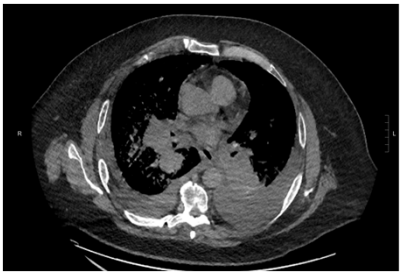
Interesting case

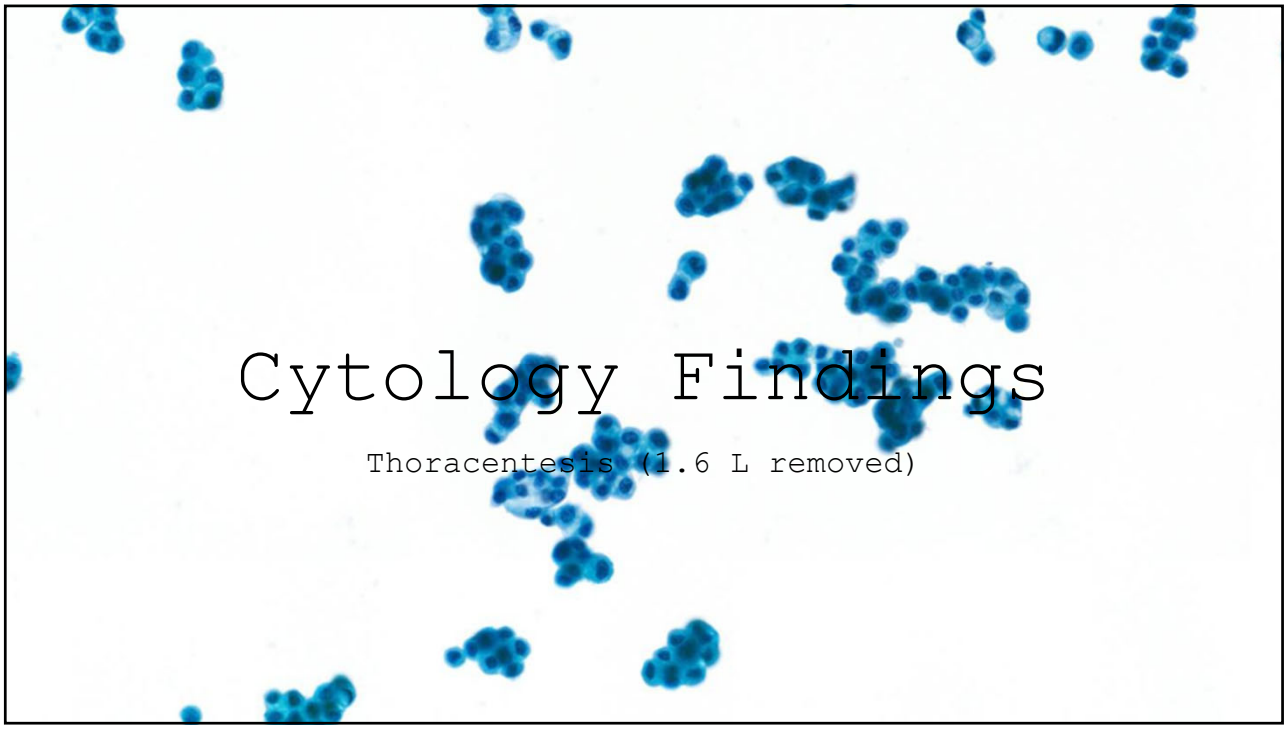
Melissa Zhao, MD, MS

6/11/2025

Clinical History

- 38-year-old male diagnosed with papillary thyroid carcinoma, classical type, status post thyroidectomy (2009)
  - 7.6 cm tumor
  - Gross extrathyroidal extension into skeletal muscle
  - Positive margins
  - 1 of 2 lymph nodes positive
  - Genotyping positive for *BRAF* p.V600E
- Developed progressive, RAI-refractory PTC (2020)
  - Started on levatinib with response
- CT showed many lung nodules (2024)
  - Shortness of breath, dry cough
  - Increasing mediastinal and hilar lymphadenopathy
  - Bilateral pleural effusions

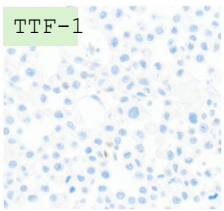


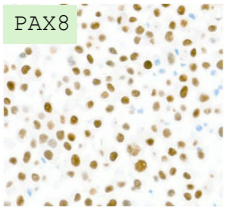


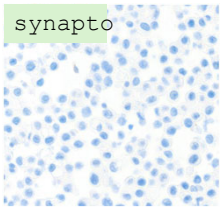
Cytology Findings

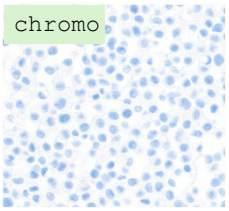
Thoracentesis (1.6 L removed)

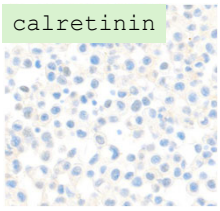
Immunohistochemistry panel

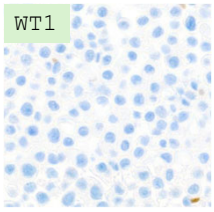


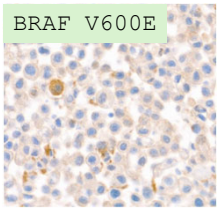


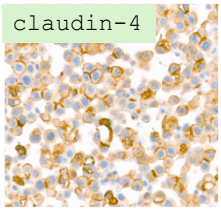


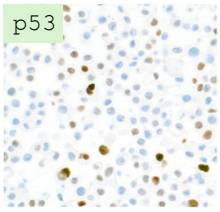


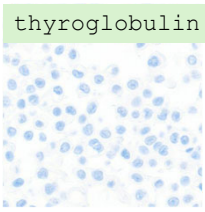












Cytology Report

- DIAGNOSIS:
  High-grade thyroid carcinoma (see note).

