Fine Needle Aspiration vs. Touch Preparation Cytology:

Insights from Selected Case Presentations

Park City Anatomic Pathology Update
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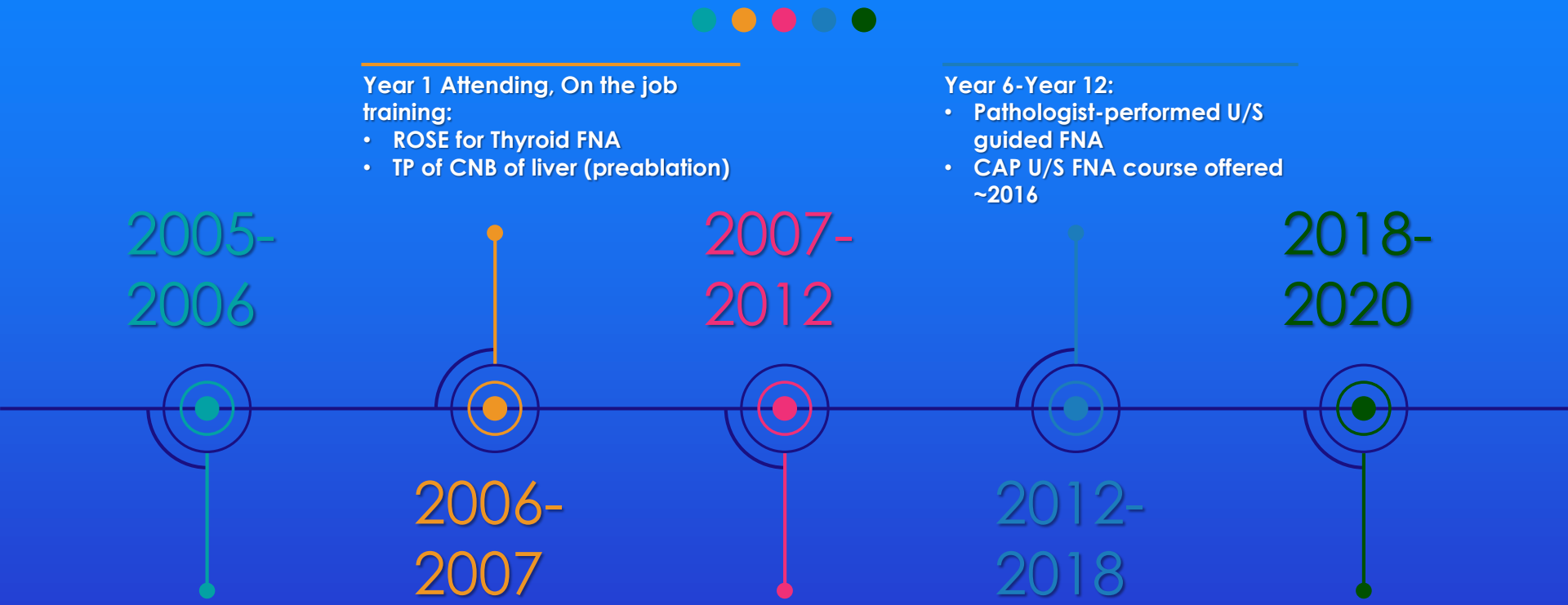
Disclosures

- I have no conflicts of interest to disclose.

Objectives

- To review the factors influencing the increasing trend toward small diagnostic biopsy procurement and diagnosis across selected tumor tissue types.
- To review various types of cytologic preparations from tissue samples, in particular touch preparations, and how they compare to fine needle aspiration biopsy.
- To enhance knowledge of the utility of ROSE in the adequacy assessment and triage of small tissue samples and how FNAB and touch preparation evaluation of core needle biopsies compare in ROSE.
- To appreciate some important insights gained from the use of ROSE illustrated by a series of selected cases.

Evolution of Pathology Practice from One Academic Cytopathologist's Perspective



Emergence of Targeted Therapies in the Treatment of Solid Malignancies

- HER2+ breast carcinoma: Trastuzumab (1998)
- CML/GIST: Gleevec/imatinib (early 2000's)
- Non-small cell lung carcinoma
  - 2004: discovery of targetable mutations for EGFR
  - 2007, 2010: identification of transforming EML4-ALK fusion gene
- Melanoma and others: Ipilimumab, nivolumab 2010's)
- Foundation One testing: FDA-approved broad companion diagnostic testing for solid tumors

Cruz & Kayser, Biol: Targets&Ther 2019

NSCLC: Targeted Therapies and Immunotherapies

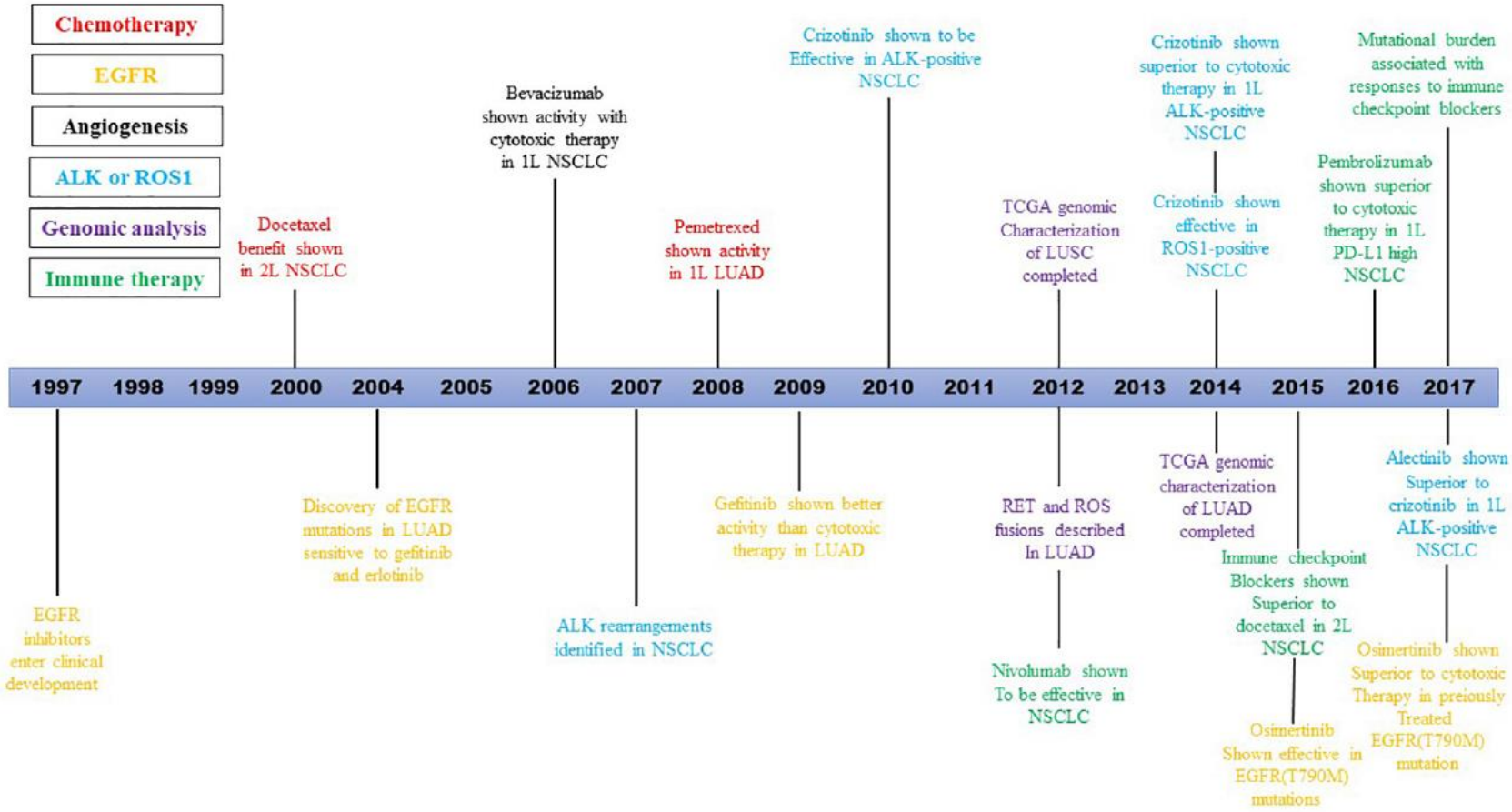


FIGURE 1 | Timeline illustrating the development of targeted therapies and immunotherapies for the treatment of NSCLC over two decades.

Dong et al. Frontiers in Pharm 2019

Changes in Treatment Paradigms of Selected Solid Tumor Types

- Increasing use of neoadjuvant treatment (breast, gynecologic malignancies, sarcoma)
- Management of some tumors similar to chronic diseases (ER+/HER2- metastatic breast cancer; monitoring change in biomarkers for recurrent disease)
- Interrogation of bulky metastatic disease with or without known primary for driver mutations (consideration for off-protocol treatments or clinical trial eligibility?)
- Subpopulation of patients with more than one primary tumor (new biomarkers to test?)

Impact on Routine Practice of Anatomic Pathology

Ultimate result: Dominance of small tissue biopsies and the need to do more with less but,

- Who should be tested?
- What are the tests?
- What material is required (preanalytic factors such as quantity, quality)?
- Who is procuring the tissue?

Proceduralists

- Interventional radiologist
- Endocrinologist
- GI endoscopist
- Interventional pulmonologist
- Pathologist (palpable and/or U/S-guided)

Point of the Spear: Tissue Procurement and the Utility of ROSE

- The “frozen section” of minimally invasive tissue procurement procedures
- Role of ROSE:
  - Ensure adequacy of tissue via real-time feedback to proceduralist (minimize non-diagnostic rate, number of sites biopsied)
  - Provide preliminary diagnosis/differential diagnosis
  - Ensure appropriate disposition of tissue for
    - Diagnostics
    - Prognostics
    - Theranostics

ROSE for Image-Guided Tissue Sampling

- Results of studies vary depending on clinical setting and investigators (proceduralists, pathologists)
- Adequacy: if a sample provides sufficient material for a diagnosis
- Diagnostic yield: rate at which a diagnosis is made
- Accuracy: concordance between cases in which a diagnosis is rendered and a gold standard

Schmidt et al. AJCP 2013, Zakowski, Cancer Cytop 2016

The Influence of Rapid Onsite Evaluation on the Adequacy Rate of Fine-Needle Aspiration Cytology

A Systematic Review and Meta-Analysis

Robert L. Schmidt, MD, PhD, MBA, Benjamin L. Witt, MD, Leslie E. Lopez-Calderon, MD, and Lester J. Layfield, MD

- Reviewed studies across all anatomic locations which included a control arm
- On average ROSE improves adequacy rate by 12%
- Considerable variability across studies noted
- Effect of non-ROSE adequacy rate is critical

Schmidt et al. AJCP 2013

ROSE for Image-Guided Tissue Sampling

- Although evidence for utility of ROSE across all anatomic locations is variable, it is generally recommended by:
  - Pulmonary Pathology Society
  - Papanicolaou Society of Cytopathology
  - European Society of Gastrointestinal Endoscopy
  - American Thyroid Association Management Guidelines (particularly after initial non-diagnostic result)

Vanderlaan et al. J Am Soc Cytopathol 2019

Results from the 2019 American Society of Cytopathology survey on rapid on-site evaluation—Part 1: objective practice patterns

- Survey of membership of American Society for Cytopathology
- 20% response rate
- US practitioners compose vast majority
- > 95% of respondents offered ROSE as a clinical service
- EBUS FNA procedure most frequently reported to employ ROSE

Vanderlaan et al. J Am Soc Cytopathol 2019

Table 1 Respondent demographics for 2019 American Society of Cytopathology rapid on-site evaluation survey.

Demographic	n (%)
Role of respondent	
Cytopathologist/pathologist	255 (47.1)
Cytotechnologist	261 (48.2)
Cytopathology trainee (fellow or resident)	19 (3.5)
Other	6 (1.1)
Total	541 (100.0)
Practice setting	
Academic medical center	304 (56.2)
Community hospital/private practice	197 (36.4)
Reference/commercial laboratory	27 (5.0)
Veteran's hospital	11 (2.0)
Unknown	2 (0.4)
Total	541 (100.0)
Annual nongynecologic cytology specimen volume	
<1000	55 (10.2)
1000-4999	205 (37.9)
5000-9999	144 (26.6)
10,000-14,999	57 (10.5)
>15,000	75 (13.9)
Unknown	5 (0.9)
Total	541 (100.0)

Variety of roles, practice settings & volumes represented

Table 2 Rapid on-site evaluation availability stratified by practice setting and annual nongynecologic cytology specimen volume.

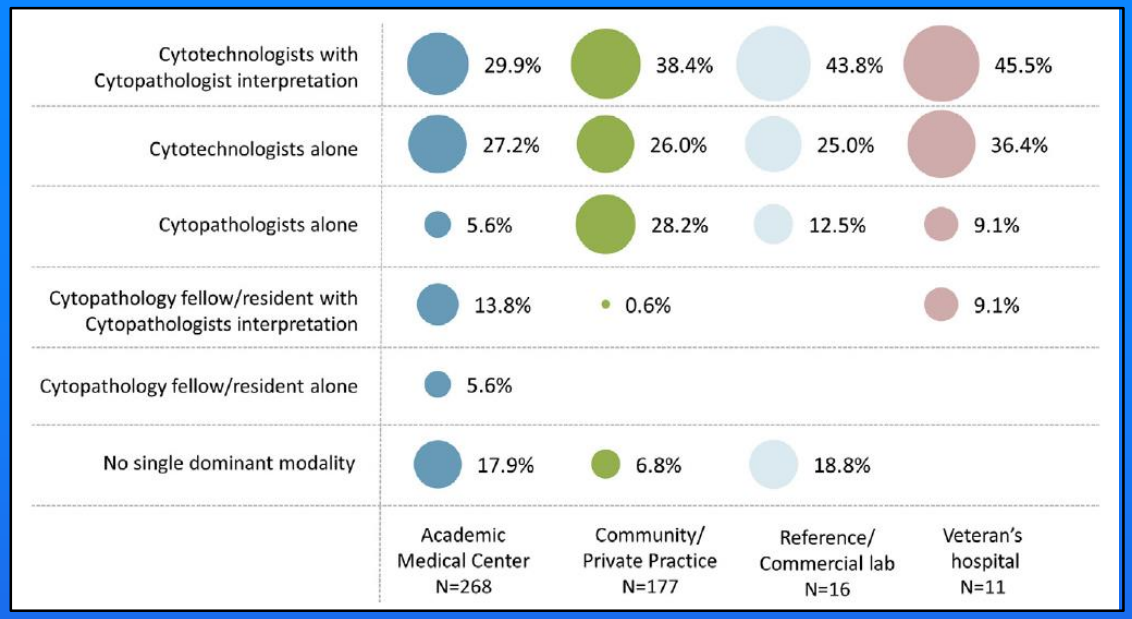
Variable	ROSE available		Total	*P* value
	Yes	No		
Practice setting				<0.001
Academic medical center	295 (97.0)	9 (3.0)	304	
Community hospital/private practice	189 (95.9)	8 (4.1)	197	
Reference/commercial laboratory	20 (74.1)	7 (25.9)	27	
Veteran's hospital	11 (100.0)	0 (0.0)	11	
Unknown	1 (50.0)	1 (50.0)	2	
Total	516 (95.4)	25 (4.6)	541	
Annual nongynecologic cytology specimen volume				0.007
<1000	47 (85.5)	8 (14.5)	55	
1000-4999	198 (96.6)	7 (3.4)	205	
5000-9999	139 (96.5)	5 (3.5)	144	
10,000-14,999	57 (100.0)	0 (0.0)	57	
>15,000	71 (94.7)	4 (5.3)	75	
Unknown	4 (80.0)	1 (20.0)	5	
Total	516 (95.4)	25 (4.6)	541	

Abbreviation: ROSE, rapid on-site evaluation.
Data presented as n (%).

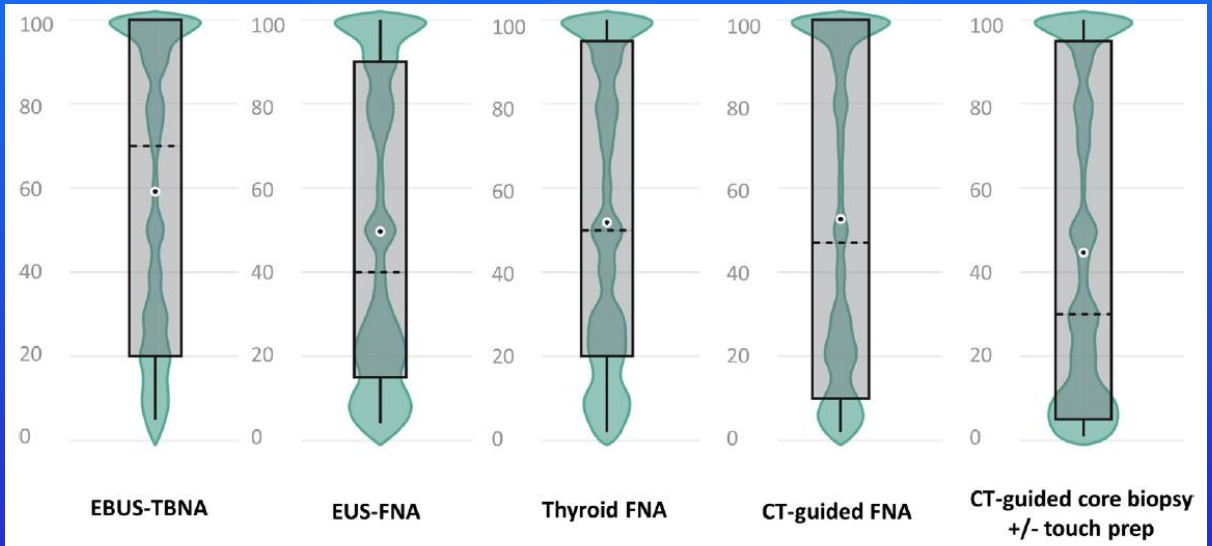
Most groups offered ROSE regardless of setting or size

	<1,000	1,000 - 4,999	5,000 - 9,999	10,000 - 14,999	>15,000	Unknown	Grand Total
Academic medical center	1.3%	15.7%	20.3%	8.3%	10.2%	0.4%	304
Community hospital/private practice	8.1%	19.0%	5.7%	1.5%	1.8%	0.2%	197
Reference/commercial laboratory	0.6%	1.3%	0.4%	0.7%	1.8%	0.2%	27
Veteran's hospital	0.2%	1.7%	0.2%	0.0%	0.0%	0.0%	11
Unknown	0.0%	0.2%	0.0%	0.0%	0.0%	0.2%	2
Grand Total	55	205	144	57	75	5	541

Vanderlaan et al. J Am Soc Cytopathol 2019



Most respondents reported presence of a cytotechnologist in a majority of cases



Procedures	Min	Q1	Median	Mean	Q3	Max	N
EBUS-TBNA	1.0	20.0	70.0	59.1	100.0	100.0	454
EUS-FNA	1.0	15.0	40.0	49.7	90.0	100.0	424
Thyroid FNA	1.0	20.0	50.0	51.9	95.0	100.0	409
CT-guided FNA	1.0	10.0	47.0	52.6	100.0	100.0	385
CT-guided core biopsy +/- touch prep	1.0	5.0	30.0	44.7	95.0	100.0	328

High variability in frequency in which ROSE was used per procedure

Vanderlaan et al. J Am Soc Cytopathol 2019

Traditional Technique: Fine Needle Aspiration

- Effective in multiple tissue sampling scenarios
- Specific lesion types: infection, lymphoma
- Aspirates are optimal specimens for flow cytometric analysis (Boyd et al. 2015)
- Specific lesion locations historically not requiring tissue biopsy before definitive treatment
  - Thyroid
  - Pancreatic adenocarcinoma
  - Salivary gland lesions
  - Confirmation of recurrence/metastasis

Fine Needle Aspiration

Advantages

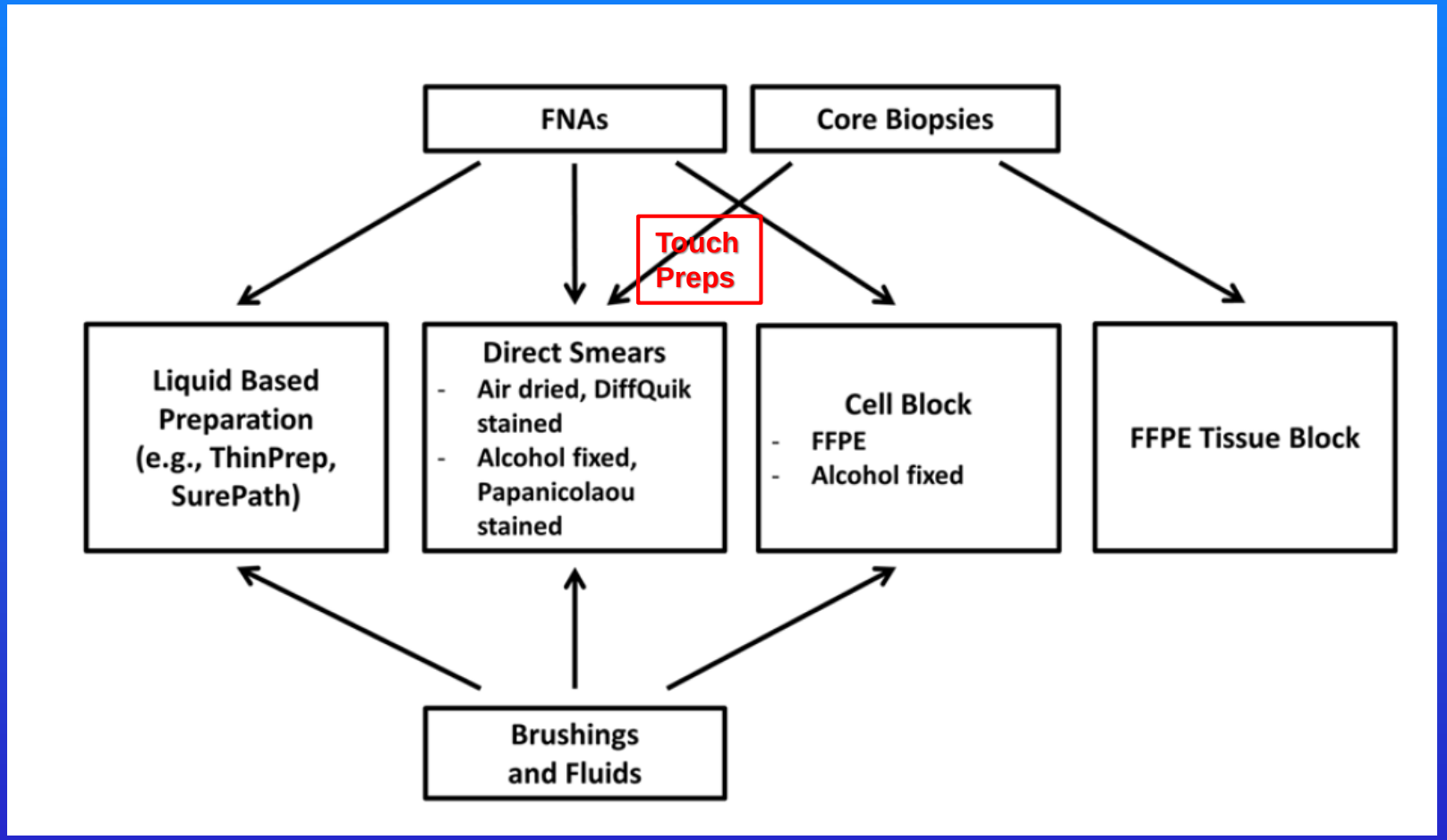
- Rapid to perform and evaluate
- Usually does not require anesthesia
- Can be performed anywhere
- Lower complication rate
- Multiple staining modalities
- Multiple collection methods
- Fresh intact viable cells
- Enhanced assessment of tumor heterogeneity

Limitations

- Good FNA performance requires skill
- U/S guidance for deep-seated lesions
- Good slide preparation requires skill
- Limited tissue architecture
- Can’t distinguish between in situ vs invasive disease
- Decreased utility in fibrotic/dense lesions
- Must validate specimens for IHC, molecular tests
- Lower rate of reimbursement

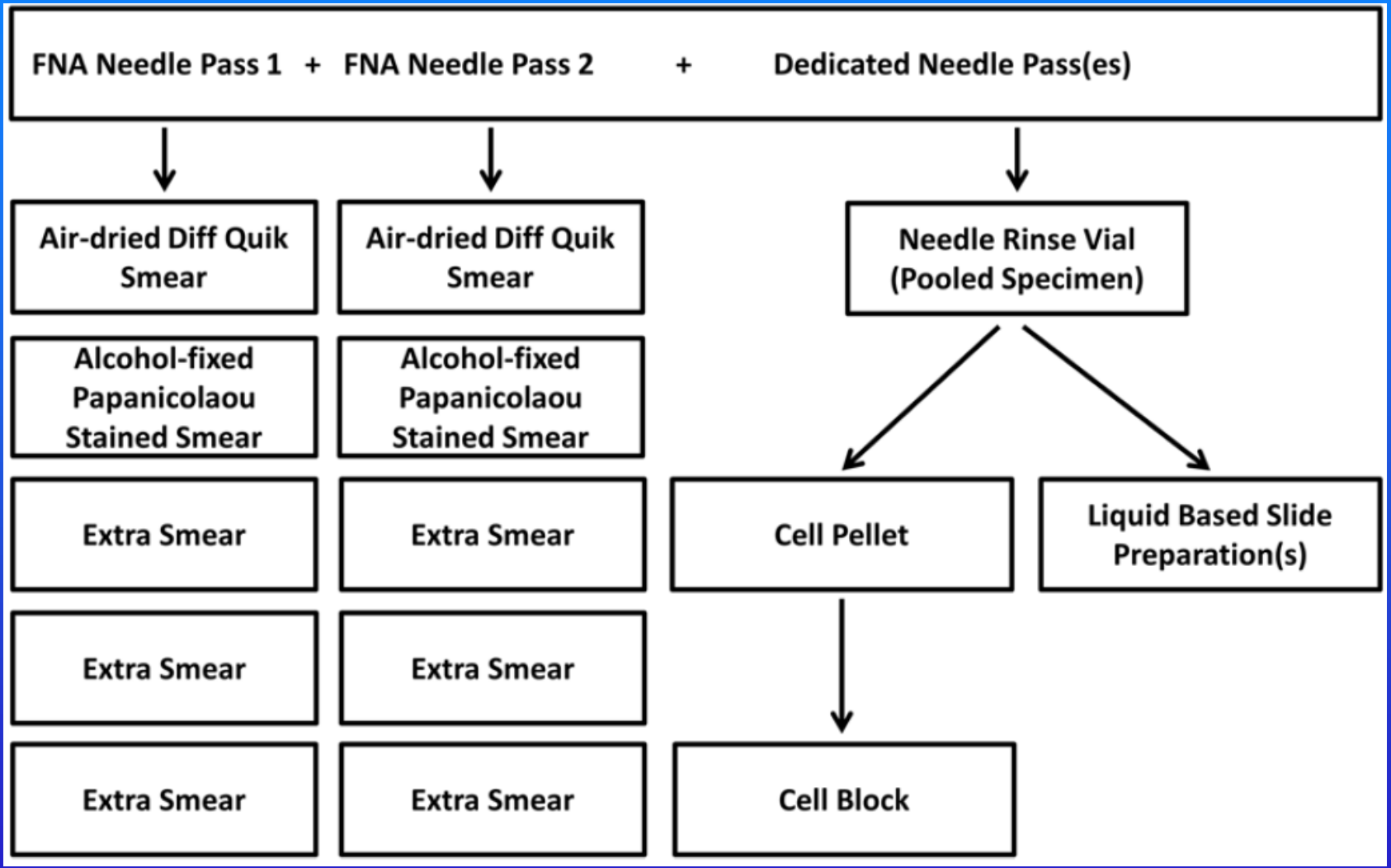
Vanderlaan P. Cancer Cytopathol 2016

Utility of Cytology Specimens for Ancillary Molecular Testing



Roh M. Mod Pathol 2019

Utility of Cytology Specimens for Ancillary Molecular Testing



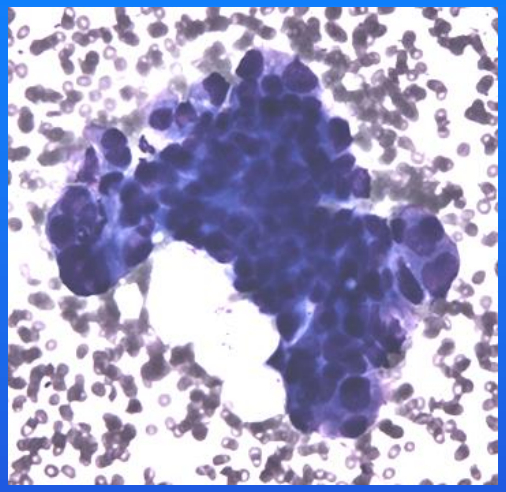
Roh M. Mod Pathol 2019

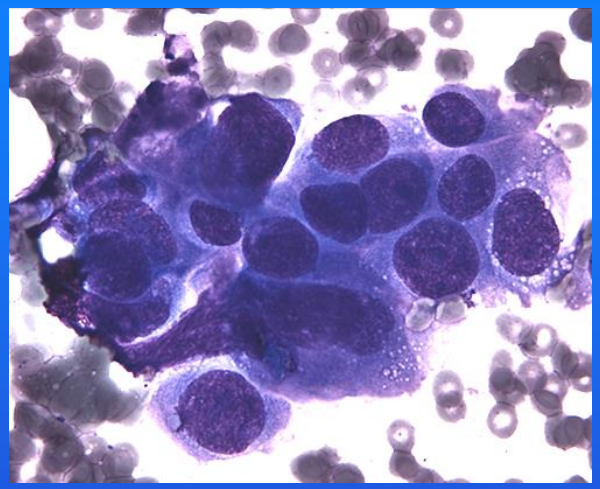
Utility of Cytology Specimens for Ancillary Molecular Testing

- Immunohistochemistry, FISH, and molecular diagnostics can be performed on a wide variety of cytologic preparations
- Multiple groups have demonstrated success with system-wide algorithms (Brainard & Farver, Mod Pathol 2019, Aisner et al, 2016)
- Molecular updates: Dr. Deftereos (2/12)

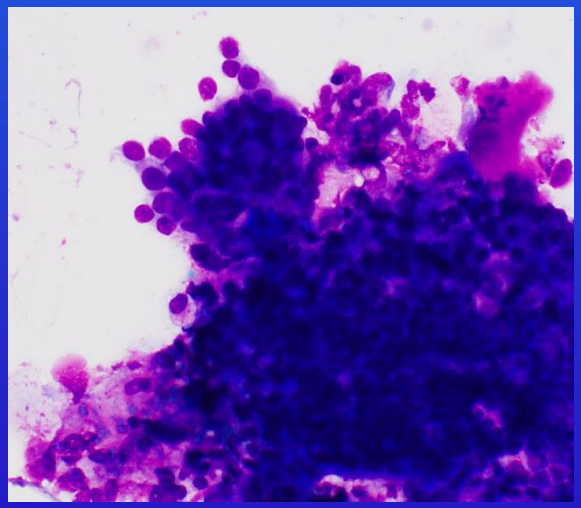
Paradigm Shift: Core Biopsies Evaluated by Touch Preparations

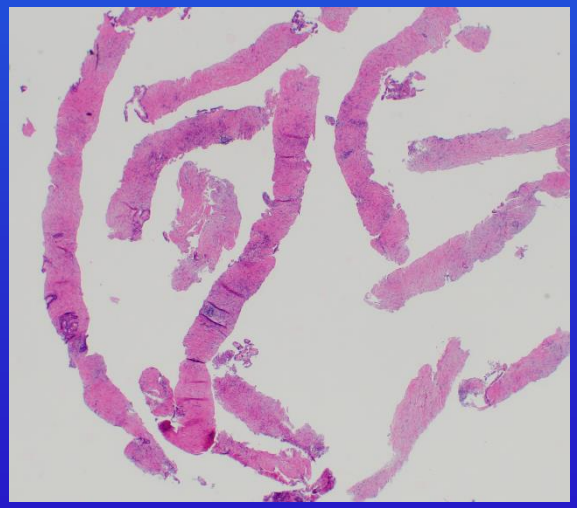
- Pancreatic head mass: FNA 2006

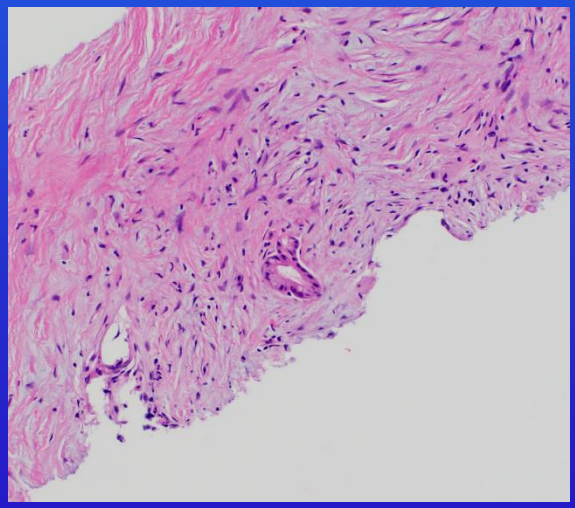




- Pancreatic mass: CNB 2019







Clinical Proceduralist Perspective:

