
به نام خدا

Diagnostic Immunohistochemistry in Hematolymphoid Pathology
A Pattern-Based, Morphology-Driven Approach

PART 1

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Learning Objectives

- By the end of this session participants should be able to apply a **structured diagnostic strategy** when approaching hematolymphoid lesions.
- Use **morphologic patterns** to guide the selection of immunohistochemical panels
- Recognize **normal lymph node architecture** and its immunophenotypic correlates
- Interpret IHC results in the context of **cell distribution, staining intensity, and internal controls**
- Distinguish **reactive lymphoid proliferations from lymphoid neoplasms**
- Recognize **common immunophenotypic pitfalls and aberrant antigen expression**

Rational marker selection is more important than ordering large antibody panels.

Role of Immunohistochemistry in Hematolymphoid Diagnosis

- Immunohistochemistry provides **essential phenotypic information** that complements **morphologic interpretation.**

Key diagnostic questions addressed by IHC:

- **Lineage determination:** Is the proliferation derived from B-cells, T-cells, NK-cells, or myeloid precursors?
- **Stage of differentiation**: Does the immunophenotype reflect precursor, germinal center, post-germinal center, or activated lymphoid cells?
- **Disease classification**: Can the immunophenotypic profile support classification into a specific lymphoma or leukemia subtype?
- **Biologic characterization:** Certain markers provide information regarding tumor biology, proliferation rate, or therapeutic targets.

Immunohistochemistry refines diagnostic probability but rarely establishes a diagnosis in isolation

Fundamental Diagnostic Principle

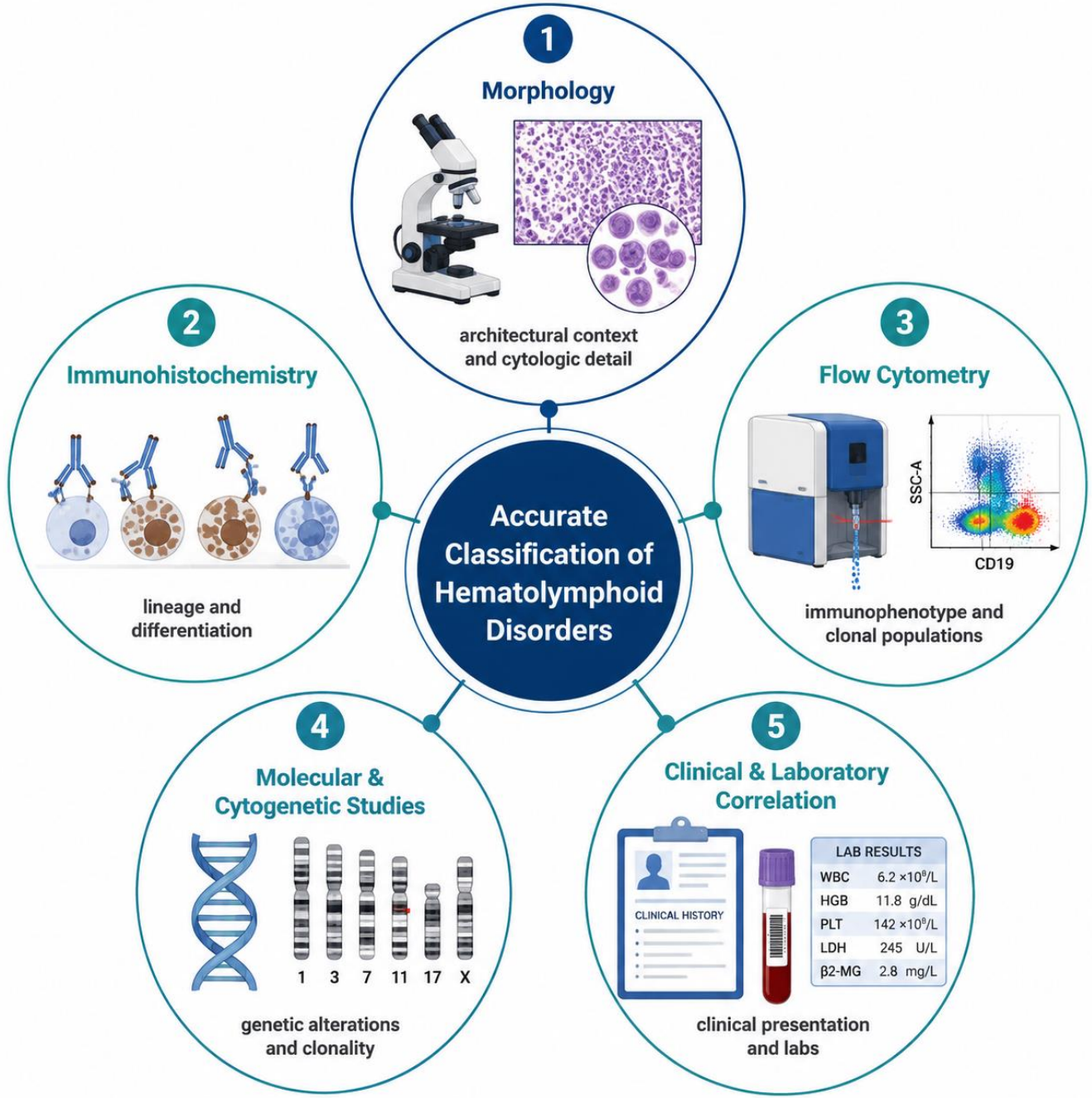
- **Morphology Remains the Foundation of Hematopathologic Diagnosis**
- The diagnostic process in lymphoid pathology follows a sequential reasoning pathway.
- **Step 1 — Morphologic assessment**
  - Evaluation of lymph node architecture, cytologic features, and distribution of the infiltrate on H&E sections.
- **Step 2 — Pattern recognition**
  - Identification of major morphologic patterns such as follicular, paracortical, diffuse small-cell, diffuse large-cell, or Hodgkin-like patterns.
- **Step 3 — Targeted immunohistochemistry**
  - Selection of a focused antibody panel based on the morphologic differential diagnosis.
- **Step 4 — Integrated interpretation**
  - Correlation of morphology, immunophenotype, molecular studies, and clinical findings to reach a final diagnosis.

The role of immunohistochemistry is to **clarify morphologic impressions rather than replace them**.

Integrated Approach in Modern Hematopathology

- Accurate classification of hematolymphoid disorders requires integration of multiple diagnostic modalities.
  - **Morphology**
    - Provides architectural context and cytologic detail.
  - **Immunohistochemistry**
    - Defines lineage, differentiation stage, and biologic phenotype.
  - **Flow cytometry**
    - Allows detailed immunophenotypic analysis and detection of clonal populations.
  - **Molecular and cytogenetic studies**
    - Identify clonality and disease-defining genetic alterations.
  - **Clinical and laboratory correlation**
    - Patient age, clinical presentation, laboratory findings, and disease distribution are critical for correct interpretation.

Modern hematopathology is **integration-driven rather than marker-driven**.

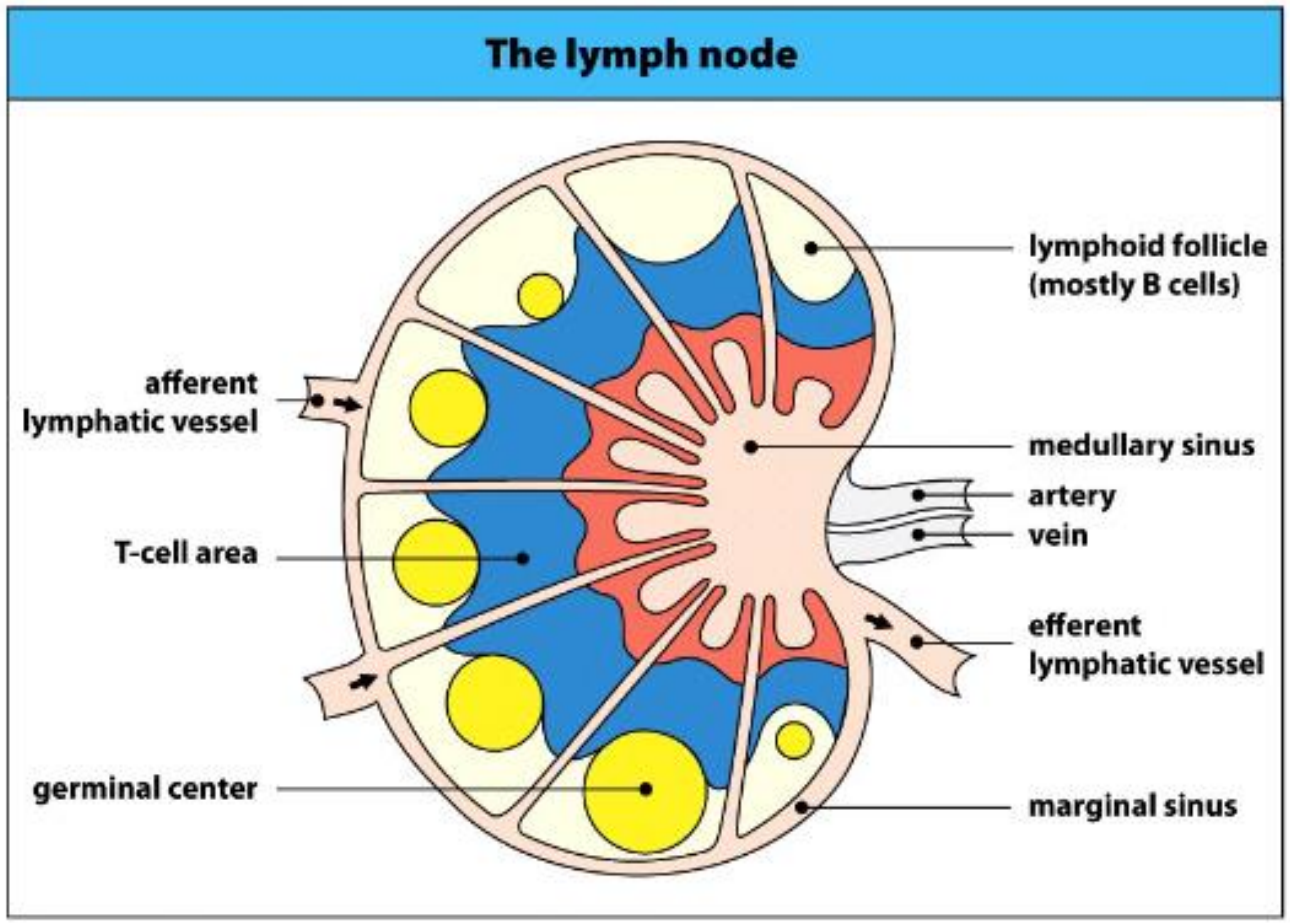

1
Morphology
architectural context
and cytologic detail
2
Immunohistochemistry
lineage and
differentiation
3
Flow Cytometry
SSC-A
CD19
immunophenotype and
clonal populations
Accurate
Classification of
Hematolymphoid
Disorders
4
Molecular &
Cytogenetic Studies
1 3 7 11 17 X
genetic alterations
and clonality
5
Clinical & Laboratory
Correlation
CLINICAL HISTORY
LAB RESULTS
WBC 6.2 ×10⁹/L
HGB 11.8 g/dL
PLT 142 ×10⁹/L
LDH 245 U/L
β2-MG 2.8 mg/L
clinical presentation
and labs

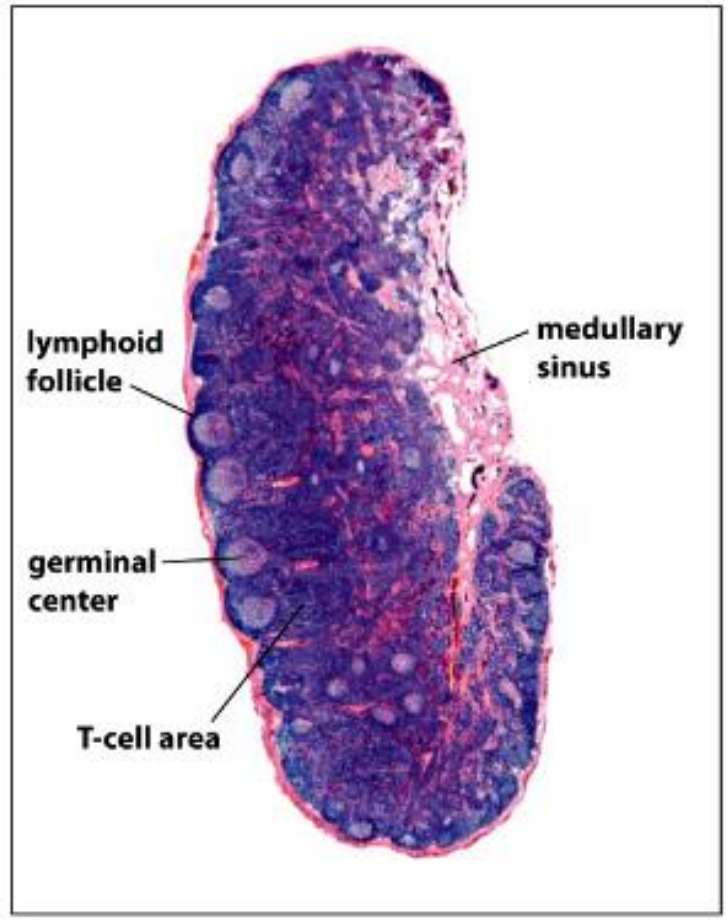
Normal Lymph Node Architecture

- Understanding **normal lymph node structure** is essential before interpreting pathologic lymphoid proliferations.
- Major anatomic compartments
  - Cortex: Contains lymphoid follicles composed predominantly of B-cells.
  - Paracortex: T-cell–rich zone where antigen presentation and T-cell activation occur.
  - Medulla: Contains plasma cells, lymphocytes, and macrophages within medullary cords and sinuses.
- Functional organization: A normal lymph node has an organized distribution of B-cells and T-cells, and recognizing this normal pattern helps pathologists detect abnormal lymphoid proliferations.

Recognition of preserved or distorted architecture is the first clue in distinguishing **reactive processes from lymphoma**.

Lymph node: Functional Histology





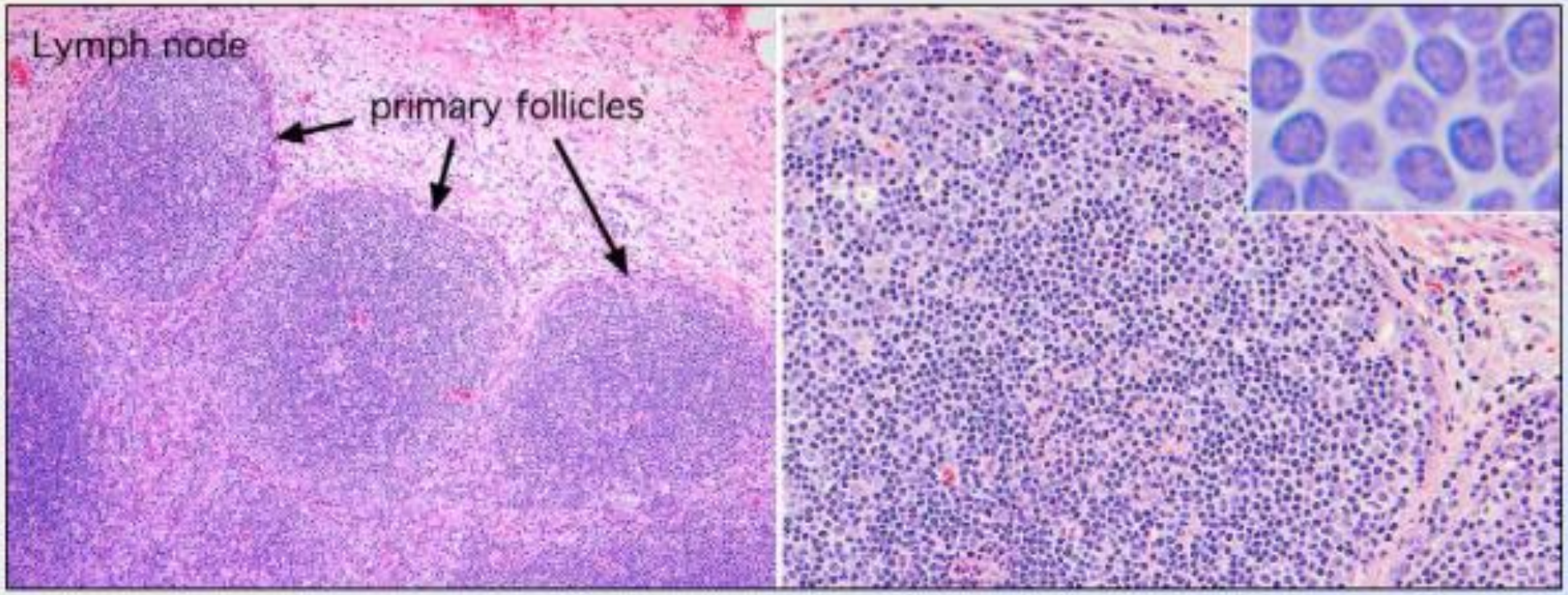
Immunophenotypic Correlates of Normal Lymph Node Compartments

Immunohistochemistry mirrors the functional organization of the lymph node.

- B-cell markers
  - CD20 : Highlights B-cells within follicles and mantle zones.
  - CD79a and PAX5: Additional markers confirming B-cell lineage.
- T-cell markers
  - CD3: Highlights T-cells predominantly in the paracortex.
  - CD5 and CD7: Support T-cell lineage and distribution.
- Practical implication
  - Evaluation of the **distribution pattern of B-cells and T-cells** is often more informative than the presence or absence of staining alone.

Normal lymphoid tissues

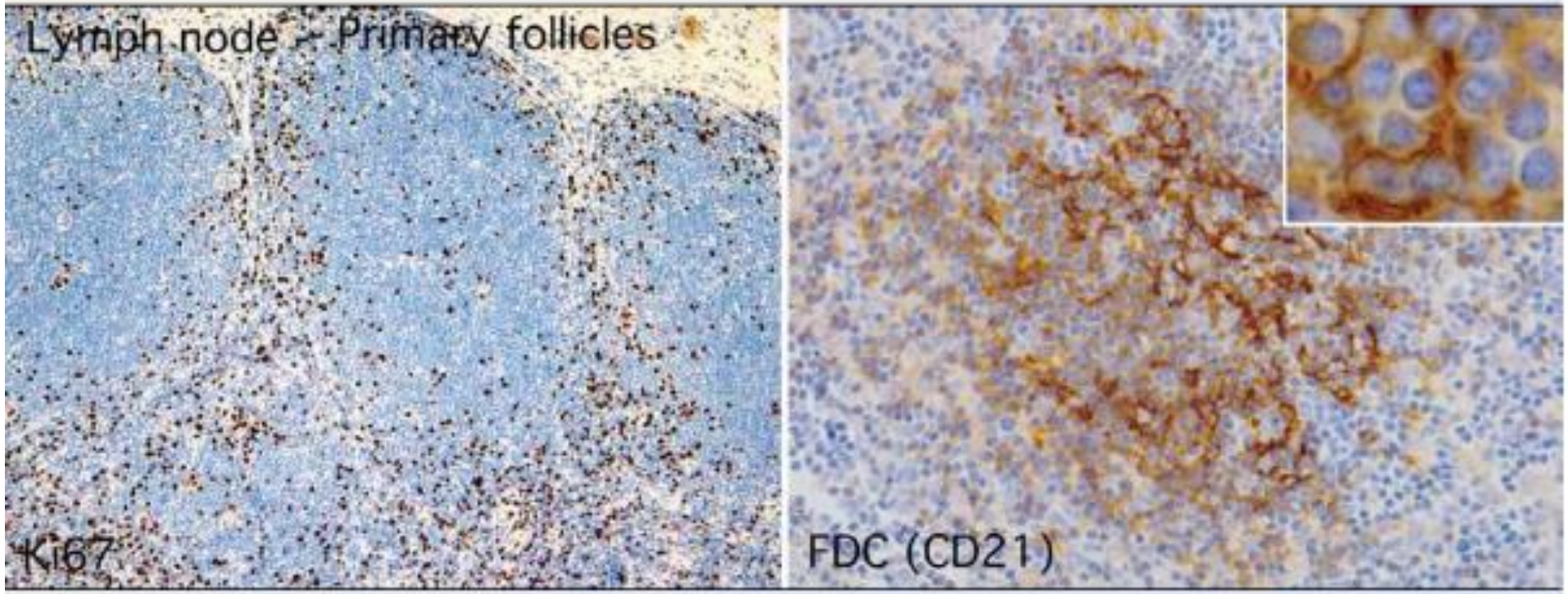
Histology of primary lymphoid follicle



Primary lymphoid follicles in the subcapsular region of an unstimulated lymph node (left). The follicles contain a monomorphous population of small naïve B lymphoid cells.