  Note: Cytology and cell block preparation show a monomorphic epithelioid malignant neoplasm with intracytoplasmic vacuoles. The nuclei are uniform, with coarse granular chromatin, and with inconspicuous nucleoli. There are no papillary structures (fibrovascular cores) or intranuclear cytoplasmic inclusions characteristic of papillary thyroid carcinoma. There is no sarcomatoid or pleomorphic morphology.

  Immunohistochemistry - tumor cells are positive for PAX8 and claudin-4. They are negative for TTF1, thyroglobulin, synaptophysin, chromogranin, calretinin, WT1, S100, SOX10, and ERG. P53 IHC shows wildtype expression (normal). BRAF V600E shows weak expression.

  Overall, the cytomorphologic features shows a high-grade thyroid carcinoma. The high-grade morphology with loss of TTF1 and thyroglobulin supports transformation to anaplastic thyroid carcinoma, epithelial pattern; however, the monomorphic morphology and wild-type p53 expression are unusual. NGS is recommended to confirm presence of *TERT* promoter and *PIK3CA/PTEN* pathway mutations to further support anaplastic dedifferentiation.

Molecular results

DNA Variants:

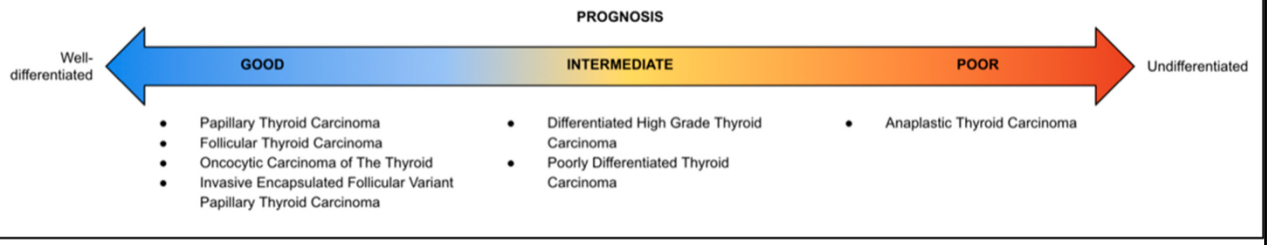
Variants of Clinical/Potential Significance:
BRAF NP_004324.2:p.Val600Glu (NM_004333.6:c.1799T>A) (54%)
TERT promoter variant (hg19 chr5:g.1295228G>A; c.-124C>T; C228T) (51%)

Variants of Unknown Significance:
ARID1B NP_001361757.1:p.Gly103Ser (NM_001374828.1:c.307G>A) (43%)
PIK3CA NP_006209.2:p.Ile1058Phe (NM_006218.4:c.3172A>T) (43%)

Copy Number Variants:
CDKN2A Loss
CDKN2B Loss

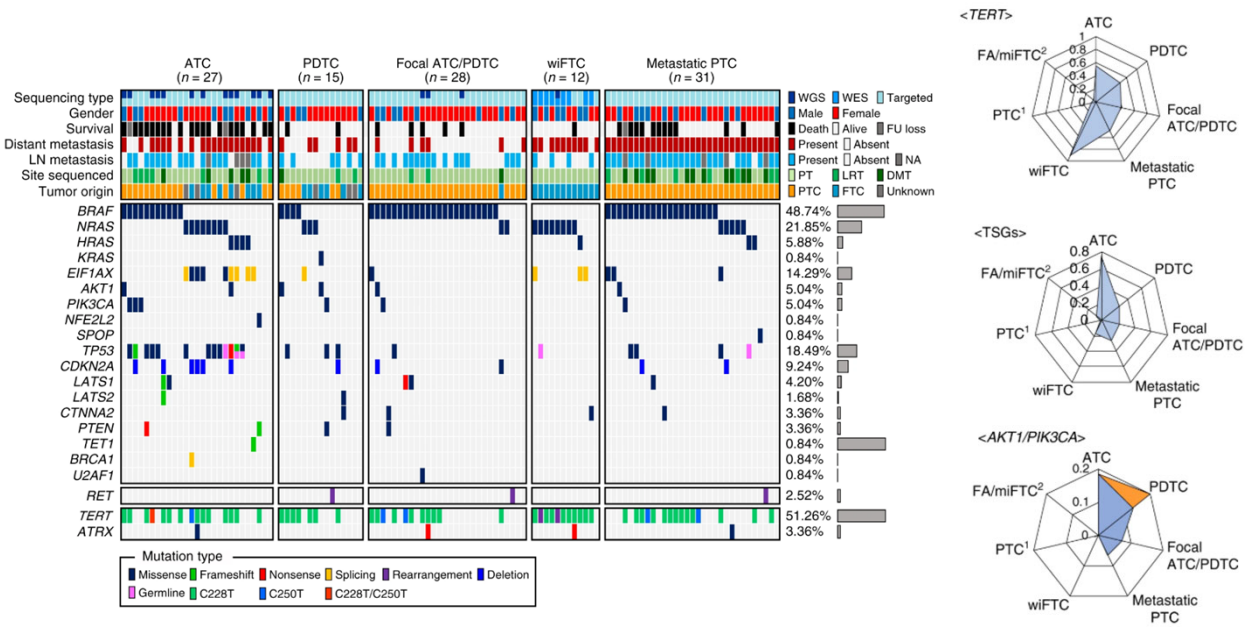
Tumor Mutation Burden:
Low (3.6 mutations per megabase (mutations/Mb))

