When morphology and immunocytochemistry align

Pleural fluid

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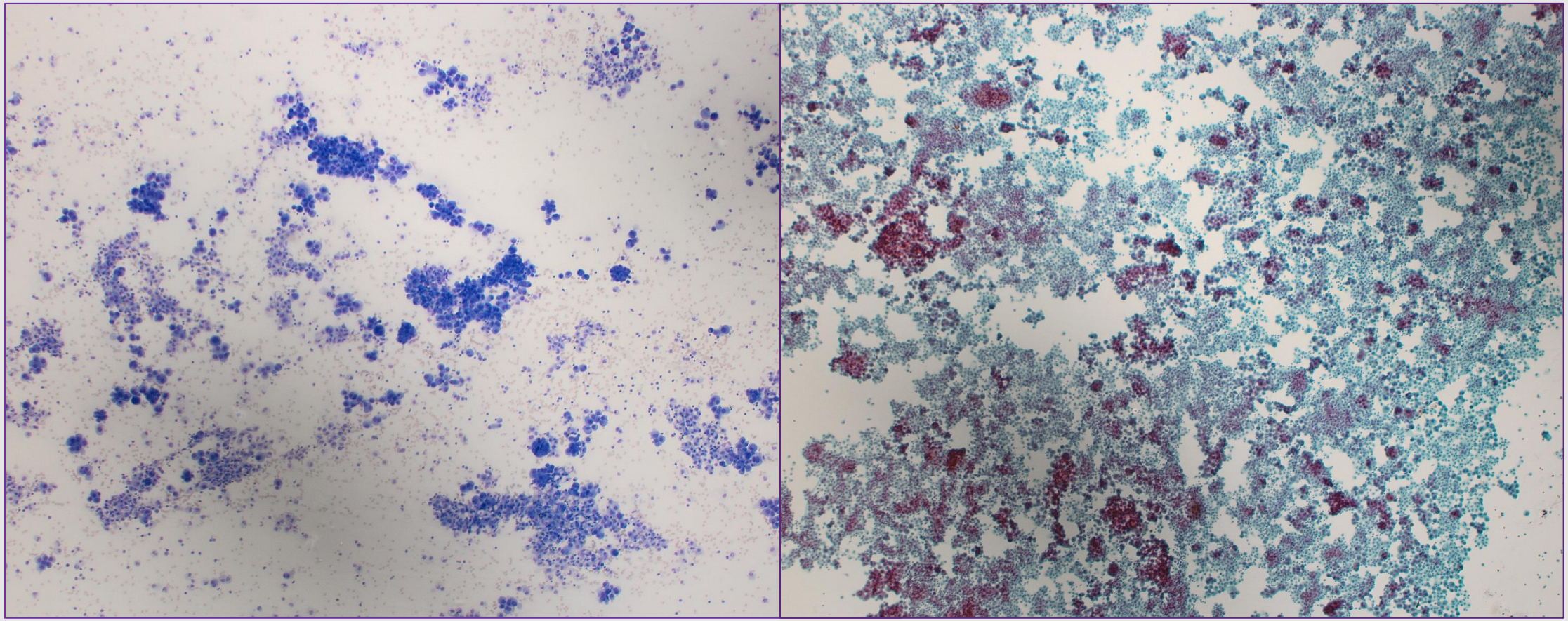
Patient

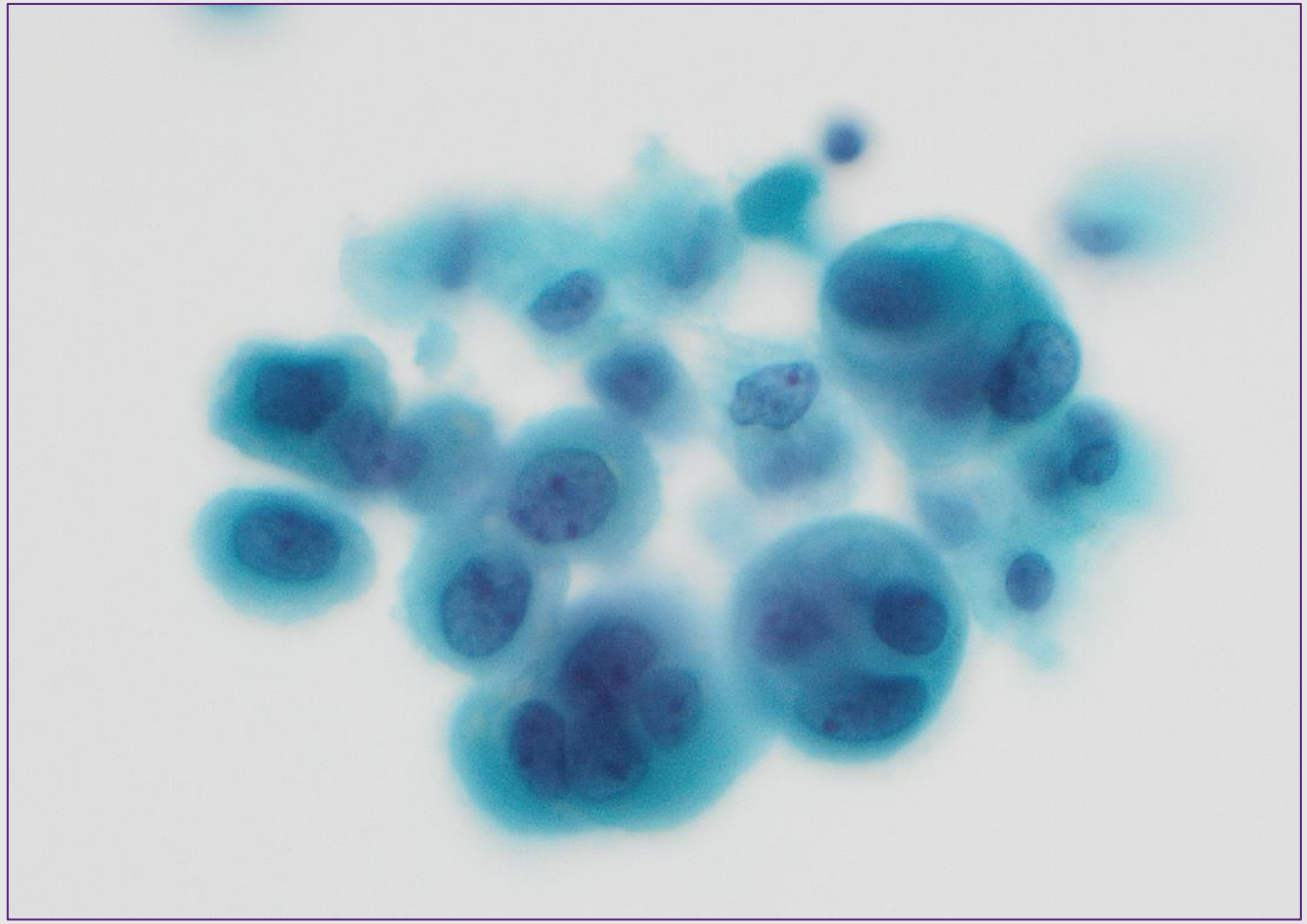
- Male 81 yrs. Smoker
- Presented in ED with shortness of breath.
- Chest Xray – large left pleural effusion suspicious of malignancy
- Clinical details on request form ? Empyema ? Malignancy