Normal lymphoid tissues

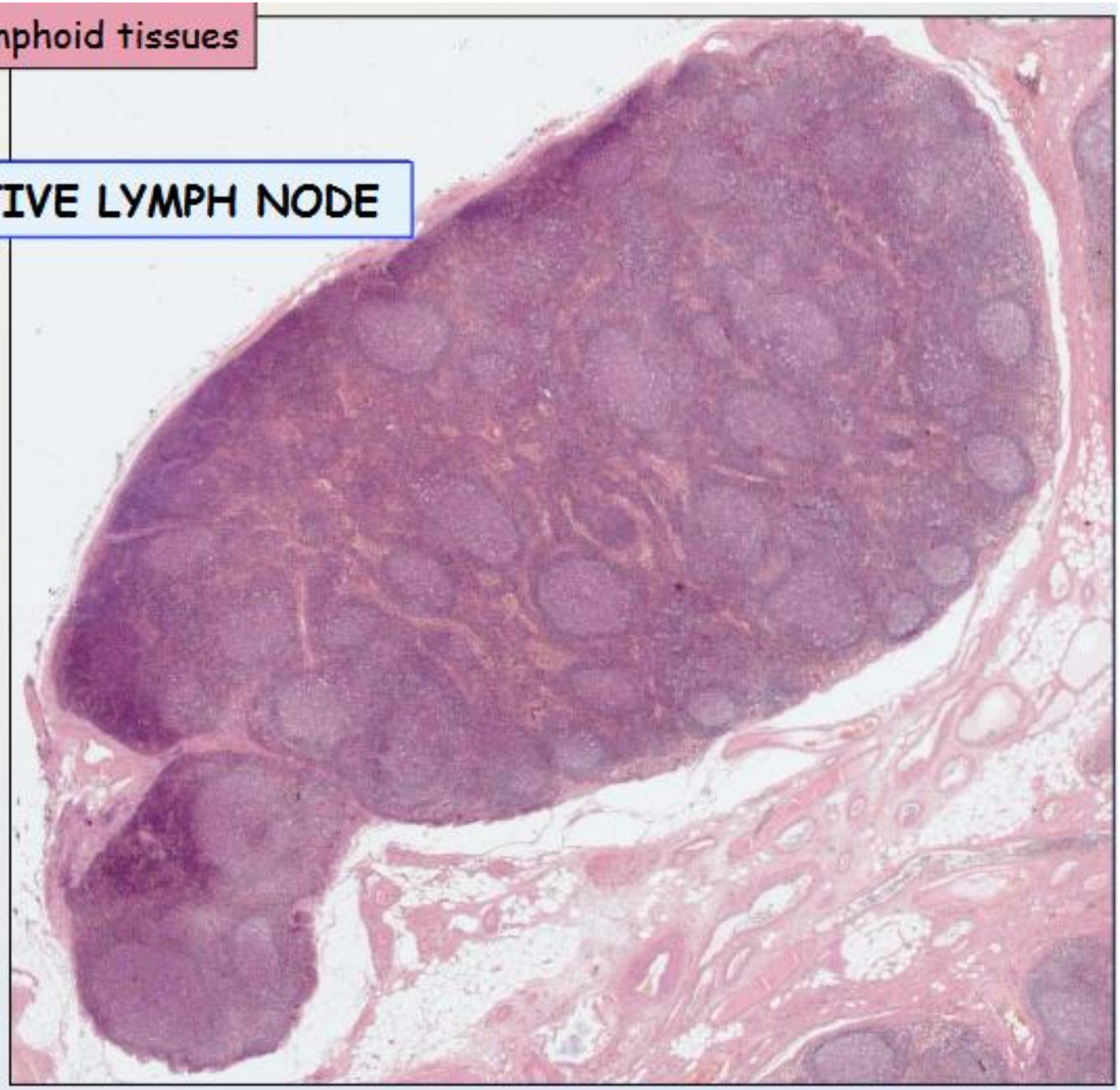
Histology of primary lymphoid follicle



Immunostaining of an unstimulated lymph node for the proliferation marker Ki67 (left) shows that very few proliferating cells in the primary follicleS. CD21 labelling highlights the follicular dendritic cell (FDC) meshwork with characteristic long dendritic processes between the lymphoid cells.

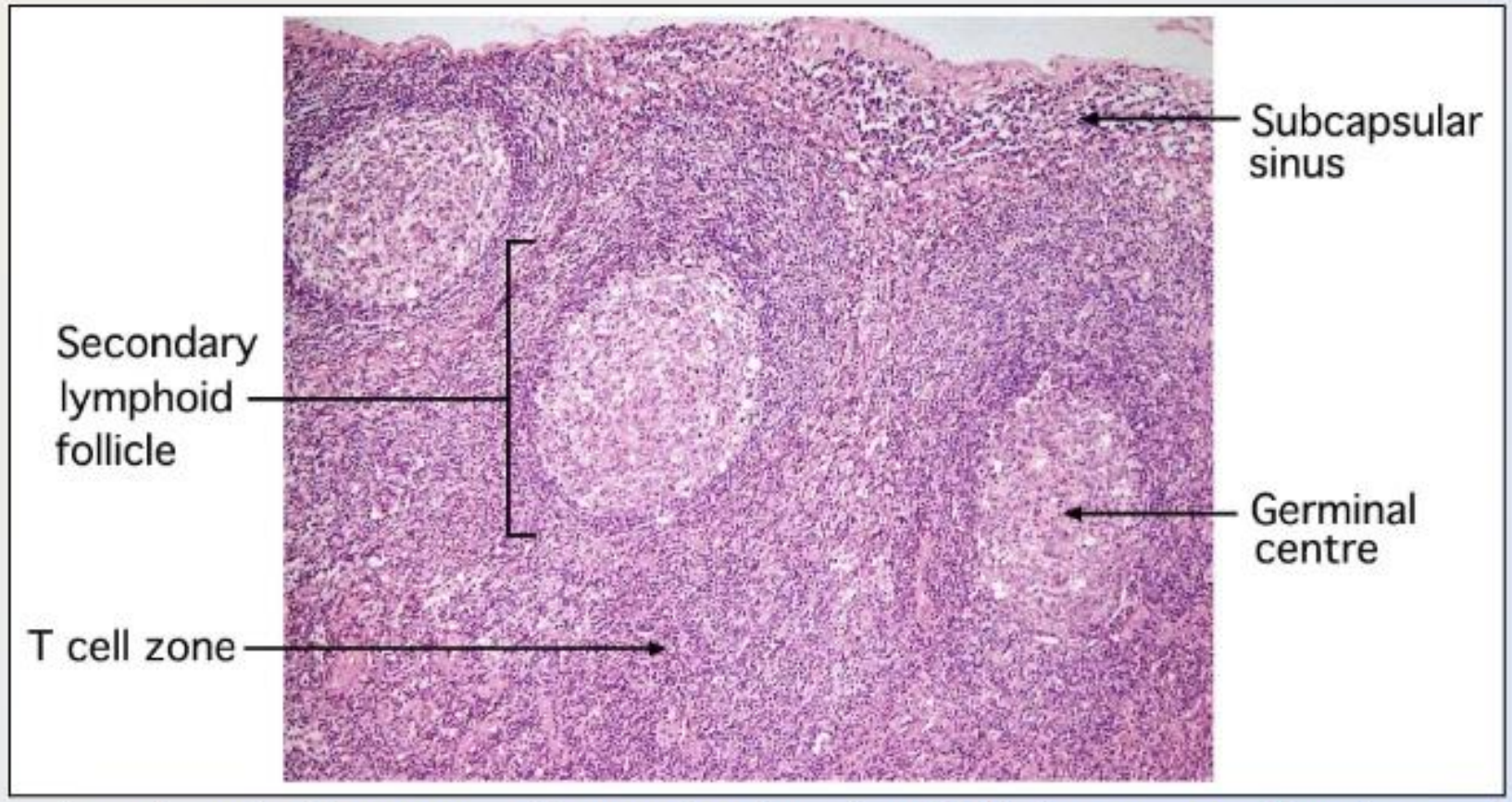
Normal lymphoid tissues

REACTIVE LYMPH NODE



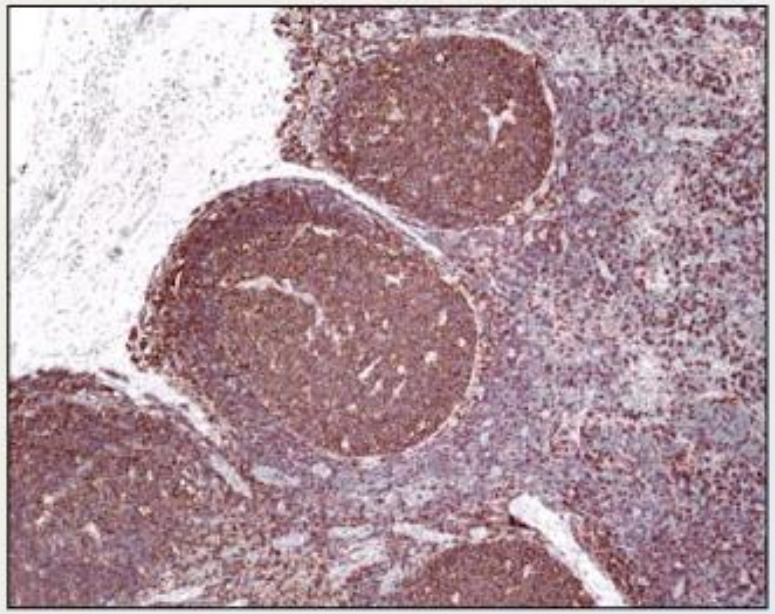
Normal lymphoid tissues

Histology of secondary lymphoid follicle



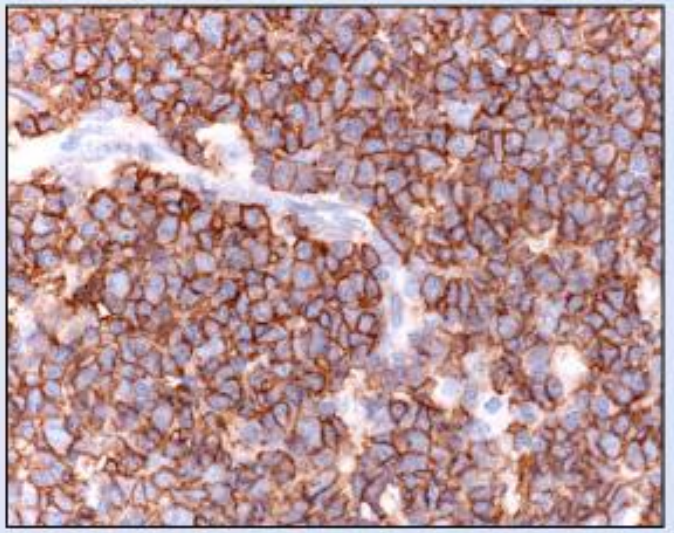
A lymph node shows several secondary lymphoid follicles separated by the T cell zone. The follicles are composed of germinal centres surrounded by mantle zone cells.

Cellular composition of a reactive lymph node

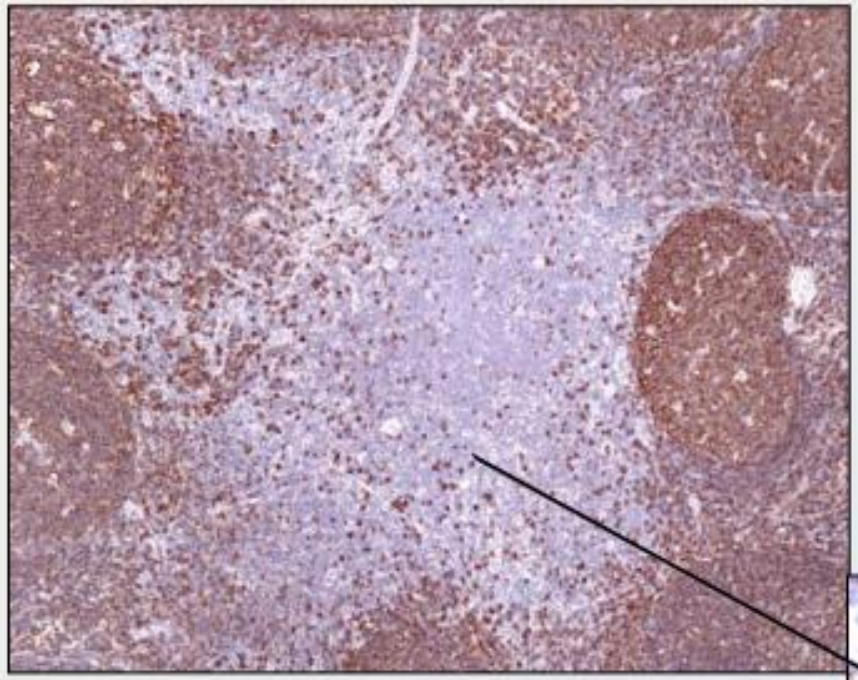


B cells (CD20 +)

All cells in secondary follicles are positive for the pan-B cell marker CD20

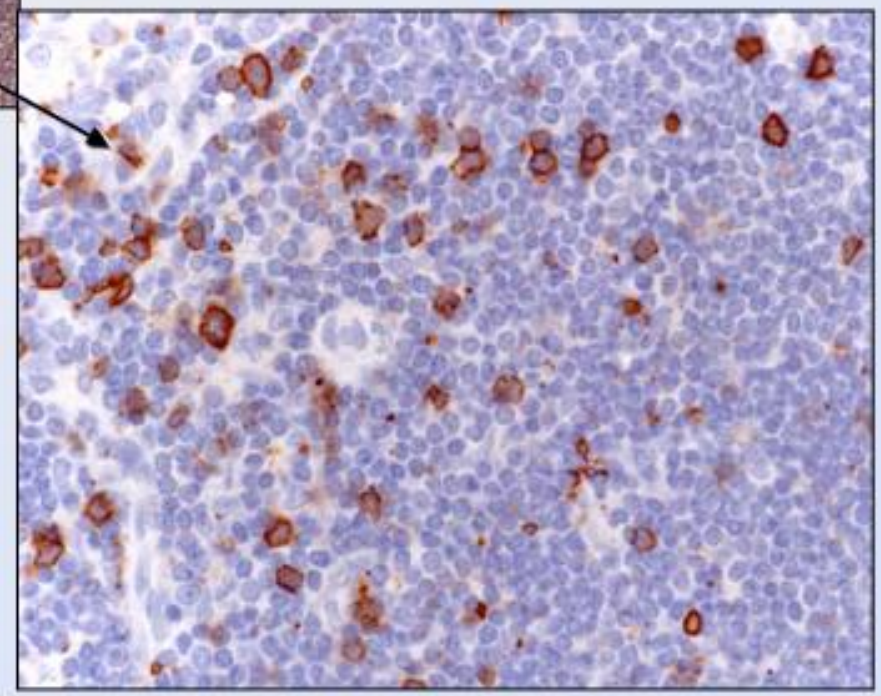


Cellular composition of a reactive lymph node

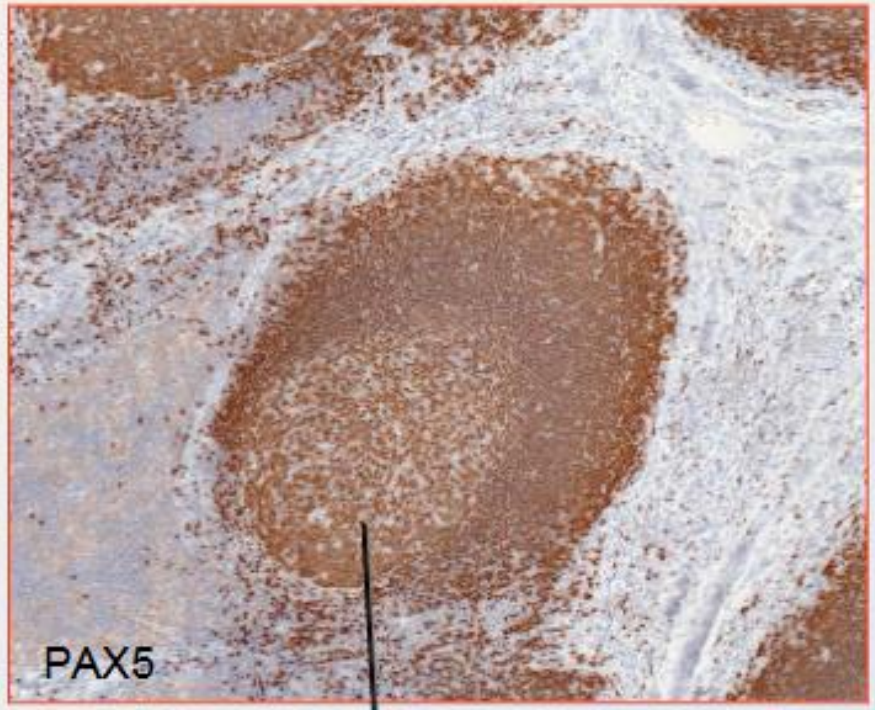


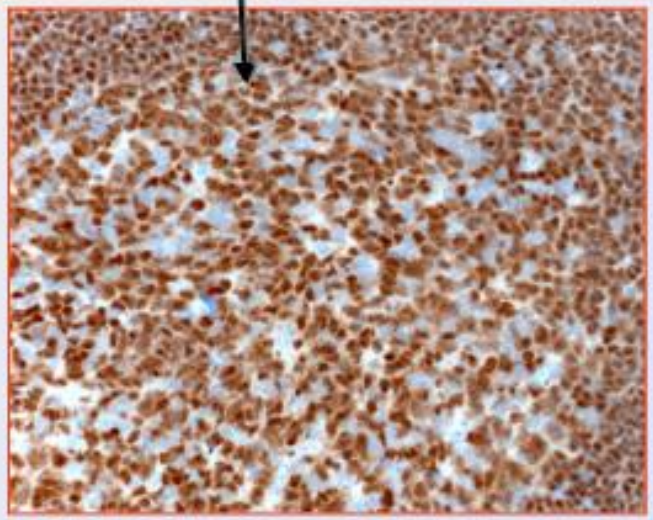
CD20 staining

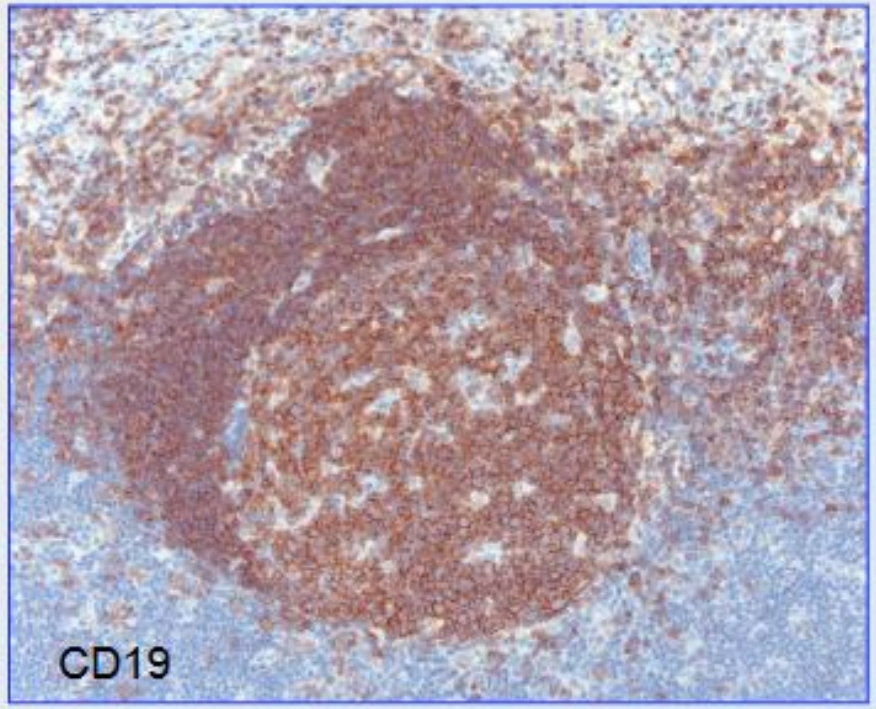
A few scattered B cells in the T cell zone



