

Lymph Node FNA

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Outline

- Preview of structured reporting system for lymph node cytology
- Diagnostic pattern-based approach with brief case presentations

WHO Structured Reporting System (A Preview)

Acta Cytologica

Fine Needle Aspiration

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A Proposal for the Performance, Classification, and Reporting of Lymph Node Fine-Needle Aspiration Cytopathology: The Sydney System

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International Agency for Research on Cancer



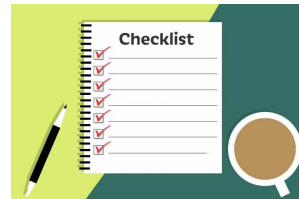
WHO Reporting System for Lymph Node, Spleen,
and Thymus Cytopathology

WHO Lymph Node international reporting system

- Improve patient care and outcomes through use of cytopathology



- Key diagnostic criteria in cytopathology for specific non-neoplastic, benign and malignant lesions to be established by international expert consensus for first time



WHO Lymph node Reporting System Categories

- Insufficient/Inadequate/Non-diagnostic
- Benign
- Atypical
- Suspicious for malignancy
- Malignant



- Each category associated with:
 - Risk of Malignancy (ROM)
 - Recommendation for steps to refine DDX or achieve specific WHO diagnosis (goal)
- Categories are used to assist communication with clinicians

Haematolymphoid Tumours, 5th edition

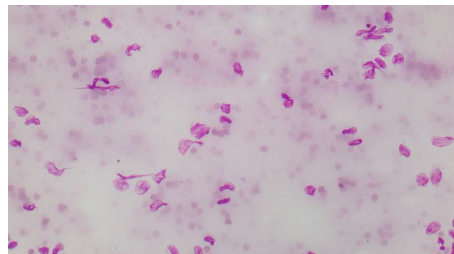
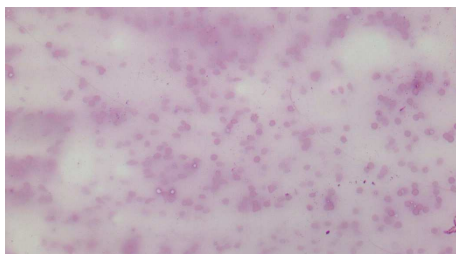
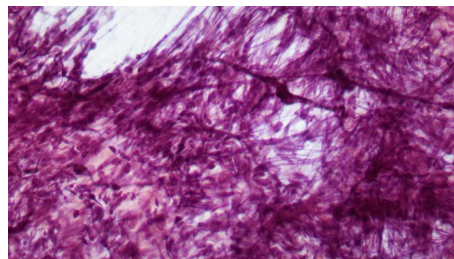
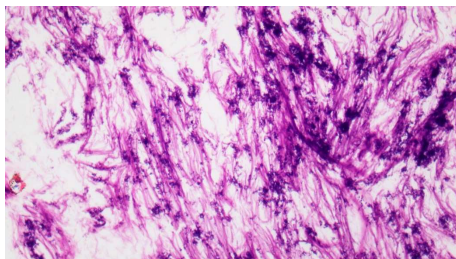
- Reliable interpretation cannot be made (qualitative or quantitative limitations)
- E.g. insufficient cellularity, poor smearing technique, air-dry artifact, fixation artifact, obscuring material
- Repeat FNAB is recommended, with ROSE if possible, and with core needle biopsy if available
- If insufficient at time of ROSE, needle rinsings may enable diagnosis when analyzed by flow cytometry, cell block with ICC, cytogenetics, FISH etc

No consensus on LN FNAB adequacy criteria

- Generally, no or very few lymphoid cells present
- Some suggested 40 lymphocytes per HPF (400x) in the most cellular areas

Karunamurthy A et al. Evaluation of EBUS-FNA: correlation with adequacy and histologic follow-up. Cancer Cytopathol. 2014. PMID: 24127207.

Insufficient/Inadequate/Non-diagnostic Category



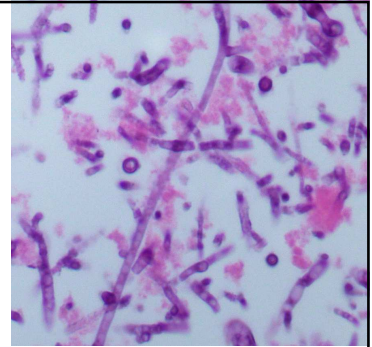
Insufficient/Inadequate/Non-diagnostic Category

- Use one term consistently for clear communication.
- Triple Test: always correlate with imaging and clinical findings.
- WHO system accepts ND diagnosis in cases where there is good lymphoid material, but clinical findings are not explained
 - Others may call these Benign, with “sample may not be representative.”

Diagnostic categories and ROM

ROM	ND	Benign	Atypical	Suspicious	Malignant
Gupta P, Cancer Cytopathol 2021	27.5%	11.5%	66.7%	88.0%	99.6%
Vigliar E, Diagnostics 2021	50%	1.92%	58.3%	100%	100%
Torres Rivas HE, Acta Cytol 2021	27%	3%	50%	100%	100%
Caputo A, Acta Cytol 2022	46%	1.05%	28.6%	100%	99.8%
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Ahuja S, Cytopathol 2022	9.1%	1.5%	37.5%	96.9%	98.2%

Benign Category



- Unequivocally benign; precise diagnosis not required
- e.g. normal lymphoid populations, necrosis, granulomas, specific infections (viral, mycobacterial, fungal)
- Microorganisms may be seen with routine or special stains (e.g. GMS)
- Support Benign diagnosis with ancillary techniques if available:
 - PCR, cultures
 - cell block +/- stains
 - flow cytometry showing reactive population