Pleural fluid

Cellular with both large fragments / 3D clusters and single cells

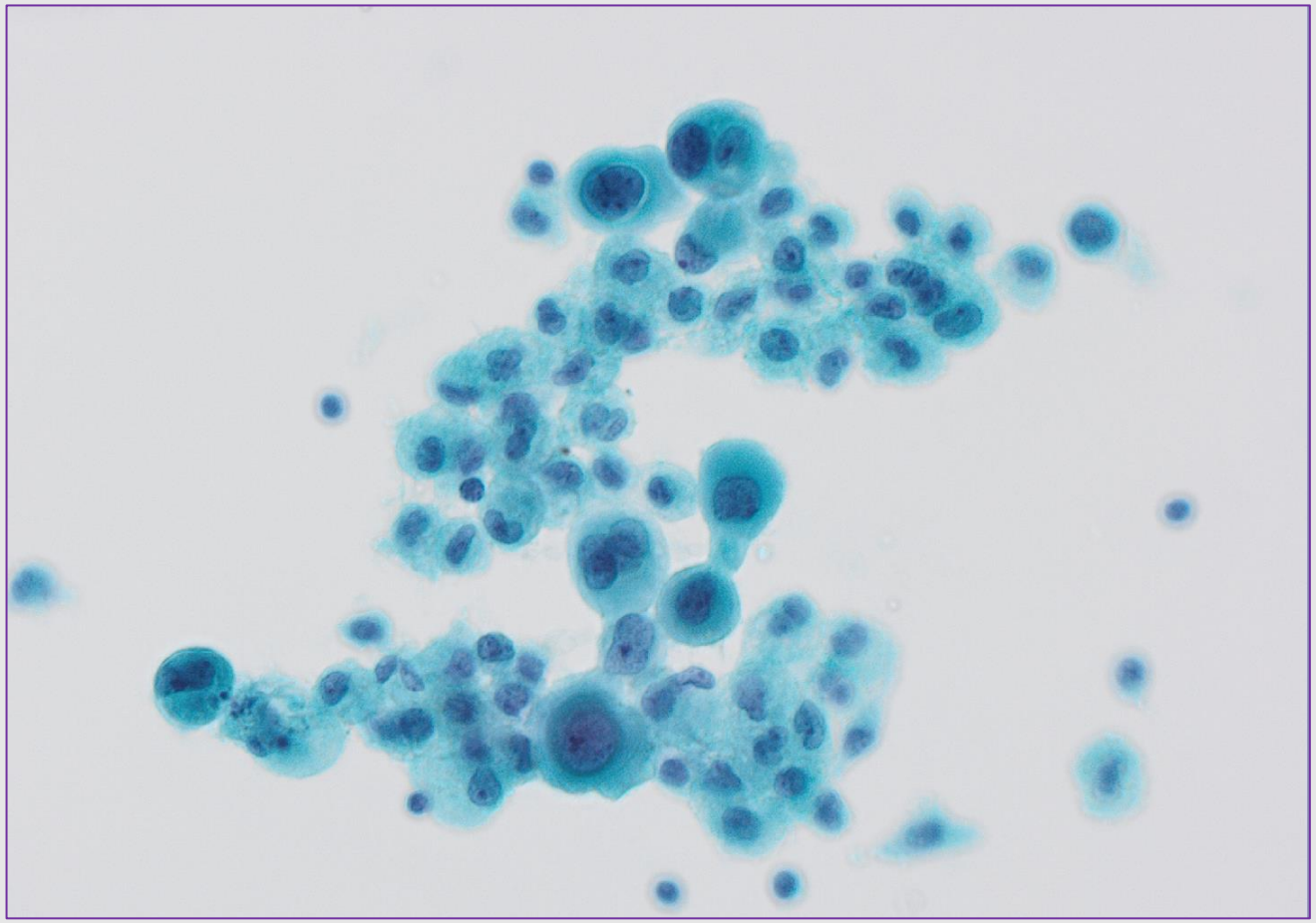


Pleural fluid



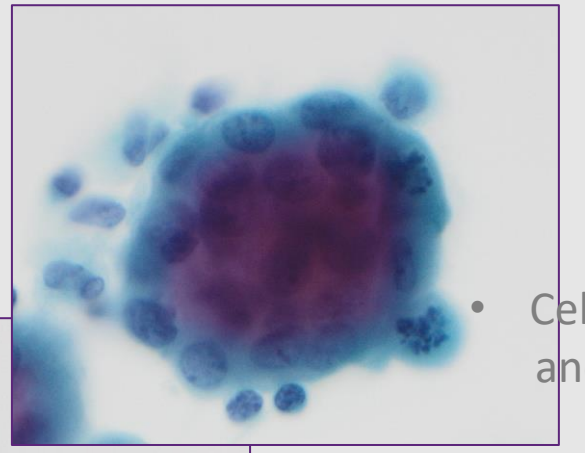
- Cellular with both large fragments / 3D clusters and single cells
- Centrally located nuclei
- Moderate amounts of cytoplasm
- Multi nucleation