Cellular composition of a reactive lymph node



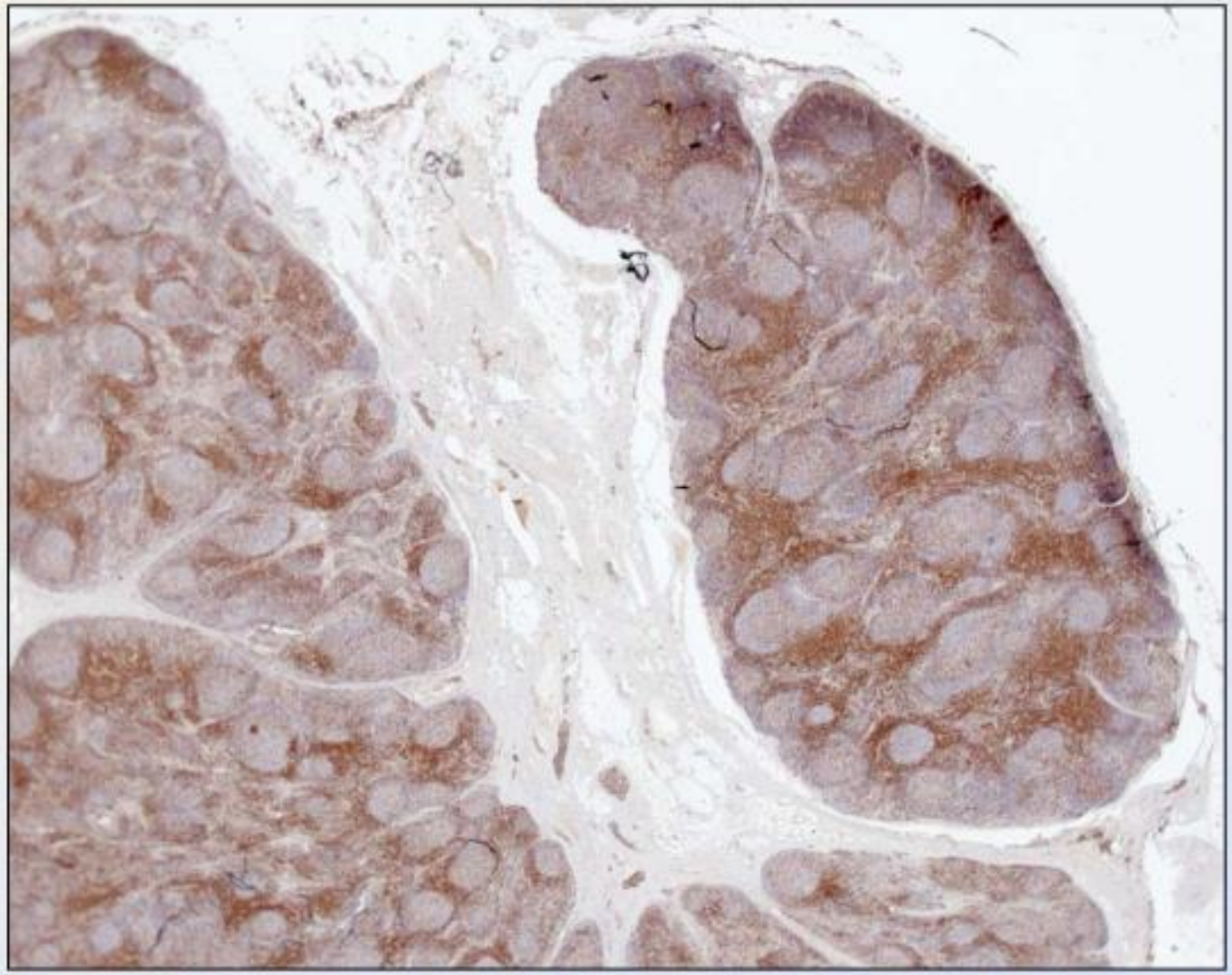




Other pan B cell markers: PAX5 and CD19 labelled mainly lymphoid follicles

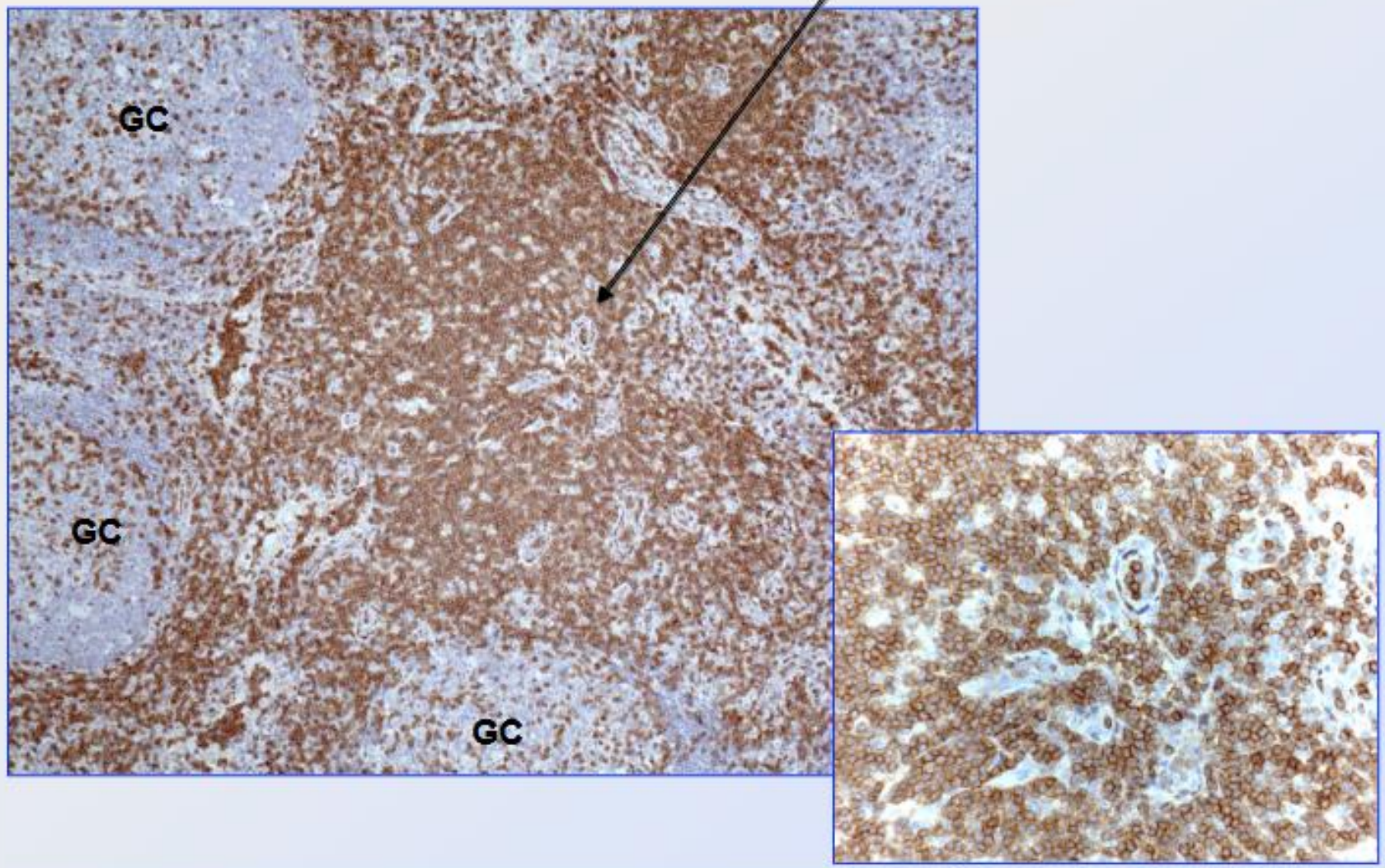
Cellular composition of a reactive lymph node

T cell zone (CD3)



Cellular composition of a reactive lymph node

T cell zone (CD3) : interfollicular areas



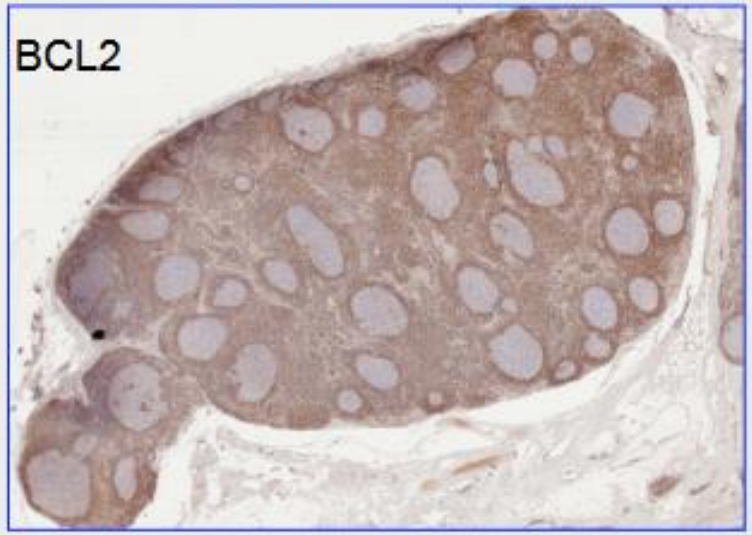
Germinal Center Biology and Immunophenotype

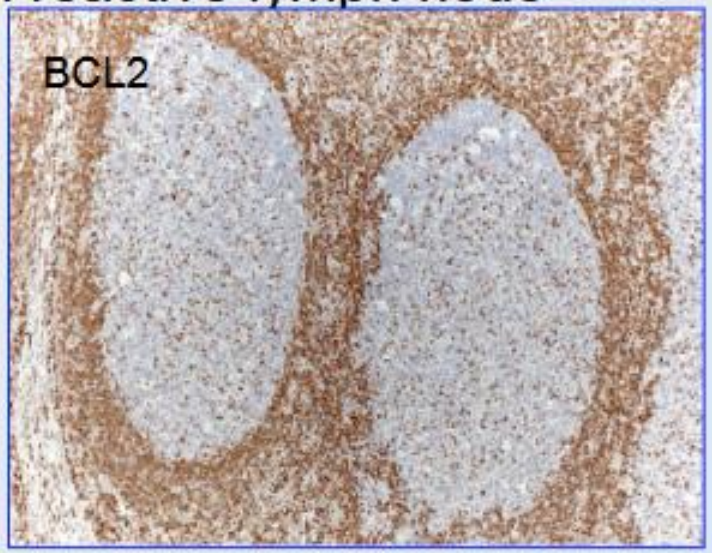
- Secondary follicles represent sites of active B-cell proliferation and maturation.
  - Key cellular processes occurring in germinal centers
  - Clonal expansion of activated B-cells
  - Somatic hypermutation
  - Affinity maturation
  - Selection of high-affinity B-cell clones
- Characteristic immunophenotype of germinal center B-cells
  - CD10 positive
  - BCL6 positive
  - High Ki-67 proliferation index
- Characteristic negative marker
  - BCL2 is typically **negative in reactive germinal centers**, reflecting physiologic apoptosis of low-affinity B-cells.

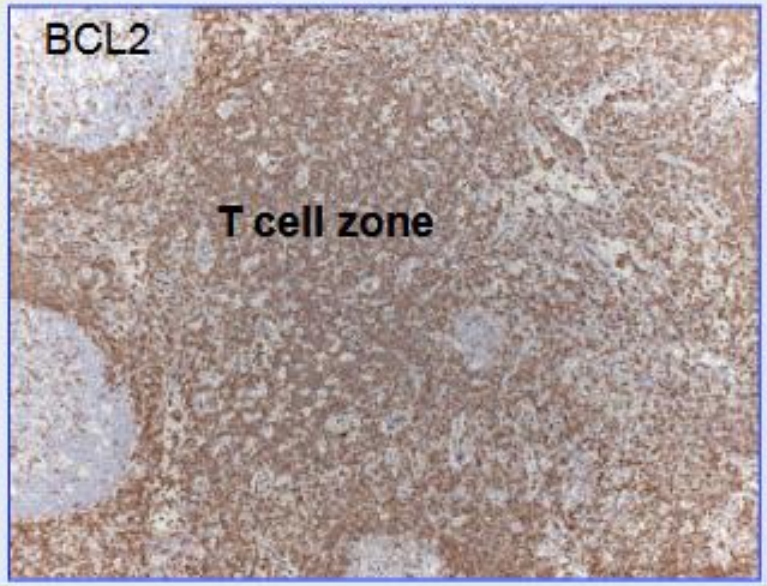
This immunophenotypic pattern is critical for distinguishing **reactive follicles from follicular lymphoma**.

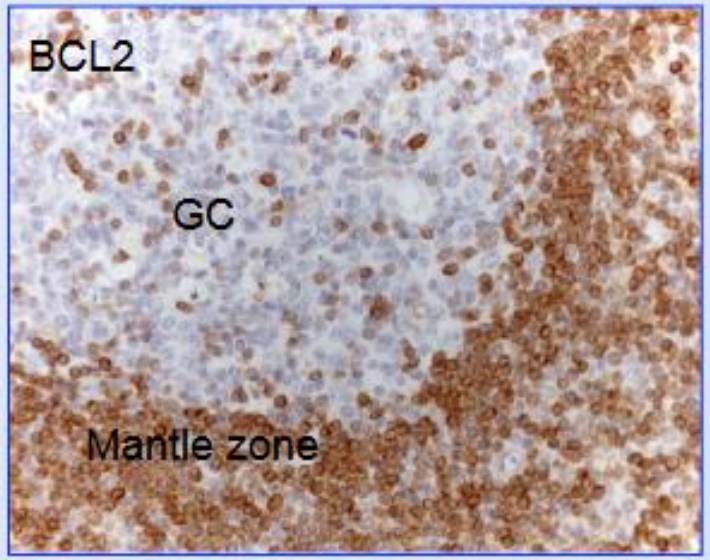
Cellular composition of a reactive lymph node

BCL2 protein expression in reactive lymph node



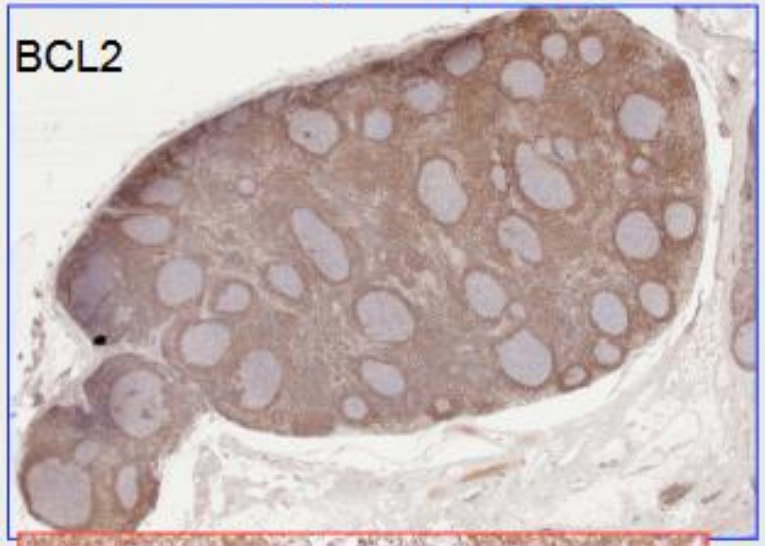


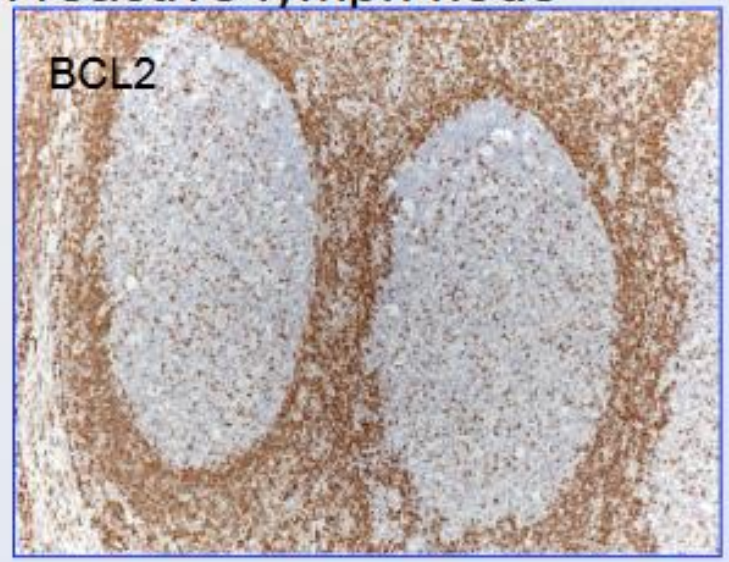


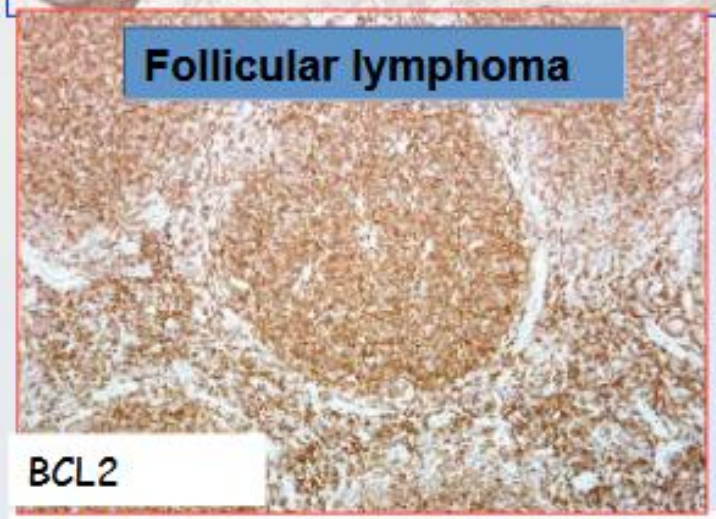


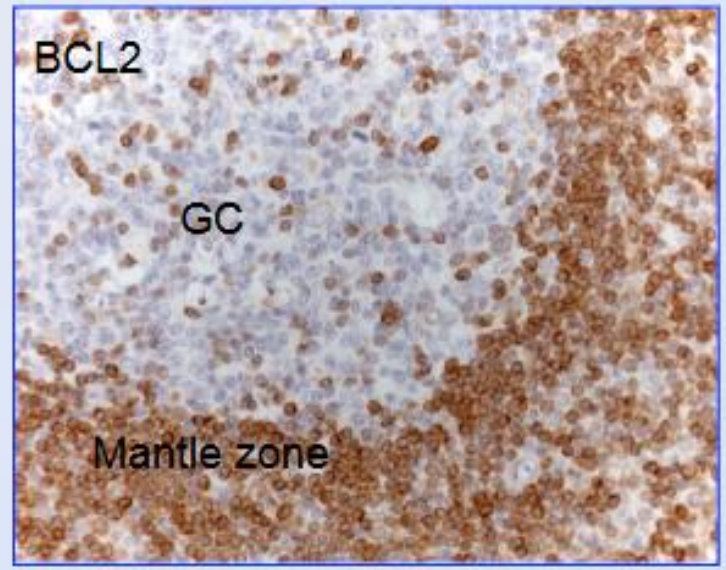
Cellular composition of a reactive lymph node

BCL2 protein expression in reactive lymph node





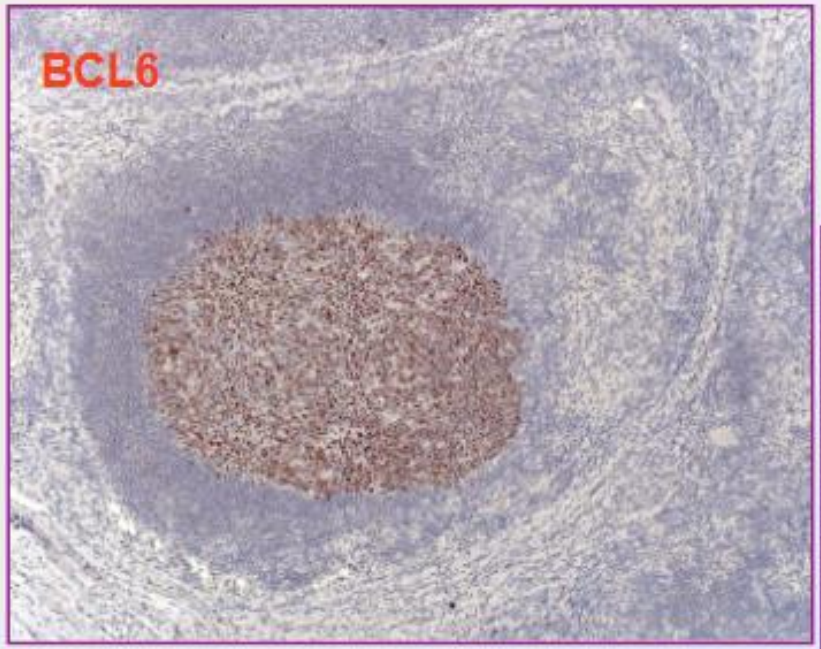


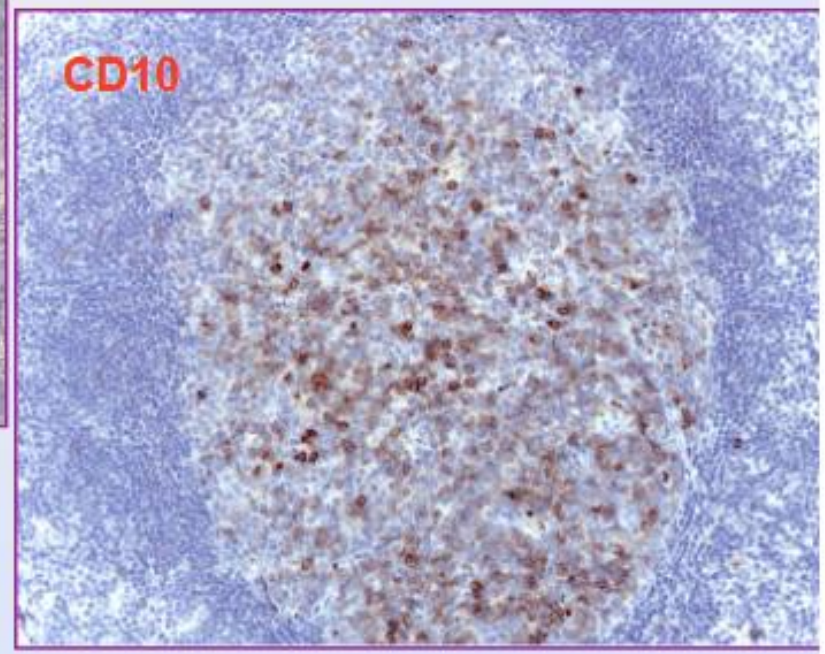


BCL2 is positive in essentially all neoplastic cells.