- Minimization of patient complications = desire for less passes, avoidance of repeat biopsies
- Improvements in fine, flexible biopsy needles compatible with endoscopes (ProCore, SharkCore)
- Requirement for more tissue volume for additional testing
- Ability to exclude benign mimics of invasive carcinoma (IgG4 sclerosing disease, chronic pancreatitis, serous cystadenoma)

Increase in CNB +/- TP Across Various Anatomic Sites

- EUS Pancreas – ProCore (Dwyer et al. 2016, Bang et al. 2016), SharkCore (Witt et al. 2018, Fitzpatrick et al. 2019)
- EBUS TBNA (mediastinal lesions) – Jayabalen et al 2014, Xing et al Acta Cytol 2016
- Salivary gland (Witt et al 2014)

Core Needle Biopsy

Advantages

- Larger intact tissue fragments with preserved architecture
- Tissue processing and histology familiar to surgical pathologists
- Tissue is validated for IHC, molecular studies
- Better visualization for fibrotic/dense lesions

Limitations

- Performed by a clinical interventionalist/team
- More expensive
- Requires anesthesia
- Higher complication rate
- ROSE requires trained cytology personnel
- Longer tissue fixation and processing time
- Potential for less extensive lesional sampling

Vanderlaan P. Cancer Cytopathol 2016

ROSE of CNB: Touch Preparations (TP)

- Use varies at different medical centers and among interventionalists:
  - FNA and CNB with TP
  - CNB with TP only
- Generally, accuracy of TP reported to be 80-92%
- Accuracy of FNA+TP reported to be about 95%

Pantanowitz et al. Atlas of Touch Preparation Cytology. 2019

Touch imprint cytology

- Smear/squash preparations in neuropathology intraoperative consultation (IOC)
- Imprint cytology for IOC tissue triage
  - Infection
  - Lymphoma
  - Sarcoma
- Cytologic preps from intact tissue samples or resection specimens

Pantanowitz et al. Atlas of Touch Preparation Cytology. 2019

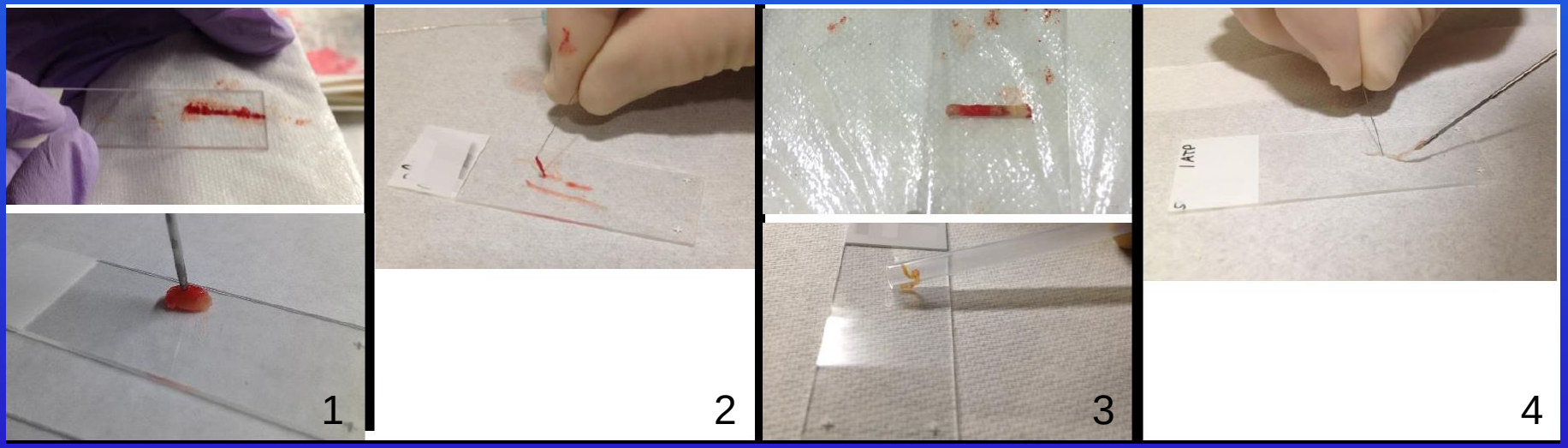
Other Cytologic Preparations

- Touch imprint:
  - Usually taken from an incisional biopsy or resection specimen
  - Commonly used during intraoperative frozen section evaluations
- Scrape or squash preparation: smear
- Touch preparation: imprint cytology of small tissue biopsy samples

Pantanowitz et al. Atlas of Touch Preparation Cytology. 2019

Touch Preparation Techniques

1. Imprint: slide is touched gently to the core
2. Drag: minimize distance to avoid tissue loss
3. Roll: can use a needle cap to gently roll core on slide
4. Touch & pick: core is picked up with needle and touched to slide without dragging



Pantanowitz et al. Atlas of Touch Preparation Cytology. 2019

Comparison of Cytomorphologic Features of FNA vs TP

Cytologic Features	Aspirate Smears	Touch Preparations
Cellularity	Usually cellular	Variable
Heterogeneity	High, especially with multiple passes	Limited
Cohesive cell groups	Present without stroma	Present without stroma
Discohesive cells	Present, including lymphocytes with LGB	May show artifactual clustering, including lymphocytes +/- LGB
Spindle cells	Easily appreciated if aspirated	Easily appreciated if present
Stroma	Not present or scant	Not present or scant
Background	e.g. ECM or disrupted cytoplasm is easy to smear	Typically present and patchy
Necrosis	Appears as granular debris aggregating in thick, curly lines	Appears as thick, granular debris
Blood dilution	Often present	Limited
Overall architecture	Disrupted	Intact clues to architecture
Artifacts	Air-drying and crushed cells	Air-drying, thick groups, stripped cells

LGB = lymphoglandular bodies

Modified from Pantanowitz et al. Atlas of Touch Preparation Cytology. 2019

TP Pitfalls

- Tissue loss:
  - avoid dragging cores > 1 cm along length of slide (Rekhtman et al. 2015)
  - FNA material helpful to bolster quantity
- Artifacts
  - Streaking artifact
  - Thick cellular clusters
  - Ultrasound gel contamination

Modified from Pantanowitz et al. Atlas of Touch Preparation Cytology. 2019

Factors Impacting Adequacy

- Type and expertise of proceduralist
  - Endocrinologist
  - GI endoscopist
  - Interventional pulmonologist
  - Interventional radiologist
  - Pathologist
- The use of ROSE
- Needle type and size
- Number of passes

Schmidt et al. 2013

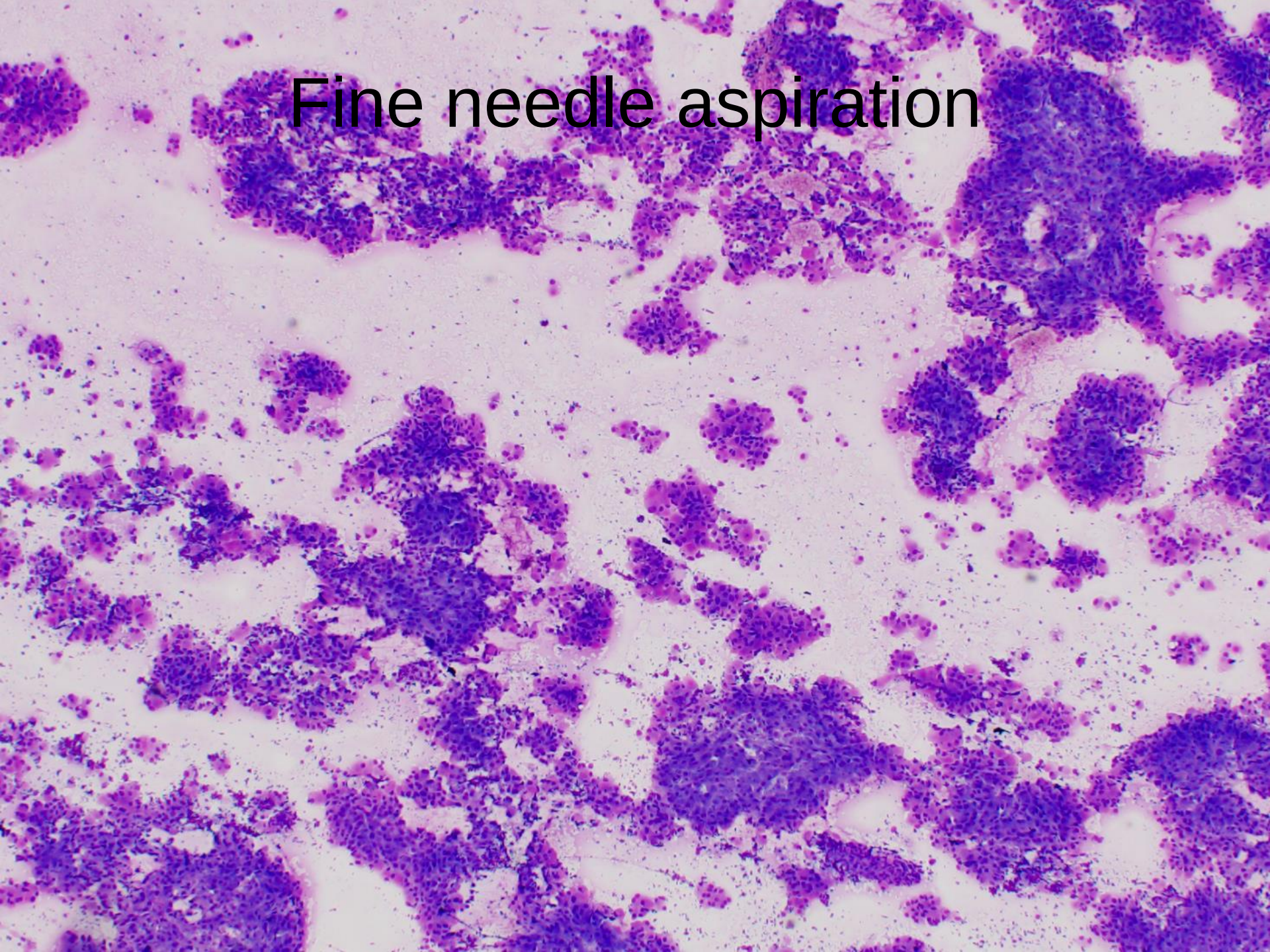
- Anatomic site of lesion
- Clinical history (prior malignancy, prior treatment, immunosuppression, radiological ddx, etc)
- Size of lesion
- Physical characteristic or consistency of the lesion
  - Solid and cystic
  - Stroma predominant-ECM
  - Stroma predominant-fibrotic (mesenchymal or epithelial + desmoplastic stroma)

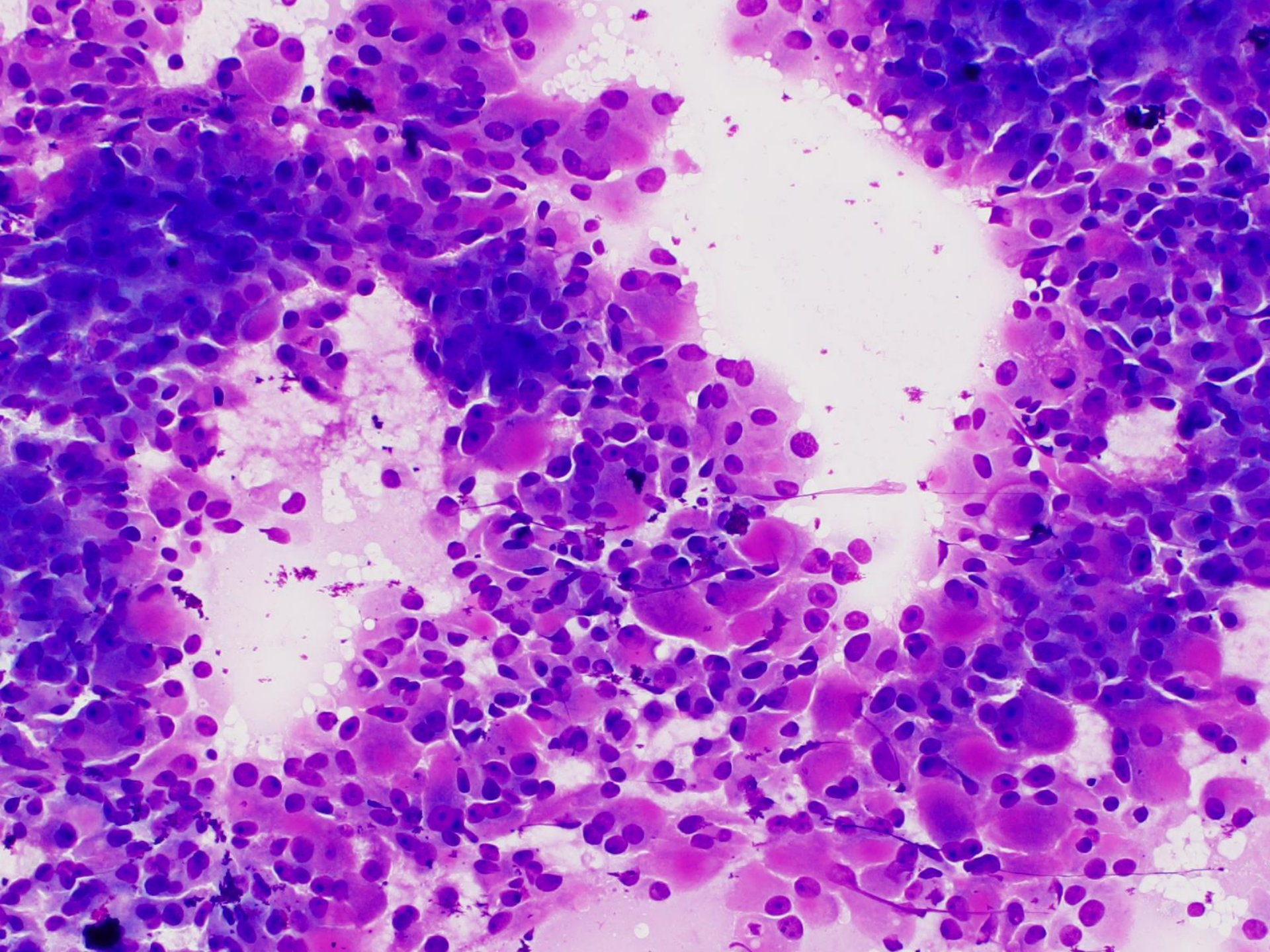
Case Presentations:
FNA and TP at ROSE

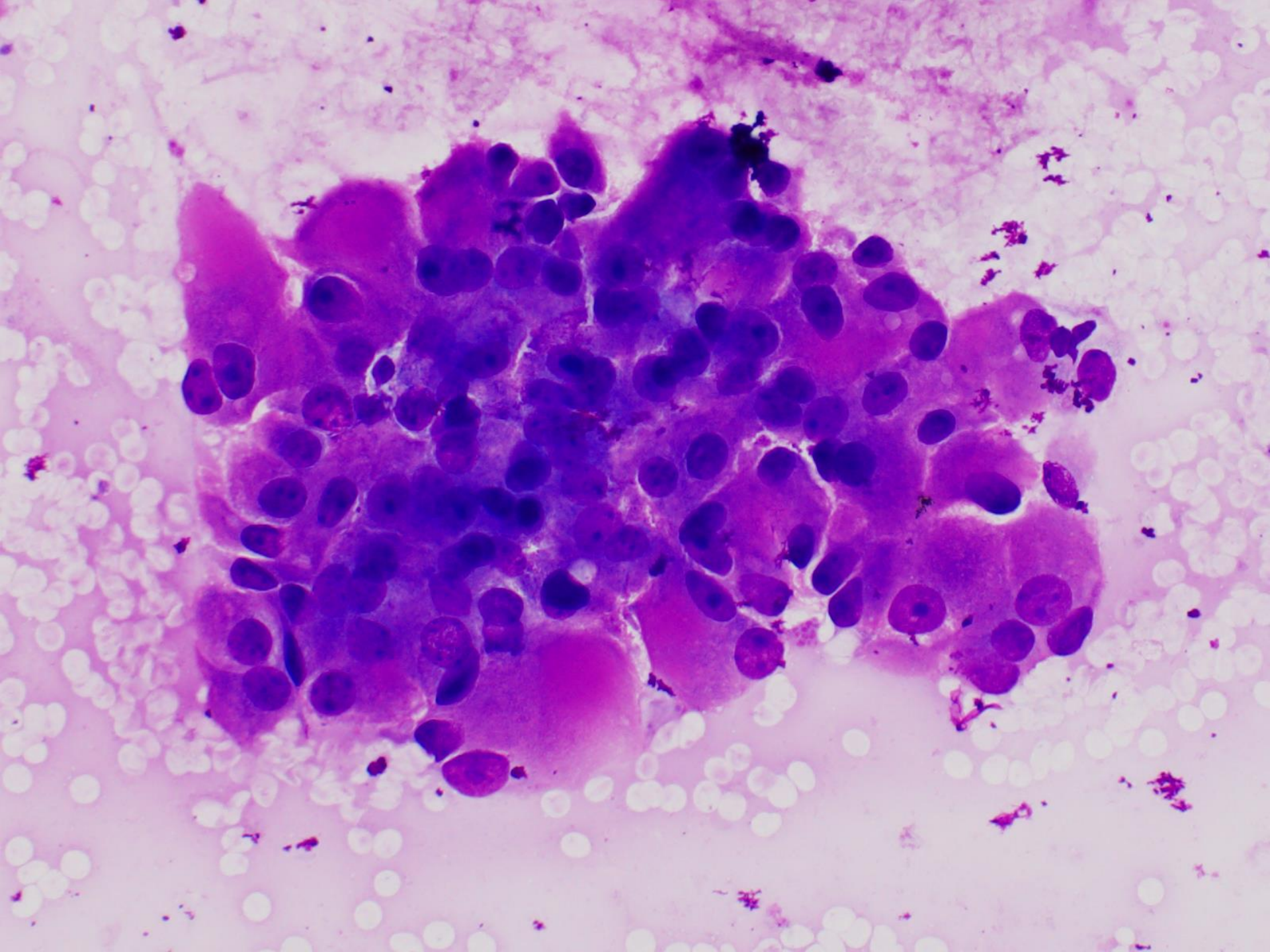
Baseline Case (Diagnostic Best Case Scenario)

- 67 year old male with innumerable liver lesions, no known primary
- Later review of OSH imaging indicated circumferential wall thickening & nodularity of proximal rectum concerning for rectal carcinoma
- IR performed U/S-guided FNA and CNB