Benign Category

- Potentially difficult DDX:
 - Follicular hyperplasia vs follicular lymphoma
 - EBV mononucleosis vs Hodgkin lymphoma
 - Partial involvement of lymph node by low grade lymphoma
- Cytopathologist categorization of inflammatory processes as Benign vs Atypical depends on skill and practice milieu
- **If the findings raise the possibility of lymphoma, do not use Benign category- use Atypical category

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Atypical Category

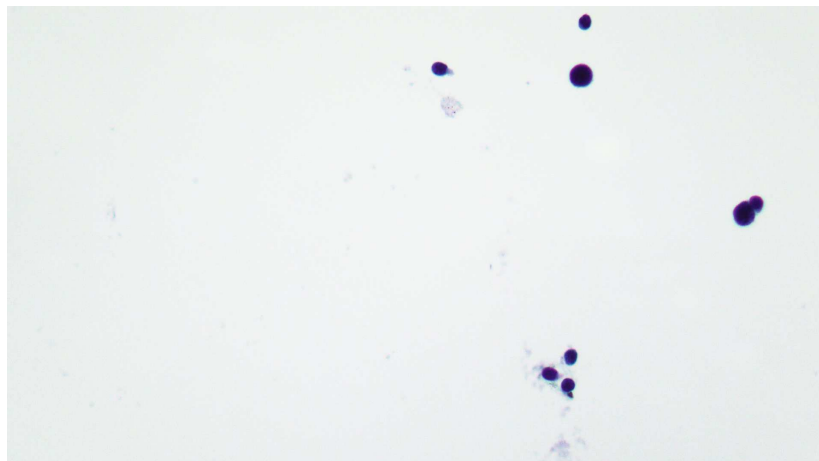
- Predominantly supports a benign process, but minimal features raise possibility of malignancy
- Insufficient quantity or quality of concerning features to diagnose as benign or malignant
- **ALUS**: “Atypical lymphoid cells of uncertain significance” (concern for lymphoma)
- **AUS**: “Atypia of uncertain significance” (concern for non-lymphoid neoplasm)
- Report should describe the specific atypical features seen, and DDX raised
- The Atypical category allows Benign category to have high negative predictive value, but do not use as a “garbage can”

Atypical Category- Next Steps

- Repeat FNAB for more material, ancillary testing by flow cytometry, or CNB which can be evaluated by IHC
- If FC or CNB not possible, excise node or closely watch for 2-4 weeks, depending on the DDX and clinical judgment
- e.g. DDX infectious mononucleosis vs Hodgkin lymphoma-- may watch patient to see if lymphadenopathy resolves
- e.g. DDX low grade B lymphoma vs benign-- lymph node should be resampled with material sent for FC or CNB/excision

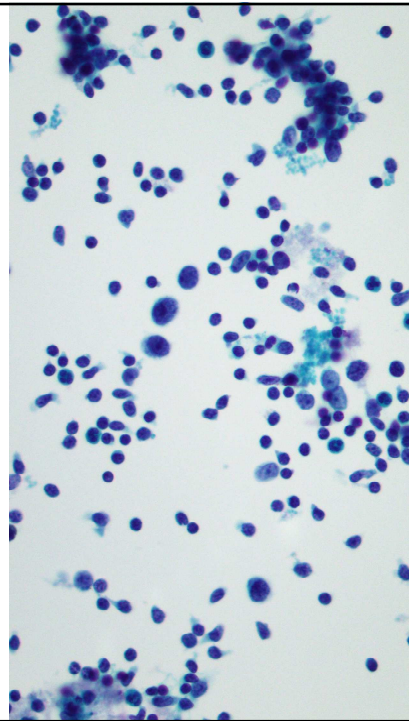
Atypical Category

- Paucicellular sample with small and atypical large lymphoid cells



Atypical Category

- Cyto slides with heterogeneous lymphoid population with tangible-body macrophages, dendritic cells, germinal center fragments – suggests reactive follicular hyperplasia; no FC available
- Can diagnose Atypical (suggestive of FH), recommend repeat sampling with FC if LAD persists



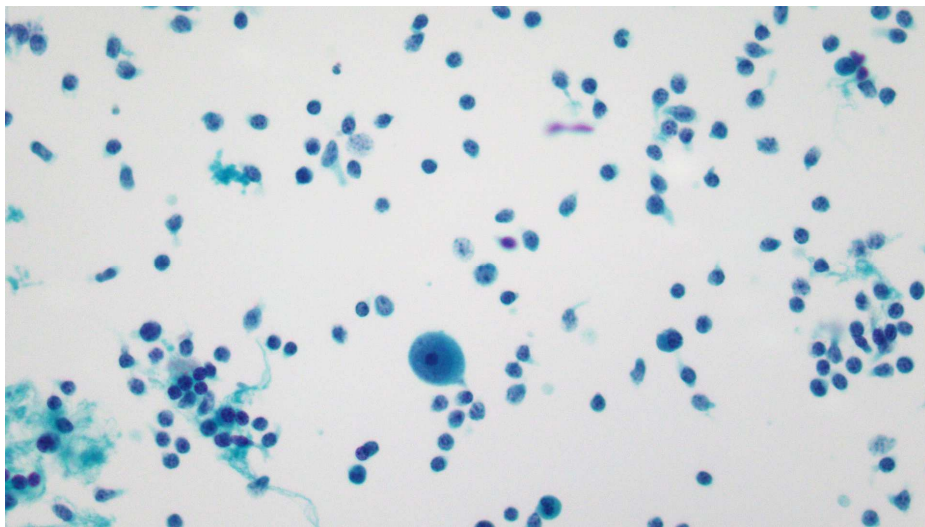
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Suspicious for Malignancy Category

