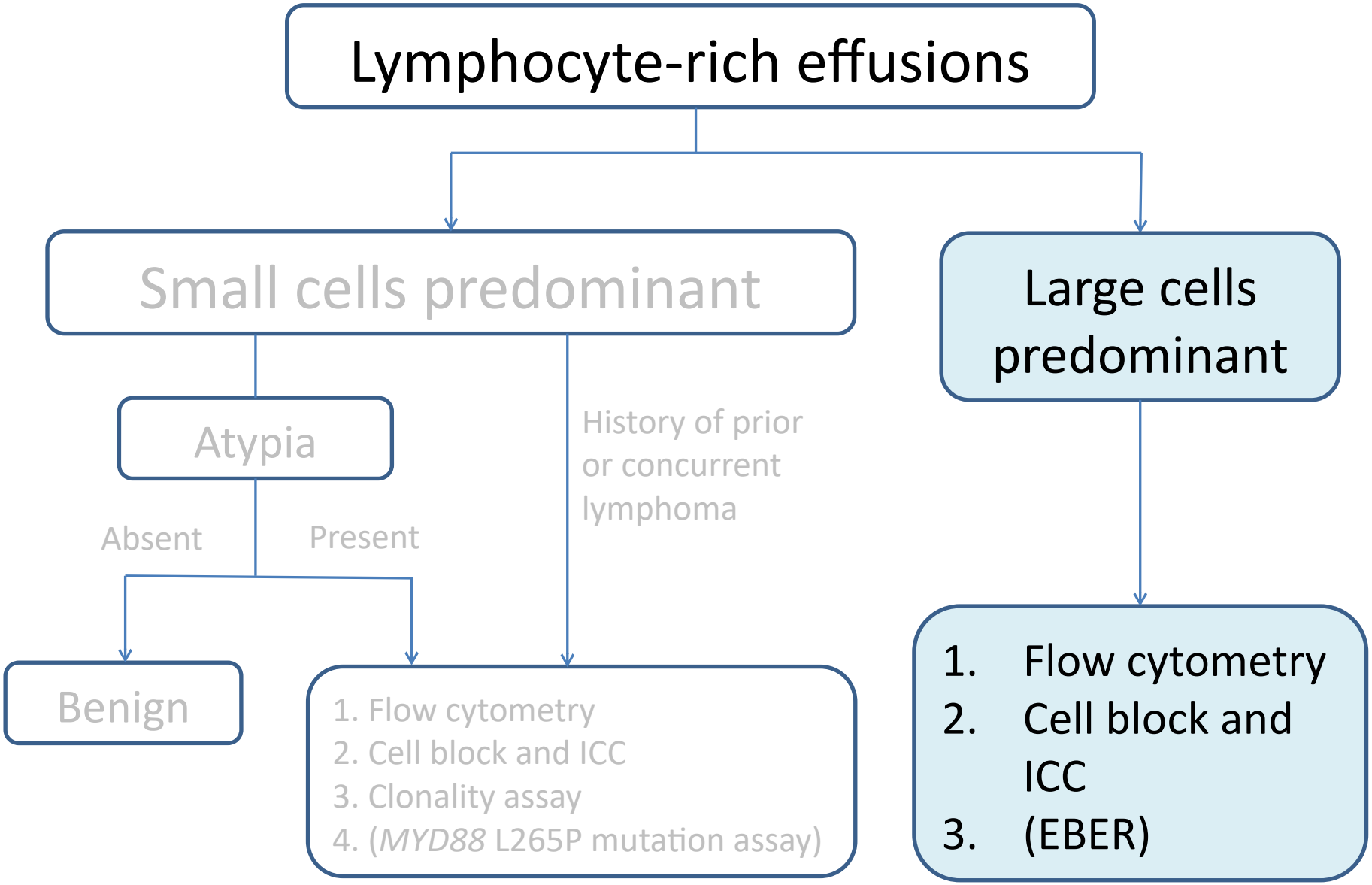
## Low/medium-power examination of the cellular pattern:

- Monomorphic or Polymorphic
- Index of suspicion: Patients with a history or a clinical suspicion of lymphoma

## High-power examination

- Cellular size: small/medium-sized or large cell predominant pattern
- Cellular features as atypia: plasmacytoid or plasmablastic morphology, nuclear pleomorphism with multilobulation

Plasmacytoid morphology, abundant eosinophilic cytoplasm with perinuclear hof and eccentric clock-faced nuclei
Clinical history and immunophenotyping




*Liu CY, Chuang SS. A Simple and Practical Guide for Triaging Lymphocyte-rich Effusions for Ancillary Studies. Adv Anat Pathol. 2021 Mar 1;28(2):94-104.*

