A Potential Pitfall in Low-grade Lymphoma

Head and neck FNAC

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Clinical information

- 46-year-old female
- 8-week history of non-fluctuating left mandibular triangle swelling
- No other symptoms
- Neck US showed 3 abnormal left level 1B lymph nodes, largest 2.1x1.5 cm and another abnormal node within the left submandibular gland – Regarded as “slightly atypical nodes”
- FNA (with ROSE) was taken from the largest node

ROSE triage for lymphocytic aspirates

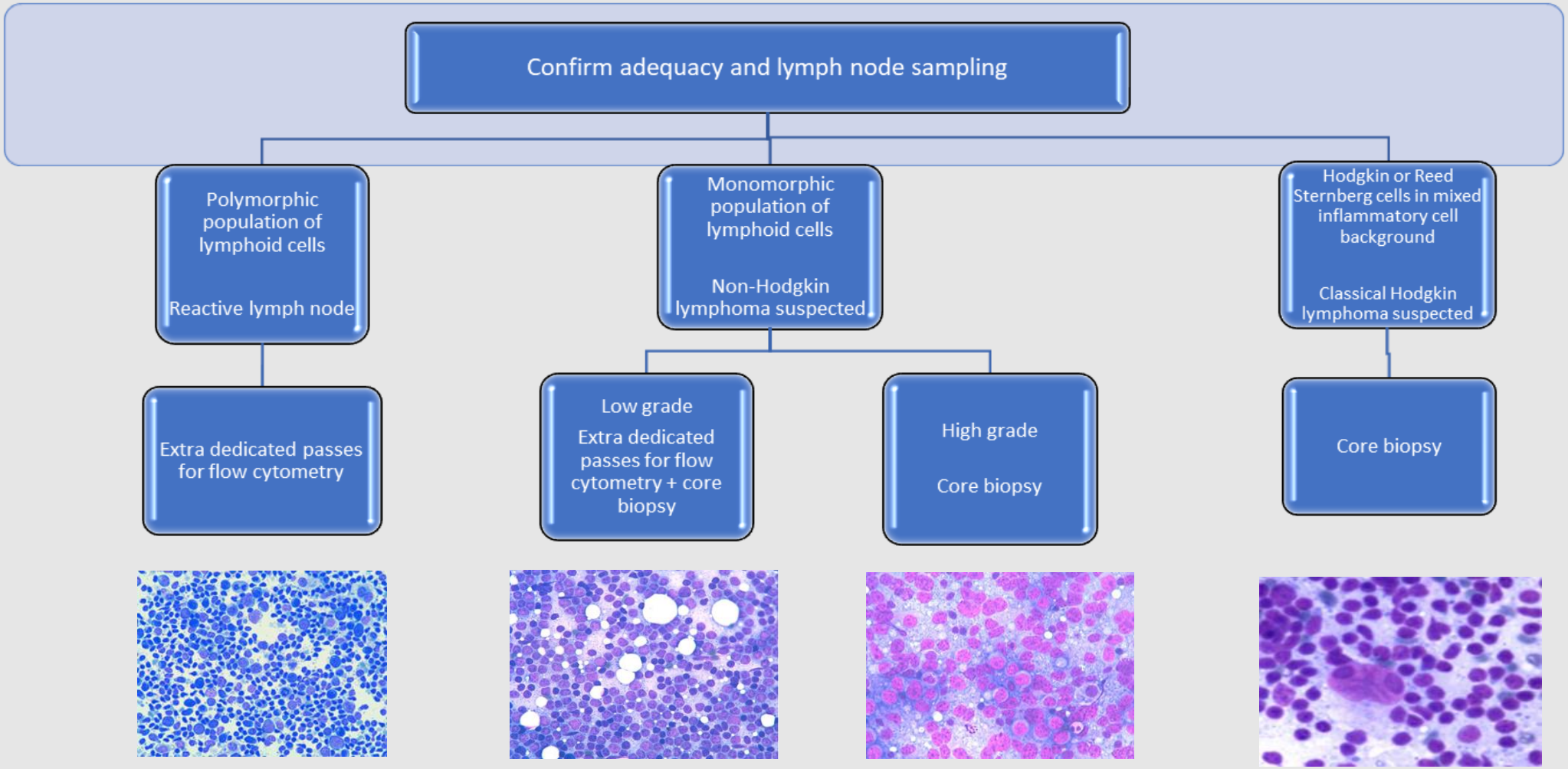
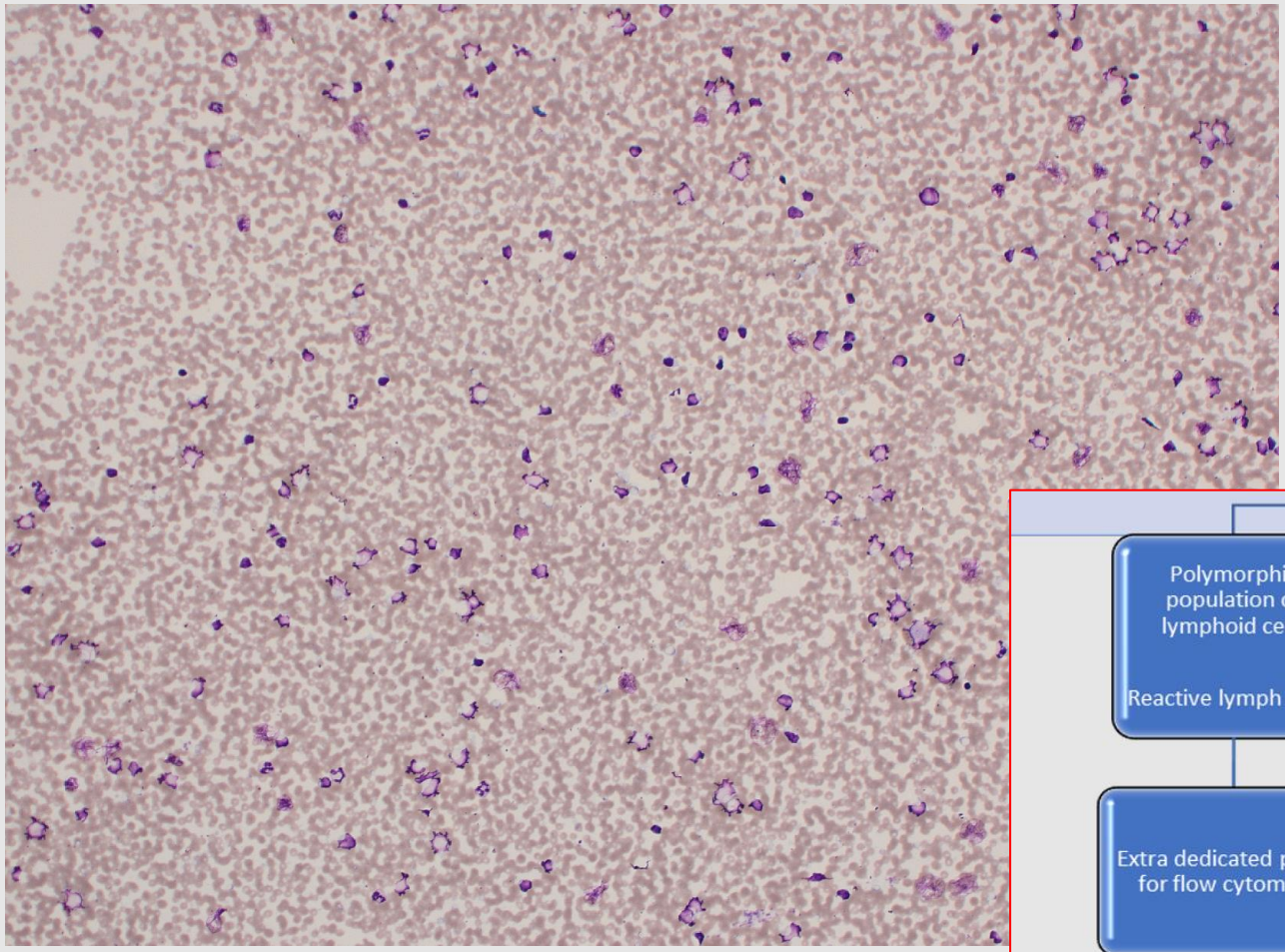
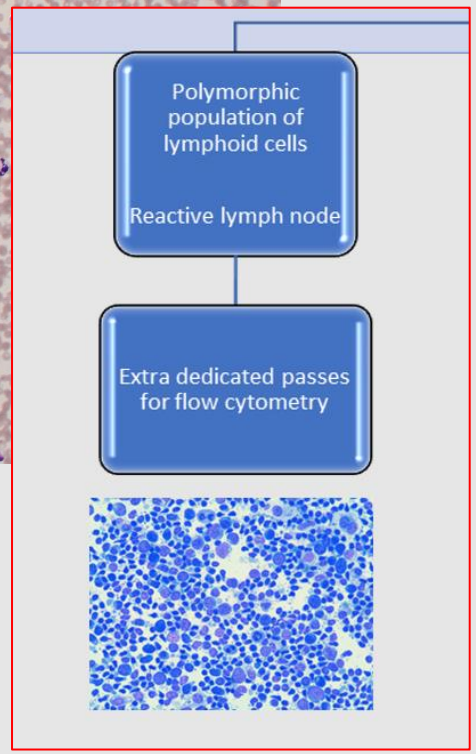