- Morphologic features concerning for malignancy
- Limited quantity/quality of findings precludes definitive Malignant dx
- High positive predictive value for Malignancy
- Includes lymphoid and non-lymphoid neoplasms

Suspicious for Malignancy



Suspicious for Malignancy Category

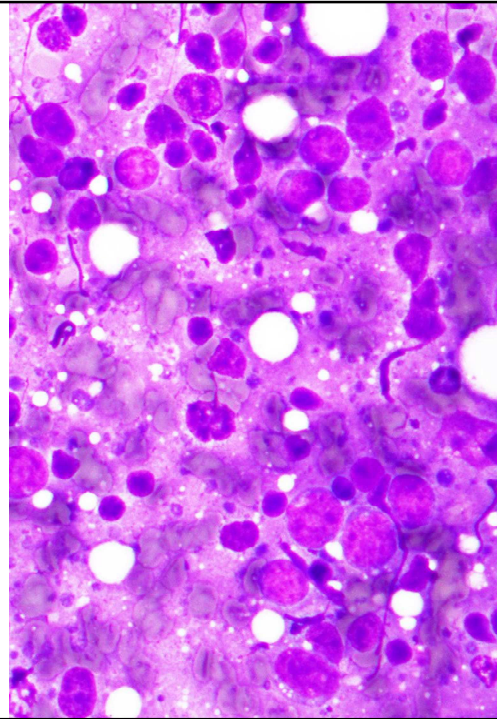
- Report describes the suspicious features, and provides DDX
- Utilize ancillary testing (FC, cell block with ICC) to try to move diagnosis to Malignant category
- Additional management required. Repeat sampling by FNAB or CNB + ancill. Excised if ancillary testing limited/not available.

Diagnostic categories and ROM

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Malignant Category

- Unequivocal features of malignancy (any type)
- Malignant diagnosis is possible without ancillary testing
- Should have low false-positive rate
- Use ancillary testing to move from Suspicious to Malignant category

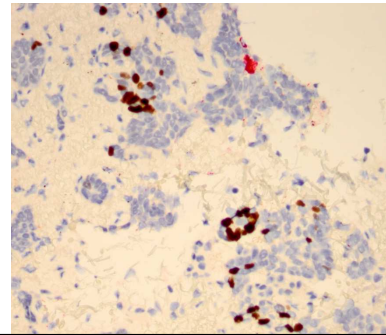
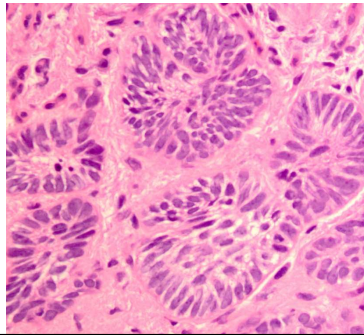
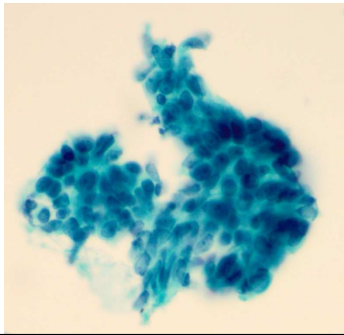


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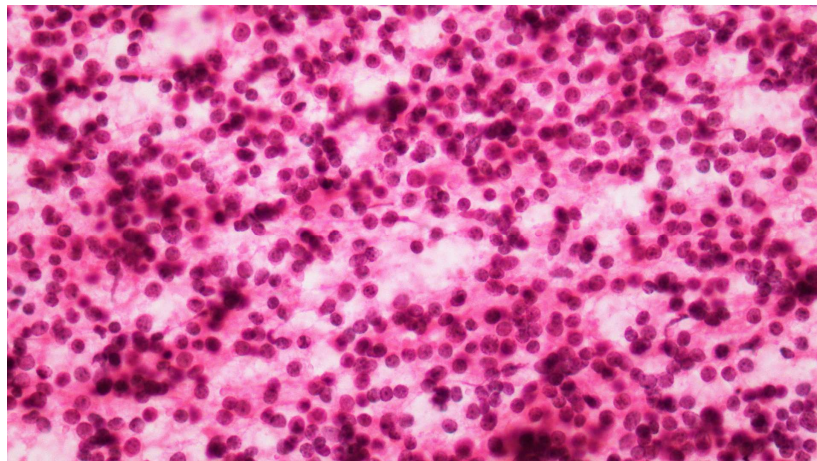
Going Beyond the Malignant Category: Ancillary testing enhances FNAB diagnostic utility

- Can provide specific diagnosis
 - “Non-small cell carcinoma” at ROSE → basaloid squamous cell carcinoma with p40+/TTF1- on cell block, PD-L1



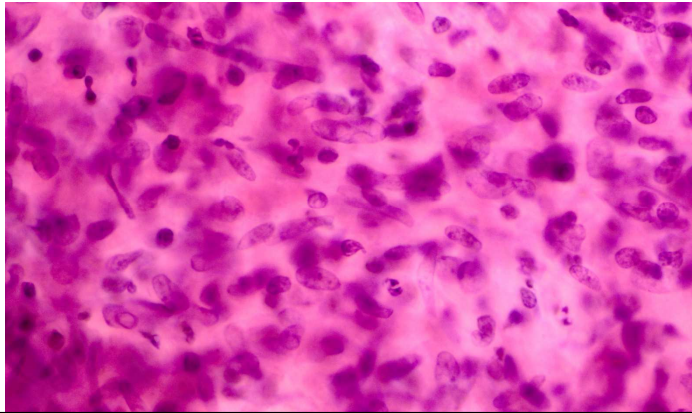
Going Beyond the Malignant Category: Ancillary testing enhances FNAB diagnostic utility

