Malignant Effusions: Primary versus Metastatic

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Objectives

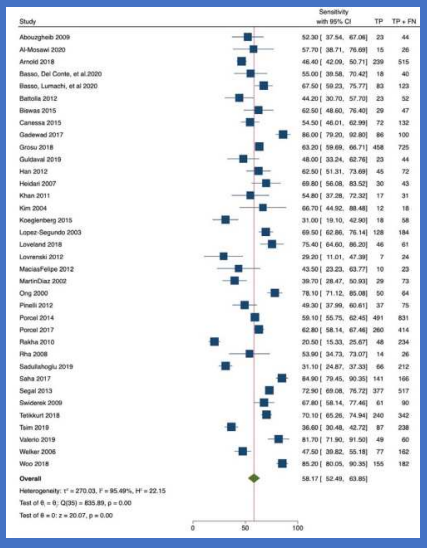
- Why are effusions important?
- Review reporting of effusion cytology
- Review the more common metastatic tumors in effusions
- Discuss diagnosis of both common and uncommon metastatic tumors in fluids with comparison to mesothelioma
- Discuss emerging issues in the cytologic diagnosis of mesothelioma

Clinical Significance of Effusions

- Benign disease
  - Heart/liver/renal failure
  - Infection
  - Autoimmune disease
  - Vasculitis
- Malignant disease
  - Metastatic disease
  - Primary malignancy (mesothelioma)
- Clinical outcomes poorer in patients with effusions in multiple clinical settings
  - Increased risk of death in inpatients with pneumonia (13.3% at 30 days)
  - Indicates advanced disease in patients with malignancy
    - Median survival 3-12 months
      - Lung cancer > cancer of unknown primary > ovarian cancer
- Treatment is palliative
  - Chemotherapy
  - Drainage and pleurodesis

Historical Accuracy of Effusion Cytology

- Overall sensitivity 58%
  - Varies by tumor type
  - **Lower for mesothelioma (32%)**
  - **High for lung adenocarcinoma (84%)**
  - Use of cell blocks and ancillary studies increases sensitivity
- Overall specificity = 97-99%
- Accuracy in tumor typing 94%



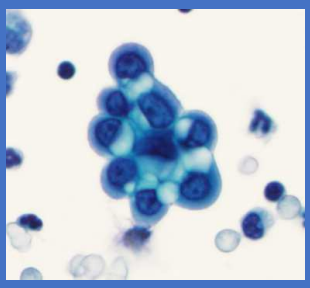
Kassirian S, Hinton SN, Cuninghame, et al. *Thorax*. 2022;78(1):32-40.

The International System for Reporting Effusion Cytology

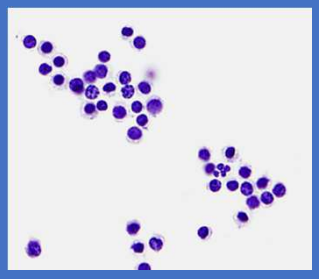
- Developed in 2019 by IAC and ASC to improve diagnostic agreement and accuracy
- Not uniformly applied
- Use 50-75 mL adequacy threshold
- Requires enough mesothelial cells for evaluation

Category	Examples
I. Nondiagnostic	<50-75 mL, acellular specimen, obscuring rbc
II. Negative for malignancy	Adequate, definite benign diagnosis
III. Atypia of undetermined significance (AUS)	Insufficient IHC evidence to prove malignancy, atypical lymphocytes, reactive atypia, degenerated cells
IV. Suspicious for malignancy	Insufficient material for definitive diagnosis
V. Malignant	Definitive evidence of malignancy

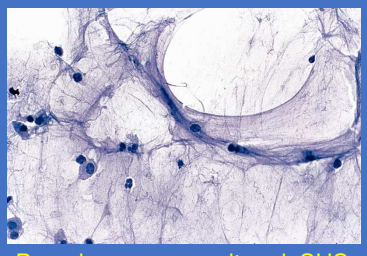
Effusion Cytology Reporting System



Serous Cystadenoma: AUS



Lymphocytic effusion + no flow data: AUS



Pseudomyxoma peritonei: SUS

Pinto D, et al. *J Am Soc Cytopathol.* 2020;9(6):469-77.

The International System for Reporting Effusion Cytology

Category	Risk of Malignancy: Pleural Effusions						
	Lobo et al.	Zhu et al.	Ahuja & Malviya	Straccia et al.	Jha et al.	Bharti et al.	Pinto et al.
Non-diagnostic	57.1%	40%	0%	18%	87.5%	30.9%	40%
Negative for malignancy	23.9%	29.8%	2.1%	15%	51.61%	12.9%	20.16%
AUS	50%	49.3%	33.3%	45.3%	88.23	100%	42.86%
SFM	76.2%	99.3%	94%	93%	87.5%	100%	78.57%
Malignant	100%	100%	100%	100%	100%	90.2%	100%

The International System for Reporting Effusion Cytology

Category	Risk of Malignancy: Peritoneal Effusions			
	Lobo et al.	Zhu et al.	Ahuja & Malviya	Straccia et al.
Non-diagnostic	100%	0%	50	19.3%
Negative for malignancy	26.3%	27.5%	4.8%	10.4%
AUS	62.5%	60.9%	22.2%	43.5%
SFM	91.7%	99.5%	83.3%	100%
Malignant	100%	100%	100%	100%

The International System for Reporting Effusion Cytology

Interobserver Agreement

- Highest for negative (76%) and malignant (81%) categories
- Lowest for the suspicious category (22%)
  - 44% of diagnoses varied by two categories

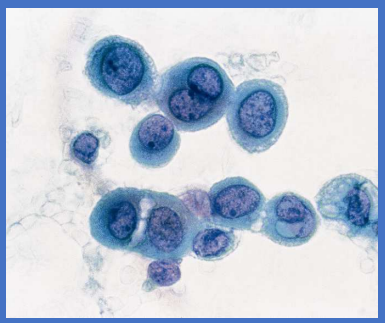
	Size of disagreement			
Category	0 category	1 categories	2 categories	3 categories
2	224/293 (76%)	58/293 (20%)	11/293 (4%)	0
3	30/94 (32%)	61/94 (65%)	3/94 (3%)	0
4	8/36 (22%)	12/36 (33%)	16/36 (44%)	0
5	143/176 (81%)	19/176 (11%)	6/176 (3%)	8/176 (5%)

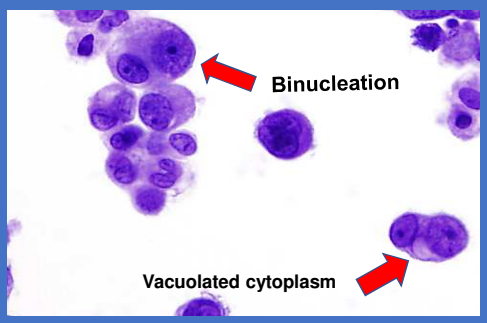
Comparable to other reporting systems

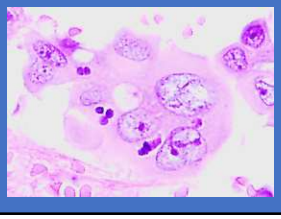
Body site	Observed agreement	Kappa	Weighted Kappa	Strength of agreement
Breast[9]	68.6%	0.69	0.91	Substantial
Salivary Gland[9]	NR	0.42	NR	Moderate
Lung[10]	49.5%	0.20	NR	Slight
Urine[11]	65%	0.32	NR	Fair
Pancreas[15]	NR	0.45	0.65	Moderate
Pleural fluid	68%	0.51	0.63	Moderate

Layfield LJ et al. *Diagn Cytopathol.* 2022;50(1):3-7.