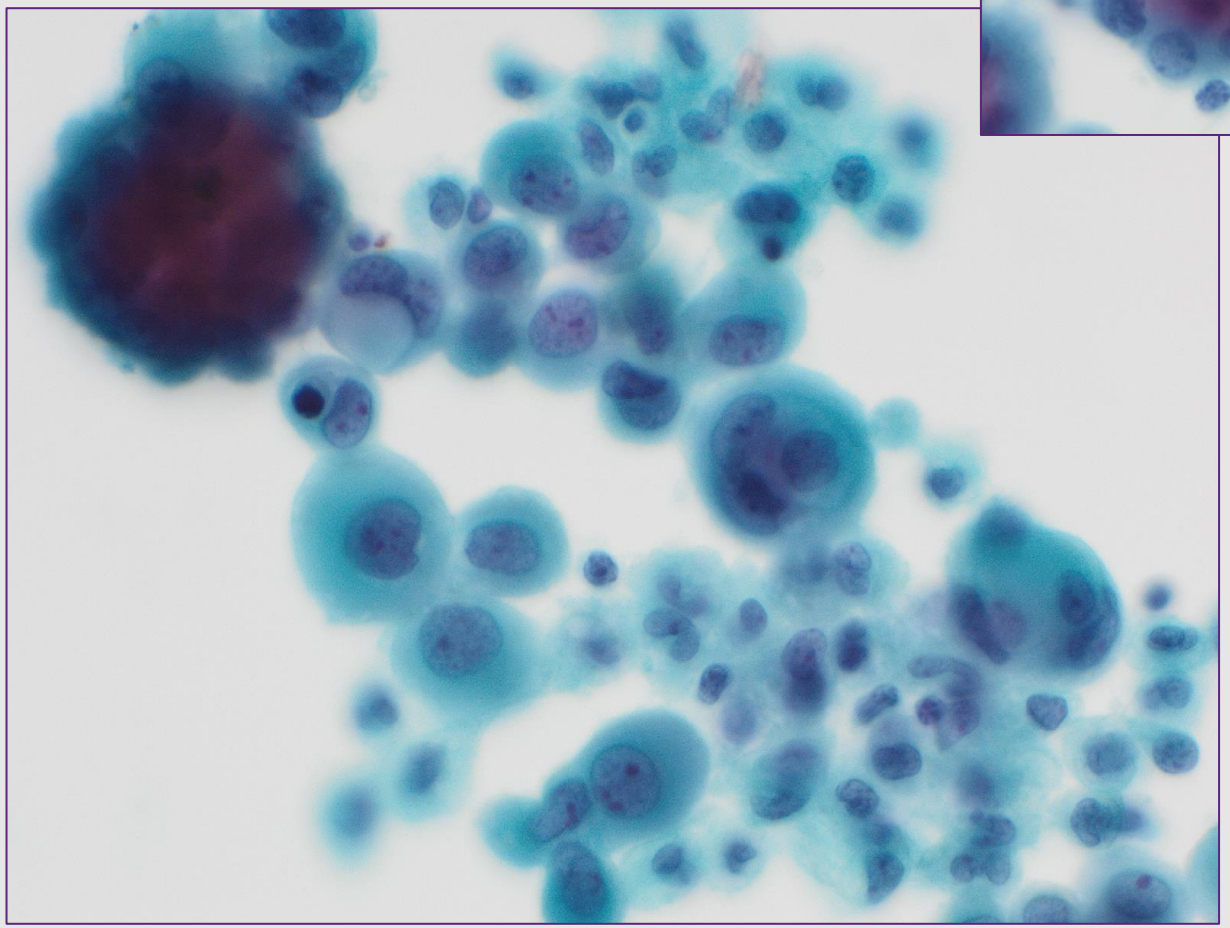
Pleural fluid



- Cellular with both large fragments / 3D clusters and single cells
- Centrally located nuclei
- Moderate amounts of cytoplasm
- Multi nucleation
- Prominent nucleoli that vary in size and shape
- Monotonous population of cells with a wide variation in size
- Enlarged nuclei with subtle atypia