- “Lymphocytes” at ROSE → Mantle cell lymphoma with FC, cell block + stains



Going Beyond the Malignant Category: Ancillary testing enhances FNAB diagnostic utility

- “Necrotizing granulomas” at ROSE → Send material for cultures to identify infectious organism



FNA Diagnostic Utility is enhanced by:

- High quality FNAB smear preparations (learn and teach good technique!)
- ROSE
 - decreases ND rates
 - supports appropriate triage for ancillaries
 - provides preliminary diagnoses
- Routine use of FC and Stains to confirm diagnoses

FNAB can triage management for the patient (even if specific diagnosis cannot be made)



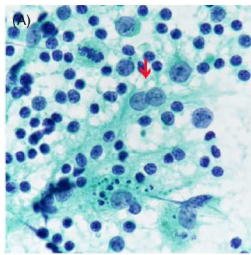
Lymph Node FNAB Sampling and Specimen Preparation

- Split sample and create multiple direct smears
- Make air-dried (Diff-Quik) and alcohol-fixed (Pap) slides, they are complementary:
 - air-dried smears better for background material and cytoplasm quality
 - alcohol-fixed better for nuclear detail
- ROSE is possible with both fixation methods
- If ROSE suggests infection, may reserve few air-dried smears for micro stains (GMS, Gram etc)
- Take additional DEDICATED passes for cultures, flow cytometry, cell block, molecular (based on ROSE)

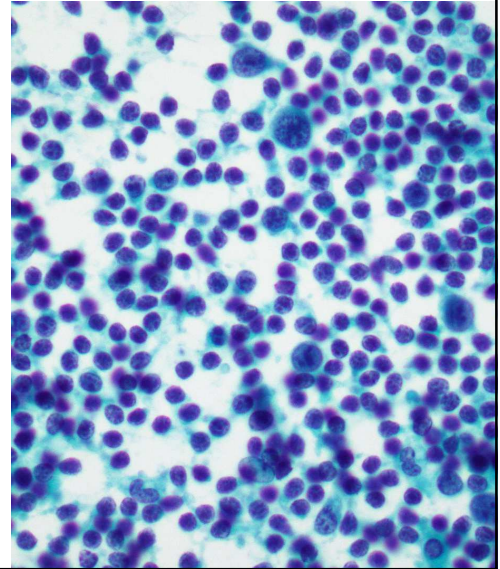
Morphologic Approach

Normal lymph node populations

- Resting small lymphocytes
- Centrocytes
- Centroblasts
- Tingible body macrophages
- Dendritic cells

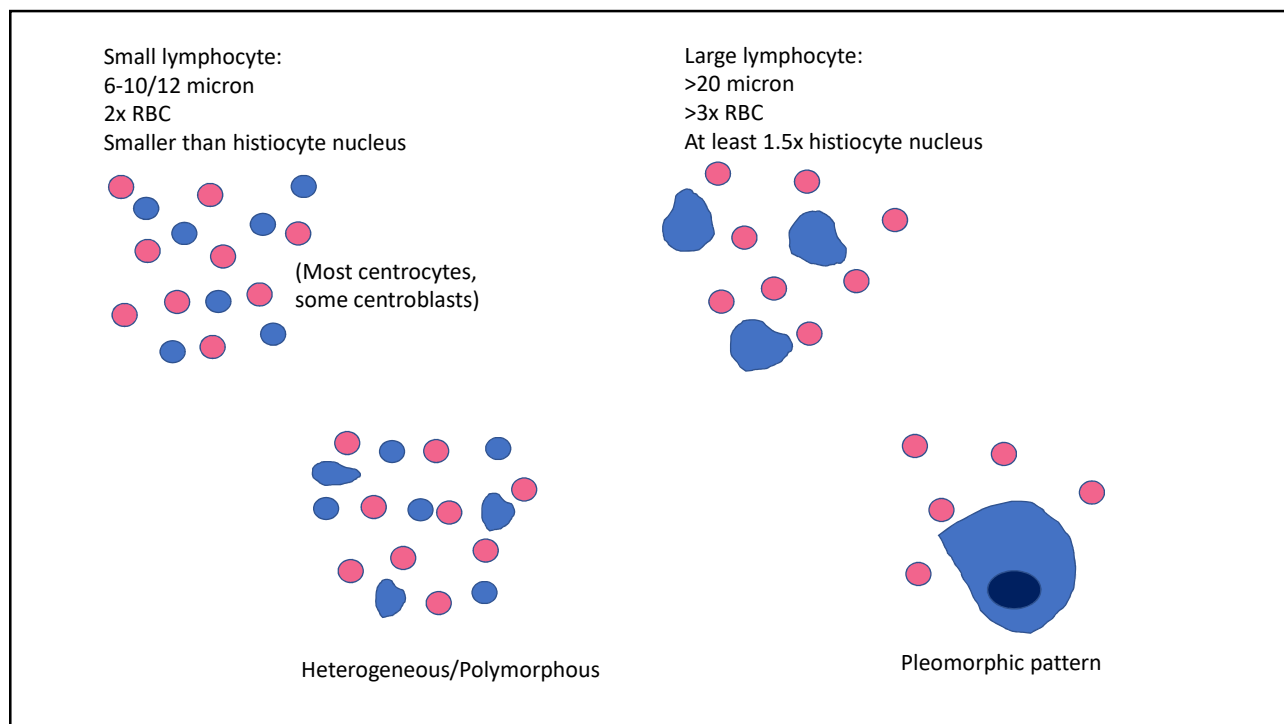


Sasaki et al. Diag Cytopathol. 2021.