Normal Elements: Mesothelial Cells



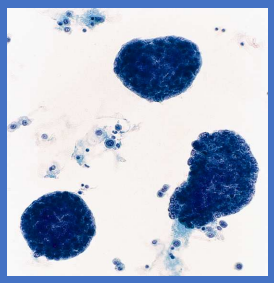


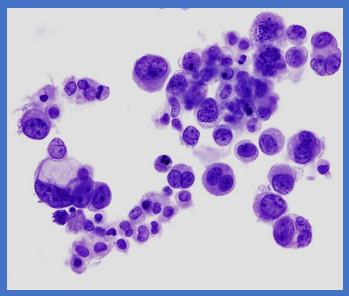


Distinction Between Primary and Metastatic Disease

1. Establish malignancy
2. Differentiate between mesothelioma and metastases
3. If metastatic disease, establish tumor lineage
4. If mesothelial, distinguish between benign reactive mesothelial cells and mesothelioma

Features of Malignant Effusions





- Increased specimen cellularity
- Morphologically distinct “Second population”
  - May not be present in mesothelioma
- Numerous large clusters with community border OR singly dispersed cells
- +/- cytologic atypia
- Don’t neglect background elements
  - Background mucin
  - Psammoma bodies
  - Necrotic debris

Primary Source in Malignant Effusions

Pleural Effusions		Peritoneal Effusions		Pericardial	
Lung ACA	29-37%	Ovarian ACA	27%	Lung	60-75%
Breast	8-40%*	Gastric	14%	Breast	25-39%±
Ovarian	18-20	Breast	13%	GI tract	9%
GI tract	5%	Pancreatic	11%	Hematolymphoid	3%
Lymphoma	3-16%	Colorectal	10%	Ovarian	4-8%
Melanoma	5-6%	Lymphoma	5-12%	Mesothelioma	3%
Mesothelioma	1-6%	Melanoma	2%	Melanoma	1%
Sarcoma	1-3%	Mesothelioma	1-8%	Mesothelioma	1%

* Higher figures are in effusions in women only
± Only women in this analysis

Metastatic Carcinoma vs. Mesothelioma: Immunohistochemistry

Epithelial/Carcinoma Markers			Mesothelioma Markers		
Marker	Sens.	Spec.	Marker	Sens.	Spec.
CEA	63-78%	98%	Calretinin	85-96%	87-100%
BerEP4	74-89%	95-98%	WT-1	78%	62%
MOC31	86-92%	87-97%	D2-40	79%	100%
Claudin-4	91-100%	99-100%	Mesothelin	75%	71%

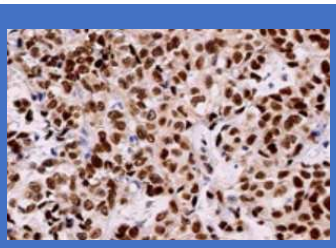
Mesothelioma vs. Metastatic Lung Adenocarcinoma: SOX6 Immunohistochemistry

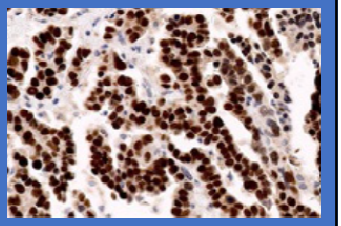
Meso vs. LUAD

98% sensitivity
93% specificity

Epithelioid Mesothelioma (54 Cases)						Lung Adenocarcinoma (69 Cases)					
		Immunohistochemical Score*						Immunohistochemical Score*			
Marker	No. Positive Cases, n (%)	0	1+	2+	3+	Marker	No. Positive Cases, n (%)	0	1+	2+	3+
SOX6	53 (98)	1	0	5	48	SOX6	5 (7)	64	2	3	0
Calretinin	53 (98)	1	4	0	49	Calretinin	15 (22)	54	9	6	0
D2-40	53 (98)	1	2	6	45	D2-40	7 (10)	62	4	3	0
WT1	42 (78)	12	10	3	29	WT1	0	69	0	0	0

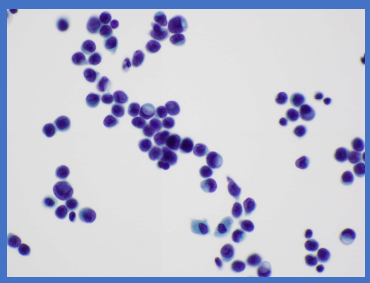
*0, negative; 1+, <10%; 2+, 10% to 50%; 3+, <50% of tumor cells with immunoreactivity.

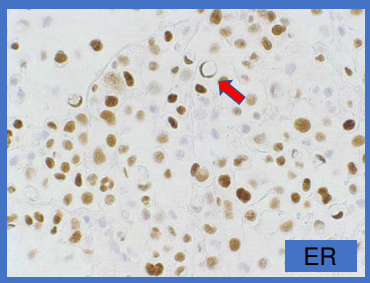




Adapted from: Kambara T, et al. *Am J Surg Pathol.* 2020;44(9):1259-1265.

Lobular carcinoma of breast

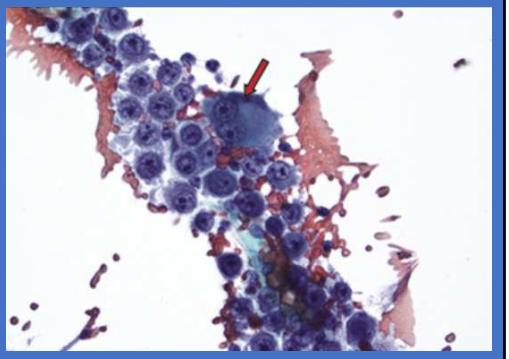




- May be extremely subtle
- Often impossible to distinguish from mesothelial cells or histiocytes
- IHC advisable in ANY effusion from a patient with a history of lobular (or unspecified) breast cancer:
  - Epithelial markers
  - ER, GATA3
    - Be aware that mesothelial cells can have patchy weak to moderate GATA3 staining

Mesothelioma vs. Melanoma

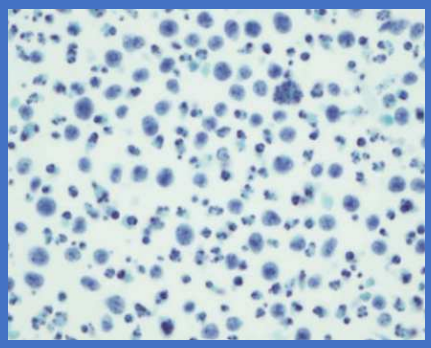
- 1-6% effusions
- Usually single cells, but can be clustered
- Shared features with mesothelioma:
  - Low NC ratio
  - Binucleation
  - Eccentric nuclei
- Melanin in 50-83% of cases
- Can show weak and focal keratin staining in rare cases