High grade follicular cell-derived thyroid carcinoma

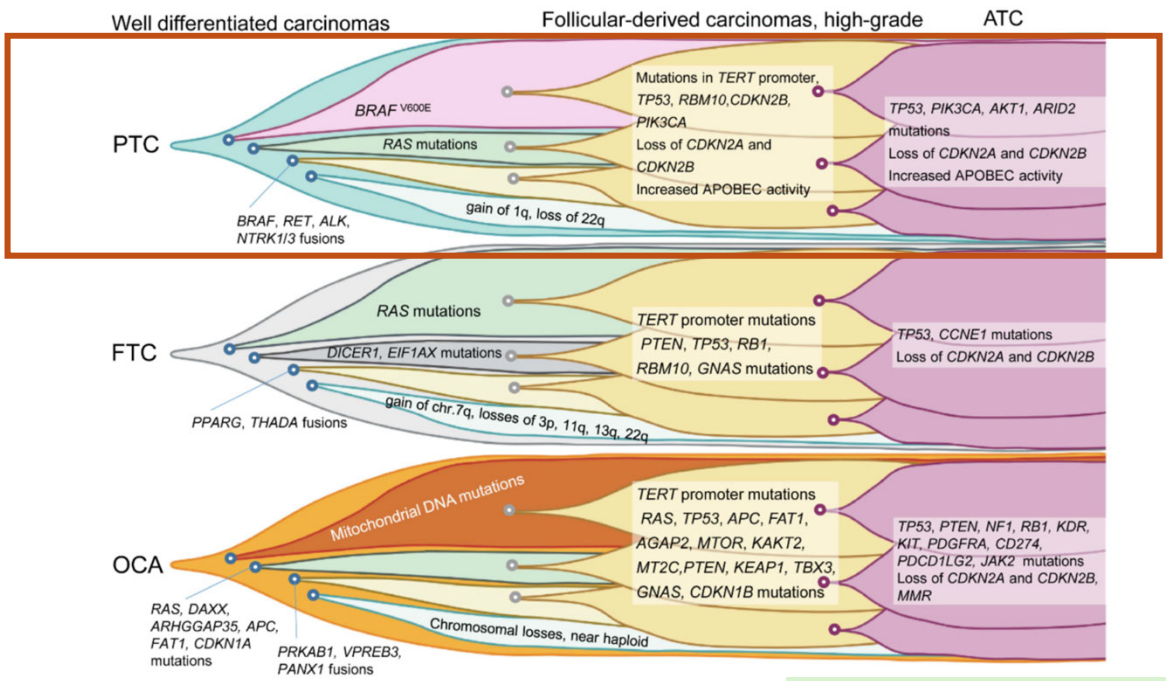


Criteria	PDTC	DHGTC
Growth Pattern	Solid/insular/trabecular	Papillary/follicular/solid
Invasion	Yes	Yes
Papillary nuclear features	No	Yes/no
High-grade features	Mitotic count ≥ 3/2 mm^2 or tumor necrosis or convoluted nuclei	Mitotic count ≥ 5/2 mm^2 or tumor necrosis
Well-differentiated carcinoma component	No or retention of some	Yes (PTC/FTC/OCA)
Anaplastic features	No	No

Harahap et al., Current Oncology, 2024



Yoo et al., Nature Comm., 2019



Argawal et al., Cancers, 2022

Summary

- While morphology was not typical for PTC or ATC on pleural fluid cytology, molecular findings of *CDKN2A/B* loss along with *TERT* promoter mutation support dedifferentiation into ATC.
- High grade follicular cell-derived thyroid lesions share common molecular alterations, involving *TERT*, *PIK3CA*, *AKT1*, *TP53*, and *CDKN2A/B*.
- *CDKN2A* loss is significantly associated with poor disease-specific survival in patients with ATC or advanced DTCs.

References

1. Harahap AS, Roren RS, Imtiyaz S. A Comprehensive Review and Insights into the New Entity of Differentiated High-Grade Thyroid Carcinoma. Curr Oncol. 2024 Jun 9;31(6):3311-3328. doi: 10.3390/curroncol31060252. PMID: 38920735; PMCID: PMC11203239.
2. Yoo SK, Song YS, Lee EK, Hwang J, Kim HH, Jung G, Kim YA, Kim SJ, Cho SW, Won JK, Chung EJ, Shin JY, Lee KE, Kim JI, Park YJ, Seo JS. Integrative analysis of genomic and transcriptomic characteristics associated with progression of aggressive thyroid cancer. Nat Commun. 2019 Jun 24;10(1):2764. doi: 10.1038/s41467-019-10680-5. PMID: 31235699; PMCID: PMC6591357.
3. Agarwal S, Bychkov A, Jung CK. Emerging Biomarkers in Thyroid Practice and Research. Cancers (Basel). 2021 Dec 31;14(1):204. doi: 10.3390/cancers14010204. PMID: 35008368; PMCID: PMC8744846.