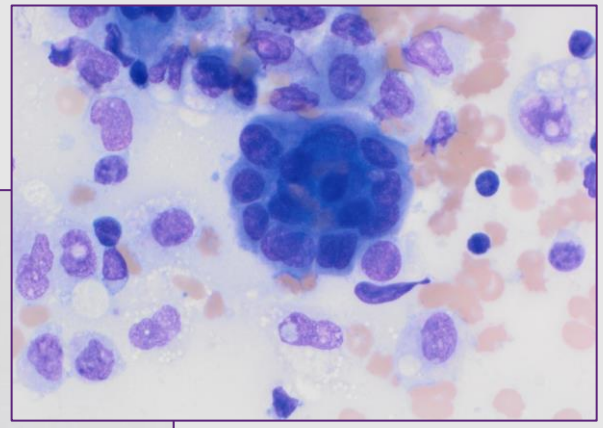
Pleural fluid

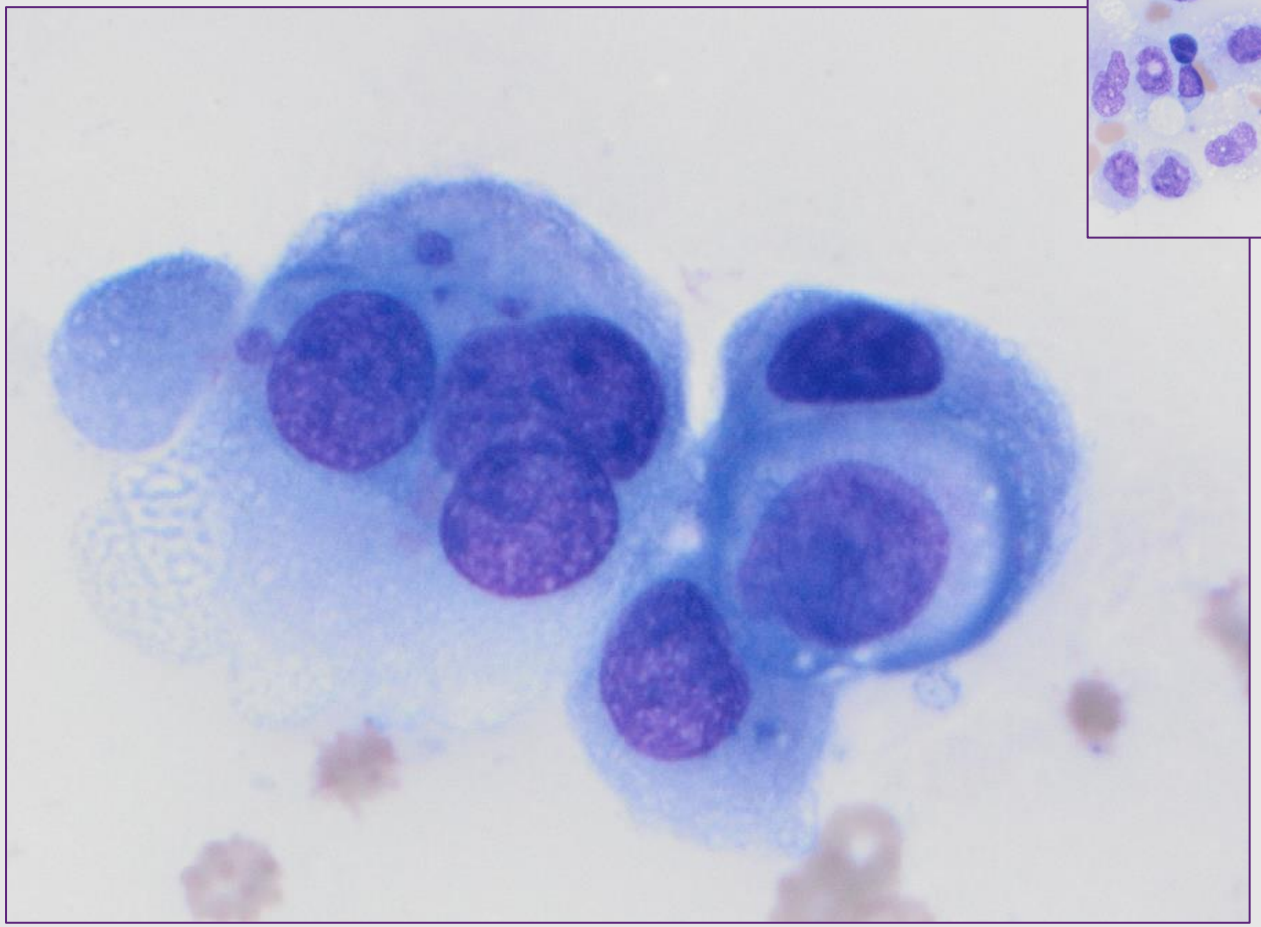




- Cellular with both large fragments / 3D clusters and single cells
- Centrally located nuclei
- Moderate amounts of cytoplasm
- Multi nucleation
- Prominent nucleoli that vary in size and shape
- Variation in cell size
- Enlarged nuclei with subtle atypia
- 3D clusters with scalloped borders