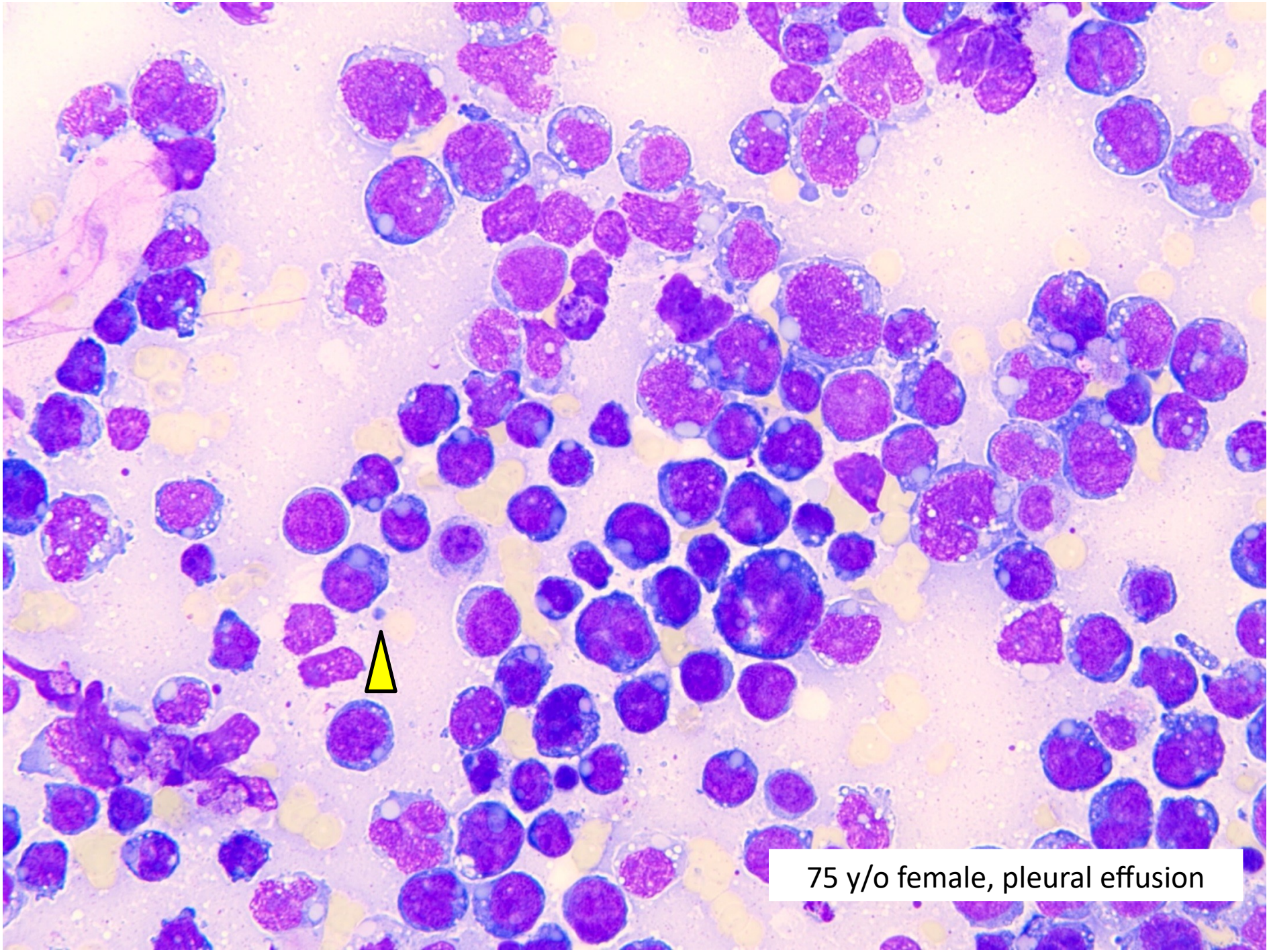
# General diagnostic principles

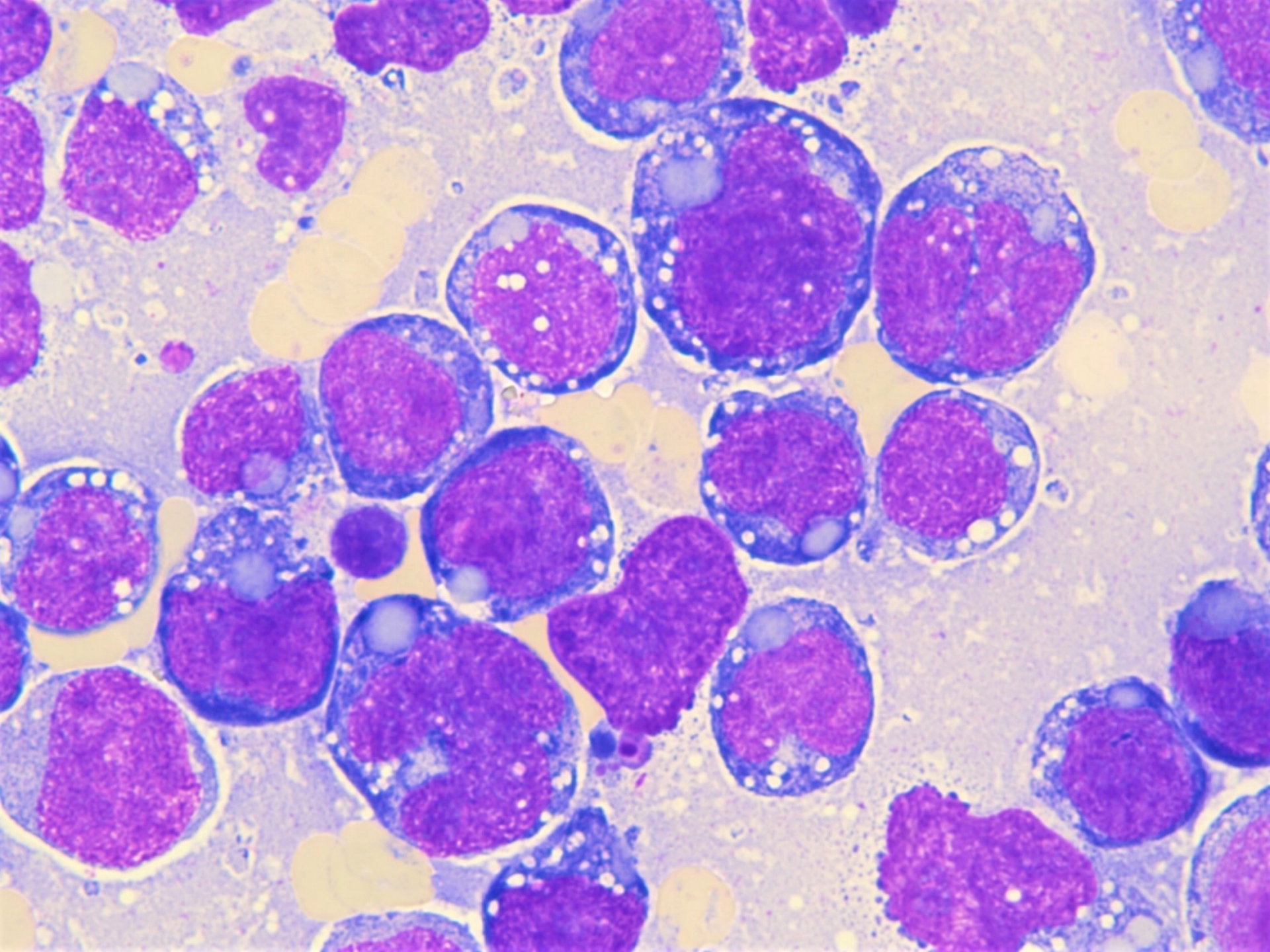
## Low/medium-power examination of the cellular pattern:

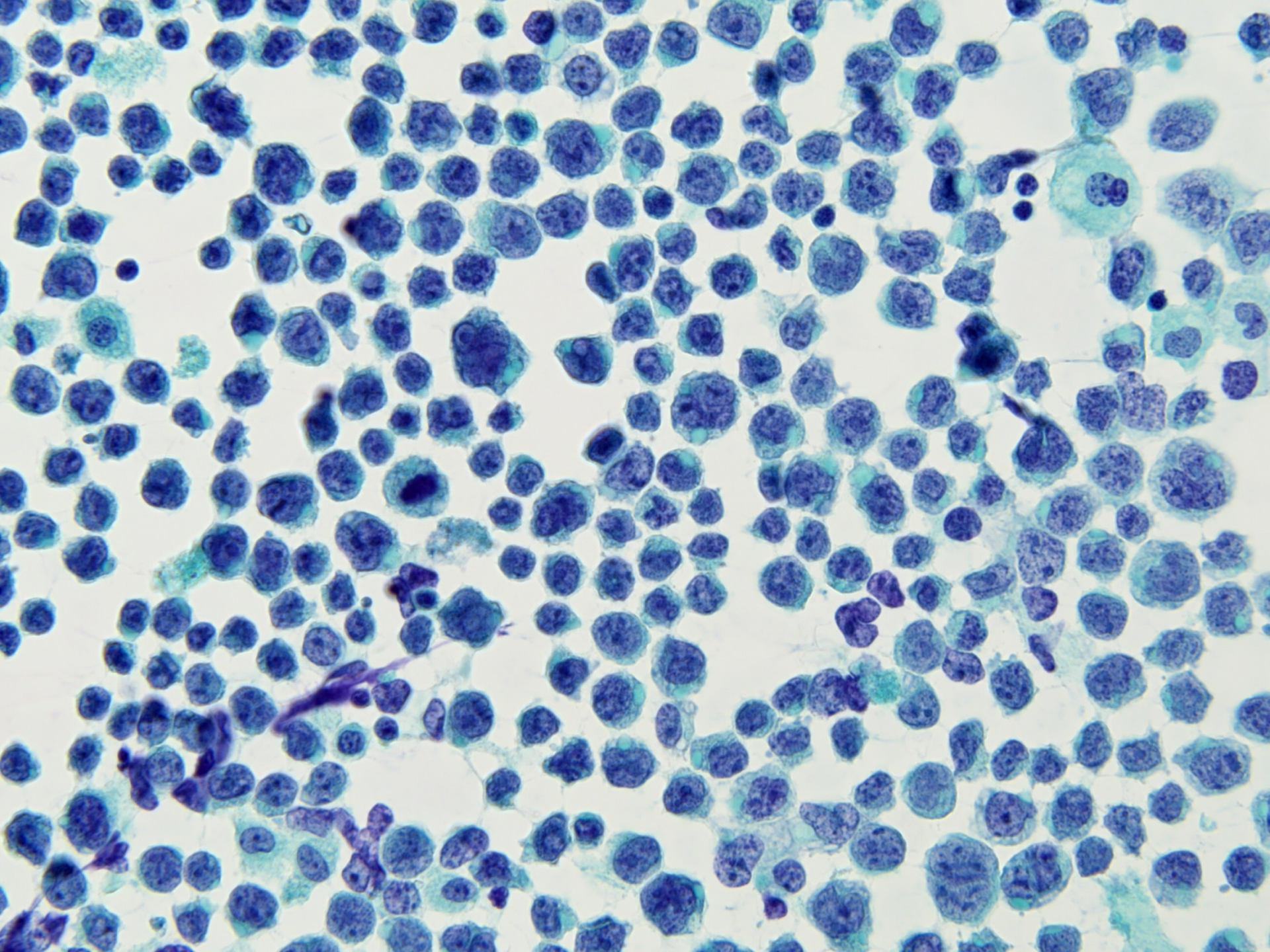
- Monomorphic or Polymorphic
- Index of suspicion: Patients with a history or a clinical suspicion of lymphoma

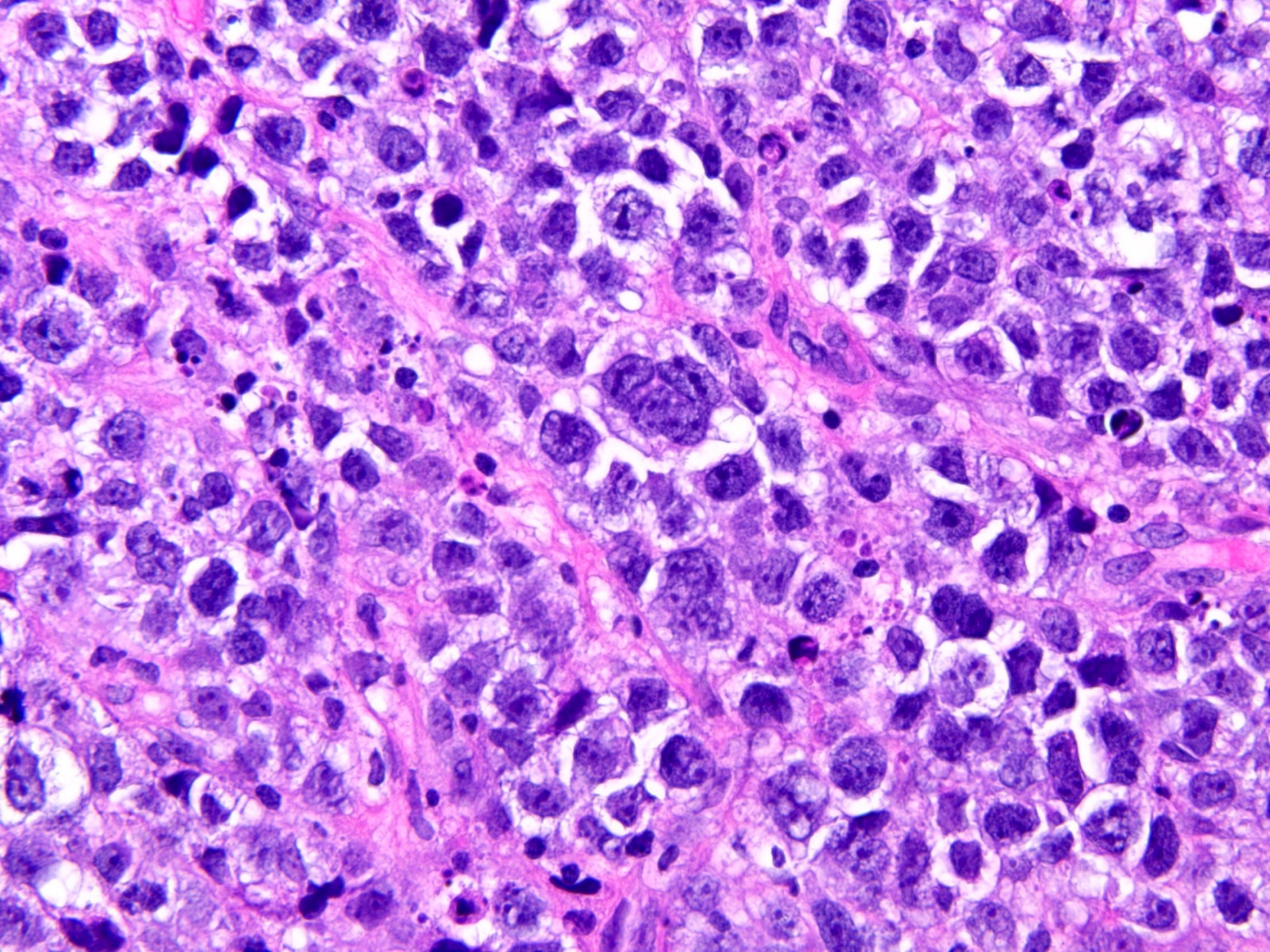
## High-power examination

- Cellular size: Large cell predominant pattern (>3-4X of a mature small lymphocyte or RBC)
- Cellular atypia: Irregular or lobulated nuclei, distinct nucleoli, anaplastic cells with kidney-shaped nuclei or multi-nucleation; cytoplasmic features (vacuoles or granules) in Romanowsky-type stains
- Cytoplasmic features: vacuoles, granules
- Background: Cellular apoptosis, necrosis or degeneration
- Lymphoglandular bodies (LGB): distinct feature from metastatic carcinomas