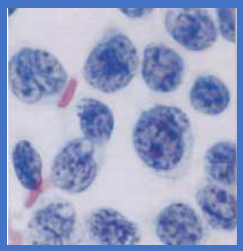


Pantanowitz, Chivukula. *Cytojournal.* 2022;19:15

Effusions in Lymphoma

- 3-16% of effusions
- Lymphoma in effusions almost always represents involvement by previously-diagnosed disease
- 75% are B-cell lymphomas
- 44-50% are large B-cell lymphomas
  - Cellular samples
  - Often smaller than mesothelial cells
  - Higher N/C ratio than mesothelial cells

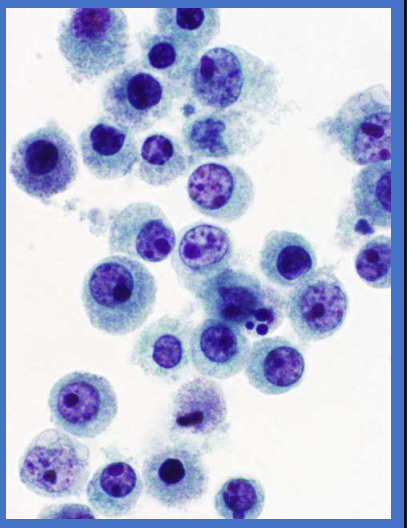




Koh J, et al. *J Pathol Transl Med.* 2022;56(4):173-86.
Das. *Diagn Cytopathol.* 2006;34(5):335-47.

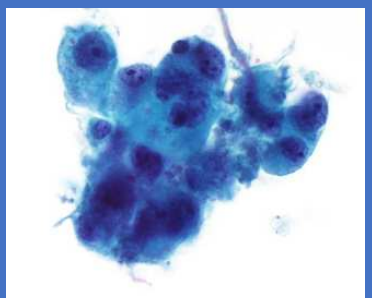
Primary Effusion Lymphoma

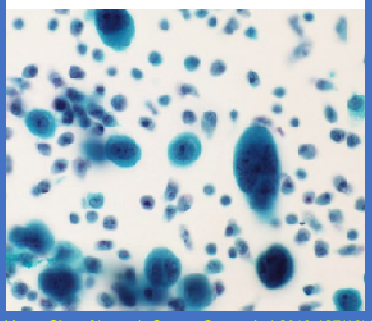
- 0.1% effusions
- Immunocompromised patients
- Most (not all) **HHV-8 +**
- B-cell lymphoma
  - **Negative for pan-B markers**
  - LCA, CD138+
  - Clonal Ig gene rearrangements
- Large, dyshesive cells with plasmablastic features
- Ancillary studies (IHC, flow cytometry) required
- Resistant to chemotherapy and fatal within 6 months



Mesothelioma vs. Sarcoma

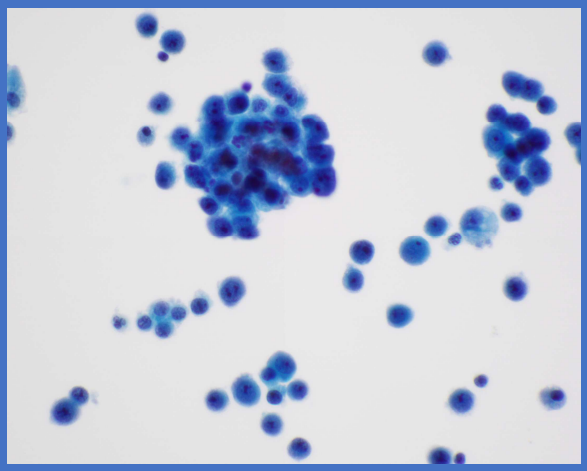
- 1-6% effusions
- Usually patients have established history
- Cells may be rounded or oval in liquid-based preparations even if spindled on histology
- Can be singly-dispersed cells, multinucleated
  - Ewing sarcoma
  - Vascular tumors (EHE, angiosarcoma)
  - Undifferentiated pleomorphic sarcoma



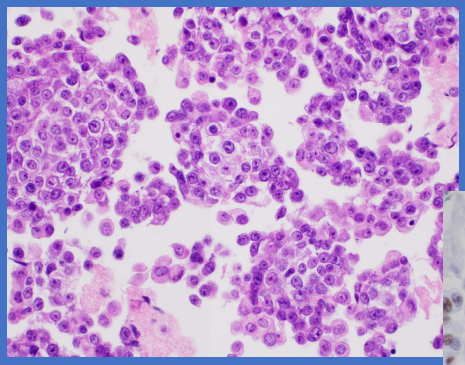


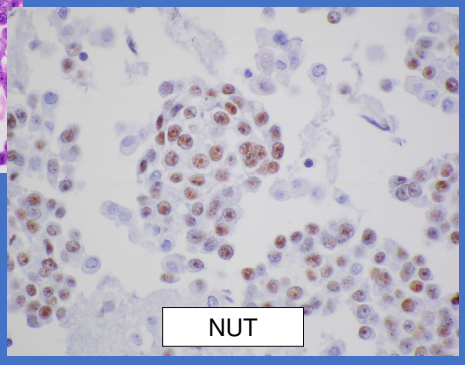
Adapted from: Chen AL, et al. *Cancer Cytopathol.* 2019;127(12):778-84.

Mesothelioma?