Low-power morphologic assessment

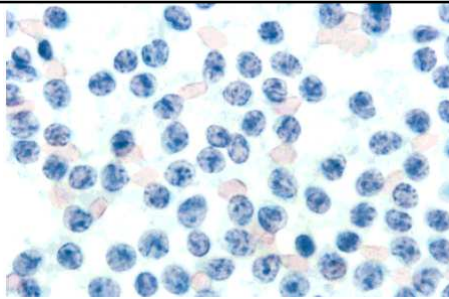
- 1. Are there cells to evaluate?
- 2. Are the cells lymphoid or non-lymphoid?
- 3. If lymphoid, identify the pattern:
 - Heterogeneous/polymorphous
 - Monotonous, small
 - Monotonous, medium
 - Monotonous, large
 - Pleomorphic



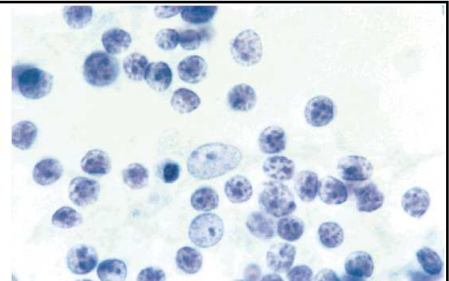
Medium-High power evaluation

- Nuclear size(s)
- Nuclear shapes (membrane irregularities)
- Amount of cytoplasm (N:C ratio)
- Chromatin quality (condensed, coarse, vesicular)
- Be aware: relative nuclear size can vary based on stain used, extent of drying artifact
- Crush artifact and thick smears present additional challenges

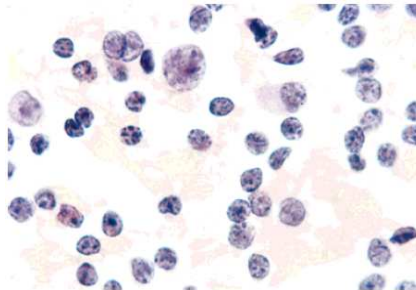
Subtyping lymphoma by cytomorphology back in the 90s...



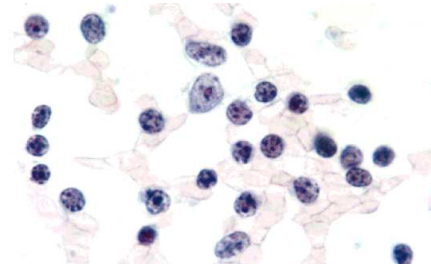
Mantle cell. Monomorphic, irregular, small/med, "clumped" chromatin.



Follicular. Mixed small and large cleaved; non-cleaved with large nucleoli.



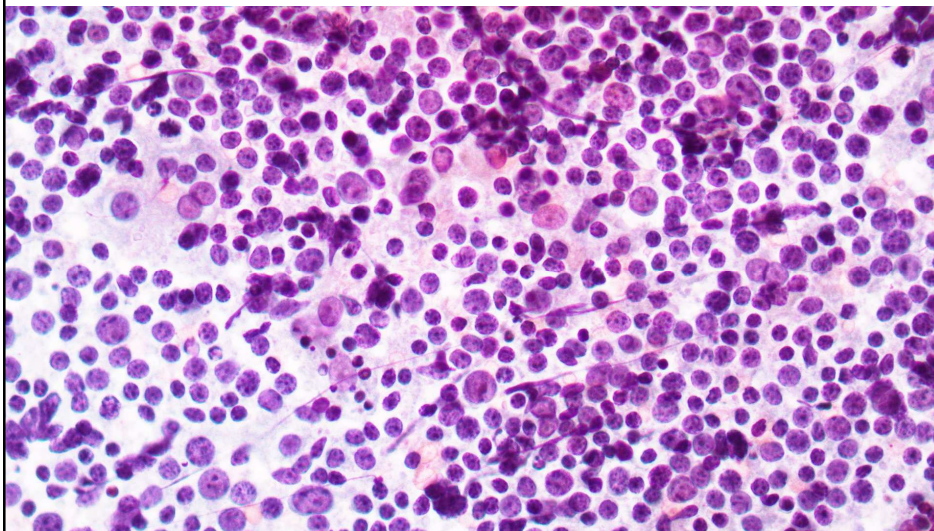
MALT/MZ. Small, round; scattered large cells with nucleoli.



SLL. Small, round, "clumped" chromatin; occasional polymorphous, paraimmunoblasts.

Diagnosis of Lymphoma by Fine-Needle Aspiration Cytology Using the Revised European-American Classification of Lymphoid Neoplasms. *Cancer Cytopathol.* 1999;87:325-45.

Case 1. Axillary node, 45yo man, HIV+



What pattern?