Image reference eurocytology.eu

ROSE Triage

- Adequate sample
- Nodal sampling confirmed
- Lymphocytes of variable sizes (mixed population)



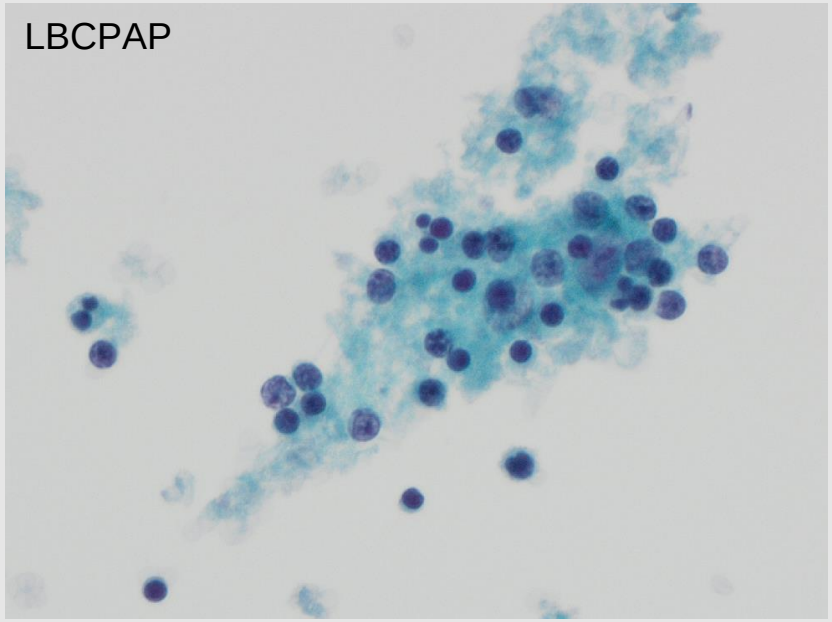


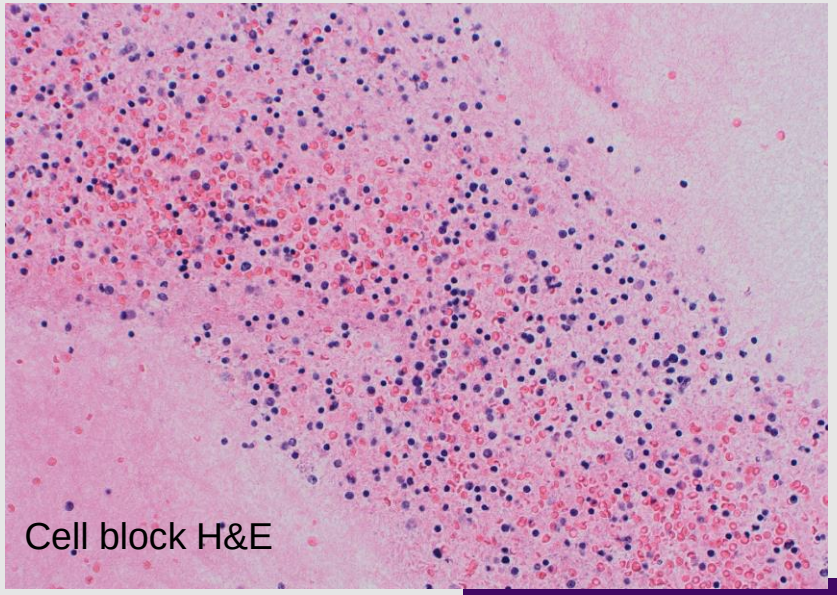
Following triage guidance, further passes were taken for flow cytometry

Natural clot obtained

Cytology

- Cellular aspirate
- Mixed population of lymphocytes
- Scattered tingible body macrophages
- No evidence of metastatic carcinoma / melanoma
- No evidence of high-grade lymphoma





Flow Cytometry – Initial Report

- 11% of lymphocytes are B cells with polyclonal light chain expression.
- 34% of lymphocytes are T cells with a CD4:CD8 ratio of 3.8.
- 47% of lymphocytes are **NK cells** expressing CD4+, CD8-,CD56+.

⇒The predominant subset was interpreted as an unusual NK cell phenotype due to the lack of expression of CD19 and CD3.

Conclusion: Non-diagnostic phenotype

(Non-diagnostic phenotype in flow cytometry is used when the significance of the results is unclear and there is caution about over interpretation. Could be a reactive phenomenon but lymphoproliferative disorder cannot be ruled out)

Conclusion.. So far…

Cytology - consistent with a reactive lymph node / low grade lymphoma cannot be excluded

Flow cytometry – non-diagnostic

But..

Does the diagnosis corroborate with the clinical and radiological picture?