75 y/o female, pleural effusion







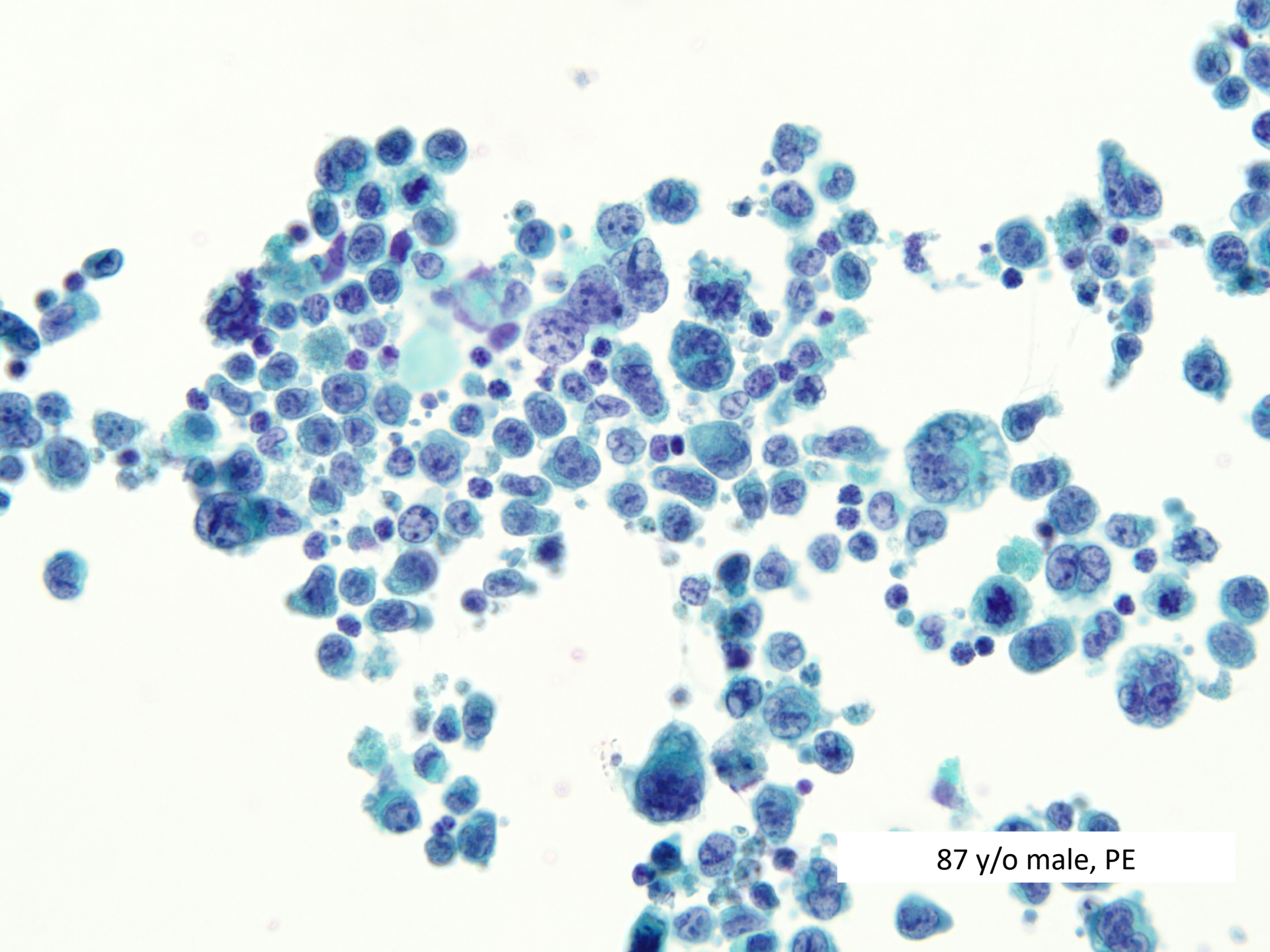
# Diffuse large B-cell lymphoma

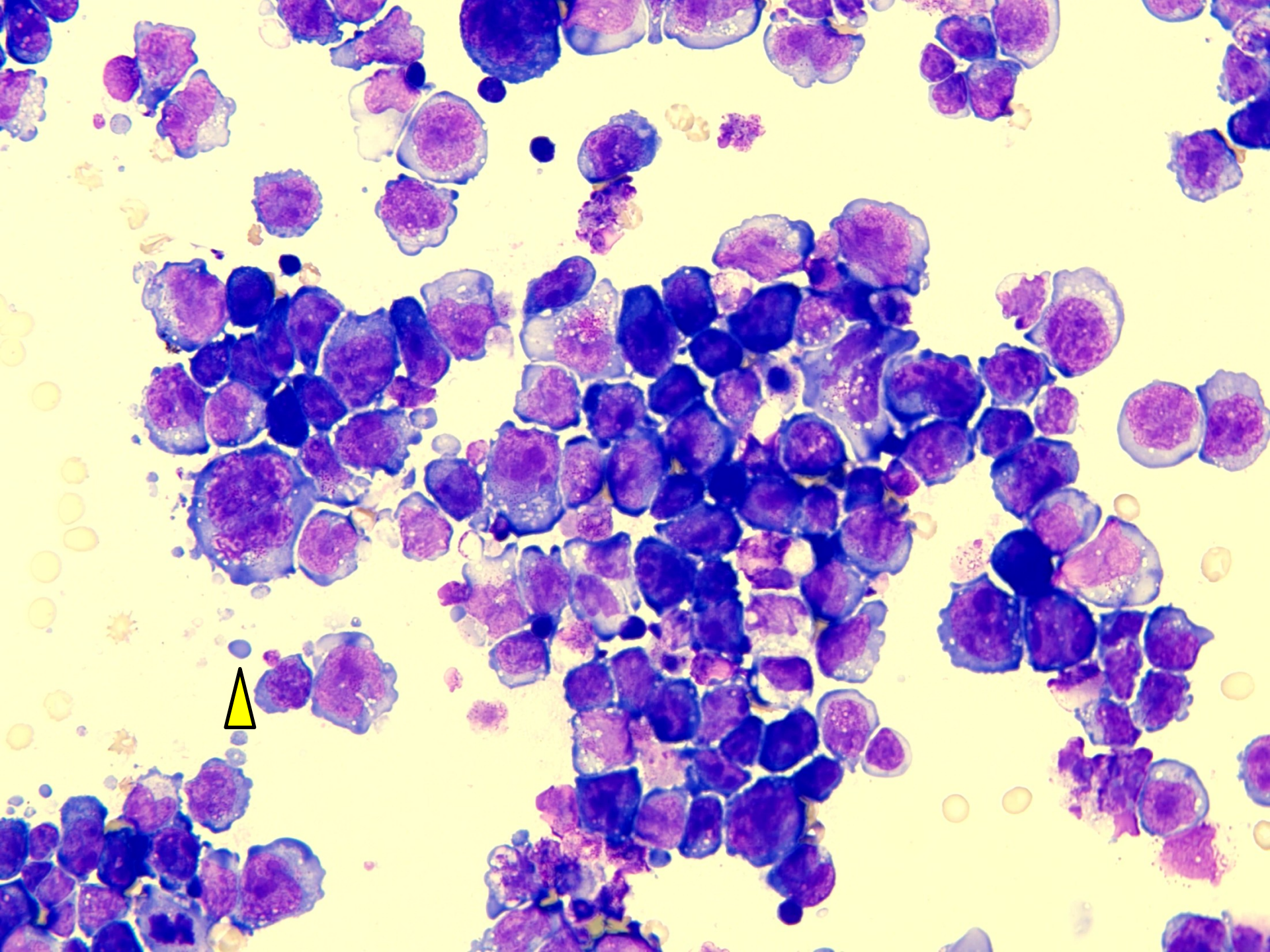
## Low/medium-power examination of the cellular pattern:

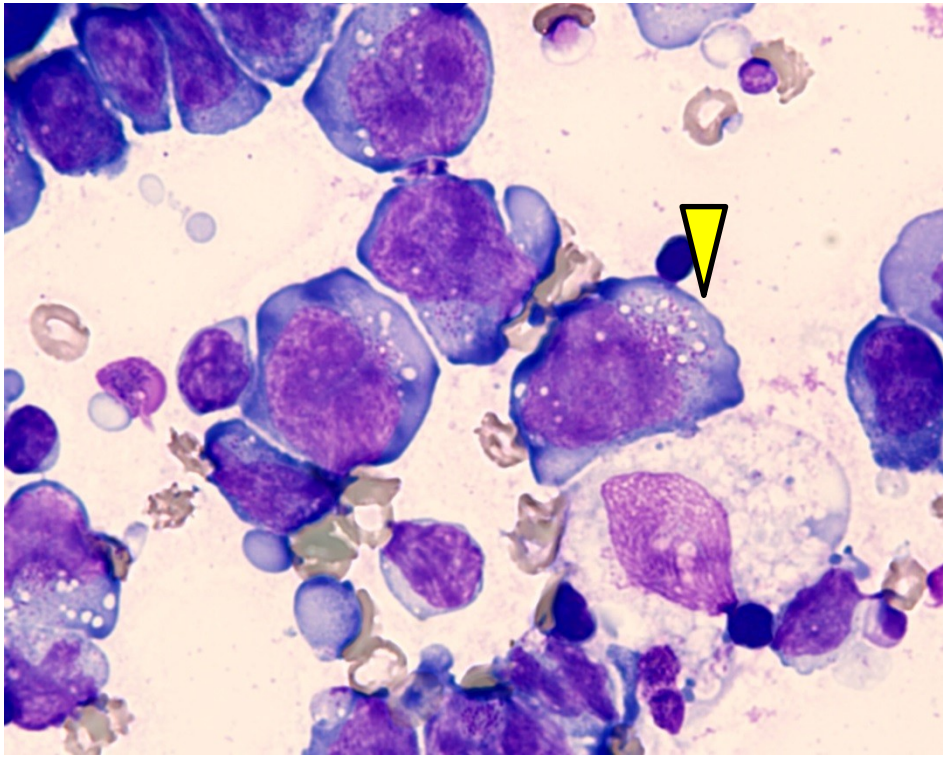
- Monomorphic or Polymorphic
- Index of suspicion: Patients with a history or a clinical suspicion of lymphoma

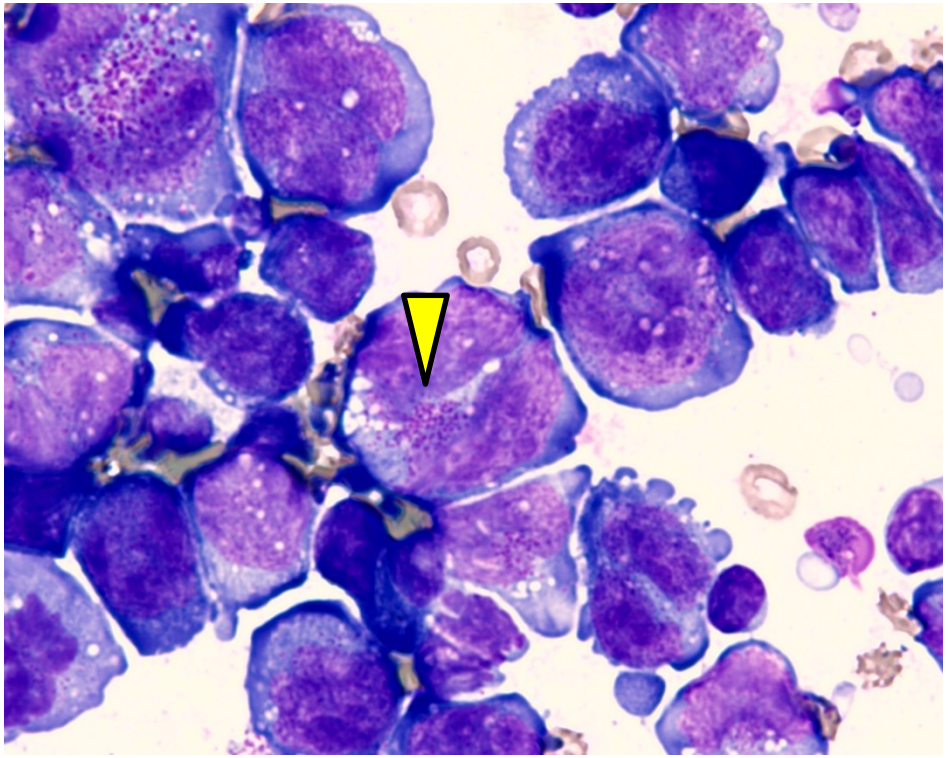
## High-power examination

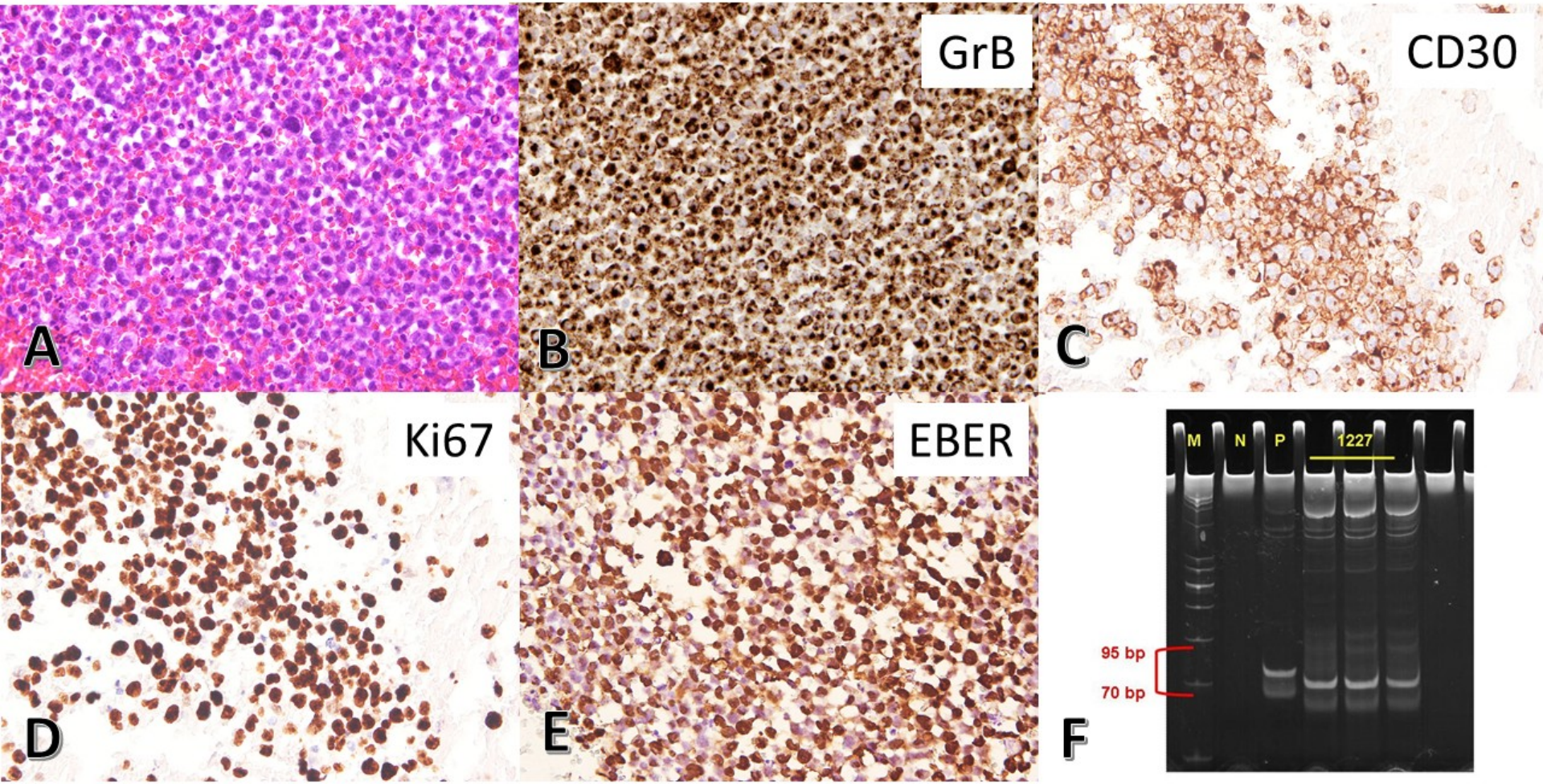
- Cellular size: Large cell predominant pattern (>3-4X of a mature small lymphocyte or RBC)
- Cellular atypia: Irregular or lobulated nuclei, distinct nucleoli, anaplastic cells with kidney-shaped nuclei or multi-nucleation; cytoplasmic vacuoles in Romanowsky-type stains
- Background: Cellular apoptosis, necrosis or degeneration
- Lymphoglandular bodies: distinct feature from metastatic carcinomas


87 y/o male, PE










*Liu CY, Chen BJ, Chuang SS; Taiwan Hematopathology Study Group. Malignant effusions from extranodal NK/T-cell lymphomas are frequently of anaplastic morphology with azurophilic granules and of T-cell lineage. Diagn Cytopathol. 2020 May;48(5):453-463.*