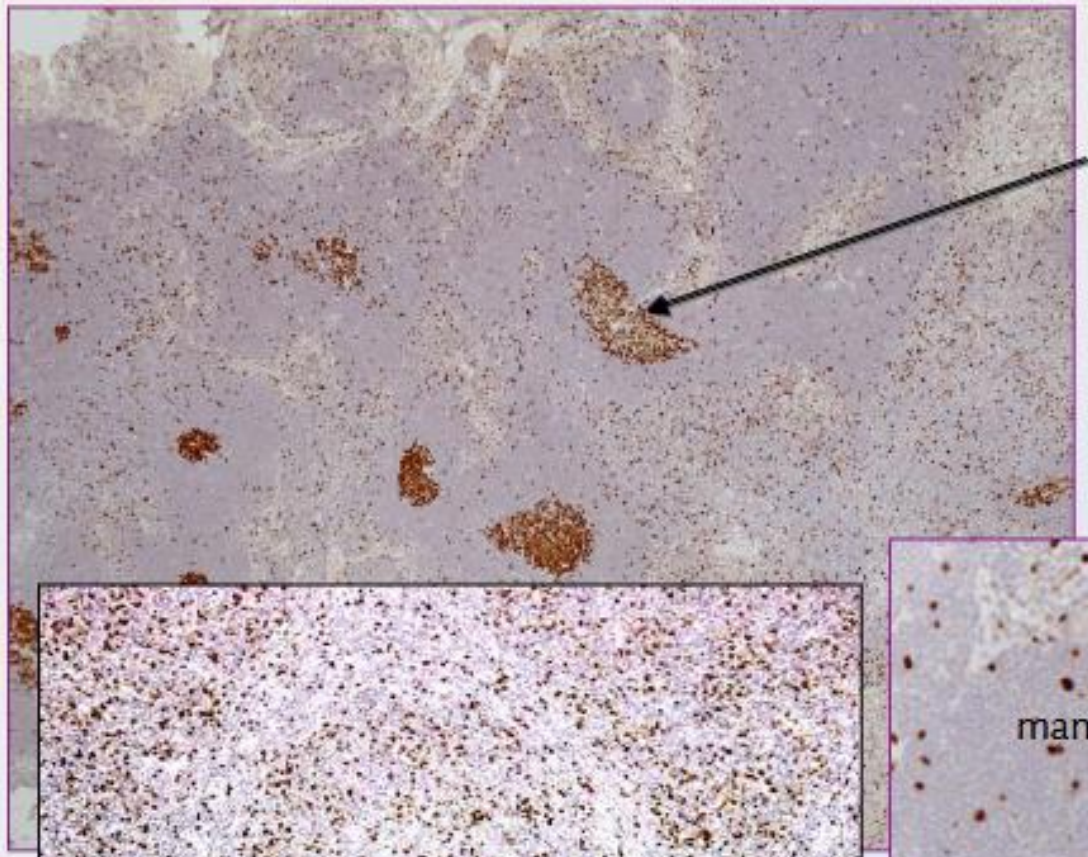
Cellular composition of a reactive lymph node

Expression of germinal centre markers: BCL6 and CD10





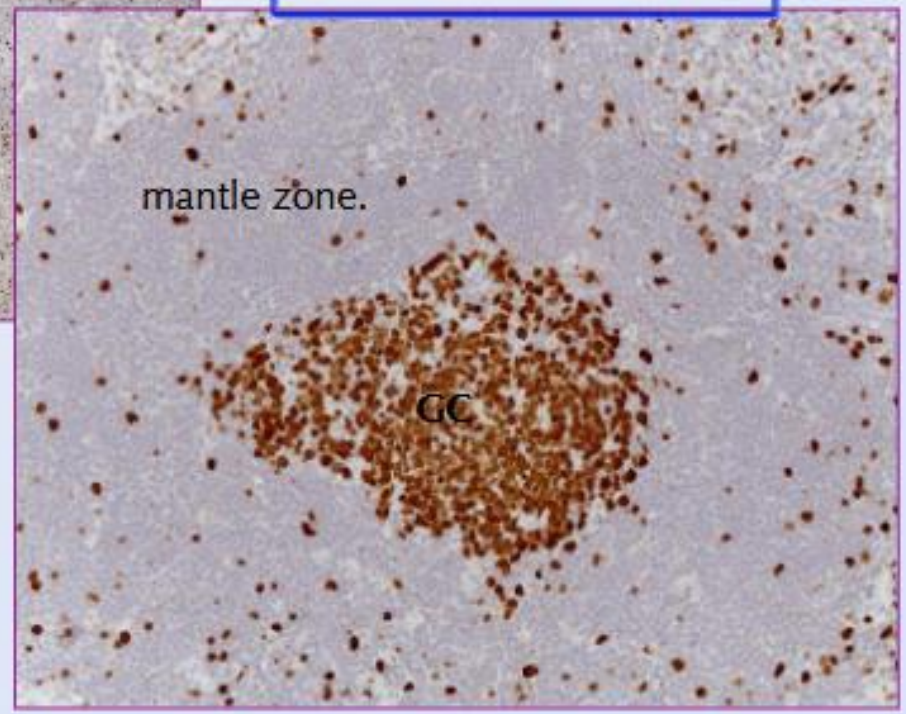
Cellular composition of a reactive lymph node



Staining for Ki67 shows that B cells in the germinal centre (GC) are highly proliferative, contrasting with the quiescent small B cells in the mantle zone.

Proliferation marker Ki67

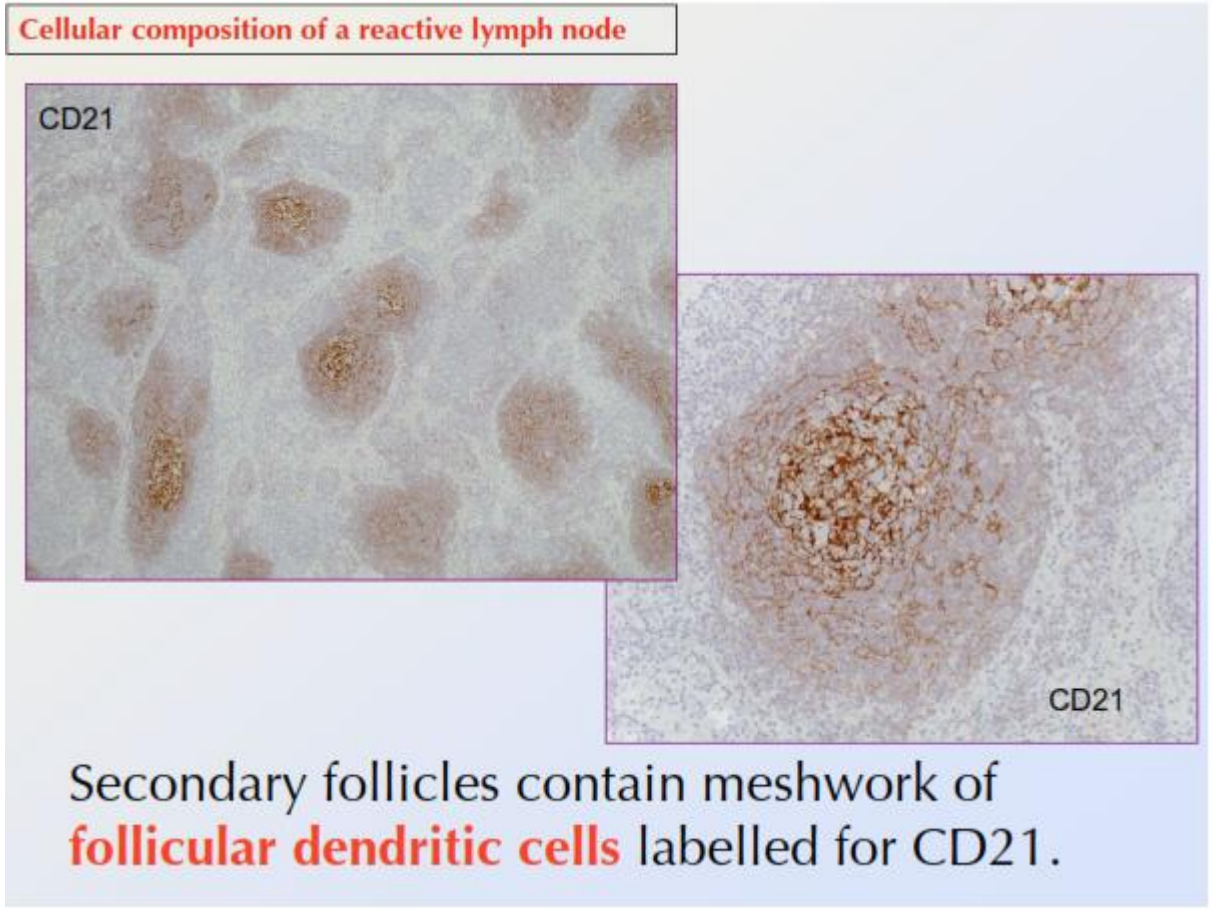
Follicular lymphoma



- **Ki67: Interpretation and Pitfalls**
  - **Value:** Reflects biologic tempo and tumor kinetics.
  - **Principle:** Ki67 alone is NOT diagnostic (e.g., reactive follicles also have high Ki67).
  - **Golden Pearl:** Ki67 reflects tempo, not specific diagnosis.

Follicular Dendritic Cell (FDC) Markers

- **Markers:** CD21, CD23, CD35.
- **Utility:**
  - **FL:** Expanded/disrupted meshworks.
  - **NLPHL:** Preserved nodular meshworks.
  - **MZL:** Follicular colonization.



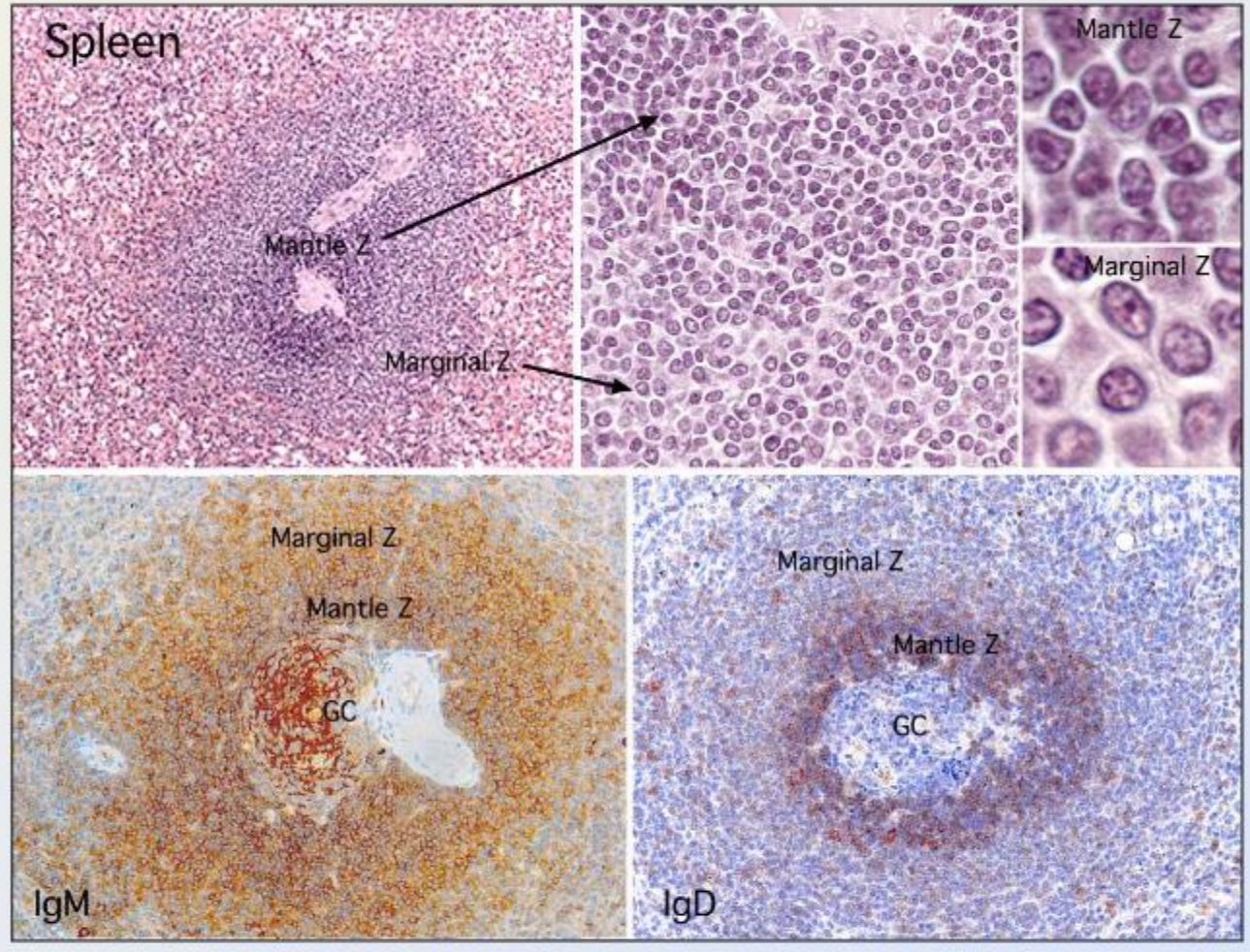
Mantle Zone and Interfollicular Regions

- The mantle zone surrounds the germinal center and contains **small naïve B-cells.**
  - Typical immunophenotype of mantle zone B-cells
    - CD20 positive
    - IgD positive
    - BCL2 positive
    - CD10 negative
- Interfollicular (paracortical) region
  - Dominated by T-cells and antigen-presenting cells.
  - Immunophenotype
    - CD3 positive T-cells
    - Variable immunoblasts and activated lymphocytes
    - Occasional CD30 positive activated cells

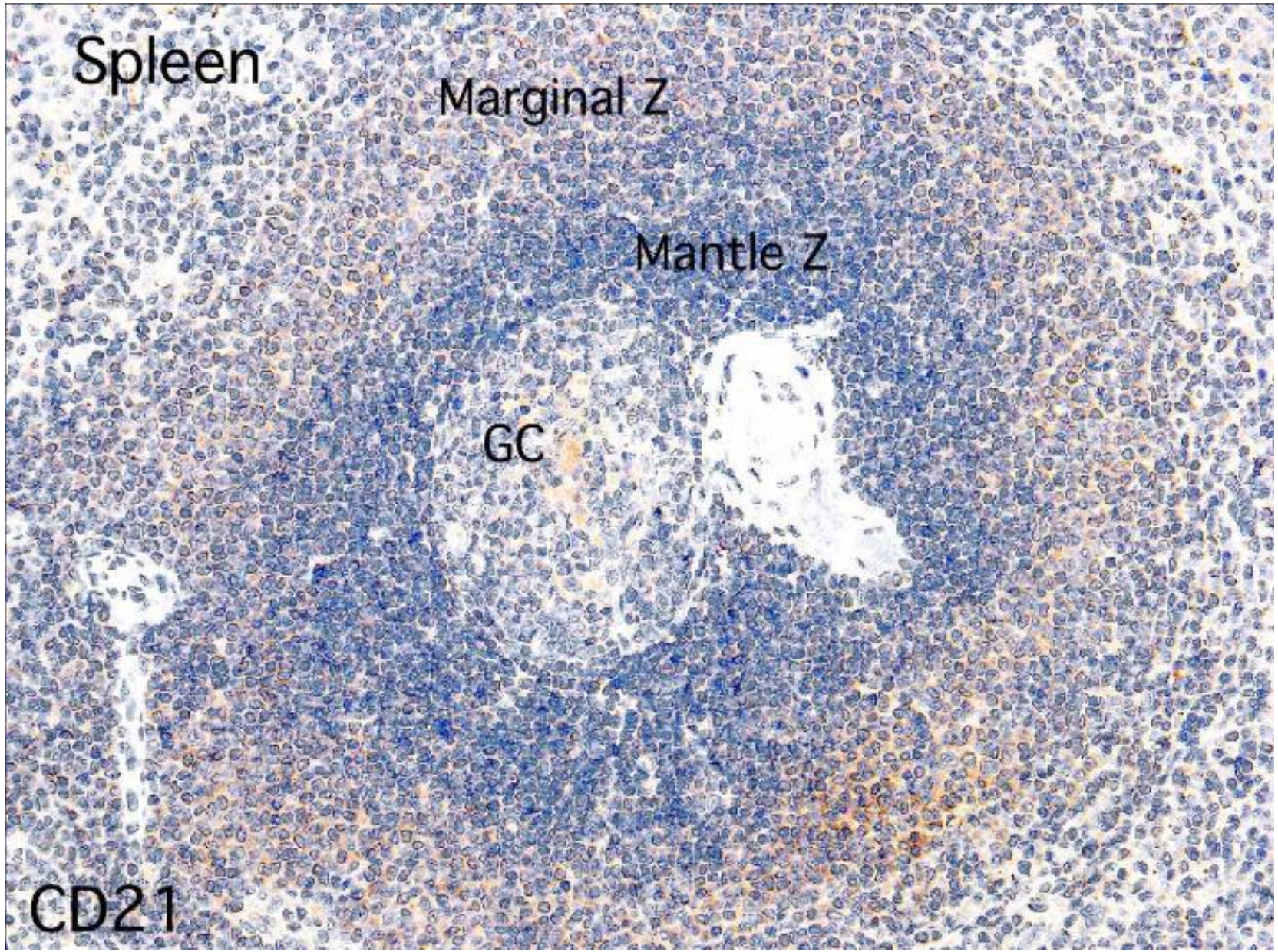
Expansion of specific compartments (follicular, mantle zone, or paracortical) may suggest particular reactive conditions or lymphoid neoplasms.

Normal lymphoid tissues

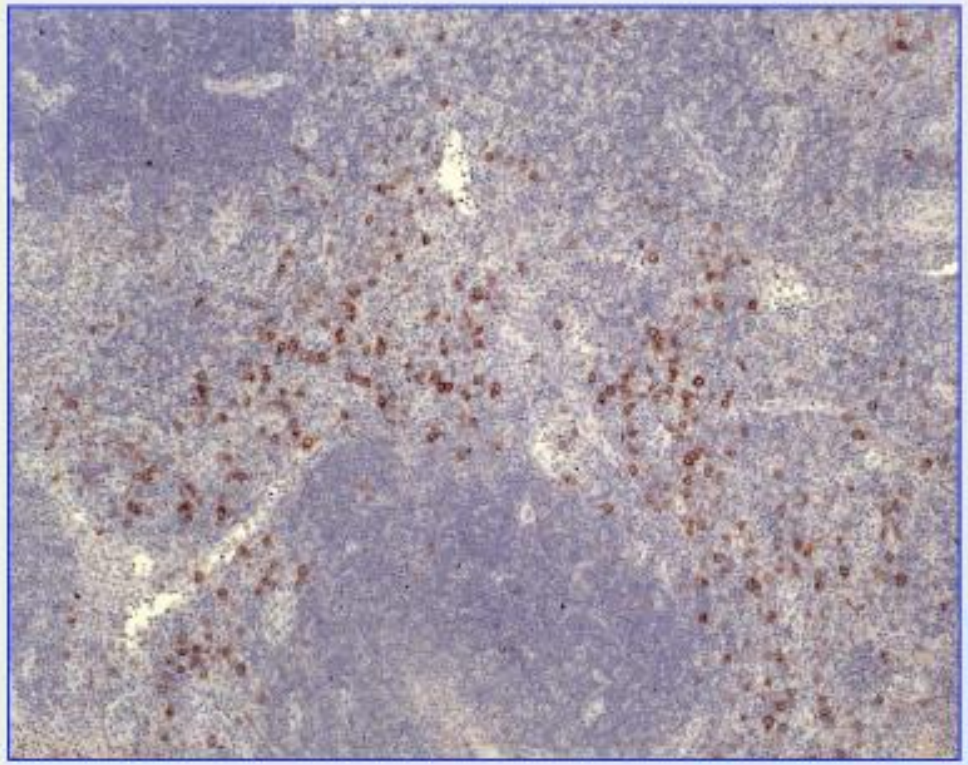
Spleen



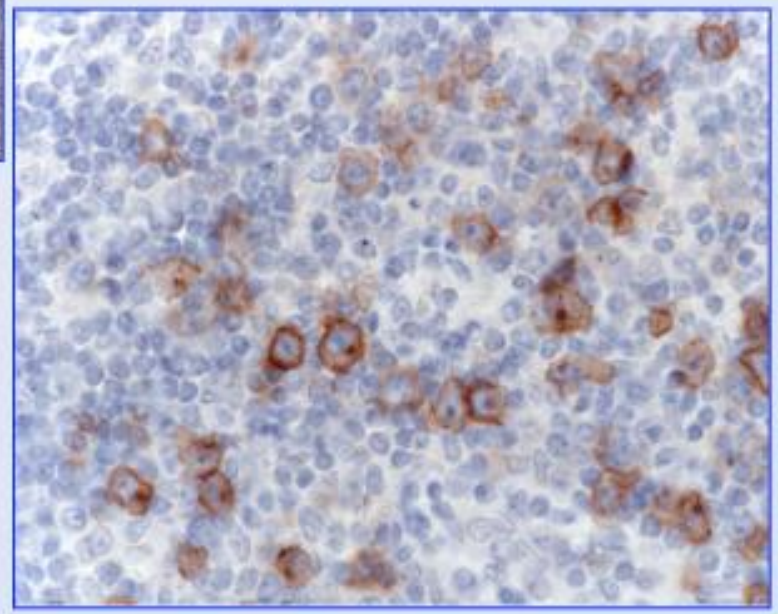
Lymphoid area of the spleen comprises a germinal centre (GC), a mantle zone and a well-defined marginal zone. The mantle zone cells are smaller and have less cytoplasm than the marginal zone cells. Immunostaining for IgM shows that both the mantle and marginal zones are strongly positive for this marker. In contrast, the marginal zone is only weakly positive or negative for IgD