Mass General Brigham

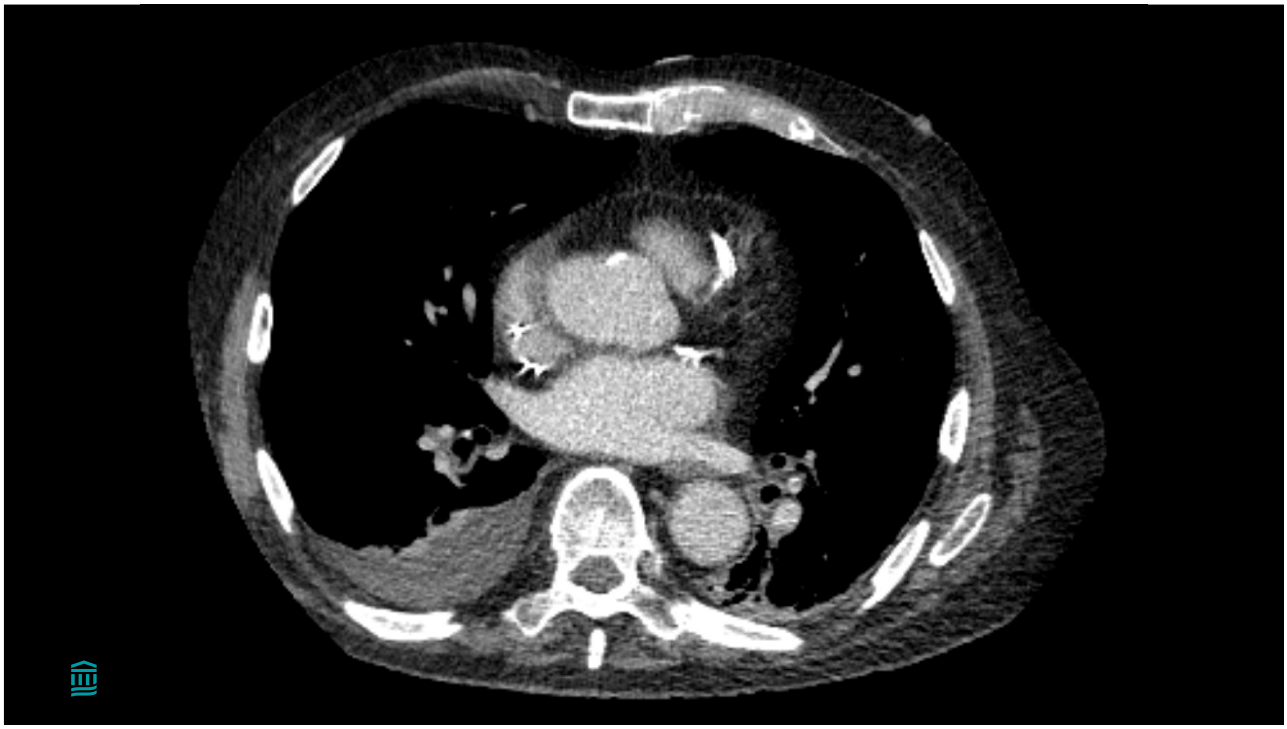
Challenges and Lessons Learned – Virtual Microscopy 3

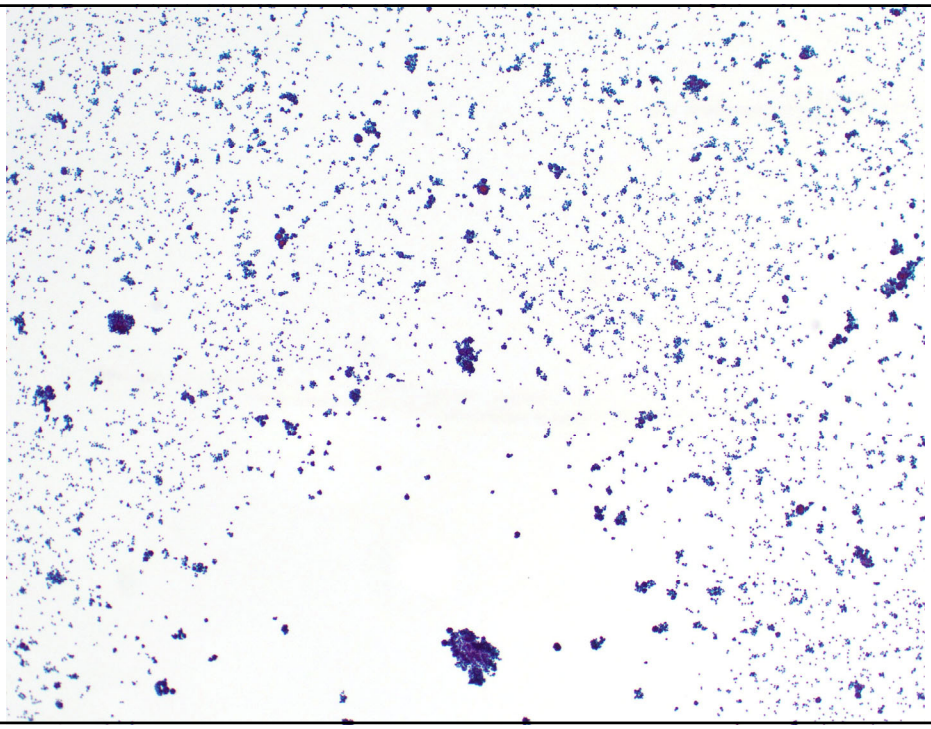
Case 2.