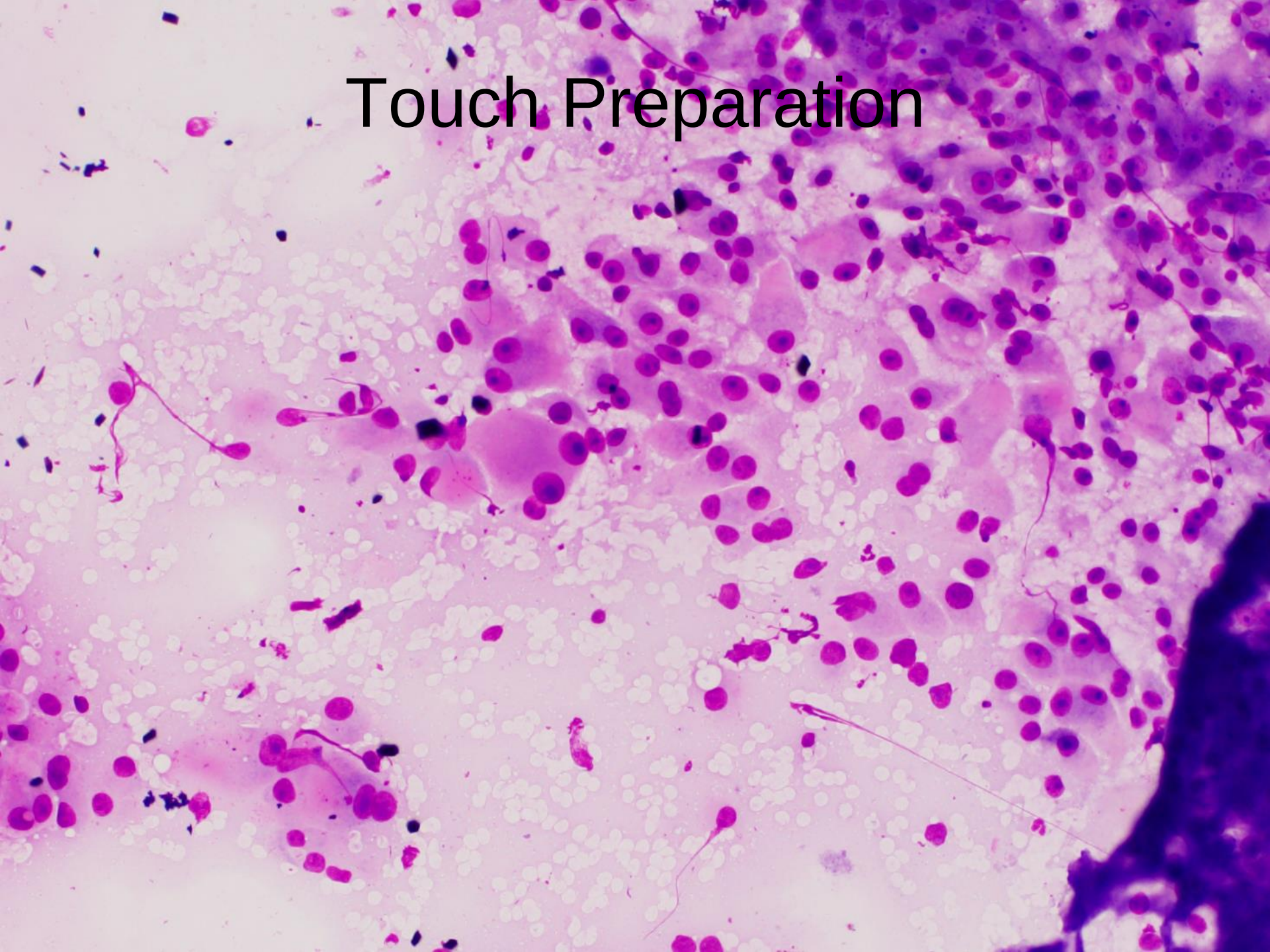
Fine needle aspiration

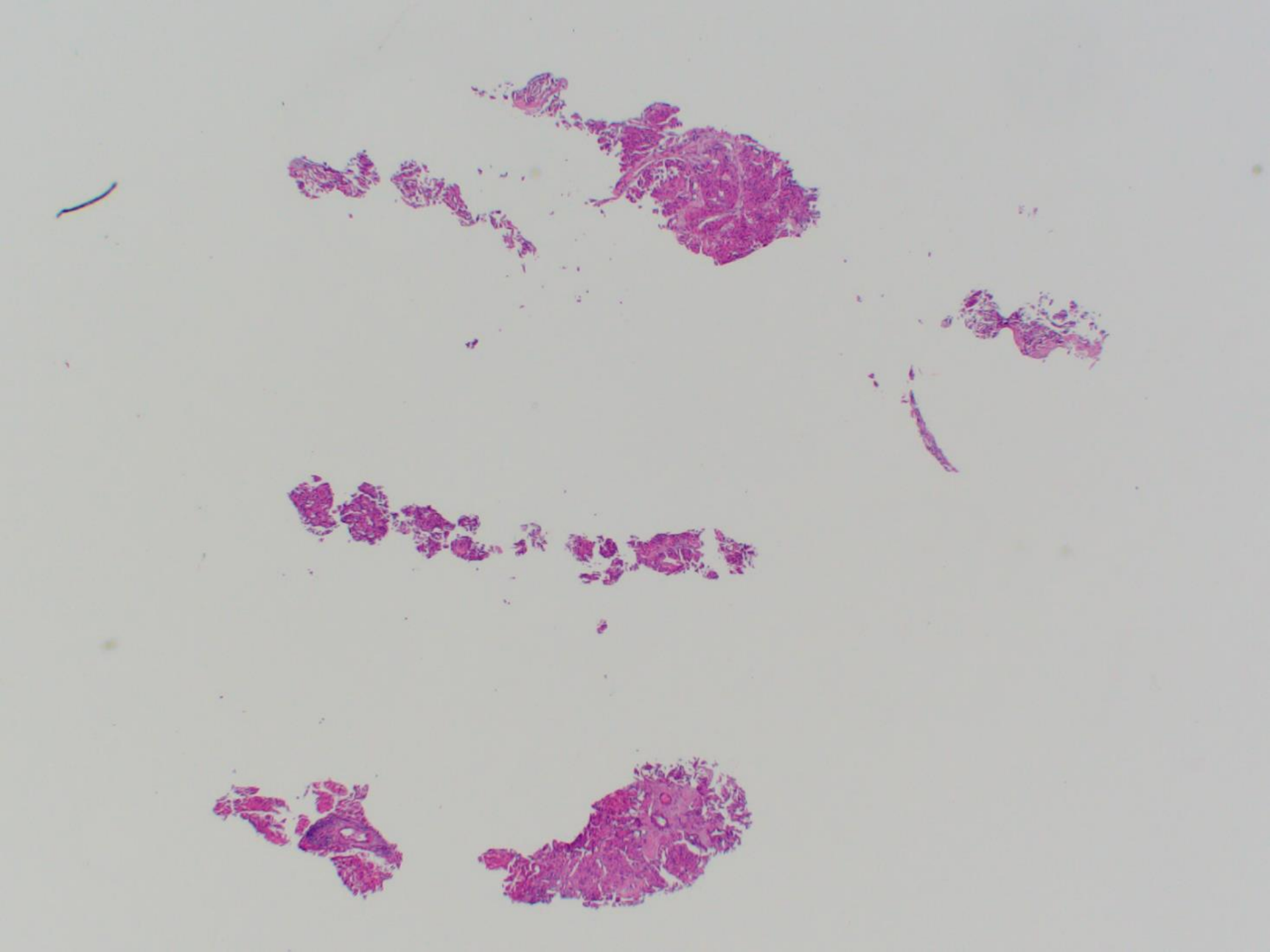


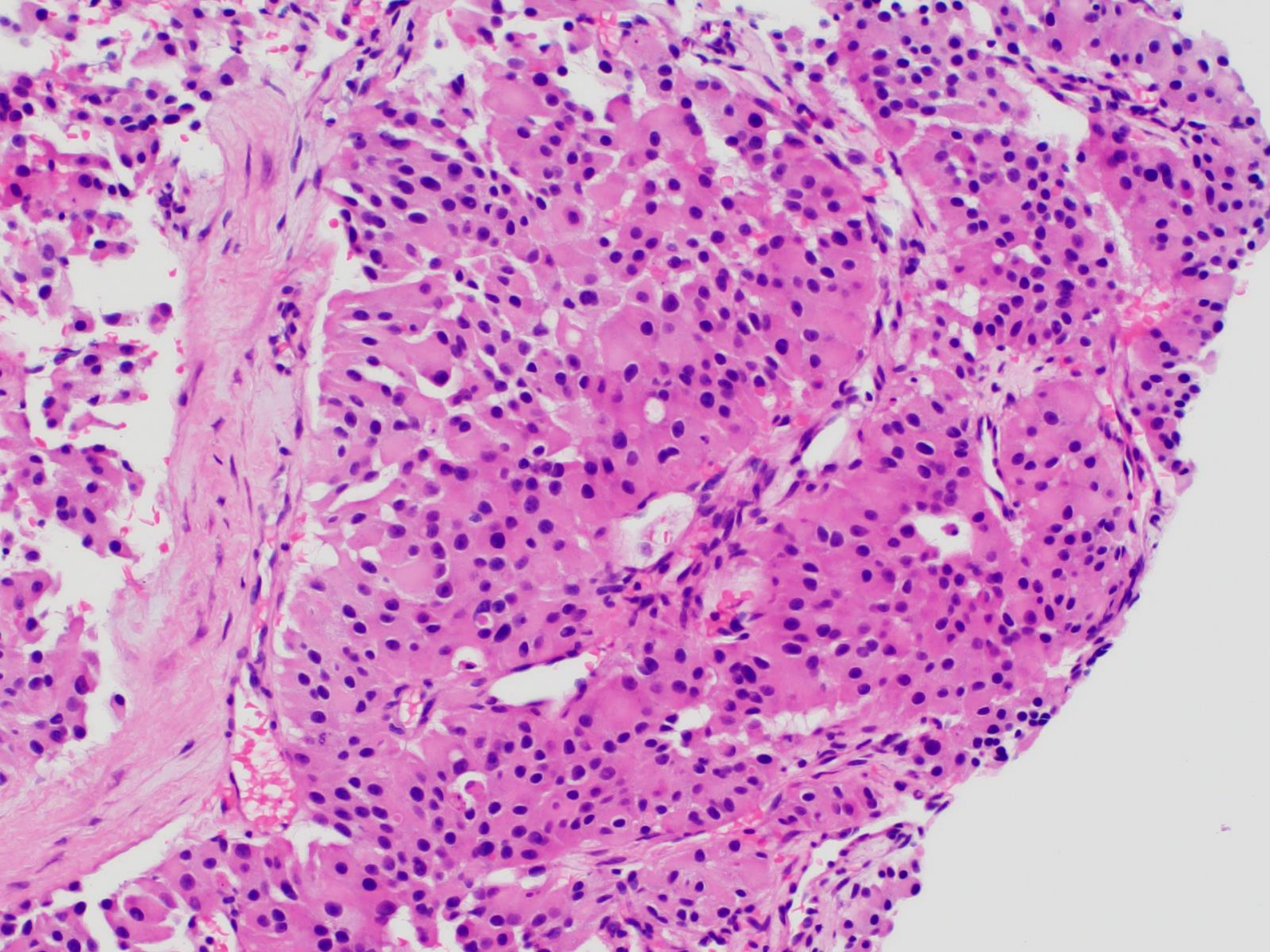


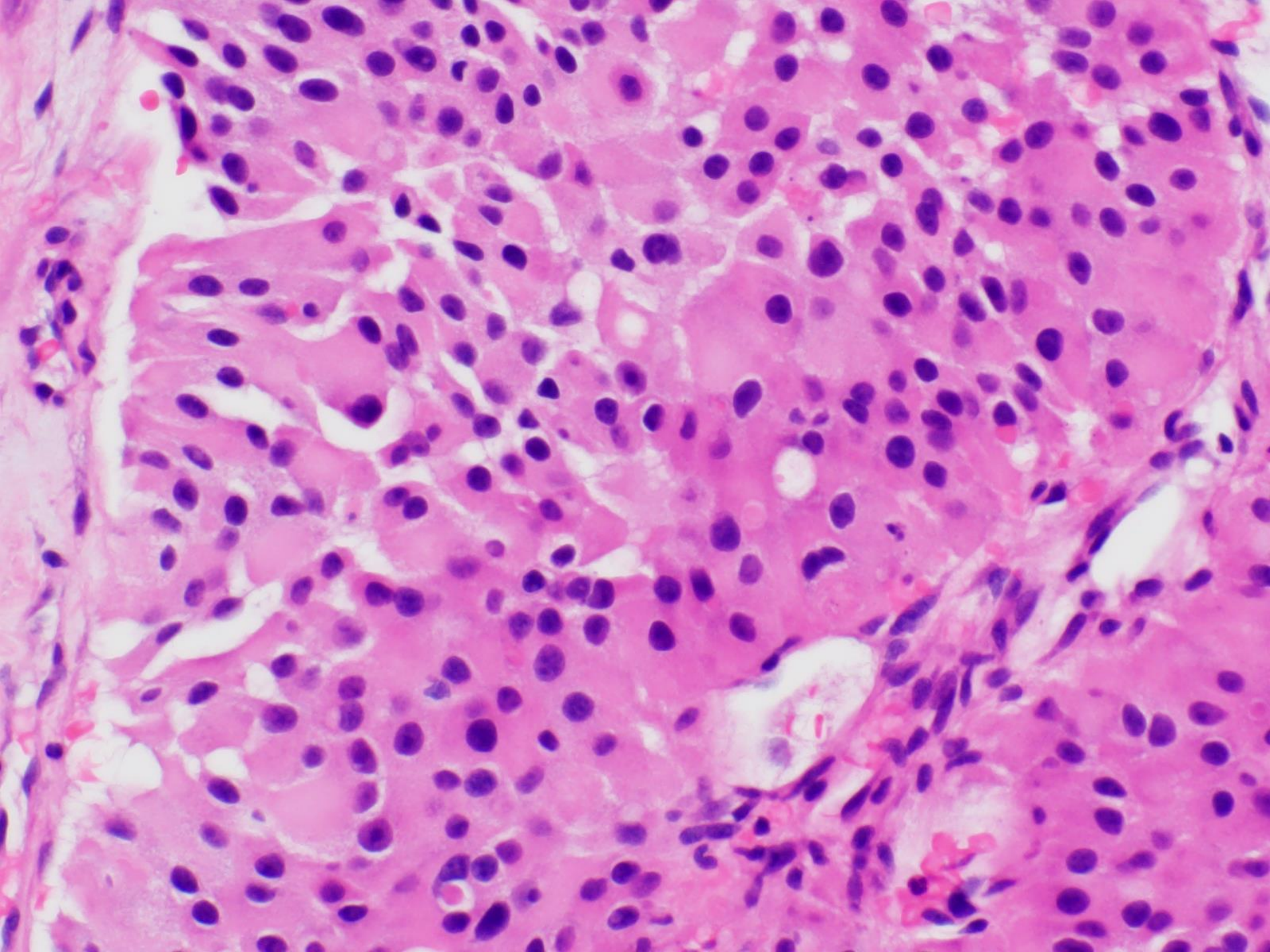


Touch Preparation

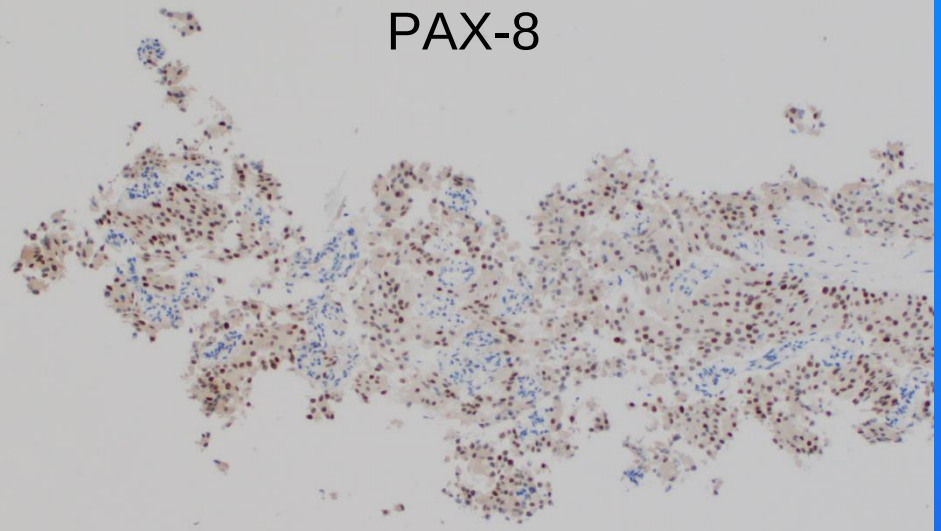




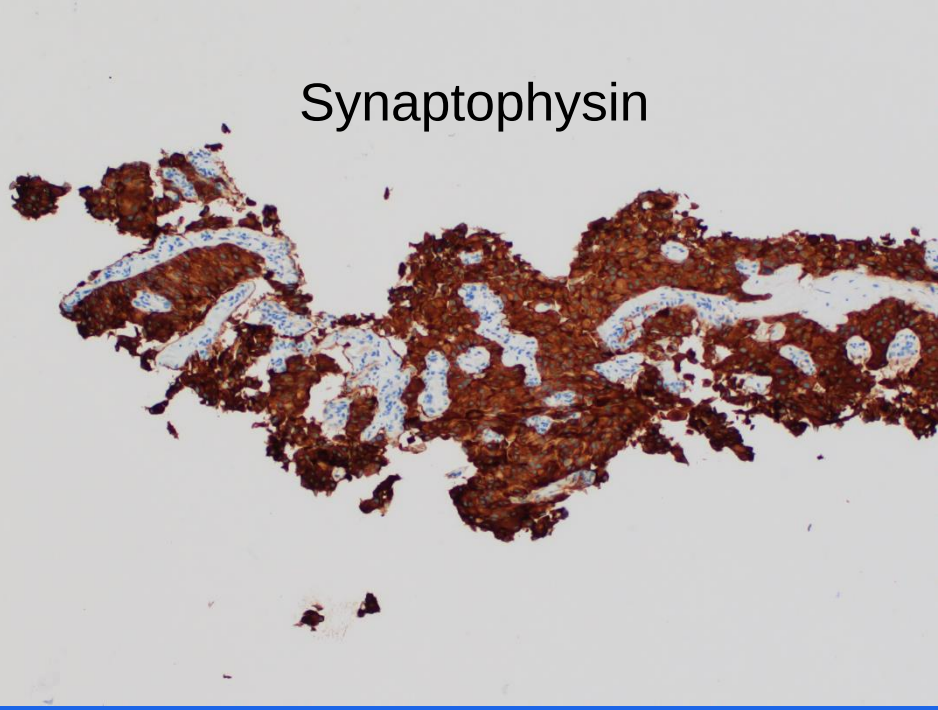




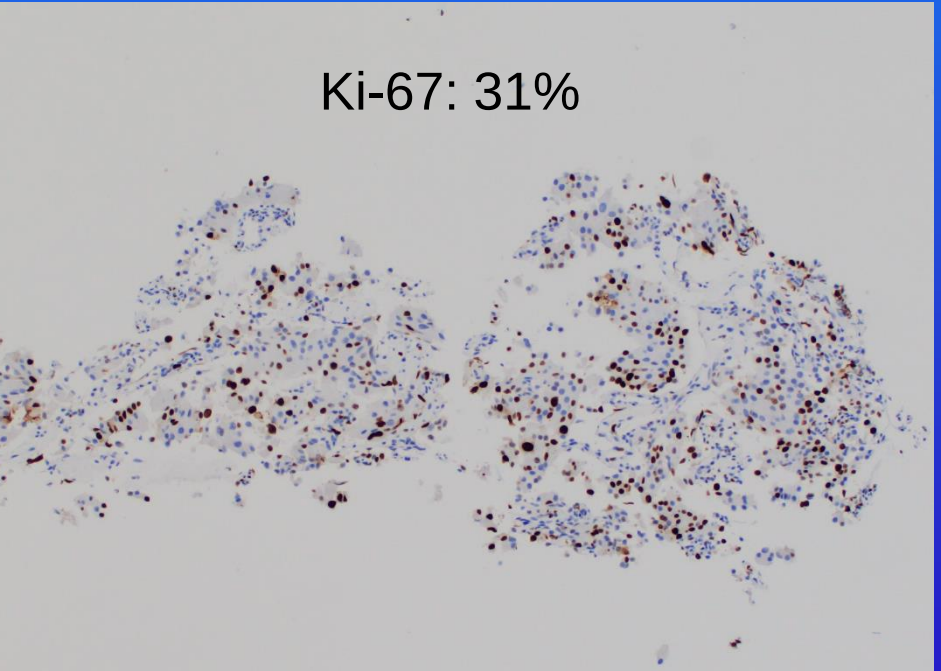
PAX-8



Synaptophysin



Ki-67: 31%

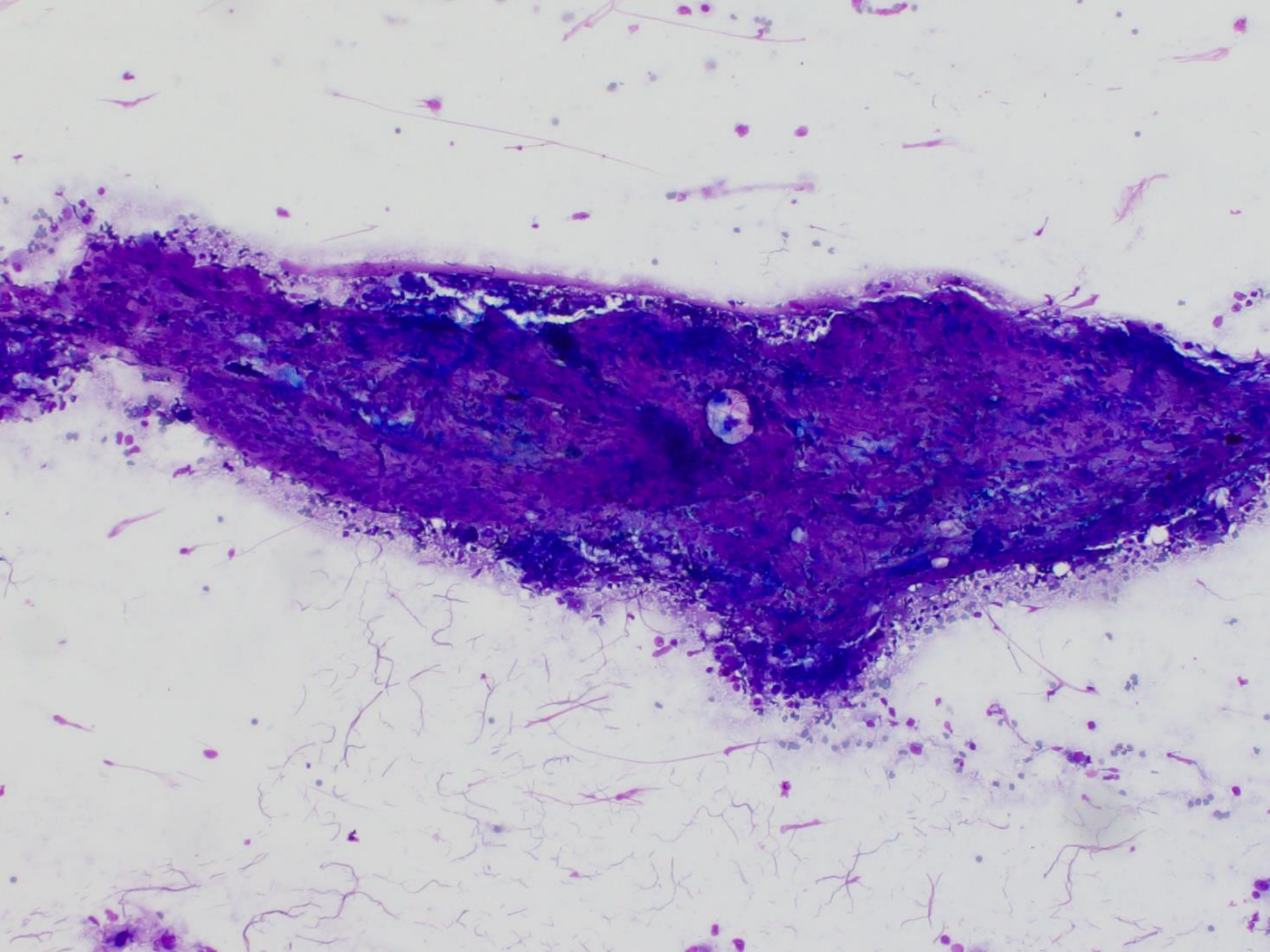


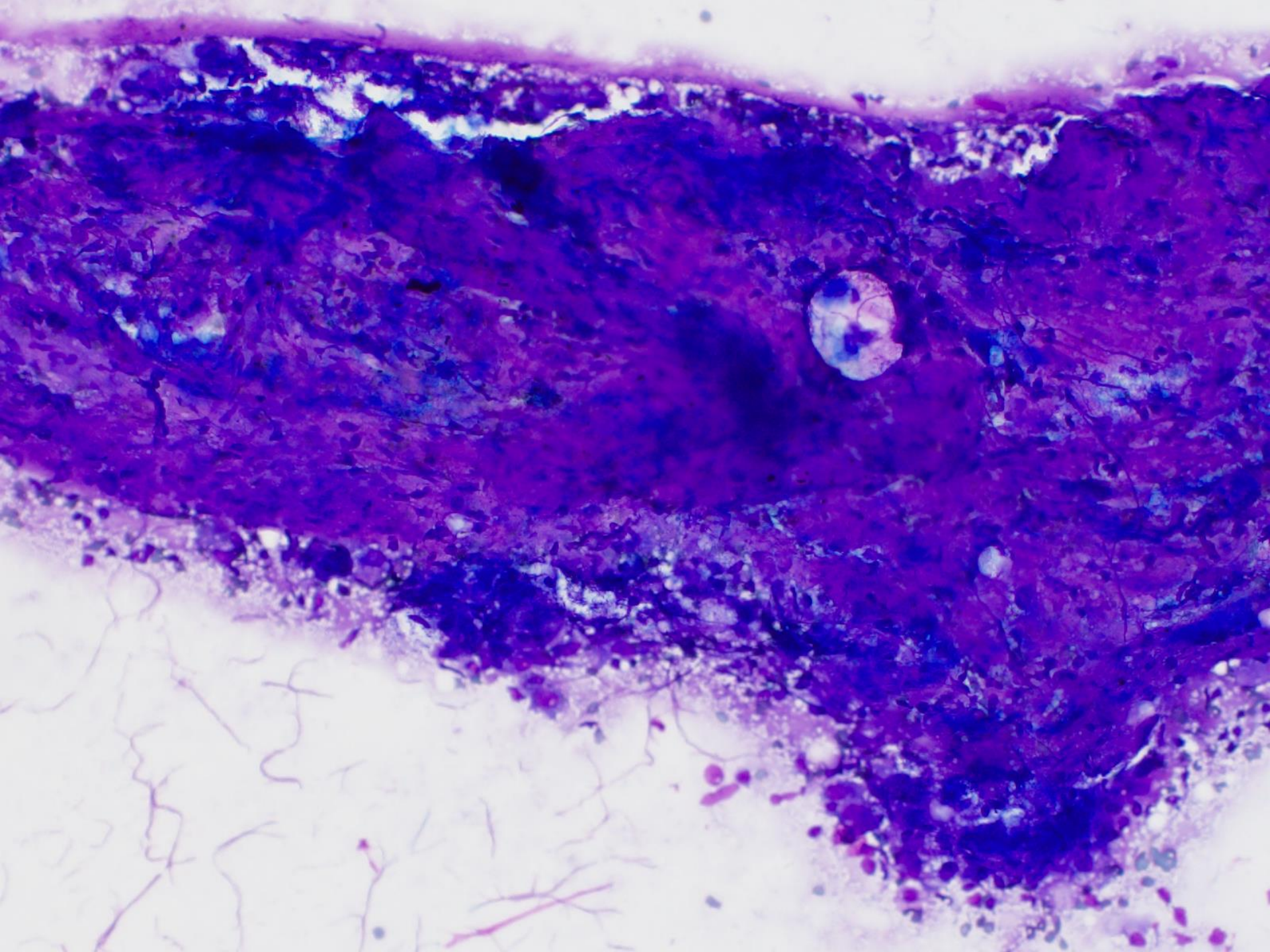
Metastatic Neuroendocrine Tumor, Provisional WHO Grade 3

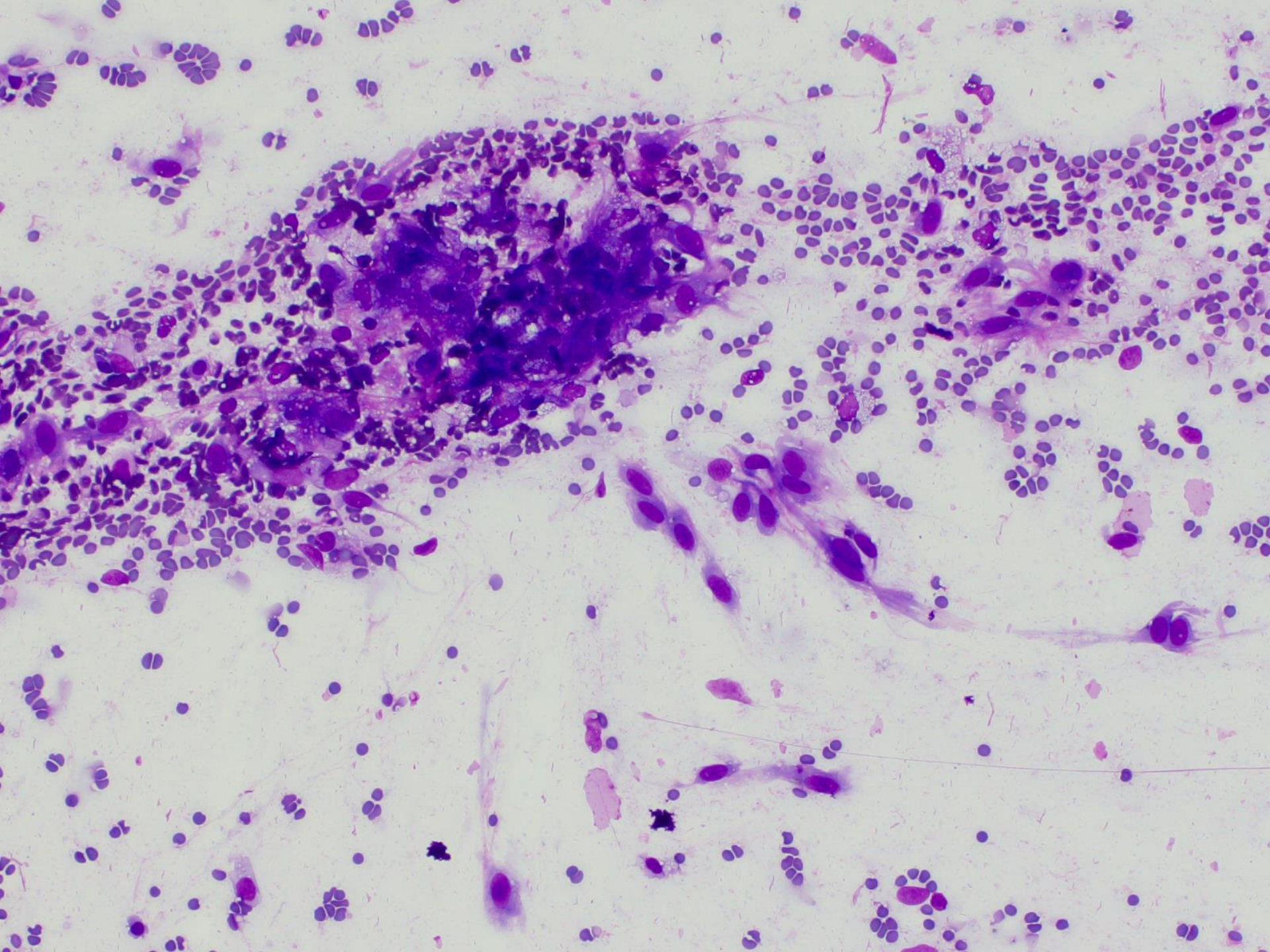
- Solid non-necrotic tumor
- Ample material on FNA, CB, CNB, TP

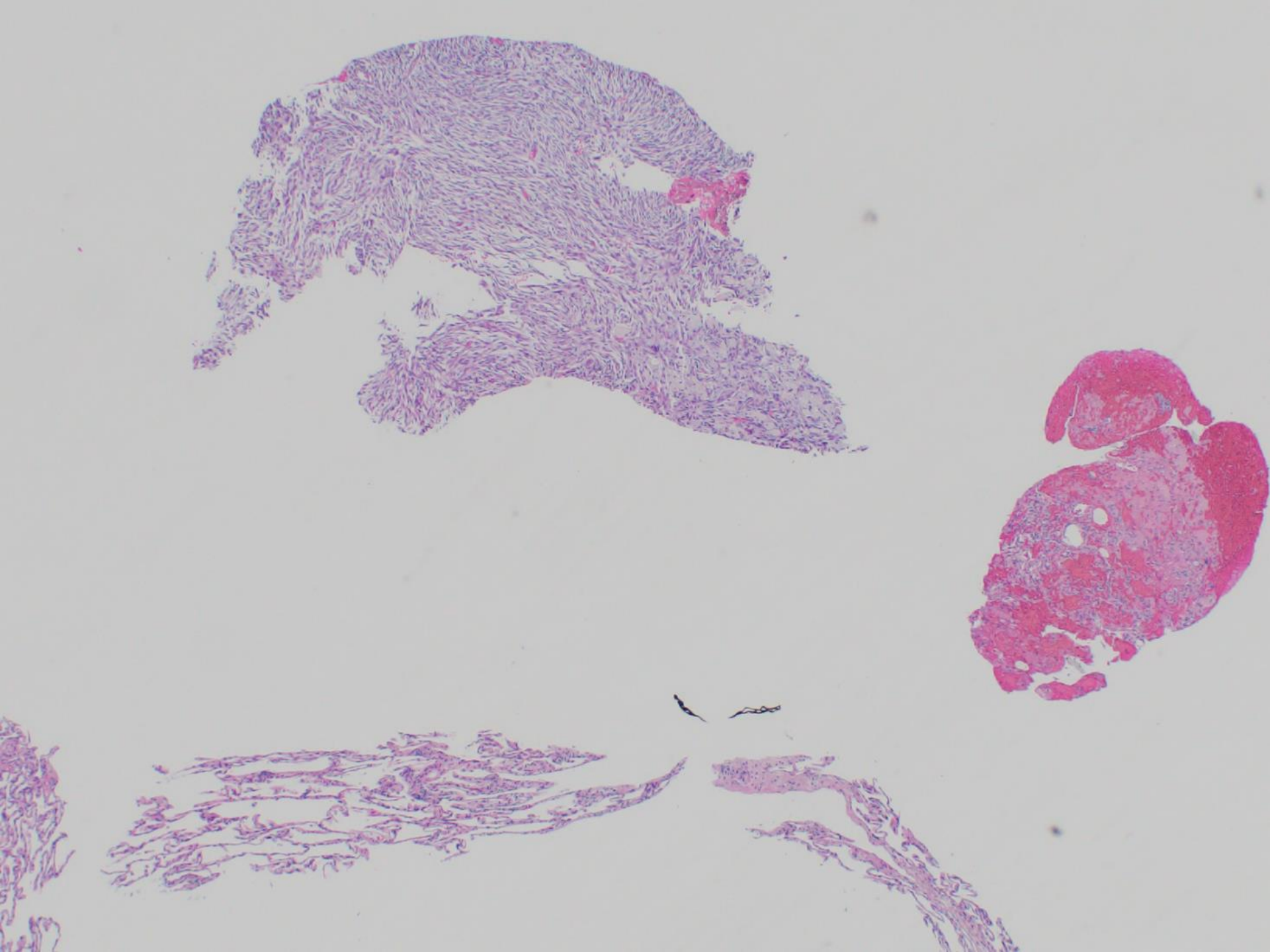
Case 1a

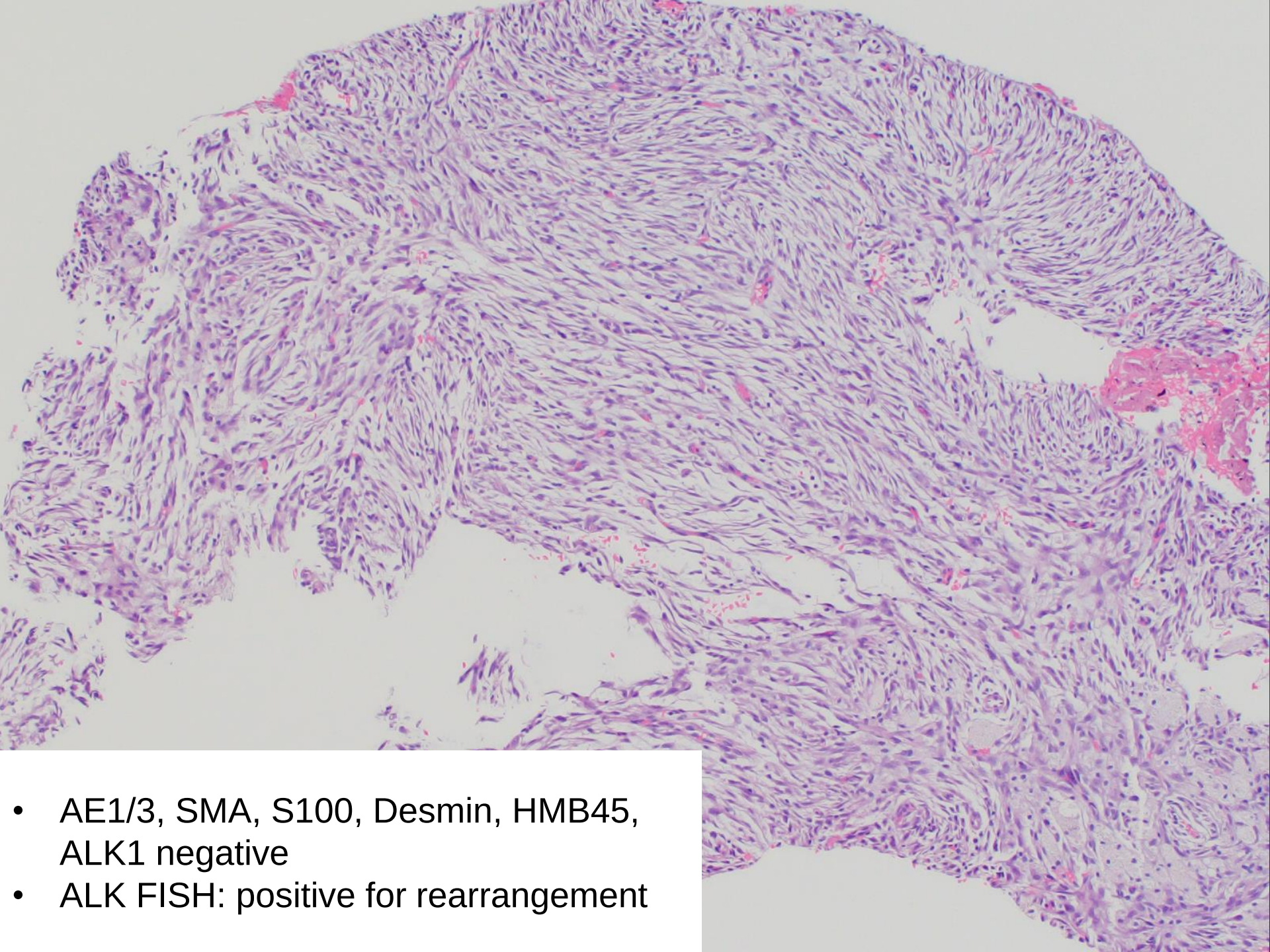
- 26 year old female with Lyme's disease presenting with cardiac symptoms
- Lung imaging disclosed a 1.6 cm well-circumscribed left upper lobe lung nodule
- IR performed CT-guided CNB



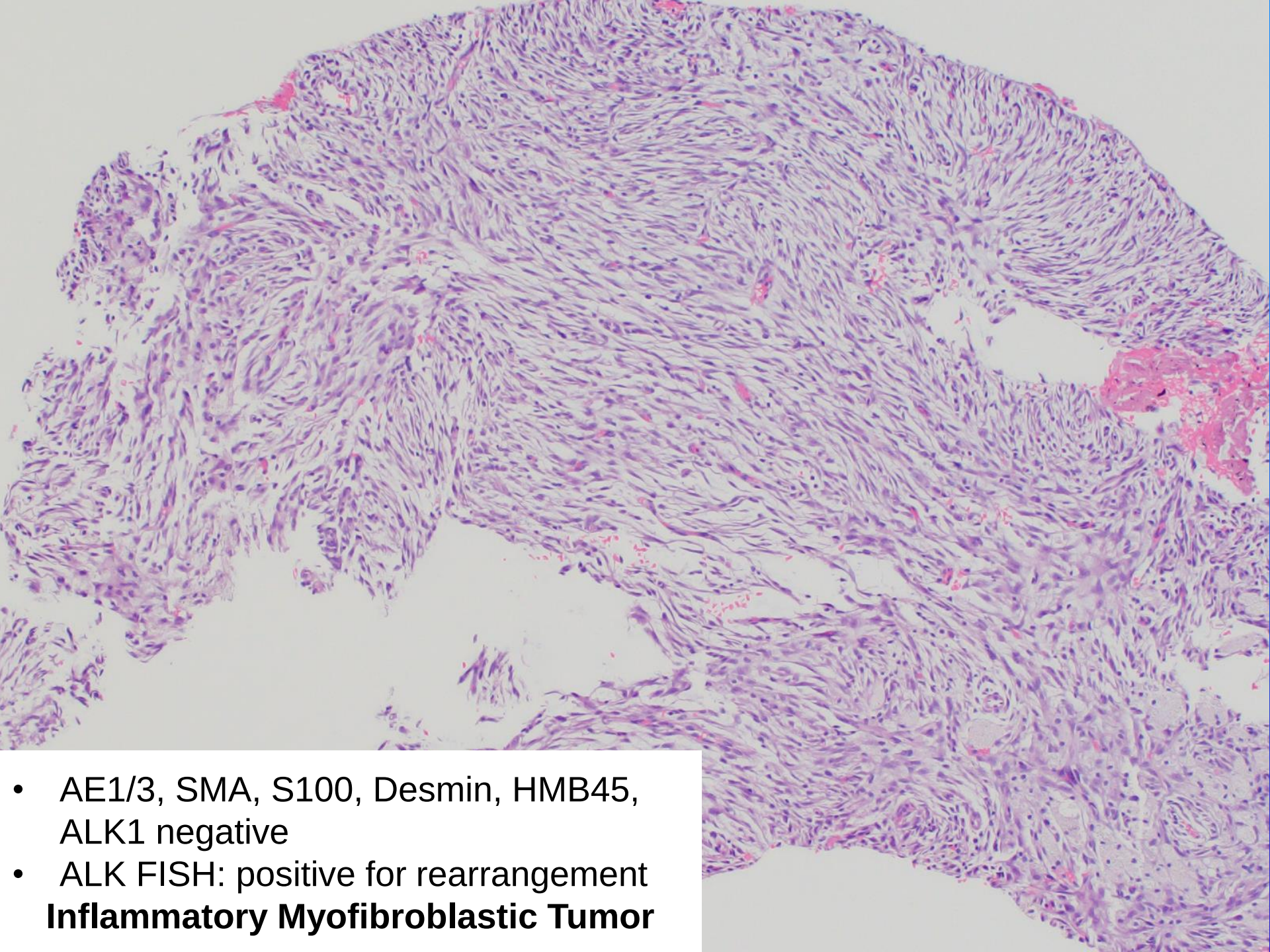








- AE1/3, SMA, S100, Desmin, HMB45, ALK1 negative
- ALK FISH: positive for rearrangement



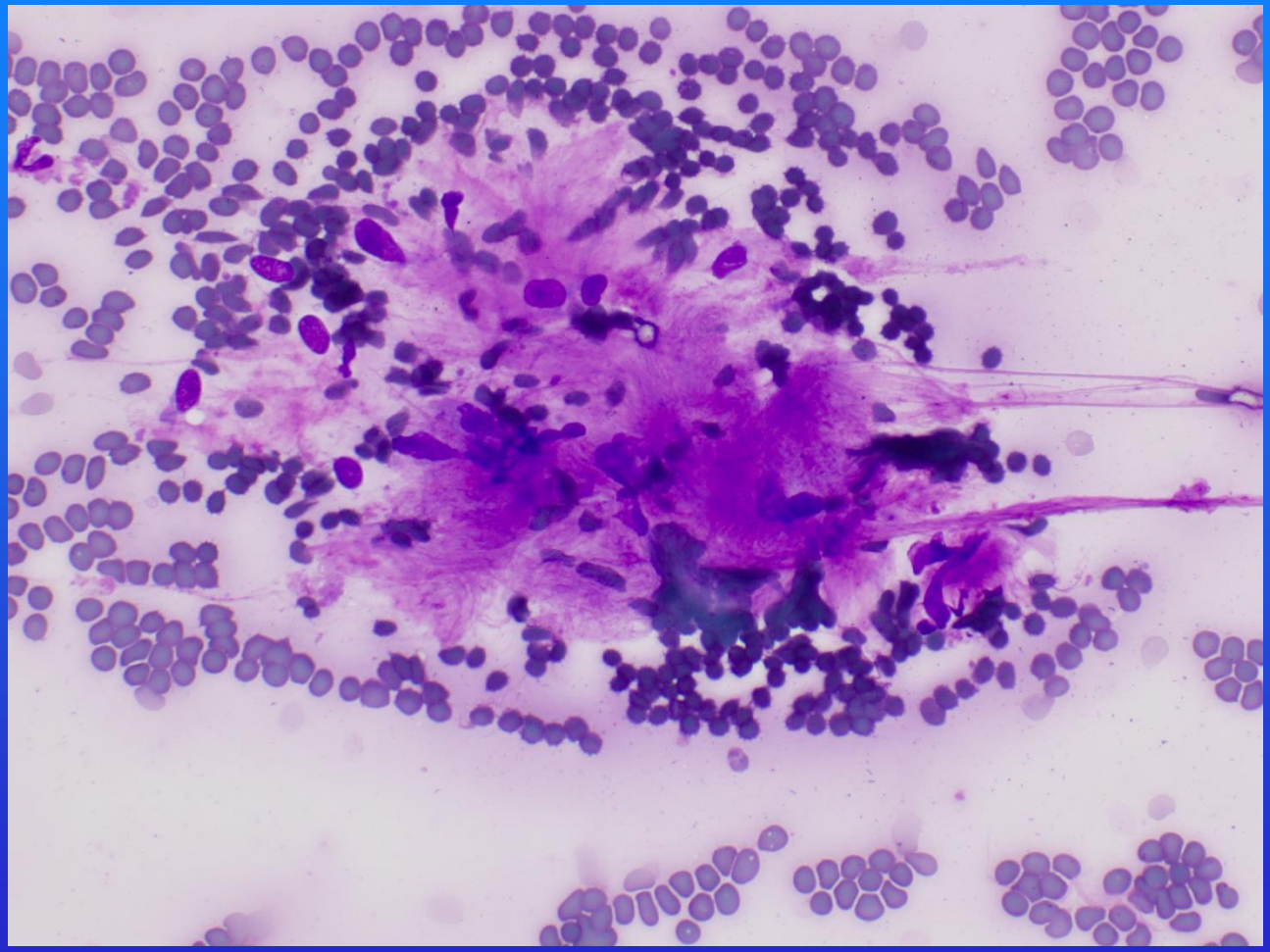
- AE1/3, SMA, S100, Desmin, HMB45, ALK1 negative
- ALK FISH: positive for rearrangement