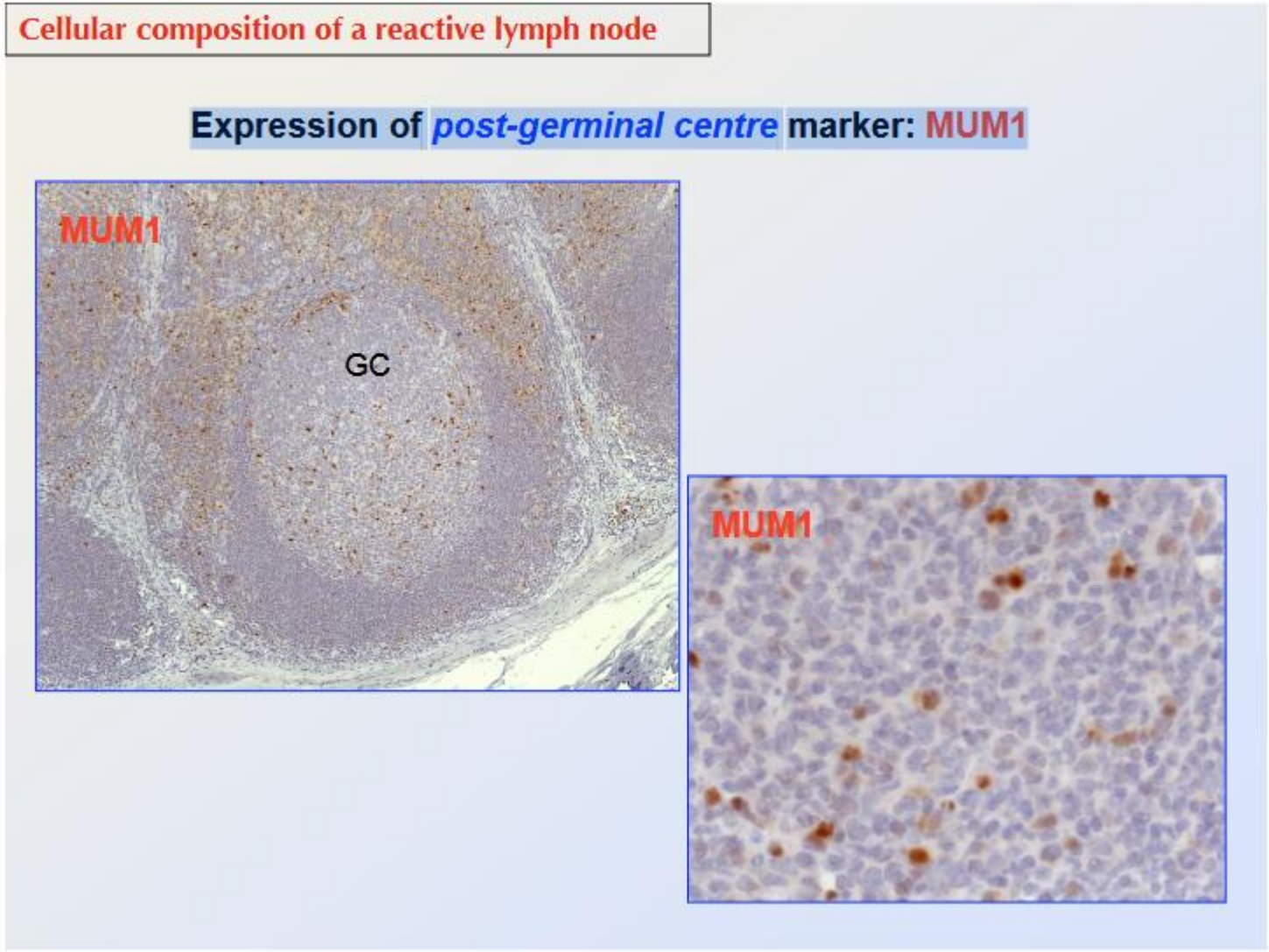

Spleen
Marginal Z
Mantle Z
GC
CD21

Cellular composition of a reactive lymph node



CD30





Post-germinal center markers

MUM1 (IRF4)

-Expressed in late germinal center and post-germinal center B-cells

-Highlights activated B-cells, plasmablasts, and plasma cell differentiation

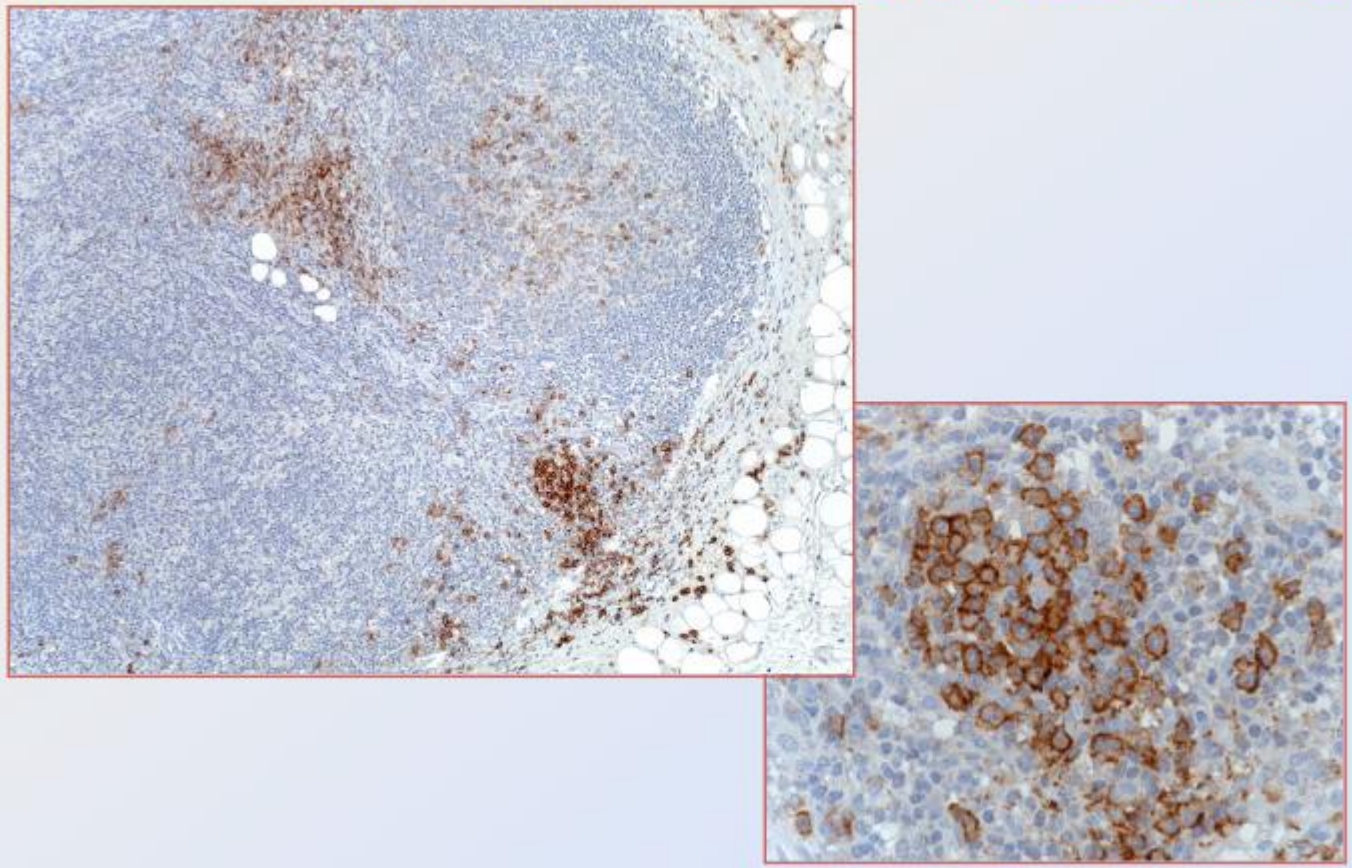
-MUM1 can be expressed in **activated T-cells**. Physiologic expression

-Activated $CD4^+$ and $CD8^+$ T-cells may show MUM1 expression during immune activation.

MUM1 positivity **does not indicate B-cell lineage**. It reflects **cell activation and late differentiation**, so lineage must always be confirmed with markers such as **CD3, CD20, or PAX5**.

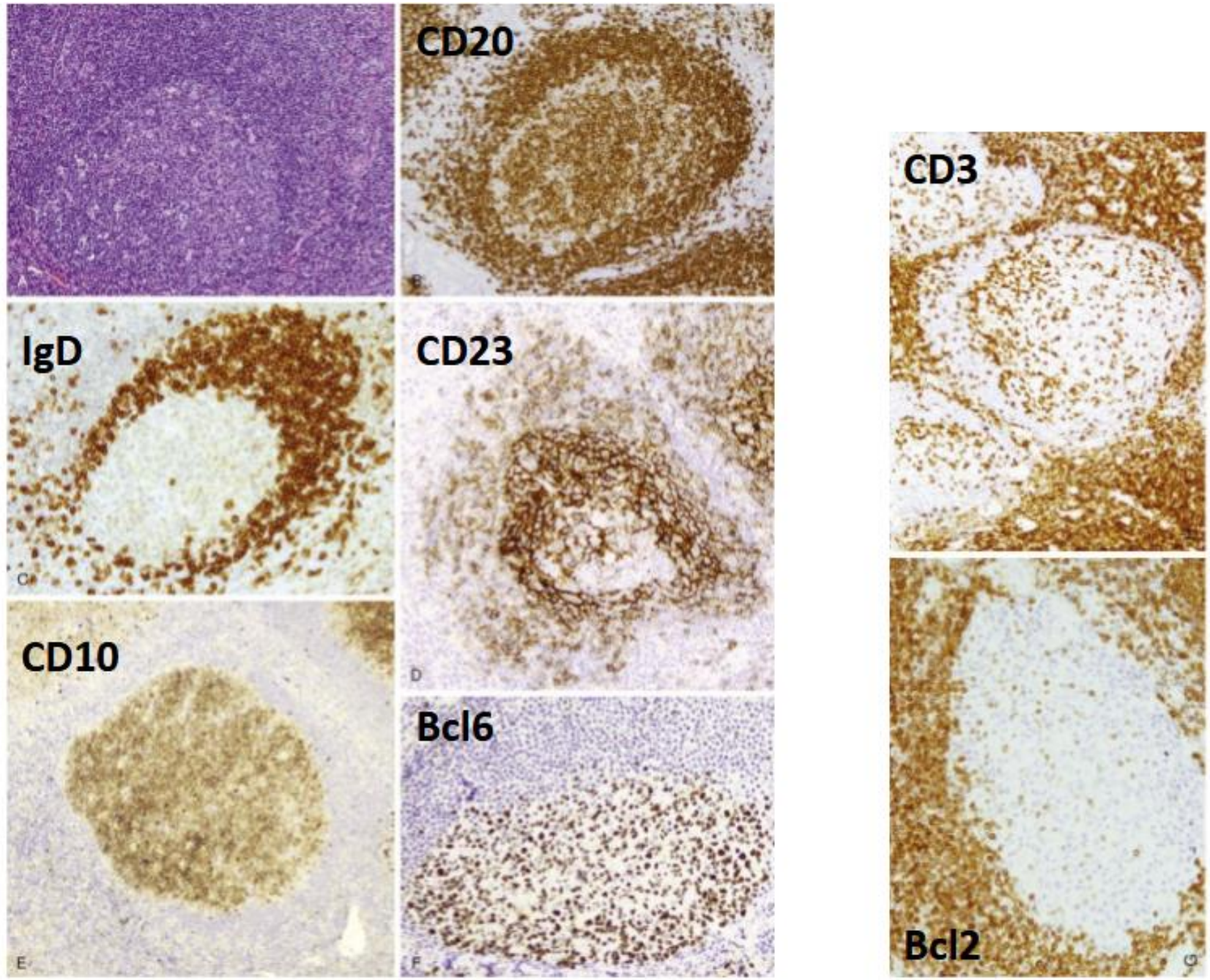
Cellular composition of a reactive lymph node

Expression of *post-germinal centre* marker: CD138 (plasma cell marker)



CD138 (Syndecan-1)Specific marker of plasma cells

Typically highlights plasma cells within medullary cords and interfollicular regions


CD20
IgD
CD23
CD10
Bcl6
CD3
Bcl2

Post-germinal center markers

- **Diagnostic relevance**
  - Detection of post-germinal center differentiation helps identify plasma cell–rich reactive conditions and contributes to the classification of lymphoid neoplasms with plasmacytic differentiation.
- **Key interpretive point**
  - In reactive lymph nodes, MUM1 and CD138 positive cells are usually scattered and polytypic, whereas extensive or monomorphic populations may suggest lymphoid neoplasia.

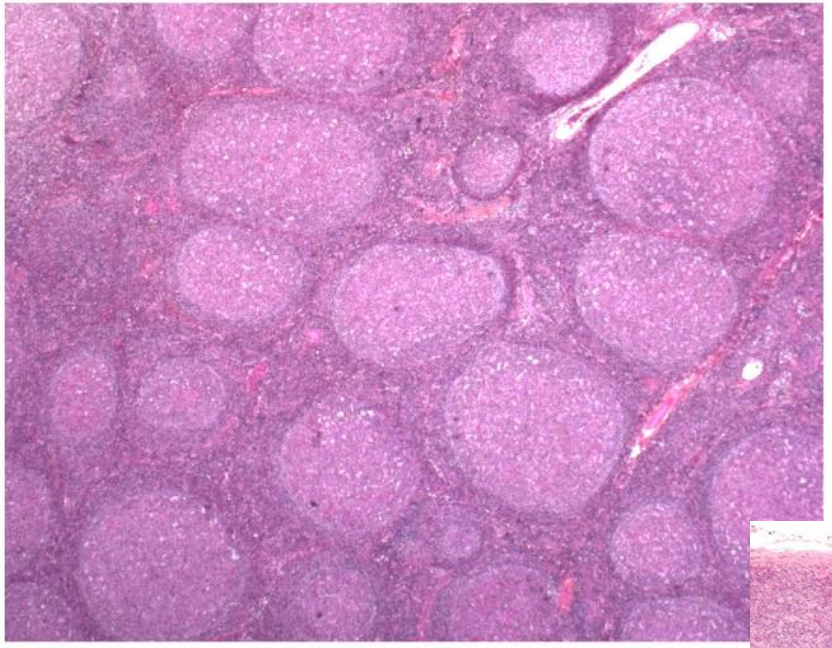
Reactive Lymph Node Patterns

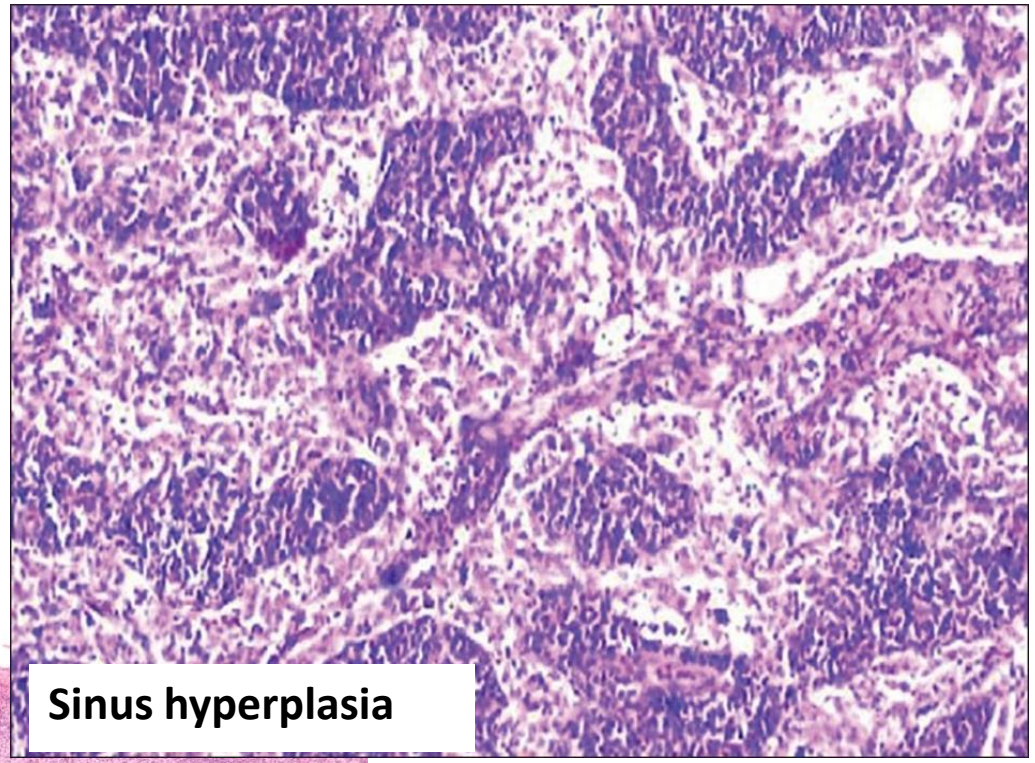
- Reactive lymph nodes may demonstrate several characteristic architectural patterns.
  - **Follicular hyperplasia**
    - Prominent secondary follicles with polarized germinal centers and preserved mantle zones.
  - **Paracortical hyperplasia**
    - Expansion of the T-cell zone with immunoblasts and activated lymphocytes.
  - **Sinus histiocytosis**
    - Prominent sinusoidal macrophages often associated with inflammatory or neoplastic conditions.
- Key morphologic clues favoring reactive processes
  - Preserved nodal architecture
  - Polymorphous cellular population
  - Presence of tingible-body macrophages
  - Polarization within germinal centers

Reactive patterns may occasionally mimic lymphoma, making correlation with immunohistochemistry essential.

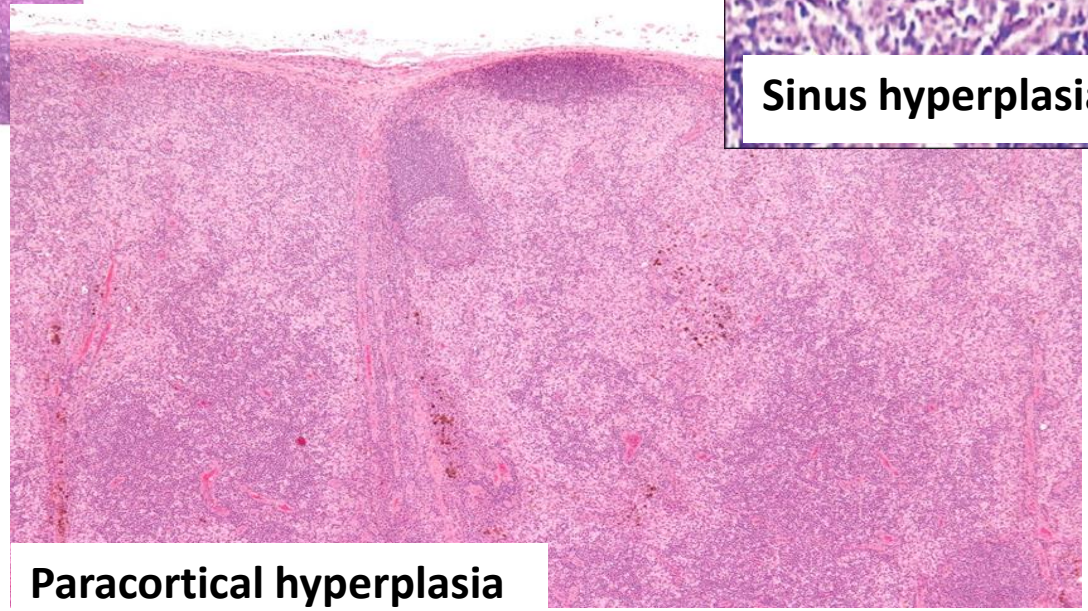
Reactive Lymph Node Patterns

Reactive Follicular Hyperplasia





Sinus hyperplasia



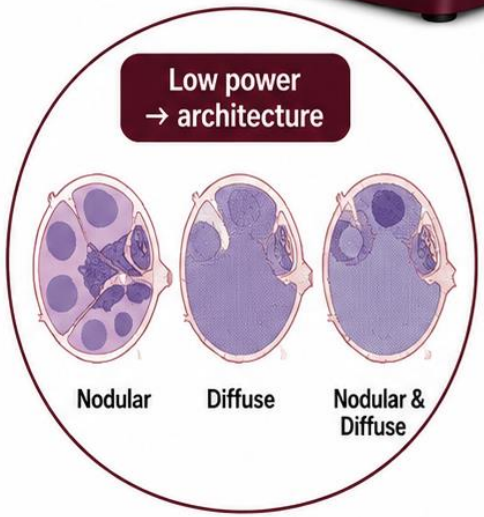
Paracortical hyperplasia

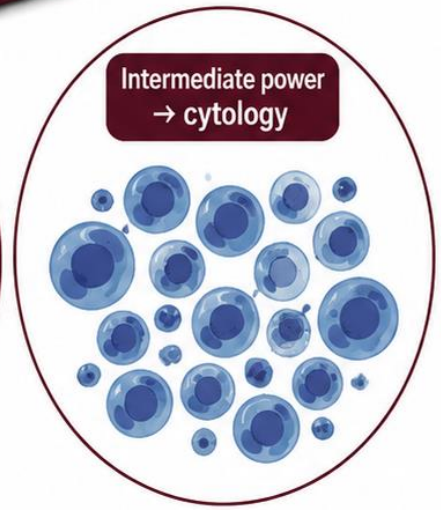
Pattern-Based Diagnostic Approach in Lymphoid Pathology

- Evaluation of lymphoid proliferations begins with recognition of **architectural and cytologic patterns on H&E sections**.
- Major morphologic patterns encountered in lymph node pathology
  - Follicular/nodular pattern
  - Diffuse small-cell infiltrate
  - Diffuse large-cell proliferation
  - Paracortical expansion
  - Hodgkin-like pattern
- Diagnostic principle
  - Each morphologic pattern suggests a **limited group of differential diagnoses** and guides the selection of the **initial immunohistochemical panel**.
- Practical workflow
  - Morphology → Pattern recognition → Targeted IHC → Integrated diagnosis

Correct identification of the **dominant morphologic pattern** significantly reduces unnecessary immunostains and improves diagnostic accuracy.

