Challenges in the Diagnosis of Hodgkin Lymphomas

Current Concepts in Hematopathology
Harvard Medical School
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DANA-FARBER CANCER INSTITUTE

Boston Children's Hospital
Until every child is well

HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

History

This entity was first described in 1832 by Thomas Hodgkin in a description of seven patients with painless lymph node enlargement

- though microscopic evaluation of these cases 100 years later showed that only 3 were Hodgkin lymphoma, with the remainder being non-Hodgkin lymphoma, tuberculosis, and syphilis

The entity was re-described in 1856 by Samuel Wilks, who in 1865 proposed the name "Hodgkin's disease"

Cytologic features of neoplastic cells described in 1898 and 1902 by Carl Sternberg and Dorothy Reed leading to the name "Reed-Sternberg" cells

- Now known as Hodgkin/Reed-Sternberg cells

Hodgkin lymphomas: common features

Hodgkin lymphomas a B cell lymphomas: now known as “Hodgkin lymphoma” instead of “Hodgkin disease” in recognition of B-cell origin

Hodgkin lymphomas are unusual in that the neoplastic cells are typically a minority of cells in the involved tissue

Majority of cellularity typically comprised of a pleomorphic inflammatory background

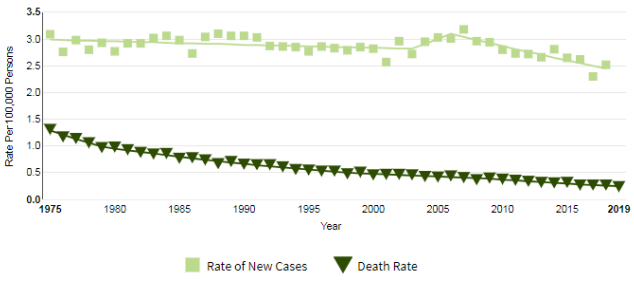
Spread is usually contiguous along lymphatic system

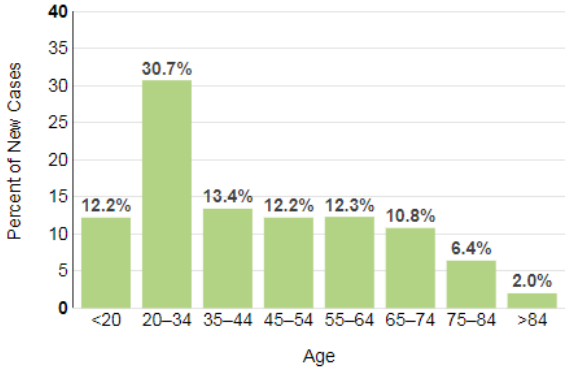
Hodgkin lymphoma epidemiology

In the United States in 2021, Hodgkin lymphoma accounted for:

- 0.5% of all new cancer cases
  - 11.4% of all lymphomas diagnosed in 2018
- 0.2% of all cancer deaths
  - 5 year survival ~88%

Occurs across age groups but most common in adolescents and young adults





SEER Cancer Stat Facts: Hodgkin Lymphoma. National Cancer Institute

Current classification Hodgkin Lymphoma

Classic Hodgkin lymphoma (CHL): ~85% of Hodgkin lymphoma

- Nodular sclerosis
- Mixed cellularity
- Lymphocyte-rich
- Lymphocyte-depleted
- Classic Hodgkin lymphoma, unspecified

Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL)

- Six nodular and diffuse patterns have been described (PMID 14508396)

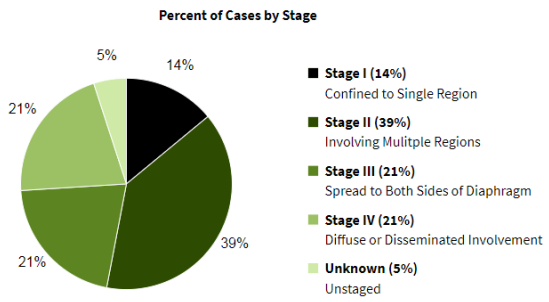
Classic Hodgkin lymphoma: presentation

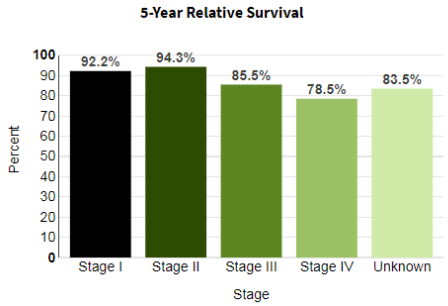
Often involves cervical and supraclavicular lymph nodes

Contiguous spread along lymphatics typical

- Non-contiguous spread more common in mixed-cellularity and lymphocyte-depleted subtypes

Abdominal nodes usually not involved unless cervical and mediastinal nodes are as well



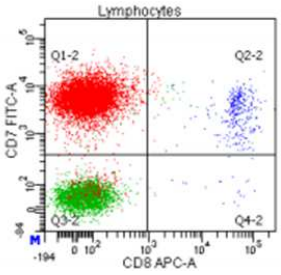


SEER Cancer Stat Facts: Hodgkin Lymphoma. National Cancer Institute

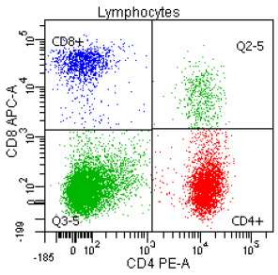
HL flow cytometric features

Most centers do not use flow cytometry to diagnose CHL

- Nevertheless, elevated CD4:CD8 ratio in T cell population has been reported in CHL (and reactive lymph nodes and NHL)
- CD4/CD8 double positive T cells common in NLPHL (and reactive lymph nodes)



CD4:CD8 ratio in this CHL case was 25:1



3.7% double positive T cells in this NLPHL case

Classic Hodgkin lymphoma: genetics

Cytogenetic and molecular studies not required for diagnosis.

- Difficult to perform due to low percentage of HRS cells

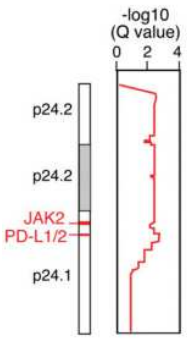
Single-cell studies have provided insight:

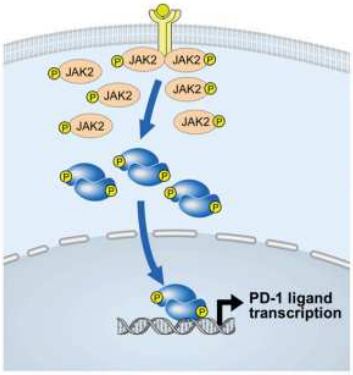
- Rearrangement of immunoglobulin genes but lack of Ig expression
- Aneuploidy
  - Recurrent gains of 2p, 9p, 17q, 19q, 20q
  - Recurrent losses of 6q, 13q
- Mutations in JAK/STAT pathway

Therapeutic significance

Gains at 9p and JAK/STAT pathway alterations increase JAK/STAT activity and PDL1/2 transcription

Clinical responses to checkpoint inhibition





Liu and Shipp, Blood, 2017

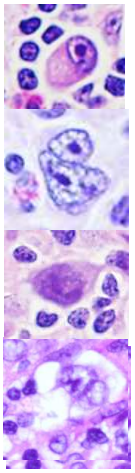
Classic Hodgkin lymphoma: common morphologic features

Neoplastic cells collectively referred to as Hodgkin/Reed-Sternberg cells

- Large cells with a variable amount of eosinophilic or amphophilic cytoplasm
- Large nuclei with a single large nucleolus
  - Hodgkin cells: mononuclear variants
  - Reed-Sternberg cells : multinucleated variants
  - Mummified cell variants: degenerated and/or apoptotic HRS cell (darkly staining with dense pyknotic nucleus)
  - Lacunar cell variants: HRS cell with abundant clear cytoplasm, artifact of formalin fixation; most common in nodular sclerosis subtype

Inflammatory background

- Differences across subtypes, but typically the vast majority of cells, comprised of a polymorphous population of small lymphocytes (mostly T-cells), plasma cells, histiocytes, eosinophils and neutrophils



Classic Hodgkin lymphoma: immunophenotypic features of HRS cells

HRS cells show common immunophenotypic features regardless of subtype

Positive for PAX5 (usually weak), but typically negative for other B cell markers

- CD20 or CD79a can occasionally be positive, but with variable staining (not diffuse positivity)

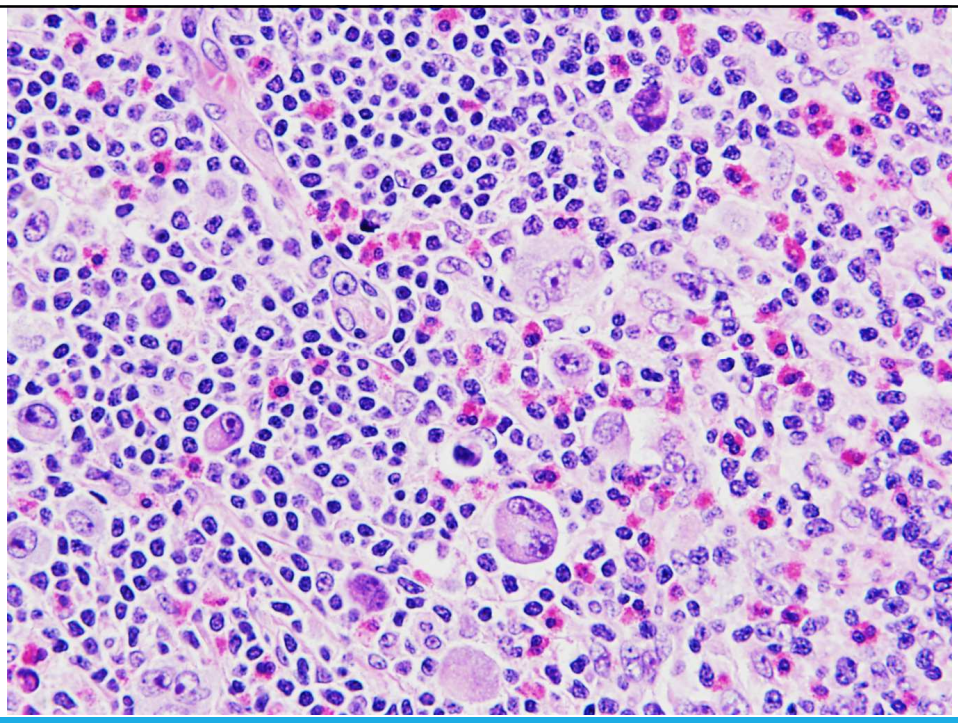
Positive for CD30 (nearly all cases) and CD15 (~75%)