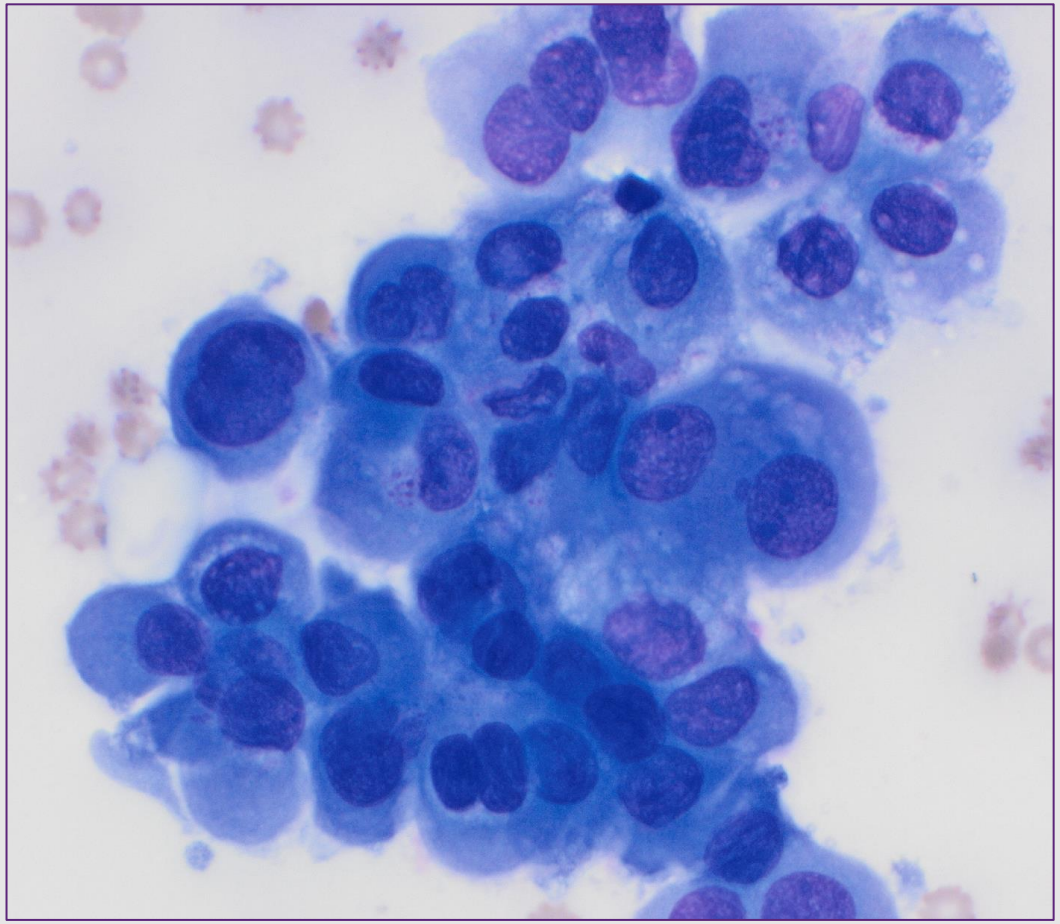
Pleural fluid





- Cell in cell arrangement – cell clasping
- 3D clusters with scalloped borders

Pleural fluid



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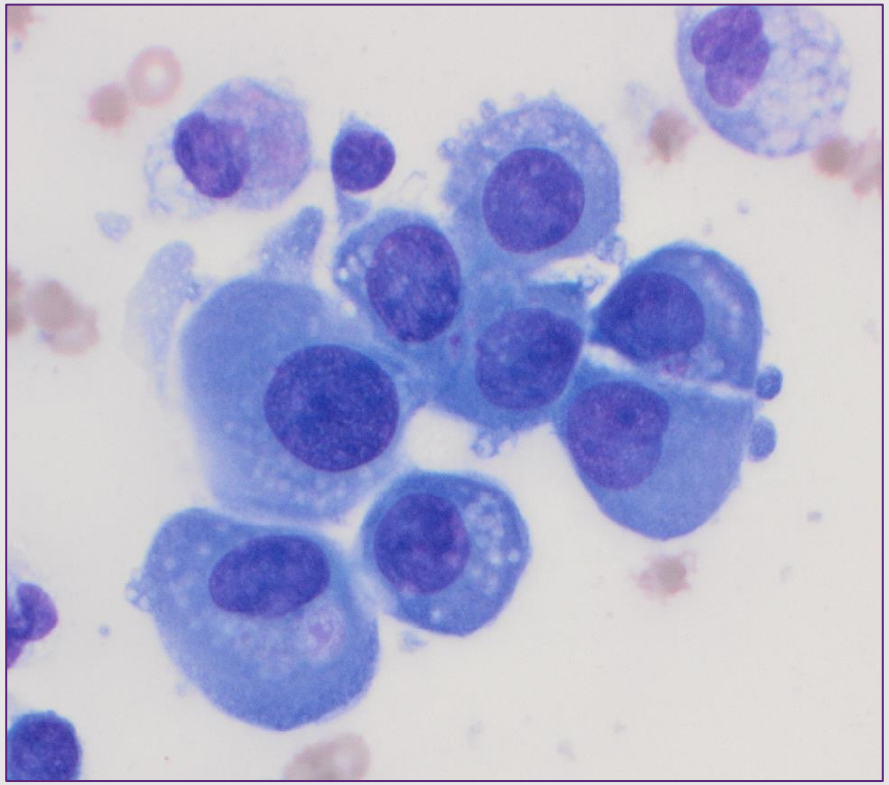
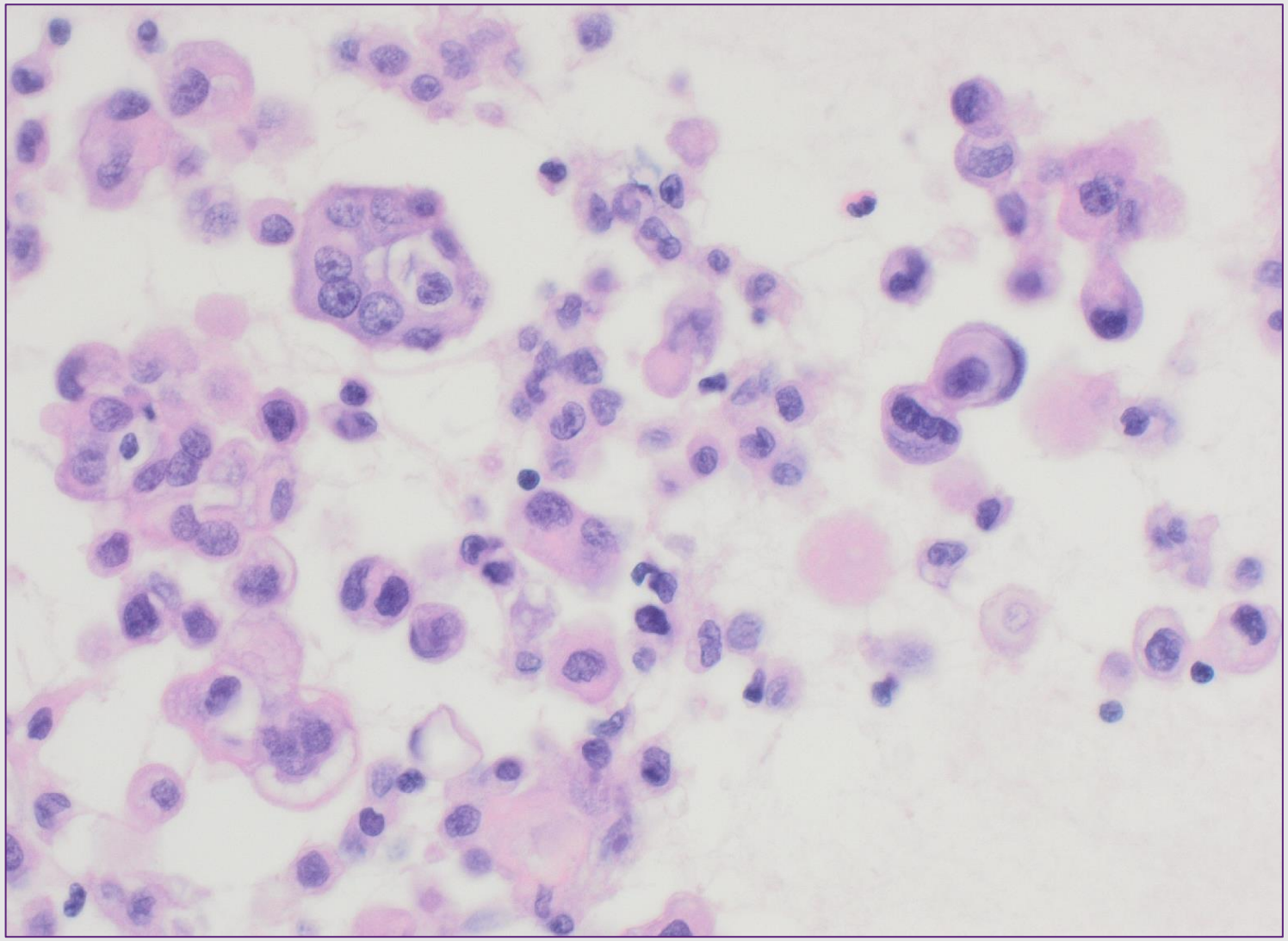
- 3D clusters with scalloped borders
- Two tone cytoplasm due to endo-ectoplasmic demarcation

Pleural fluid



- Two tone cytoplasm due to endo-ectoplasmic demarcation
- Cell in cell arrangement – cell clasping
- 3D clusters with scalloped borders
- Windowing