Integrated Morphology-Driven Diagnosis





H&E evaluation

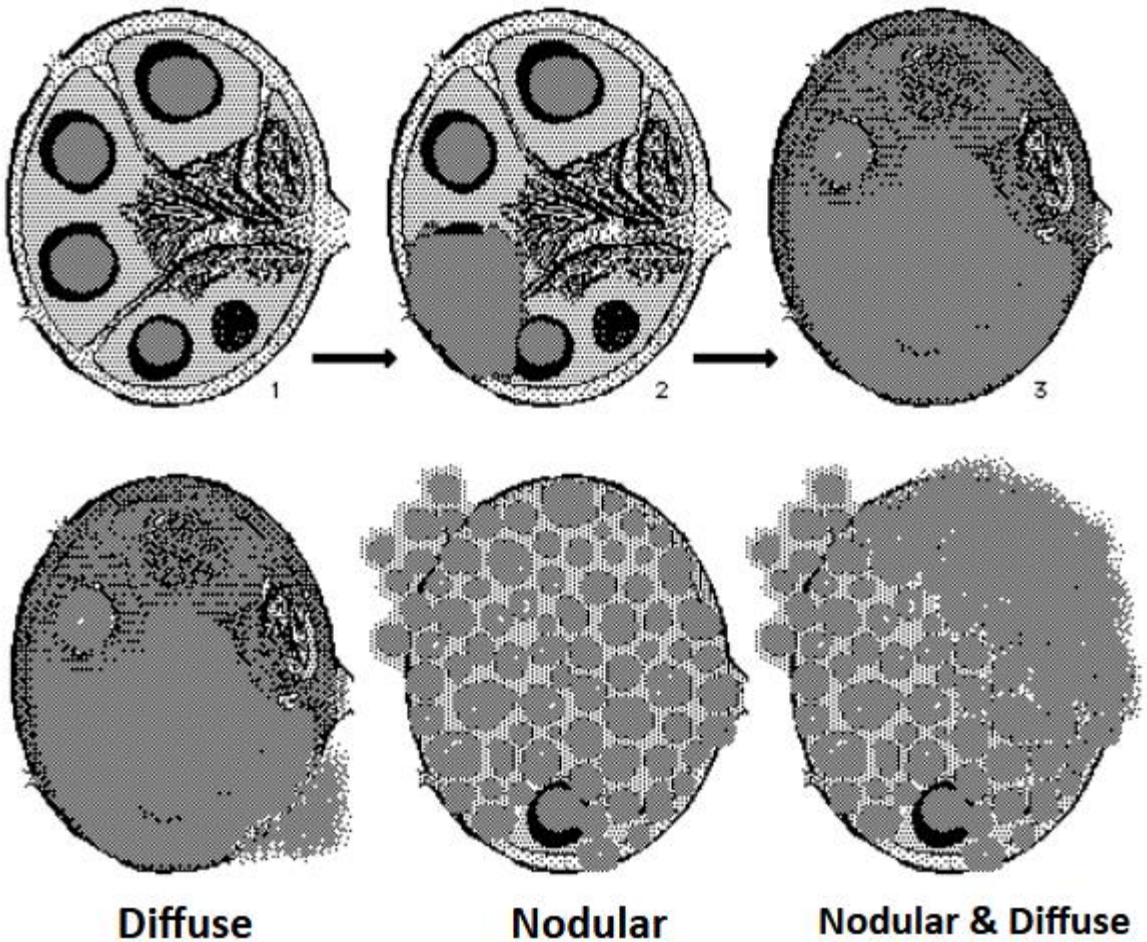
Identify architectural pattern

Generate differential diagnosis

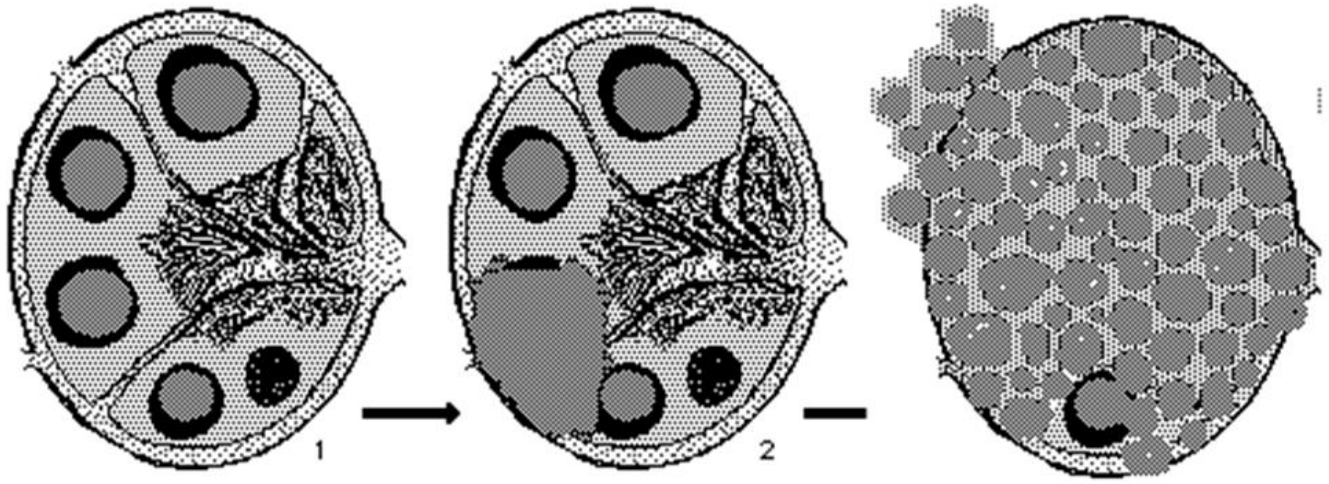
Targeted IHC panel

Integrated diagnosis

Pattern of nodal involvement



Pattern of nodular proliferation



Differential diagnosis

Benign	Malignant
Reactive Follicular Hyperplasia	Follicular lymphoma
Progress. Tranf. of GC (PTGC)	Mantle cell lymphoma
Follicle lysis	Marginal zone lymphoma
Castle man's disease	CLL/SLL
	NLP Hodgkin lymphoma

Definition:

Nodular / follicle-like structures within the lymph node.

Reactive Follicular Hyperplasia vs FL

- **Key Morphologic Questions:**
  - Follicles preserved?
  - Polarization present?
  - Mantle zones intact?
  - Tingible-body macrophages?
  - Follicles uniform/back-to-back?
- If doubtful, use clinical correlation & ancillary techniques
- **Initial IHC Panel:**
  - CD20, CD10, BCL6, BCL2, Ki-67, CD21
- **Critical Point:**

  Germinal center BCL2– → reactive
  Germinal center BCL2+ → suspicious for FL

Follicular Lymphoma:

Morphology:

- **Features:** Nodular architecture, “back-to-back” follicles.
- **Findings:** Reduced tingible body macrophages, loss of polarization.
- **Cytology:** Centrocytes and Centroblasts.

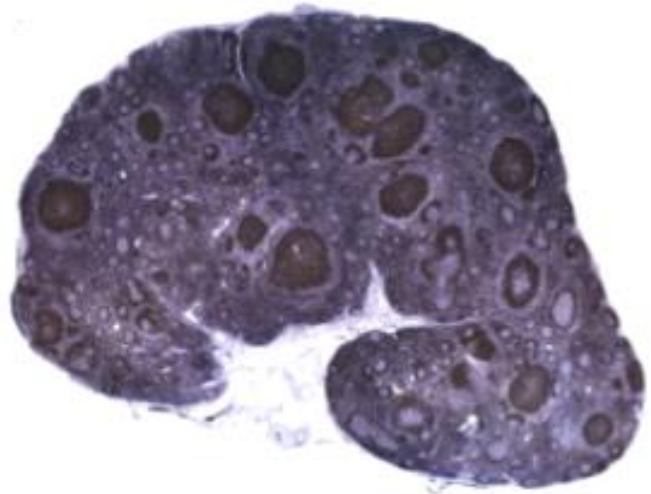
Immunophenotype:

- **Markers:** CD10 (+), BCL6 (+), **BCL2 (Aberrantly positive in follicles)**.
- **Molecular Hallmark:** t(14;18) causing BCL2 rearrangement (anti-apoptotic).

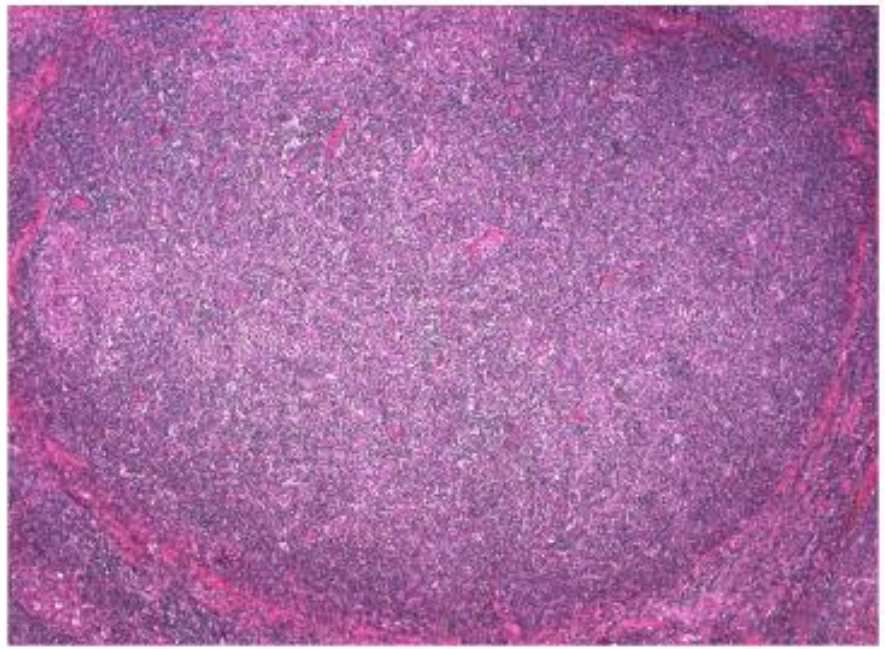
Follicular Lymphoma:

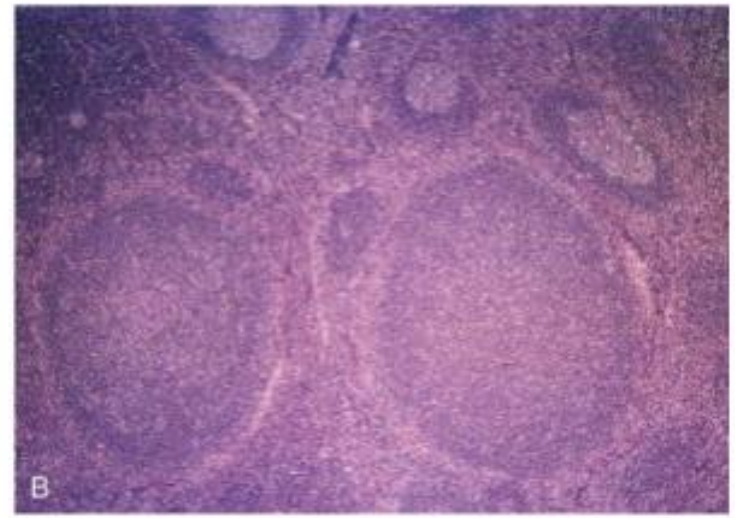
- **BCL2 and Ki67 Patterns in FL**
  - **Reactive Follicle:** BCL2 negative, **Polarized** Ki67.
  - **Follicular Lymphoma:** BCL2 positive, **Loss of Ki67 polarization**.
- **Concept:** Pattern interpretation is more important than isolated positivity.

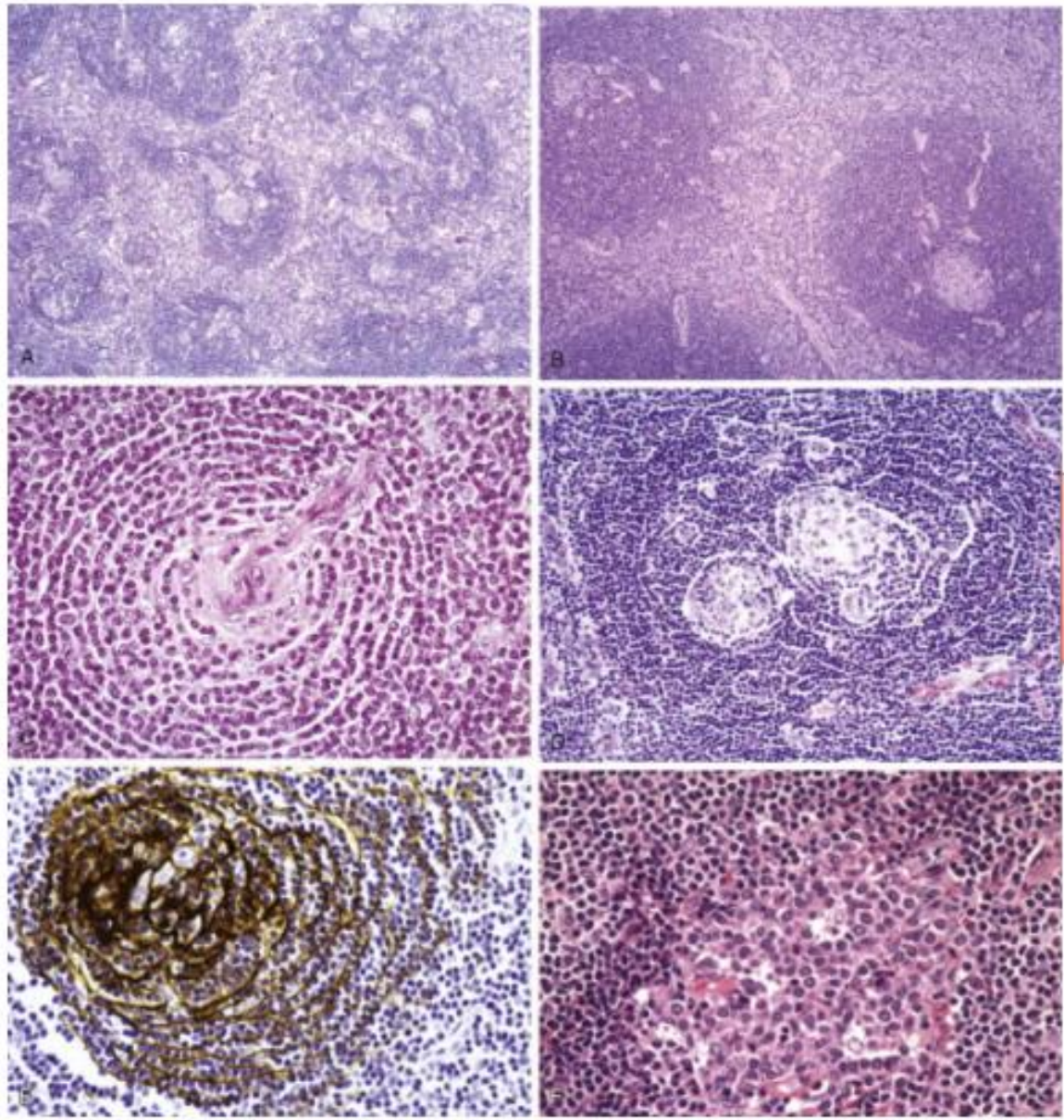
- **Follicular Lymphoma Pitfalls**
  - **Grade 3 FL:** May lose CD10.
  - **Pediatric-type FL:** Often BCL2 negative.



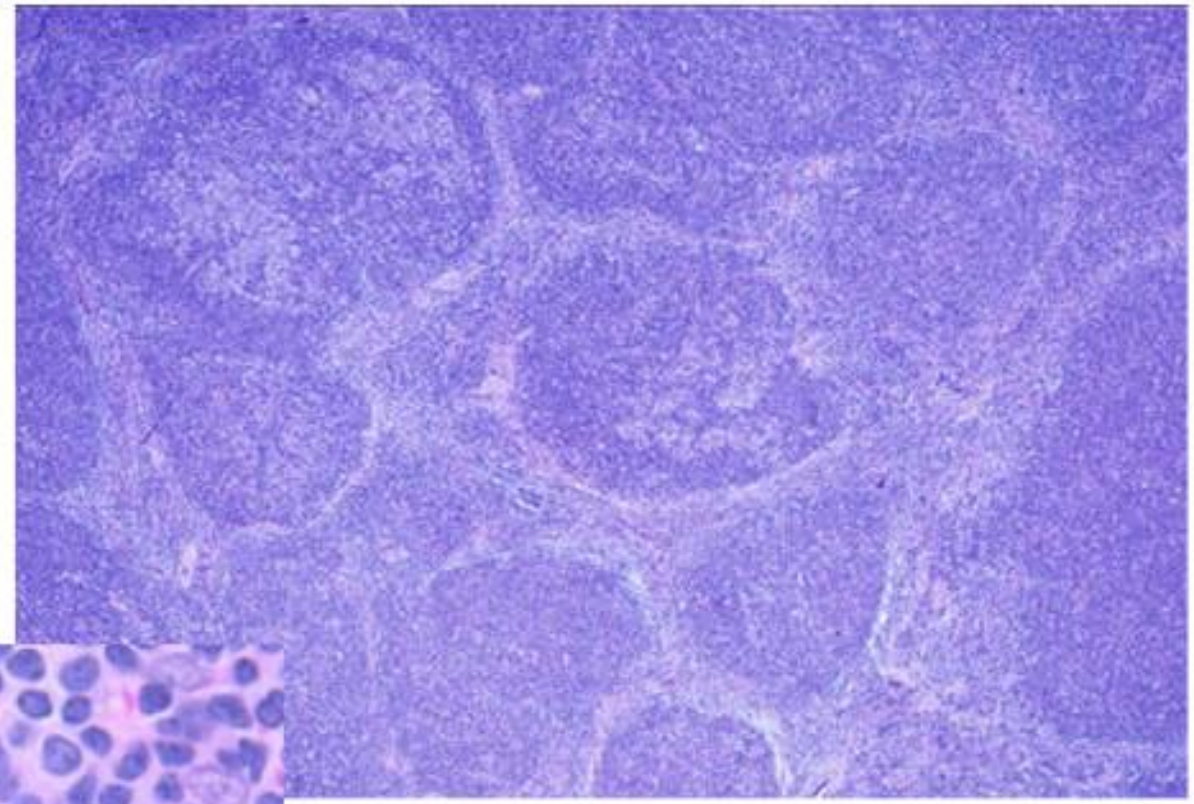
Progressive Transformation of Germinal centre (PTGC)

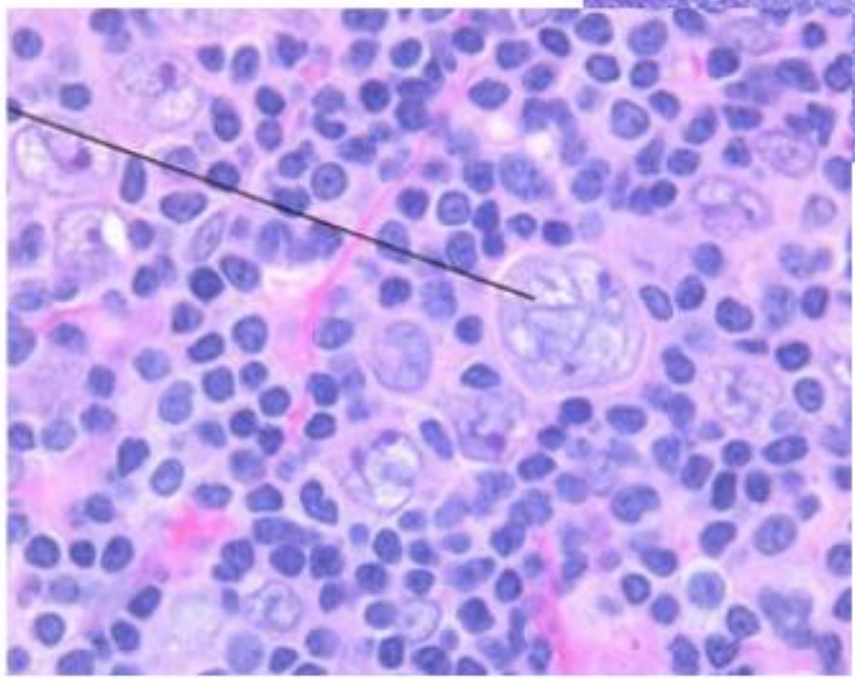






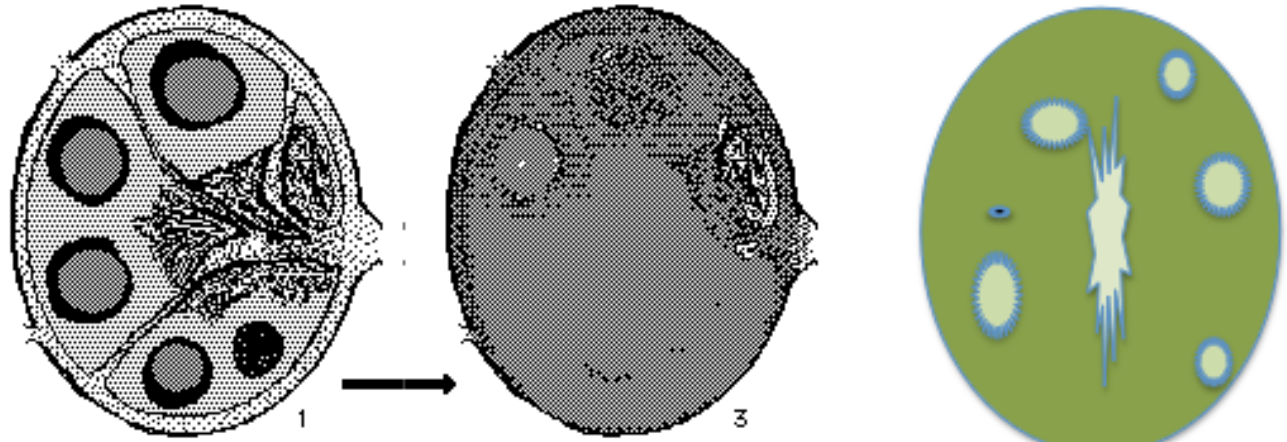
Castle man’s disease





Nodular Lymphocyte Predominant Hodgkin lymphoma

Pattern of diffuse proliferation



Differential diagnosis

Diffuse Proliferation

Small cells (CLL/SLL; MZL; LPL; MCL)