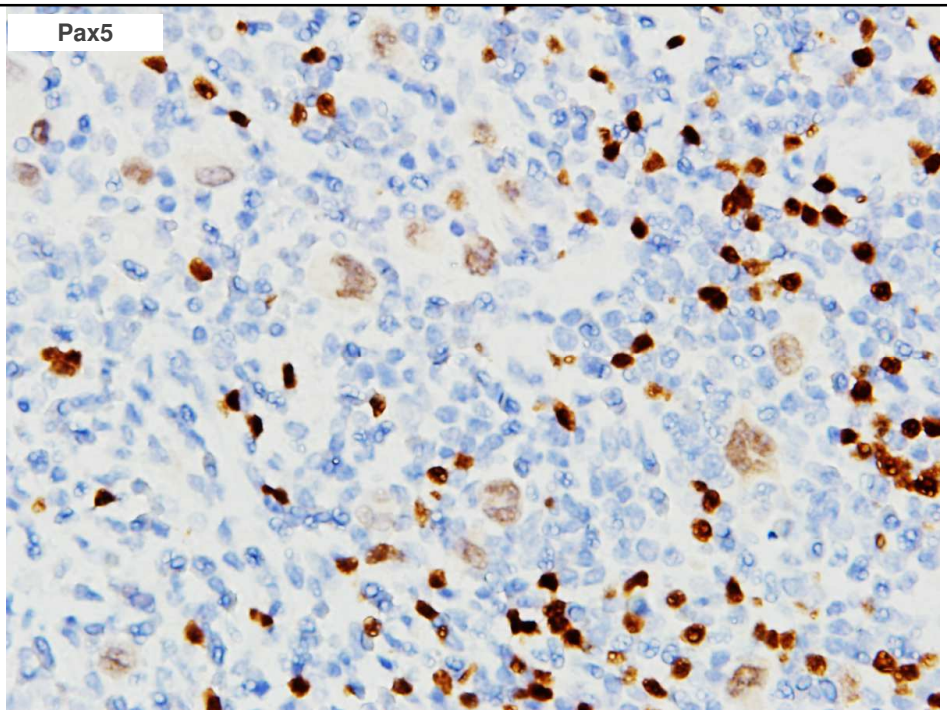
- Membrane and golgi staining pattern

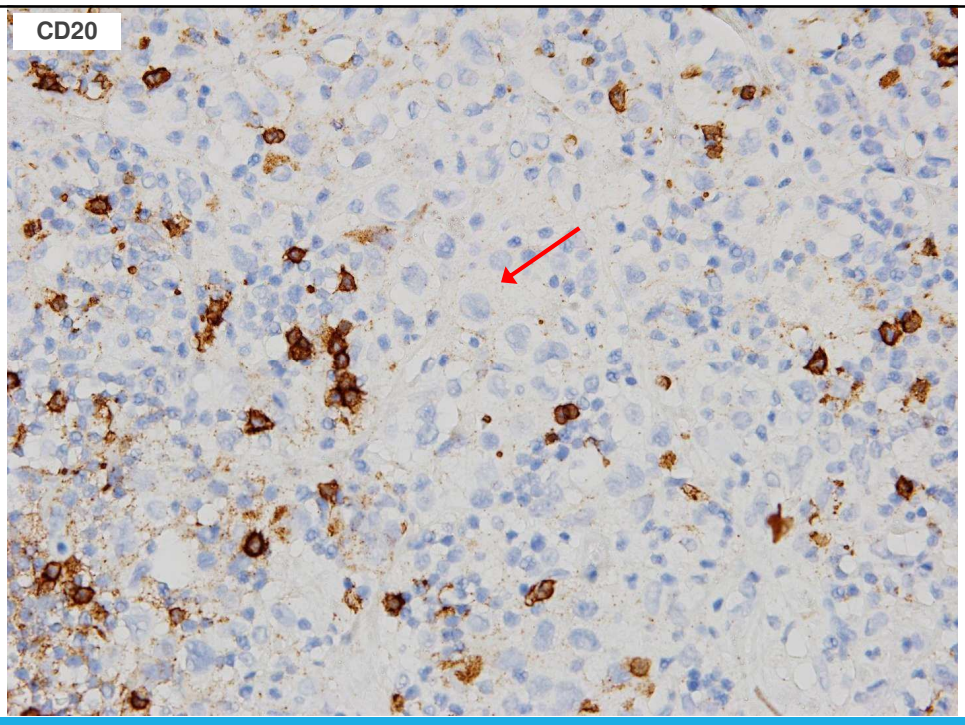
Typically negative for CD45, T cell markers, J chain, IG heavy and light chains, and the B cell transcription factors OCT2 and BOB1

Markers of EBV infection: EBER in situ hybridization or immunostain for EBV-LMP

- Less common in nodular sclerosis, more common in mixed cellularity (~75%)




Pax5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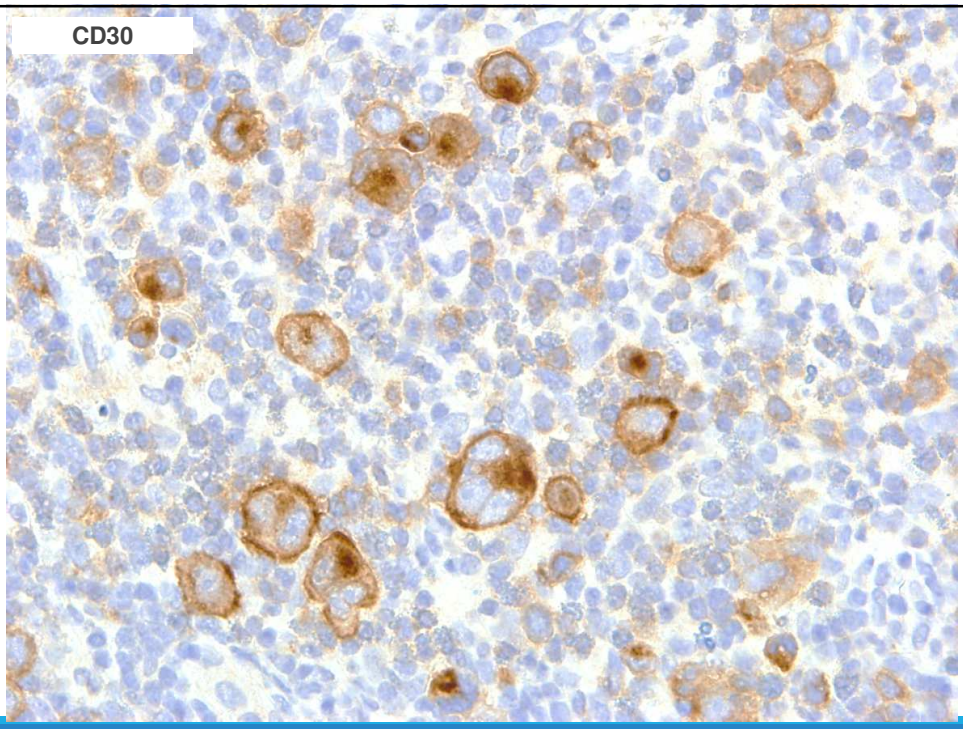
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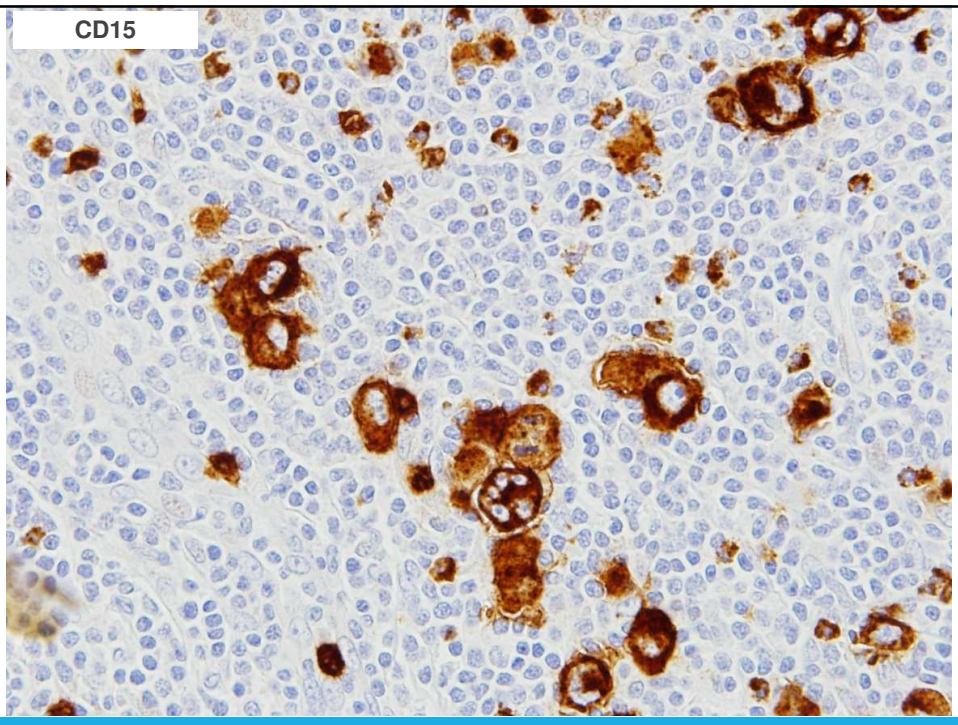
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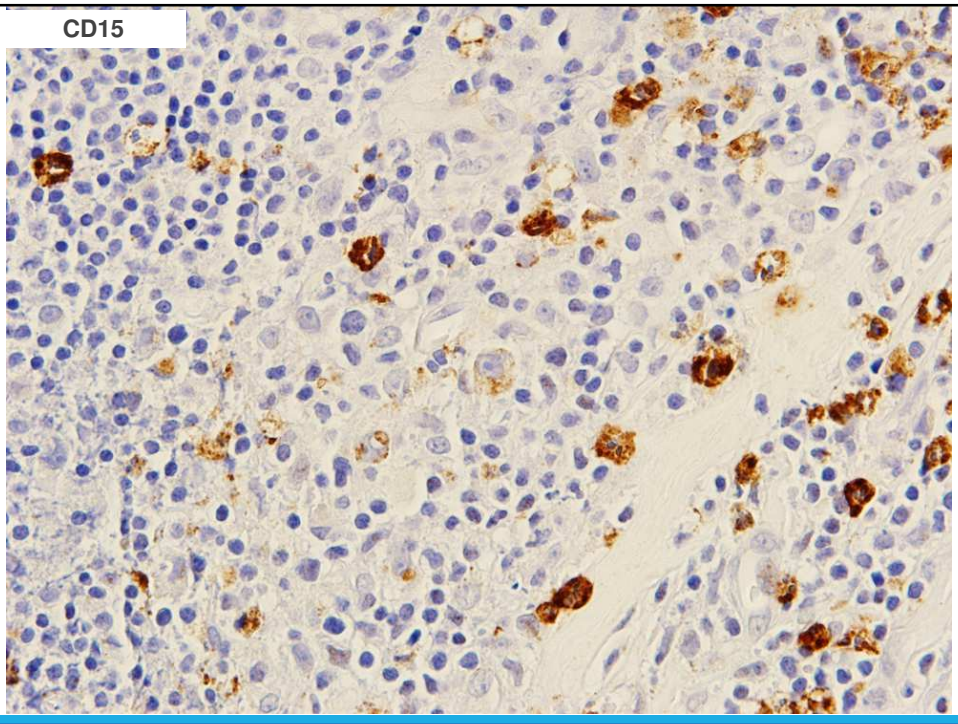
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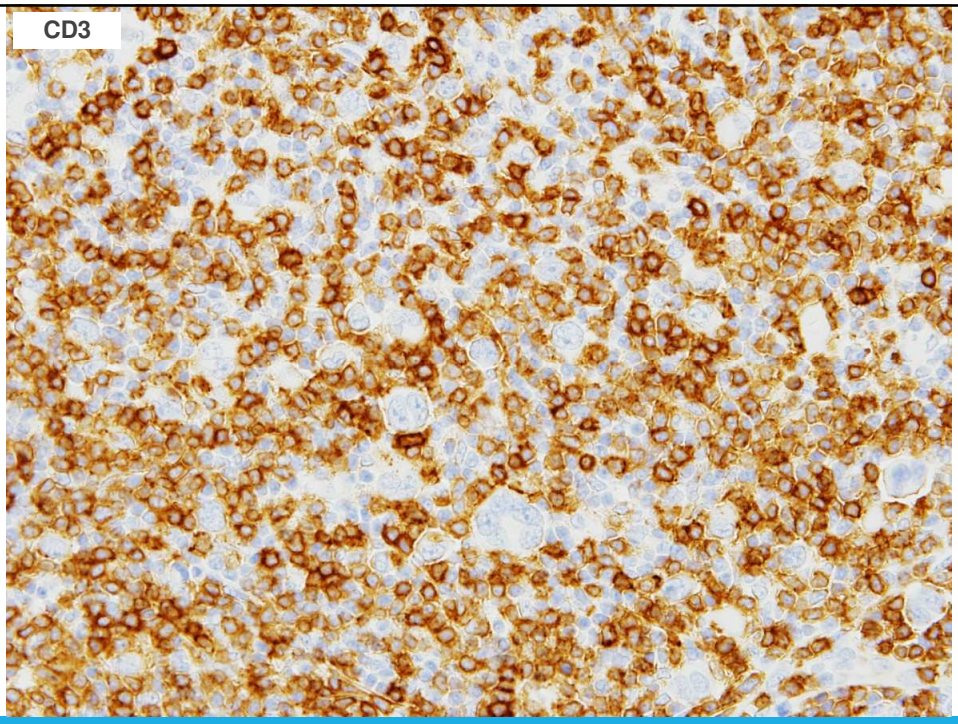