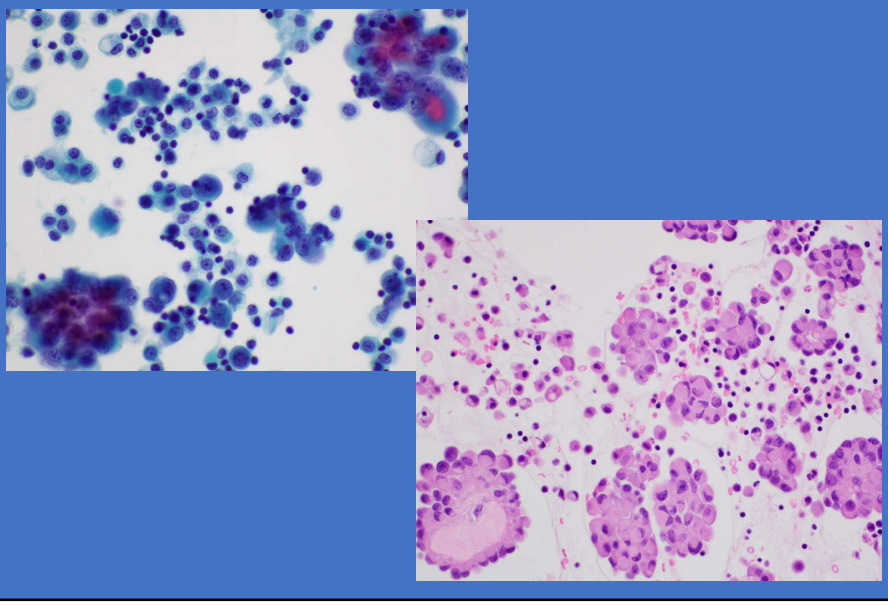


NUT Carcinoma





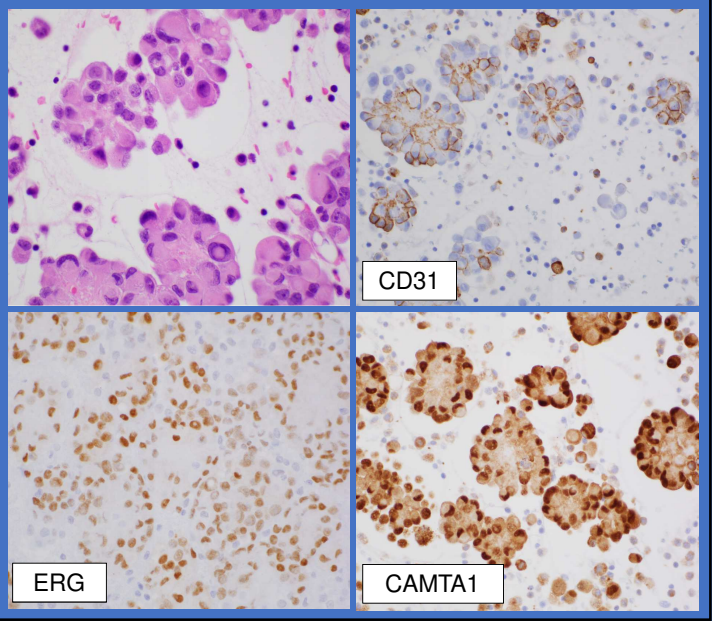
Mesothelioma?



Epithelioid Hemangioendothelioma

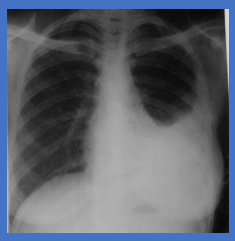
90% WWTR-CAMTA1

<5% YAP1-TFE3



Malignant Mesothelioma

- <2% of all malignant effusions
- Sites: pleura > peritoneum > pericardium
- 80% of cases linked to asbestos exposure
  - Latency of 2-4 decades
  - Mantle radiation for Hodgkin lymphoma, thorotrast
- Incidence in the US peaked in the 1990's
  - Decline in the US, continues to be a health issue worldwide
- Radiology
  - Unilateral pleural effusion (usually right-sided)
  - Pleural thickening
  - Pleural nodularity
  - Rarely a single mass



Malignant Mesothelioma

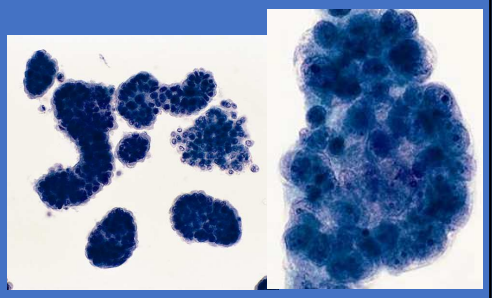
- Distribution of types in fluids: epithelioid > biphasic > sarcomatoid
- Rule out metastasis
  - Pay attention to the radiology if available
  - Effusions with large clusters more likely to be non-small cell carcinoma (adenocarcinoma) than mesothelioma
- Most objective diagnostic feature is invasion into fibroadipose tissue on pleural biopsy
- Challenges in cytologic diagnosis of mesothelioma
  - Cannot assess for invasion
  - Morphologic overlap between benign and malignant proliferations
  - Bland – reactive proliferations are often more pleomorphic than mesothelioma

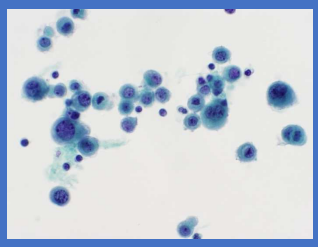
Malignant Mesothelioma: Low Power

- Large clusters with scalloped borders ("mulberry clusters")
  - Retain windows and lacy skirts seen in normal mesothelial cells

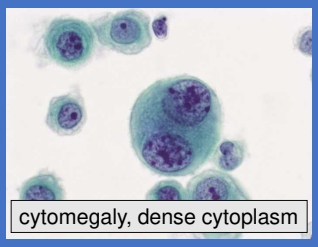
OR

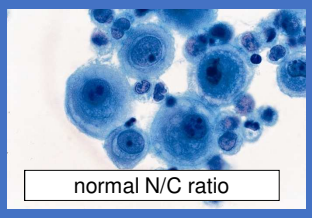
- Numerous dyscohesive cells
  - Diagnostically challenging
  - Radiology should prompt consideration
  - Rely on severe cytologic atypia and ancillary studies

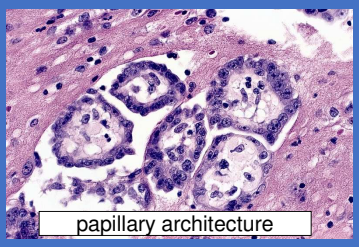




Malignant Mesothelioma: High Power

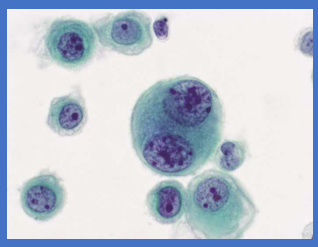


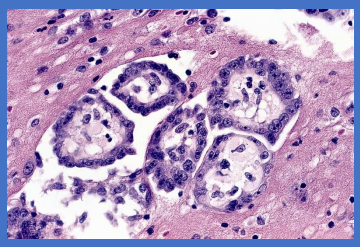




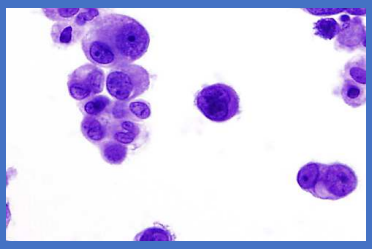
Malignant Mesothelioma: High Power

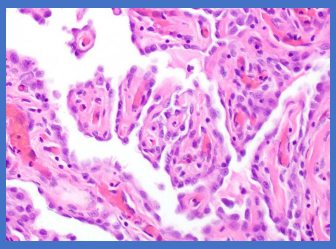
Malignant





Benign





Morphologic Features Favoring Malignant Mesothelioma

- High cellularity
- Numerous large “mulberry” clusters
  - Clusters of >20-40 cells are indicative of malignancy
  - Reactive mesothelial cells do not form large groups
  - Adenocarcinoma is more likely to have a “community border”
- Marked cytomegaly
- Severe cytologic atypia
- Typical clinical and radiographic features

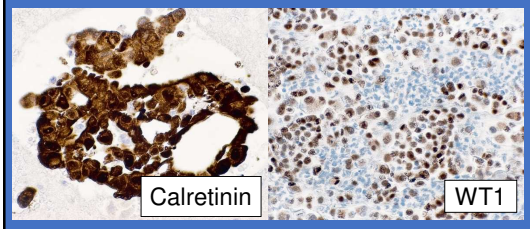
Diagnostic Immunohistochemistry for Malignant Mesothelioma

Sensitivity of effusion cytology historically only ~32%

4-stain panel

Keratin	Claudin-4
Calretinin	TTF-1
WT1	MOC31, CEA, etc.
D2-40	Other lineage markers

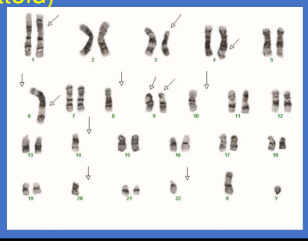
Only confirms mesothelial differentiation, not malignancy



What ancillary studies distinguish between benign and malignant mesothelial cells?