Large cells (DLBCL)

Para-cortical expansion

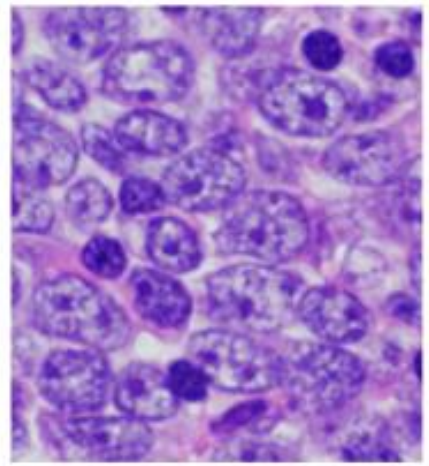
T-cell lymphoma, AILT

Cell Size & Cellularity in Lymph Node Evaluation

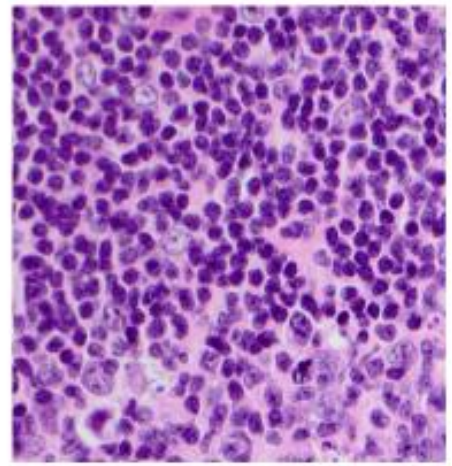
- **Cell Size Comparison**
  - Tissue fixation and processing can cause shrinkage; therefore, cell size should be compared **within the same tissue section**.
- **Internal controls for size comparison:**
  - Red blood cells (RBCs)
  - Macrophages
  - Endothelial cells
- **Definition of a Large Cell**
  - A lymphoid cell is considered **large** if its nucleus is:
    - Larger than the diameter of **endothelial cells or macrophages**, or
    - **At least twice the diameter of an RBC**

Cell Size & Cellularity in Lymph Node Evaluation

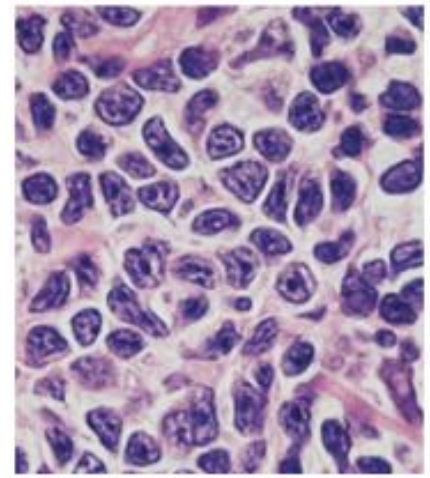
- **Hypercellularity**
  - A common feature of many **malignant proliferations**.
- **Morphologic Indicators**
  - Obliteration of **lymph node sinuses**
  - Increased number of **follicles**
  - Expansion of **cortex and/or paracortex**
  - **Engorgement of nodal sinuses**
  - **Pericapsular infiltration**
  - Increased **cellular density**
  - Proliferation of a **single cell type**, producing a **monotonous infiltrate**



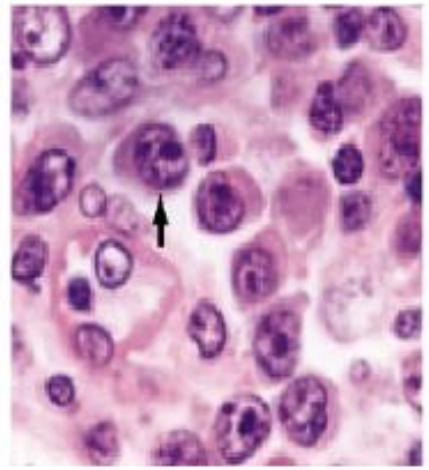
Centroblasts



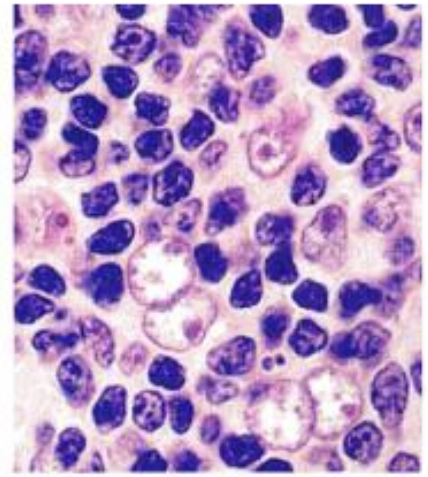
Naïve B-cells



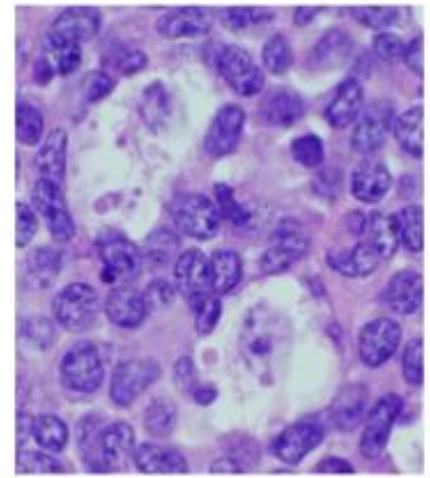
Centrocytes



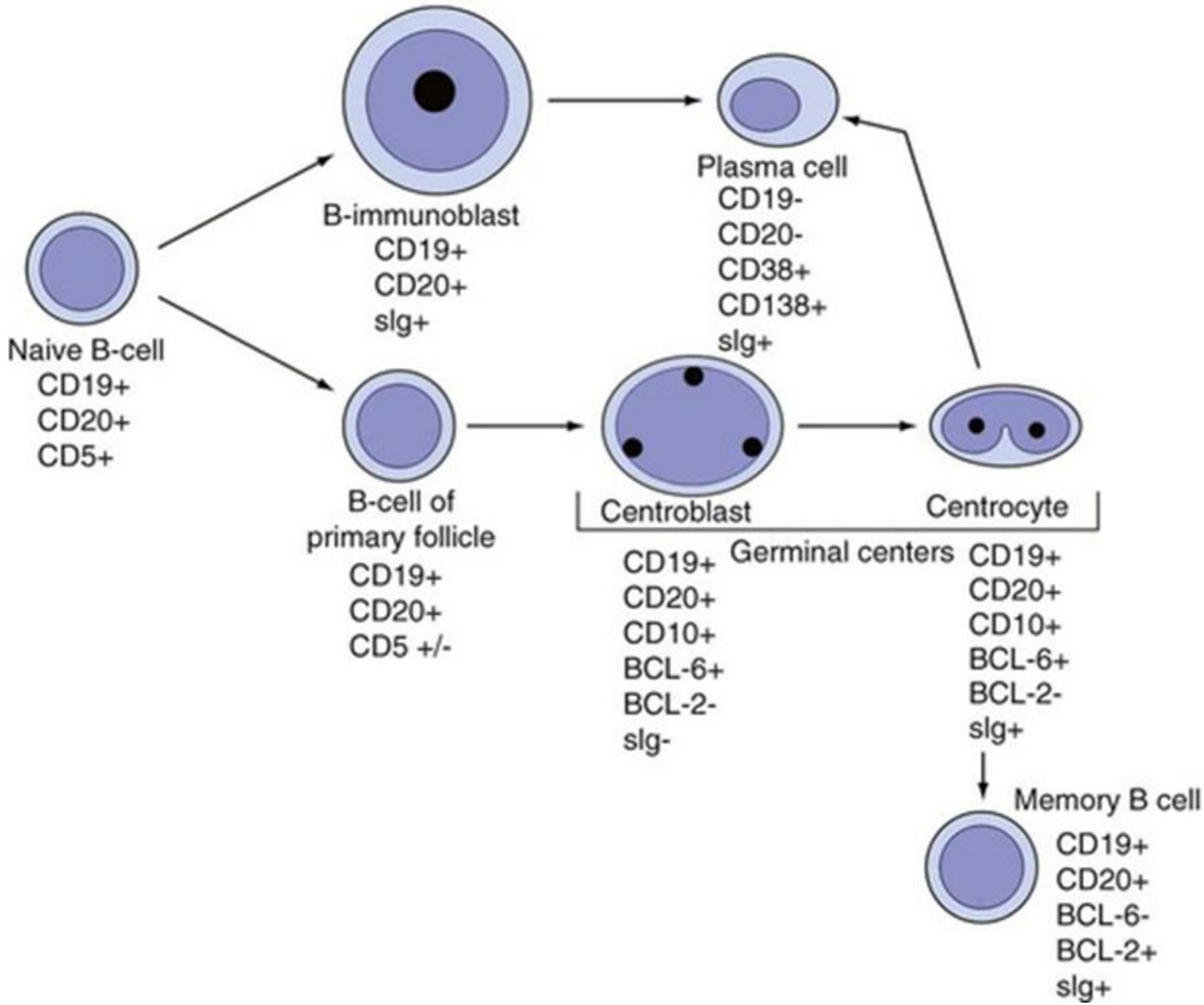
Immunoblasts



Follicular Dendritic cells



Macrophages

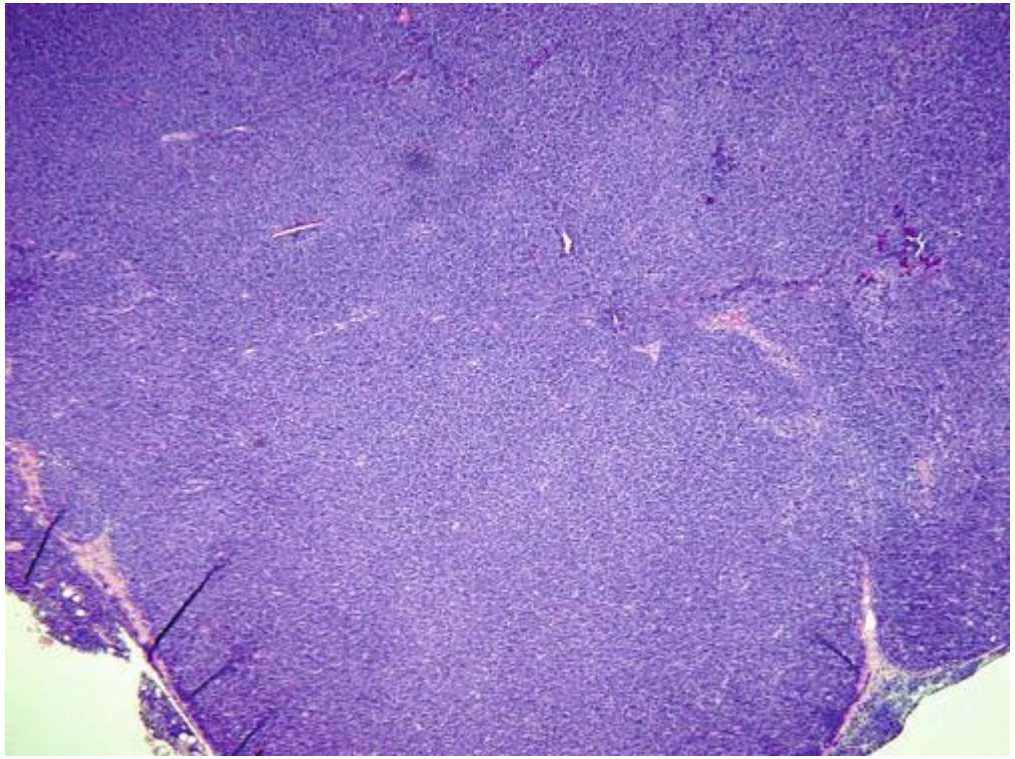

Naive B-cell
CD19+
CD20+
CD5+
B-immunoblast
CD19+
CD20+
sIg+
Plasma cell
CD19-
CD20-
CD38+
CD138+
sIg+
B-cell of
primary follicle
CD19+
CD20+
CD5 +/-
Centroblast
CD19+
CD20+
CD10+
BCL-6+
BCL-2-
sIg-
Germinal centers
Centrocyte
CD19+
CD20+
CD10+
BCL-6+
BCL-2-
sIg+
Memory B cell
CD19+
CD20+
BCL-6-
BCL-2+
sIg+

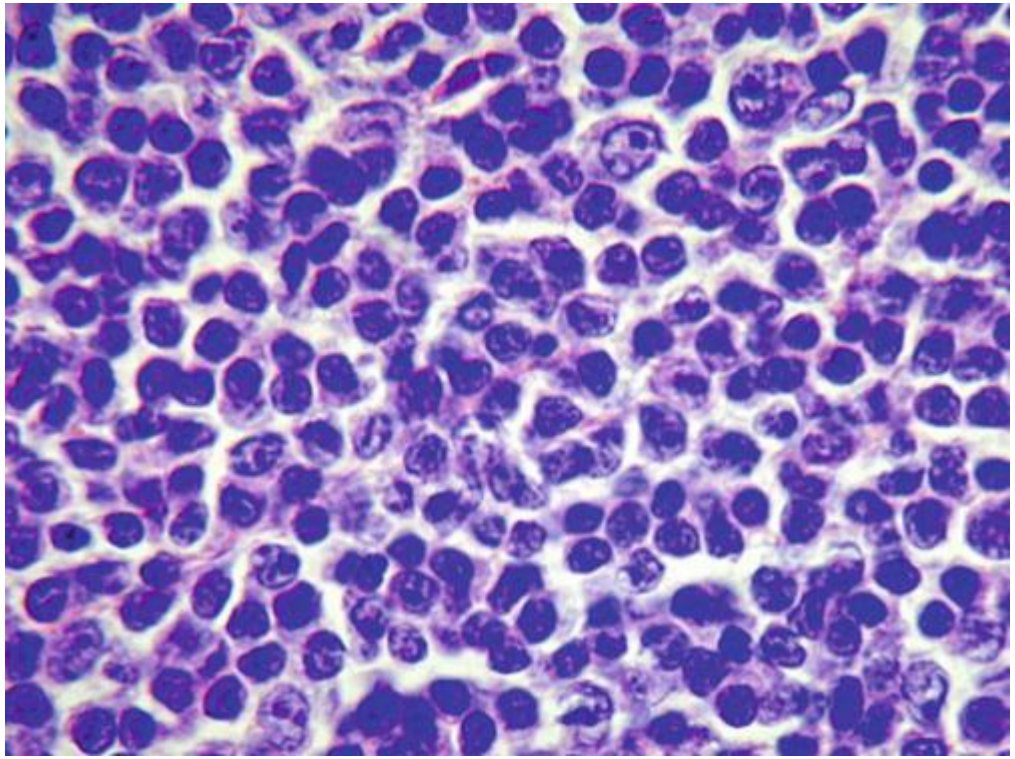
Diffuse Small-Cell Pattern

- **Definition**
  - Diffuse effacement of nodal architecture by small mature lymphocytes.
- **Key Morphologic Features**
  - Loss of follicular structure
  - Monotonous small lymphoid cells
  - ± Proliferation centers
- **Main Differential**
  - CLL/SLL
  - Mantle cell lymphoma
  - Marginal zone lymphoma
  - Lymphoplasmacytic lymphoma
  - FL diffuse pattern

- **Initial Screening Panel**
  - CD20, CD3, BCL6, CD5, CD23, Cyclin D1, BCL6, CD10
- **Diagnostic Clue (CD5+ B-cells)**
  - **CD23+ → CLL/SLL**
  - **Cyclin D1+ → Mantle cell lymphoma**
- **Pitfall**
  No single marker is diagnostic; aberrancies occur.

Diffuse Small-Cell Pattern





CD5-Positive B-Cell Lymphomas

- **Main Entities:** CLL/SLL & Mantle Cell Lymphoma (MCL).
- **Caveat:** CD5 can occasionally be positive in DLBCL or Follicular Lymphoma.
- **Suggested Panel:** CD23, CyclinD1, SOX11, LEF1.
- **Teaching Pearl:** CD5+ B-cell lymphoma is NOT always Mantle Cell Lymphoma.

Immunohistochemistry Panel for Small B-Cell Lymphomas

	CD43	CD5	CD23	Cyclin D1	CD10	BCL6	CD38
SLL/CLL	+	+	+	–	–	–	–
MCL	+	+	–	+	–	–	–
MZL	+/–	–	–	–	–	–	–
LPL	+	–	–	–	–	–	+
FL	–	–	–	–	+	+	–

Summary IHC panel for small B-cell lymphomas (educational use).

CLL/SLL

Morphology

- **Features:** Diffuse small mature lymphocytes, clumped (soccer-ball) chromatin.
- **Proliferation Centers (Pseudofollicles):**
- Pale areas within the infiltrate.
- Contain larger activated cells and paraimmunoblasts.

Immunophenotype

- **Markers:** CD5 (+), **CD23 (+)**, **LEF1 (+)**, CD20 (Dim), CyclinD1 (-).
- **LEF1:** Very useful supportive marker.
- **Dim CD20:** A helpful clue supporting CLL/SLL over other lymphomas.

Mantle Cell Lymphoma

Morphology:

- **Typical:** Monomorphic small-to-medium lymphocytes, irregular nuclear contours.
- **Growth Pattern:** Diffuse or Mantle-zone.
- **Aggressive Variants:** Blastoid and Pleomorphic variants.

Immunophenotype:

- **Markers: CyclinD1 (Nuclear +)**, **SOX11 (+)**, CD5 (+), CD23 (Usually -).
- **Molecular Hallmark:** t(11;14) causing CCND1 rearrangement and CyclinD1 overexpression

- **SOX11 in Mantle Cell Lymphoma**
- **Utility:**
  - CyclinD1-negative MCL cases.
  - Blastoid variants.
- **Teaching Pearl:** SOX11 improves recognition of aggressive and unusual MCL.

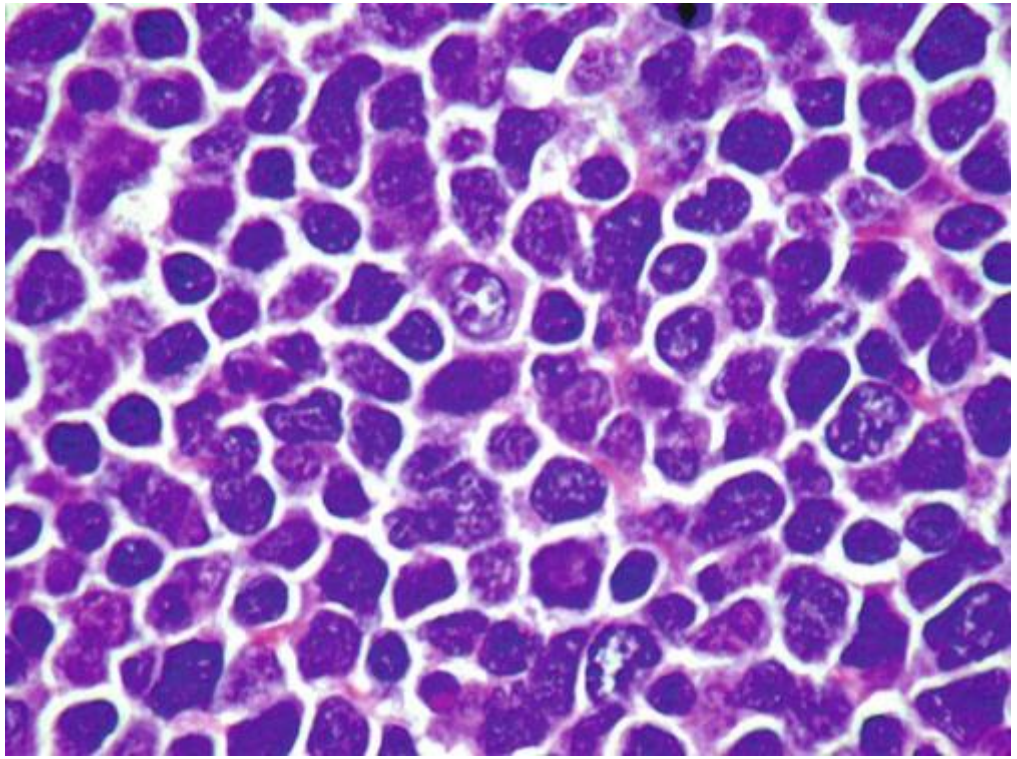
- **Blastoid Mantle Cell Lymphoma**
  - **Morphology:** Blastoid appearance, high mitoses, aggressive behavior.
  - **Differential:** B-ALL/LBL, Burkitt Lymphoma.
  - **Key Markers:** CyclinD1, SOX11, TdT (to rule out ALL).