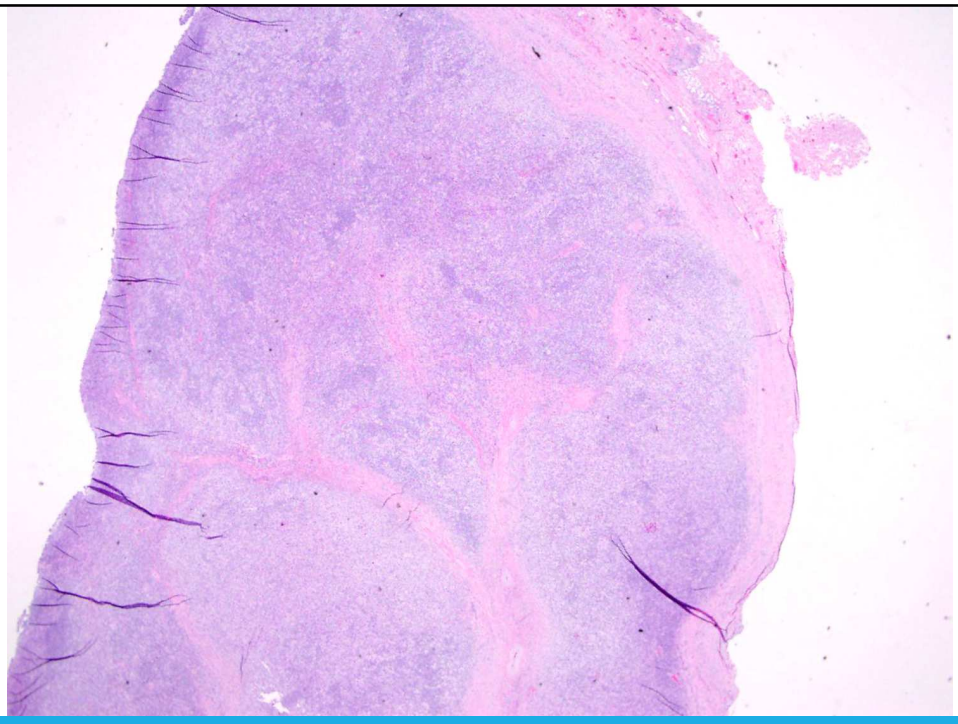
Nodular sclerosis CHL

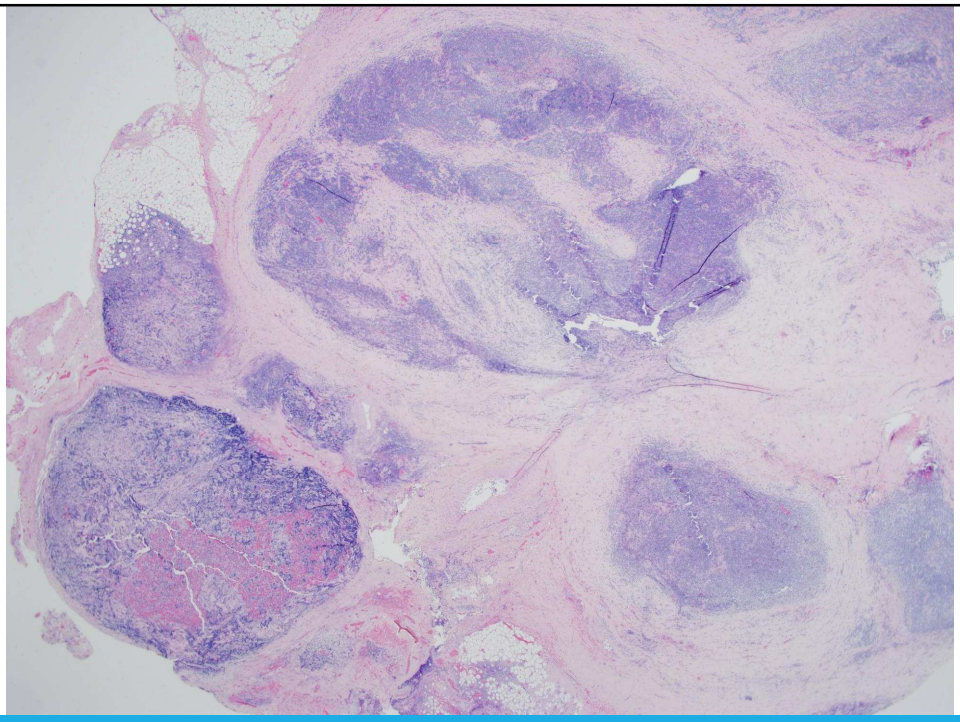
Most often involves mediastinal, supraclavicular, and/or cervical lymph nodes

Subtly to grossly nodular; cellular nodules separated by collagen bands with thickened capsule

Cellularity of nodules comprised of inflammatory background and variable numbers of HRS cells

- Lacunar cells particularly common in this subtype
- Foci of necrosis often present
- A syncytial variant has been proposed, in which HRS cells occur in sheets