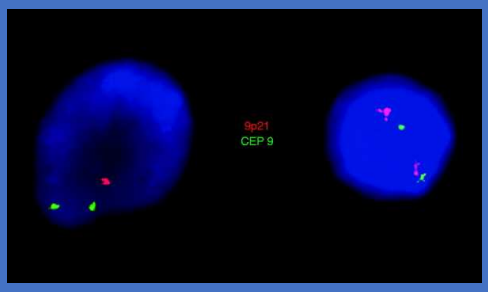
Malignant Mesothelioma: Cytogenetics

- Karyotype or FISH traditionally the only ancillary method that confirmed malignancy in cytology specimens
- Karyotype characterized by multiple chromosome and arm-level losses
- Deletion of 9p21 (CDKN2A)
  - 70% pleural epithelioid mesotheliomas
  - >95% sarcomatoid mesotheliomas
- Deletion of 22q (NF2)
  - 60% mesotheliomas (epithelioid>sarcomatoid)
- Deletion 3p21 (BAP1)
  - 20% mesotheliomas



Malignant Mesothelioma: Cytogenetics

- High sensitivity and specificity
- Can be performed on FFPE
- 2 commercially available probes (9p and 22q)
- Homozygous 9p21 deletion has 100% specificity
  - ~35% have homozygous deletion; another 3-35% heterozygous
- However: time-consuming and requires expertise for interpretation



Factor RE et al. Cancer Cytopathol .2009;117(4) 247-53.

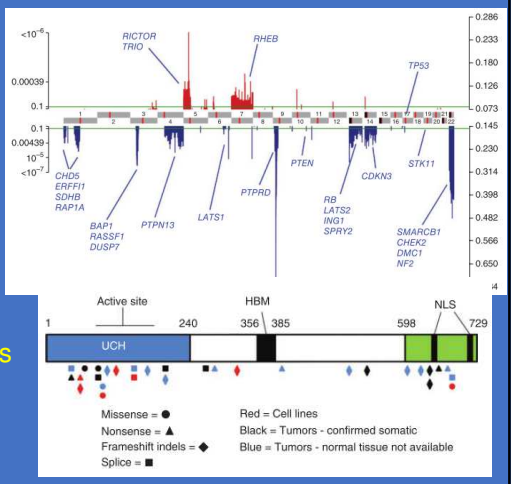
Immunohistochemistry for Distinction Between Benign and Malignant Mesothelial Proliferations

Marker	Reactive %	Mesothelioma %	Sens. (%)	Spec. (%)
Desmin	84-86	0-10	48	97
EMA	4-6	71-100	68-99	74-97
GLUT-1	0-37	40-100	40-99	80-100
P53	0-14	16-86	41-61	91
IMP3	0-27	36-91	36-77	73-100
BAP1	0	57-80	57-67	100
MTAP	0	45	45	100
NF2	0	35-65	35-65	100

- Many markers proposed based on preferential expression
- Either alone or in combination, not proven to reliably distinguish between benign and malignant mesothelial cells
  - Benign mesos may express any of these markers

Immunohistochemistry Surrogates for Genetic Alterations

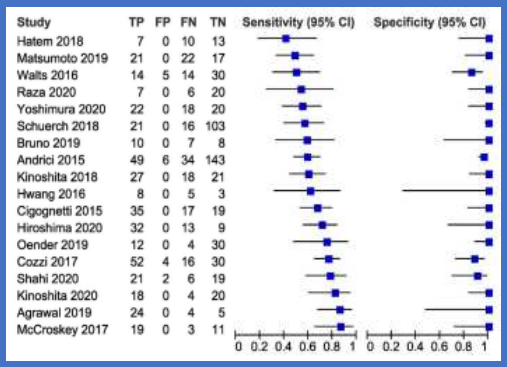
- At least single copy loss of *BAP1* locus at 3p21 in 30%
- 18-63% have mutations or translocations involving *BAP1*
- In total, approximately **60-79%** malignant mesotheliomas have *BAP1* alterations
- Loss of nuclear BAP1 expression reflects underlying *BAP1* alterations



Bott et al. Nat Genet. 2011;43(7):668-72.

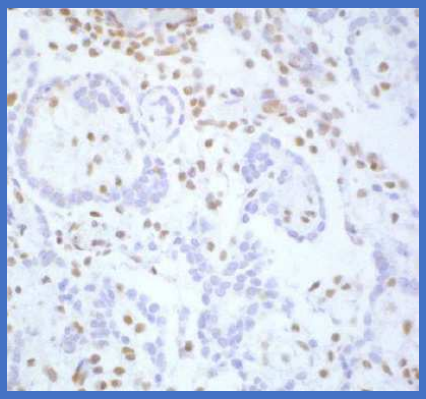
Immunohistochemistry Surrogates for Genetic Alterations

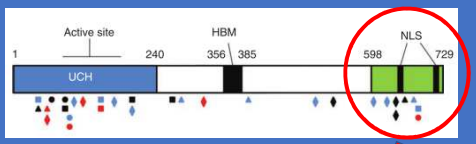
- Up to 70% of mesotheliomas show loss of nuclear BAP1 expression
  - 70% epithelioid mesotheliomas
  - 15-25% sarcomatoid mesotheliomas
- Sensitivity +/-, specificity high
  - Loss of BAP1 is NOT seen in benign mesothelial cells
- Most studies require loss in 100% of tumor cells



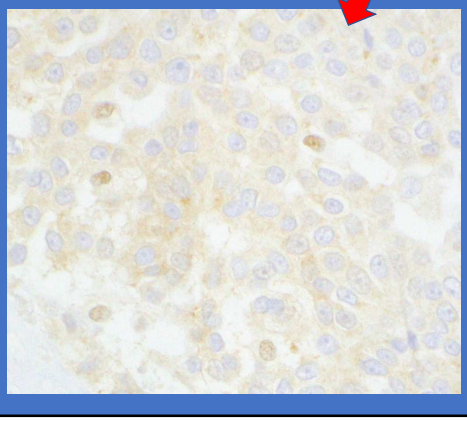
Adapted from Girolami I, et al. Cancer Cytopathol. 2021. doi:10.1002/cncy.22509 [online ahead of print]

BAP1 Immunohistochemistry





Cytoplasmic staining counts!