Pleural fluid – clot sample

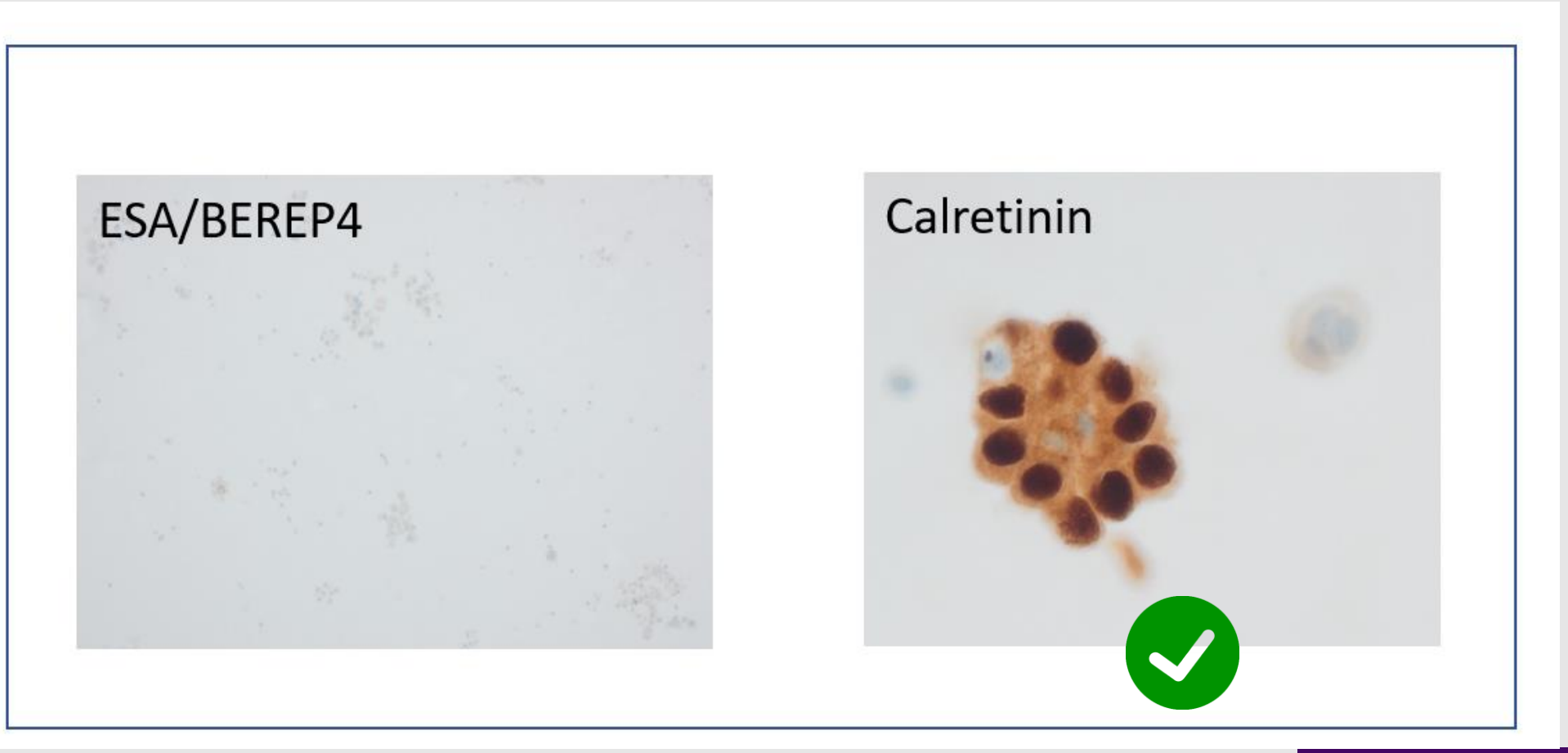


Morphological features are those of an atypical mesothelial proliferation

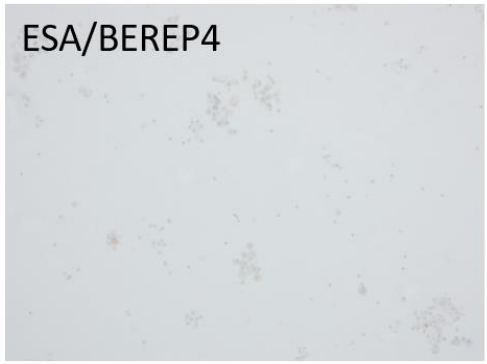
Leading to selection of a mesothelial immunocytochemistry panel