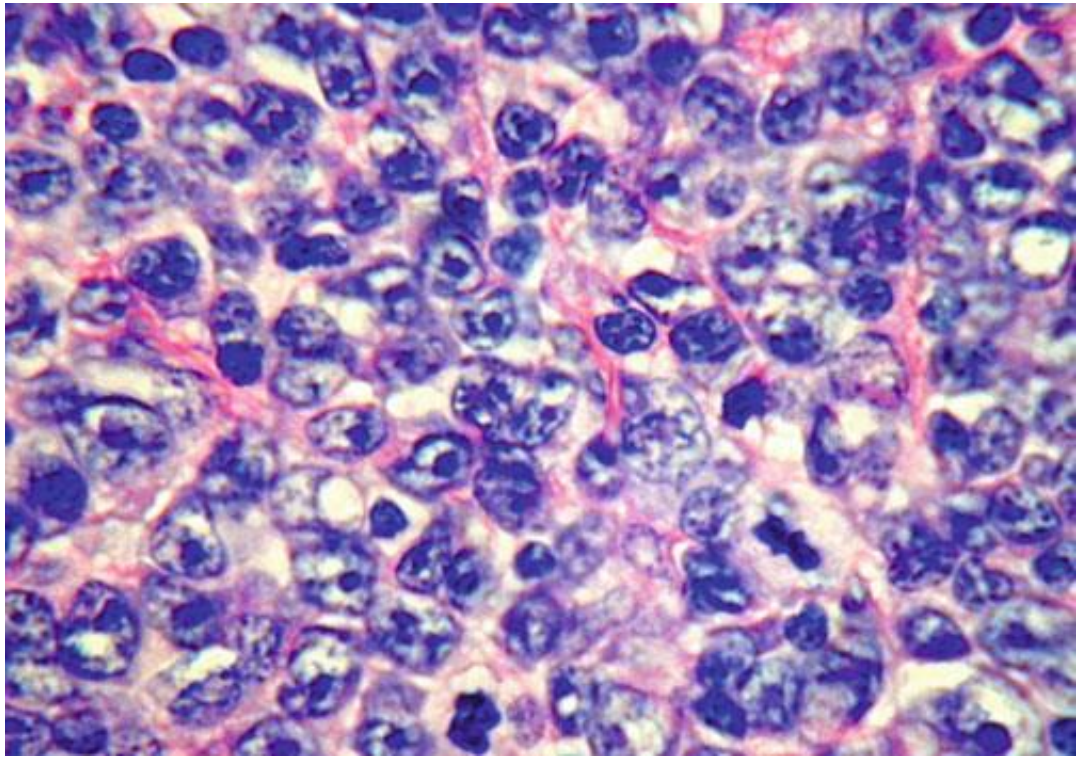
Diffuse intermediate to large-Cell Pattern

- **Definition**
  - Diffuse effacement by large atypical lymphoid cells.
- **Morphologic Clues**
  - Large nuclei, vesicular chromatin
  - Prominent nucleoli
  - High mitotic rate
  - Apoptosis/necrosis frequent
- **Main Diagnostic Considerations**
  - DLBCL
  - Burkitt lymphoma
  - High-grade B-cell lymphoma
  - Anaplastic large cell lymphoma
  - Blastoid lymphoma
- **Initial IHC Approach**
  - **Lineage:** CD20, PAX5; CD3
  - **Proliferation/Biology:** Ki-67, MYC, BCL2, BCL6, CD10, TdT, Cyclin D1

Key Concept
Determine lineage first, then subclassify based on phenotype and genetics.

Diffuse Large-Cell Pattern





Diagnostic Approach to Aggressive B-Cell Neoplasms

- **Entities:** DLBCL, Burkitt, High-grade B-cell lymphoma, B-ALL.
- **Core Panel:** CD20, PAX5, TdT, CD10, BCL6, MUM1, MYC, BCL2, Ki67.
- **DLBCL: Morphology**
  - **Features:** Diffuse architecture, large atypical cells, vesicular chromatin, prominent nucleoli.
- **DLBCL: Immunophenotype**
  - **Markers:** CD20 (+), PAX5 (+), CD10 (Var), BCL6 (Var), MUM1 (Var), Ki67 (High).

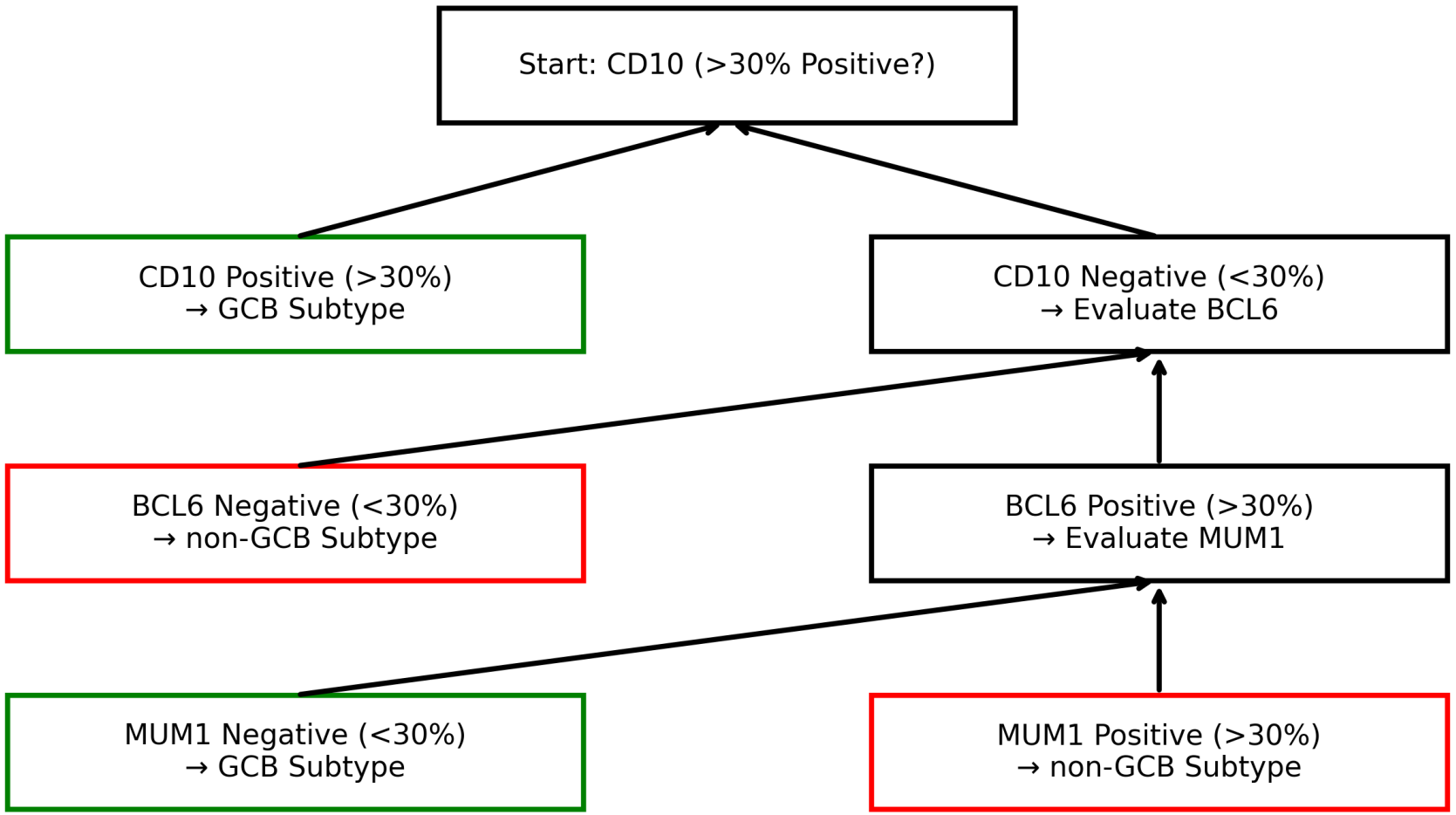
Concept: DLBCL is biologically heterogeneous.

GCB vs ABC/Non-GCB DLBCL (Hans Algorithm)

- **GCB:** CD10+ or (CD10-, BCL6+, MUM1-).
- **Non-GCB/ABC:** BCL6+,MUM1+ or CD10-,BCL6-

Significance: Reflects prognostic differences and underlying biology.

Hans Algorithm



MYC and BCL2 Biology

- **MYC:** Aggressive biology, proliferation, genomic instability.
- **BCL2:** Apoptosis inhibition, cell survival.

Double Expressor Lymphoma
Standard IHC Cut-offs

- MYC ≥ 40% (IHC)
- BCL2 ≥ 50% (IHC)

- **Double Expressor (IHC):** defined by MYC+ and BCL2+ by protein expression.
- **Double Hit (Genetic):** defined by **gene** MYC rearrangement PLUS BCL2 and/or BCL6 rearrangement (FISH).
- **High-yield:** These are NOT synonymous.
- Double expressor lymphomas usually have **worse prognosis than standard DLBCL**, but **not as poor as double hit lymphomas**.

Burkitt Lymphoma

- **Features:** Monomorphic medium-sized cells, “Starry-sky” pattern (due to tingible body macrophages).
- **Clinical:** Highly aggressive, rapidly progressive.
- **Immunophenotype:**
  - **Markers:** CD20 (+), CD10 (+), BCL6 (+), MYC (+), **Ki67 (~100%)**, BCL2 (Usually -).
- **Molecular:** t(8;14) MYC rearrangement.

- **High-Grade B-Cell Lymphoma**
- **Features:** Features intermediate between DLBCL and Burkitt.
- **Classification:** Recognized as a biologically distinct category, often with MYC and BCL2/BCL6 rearrangements.

- **Primary Mediastinal Large B-Cell Lymphoma (PMBL)**
- **Profile:** Young adults, female predominance, mediastinal mass.
- **Markers:** CD20 (+), PAX5 (+), **CD23 (Often +)**, CD30 (Var/Weak).
- **Concept:** Shares biologic overlap with CHL.

- **B-Lymphoblastic Leukemia/Lymphoma (B-ALL)**
- **Morphology:** Diffuse blasts, fine chromatin, scant cytoplasm.
- **Setting:** Bone marrow or mediastinal/nodal masses.

- **Immunophenotype B-ALL:**
- **Markers: TdT (+)**, **CD34 (+)**, PAX5 (+), CD79a (+), CD10 (Often +).

TdT: Interpretation and Pitfalls

- **Biology:** Marker of precursor/blastic differentiation (immaturity).
- **Not Lineage-Specific:** Positive in B-ALL, T-ALL, and some AMLs.
- **Golden Pearl:** TdT indicates immaturity, NOT lineage.

Blastoid Differential Diagnosis

- **B-ALL:** TdT+, PAX5+
- **Blastoid MCL:** CyclinD1+, SOX11+
- **Burkitt:** MYC+, Ki67 ~100%, TdT-
- **AML:** MPO+
- **Important Principle:** Blastoid morphology alone does not establish lineage; IHC is essential.

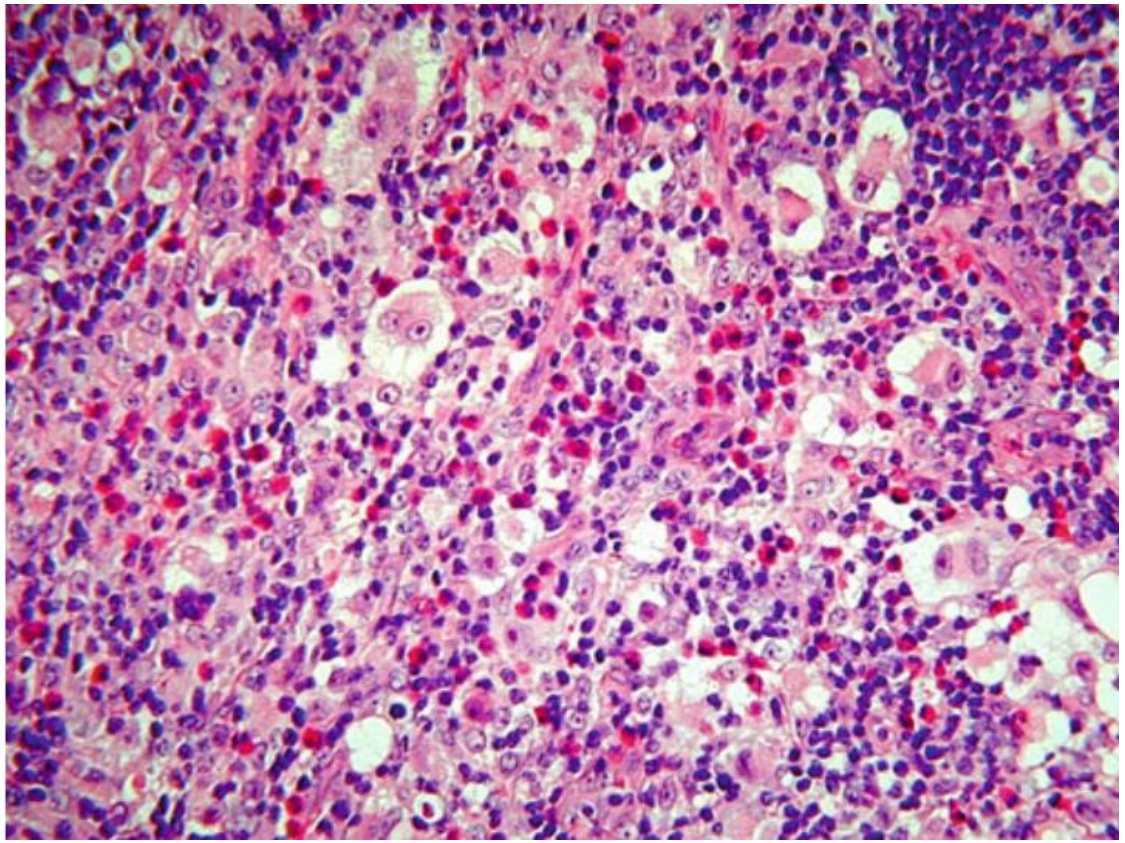
Hodgkin-Like Pattern

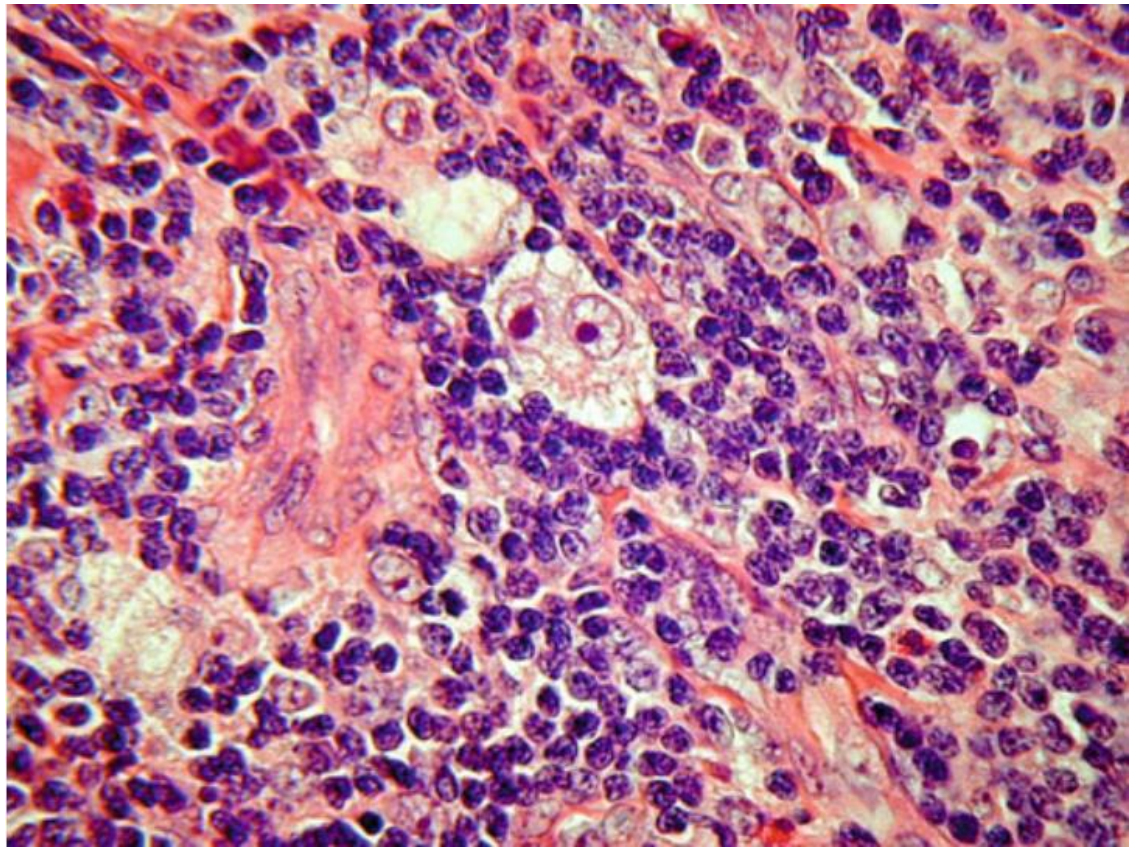
- **Definition**
  - Scattered large atypical cells in a mixed inflammatory background.
- **Morphologic Features**
  - RS-like cells
  - Mixed infiltrate with eosinophils, plasma cells, histiocytes
- **Major Diagnostic Considerations**
  - Classical Hodgkin lymphoma
  - NLPHL
  - T-cell/histiocyte-rich large B-cell lymphoma
  - EBV-related LPDs
- **Initial IHC Panel**
  - CD30, CD15, PAX5, CD20, CD45, EBER

Key Principle

Tumor cells are few; accurate diagnosis requires precise identification and use of internal controls.

Hodgkin-Like Pattern





Application of IHC in lymphoma diagnosis

Abnormal lymphocyte cytology

Plasmacytoid cells
CD138, CD38,
kappa/lambda, CD79a

Blast cells
CD79a, CD3, TdT, MPO,
CD34, CD117, add. lineage

Lymphocyte predominant cells
CD20, CD30, CD15, CD45
PAX5/OCT2/BOB1

Classical Hodgkin cells
CD20, CD30, CD15, CD45
PAX5/OCT2/BOB1, EBV

Hallmark cells
CD30, CD15, CD20,
ALK, CD2, CD5, EMA

Classical Hodgkin Lymphoma: Immunophenotype

- **Marker Profile:**
  - **CD30:** Strong membranous/Golgi (Most sensitive)
  - **CD15:** Usually positive
  - **PAX5:** Weak (Nuclear)
  - **CD20:** Weak / Focal
  - **CD45:** Negative

- **Biologic Concept:**
  - CHL is a B-cell lymphoma with **partial loss** of the B-cell program.

  Pitfalls:
  - CD15 may be negative in some cases.
  - CD20 may be focally positive.
  - CD30 is **not** specific to CHL.

CD30 Pitfalls

- **Nature:** CD30 is an activation marker, not specific for Hodgkin.
- **Conditions with CD30 Positivity:**
  - CHL & ALCL
  - Activated immunoblasts
  - EBV-associated proliferations
  - DLBCL (subset) & PTLD

Practical Point: Interpret CD30 in the context of morphology, distribution, and lineage markers.

Weak PAX5: Why It Matters

- **Biology:** PAX5 is a nuclear B-cell transcription factor, normally strong in mature B-cells.
- **Diagnostic Clue:** In CHL, weak staining reflects the partial loss of phenotype.
- **Differential Significance:**
  - **CHL:** Weak PAX5
  - **DLBCL:** Strong diffuse PAX5
  - **NLPHL:** Strong PAX5

Golden Pearl: Weak PAX5 is one of the most useful clues to differentiate CHL from its mimics.

NLPHL

Morphology

- **Architecture:** Nodular architecture.
- **Cytology:** LP (popcorn) cells with multilobulated nuclei and small nucleoli.
- **Background:** Small B-cell-rich background with preserved FDC meshworks.

Immunophenotype

- **Pattern:**
  - **CD20:** Strong positive
  - **BCL6:** Positive
  - **PAX5:** Strong positive
  - **CD30 / CD15:** Negative
- **Concept:** Unlike CHL, NLPHL retains a mature germinal center B-cell phenotype.

NLPHL vs THRLBCL

- **Key Differences:**
  - **NLPHL:** Favors nodular architecture, preserved FDC meshworks, LP cells.
  - **THRLBCL:** Favors diffuse pattern, absence of nodularity, more aggressive behavior.

- **Shared Features:** Scattered large atypical B-cells in a T-cell-rich background.

Marginal Zone Lymphoma (MZL)

- **Morphology:** Monocytoid B-cells, plasmacytic differentiation.
- **Immunophenotype:** CD20 (+), CD5 (-), CD10 (-), CyclinD1 (-).
- **Common Sites:** Stomach (MALT), Salivary gland, Thyroid.

- **Follicular Colonization**
  - **Definition:** Neoplastic MZL cells infiltrate reactive follicles.
  - **Diagnostic Challenge:** May mimic Reactive Hyperplasia or FL.
- **Teaching Pearl:** MZL is often a diagnosis of **integration and exclusion**.

Practical IHC Algorithm for Lymphoma

Morphologic Pattern	Main Differential Diagnoses	First-Line IHC Panel	Key Diagnostic Pearl
 **Follicular pattern**	Reactive follicular hyperplasia vs Follicular lymphoma	**CD10, BCL6, BCL2, Ki67**	Loss of Ki67 polarization and BCL2 in germinal centers favors FL.
 **Diffuse small B-cell (CD5+)**	CLL/SLL vs Mantle cell lymphoma vs Marginal zone lymphoma vs LPL	**CD5, CD23, Cyclin D1, LEF1, SOX11**	LEF1 and dim CD20 → CLL; Cyclin D1/SOX11 → MCL.
 **Hodgkin-like scattered large cells**	Classical HL vs NLPHL vs THRLBCL vs EBV LPD	**CD30, CD15, PAX5, CD20, CD45, EBER**	Weak PAX5 typical of classical HL.
 **Diffuse large B-cell / aggressive**	DLBCL vs Burkitt vs High-grade B-cell lymphoma	**CD20, CD10, BCL6, MUM1, MYC, Ki67**	Burkitt Ki67 ~100%; double expressor vs double hit.
 **Blastoid morphology**	B-ALL/LBL vs Blastoid MCL vs AML	**TdT, PAX5, Cyclin D1, SOX11, MPO**	TdT = immaturity; Cyclin D1 supports blastoid MCL.

With Deep Appreciation to My Hematopathology Mentors

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