Heterogeneous/polymorphous

Monotonous, small
Monotonous, medium
Monotonous, large

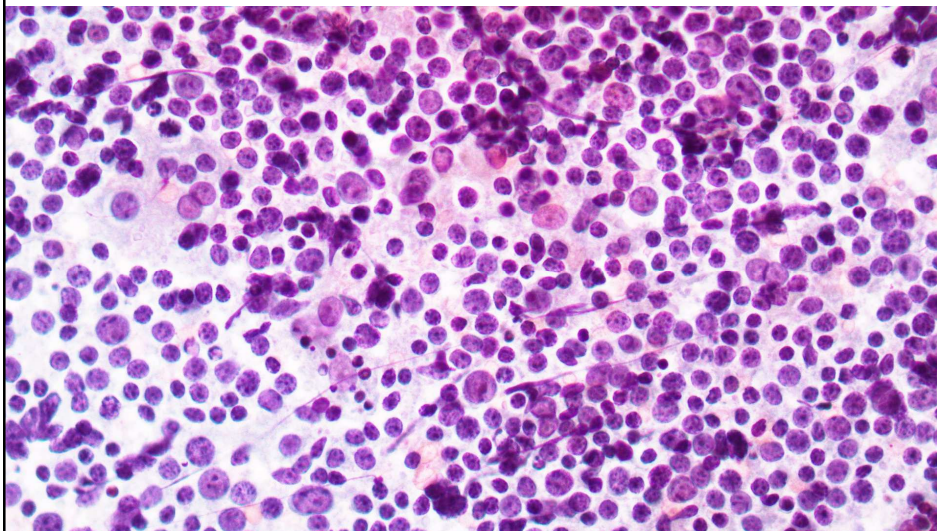
Pleomorphic

Case 1. Heterogeneous/Polymorphous pattern

DDX:

- Reactive lymphoid hyperplasia (non-specific)
- Infection
- Small B cell lymphomas (e.g. follicular, mantle, marginal zone)

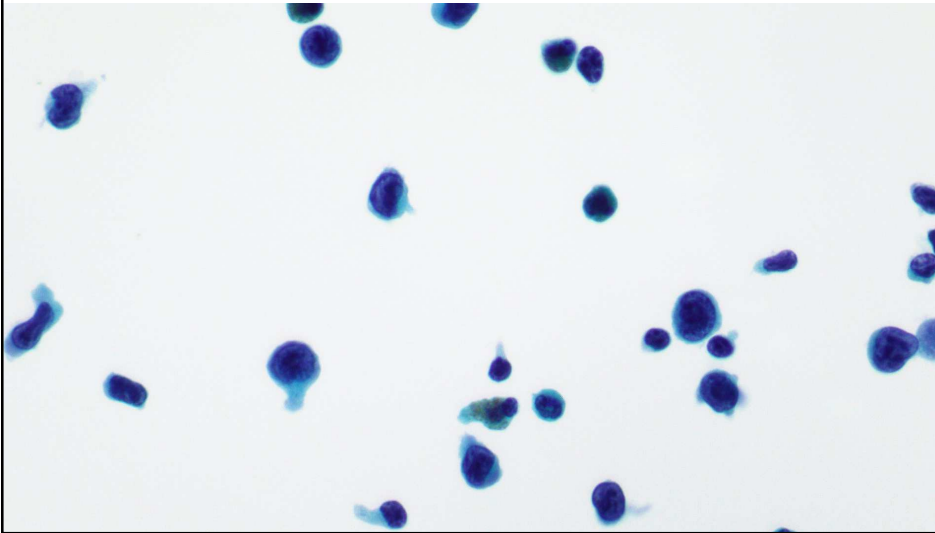
Case 1. Axillary node, 45yo man, HIV+



Diagnosis: Reactive hyperplasia

Flow cytometry negative

Case 2. Axillary node, 80 yo woman



What pattern?

Heterogeneous/polymorphous

Monotonous, small

Monotonous, medium

Monotonous, large

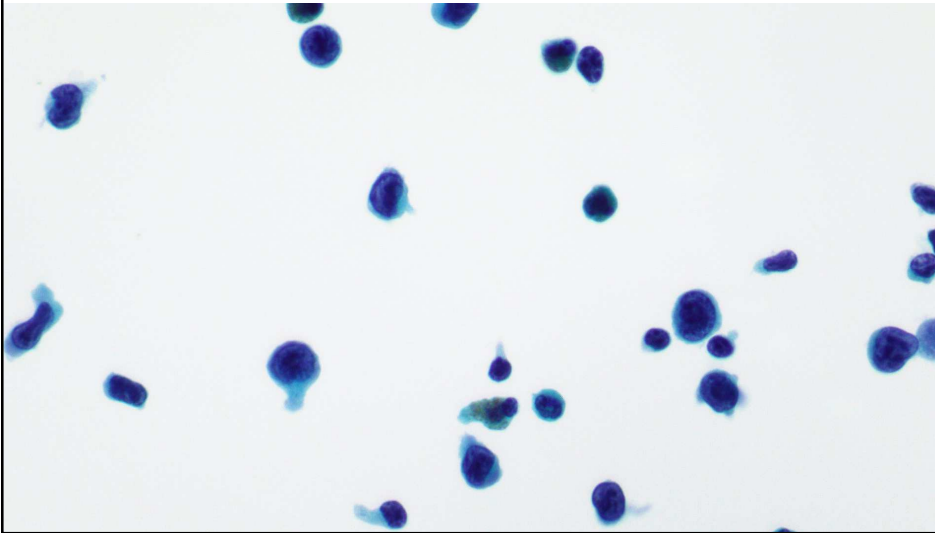
Pleomorphic

Case 2. Monotonous, Large cell pattern

DDX:

- DLBCL
- Grade 3 follicular lymphoma
- Transformation of low grade B lymphomas
- T cell lymphomas
- Blastoid mantle cell lymphoma
- Non-lymphoid metastases

Case 2. Axillary node, 80 yo woman



Diagnosis: Peripheral T cell lymphoma

Aberrant population (15%) on flow cytometry:

CD2+/CD3+/CD4+/CD5+
CD7-/CD8-/CD56-

IHC on subsequent excision:

CD4+ (weak)