Diagnostic MTAP Immunohistochemistry

- *CDKN2A* deleted in 60-70% mesotheliomas
  - Sarcomatoid > epithelioid
  - Traditionally queried only by FISH
- *MTAP* gene co-deleted in 75% of cases with *CDKN2A* deletions
- MTAP immunohistochemistry is ~75% sensitive for *MTAP* deletion
- 100% of cases with *MTAP* deletions have *CDKN2A* deletions
- MTAP itself may be a target of therapy

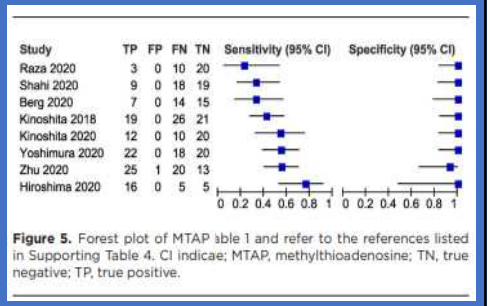
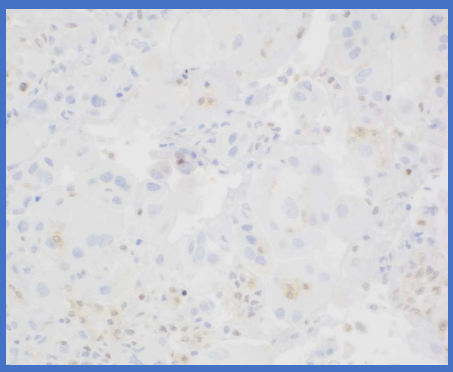


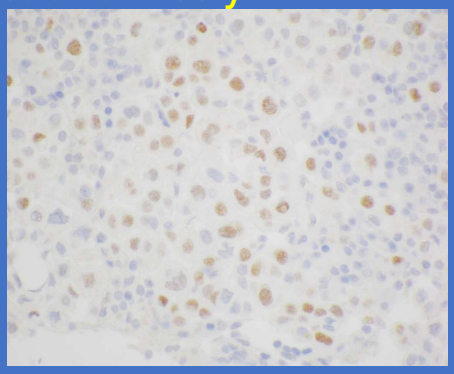
Figure 5. Forest plot of MTAP able 1 and refer to the references listed in Supporting Table 4. CI indicae; MTAP, methylthioadenosine; TN, true negative; TP, true positive.

Adapted from Girolami I, et al. Cancer Cytopathol. 2021;129(7):506-16.

Diagnostic MTAP Immunohistochemistry

Complete loss OR cytoplasmic loss only





Status of nuclear expression not reliable correlate of gene status

BAP1/MTAP Immunohistochemistry for Mesothelioma

	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

Kinoshita Y, et al. Cancer Cytopathol. 2018;126(1):54-63.

TABLE 1. Correlation Between MTAP and BAP1 Immunohistochemistry Results on the Cytology and Surgical Specimens in Cases for Which Paired Specimens Were Available

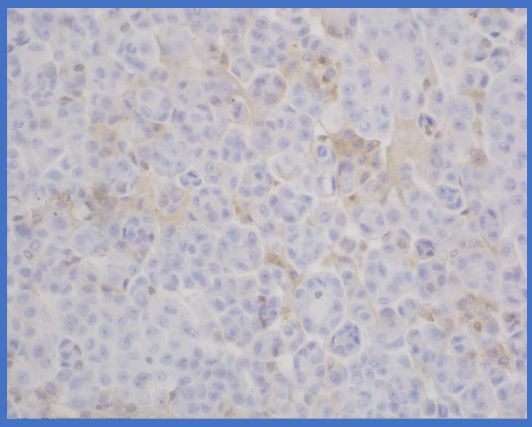
	MTAP			BAP1			
Case No.	Cytology Specimen	Surgical Specimen	Agreement	Cytology Specimen	Surgical Specimen	Agreement	MTAP or BAP1 Loss on Cytology
1	Partial[a]	Intact	Agree[b]	Intact	Intact	Agree	No
2	Lost	Lost	Agree	Lost	Lost	Agree	Yes
3	Partial[a]	Intact	Agree[b]	Lost	Lost	Agree	Yes
4	Partial[a]	Intact	Agree[b]	Lost	Lost	Agree	Yes
5	Lost	Lost	Agree	–	–	–	Yes
6	Intact	Intact	Agree	Intact	Intact	Agree	No
7	Lost	Lost	Agree	Lost	Lost	Agree	Yes
8	Lost	Lost	Agree	Intact	Intact	Agree	Yes
9	Lost	Lost	Agree	Lost	Lost	Agree	Yes
10	Intact	Intact	Agree	Lost	Lost	Agree	Yes
11	Intact	Intact	Agree	Lost	Lost	Agree	Yes
12	Intact	Intact	Agree	Lost	Lost	Agree	Yes
13	Intact	Intact	Agree	Lost	Lost	Agree	Yes
14	Lost	Lost	Agree	Lost	Lost	Agree	Yes
15	Intact	–	–	Intact	–	–	No
16	Intact	–	–	Intact	–	–	No
17	Intact	–	–	Intact	–	–	No
18	Intact	–	–	–	–	–	No
19	Intact	–	–	Lost	–	–	Yes
20	Intact	–	–	Lost	–	–	Yes
21	Lost	–	–	Lost	–	–	Yes
Total	33%	43%	100%	63%	77%	100%	71%

Abbreviations: BAP1, BRCA-associated protein 1; MTAP, methylthioadenosine phosphorylase.
[a]Case 1 demonstrated 80% intact MTAP staining and cases 2 and 3 demonstrated 40% intact MTAP staining.
[b]Threshold for loss was set at 25% staining.

Kinoshita Y, et al. Cancer Cytopathol. 2018;126(1):54-63.

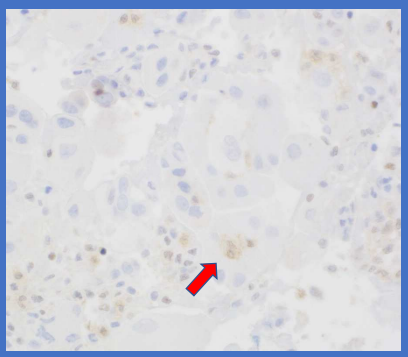
Diagnostic NF2 Immunohistochemistry

- *NF2* mutations or deletions in 60% mesotheliomas
- NF2/Merlin loss in 77% tumors with underlying *NF2* alterations
  - 96% in cases with homozygous deletion, structural variants or mutations
- IHC: loss of membranous/cytoplasmic staining



Challenges in Interpretation of Diagnostic IHC

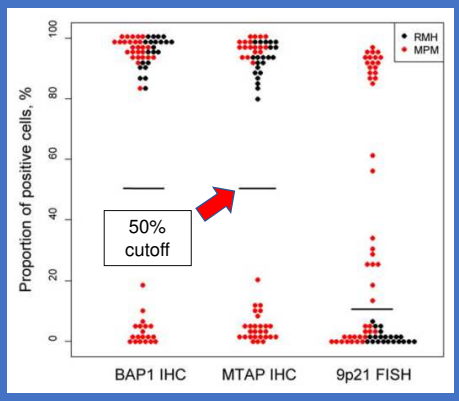
- Poor internal control
- Scant tumor cellularity
- Diagnostic Thresholds
  - Partial loss only – positive? Negative?



Diagnostic MTAP Immunohistochemistry

No established standard threshold (number of cells) to confirm malignancy

- Kinoshita et al. arbitrarily propose a 50% cutoff due to bimodal distribution of staining
- Berg et al. suggest using a 75% cutoff based on cutoff in surgical specimens



Kinoshita Y, et al. Cancer Cytopathol. 2018;126(1):54-63.