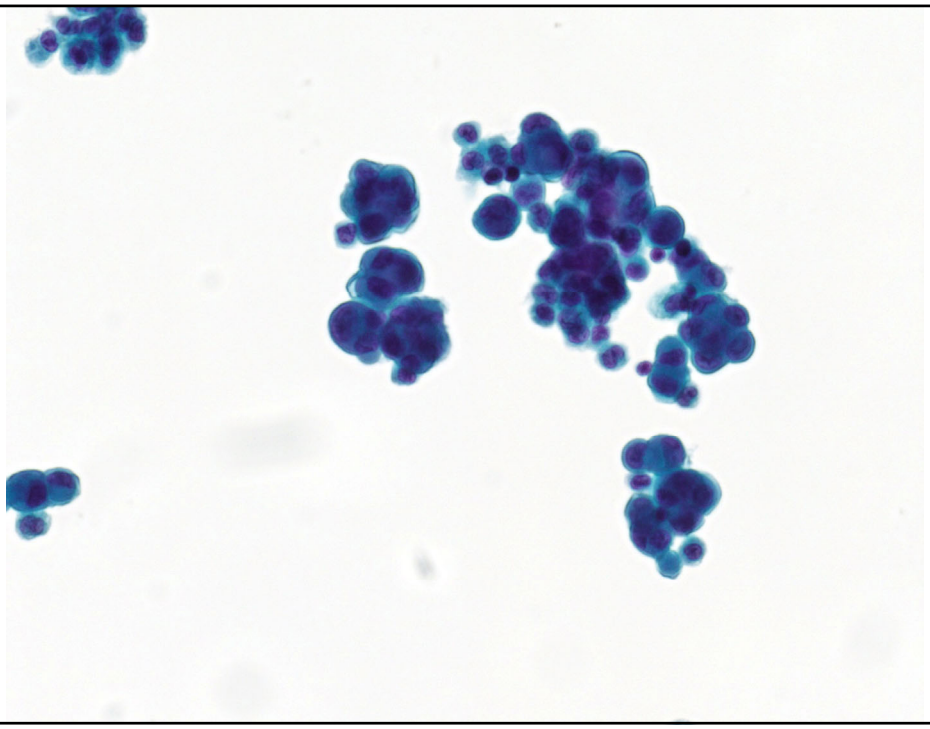
Ivan Chebib

Month, year

>89-year-old male with recurrent pleural effusion







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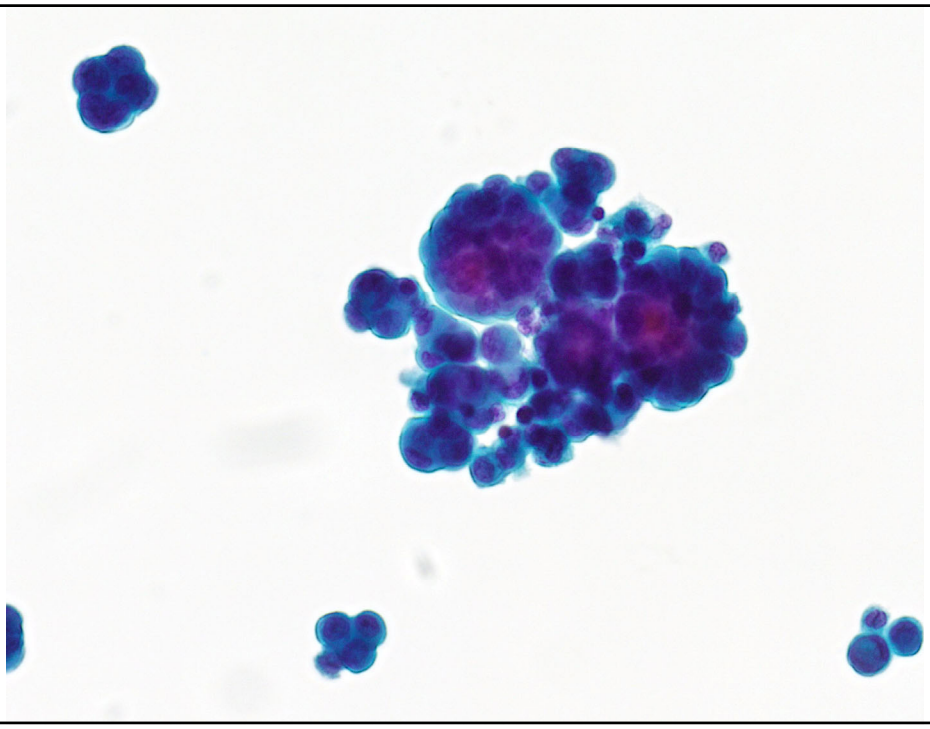