MDT decided to perform an MRI

Further investigation

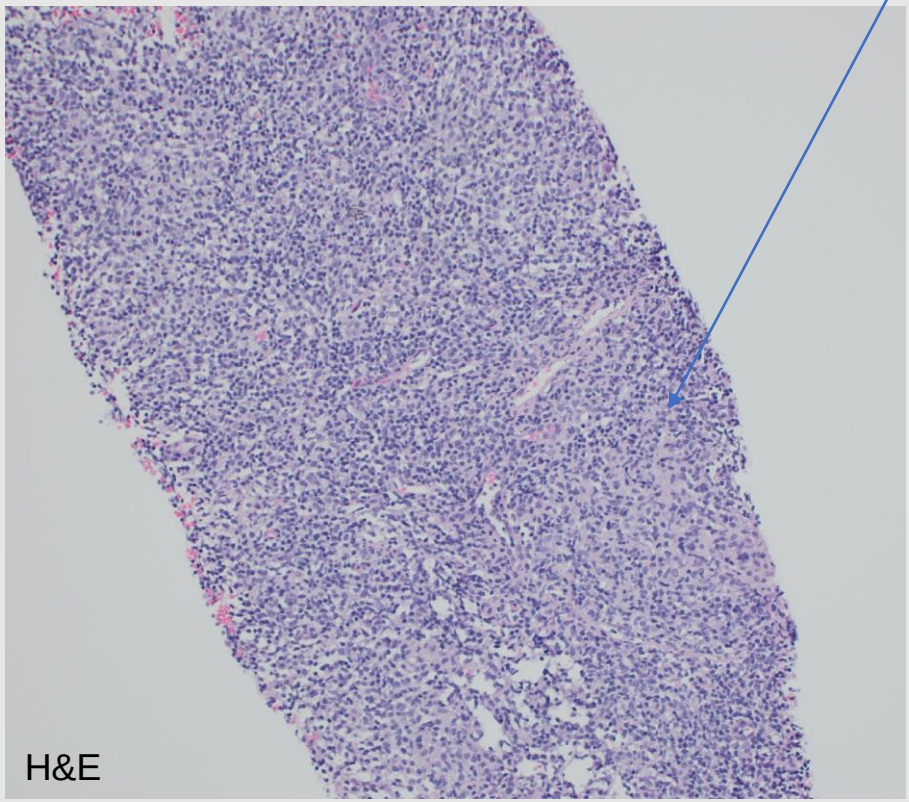
- Neck MRI revealed numerous enlarged lymph nodes involving left levels IB-IV and the superficial lobe of the left parotid most consistent with lymphoma or malignant spread from unknown primary.

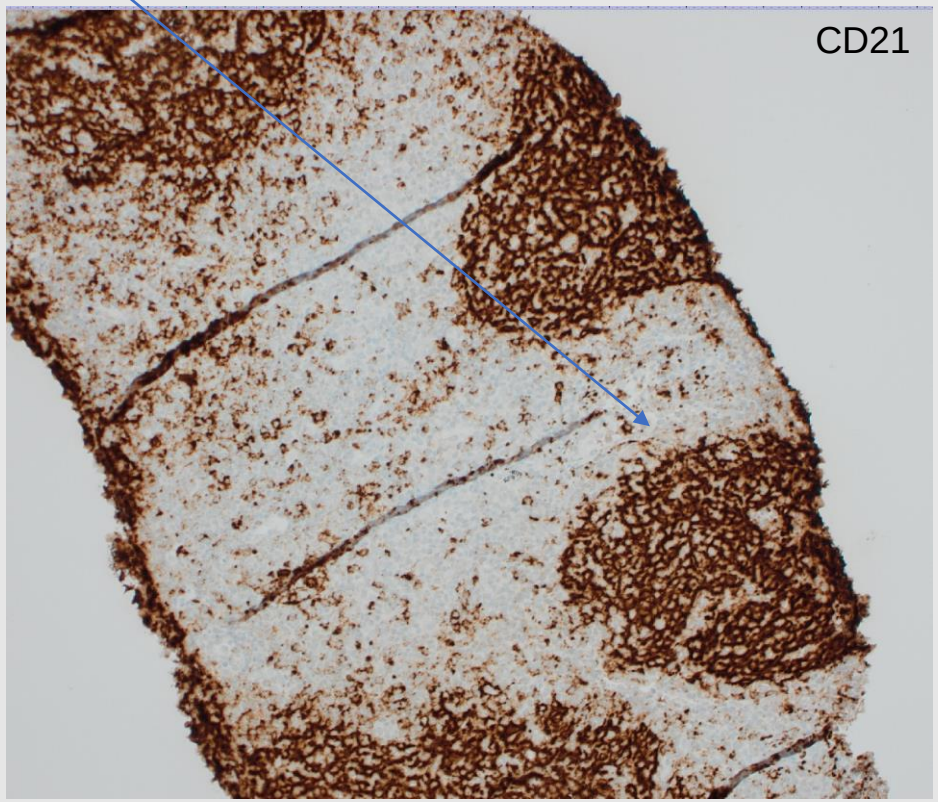
⇒ Core biopsy was undertaken considering the high suspicion

Histology Findings

CD21 highlighting the neoplastic follicles

Low grade follicular lymphoma – grade 1





Also positive for CD20, CD79a, CD10, BCL6, BCL2, PAX5. Negative for CD5.