MTAP and Tumor Heterogeneity

Tumors can show heterogeneous MTAP expression/subclonal loss

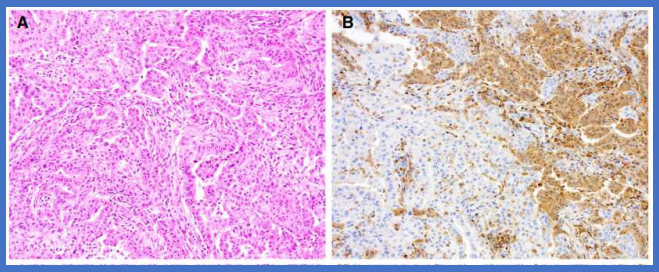


Table 6. *MTAP* and *CDKN2A* copy numbers and MTAP immunohistochemistry findings in nine mesotheliomas with heterogeneous MTAP expression

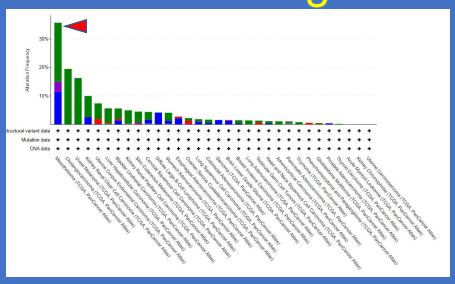
				MTAP immunostaining pattern		
Case	Histotype	*MTAP* copy number	*CDKN2A* copy number	Epithelioid component	Sarcomatoid component	Spatial distribution of foci with MTAP loss and retention
6	Biphasic	1D	2D	Loss in 30% of cells	Loss in 30% of cells	Admixed
8	Epithelioid	1D	2D	Loss in 10% of cells	NA	Admixed
15	Biphasic	1D	1D	Loss in 50% of cells	Loss in 100% of cells	Admixed
16	Epithelioid	1D	1D	Loss in 80% of cells	NA	Spatially discrete
24	Epithelioid	Neutral	1D	Loss in 50% of cells	NA	Spatially discrete
26	Biphasic	Neutral	1D	Loss in 5% of cells	Loss in 100% of cells	Spatially discrete
30	Biphasic	Neutral	Neutral	Loss in 5% of cells	Not stained	Spatially discrete
33	Epithelioid	Neutral	Neutral	Loss in 5% of cells	NA	Spatially discrete
39	Epithelioid	Neutral	Neutral	Loss in 1% of cells	NA	Admixed

1D, Single-copy (heterozygous) deletion; 2D, Two-copy (homozygous) gene deletion; MTAP, Methylthioadenosine phosphorylase; NA, Not applicable; Neutral, NO copy number alteration detected.

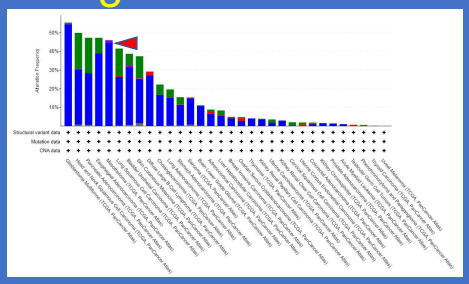
Loss in as few as 5% of cells can be seen in tumors with genetic alterations

Chapel D, et al. Histopathology. 2021;78(7):1032-42.

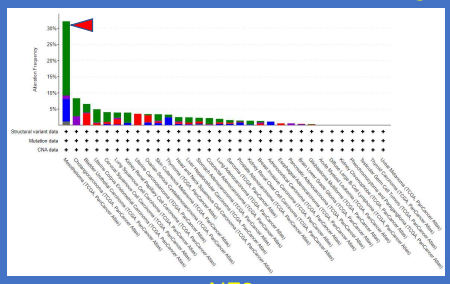
BAP1, CDKN2A, and NF2 Mutations Among Solid Malignancies



BAP1



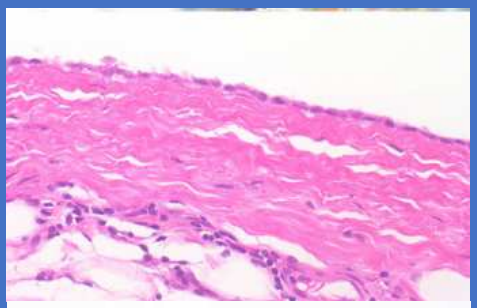
CDKN2A



NF2

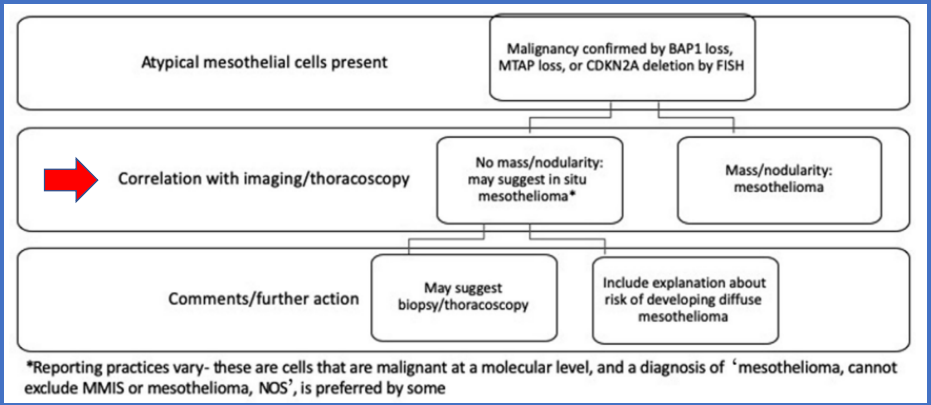
Mesothelioma In-Situ

- Pre-invasive lesion
- Entire specimen submitted
- Evidence of oncogenic genetic abnormalities
- <u>Absence of radiologic evidence for disease</u>



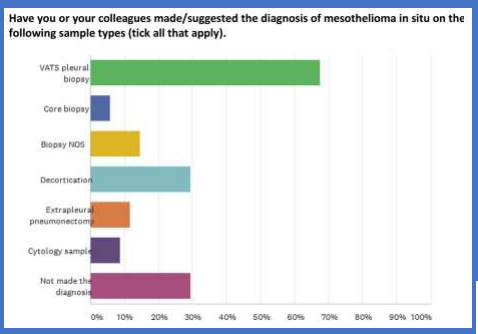
Adapted from: Michael CW, et al. *Diagn Cytopathol.* 2023;51(6):374-88.

Mesothelioma In-Situ in Cytology Specimens?



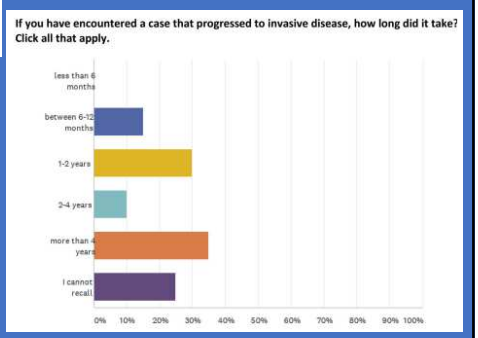
Klebe S, et al. Cancer Cytopathol. Pathology. 2021;53(4):446-53.

Mesothelioma In-Situ



Survey of 34 (heavily selected) thoracic pathologists: ~70% have made the diagnosis

Some evidence that some cases progress to invasive disease



Klebe S, et al. Cancer Cytopathol. Pathology. 2021;53(4):446-53.

Is it possible to make a definitive diagnosis of malignant mesothelioma on effusion cytology?