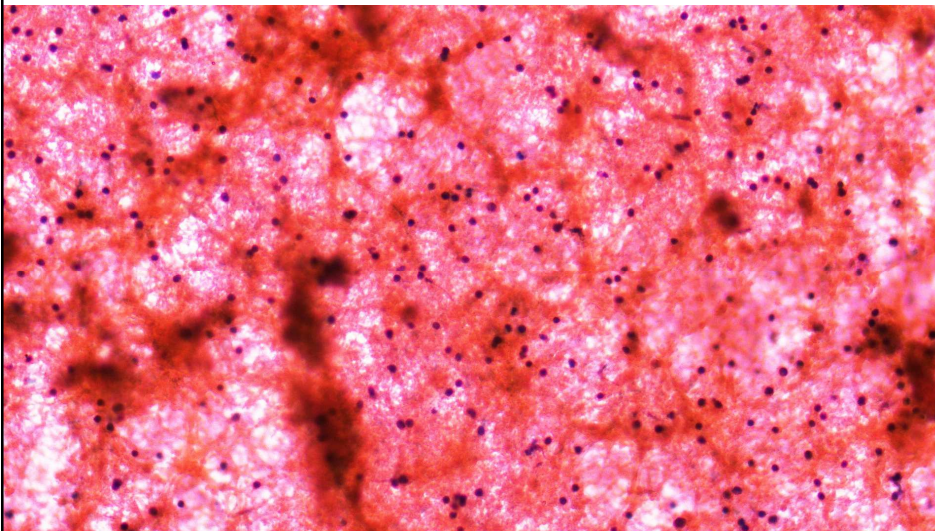
CD25+

CD30+

MUM1+

PAX5-/ALK-/CD15-/EMA-
/perforin-

Case 3. Retroperitoneum, 62 yo man



What pattern?

Heterogeneous/polymorphous

Monotonous, small

Monotonous, medium

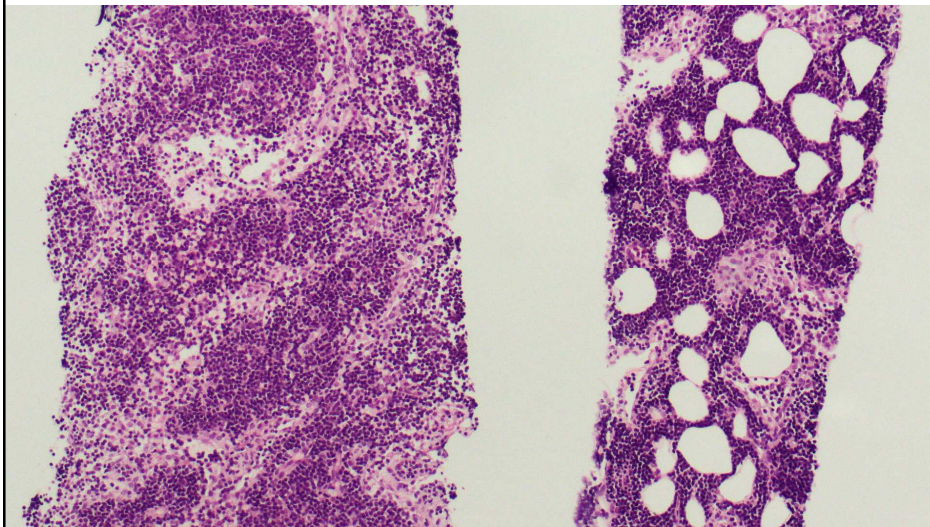
Monotonous, large

Pleomorphic

Case 3. Monotonous, small cell pattern

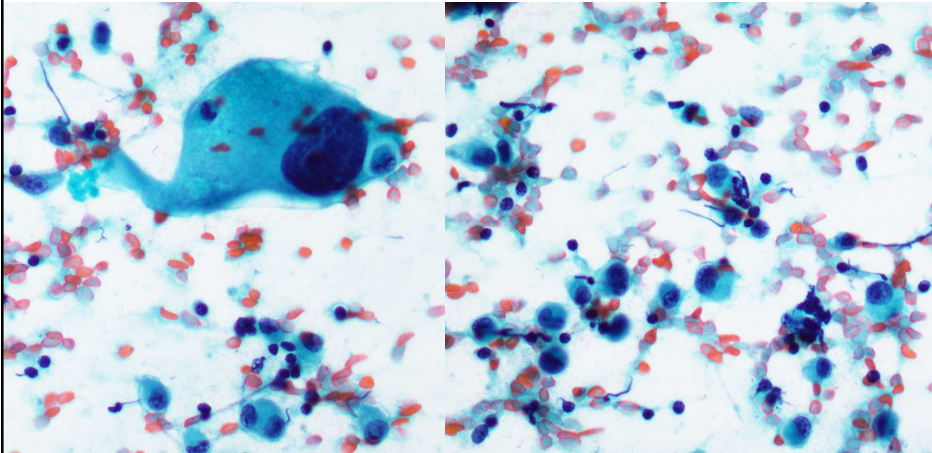
- Reactive lymphoid hyperplasia
- Small B cell lymphomas (e.g. SLL/CLL, follicular, mantle cell, marginal zone)
- Small cell/neuroendocrine metastases

Case 3. Retroperitoneum, 62 yo man



Follicular lymphoma, grade 1

Case 4. Axillary node, 45yo man



What pattern?