- Desmin
- EMA
- BAP1
- BerEP4

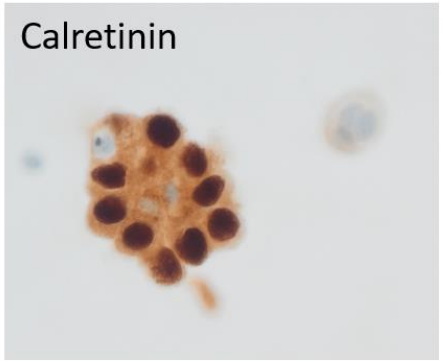
Immunocytochemistry



Immunocytochemistry

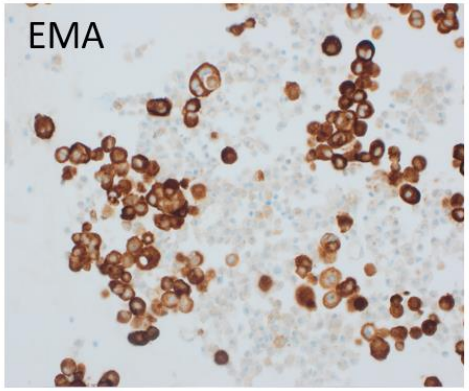


Negative

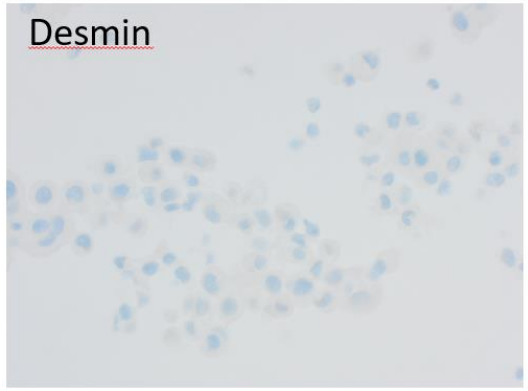


Positive

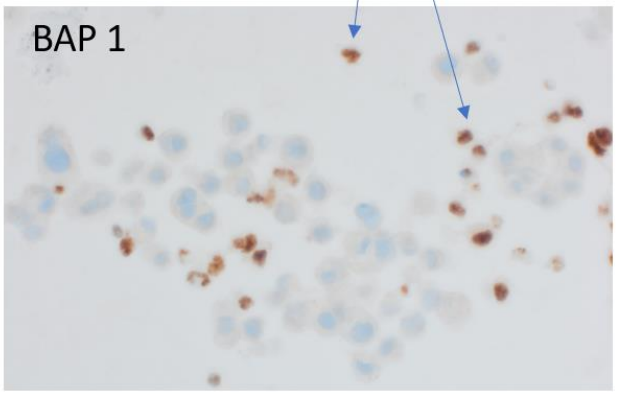
Inflammatory cells
- internal control



Positive



Negative



Loss of expression

The international reporting system for serous fluid cytology

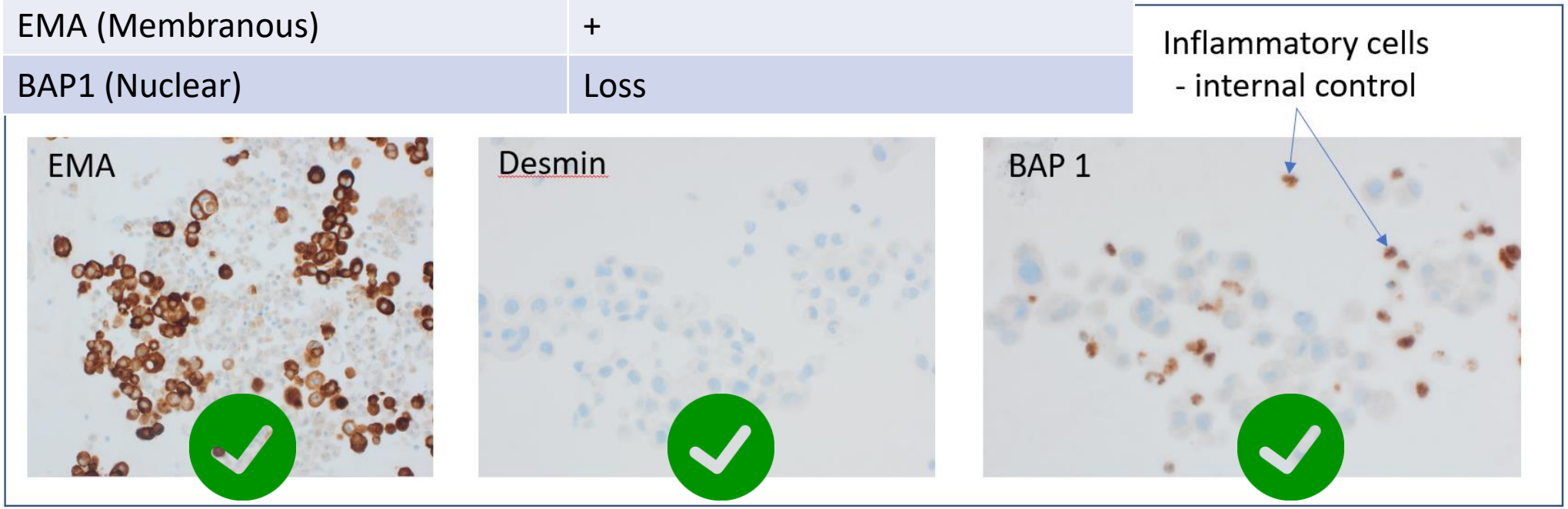
	Negative for Mesothelioma	Mesothelioma
Desmin (Cytoplasmic)	+	-
EMA (Membranous)	-	+
BAP1 (Nuclear)	+	Loss
MTA (IHC nuclear / FISH)	IHC - / FISH no deletion	IHC + / FISH: deletion detected
P16/CDKN2A (FISH)	No deletion	Deletion detected

* If Desmin, EMA and BAP1 are inconclusive then utilise MTA and P16/CDKN2A

BAC British Association for Cytopathology

The international reporting system for serous fluid cytology

	Mesothelioma
Desmin (Cytoplasmic)	-
EMA (Membranous)	+
BAP1 (Nuclear)	Loss



EMA, Desmin and BAP1 are conclusive

The role of BAP1 in mesothelioma

BAP1: controls DNA repair and genes related to cell proliferation, cell cycle, cell death. Function as a tumour suppressor gene.

- Wild type BAP1 protein is detectable by ICC.
- Deletions or mutations in BAP1 lead to loss of ICC detectable nuclear protein (protein either lost or sequestered in cytoplasm)
- Loss of nuclear BAP1 is always a marker of malignancy – Mesothelioma, Melanoma, some renal carcinomas (i.e. confirm mesothelial before Bap1)

Assessing BAP1 loss

- Confirm cell population is mesothelial first!
- BEWARE - 30% of mesotheliomas will NOT demonstrate loss of BAP1
- Reactive mesothelial proliferation never lose nuclear BAP1 staining
- Inflammatory / stromal material acts as internal positive control

SAMPLE REPORTS FOR A MESOTHELIAL PROLIFERATION

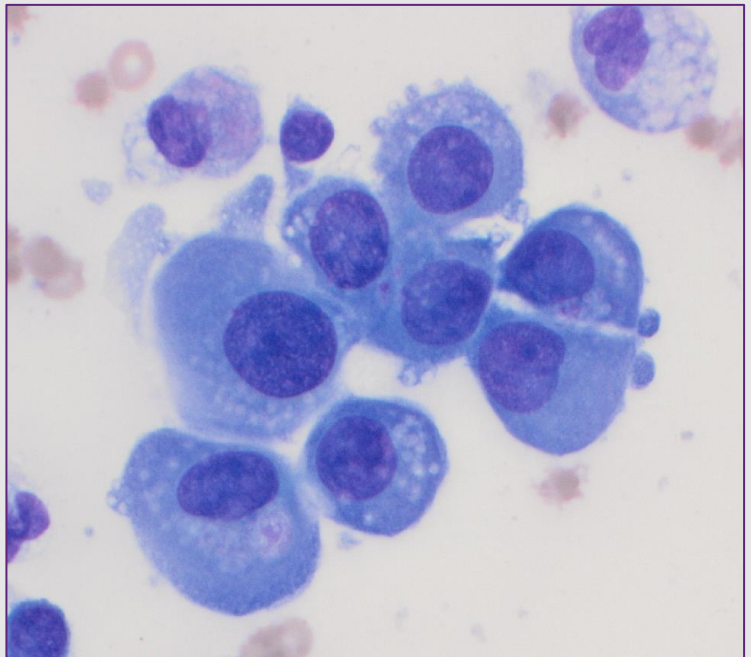
- Satisfactory for evaluation.
- Small spherical groups and dispersed mesothelial cells with mild nuclear pleomorphism are present suspicious for mesothelioma.
- Immunostains requested for confirmation (on cell block or biopsy).
- If immunostains confirmatory- MALIGNANT (PRIMARY): MESOTHELIOMA. Clinical correlation essential.
- If morphology classic but immunostains not confirmatory: SUSPICIOUS FOR MESOTHELIOMA
- If morphology not classic and immunostains not confirmatory: ATYPICAL MESOTHELIAL PROLIFERATION. Further investigation advised.