Mixed cellularity CHL

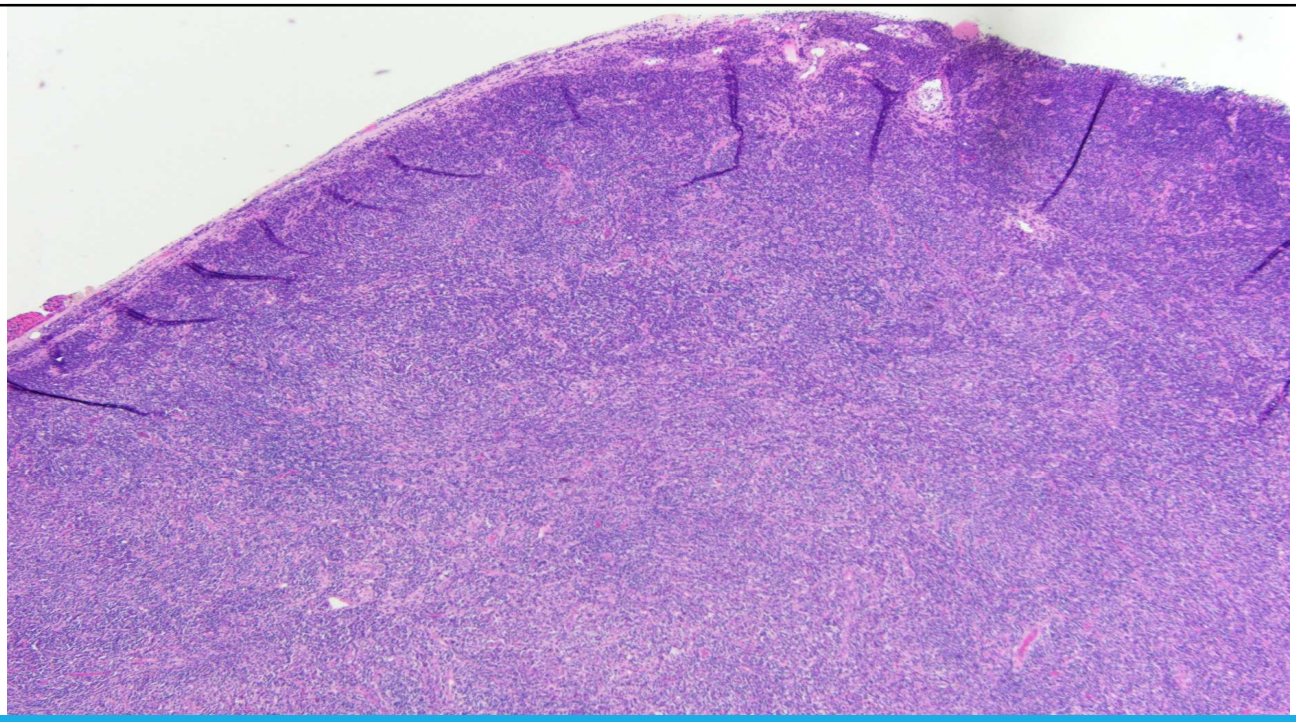
Involved lymph nodes without nodularity or collagen bands
- Typically diffuse effacement of node, though may also show interfollicular pattern

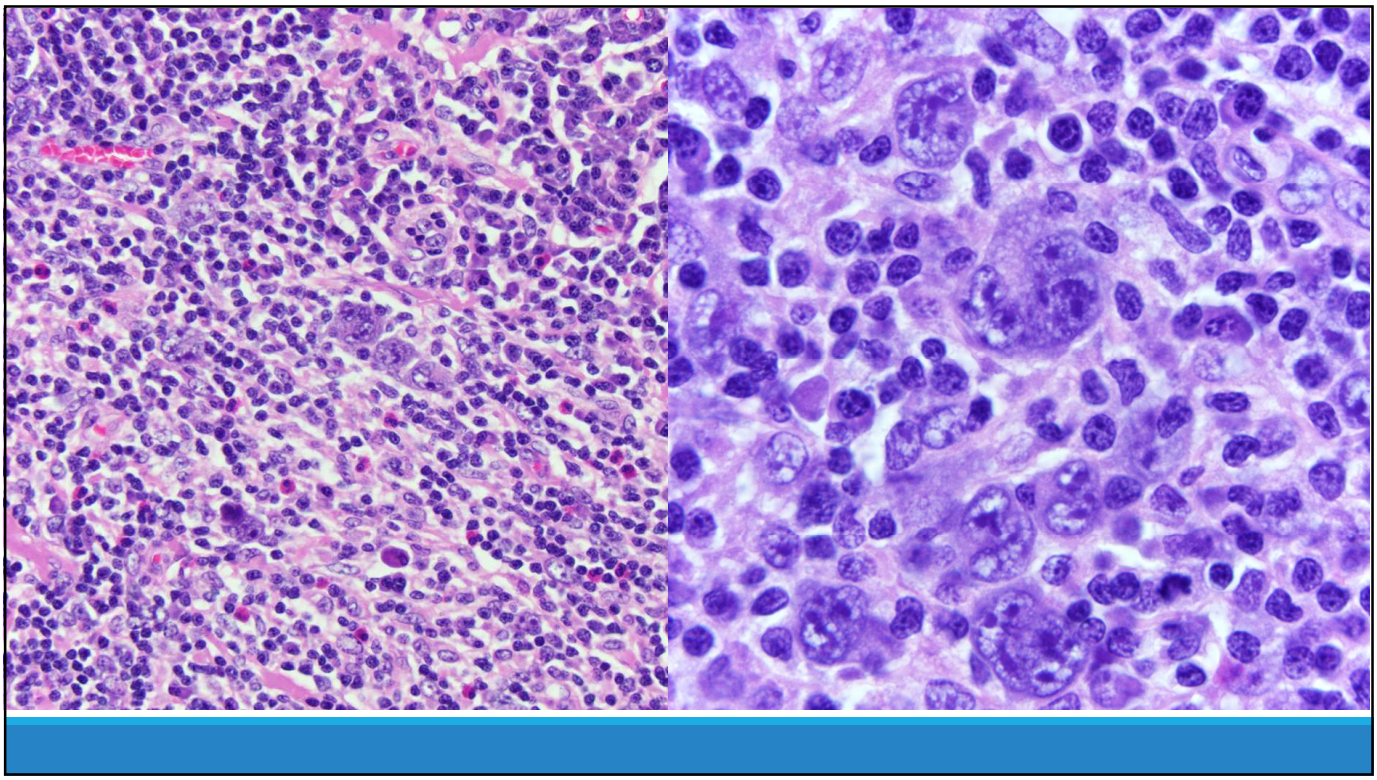
HRS cells present within mixed inflammatory background

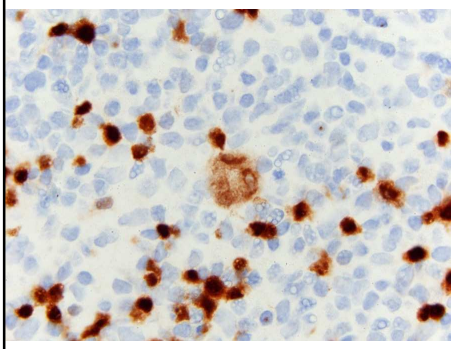
Epithelioid histiocytes or granulomas may be seen

More often EBV-positive

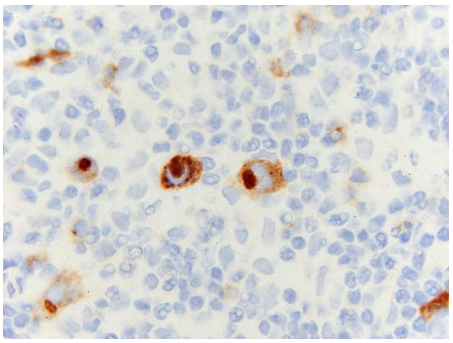
Most common subtype in patients with HIV/AIDS



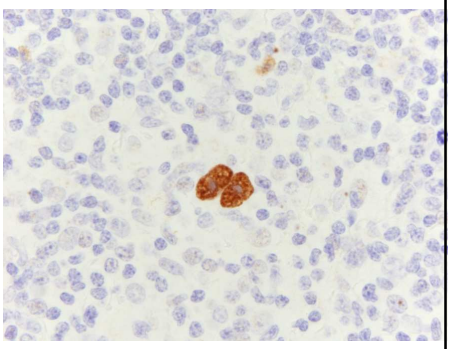




PAX5



CD30



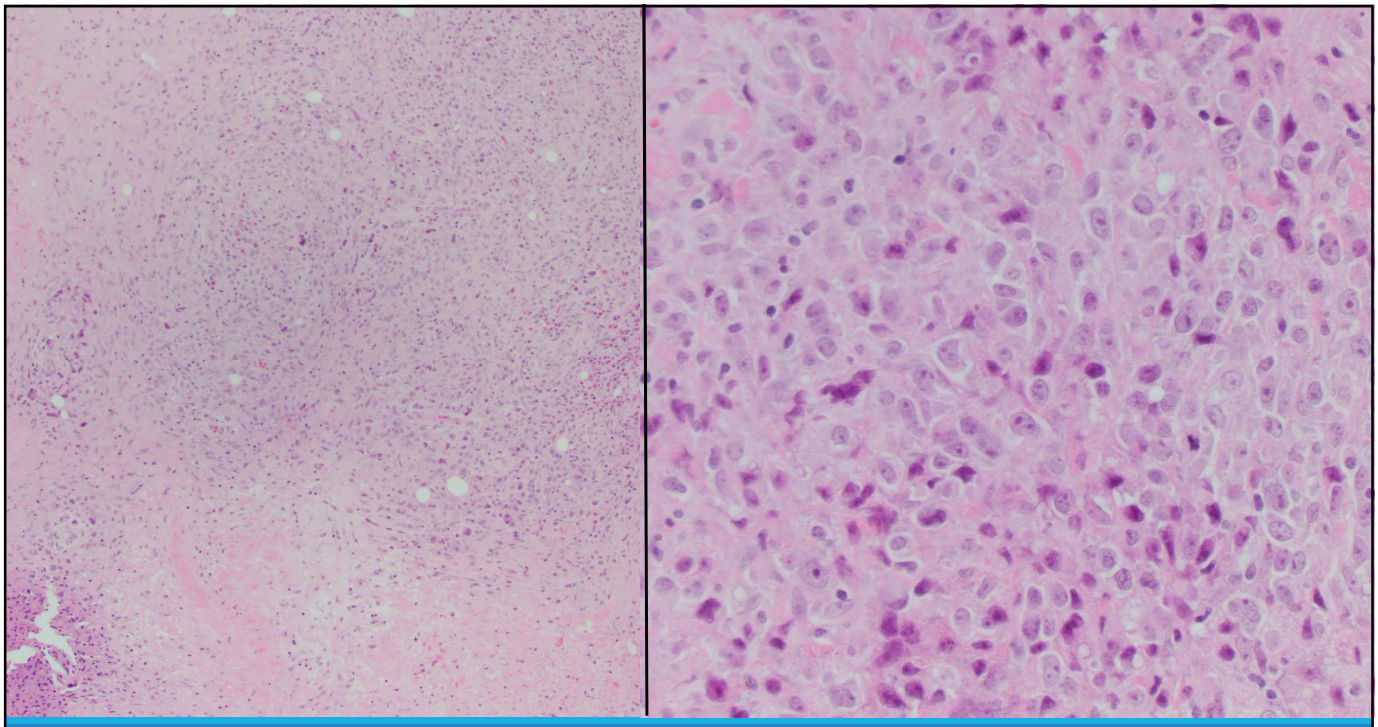
EBER

Lymphocyte-depleted CHL

Greater proportion of neoplastic cells with fewer lymphocytes in reactive background