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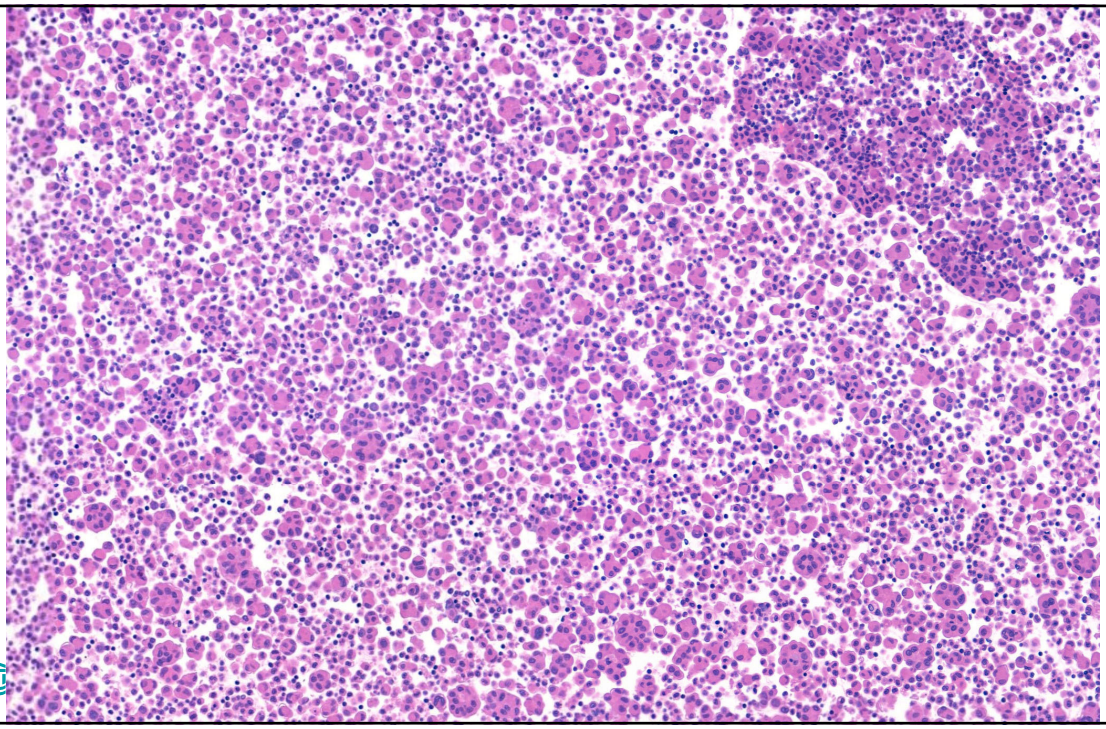
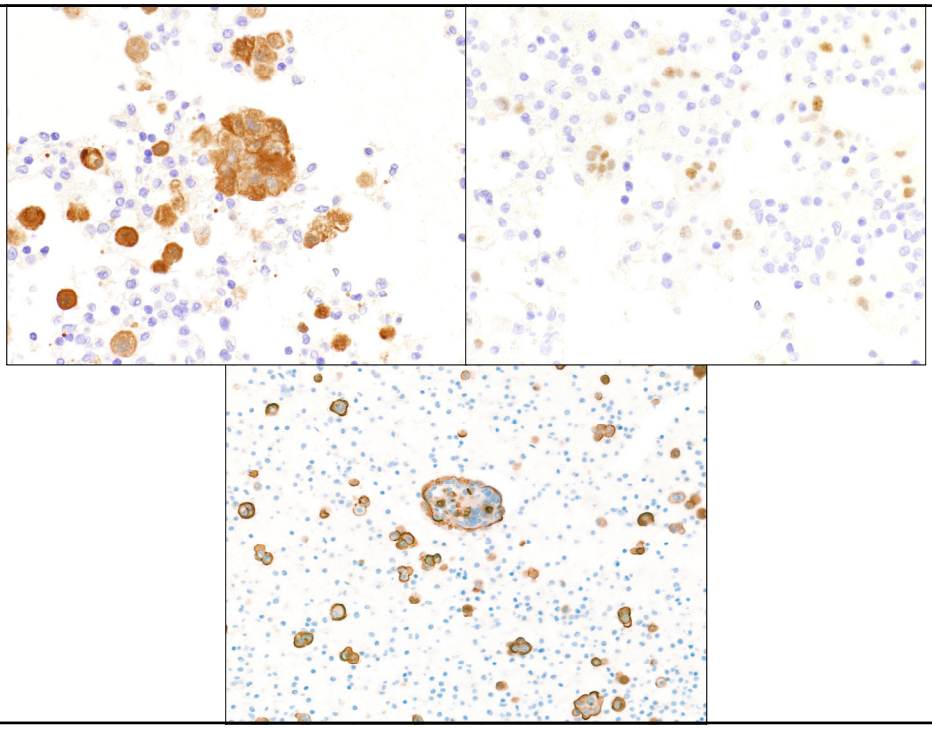
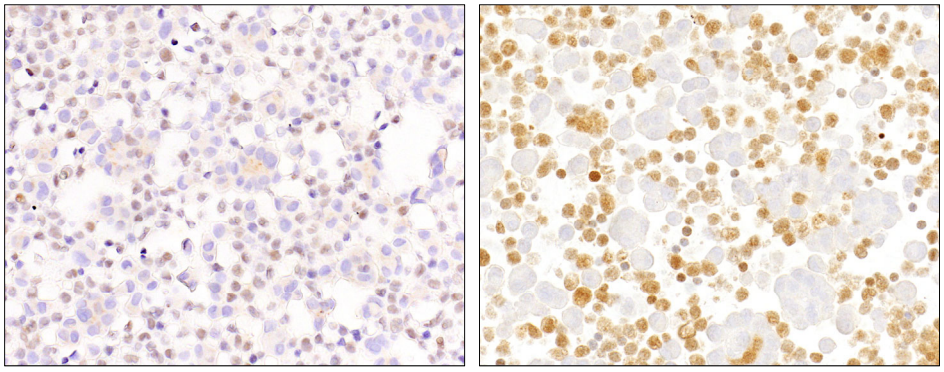






Diagnosis

Pleural Fluid:
- Epithelioid mesothelioma.

Note: Immunohistochemistry - tumor cells are positive for calretinin, WT1, and HEG1. They are negative for claudin-4 and MOC31. MTAP and BAP1 show loss of expression (abnormal).

Metastatic Adenocarcinoma versus Mesothelioma

Adenocarcinoma
- Claudin-4
- MOC31
- BerEP4
- B72.3
- CEA
- CD15 (LeuM1)
- BG8
- EMA

Mesothelioma
- HEG1
- Calretinin
- WT1
- D2-40 (podoplanin)
- CK5/6
- EMA
- Desmin

HEG1

TABLE 1. Diffuseness of Positive HEG1 Immunohistochemical Staining in Mesothelioma and Reactive Mesothelial Hyperplasia

	Mesothelioma, n (%)		Reactive Mesothelial Proliferations, n (%)	
Percent of Cells With Positive Staining	Epithelioid (N = 69)	Sarcomatoid (N = 32)	Epithelioid (N = 40)	Spindle Cell (N = 32)
0	4 (6)	18 (56)	5 (13)	21 (66)
1%-25%	3 (4)	2 (6)	8 (20)	6 (19)
25%-50%	4 (6)	4 (13)	10 (25)	2 (6)
> 50%	58 (84)	8 (25)	17 (43)	3 (9)
Total ≥ 1%	65 (94)	14 (44)	35 (88)	11 (34)