Inflammatory Myofibroblastic Tumor

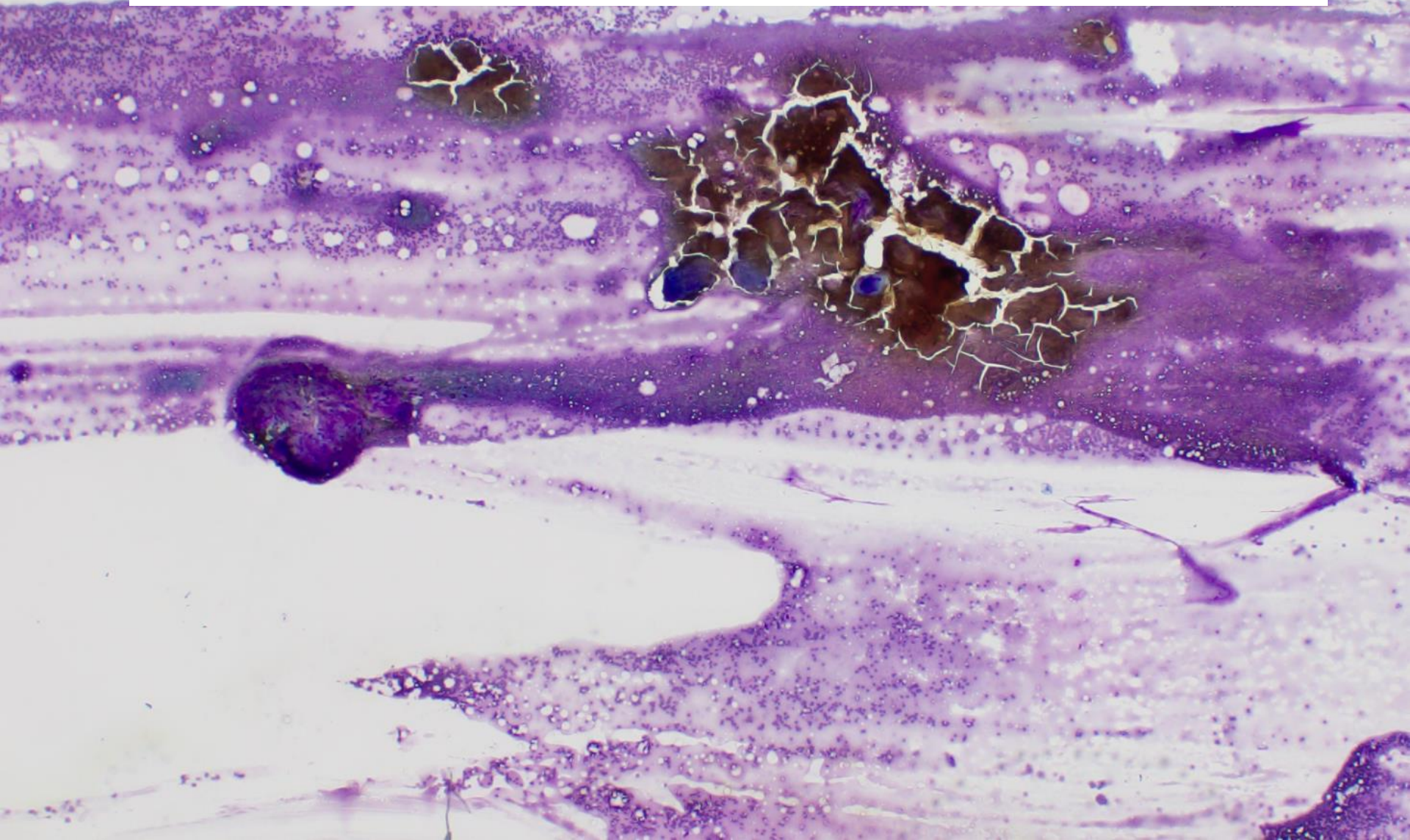
Case 1b

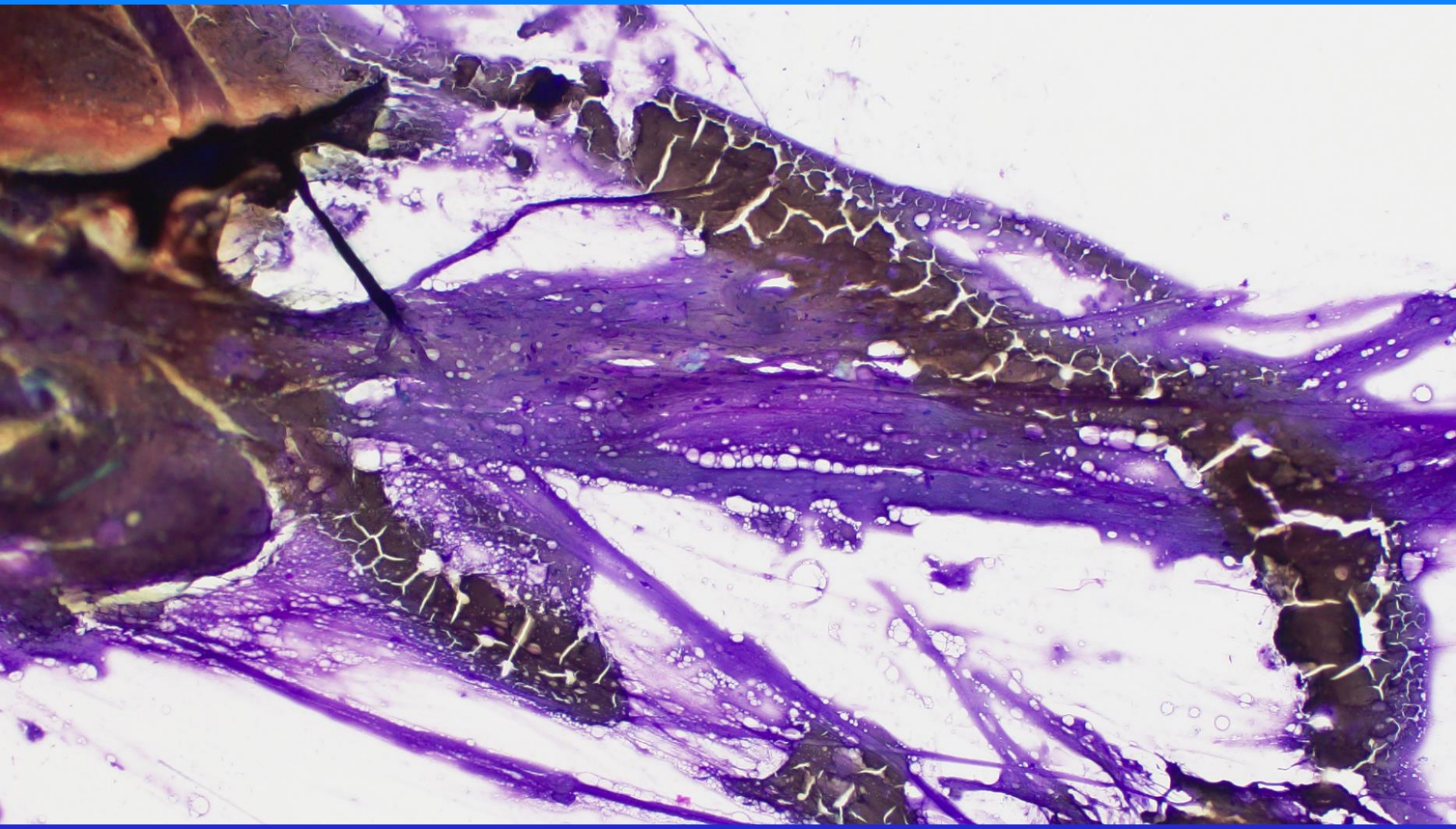
- 58 year old male with a history of p16-positive right pharyngeal squamous cell carcinoma
- Staging workup showed a mildly PET-avid right neck lymph node
- Radiologist performed U/S-guided FNA followed by CNB

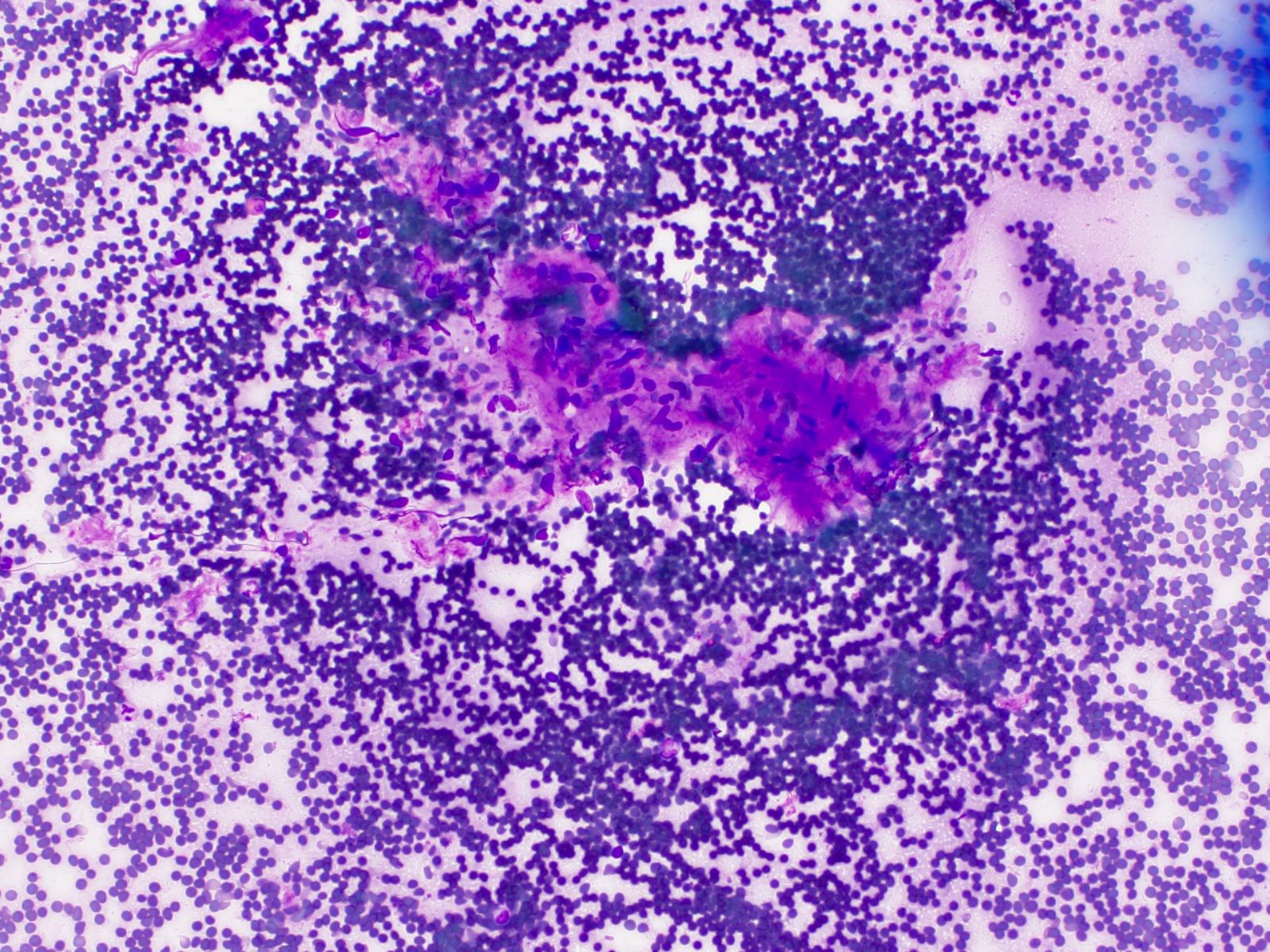
FNA: hypocellular, rare clusters

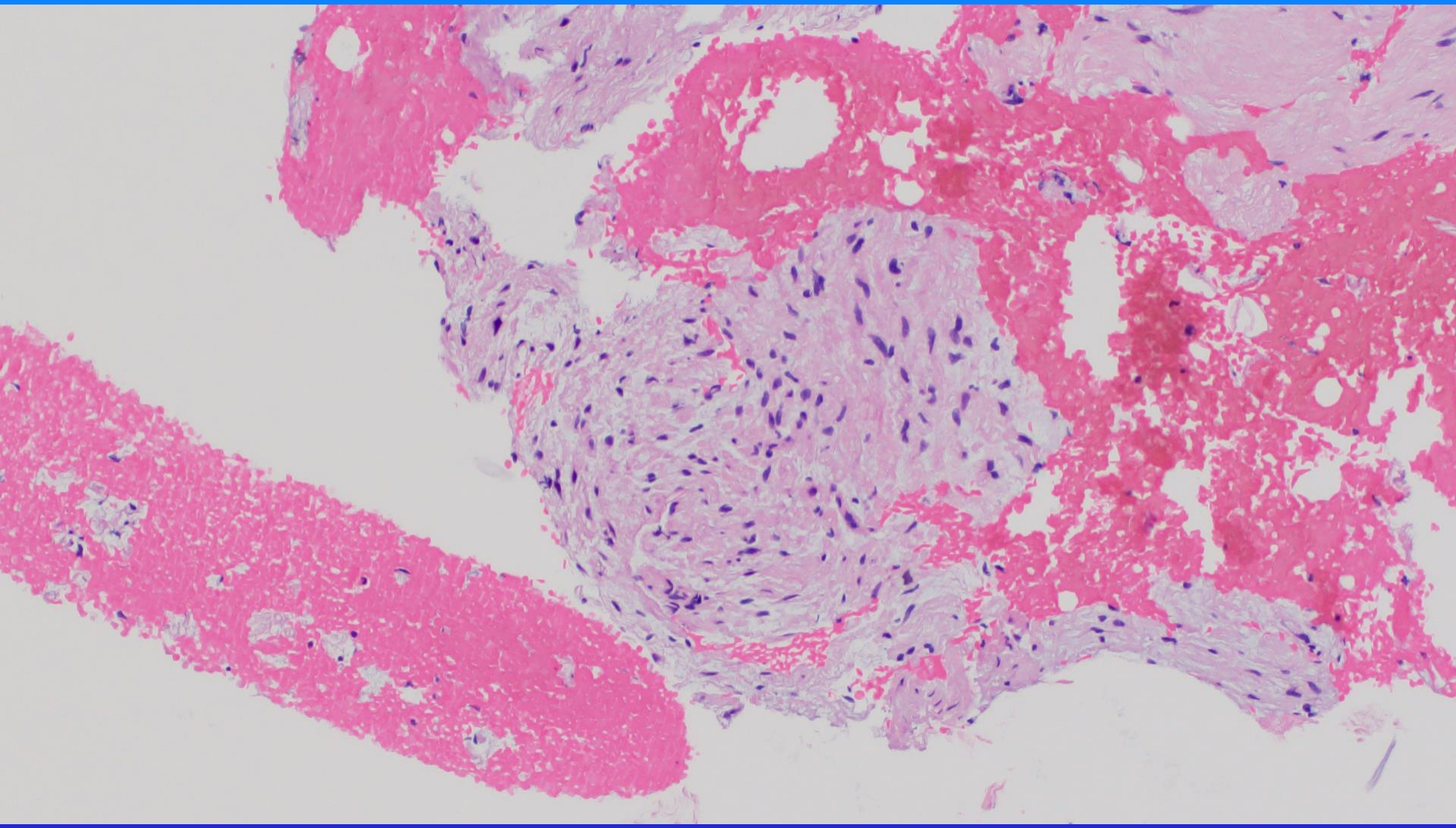


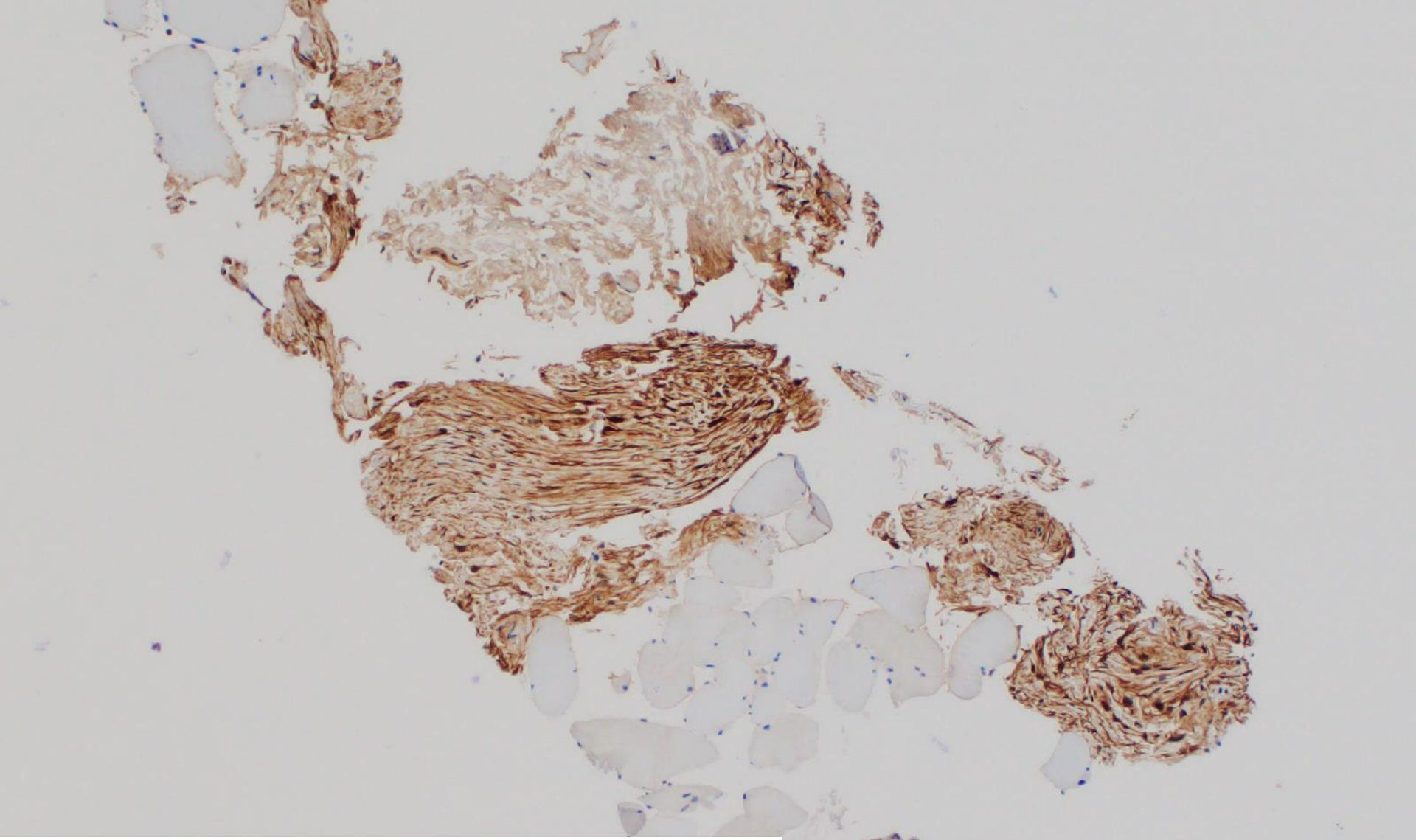
TP: Hypocellular



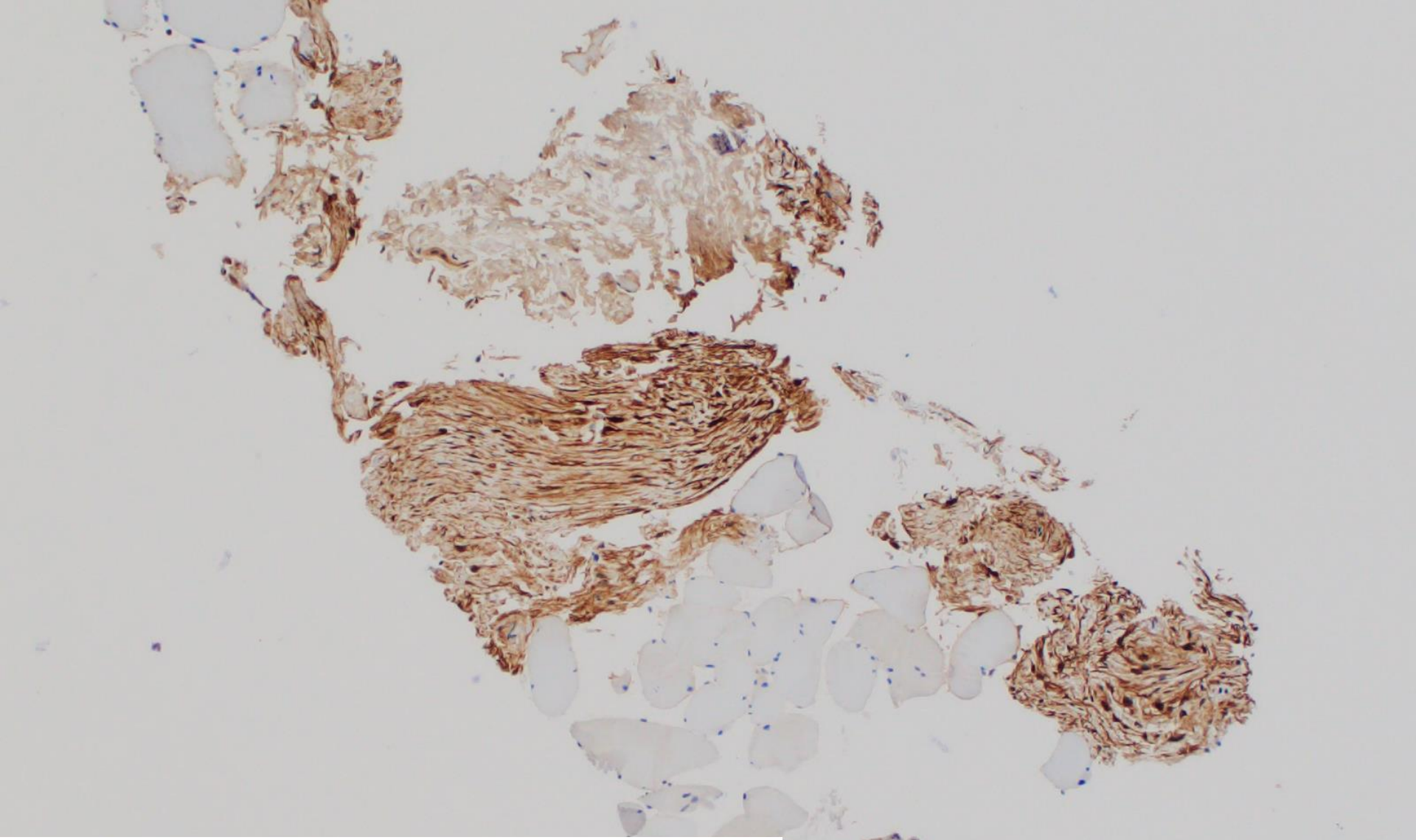








- S100-positive
- No lymphoid tissue identified
- IR: lesion is only “mildly PET-positive”



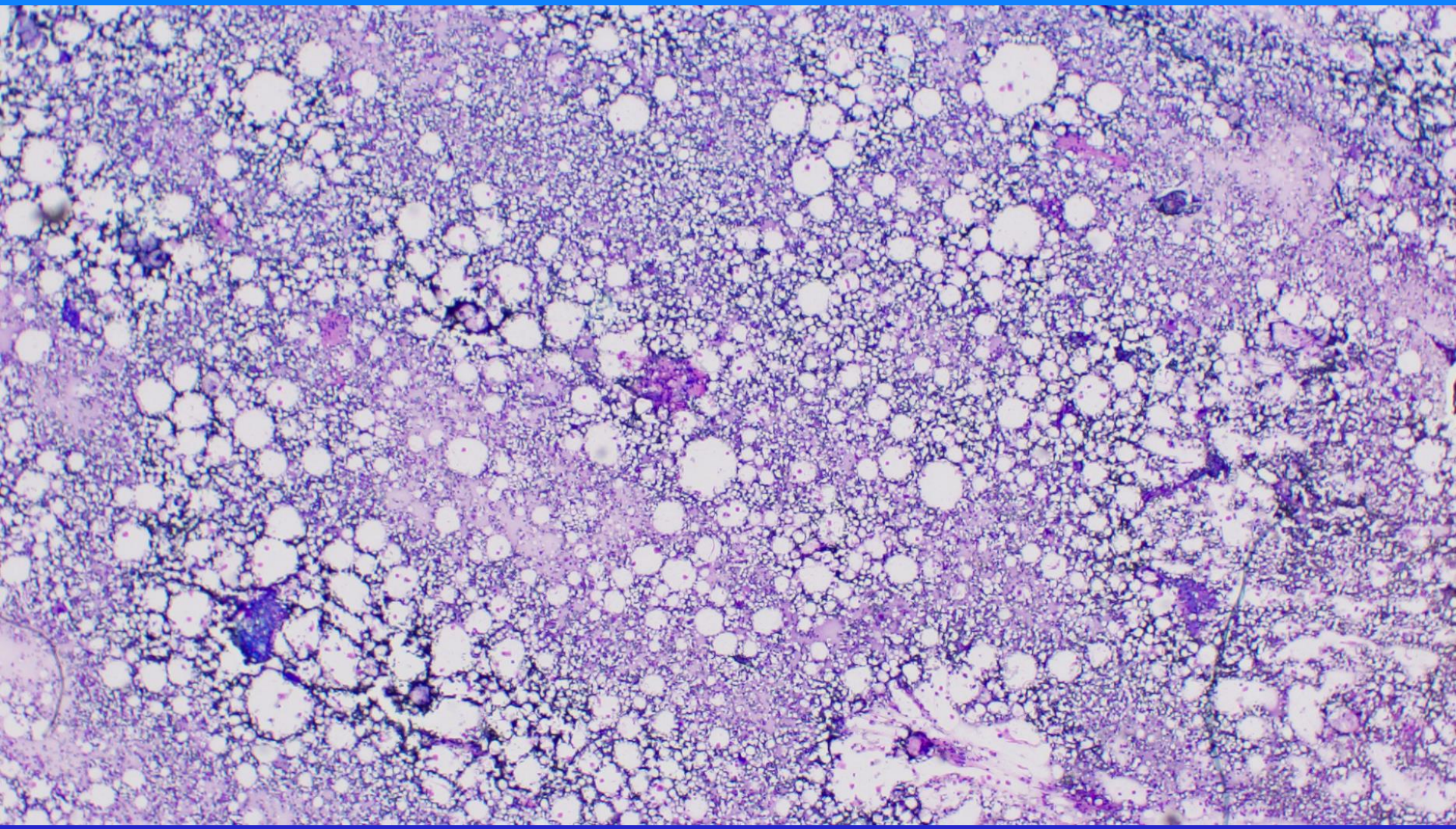
Bland smooth muscle lesion

- S100-positive, ddx: schwannoma
- No lymphoid tissue identified
- IR: lesion is only “mildly PET-positive”

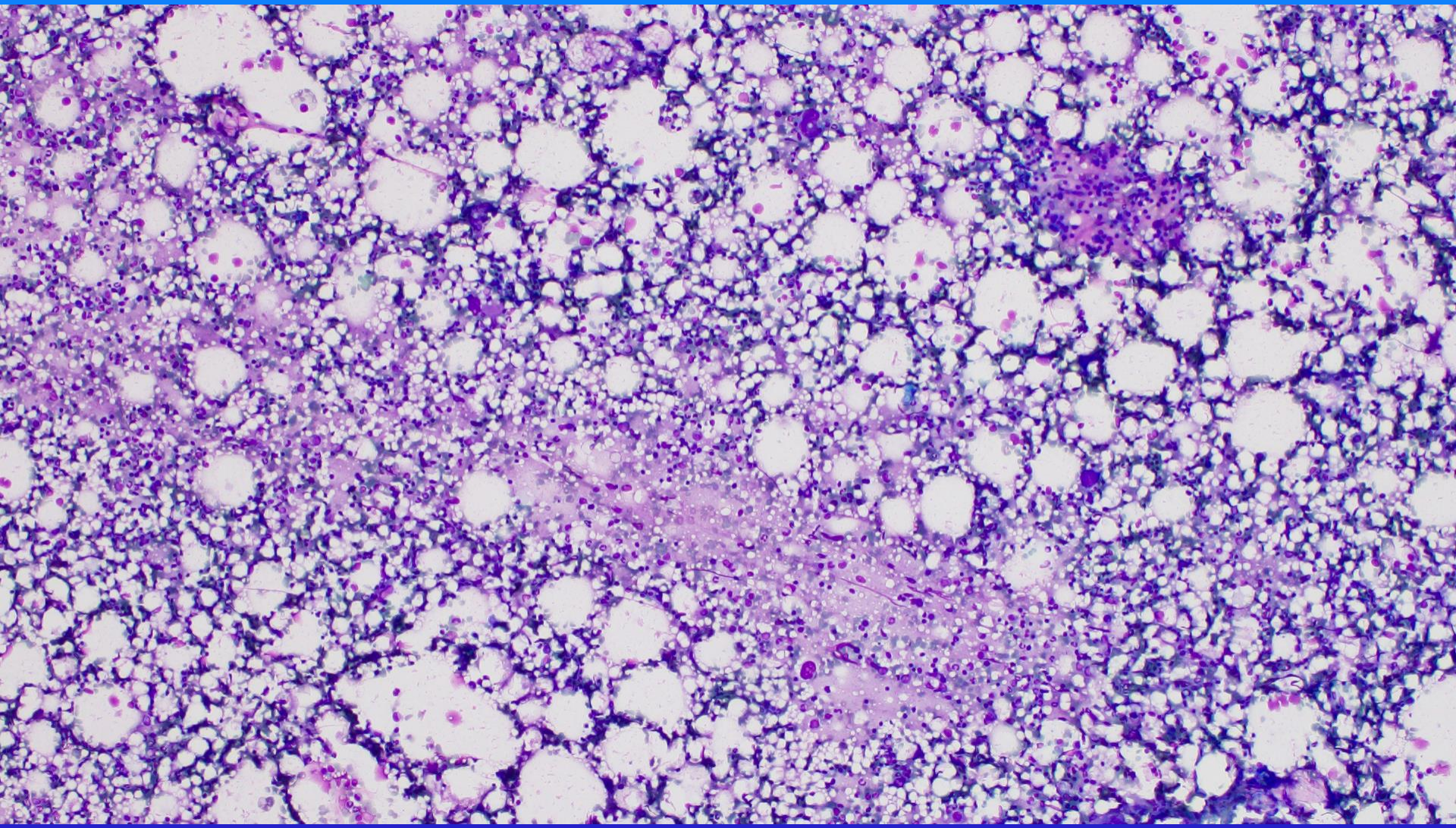
Case 1c

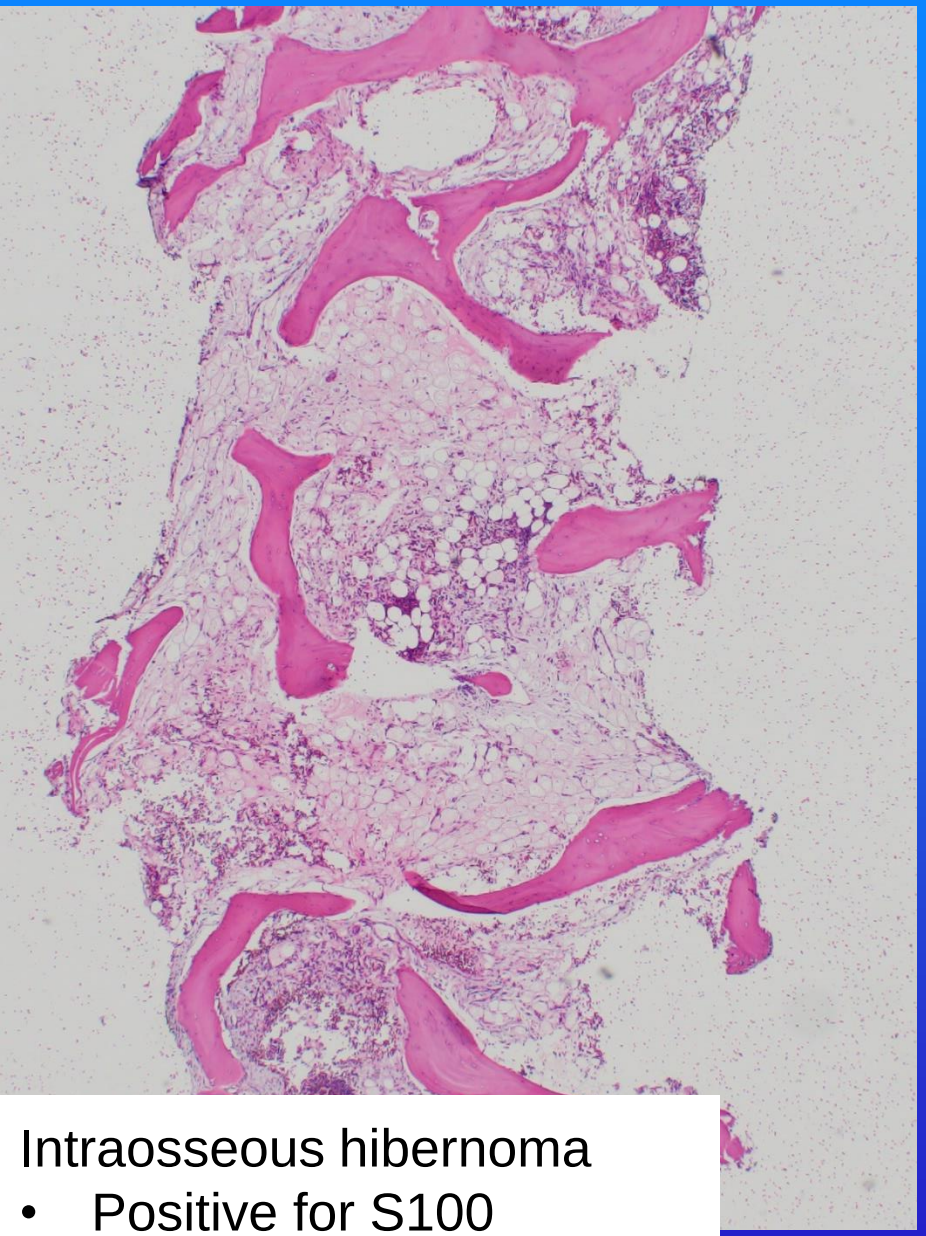
- 62 year old male with lung carcinoid tumor s/p lobectomy 3 months prior
- Now presenting with an enhancing pelvic bony lesion radiologically c/w metastasis first identified on PET scan
- IR opted to perform CT-guided FNA followed by CNB