# Extranodal NK/T-cell lymphoma

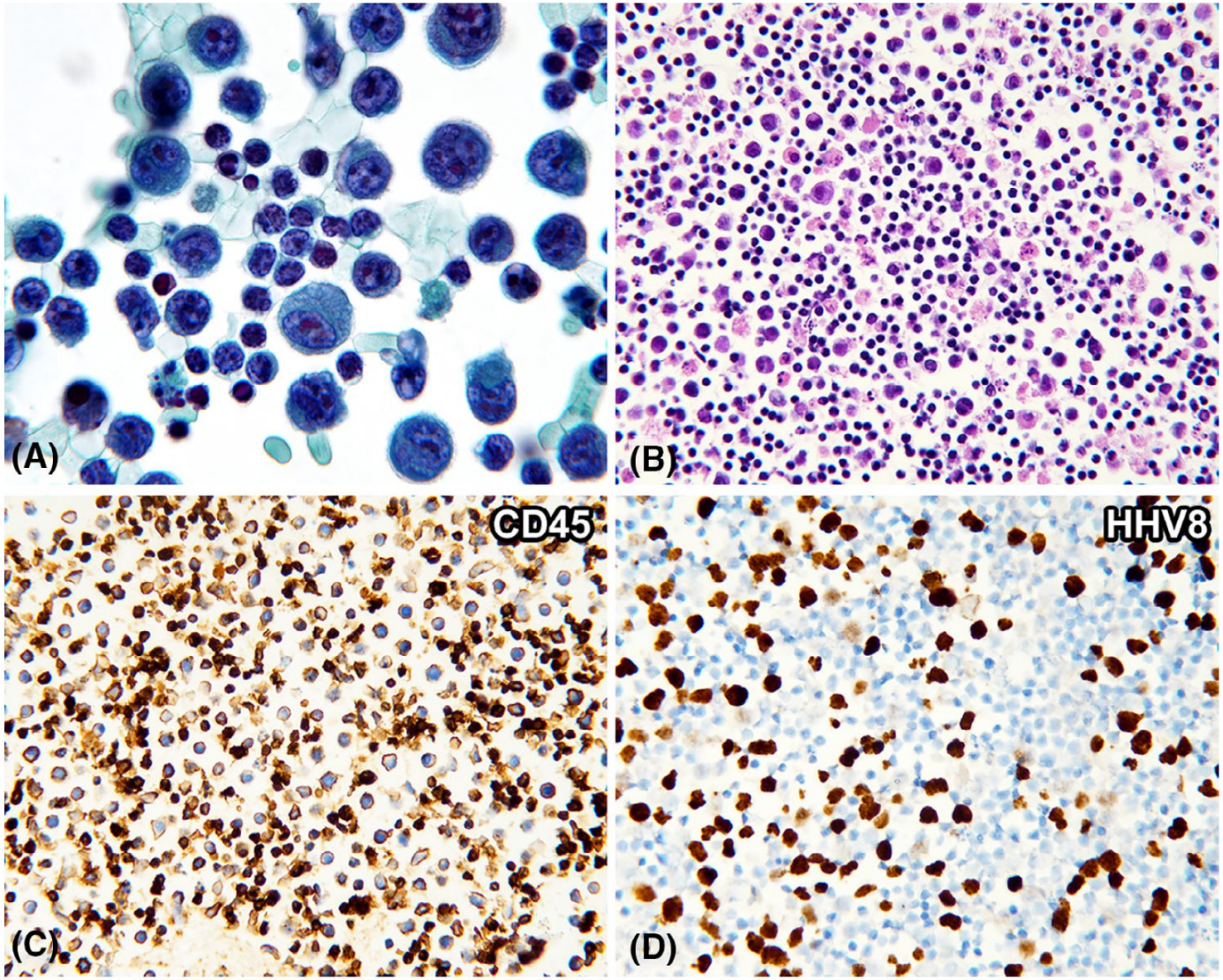
## Low/medium-power examination of the cellular pattern:

- Monomorphic or Polymorphic
- Index of suspicion: Patients with a history or a clinical suspicion of lymphoma

## High-power examination

- Cellular size: Large cell predominant pattern (>3-4X of a mature small lymphocyte or RBC)
- Cellular atypia: Irregular or lobulated nuclei, distinct nucleoli, anaplastic cells with multi-nucleation; cytoplasmic features (azurophilic granules) in Romanowsky-type stains
- Background: Cellular apoptosis, necrosis or degeneration

# Primary effusion lymphoma




Wang RC, Chen YH, Chen BJ, Chuang SS. The cytopathological spectrum of lymphomas in effusions in a tertiary center in Taiwan. Diagn Cytopathol. 2021 Feb;49(2):232-240.

# Pleomorphic lymphomatous cells

Pleomorphic lymphomatous cells may represent various aggressive lymphoma types such as PEL, EBL, DLBCL, anaplastic large cell lymphoma (ALCL), and T and NK/T-cell lymphomas.

Cytoplasmic characteristics may provide additional clues to the identification of tumor cells.

Increased mitotic figures, tingible body macrophages, and abundant apoptotic bodies or nuclear debris indicate rapid cellular turnover and favor a neoplastic process.

# Ancillary tests

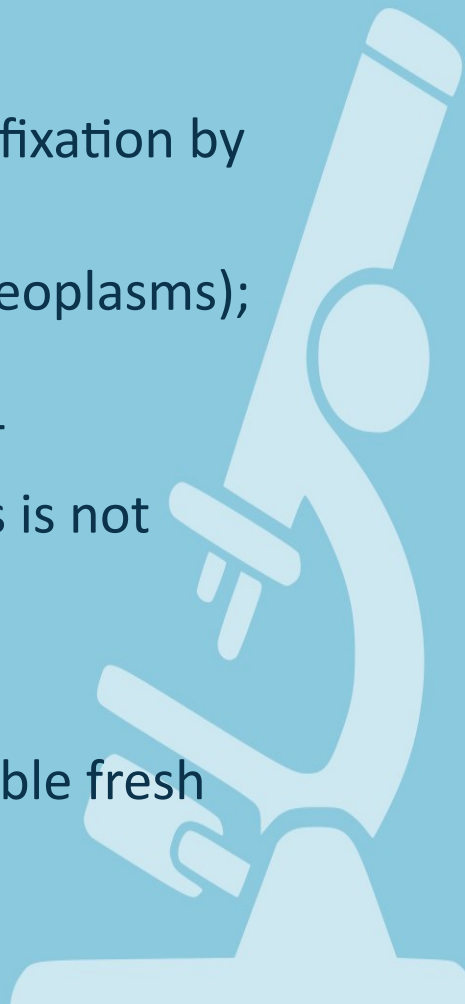
- Appropriate specimens to determine specimen adequacy for ancillary tests
- Immunophenotyping by flow cytometry:

  The prerequisite for this assay is viable cells without fixation by alcohol or formalin.

  Monotypic light chain expression (low-grade B-cell neoplasms); clonality in T-cell neoplasms

  Reactive effusions consists practically exclusively of T lymphocytes; a large population of CD3-positive cells is not indicative of a T-cell lymphoma
- Immunocytochemistry using cell block:

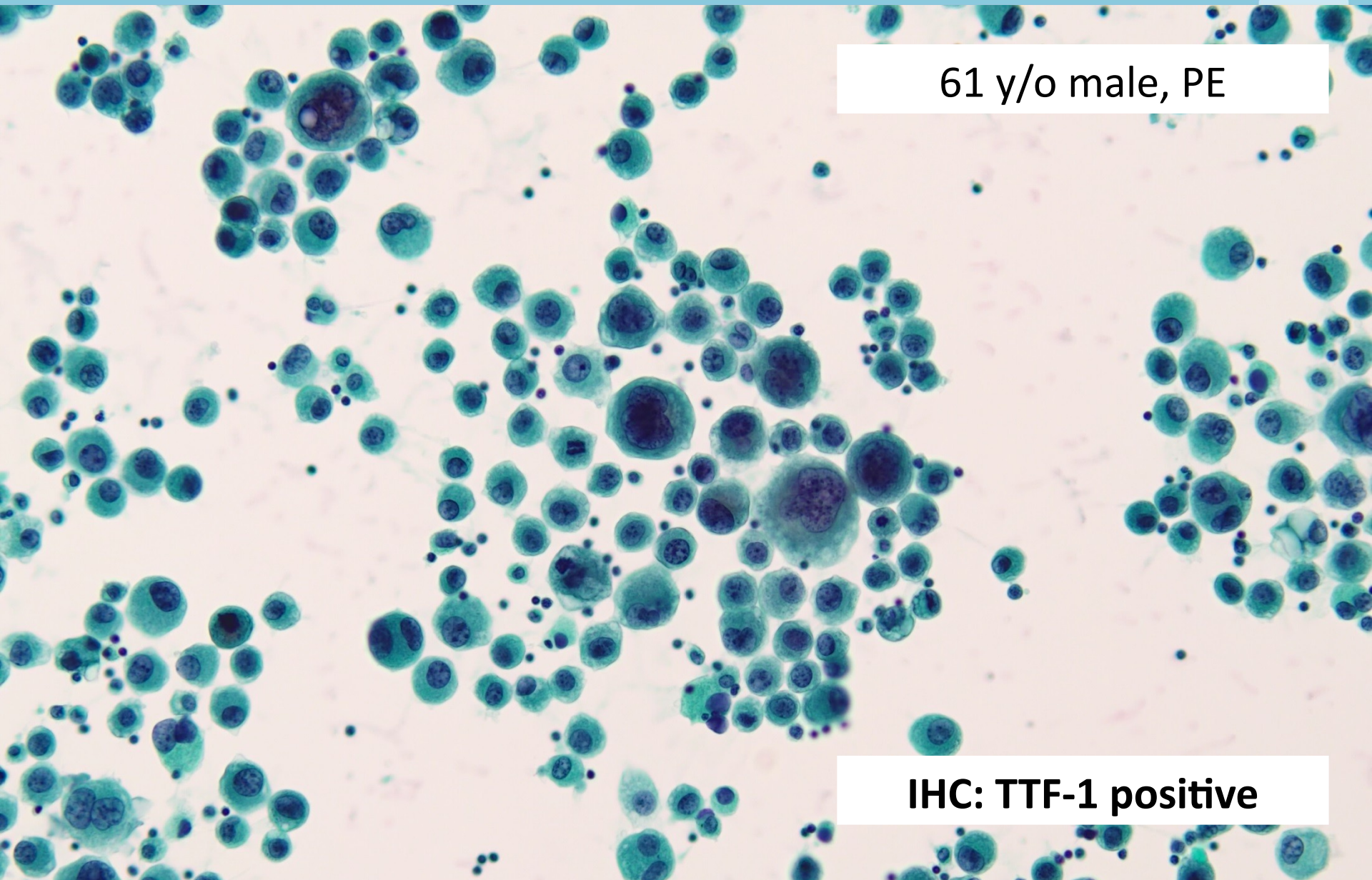
  Preliminary/early diagnosis when there are no available fresh samples for FC analysis