Like mixed cellularity CHL, lymphocyte-depleted CHL is often EBV-positive and can occur in patients with HIV/AIDS

Immunophenotyping may be needed to distinguish from other large cell lymphomas



Lymphocyte-rich CHL

HRS cells in a predominantly lymphocytic background, with greater numbers of small B cells

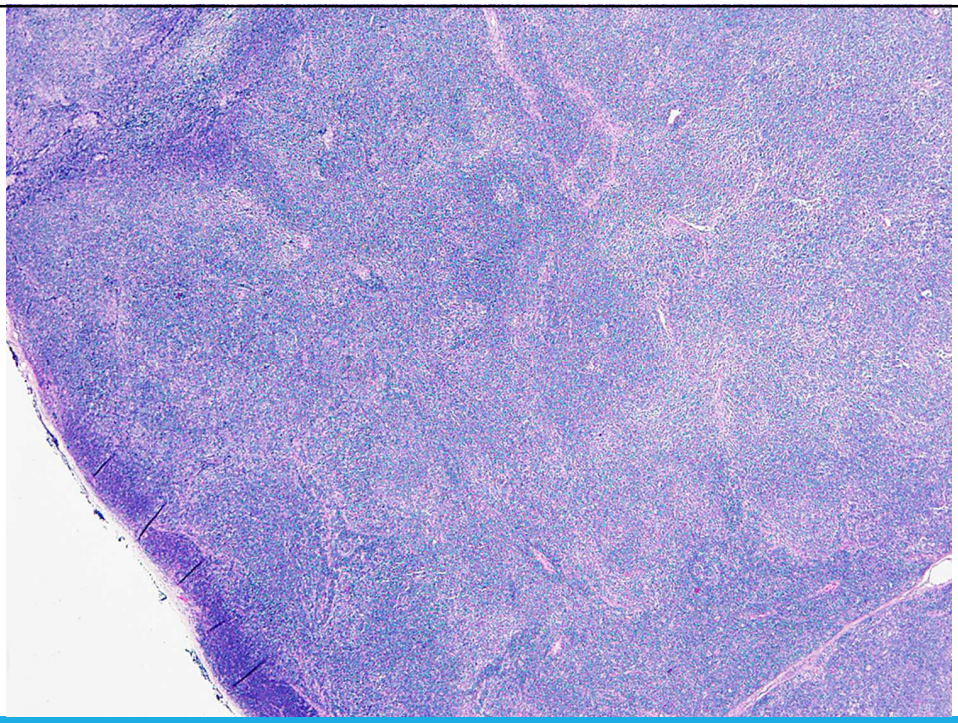
- Background lymphocytes may show nodular pattern
- May show reactive follicles and/or regressed germinal centers
- Few eosinophils and neutrophils

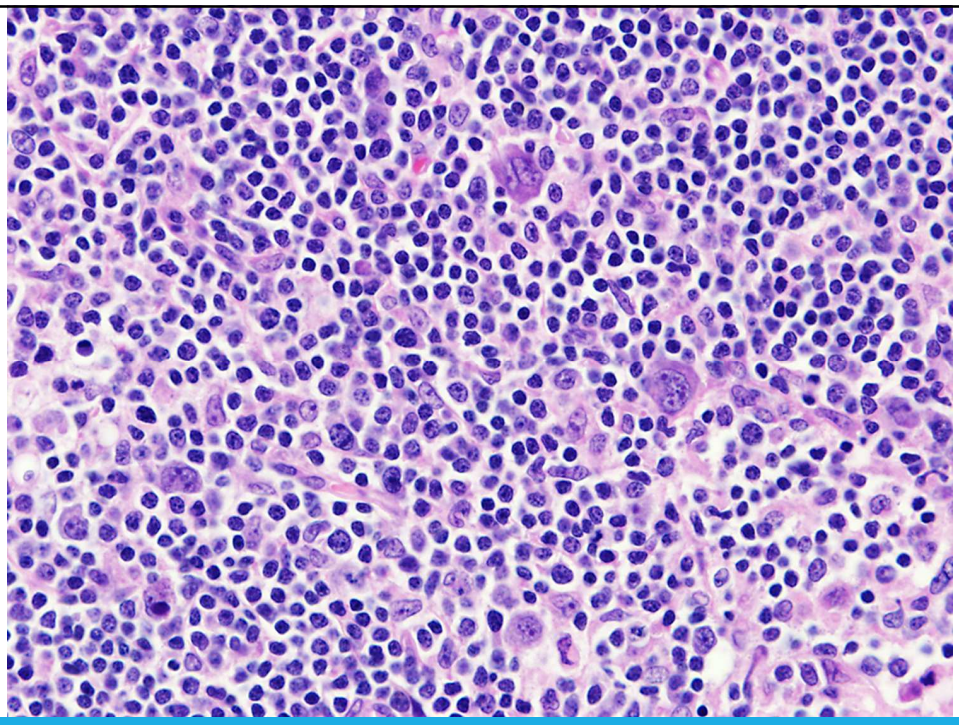
Overall histology may resemble nodular lymphocyte predominant HL

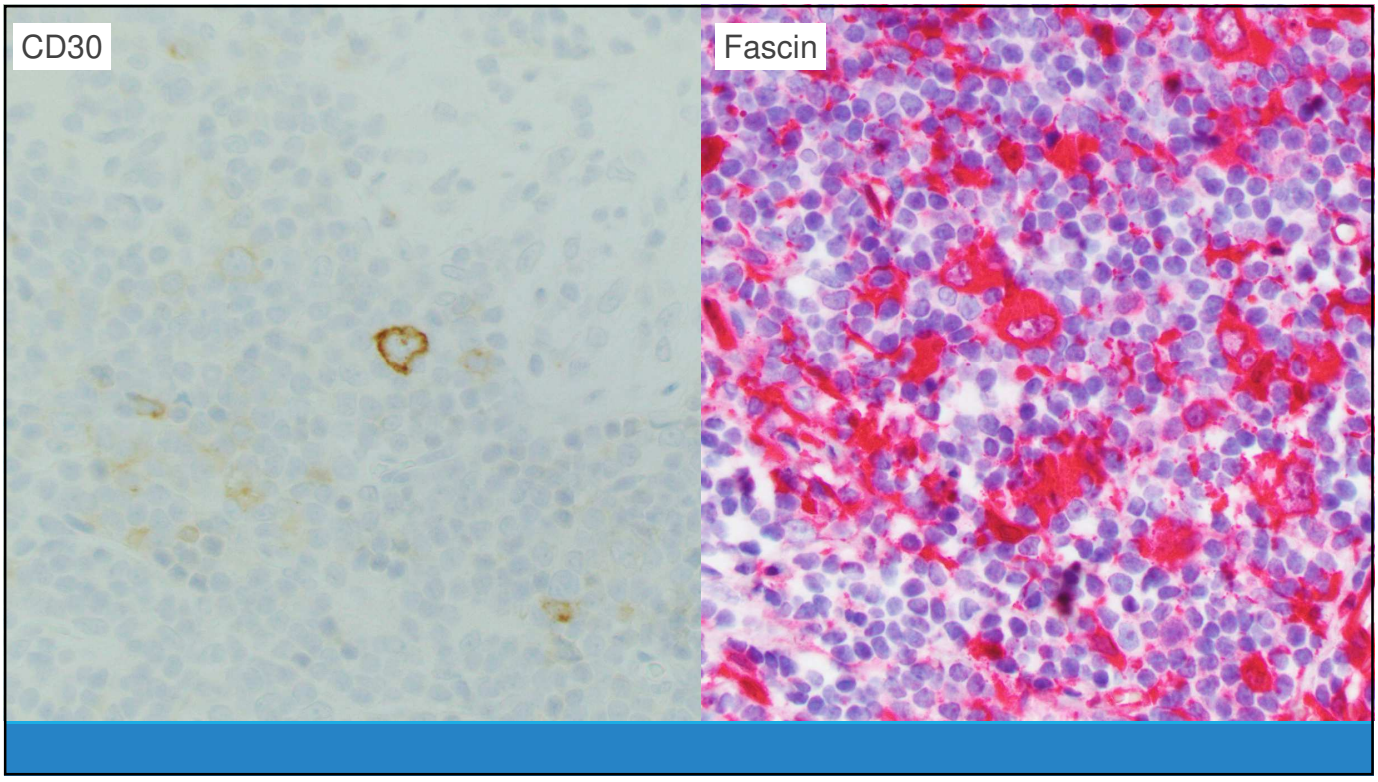
- Distinguished by typical immunophenotype of HRS cells
- Fascin may useful for this differential:
  - positive in CHL; negative in NLPHL
- OCT2 and BOB1
  - One or both lost in CHL; preserved in NLPHL

	NLPHL	CHL
CD20	+	-
BSAP (PAX5)	+	weak
OCT2	+	-
BOB1	+	-
CD45	+	-
CD30	-	+
CD15	-	+
Fascin	-	+