Flow Cytometry – Amended Report

Feedback prompted a flow cytometry team review which concluded

- Atypical case due down regulation of CD19 in the B cells.

Considering the histological diagnosis of low-grade follicular lymphoma, the original population categorised as NK cell population due to lack of CD19 and CD3 expression) was reclassified as a clonal B cells population.

Flow cytometry lymphoid screening tube

1st identifies the major lymphocyte subsets with dedicated fluorochromes

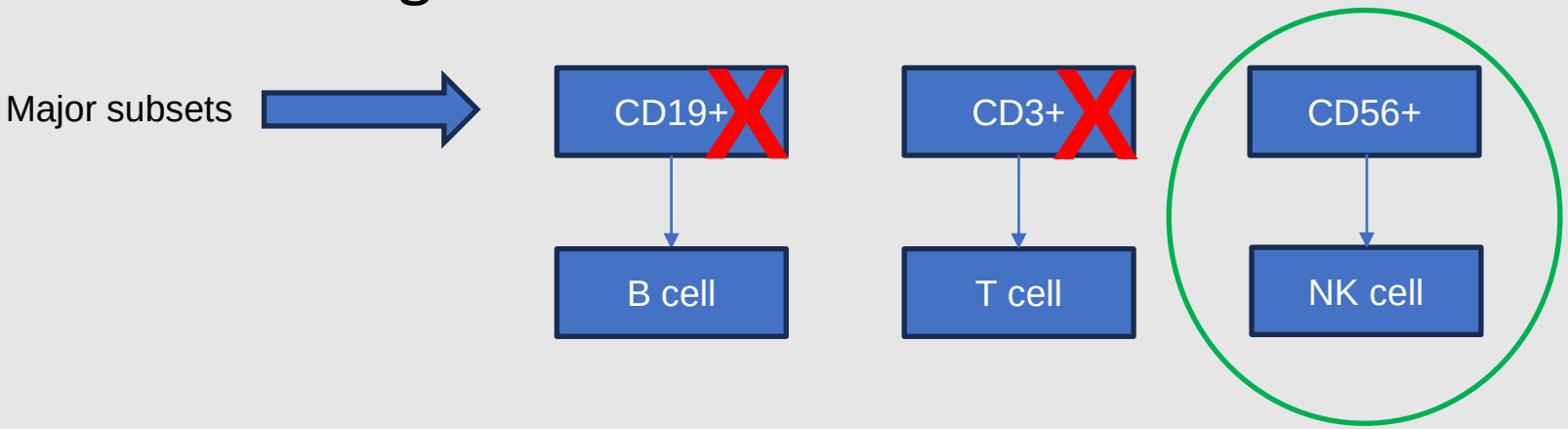
- B cells identified by CD19+
- T cell identified by CD3+
- NK cells CD56+

Once B cells are identified they are then gated for other B cell markers (further characterisation) e.g. CD20.

Subtyping heavily relies on correct delineation of the major lymphocyte subsets because the 2nd markers share fluorochromes – CD20 & CD4