Yes, if:

- Appropriate clinical and radiologic context, and:
  - Numerous large groups of cells with proven mesothelial differentiation (IHC)
  - Presence of one or more of the following:
    - FISH (9p, 22q) shows typical chromosomal deletions
    - Nuclear BAP1 loss by immunohistochemistry
    - Cytoplasmic MTAP loss by immunohistochemistry
    - Loss of Merlin expression by immunohistochemistry
- Without all supporting evidence, can interpret as "Suspicious for malignant mesothelioma"
  - Prompts **pleural biopsy** or planned **pleurectomy/decortication with frozen section**
  - If surrogate markers show loss of expression, can raise possibility of MIS/low-volume disease

Malignant Effusions: Summary

- Current reporting system
  - ROM for each category is variable between studies
  - Interobserver agreement is greatest for negative and malignant categories
  - Comparable to other reporting systems
- Most malignant effusions represent metastatic adenocarcinoma
  - Most appear as a morphologically distinct "second population"
  - Background elements: mucin, necrosis
- Judicious use of ancillary testing clarifies most diagnostic issues
  - Context: carcinoma > lymphoma > melanoma > sarcoma, mesothelioma
  - IHC panel of 4 stains suggested
  - Cytogenetics, molecular testing, flow cytometry in select circumstances
- Diagnosis of "malignant mesothelioma"
  - Requires appropriate clinical and radiographic context
  - Confirm mesothelial differentiation and exclude metastasis
  - Immunohistochemical surrogates for genetic alterations facilitate diagnosis
    - BAP1
    - MTAP
    - NF2
- Consider mesothelioma in-situ/low-volume disease if convincing evidence in effusion but no radiologic correlate
  - Lag time to development of mesothelioma and treatment implications need further study

References

Roberts ME, Neville E, Berrisford RG, et al. Management of a malignant pleural effusion: British Thoracic Society Pleural Diseases Guideline 2010. *Thorax*. 2010;65 Suppl 2:ii32-40.

Kassirian S, Hinton SN, Cuninghame, et al. Diagnostic sensitivity of pleural fluid cytology in malignant pleural effusions: systematic review and meta-analysis. *Thorax*. 2022;78(1):32-40.

Zhu Y, Ren W, Wang Q, et al. A retrospective analysis of serous effusions based on the newly proposed international system for reporting serous fluid cytopathology: a report of 3633 cases in an oncological center. *Diagn Pathol*. 2022;17(1):56.

Ahuja S, Malviya A. Categorization of serous effusions using the International System for Reporting Serous Fluid Cytopathology and assessment of risk of malignancy with diagnostic accuracy. *Cytopathology*. 2022;33(2):176-84.

Straccia P, Chiappetta M, Magnini D, et al. Application of the International System for Reporting Serous Fluid Cytopathology (TIS): a retrospective institutional study. *Cytopathology*. 2022;33(3):305-11.

Jha S, Sethy M, Adhya A. Application of the International System for Reporting Serous Fluid Cytopathology in routine reporting of pleural effusion and assessment of the risk of malignancy. *Diagn Cytopathol*. 2021;49(10):1089-98.

Bharti S, Nalwa A, Elhence PA, et al. Risk stratification of pleural fluid cytology based on the International System for Reporting Serous Fluid Cytology in a tertiary care center. *Acta Cytol*. 2022;66(5):449-56.

Pinto D, Cruz E, Branco D, et al. Cytohistological correlation in pleural effusions based on the International System for Reporting Serous Fluid Cytopathology. *Diagnostics (Basel)*. 2021;11(6):1126.

Layfield LJ, Yang Z, Vazmitsel M, et al. The international system for serous fluid cytopathology: interobserver agreement. *Diagn Cytopathol*. 2022;50(1):3-7.

Sura GH, Tran K, Fu C, et al. Molecular testing opportunities on cytology effusion specimens: the pre-analytic effects of various body fluid cytology preparation methods on RNA extraction quality and targeted sequencing. *J Am Soc Cytopathol* 2022; doi: 10.1016/j.jasc.2022.09.003 [online ahead of print]

Lepus CM, Vivero M. Updates in Effusion Cytology. *Surg Pathol Clin*. 2018;11(3):523-44.

References

Dragoescu EA, Liu L. Pericardial fluid cytology: an analysis of 128 specimens over a 6-year period. *Cancer Cytopathol*. 2013;121(5):242-51.

Song MJ, Jo U, Jeong JS, et al. Clinico-cytopathologic analysis of 574 pericardial effusion specimens: application of the international system for reporting serous fluid cytopathology (ISRSFC) and long-term clinical follow-up. *Cancer Med*. 2021;10(24):8899-8908.

Zhu H, Booth CN, Reynolds JP. Clinical presentation and cytopathologic features of malignant pericardial cytology: a single institution analysis spanning a 29-year period. *J Am Soc Cytopathol*. 2015;4(4):203-9.

Kambara T, Amatya VJ, Kushitani K, et al. SOX6 is a novel immunohistochemical marker for differential diagnosis of epithelioid mesothelioma from lung adenocarcinoma. *Am J Surg Pathol*. 2020;44(9):1259-1265.

Pantanowitz, Chivukula. Serous fluid: metastatic sarcomas, melanoma, and other non-epithelial neoplasms. *Cytojournal*. 2022;19:15

Chen AL, Janko E, Pitman MB, Chebib I. Clinical, cytologic, and immunohistochemical features of sarcomas involving body cavity fluids. *Cancer Cytopathol*. 2019;127(12):778-84.

Doyle LA, Fletcher CDM, Hornick JL. Nuclear expression of CAMTA1 distinguishes epithelioid hemangioendothelioma from histologic mimics. *Am J Surg Pathol*. 2016;40(1):94-102.

Koh J, Shin SA, Lee JA, Jeon YK. Lymphoproliferative disorder involving body fluid: diagnostic approaches and roles of ancillary studies. *J Pathol Transl Med*. 2022;56(4):173-86.

Das. Serous effusions in malignant lymphomas: a review. *Diagn Cytopathol*. 2006:34(5):335-47.

Factor RE, Dal Cin P, Fletcher JA, Cibas ES. Cytogenetics and fluorescence in situ hybridization as adjuncts to cytology in the diagnosis of malignant mesothelioma. *Cancer Cytopathol*. 2009;117(4) 247-53

Bott M, Brevet M, Taylor BS, et al. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. *Nat Genet*. 2011;43(7):668-72.

References

Eccher A, Girolami I, Lucenteforte E, Troncone G, Scarpa A, Pantanowitz L. Diagnostic mesothelioma biomarkers in effusion cytology. *Cancer Cytopathol*. 2021;129(7):506-16.

Kinoshita Y, Hida T, Hamasaki M, et al. A combination of MTAP and BAP1 immunohistochemistry in pleural effusion cytology for the diagnosis of mesothelioma. *Cancer Cytopathol*. 2018;126(1):54-63.

Chapel DB, Dubuc AM, Hornick JL, Sholl LM. Correlation of methylthioadenosine phosphorylase (MTAP) protein expression with MTAP and CDKN2A copy number in malignant pleural mesothelioma. *Histopathology*. 2021;78(7):1032-42.

Michael CW, Bedrossian CCWM, Sadri N, Klebe S. The cytological features of effusions with mesothelioma in situ: a report of 9 cases. *Diagn Cytopathol*. 2023;51(6):374-88.

Klebe S, Nakatani Y, Dobra K, et al. The concept of mesothelioma in situ, with consideration of its potential impact on cytology diagnosis. *Pathology*. 2021;53(4):446-53.