TABLE 2. HEG1 Immunostaining in NSCLC and High Grade Serous Ovarian Carcinoma

	Positive HEG1 Staining, n (%)	
Diagnosis Subtype	Membranous	Cytoplasmic
NSCLC		
Adenocarcinoma (N = 73)	0	3 (4)
Squamous cell carcinoma (N = 60)	0	16 (27)
Large cell carcinoma (N = 13)	0	2 (15)
Sarcomatoid carcinoma (N = 21)	0	0
Ovarian carcinoma		
High grade serous (N = 17)	3 (18)	0
Thymoma		
B3 (N = 10 whole sections)	0	0

Am J Surg Pathol 2020;44:1143–1148

HEG1 in Effusion Cytology

TABLE 2 Expression rate of HEG1 and traditional mesothelial markers in mesothelioma and RMCs

	EM		BM		NOS		Total		RMCs	
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
HEG1	18/18	(100)	4/4	(100)	19/19	(100)	41/41	(100)	20/26	(76.9)
Calretinin	14/15	(93.3)	2/4	(50)	20/20	(100)	36/39	(92.3)	20/20	(100)
WT1	14/15	(93.3)	3/4	(75)	19/19	(100)	36/38	(94.7)	20/20	(100)
Podoplanin	16/18	(88.9)	2/3	(66.7)	20/20	(100)	38/41	(92.7)	20/20	(100)

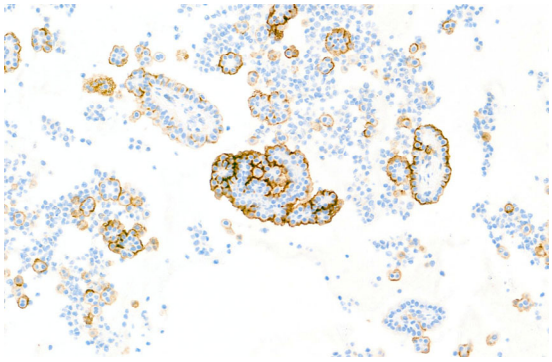
Abbreviations: BM, biphasic mesothelioma; EM, epithelioid mesothelioma; NOS, not otherwise specified; RMC, reactive mesothelial cell.

Diagnostic Cytopathology. 2021;49:622–632.

HEG1 and Claudin-4

Table 3. Suggested scheme for interpretation of cases stained for HEG1 and claudin-4

HEG1	Claudin-4	Interpretation
Positive	Negative	Mesothelioma
Negative	Positive	Carcinoma
Positive	Positive	Carcinoma, serous most common
Negative	Negative	Non-informative. Run other markers



Histopathology. 2023 Feb;82(3):385-392.

The cytologic diagnosis of mesothelioma: are we there yet?

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Table 1 International guidelines for reporting serous effusions (mesothelioma).

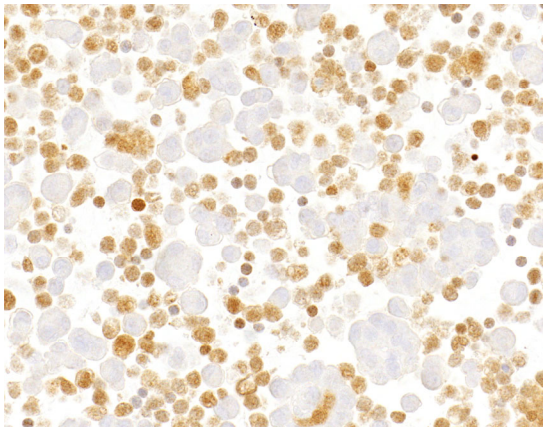
- Evaluate the fluid for mesothelial origin and presence or absence of atypia. If mesothelioma is suspected, mesothelial origin should be confirmed by IC.
- Report as "Mesothelioma" in the following conditions:
  - Morphologically malignant mesothelium (high cellularity and atypia) and supportive radiological findings. Additional biomarkers are optional. For pericardial fluids always confirm malignancy with biomarkers.
  - Morphologically malignant but of moderate cellularity and supportive radiological findings. Perform biomarkers to support malignancy.
  - Morphologically malignant but radiological findings are not available. Perform biomarkers to support malignancy.
  - Morphologically malignant but radiological findings are negative. Perform biomarkers to support malignancy and include a comment to raise concern for MIS.
- Report as "Suspicious for mesothelioma" in the following conditions:
  - Morphologically malignant cases and negative radiology if biomarkers were not supportive of malignancy.
  - Morphologically malignant pericardial fluids if biomarkers are not supportive of malignancy.
  - Morphologically malignant but fluid is of low to moderate cellularity, positive radiological findings but negative biomarkers.
- Report as "Atypical mesothelial cell proliferation"
  Only when the cytologic atypia exceeds that expected in reactive conditions or if reactive mesothelium cannot be supported by ancillary tests but the findings are not enough to raise the concern for mesothelioma. The term "Atypical" should not be used in the context of reactive mesothelium which instead should be diagnosed as "negative for malignancy)".

Some mesotheliomas will not exhibit any abnormal biomarker results and consequently negative biomarkers do not exclude the diagnosis of mesothelioma.

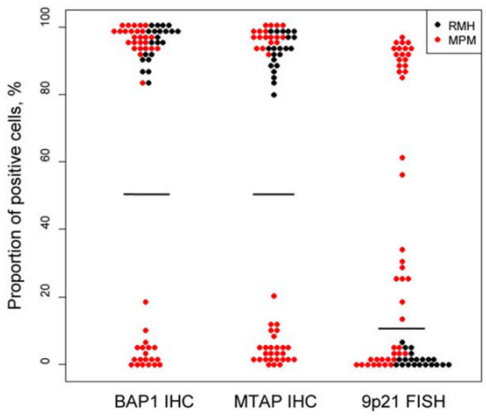
J Am Soc Cytopathol. 2023 Mar-Apr;12(2):89-104.