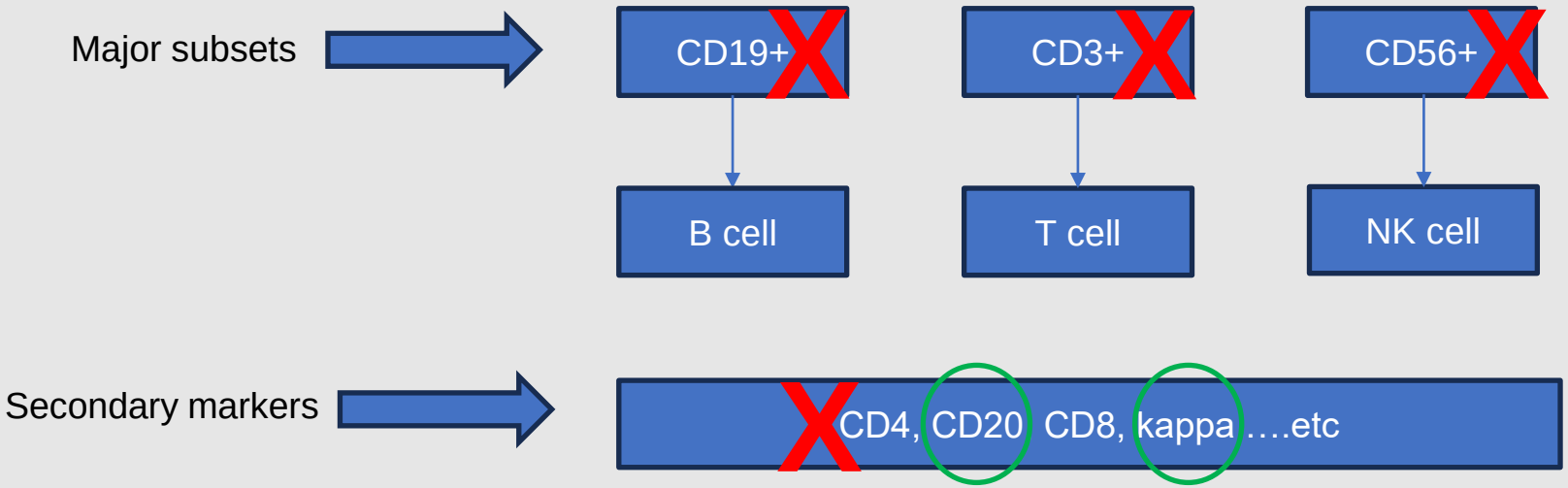
Flow lymphoid screening tube

- Dim CD19 expression is more common. Negative expression for CD19 is rare
- In this case the B cells were negative for CD19 expression. This affected the major lymphocyte subtyping in flow cytometry.
- CD3 was also negative



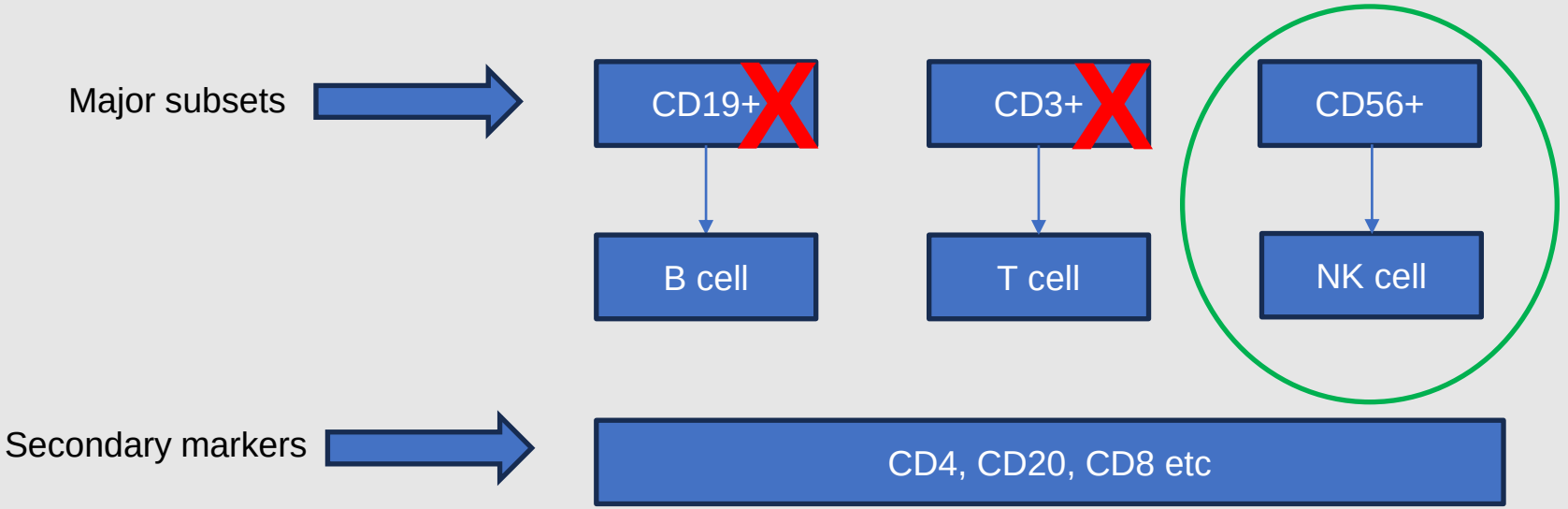
Flow lymphoid screening tube

- Misinterpreted as CD4+ and CD56 + NK cells
- On review the CD4+ was actually dim CD20 (CD4 and CD20 share the same fluorochrome) and CD56 + was actually kappa.