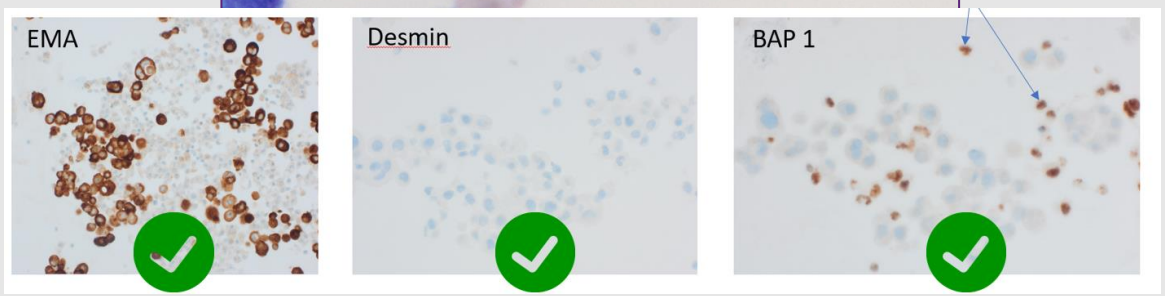
Presentation slide - Dr Ash Chandra – The international reporting system for serous fluid Cytopathology

When morphology and immunology align

- Sample is satisfactory for evaluation
- Morphology is classic
- Immunostains are confirmatory

= Malignant Mesothelioma





Clinical and radiological correlation essential

- CT chest and liver

Large left pleural effusion.

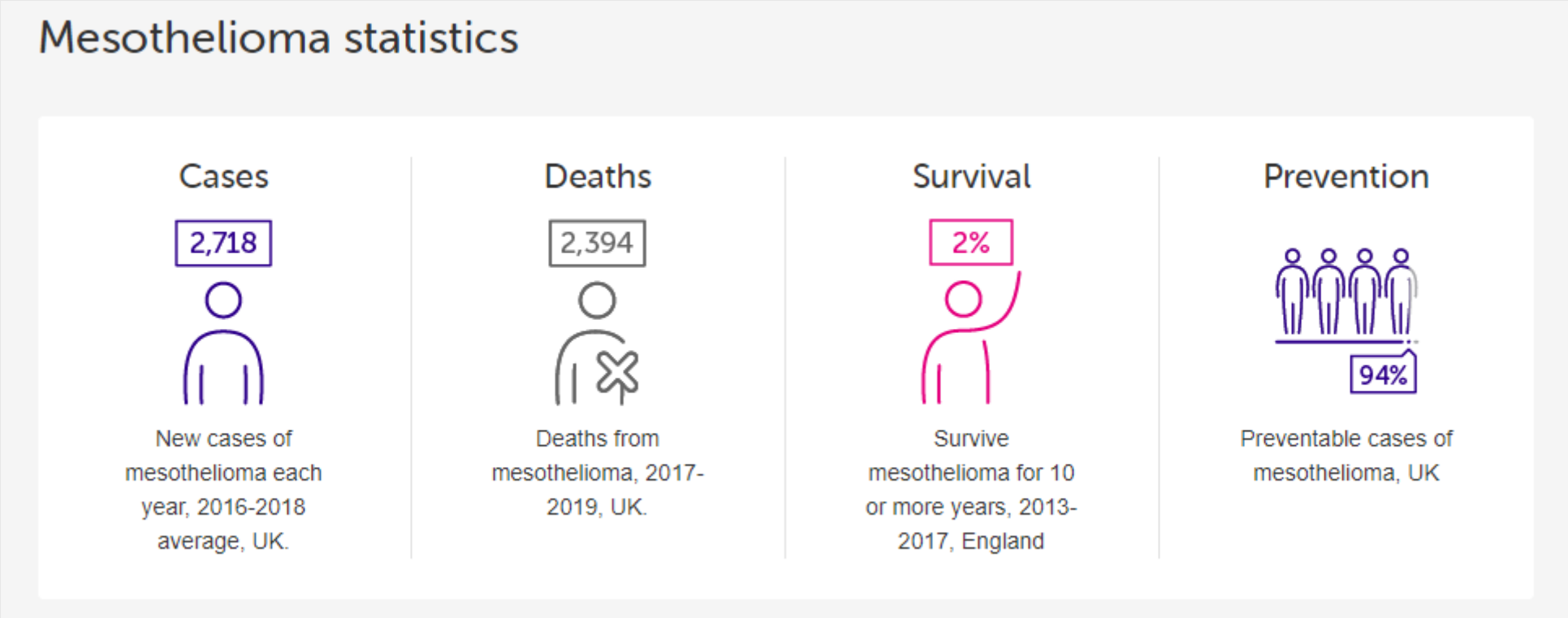
Likely malignant. Evidence of previous asbestos exposure

MDT discussion: Imaging is also in keeping with mesothelioma

Mesothelioma

- Mesothelioma – Malignant mesothelial proliferation
- Often presents at an advanced stage with poor prognosis
- Can manifest as an effusion
- 30,000 globally in 2018
- Asbestos exposure documented in 80% of cases – rarer causes include radiation, fibreglass, nickel, beryllium and silica dust
- Latent period >20 years
- More common in males
- Majority occurring in the pleura
- Epithelioid and biphasic variants can be observed in cytology preparations due to tumour shedding. Sarcomatoid variants rarely exfoliate and therefore not seen in cytology preparations

Mesothelioma statistics Cancer Research UK



https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/mesothelioma#:~:text=Mesothelioma%20risk&text=1%20in%20212%20UK%20males,are%20caused%20by%20workplace%20exposures.

BAC British Association for Cytopathology

Key things to remember

- Morphology first to guide immunocytochemistry panel
- Confirm cell population is mesothelial to avoid pitfalls such as melanoma and renal carcinoma
- No loss of BAP1 - doesn’t mean its not Mesothelioma
- Reactive mesothelial cells will not lose expression of BAP1
- Inflammatory cells act as an internal control for BAP1
- Clinical and radiological correlation is essential

References

1. The international reporting system for serous fluid cytopathology
2. Cancer Research UK