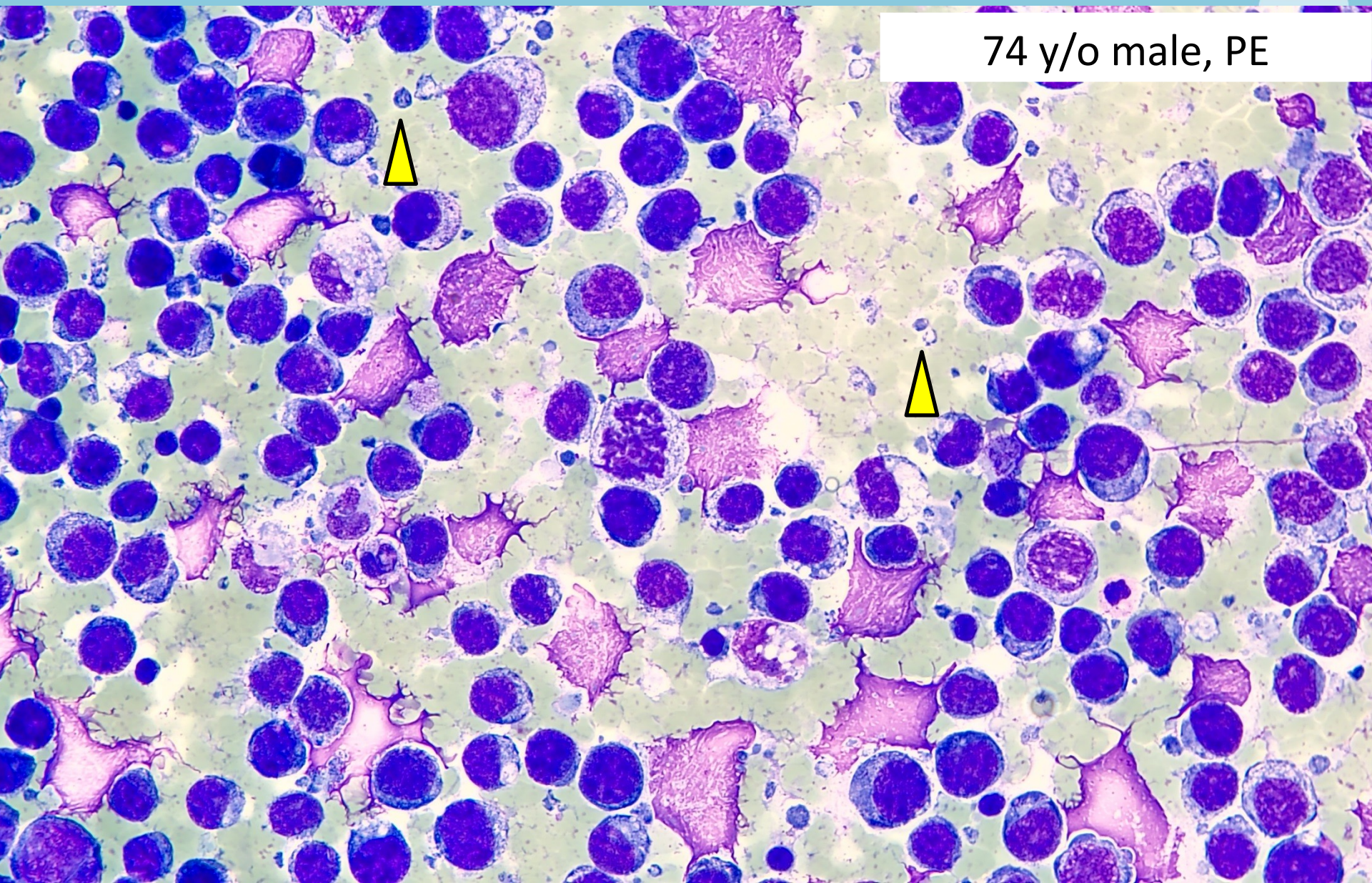
# Ancillary tests

- Fluorescence in situ hybridization (FISH) :
  MYC translocation in patients with a history of B-cell lymphoma or Burkitt lymphoma
- In situ hybridization for EBV-encoded mRNA (EBER) :
  Characterization of certain types of large cell lymphomas such as PEL, EBV-positive DLBCL and ENKTL
- Allele-specific polymerase chain reaction for MYD88 L265P mutation analysis:
  Distinguishing benign from lymphomatous effusion by lymphoplasmacytic lymphoma