Flow cytometry intepretation

- **Key learning point: CD4+ + CD8- NK cell population should raise suspicion of a misclassified rare CD19- B cell clone**



FNAC and Low-grade Lymphoma

- Diagnosing low-grade non-Hodgkin lymphoma cytologically poses a challenge due to the overlapping features with benign reactive lymphadenopathy.
- Incorporating flow cytometry has notably enhanced the diagnostic accuracy combining the cytological features and specific phenotypic profiles.
- Adopting the current ROSE triage approach with early flow cytometric analysis of lymphocytic aspirates improves turnaround times.
- However, interpretation of the results within the clinical and radiological context is essential prevent misdiagnosis.

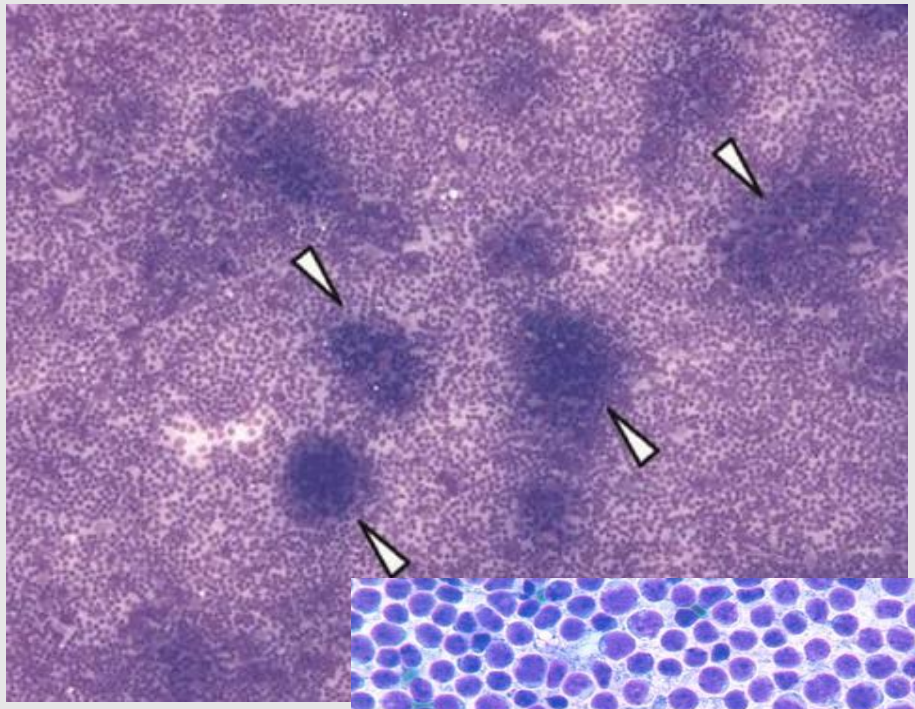
Follicular Lymphoma

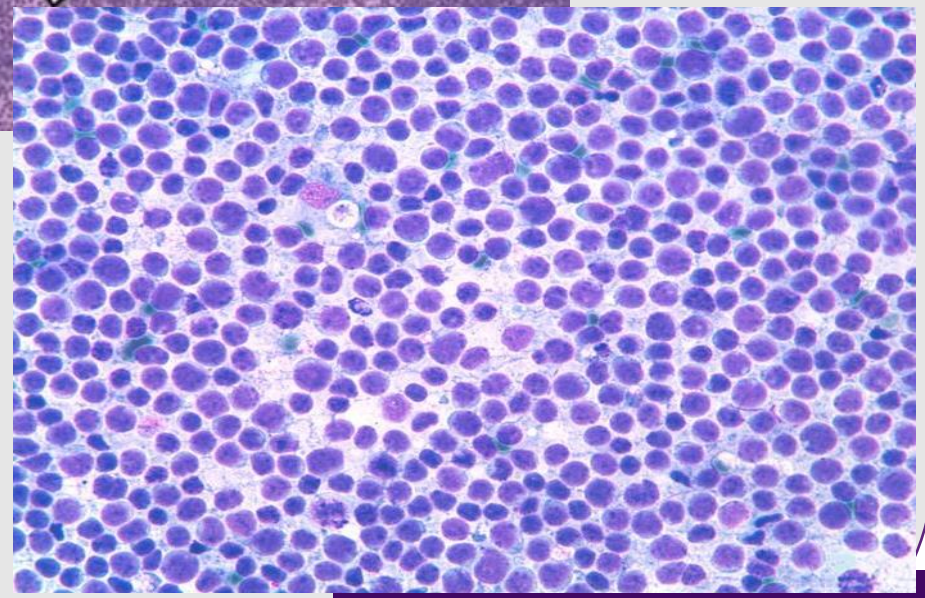
- 30% of all lymphoma cases
- B cell lymphoma
- High incidence in Caucasians when compared to Asian and African ethnicities
- Affects adults and elderly, rarely under age 20 years
- Characteristically presents with generalised painless lymphadenopathy. Symptoms may include; fatigue, fever or night sweats, weight loss or recurrent infections – however most patients have no obvious symptoms of the disease
- Known association with translocation (14;18)(q32;q21)