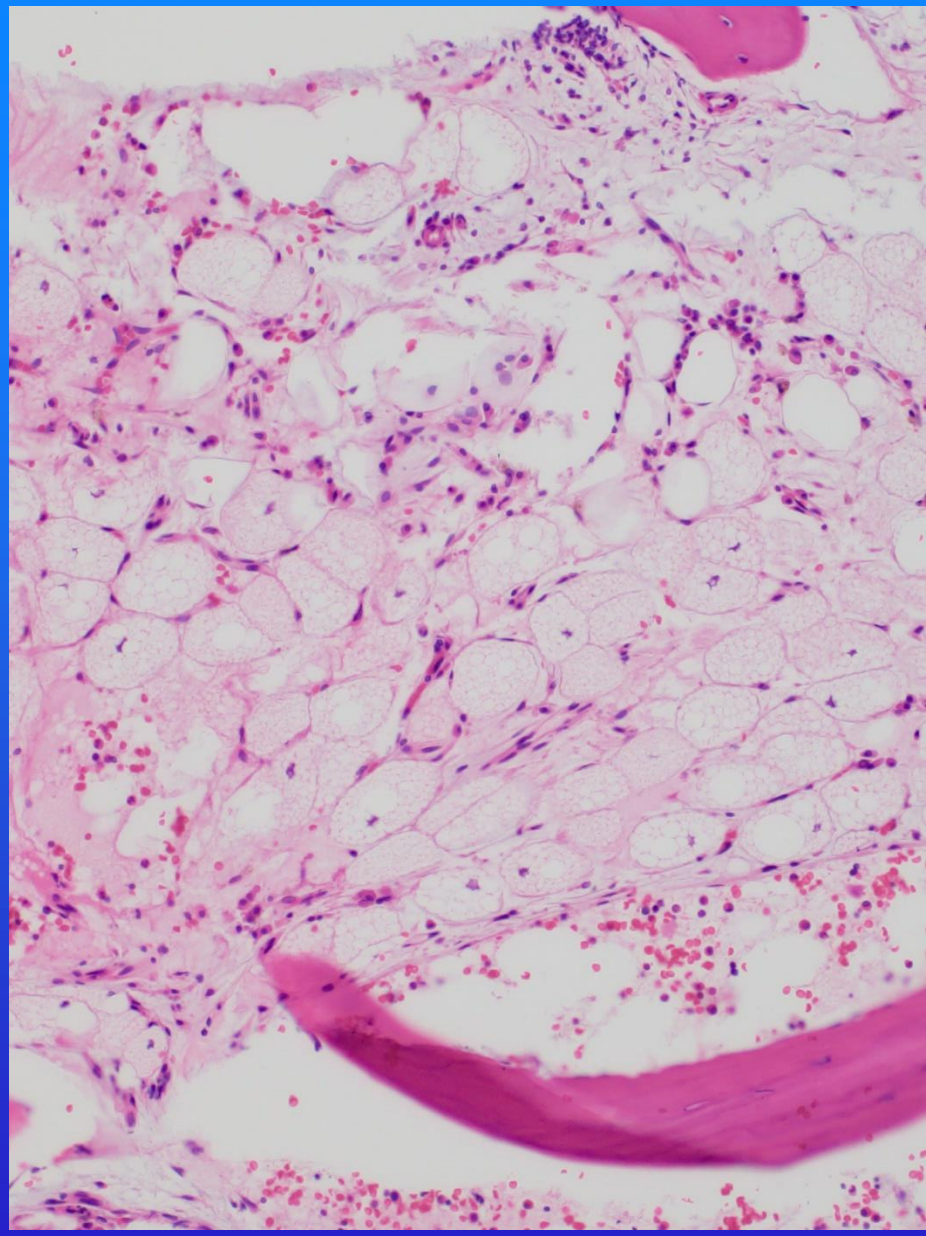
FNA



TP of CNB

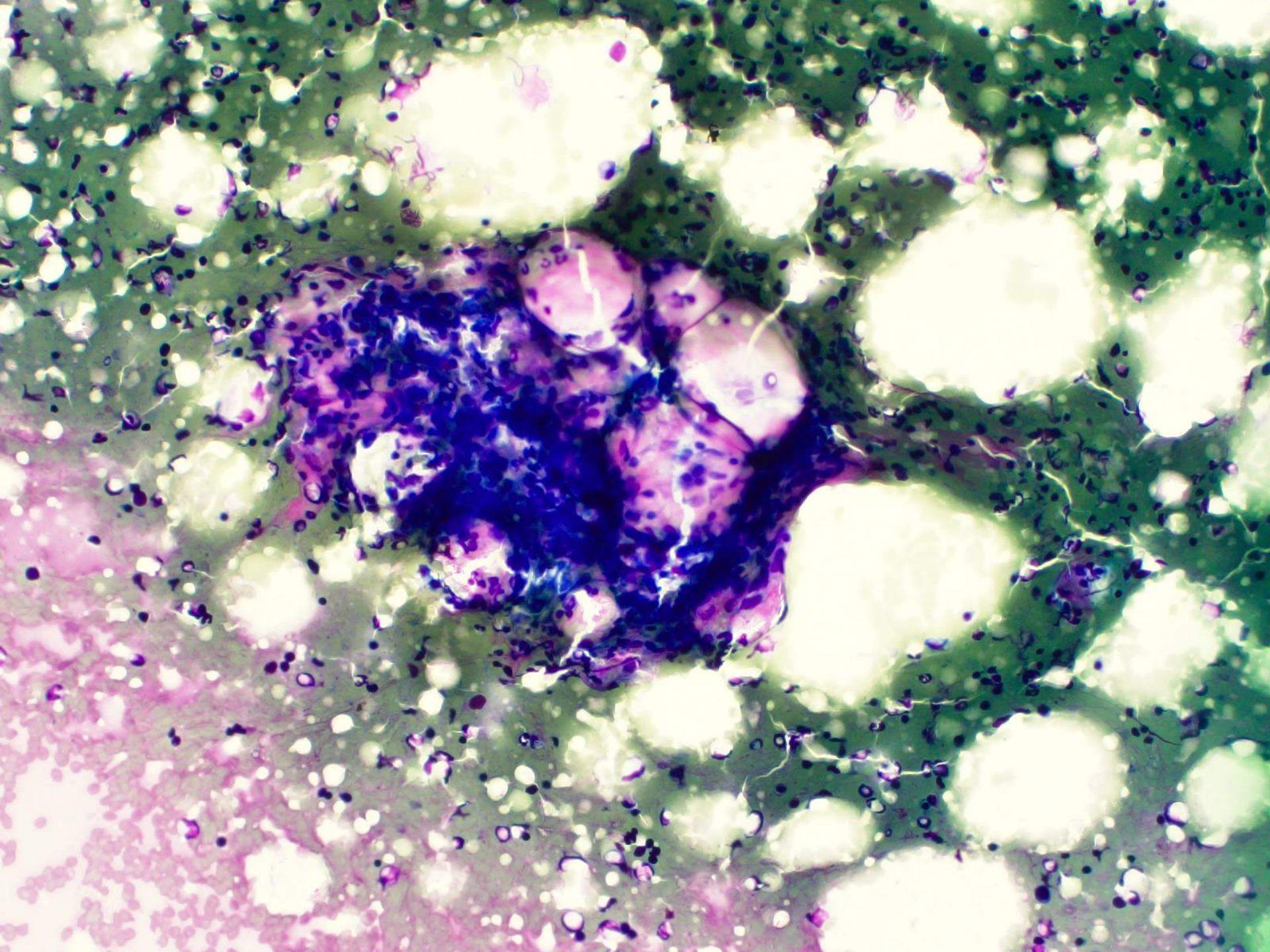


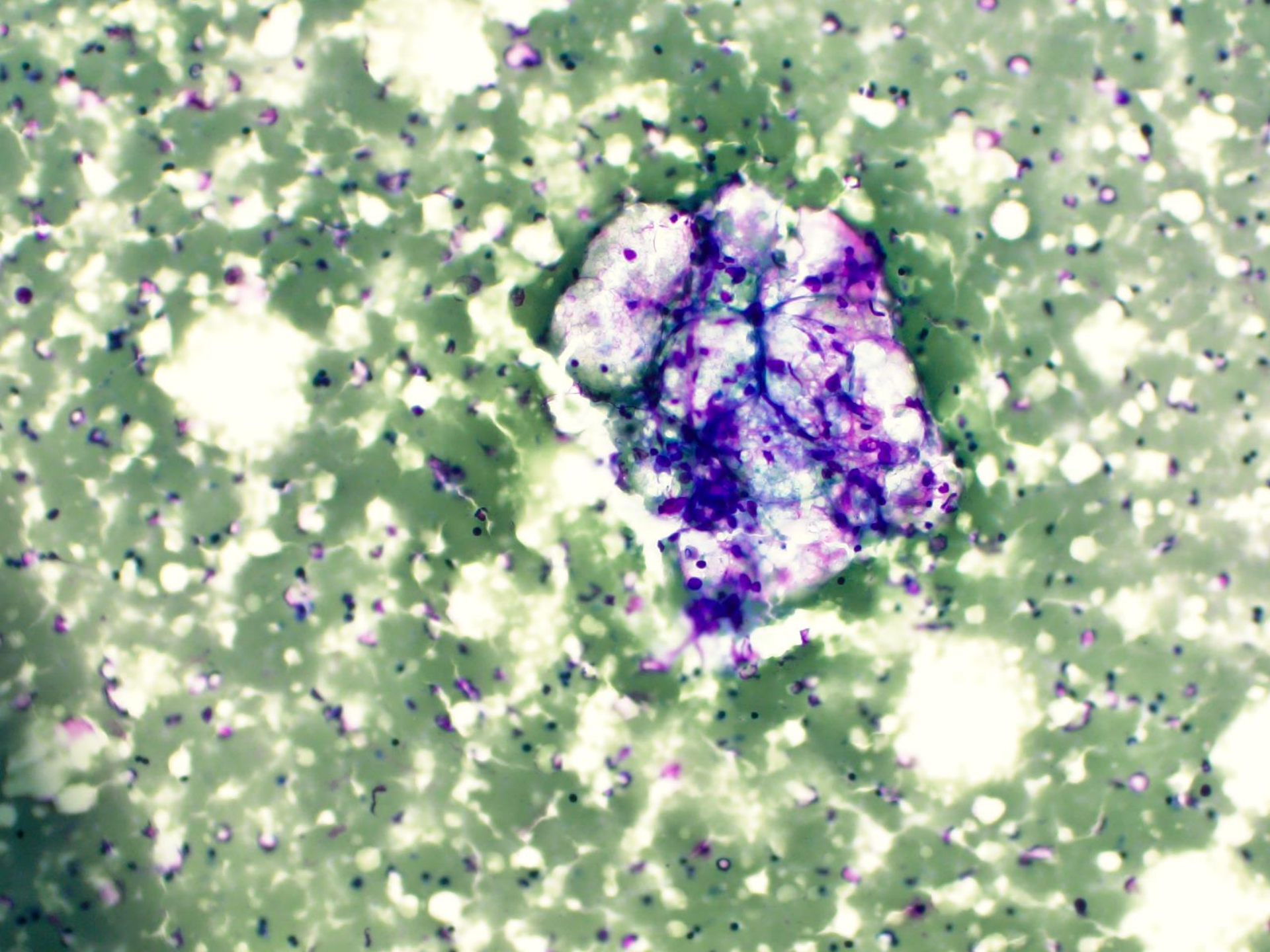




Intraosseous hibernoma

- Positive for S100
- Negative: CD68, AE1/3





Intraosseous hibernoma: a potential mimic of metastatic carcinoma

Shuting Bai, MD, PhD [a,*], Carolyn Mies, MD [a], Jason Stephenson, MD [b], Paul J. Zhang, MD [a]

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[b] *Department of Radiology, Hospital of the University of Pennsylvania, Philadelphia, PA 19104*

- Hibernomas are rare benign tumors
- Intraosseous location even more uncommon
- Case reports document primarily pelvic or lower extremity bony locations
- Can be radiologically suspicious

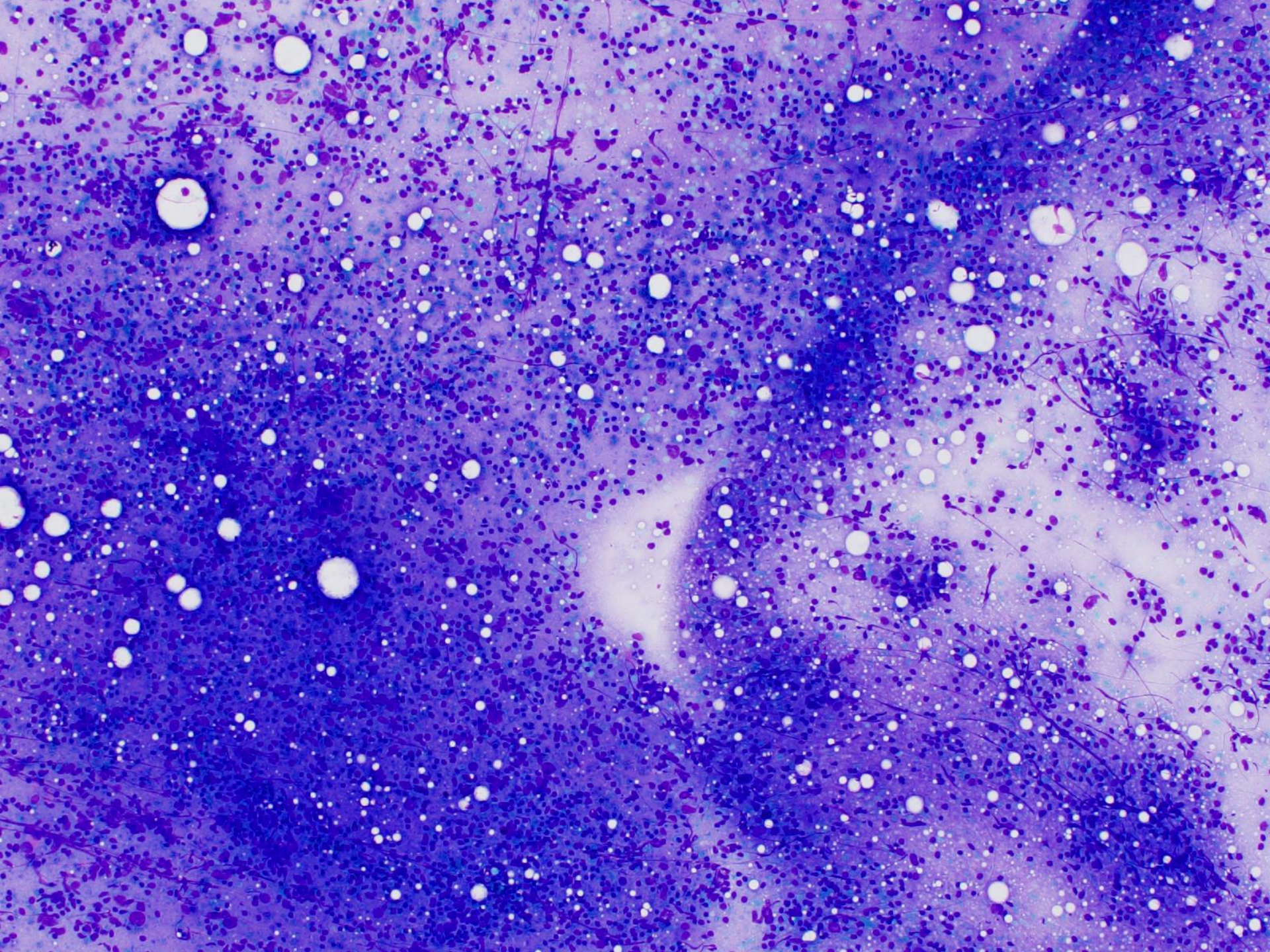
Bai et al. Annals Diag Pathol 2013

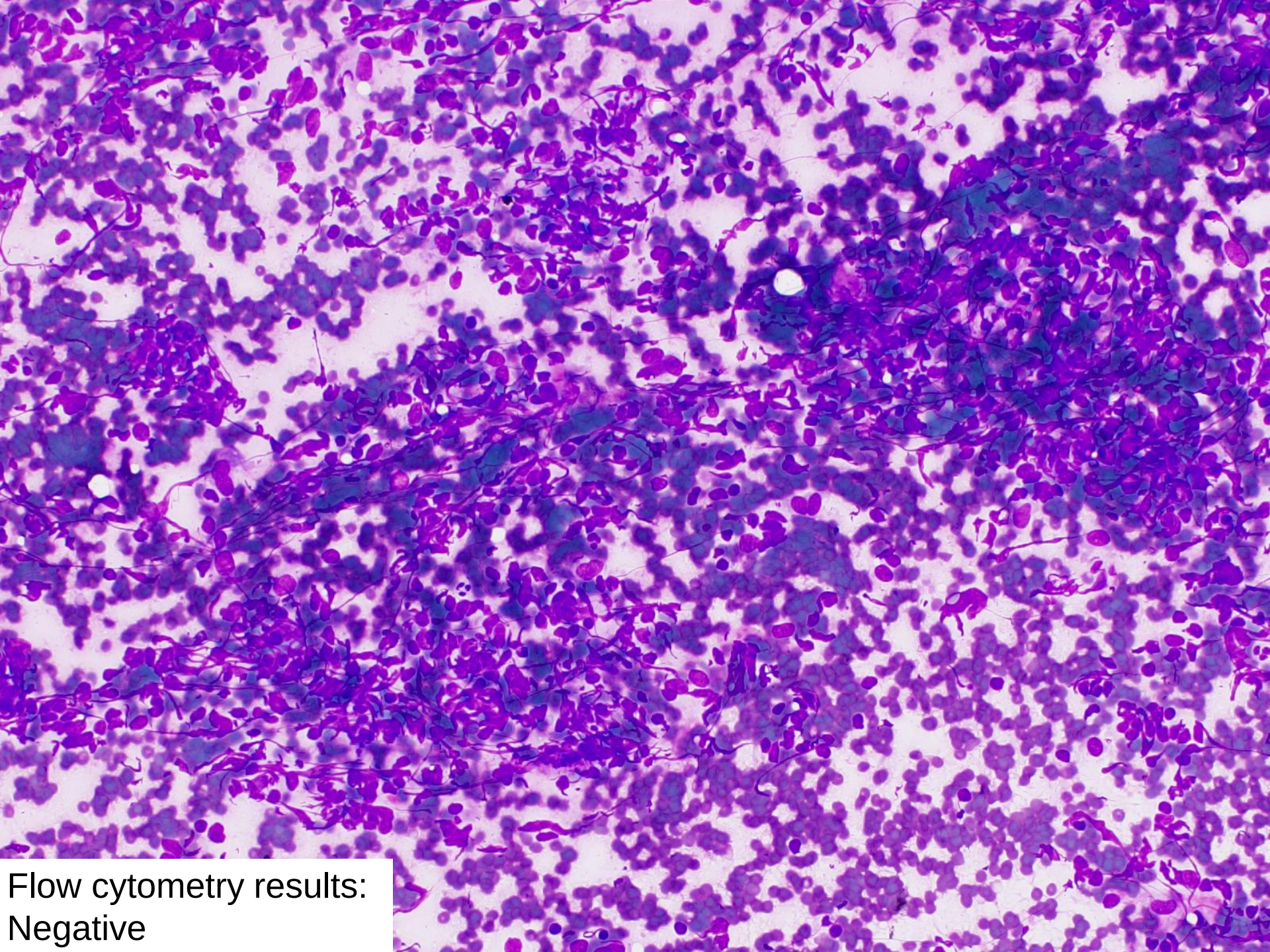
Take Home Messages #1

- Quality of lesion matters: fibrotic lesions (carcinomas with desmoplasia, mesenchymal lesions) may not yield much material on FNA or TP-CNB
- Discussion with IR may guide trajectory of needle and therefore procedure
- Clinical history important but can be misleading (case 1b, 1c)

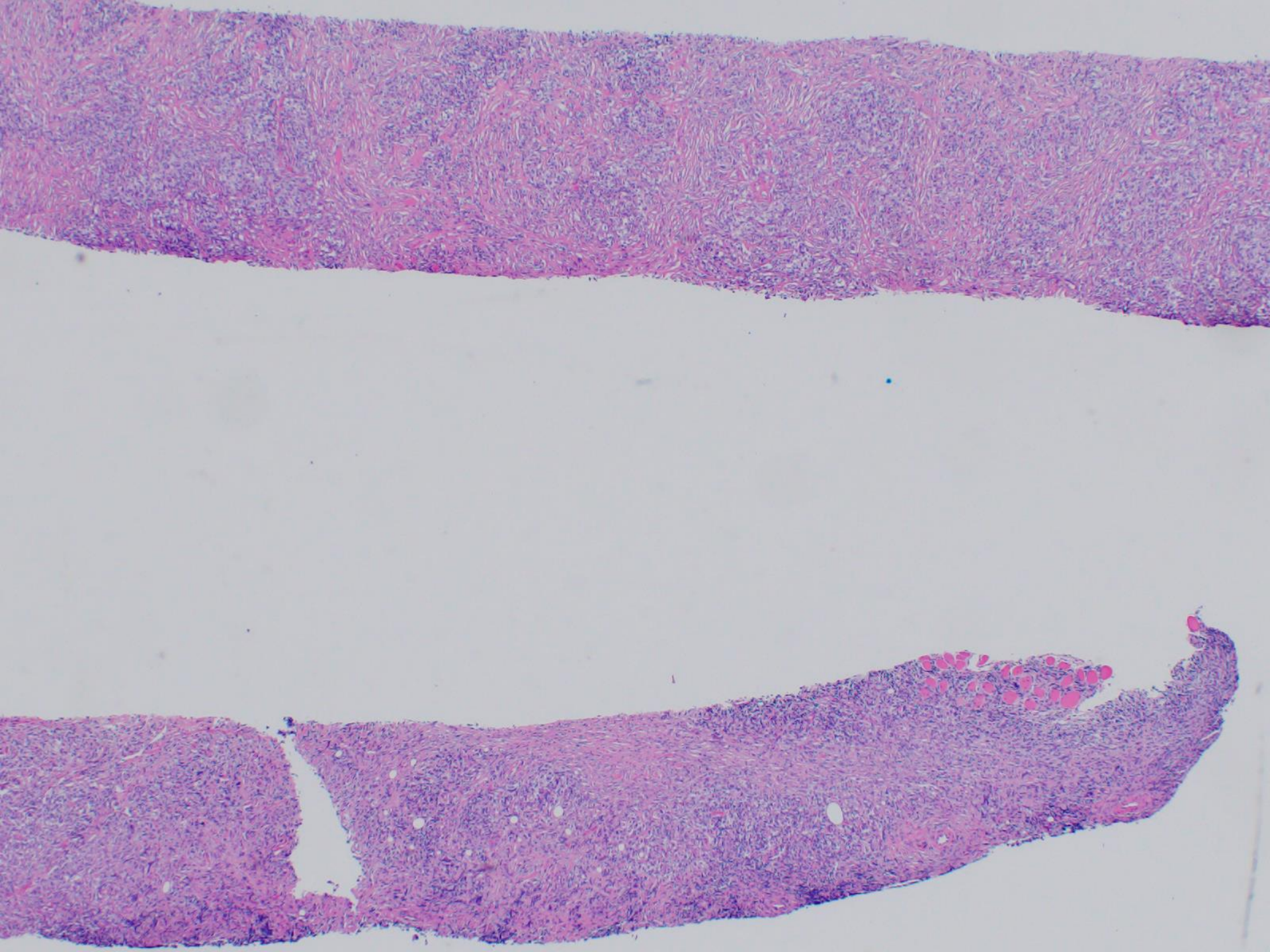
Case 2a

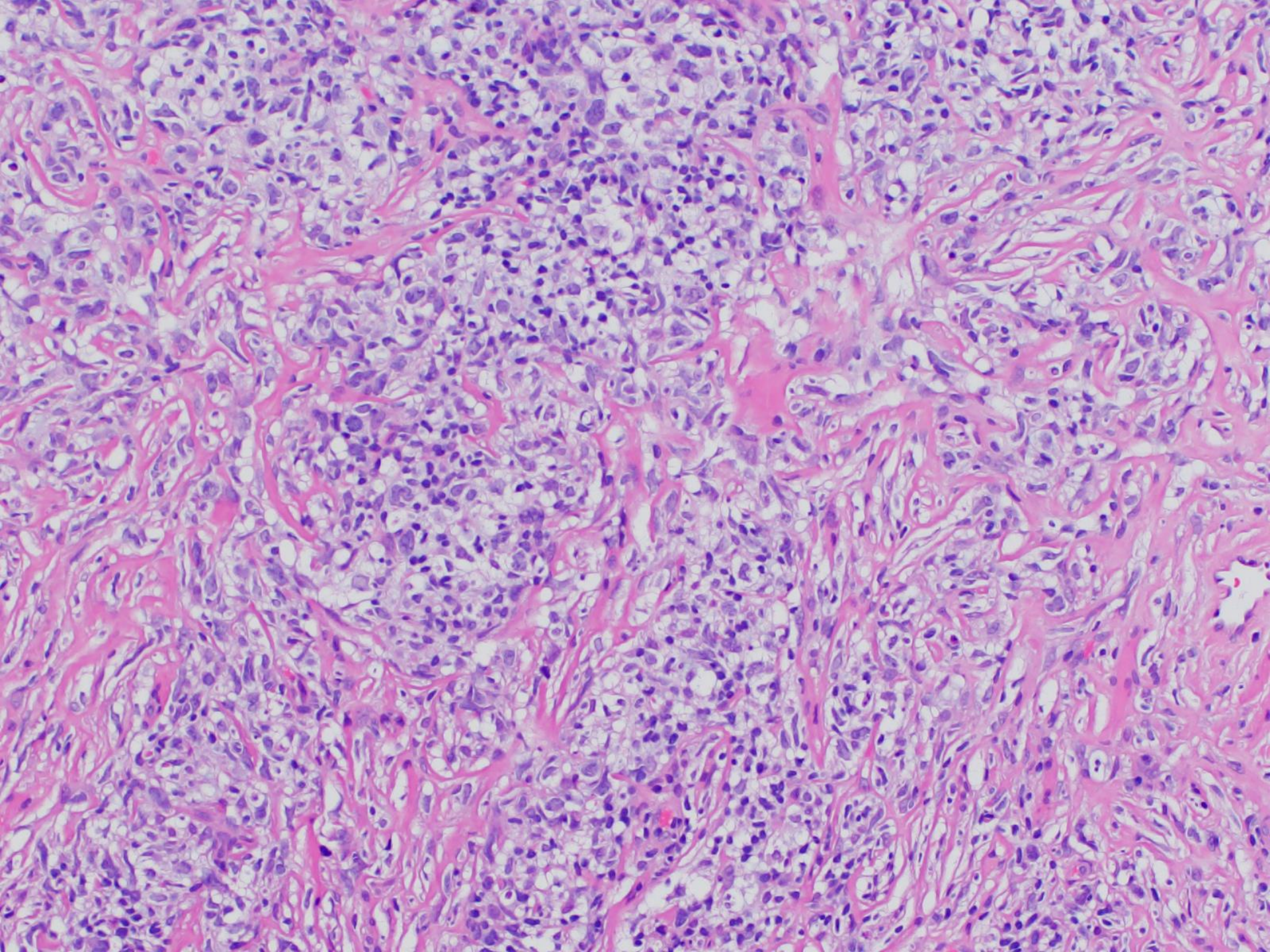
- 82 year old male presents with recent onset of right lower extremity sciatica-type pain
- Imaging revealed an extensive presacral and retroperitoneal soft tissue mass encasing the abdominal aorta
- Patient had a remote history of a right calf soft tissue sarcoma
- IR performed CT-guided CNB