PMIDs: 9033270, 19550297, 31075666






CD30
Fascin

CHL challenges

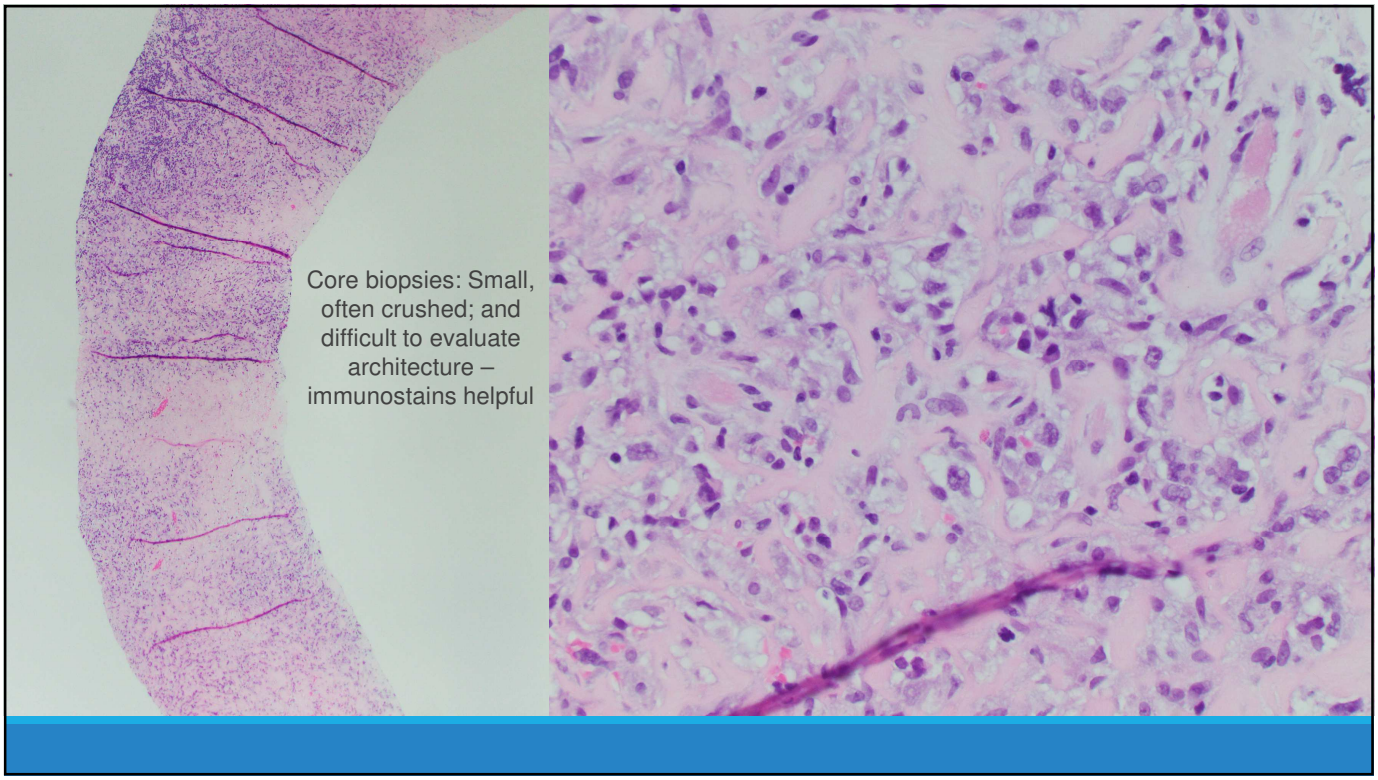
Specimen-related: need a good amount of tissue to evaluate architecture and sparse HRS cells

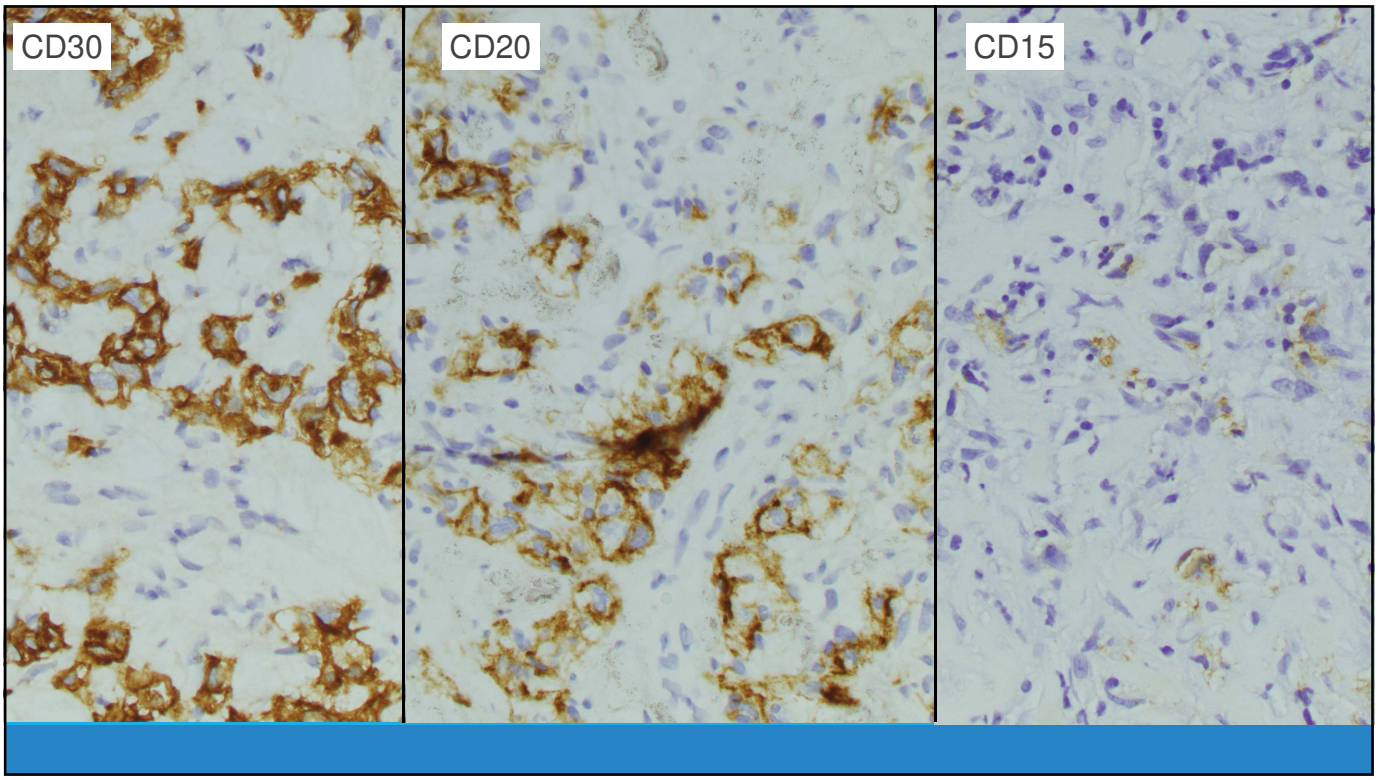
- Core biopsies
- Partial involvement of node
- Smears
- Frozen sections

Challenging differentials

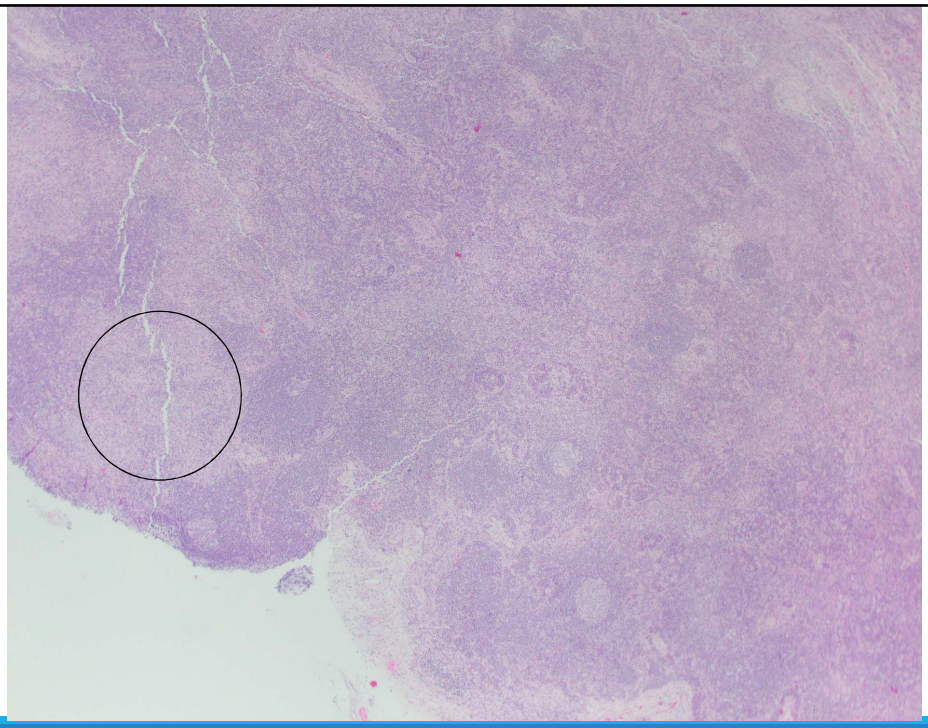
- CD30+ immunoblasts in various contexts
- EBV infections: infectious mononucleosis, post-transplant lymphoproliferative disorders
- Lymphocyte-rich CHL vs NLPHL
- Grey zone lymphomas

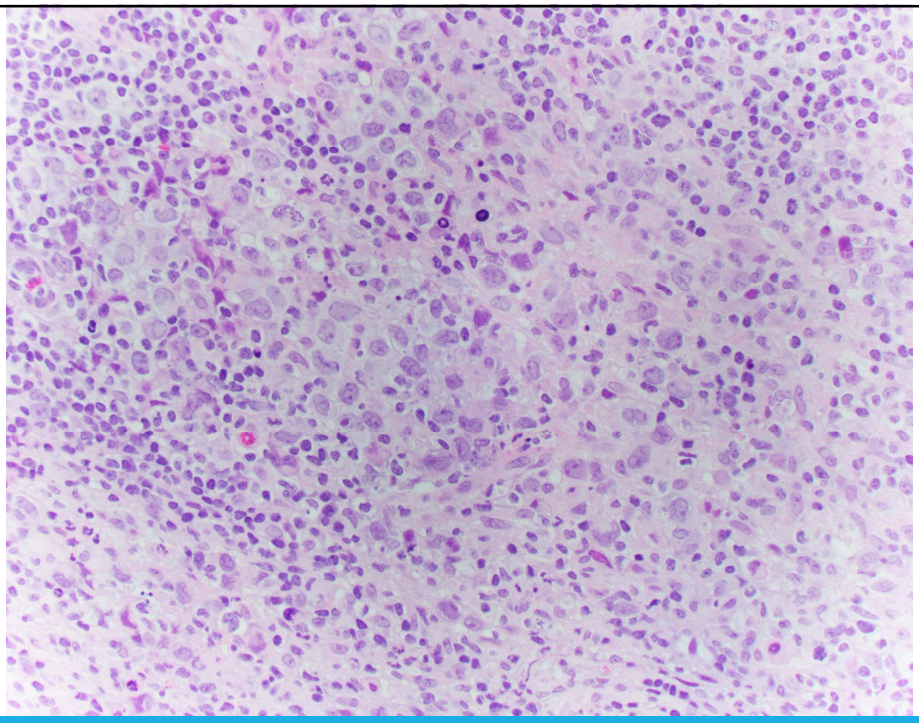
Use clinical context, tissue architecture, morphology, and immunophenotype to arrive at diagnosis