Heterogeneous/polymorphous

Monotonous, small
Monotonous, medium
Monotonous, large

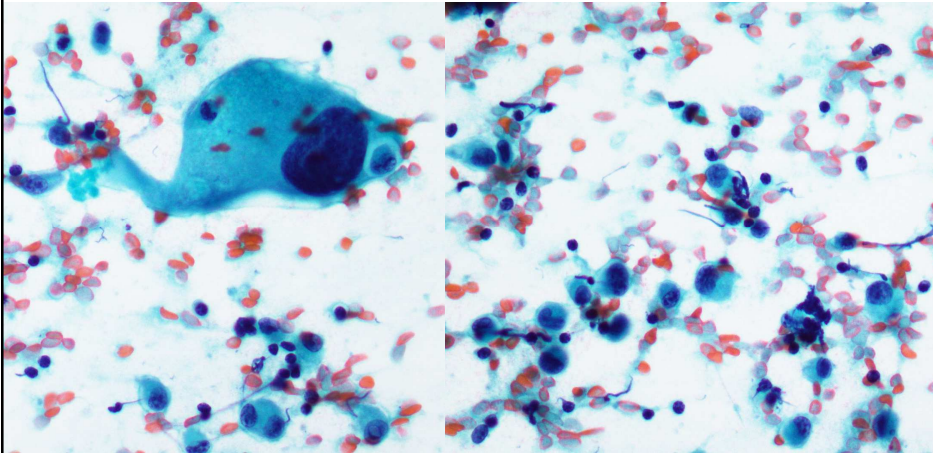
Pleomorphic

Case 4. Pleomorphic pattern

DDX:

- Hodgkin lymphoma
- Anaplastic large cell lymphoma
- DLBCL
- Transformation of low grade B lymphoma
- T cell lymphomas
- Metastatic malignancies

Case 4. Axillary node, 45yo man



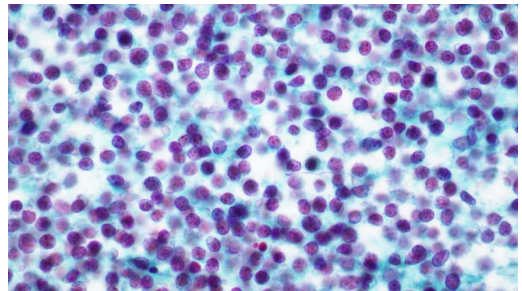
Diagnosis:
Melanoma

Tailor IHC panel to morphologic DDX

- Small B-cell lymphomas
- Hodgkin lymphoma
- Large cell lymphomas
- T-cell lymphomas

“Small B-cell lymphomas” suggested panel

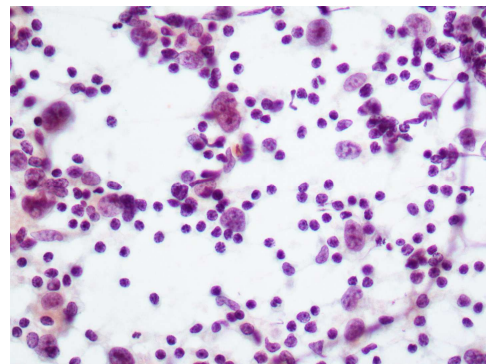
CD3	CLL/SLL
CD20	Follicular lymphoma
CD5	Mantle cell lymphoma
Cyclin D1	Marginal zone lymphoma
Sox11	Lymphoplasmacytic lymphoma
BCL2	
BCL6	
Ki67	
LEF1	



Mantle cell lymphoma

“Hodgkin” suggested panel

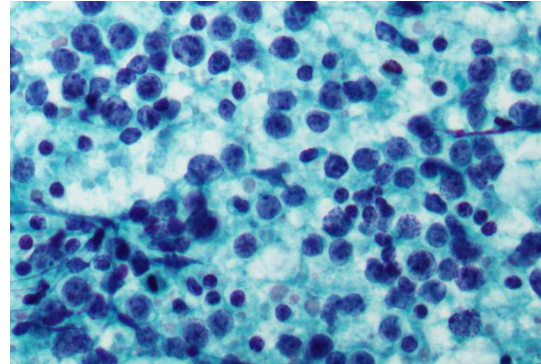
CD3	Classic Hodgkin lymphoma
CD20	NLPHL
CD30	T cell/histiocyte-rich large B cell lymphoma
CD15	Reactive node (e.g. with many immunoblasts)
EBER	
PAX5	



Hodgkin lymphoma

“Large cell lymphomas” suggested panel

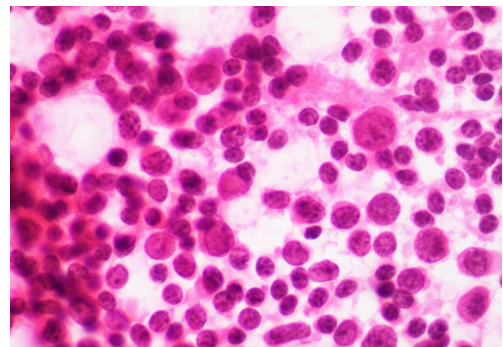
CD3, CD5	DLBCL
CD10, CD20	High grade B cell lymphoma
Cyclin D1, MUM1	Burkitt lymphoma
BCL2, BCL6	
CD30, EBER	
Ki67, C-MYC, p53	



DLBCL

“T cell lymphomas” suggested panel

CD2, CD3, CD4, CD5, CD7, CD8	Peripheral T cell lymphoma, NOS
CD25, CD30, CD56	ALCL (+/- ALK subtypes)
CD10, BCL2, BCL6	Reactive hyperplasia
CD21/CD23	Nodal T-follicular helper cell lymphomas (e.g. AITL)
Ki67, ALK, EBER, Ki67, PD-1	
Perforin, granzyme B	
TCRs (gamma, delta)	



Peripheral T cell lymphoma, NOS

Thank you