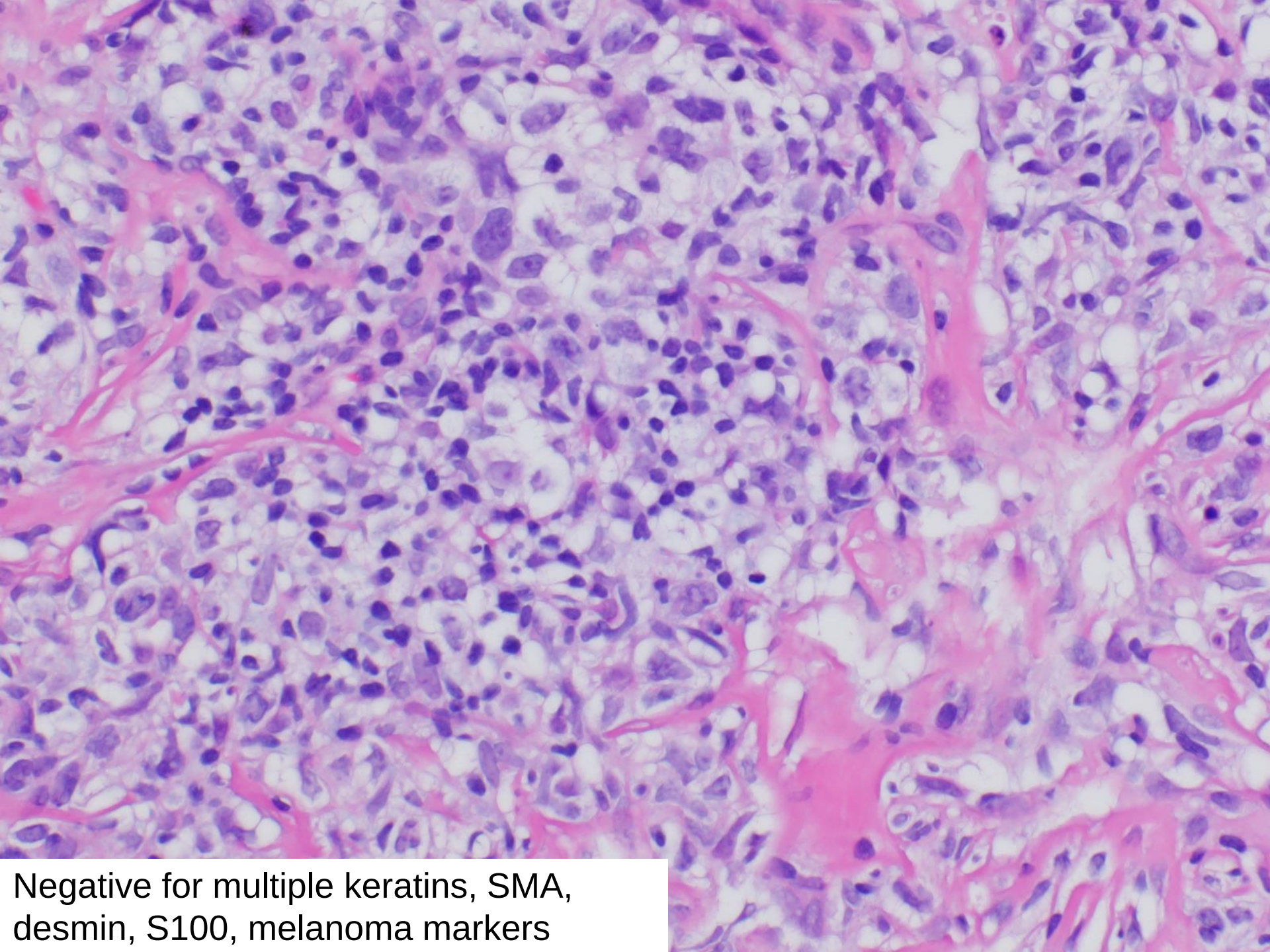




Flow cytometry results:
Negative

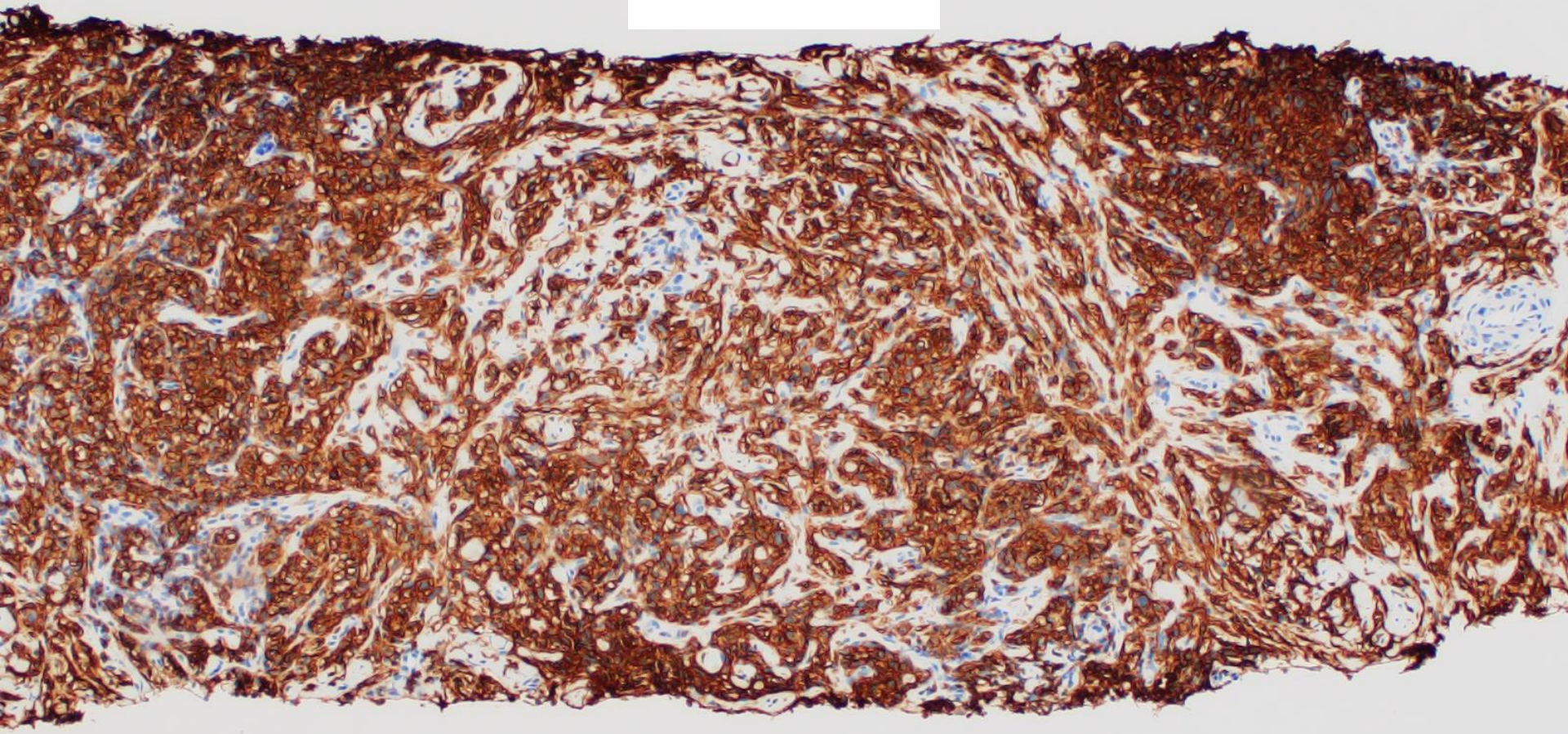






Negative for multiple keratins, SMA, desmin, S100, melanoma markers

CD20

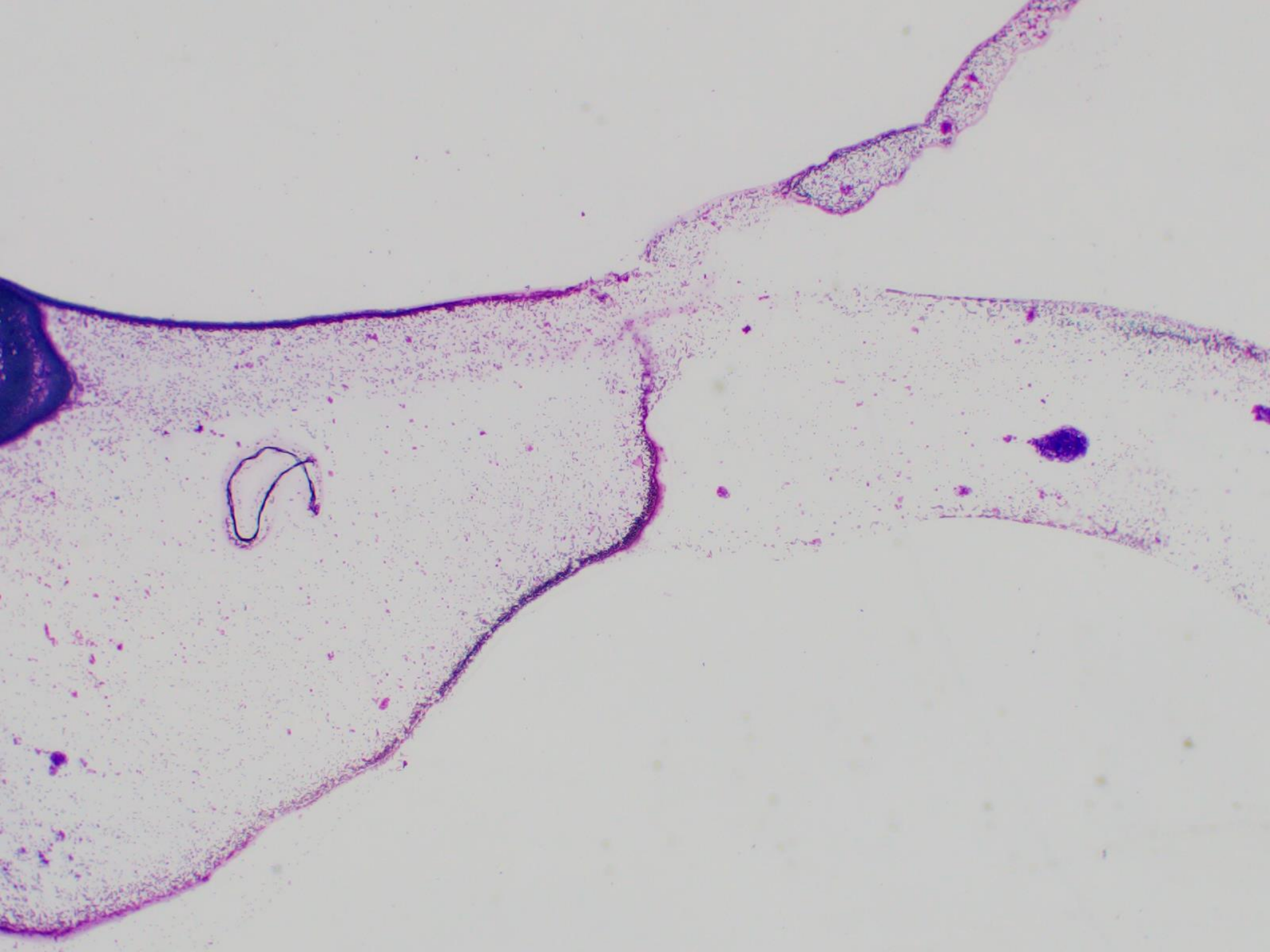


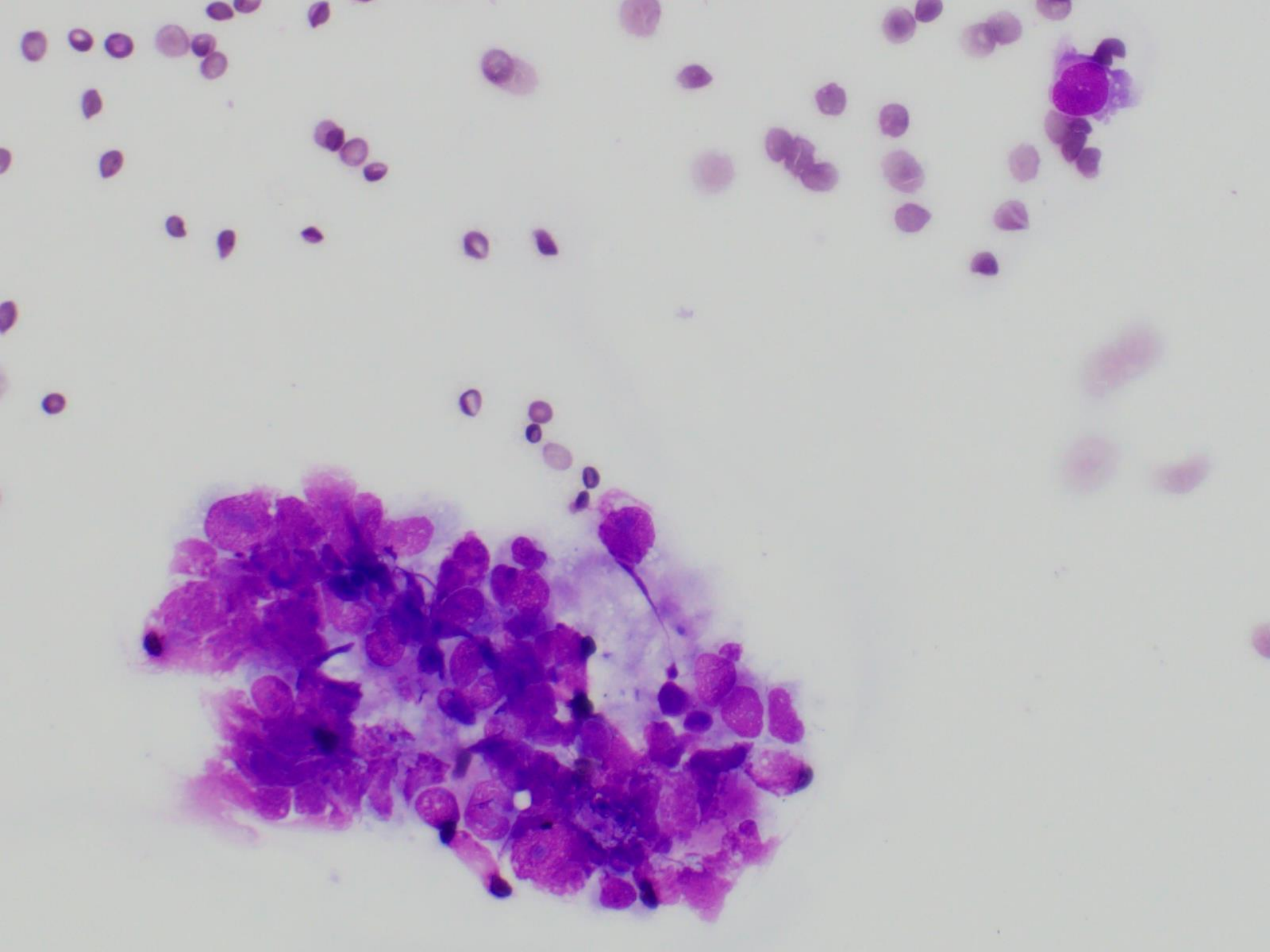
Consistent with Low grade B cell lymphoma
Positive: CD20, PAX-5, BCL6 ; Negative: BCL2, CD10, CD30

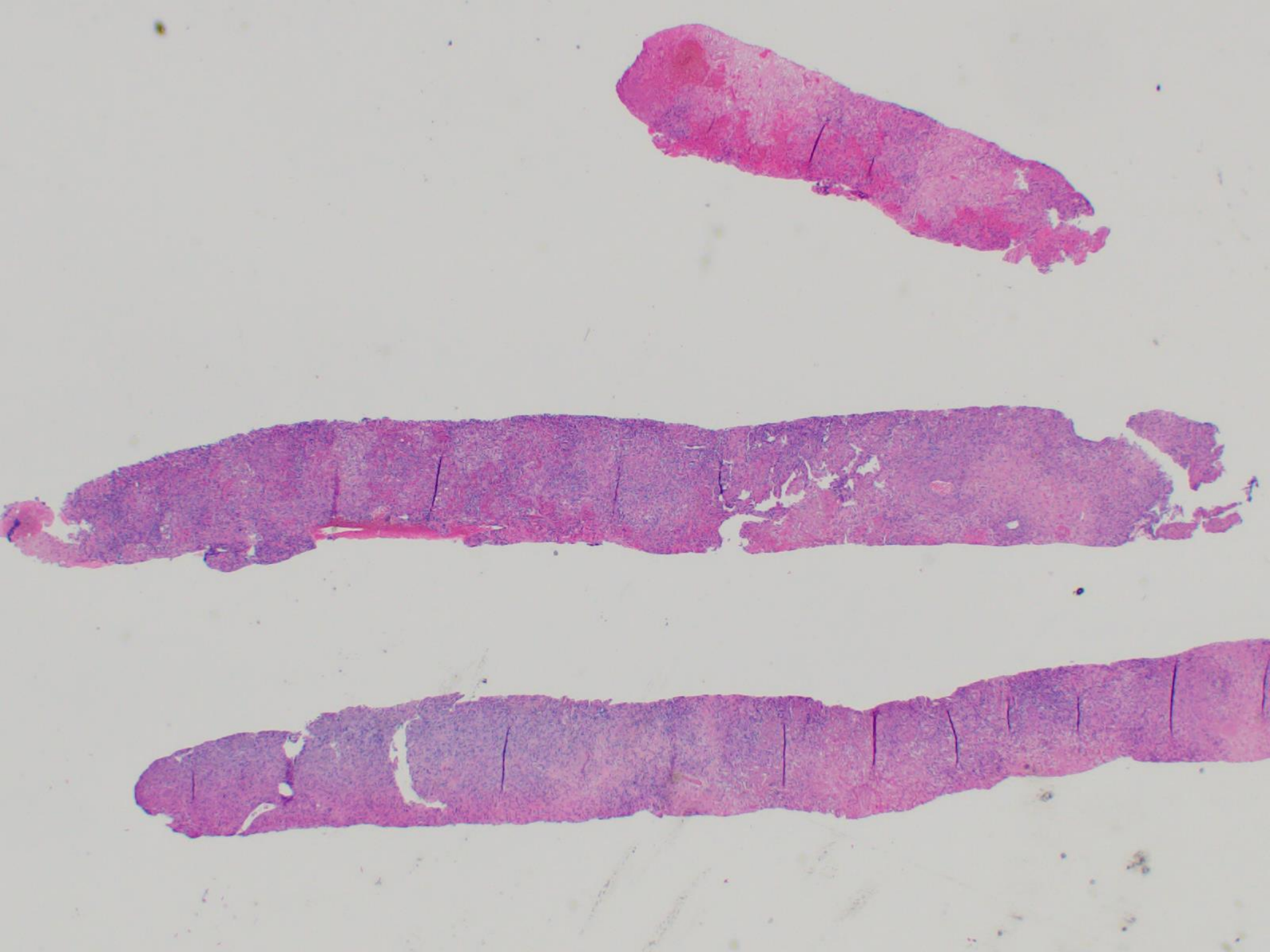
Re-review of flow cytometry report: Negative; cellular viability could not be assessed due to extensive cellular degeneration

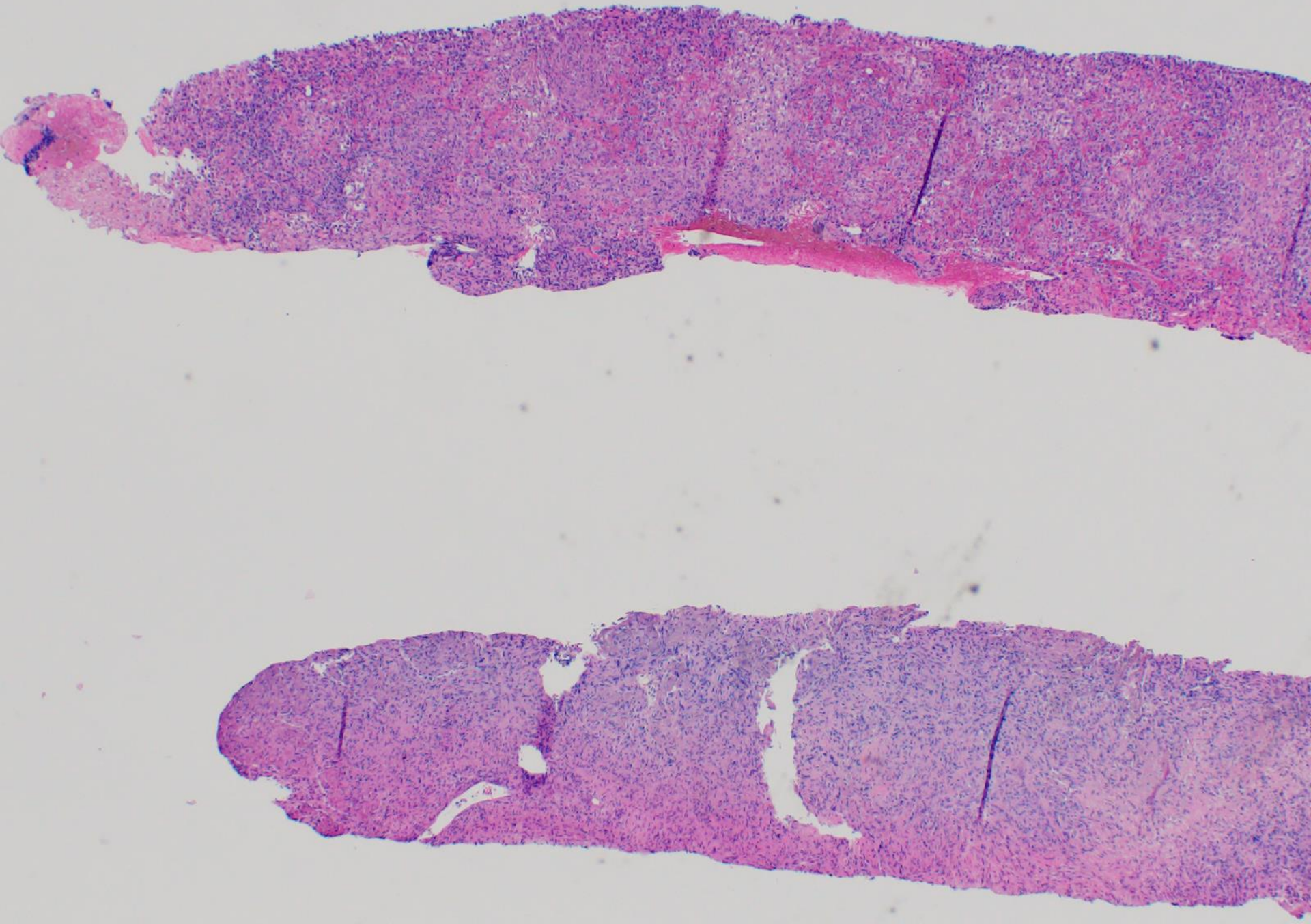
Case 2b

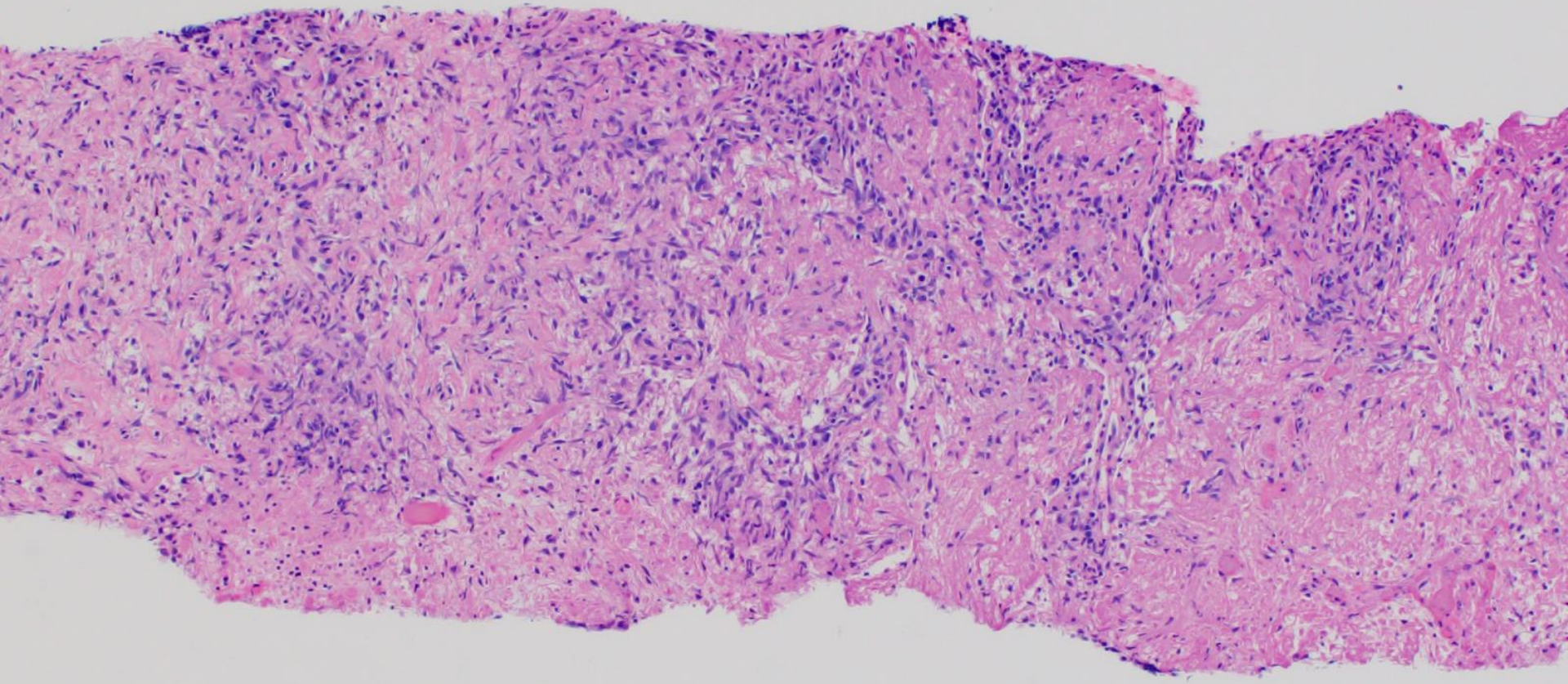
- 70 year old with a female with a 6.7 cm right upper lobe mass incidentally found on workup for acute dyspnea.
- She recently underwent an EBUS-guided CNB of a right paratracheal mass 1 month prior read out as necrotizing granulomatous inflammation.
- IR performed CT-guided CNB of the lung mass.