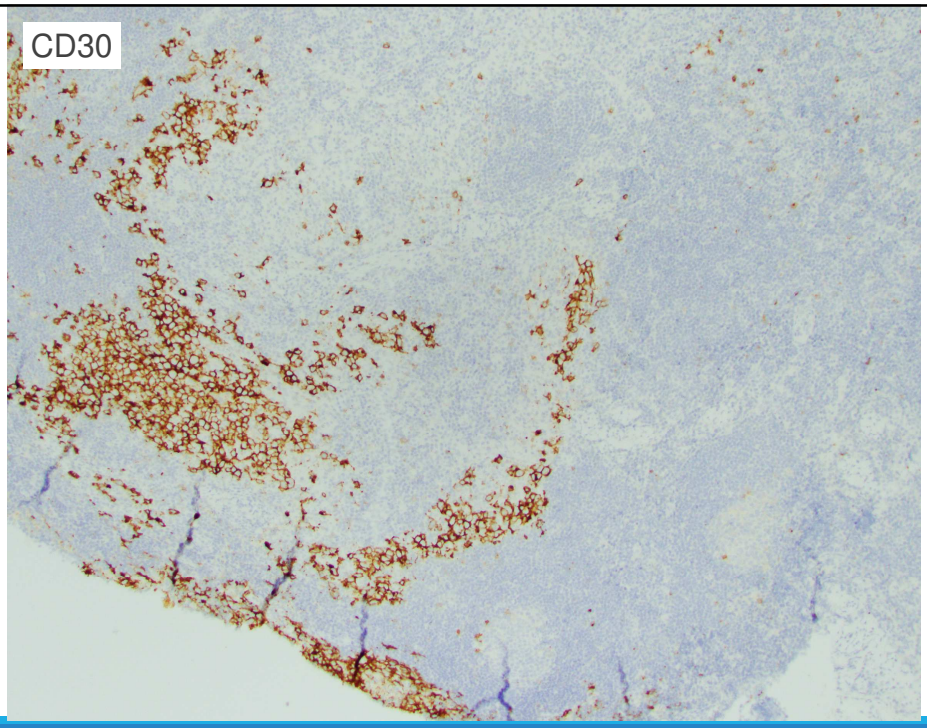




Partial involvement

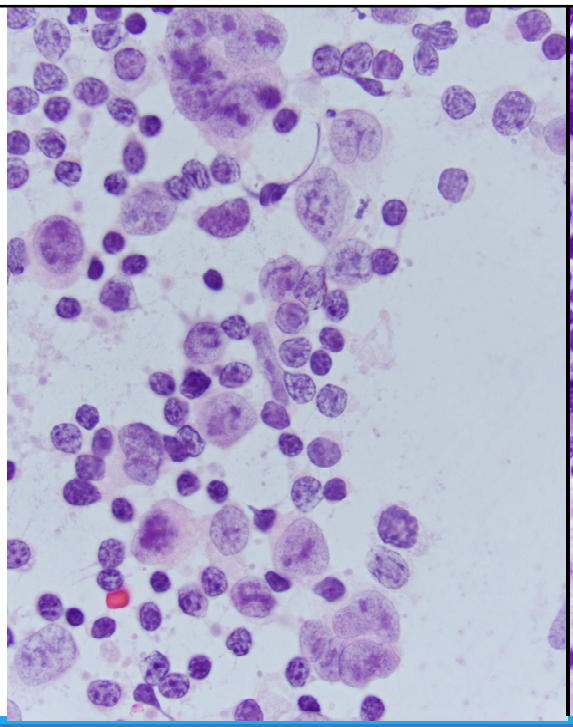


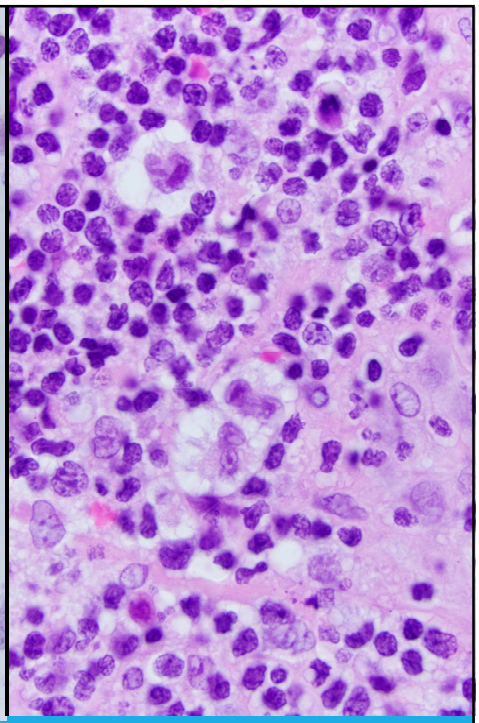




CHL smear

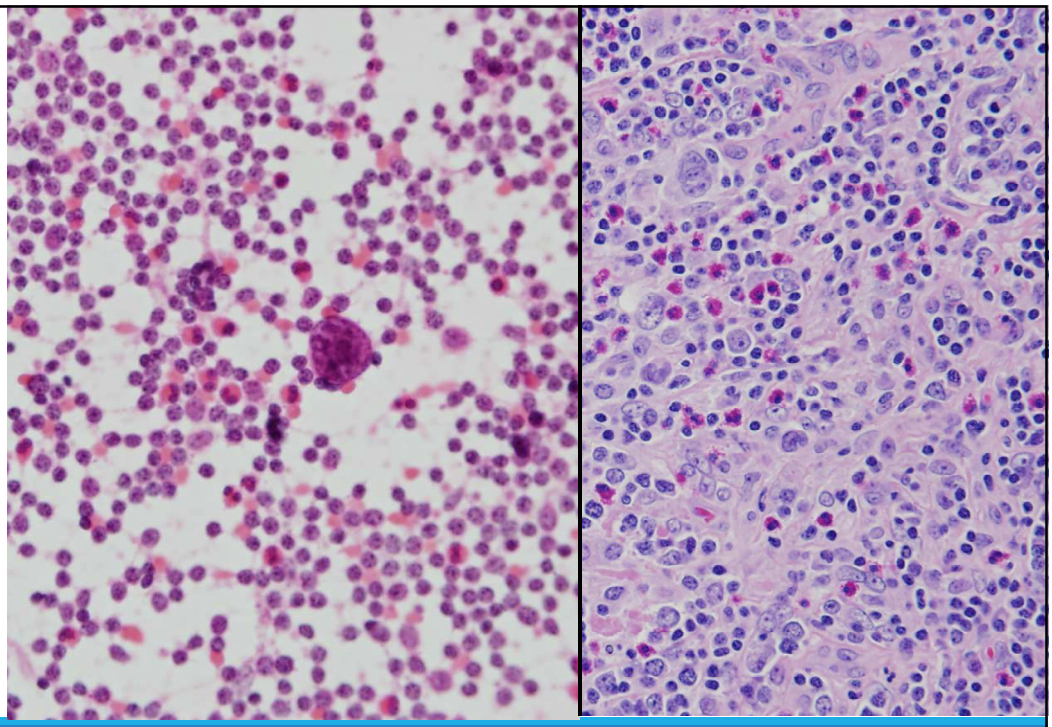
Frequent atypical cells





CHL smear

Polymorphous background and atypical cell



Nodular lymphocyte-predominant HL

5-10% of all Hodgkin lymphoma are NLPHL
- Distinct entity from classic Hodgkin lymphoma
- Male predominance with wide age distribution

Common presentation of isolated, often longstanding, lymphadenopathy
- ~40% cervical or axillary; ~20% iliac or inguinal

Neoplastic cells called LP cells ("lymphocyte predominant")
- Also known as "popcorn cells" or L&H cells ("lymphocytic and histiocytic")
- Large, multilobated cells with single or multiple nucleoli and pale cytoplasm

Partial or complete architectural effacement by nodular proliferation of lymphocytes and histiocytes, with scattered, usually single, LP cells
- Unlike CHL, granulocytes do not form part of the background of NLPHL

NLPHL: immunophenotype

LP cells are generally positive for B cell markers, including CD20 (unlike CHL), CD22, and CD79a, though may be negative for CD19

Positive for B cell transcription factors PAX5, OCT2, BOB1, PU.1

Positive for CD45 and BCL6, but not CD10

Typically negative for CD30 (though have focal/weak positivity), almost always negative for CD15

T-follicular helper (T_{FH}) cells form rosettes around LP cells

- T_{FH} cells positive for CD3, PD1, CD57

NLPHL: growth patterns

Six immuno-architectural patterns have been described: A-F

Patterns A and B are B cell-rich and comprise ~75% of cases

- Classic nodular pattern
- Serpiginous/interconnected nodular pattern

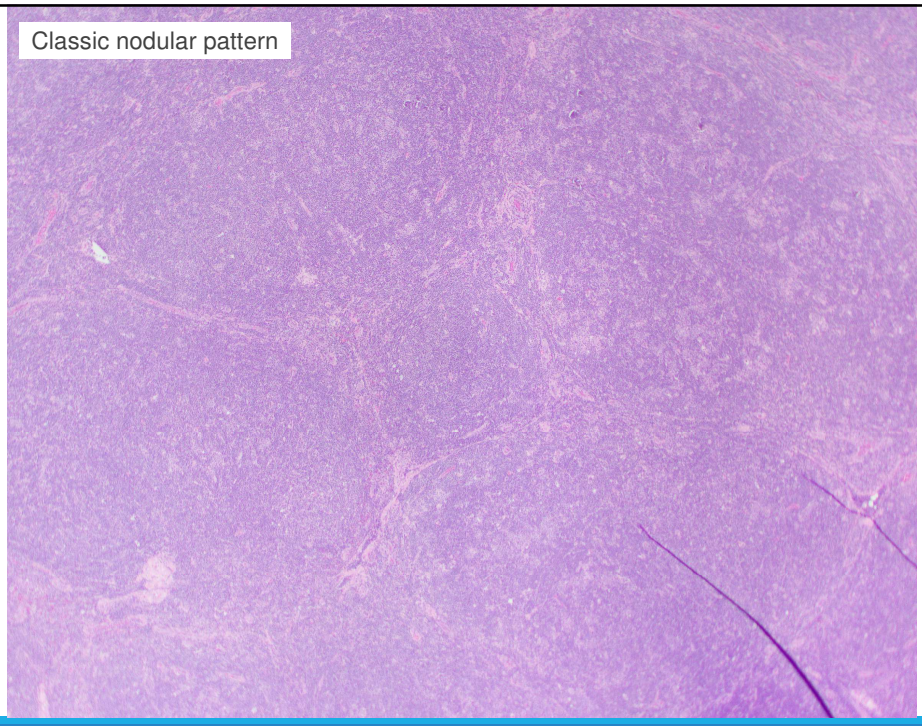
Patterns C-F are variant patterns (~25% cases)