# Non-cohesive and large atypical cells in effusions

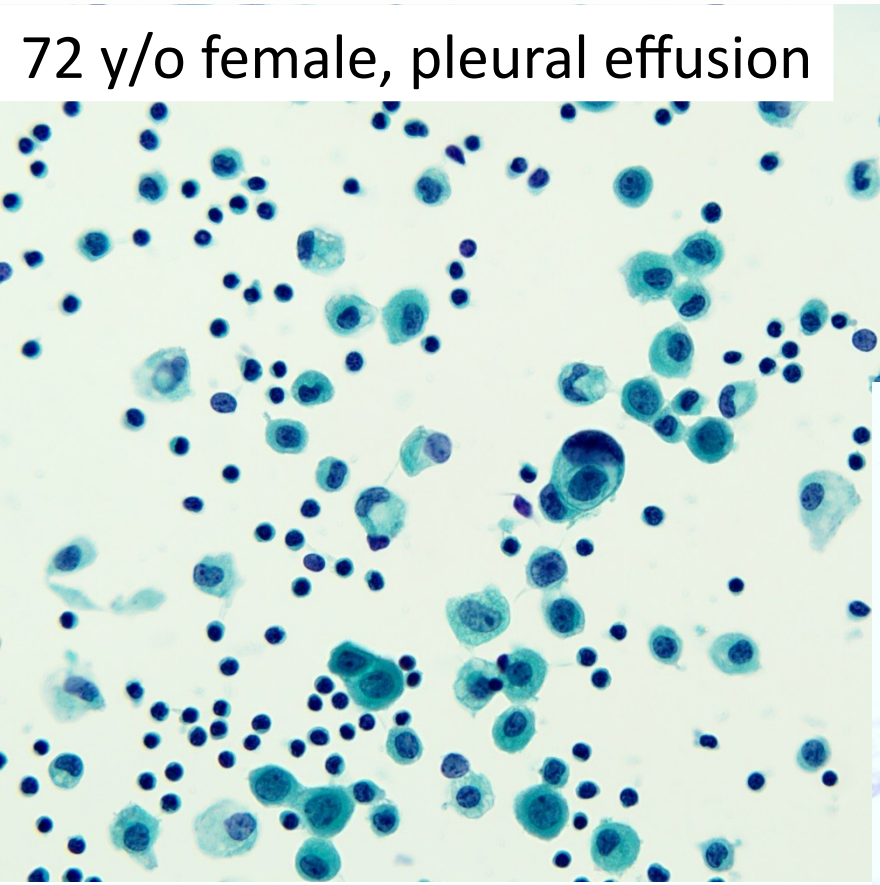


# Non-cohesive and large atypical cells in effusions

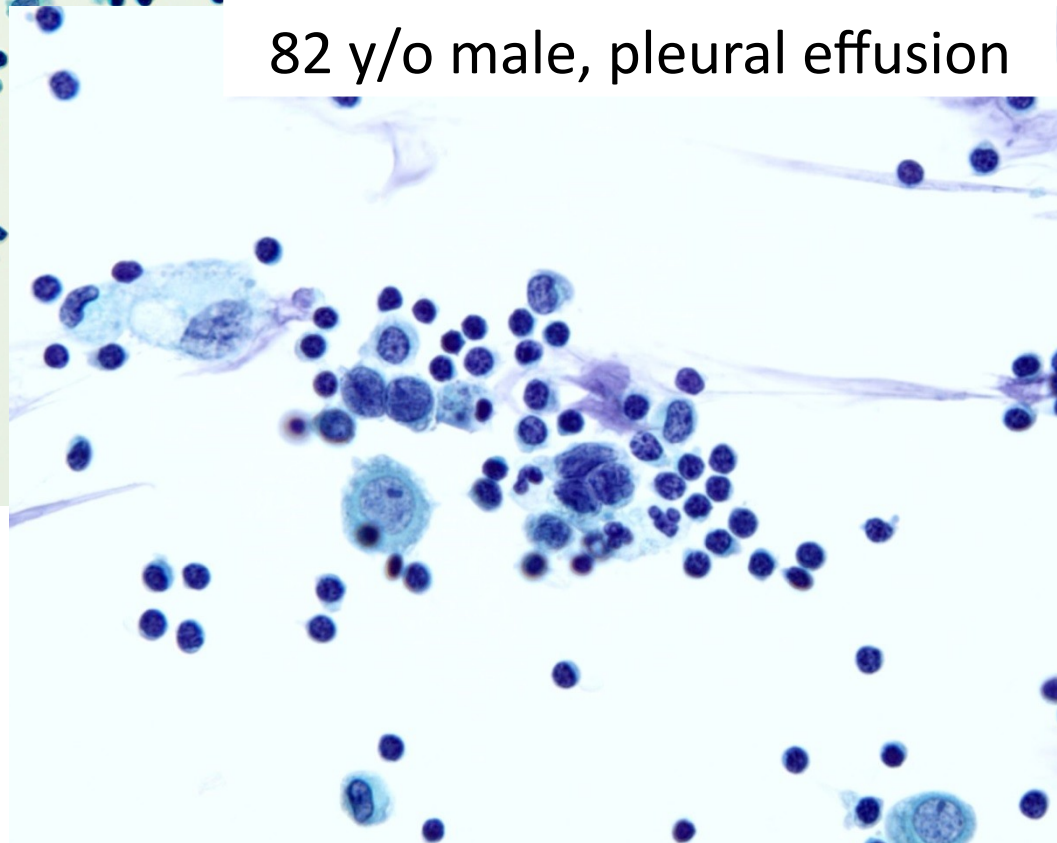


# Non-cohesive and small atypical cells in effusions

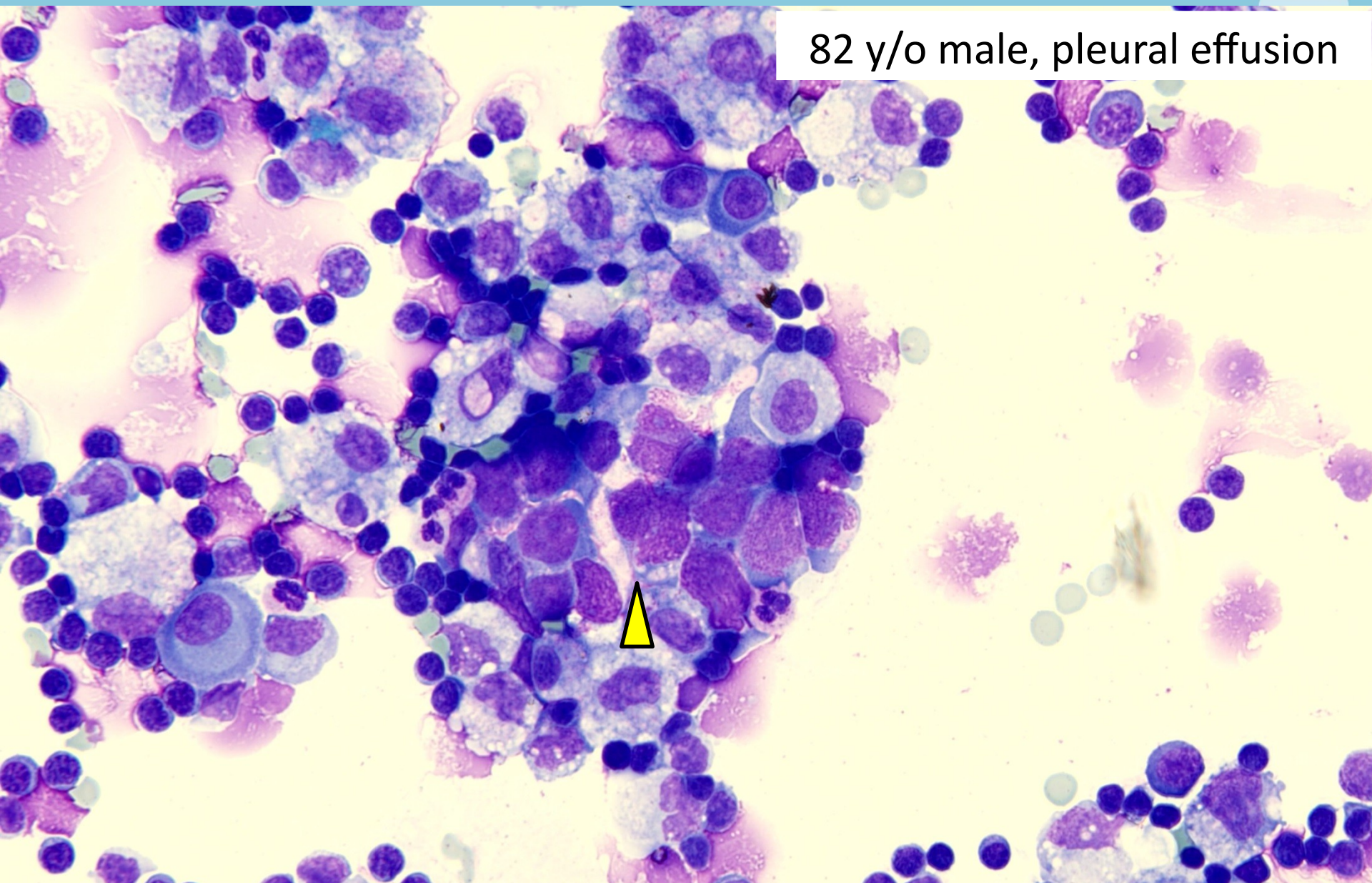
72 y/o female, pleural effusion



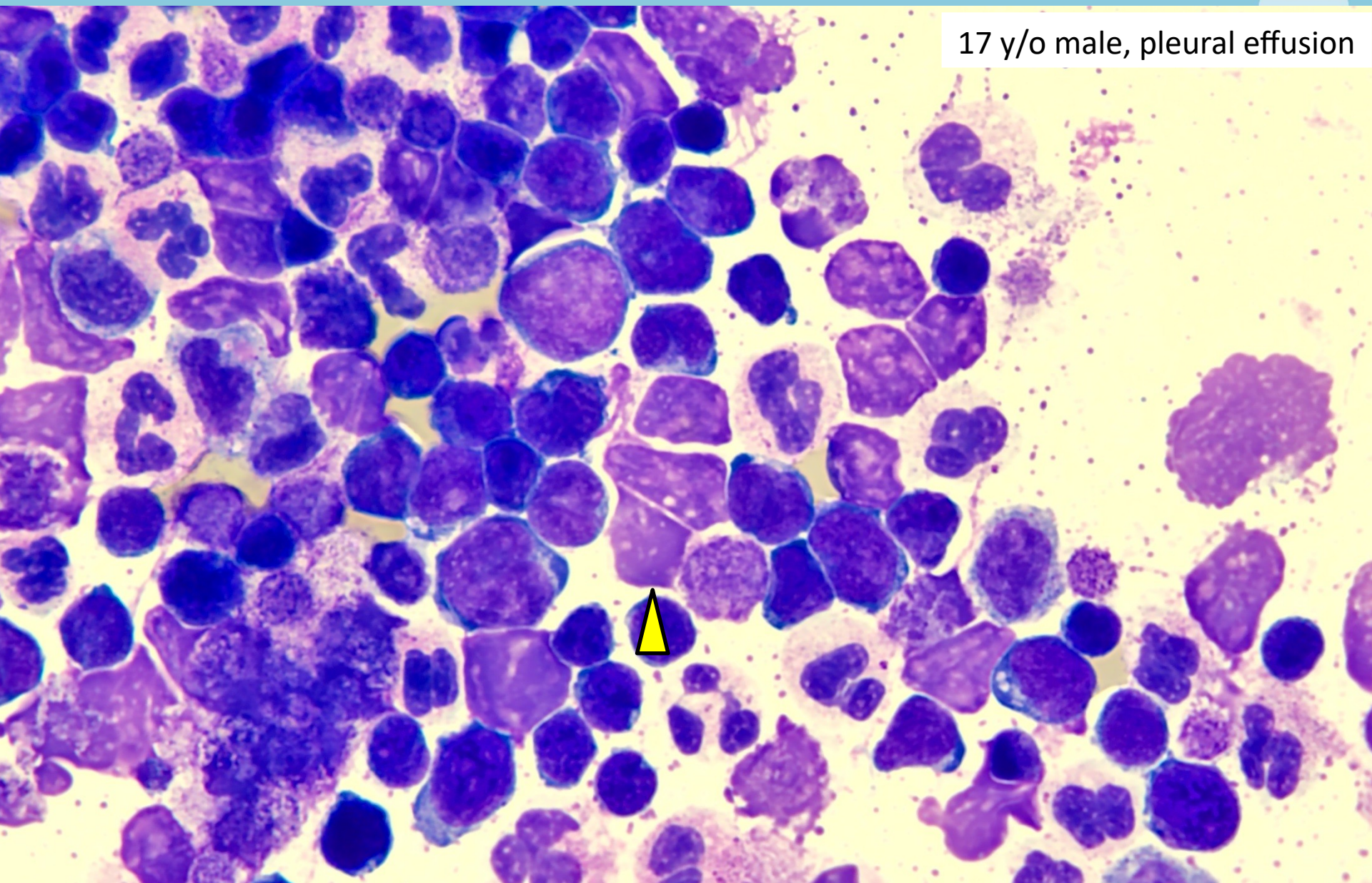
82 y/o male, pleural effusion



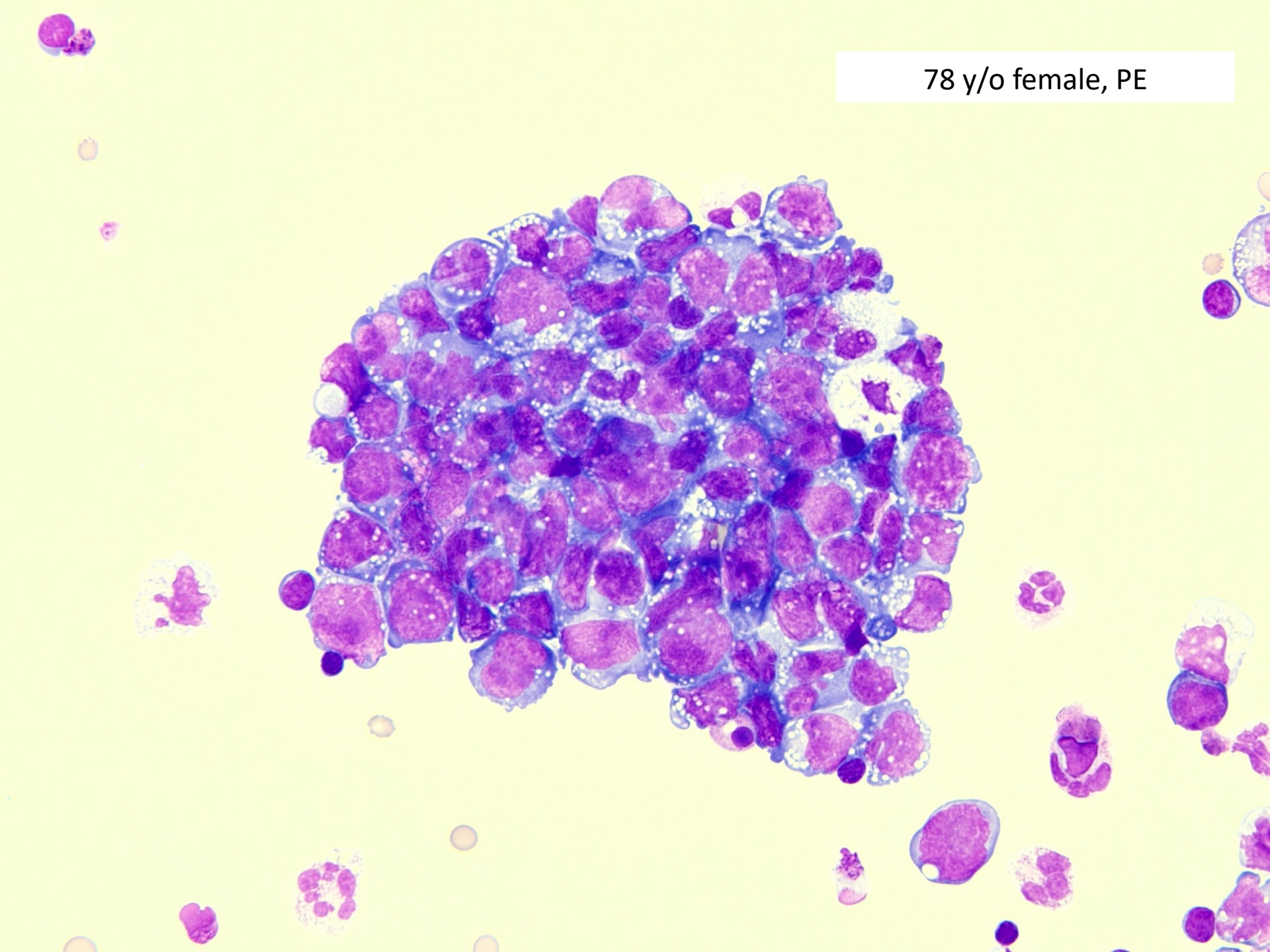
# Non-cohesive and small atypical cells in effusions