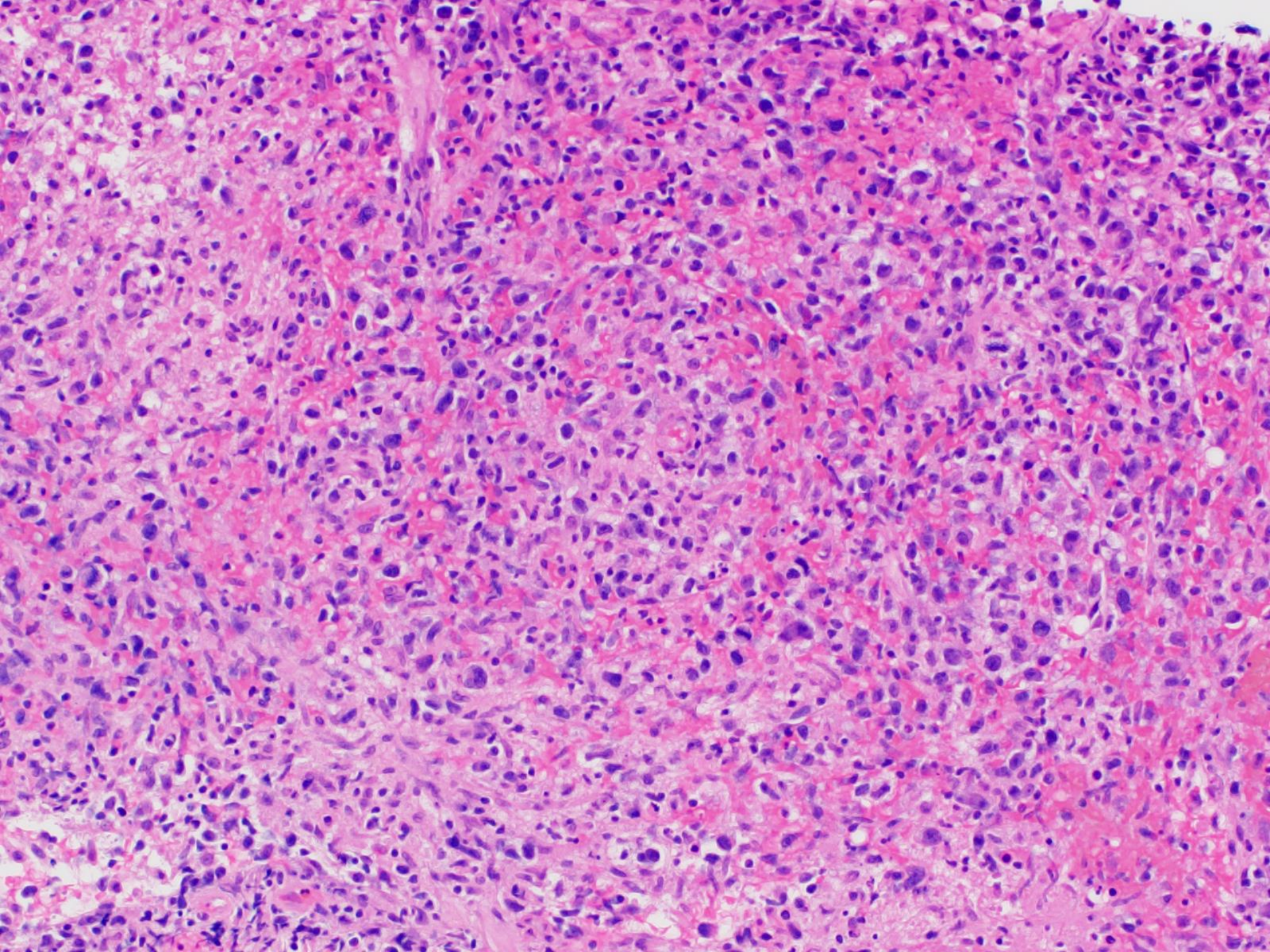


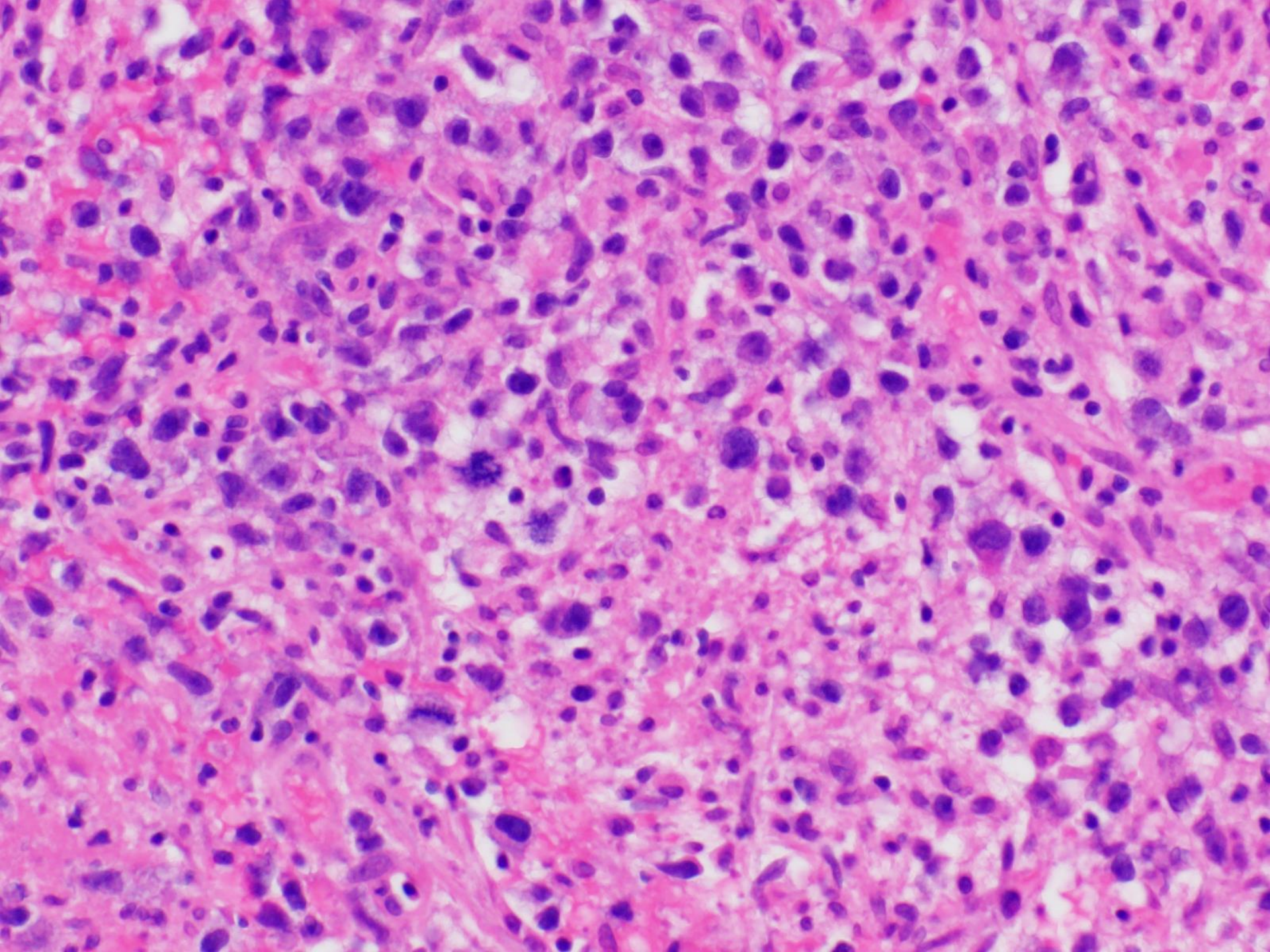




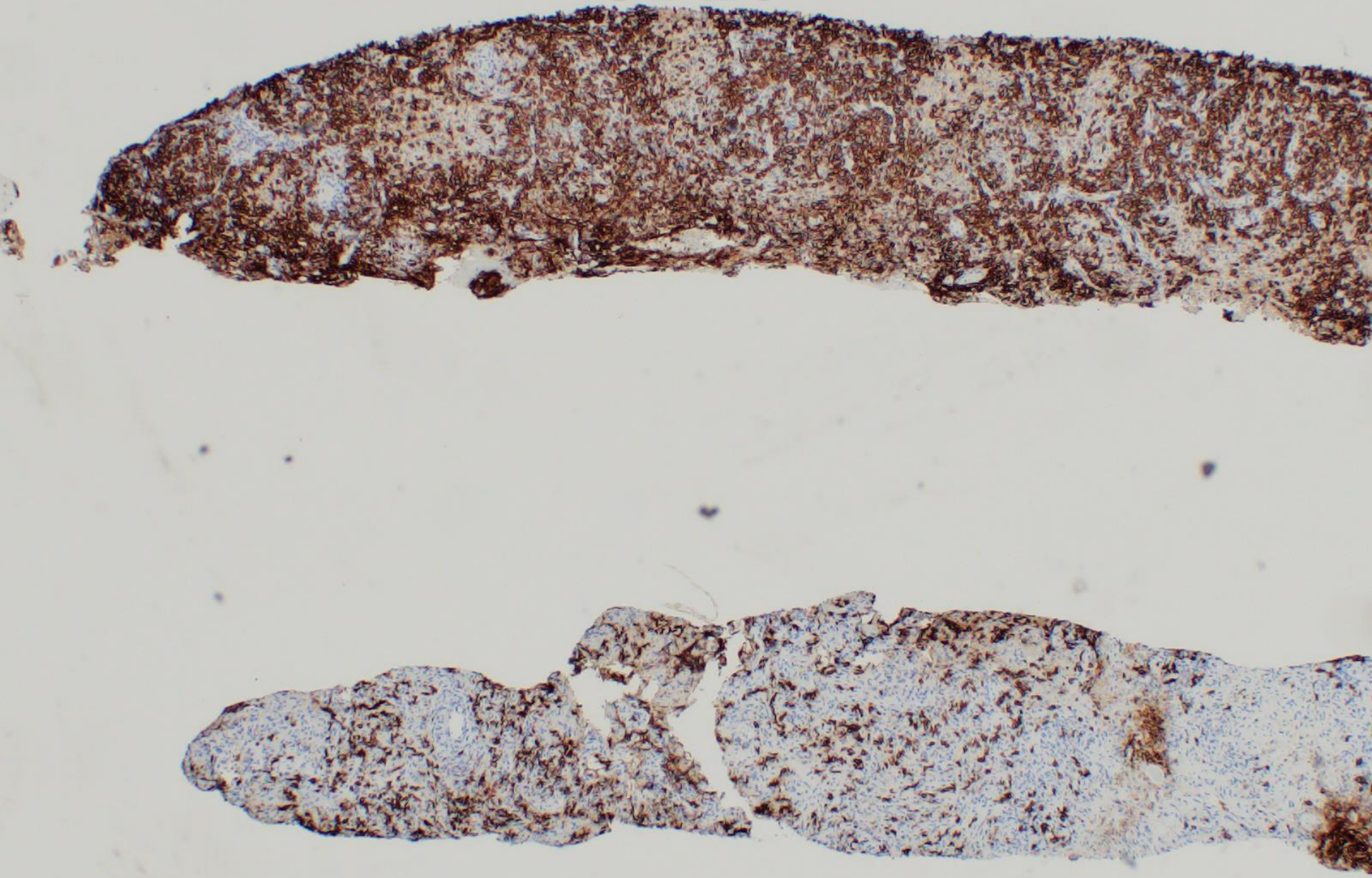


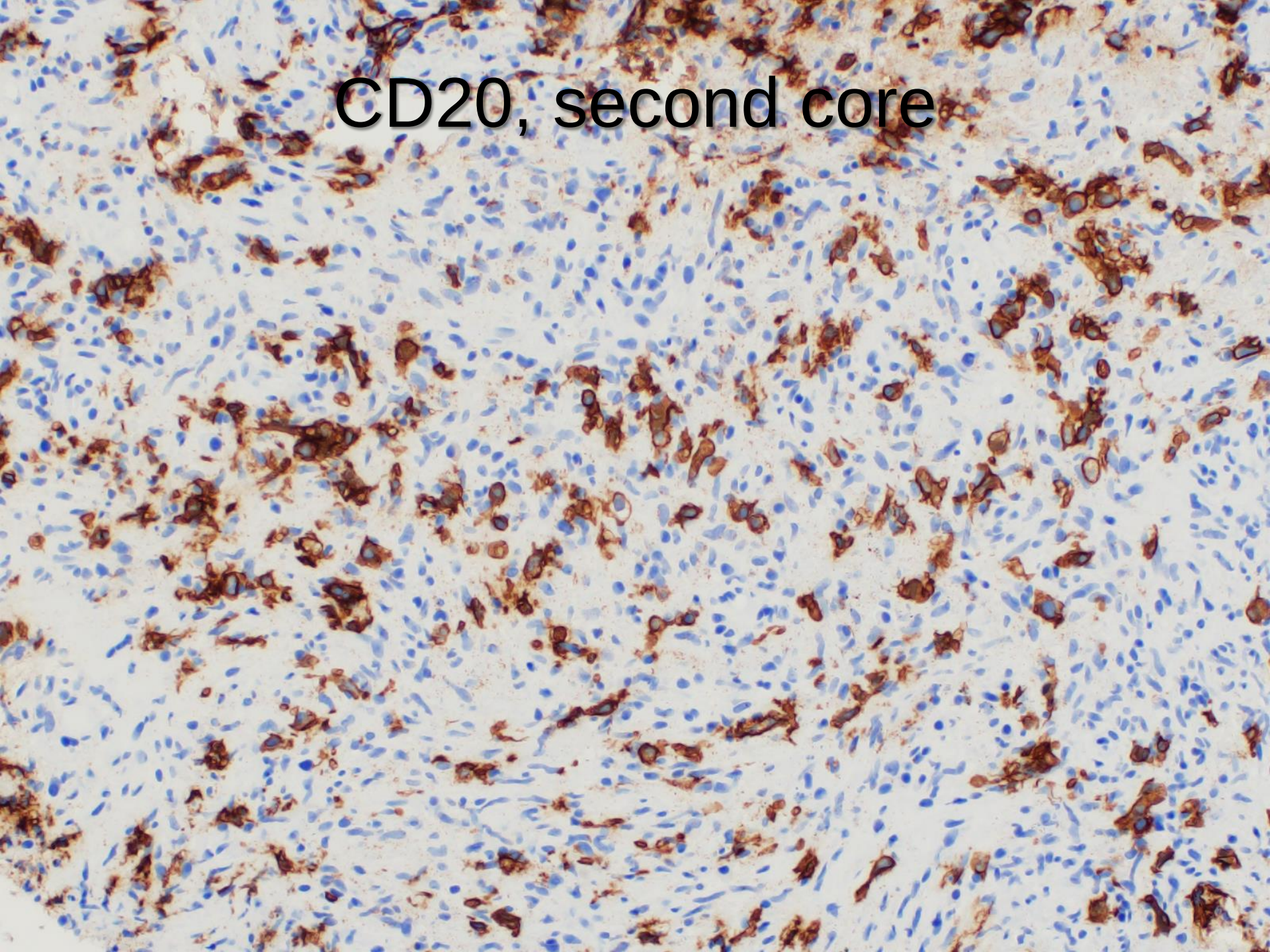


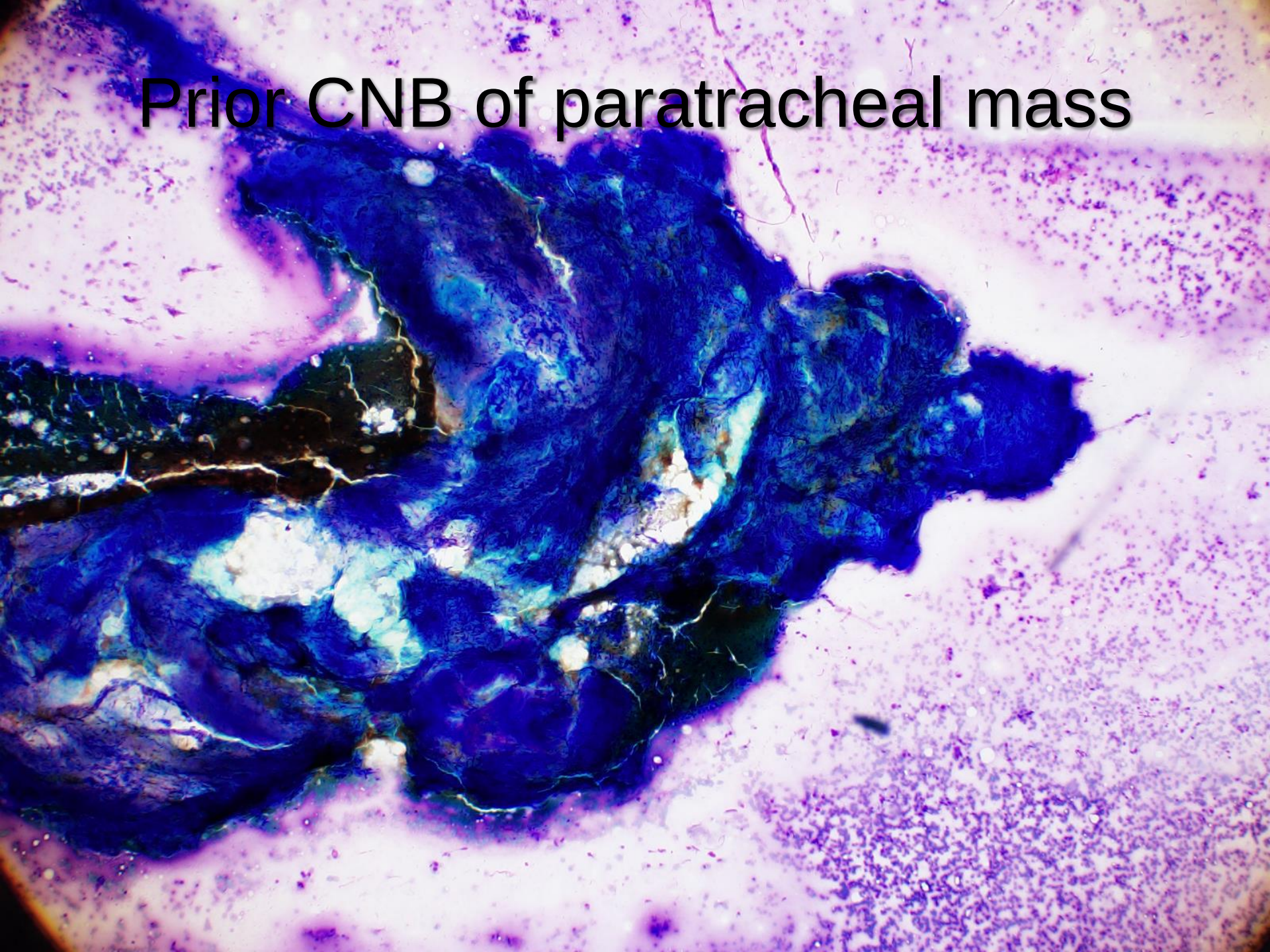


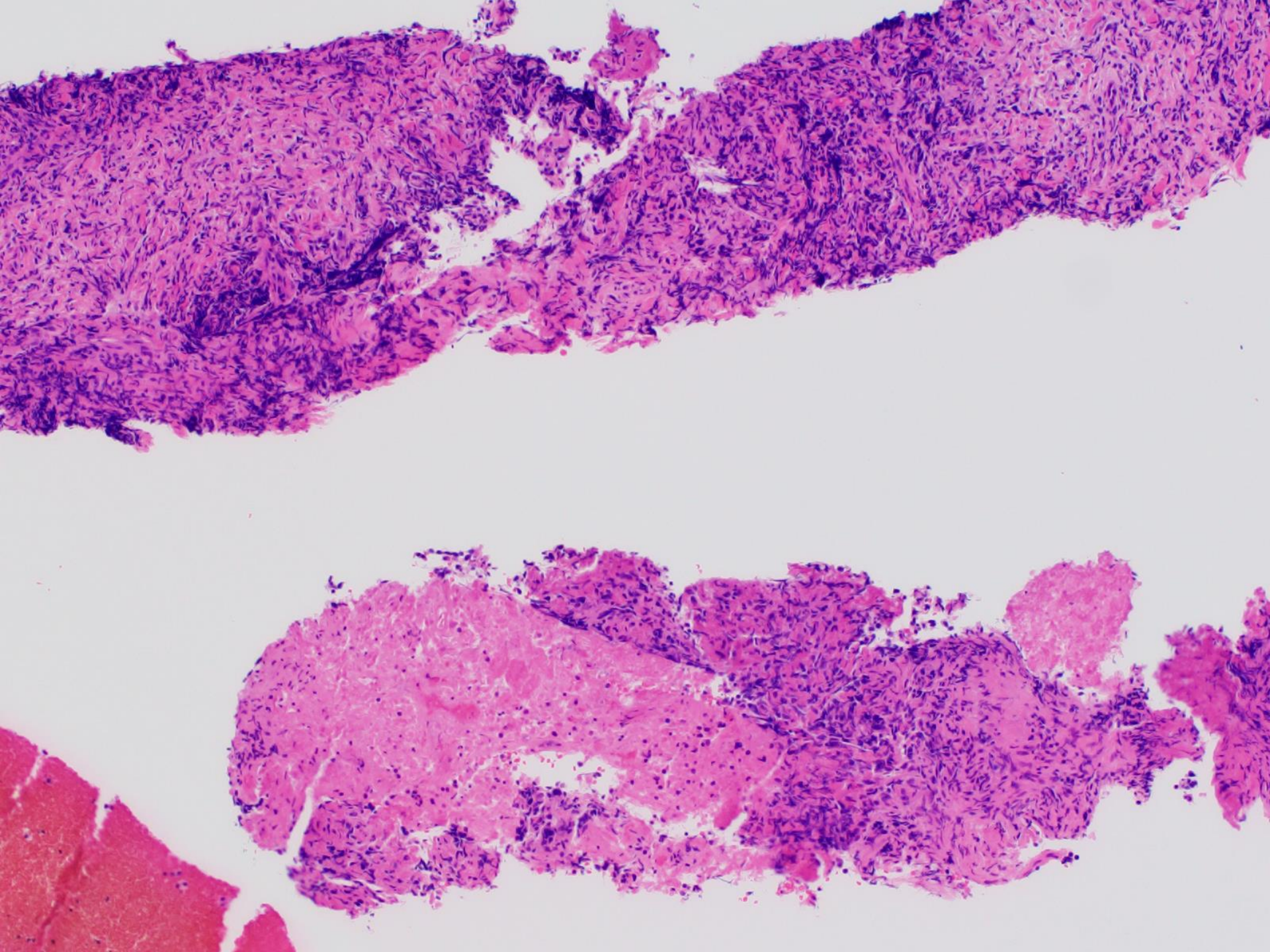


CD20




CD20, second core


Prior CNB of paratracheal mass

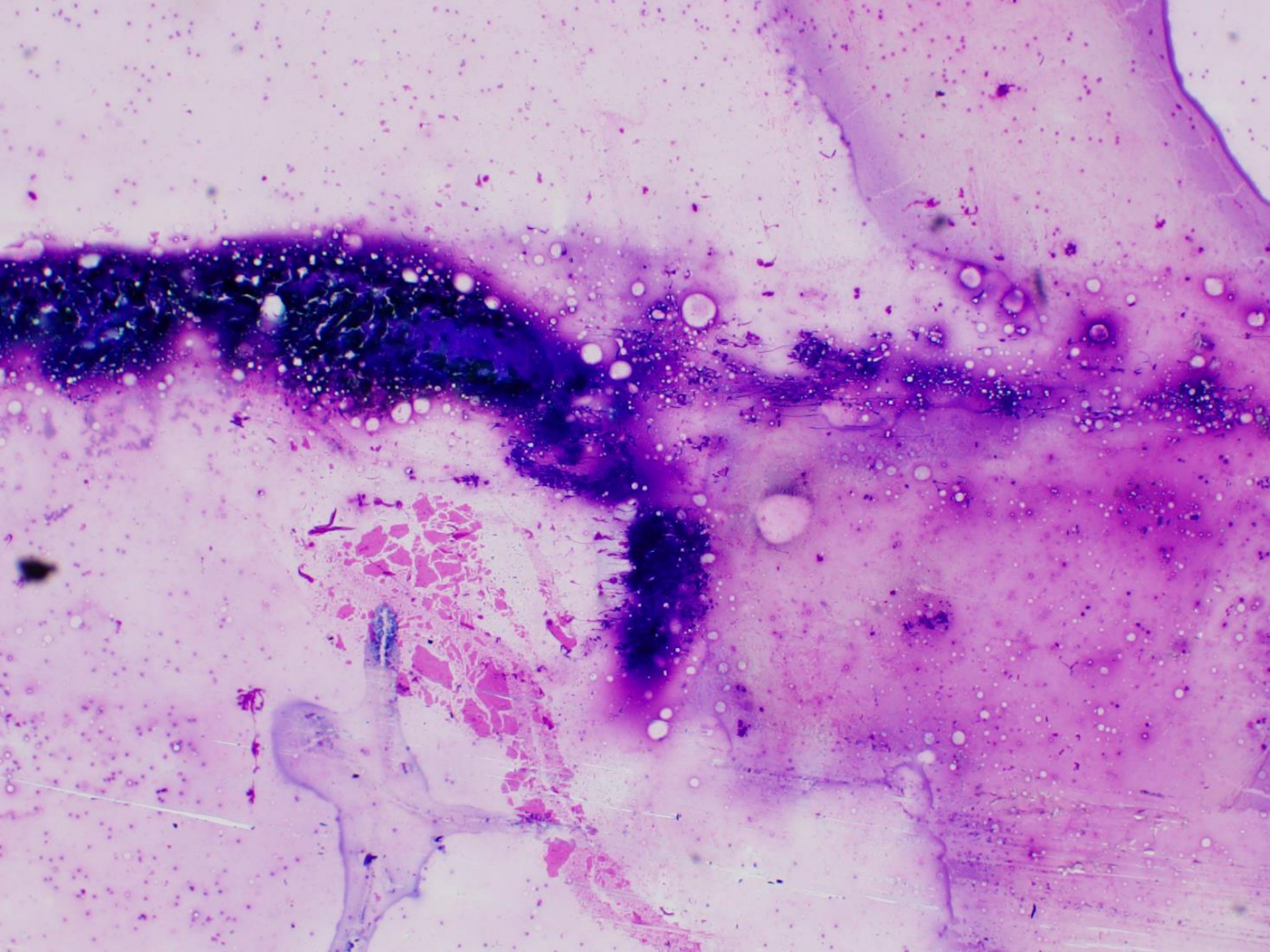


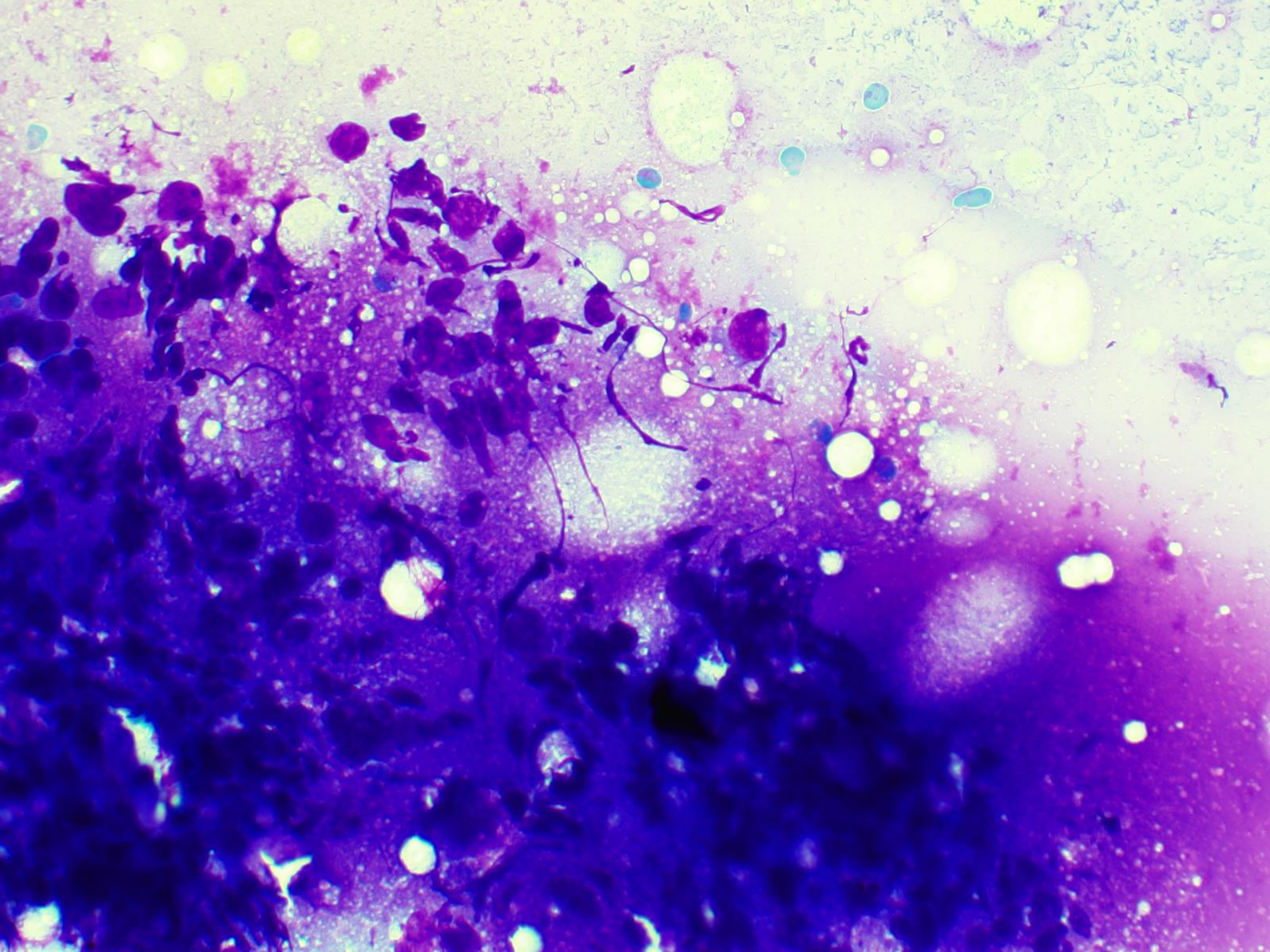
Diffuse Large B Cell Lymphoma

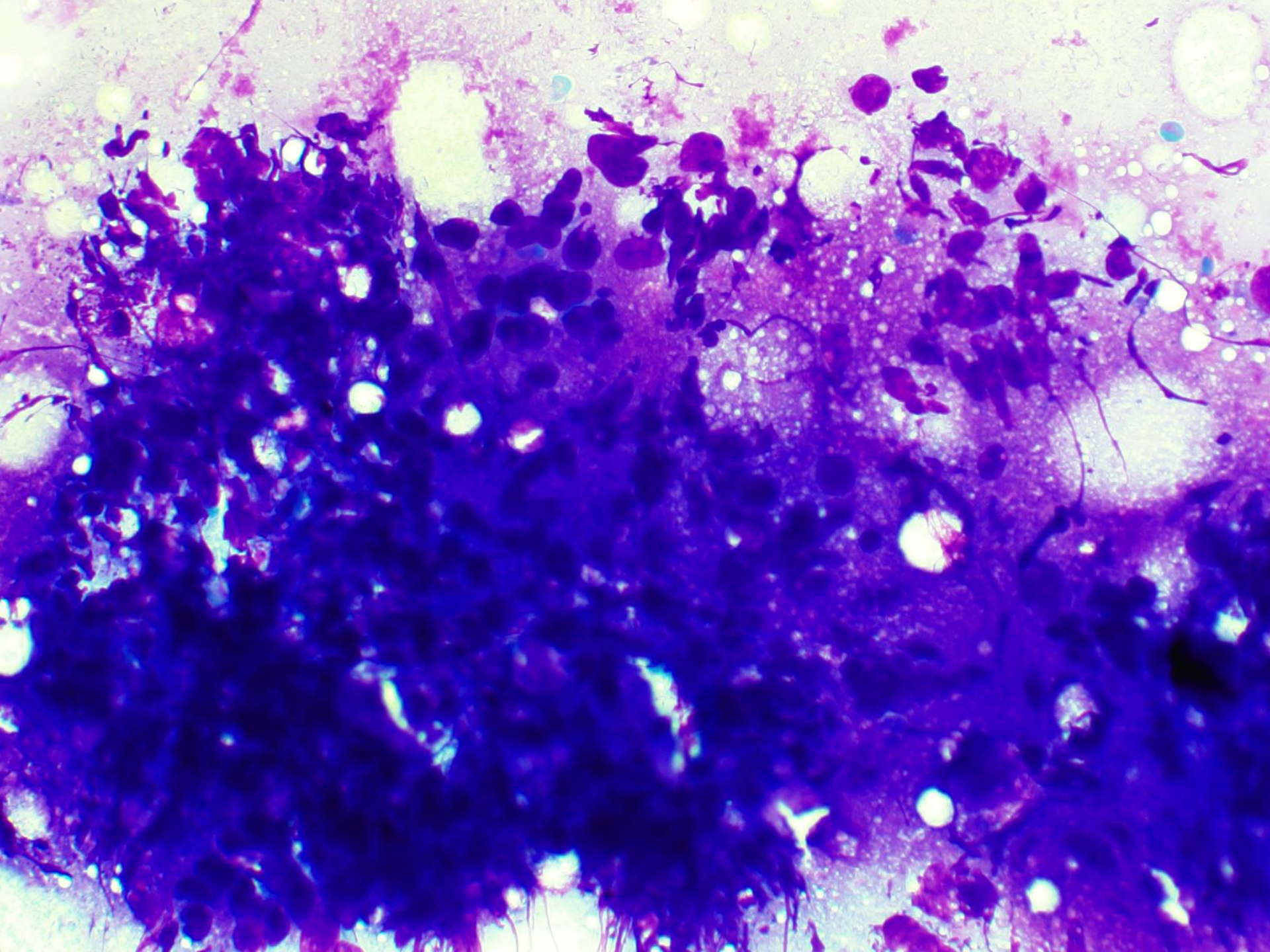
- Non-germinal center subtype, double-expressor phenotype
- Negative for LSI MYC (FISH)

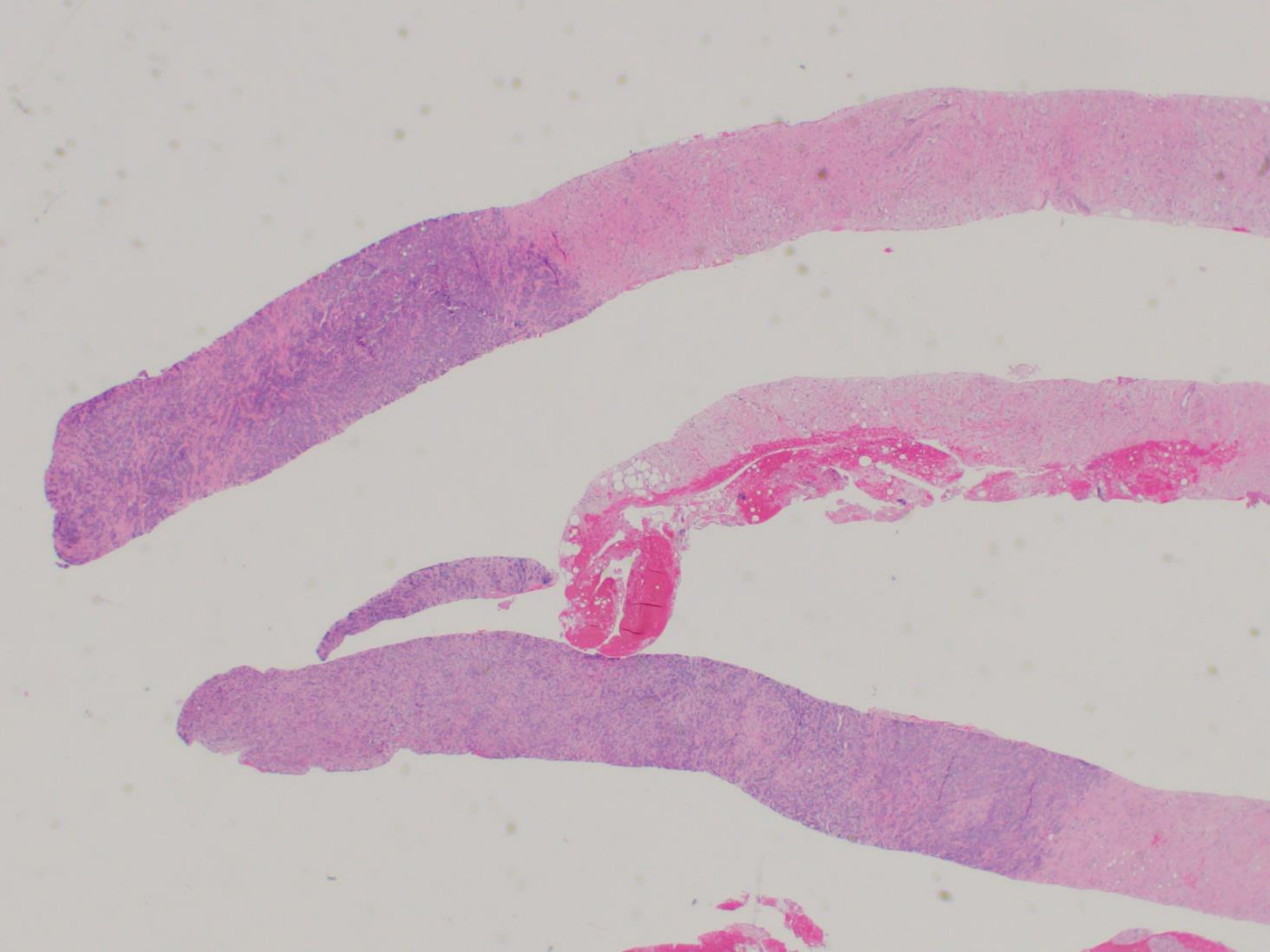
Case 2c

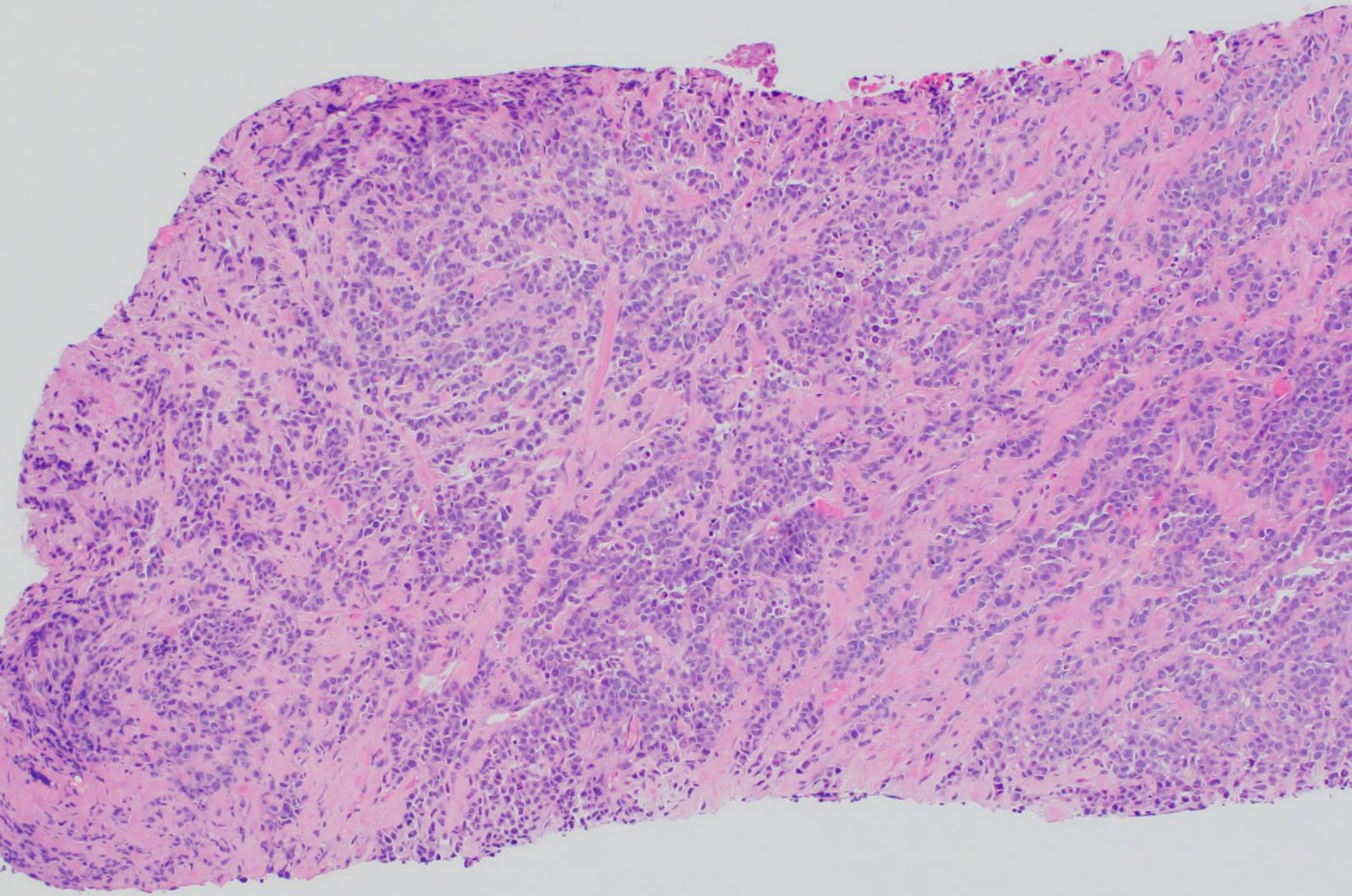
- 79 year old male presenting with a large posterior mediastinal mass
- No other significant PMH
- IR performed CT-guided CNB