Follicular Lymphoma - Cytology

Low power:

- Characterised by a monotonous and dimorphic pattern, respectively.
- The dimorphic feature can be initially misinterpreted as the mixed/polymorphous pattern seen in reactive lymph nodes.
- Smears may give an impression of nodular pattern (arrow heads)



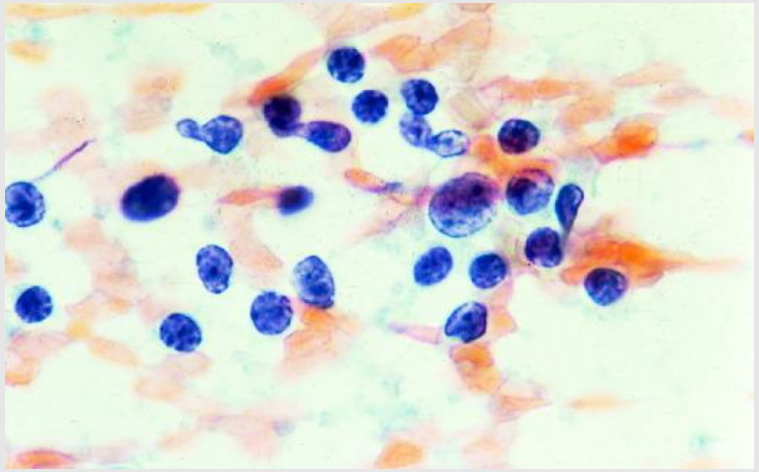


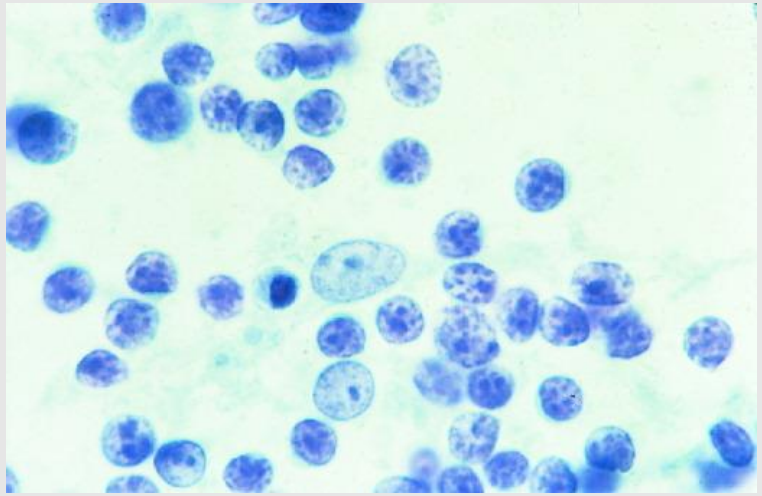
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3368210/
Eurocytology.eu

Follicular Lymphoma - Cytology

High power:

- Small-intermediate size centrocytes with cleaved nuclei (typically larger than a mature lymphocyte) with clumped chromatin and irregular nuclear borders.
- Larger noncleaved centroblasts (occurring in higher frequency in grade 2 FL) with vesicular chromatin and small, occasionally multiple, nucleoli
- Occasional tingible body macrophages
- Infrequent mitoses
- Large dendritic cells in the background





https://acsjournals.onlinelibrary.wiley.com

Follicular lymphoma - Immunophenotype

Immunophenotyping of FL cell is typically positive for:

CD20, **CD19 (dim),**CD79a, CD10, BCL2 and BCL6 with variable expression of CD23, CD38, CD11c,CD43 and surface immunoglobulins.

T-cell markers including CD3 and CD5 and Cyclin-D1 are negative.

However, CD19 expression was not detected on flow in this atypical case

Discussion

- In this scenario, the combination of the patient's age and clinical presentation with multiple painless enlarged cervical lymph nodes prompts concern for a lymphoproliferative disorder.

- Limitations of cytology and flow cytometry were demonstrated. A comprehensive review of investigations, imaging and clinic letters was warranted and revealed inconsistencies.

- A collaborative MDT review confirmed the need to proceed with further imaging which led to a core tissue biopsy and ultimately to the final diagnosis of follicular lymphoma.

Key Learning Points

- Correlation with clinical and radiological information is paramount!
- Maintain a high level of suspicion is crucial when clinical and morphological/flow cytometric features do not align.
- Discussion of flow cytometry results with an immunologist can provide a better understanding, especially when the results are inconclusive or inconsistent with any of the differential diagnoses.
- Bring such cases up for discussion with clinicians and radiologists at MDMT ensures clear guidance on further investigations and management, exemplified by the decision to proceed with a core biopsy in this case.

References

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