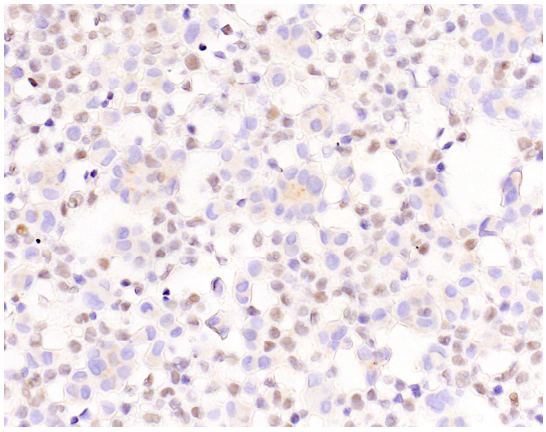
Mesothelioma versus Reactive Mesothelial Cells

Mesothelioma
- TP53 (rare)
- CDKN2A FISH
- SNP array
- NGS panels
- Loss of BAP1
- Loss of MTAP
- Loss of Merlin



BAP1, MTAP, and 9p21 FISH on Effusion Cytology





Cancer Cytopathol. 2018 Jan;126(1):54-63.

BAP1, MTAP, and 9p21 FISH on Effusion Cytology

TABLE 3. Diagnostic Usefulness of MTAP IHC, BAP1 IHC, and 9p21 FISH in Cell Blocks for Distinguishing MPM From Non-Neoplastic RMH

	MPM N=45		RMH N=21			
	Positive[a]	Negative[a]	Positive[a]	Negative[a]	Sensitivity, %	Specificity, %
MTAP IHC	19	26	0	21	42.2	100
BAP1 IHC	27	18	0	21	60.0	100
9p21 FISH	28	17	0	21	62.2	100
BAP1/MTAP IHC	35	10	0	21	77.8	100
BAP1 IHC/9p21 FISH	38	7	0	21	84.4	100

Abbreviations: BAP1, BRCA1-associated protein 1; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; MPM, malignant pleural mesothelioma; MTAP, methylthioadenosine phosphorylase; RMH, reactive mesothelial hyperplasia.
[a] For IHC, positive indicates expression loss and negative indicates negative expression loss, whereas for FISH, positive indicates positive for homozygous deletion (HD) and negative indicates negative for HD.

Cancer Cytopathol. 2018 Jan;126(1):54-63.

4 Panel Marker (BAP1, MTAP, Merlin, p53)

Table 3. Comparative sensitivities of immunomarkers, immunopanels, and panel NGS for diagnosis of malignant mesothelioma.

Marker/panel	Sensitivity for diagnosis of malignant mesothelioma, % (95% CI)			
	All MM	EMM	BMM	SMM
BAP1	54 (44–65)	59 (45–72)	52 (33–71)	17 (0–46)
MTAP	46 (36–57)	35 (22–48)	59 (41–78)	83 (54–100)
Merlin	52 (42–63)	41 (28–55)	70 (53–88)	67 (29–100)
P53	7 (2–13)%	10 (2–18)	0%	17 (0–46)
BAP1 + MTAP	79 (70–87)	75 (63–86)	85 (72–99)	83 (54–100)
BAP1 + Merlin	85 (75–93)	80 (69–92)	93 (83–100)	83 (54–100)
MTAP + Merlin	71 (62–81)	63 (49–76)	85 (72–99)	67 (29–100)
BAP1 + p53	60 (50–71)	67 (54–79)	52 (33–71)	33 (0–71)
MTAP + p53	49 (38–59)	39 (26–53)	59 (41–78)	83 (54–100)
Merlin + p53	62 (52–72)	49 (35–63)	70 (53–88)	67 (29–100)
BAP1 + MTAP + Merlin	90 (84–97)	88 (79–97)	96 (89–100)	83 (54–100)
BAP1 + MTAP + p53	81 (73–89)	78 (67–90)	85 (72–99)	83 (54–100)
BAP1 + Merlin + p53	89 (82–96)	88 (79–98)	93 (83–100)	83 (54–100)
MTAP + Merlin + p53	74 (64–83)	67 (54–80)	85 (72–99)	67 (29–100)
BAP1 + MTAP + Merlin + p53	93 (87–98)	92 (85–100)	96 (89–100)	83 (54–100)
OncoPanel	95 (91–100)	94 (88–100)	96 (89–100)	100%
OncoPanel + BAP1 + MTAP + Merlin + p53	99 (96–100)	98 (94–100)	100%	100%

Mod Pathol. 2022 Oct;35(10):1383-1397.

Mesothelioma *in situ* (MIS)

- Rare entity, considered precursor of pleural mesothelioma
- Preinvasive single-layer surface proliferation of neoplastic mesothelial cells.
- Histologically, MIS shows a layer of flat or cuboidal cells with or without cytopathological atypia and/or small papillary projections, or small nodules that may show moderate to severe atypia
- Loss of BAP1 and/or MTAP and/or show CDKN2A homozygous deletion

Mesothelioma *in situ* (MIS)

- Not possible to exclude MIS from early stages of invasive diffuse mesothelioma
- Cytopathology may represent the first clue towards the **histopathological diagnosis** of MIS.
- If either diagnostic or very suspicious for pleural mesothelioma
  - With confirmatory surrogate findings (e.g. loss of BAP1 and/or MTAP expression)
  - But imaging of typical diffuse pleural mesothelioma is absent
  - Possibility of early stage mesothelioma should be considered and diagnosis of MIS could be raised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