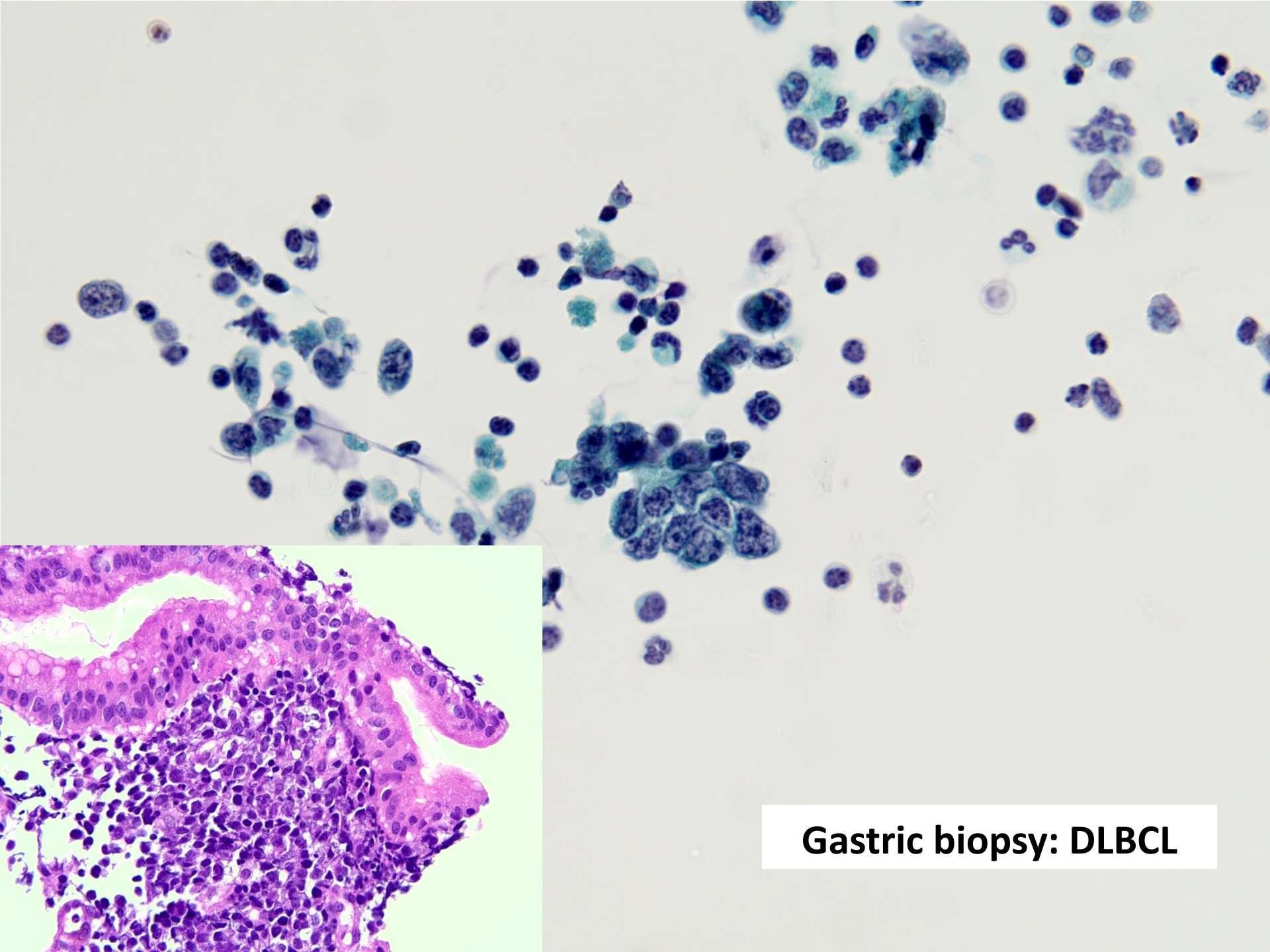


# Non-cohesive and Small Atypical Cells in Effusions



78 y/o female, PE

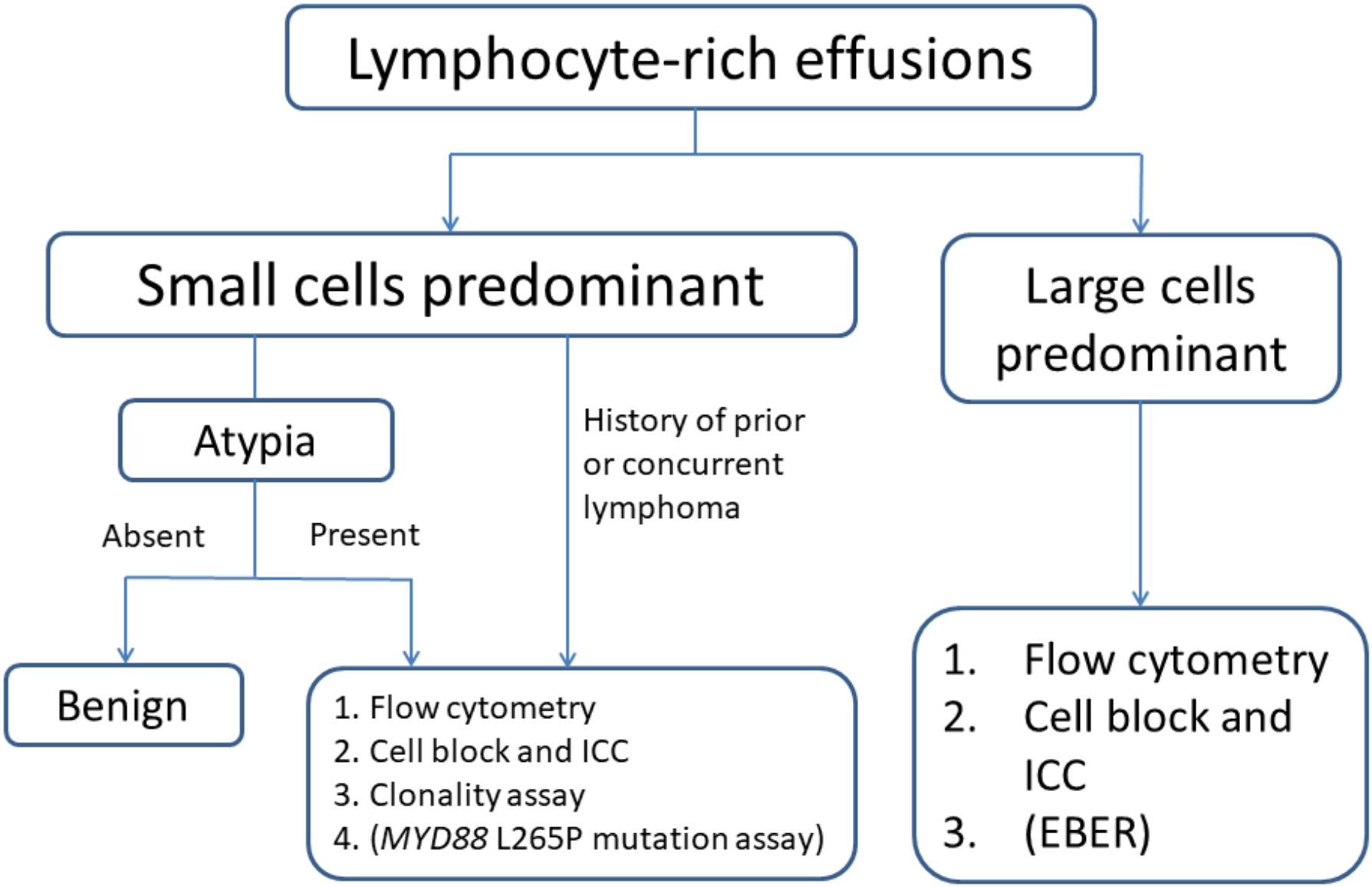




Gastric biopsy: DLBCL

# Take home messages

- Malignant effusion as a complication of lymphoma / leukemia is not uncommon.
- The assessment of lymphocyte-rich effusions depends on evaluation of morphological, immunophenotypic, genetic features and clinical information.
- When the etiology of a microscopically non-neoplastic effusion remains unexplained, and particularly if it is accompanied by lymphadenopathy, ancillary tests should be performed to rule out or prove the presence of an indolent lymphoma.
- The ancillary studies are guided by the initial cytological evaluation and clinical information.




*Liu CY, Chuang SS. A Simple and Practical Guide for Triaging Lymphocyte-rich Effusions for Ancillary Studies. Adv Anat Pathol. 2021 Mar 1;28(2):94-104.*

感謝奇美醫院莊世松主任指導

Special thanks to Taiwan Hematopathology Study Group