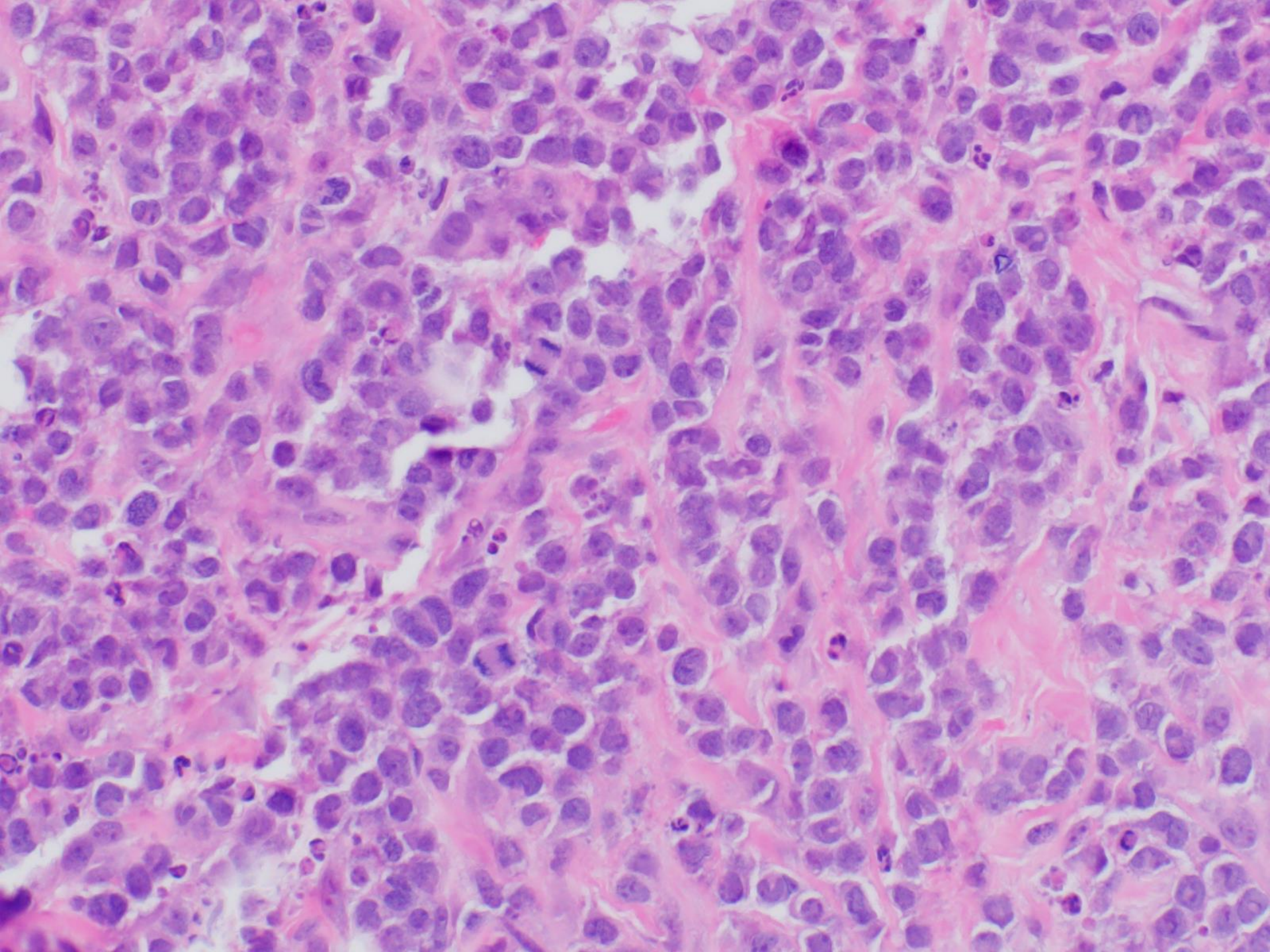


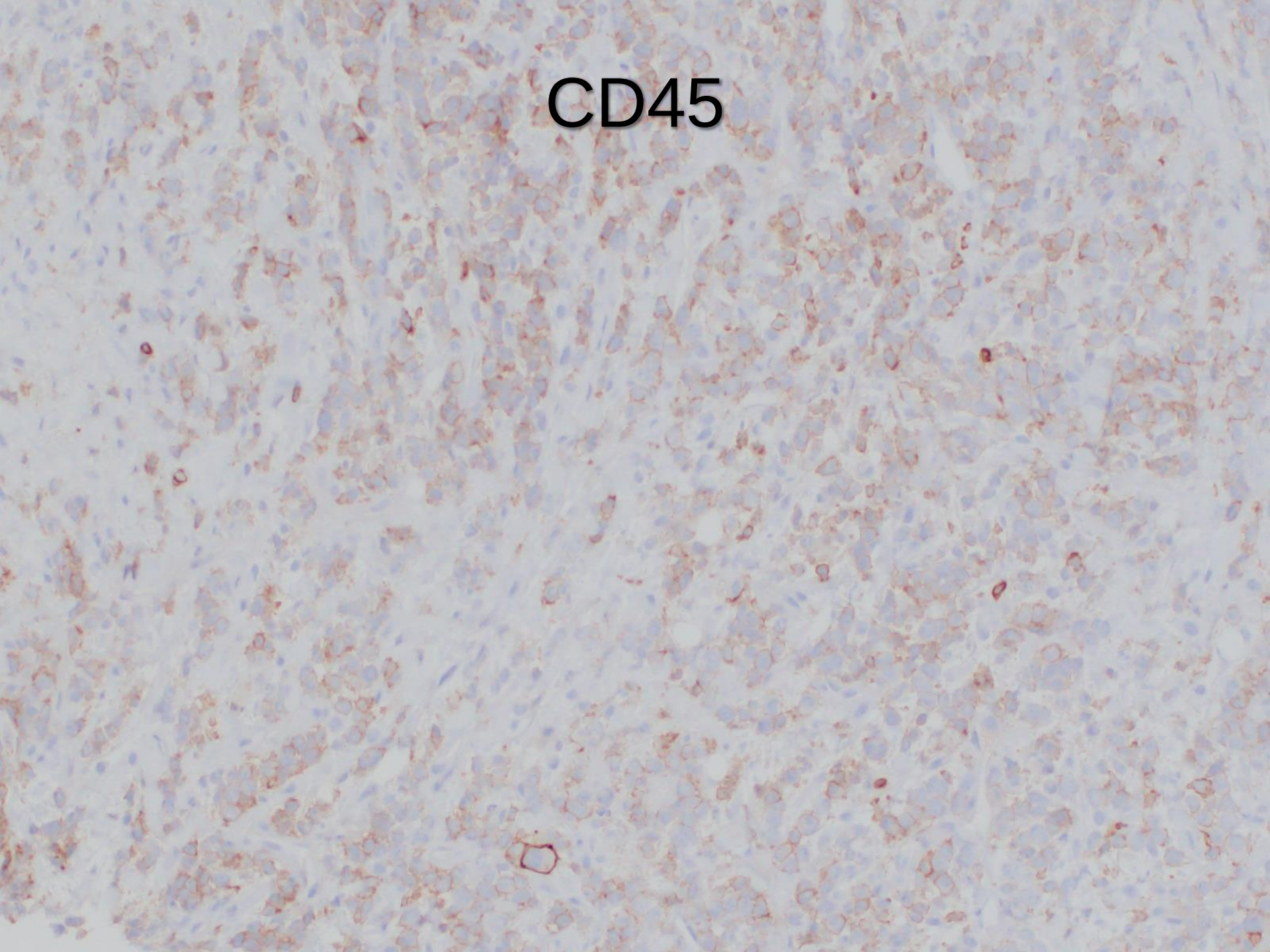


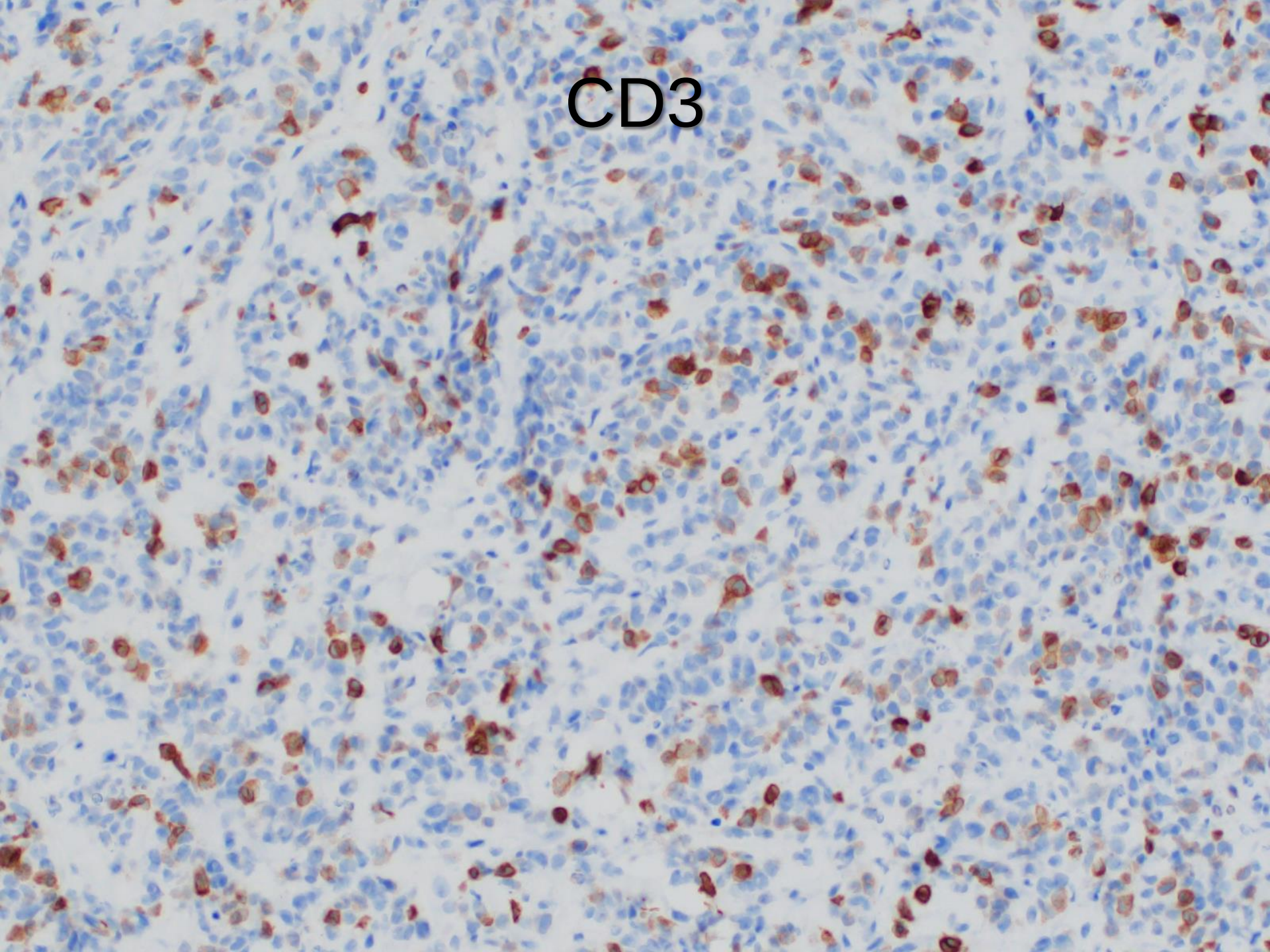


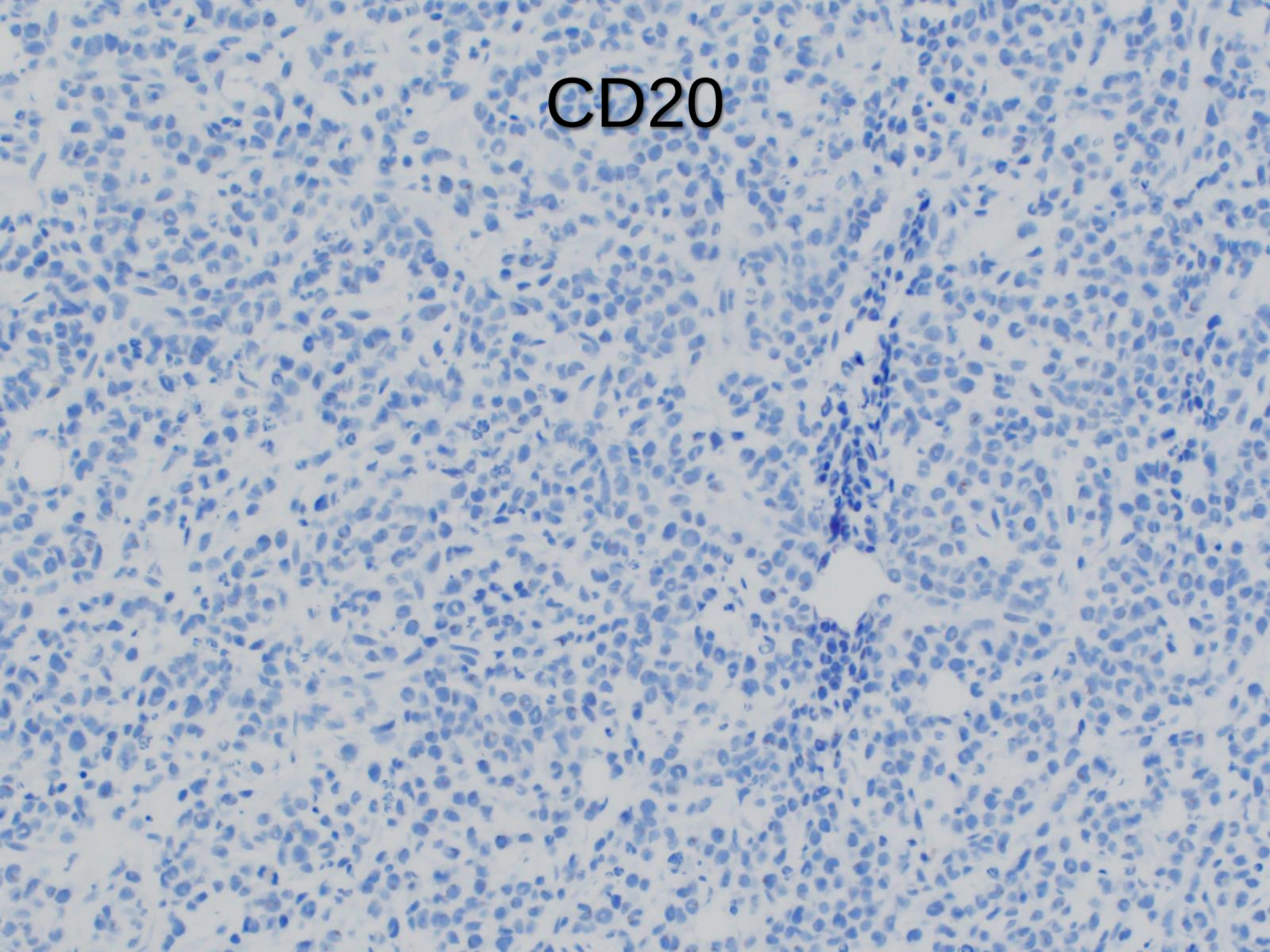


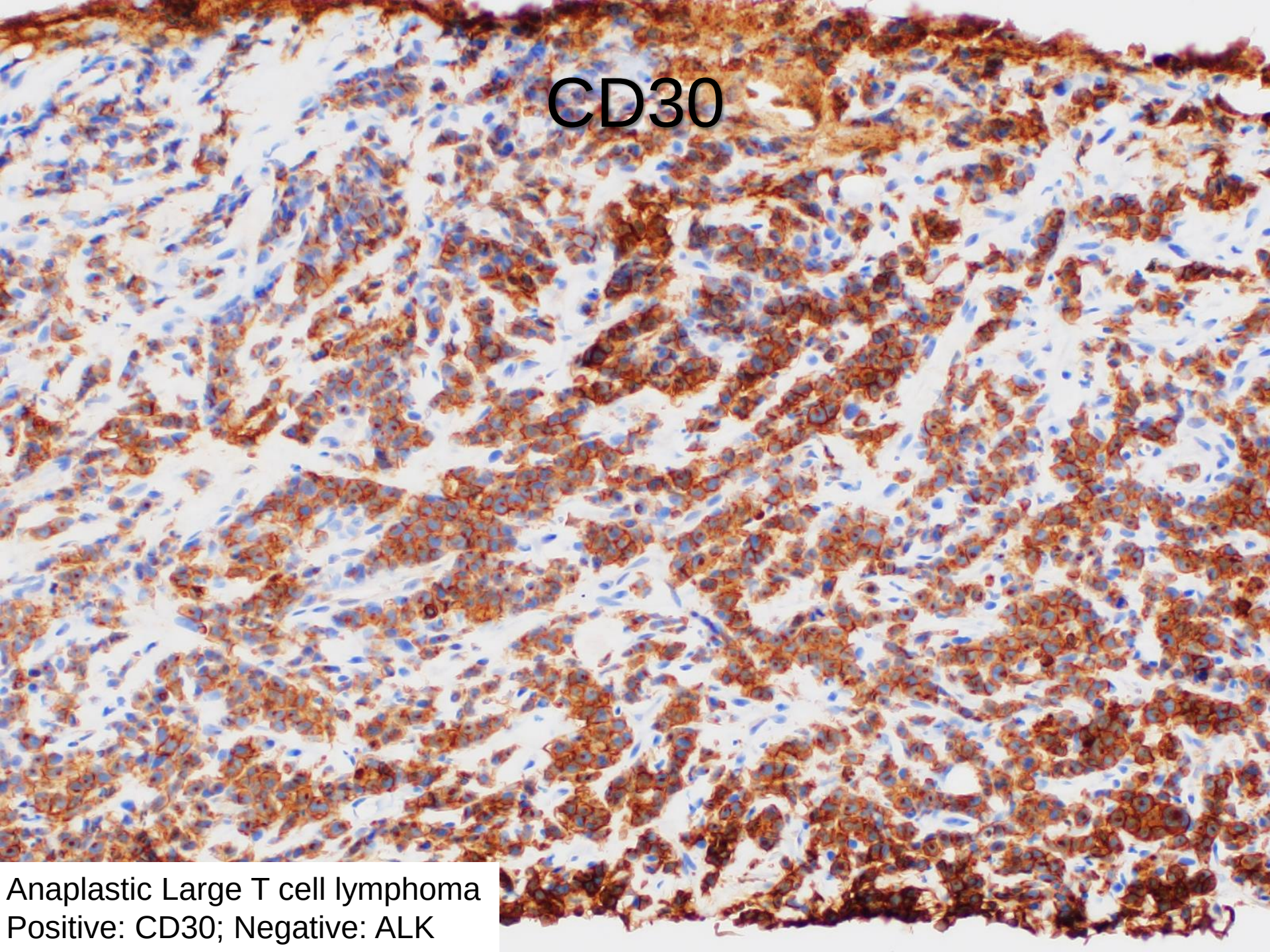





CD45


CD3


CD20



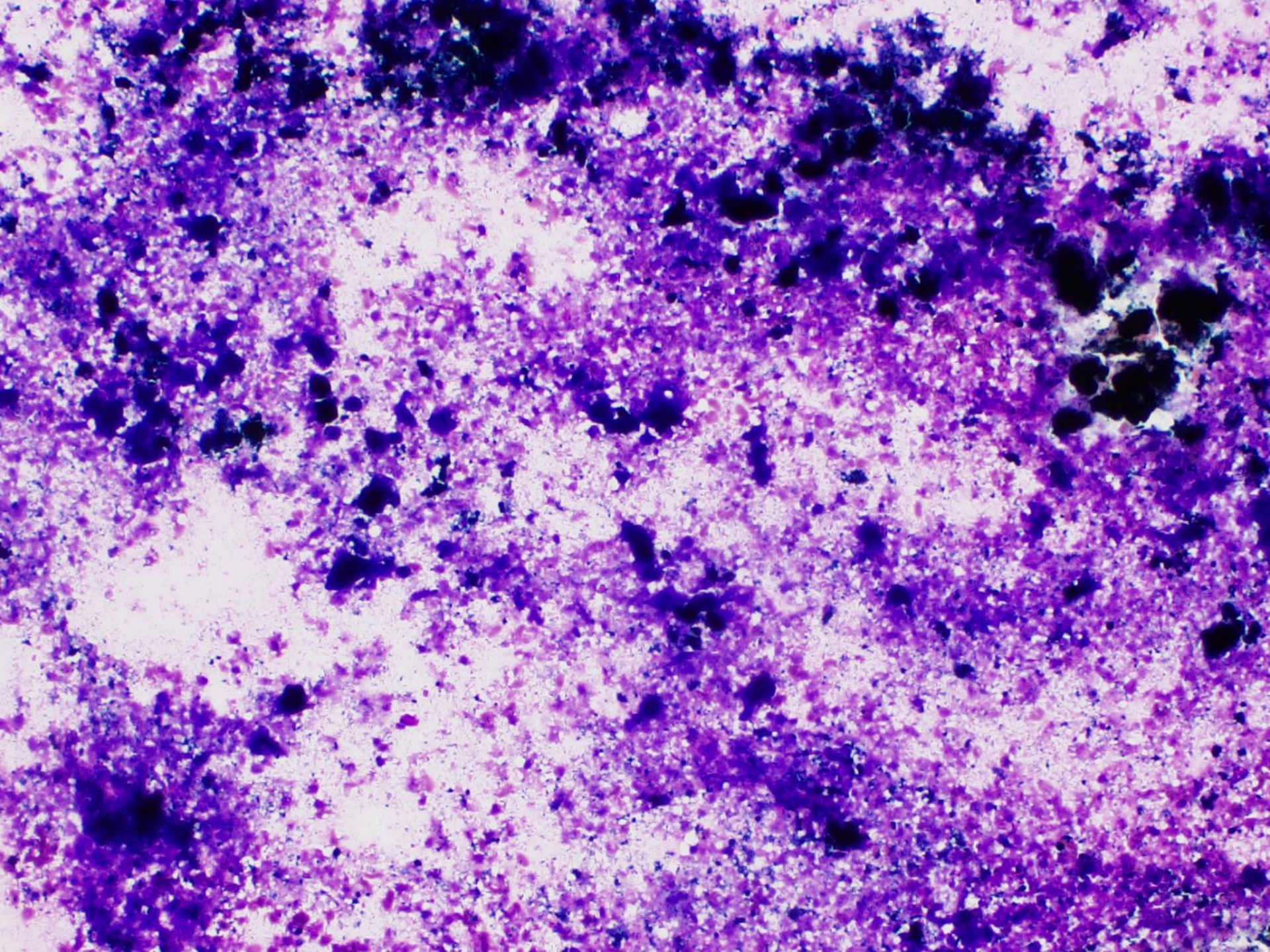
Anaplastic Large T cell lymphoma
Positive: CD30; Negative: ALK

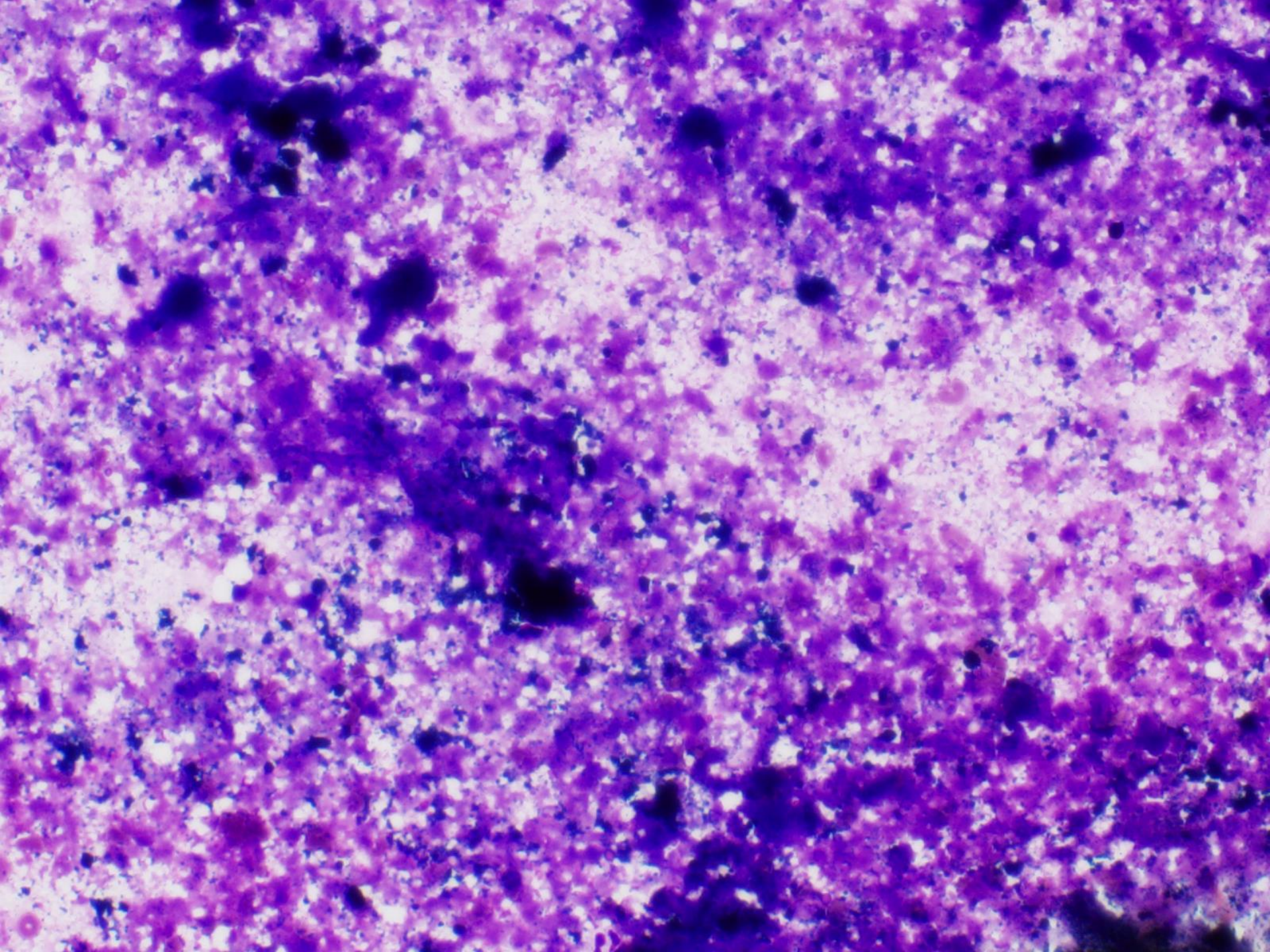
Take home messages #2

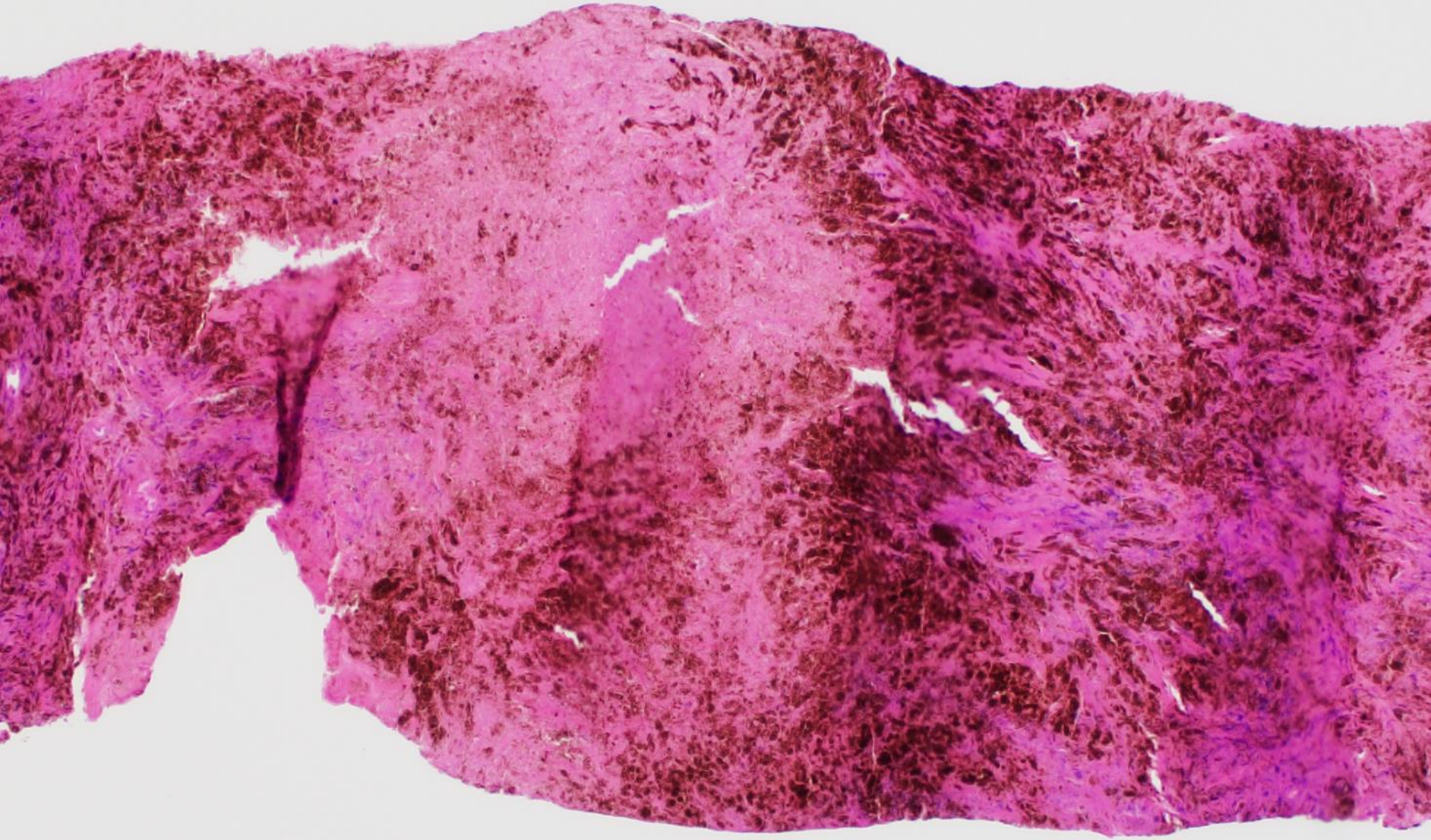
- Clinical history important but can be misleading (case 2a, 2b)
- Lymphomas remain firmly in the ranks of diagnostically challenging lesions
- Challenging subtypes of lymphomas
  - T cell lymphomas
  - Associated sclerosis
  - Tumors that won't flow well (HD, DLBCL)

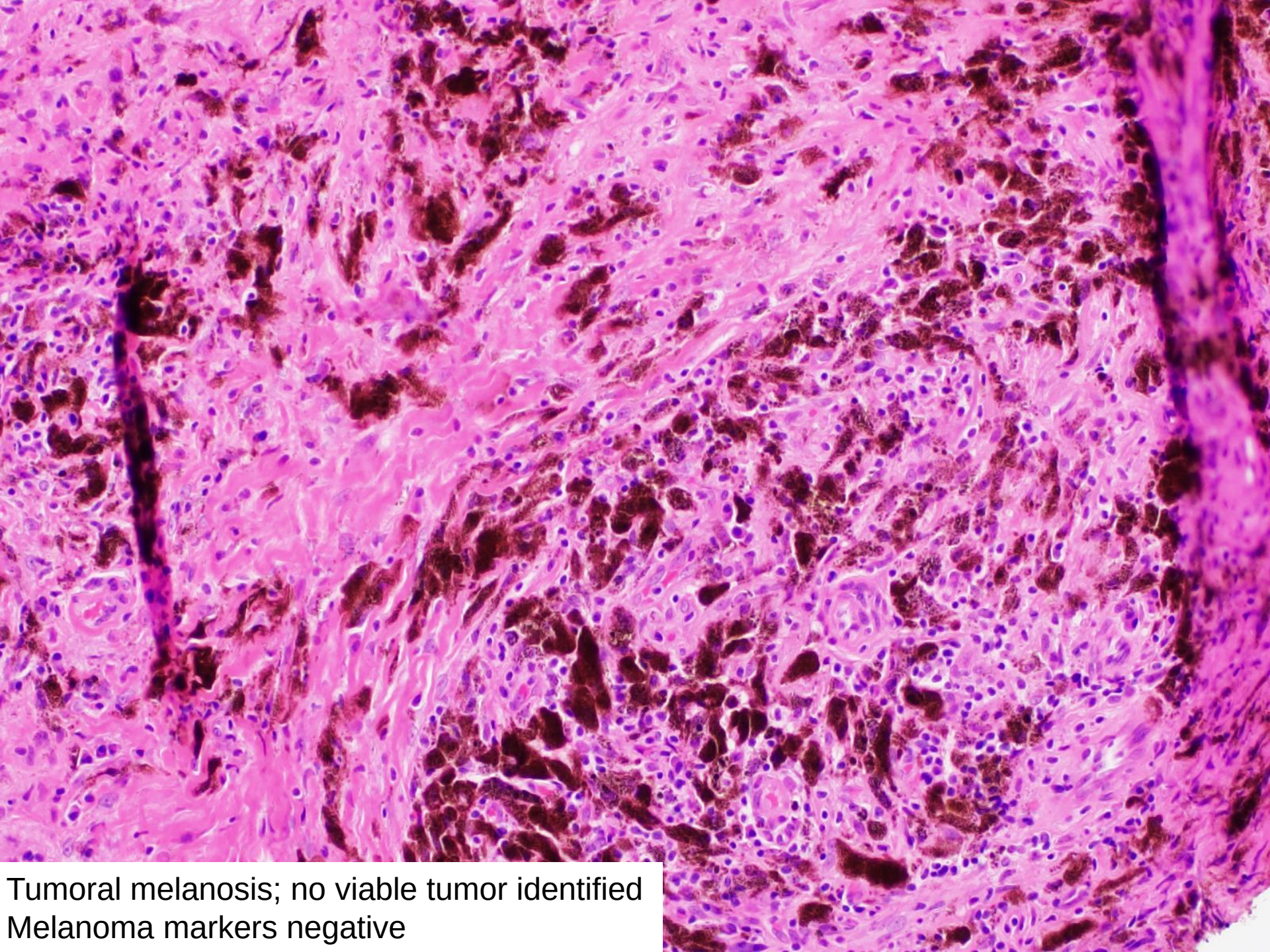
Case 3

- 73 year old male with a history of melanoma of the right upper back diagnosed in 2015 s/p radical resection and lymphadenectomy 2016
- Biopsy from outside hospital not reviewed; reported history of metastatic melanoma to LLL lung s/p pembrolizumab 1 year ago
- Now presenting with multiple small non-calcified pulmonary nodules bilaterally
- IR performed CT-guided CNB









Tumoral melanosis; no viable tumor identified
Melanoma markers negative

Take Home Messages #3

- Abundant necrosis (lesional, but not viable) may be non-diagnostic at ROSE but not necessarily at final sign-out
- Infection is typical consideration but don’t forget neoadjuvant chemotherapy or prior ablation therapy

FNA vs TP/CNB: What is the Verdict?

- The debate remains ongoing and strong proponents for FNA remain (van Zante & Ljung 2016)
- CNB in part driven by external factors such as proceduralist preference and increasing demand for ancillary testing
- Studies are ongoing pertaining to each modality and likely will be impacted by proceduralist, needle type and anatomic location

FNA vs TP/CNB

- FNA offers distinct advantages of minimal complications, enhanced assessment of tumor heterogeneity and flow cytometric analysis
- CNB provides increased tissue quantity and architectural context
- Ancillary studies can be applied to samples from either modality

Vital Considerations

- The presentation of patients presenting with clinicoradiologically suspicious lesions is increasing in complexity
- Heightened knowledge of preanalytic factors ensuring integrity of quality and quantity of tissue for diagnostic, prognostic and theranostic purposes is needed
- Awareness of the utility of ROSE and advantages and limitations of FNA and TP can help empower pathologists to advocate for what is needed to ensure success of the biopsy procedure and proper triage of biopsy material

Where Are We Headed?

- Updates in lymphoid lesions: Dr. R. Miles
- Molecular diagnostics: Dr. G. Deftereos
- Telecytology