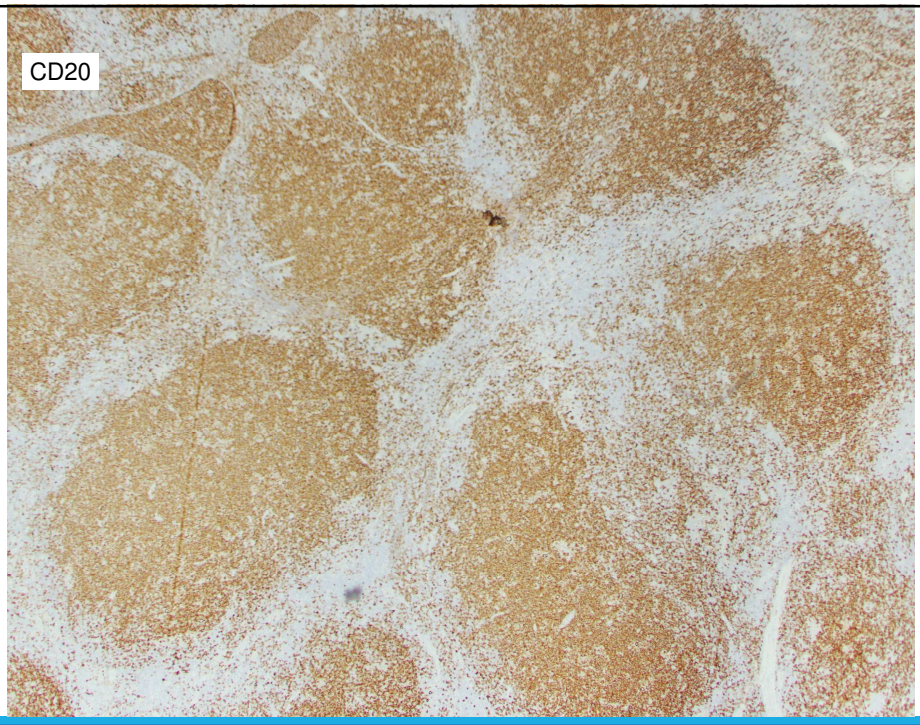
- Nodular with prominent extranodular LP cells
- Nodular with T-cell rich backbround
- Diffuse pattern (THRLBCL-like)
- Diffuse, “moth-eaten” with B-cell rich background

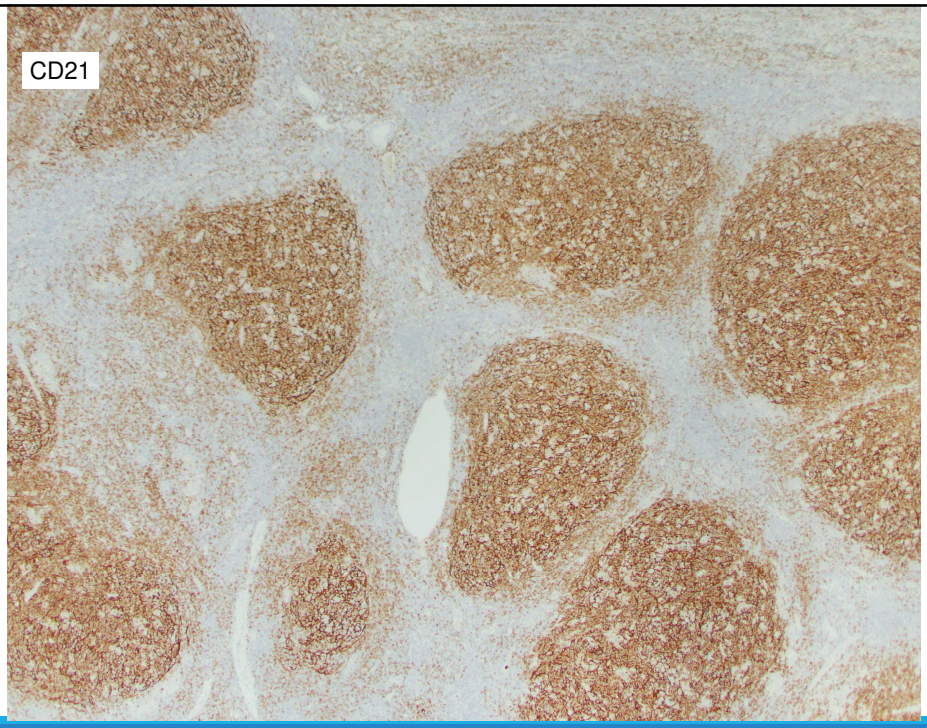
Challenging differentials:

- NLPHL vs T cell/histiocyte-rich large B-cell lymphoma (THRLBCL)
- Progressive transformation of germinal centers (PTGC) vs NLPHL

Fan et al, Am J Surg Path, 2003


Classic nodular pattern


CD20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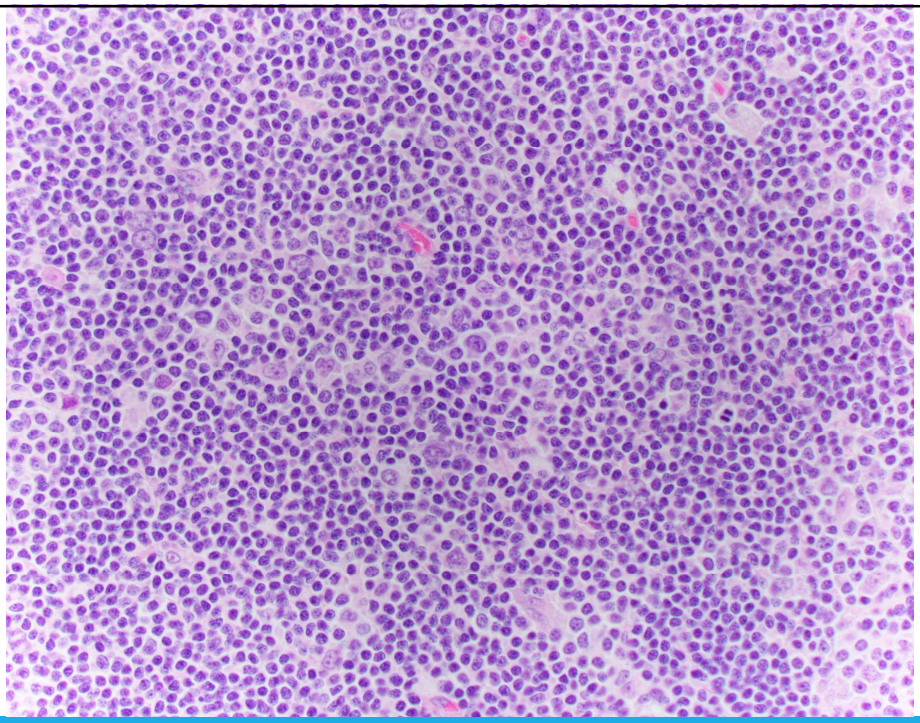

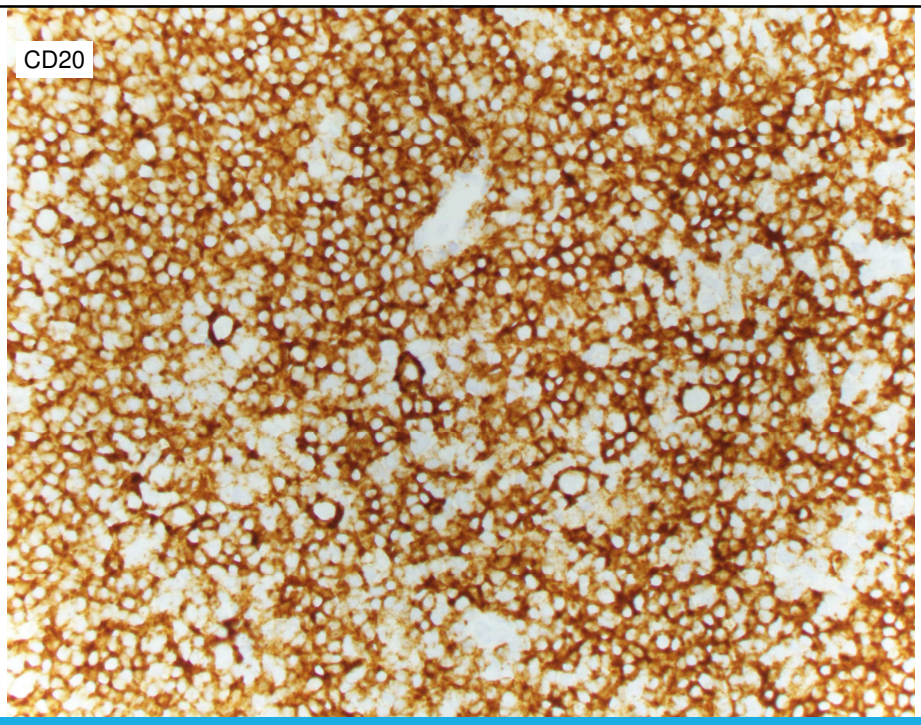
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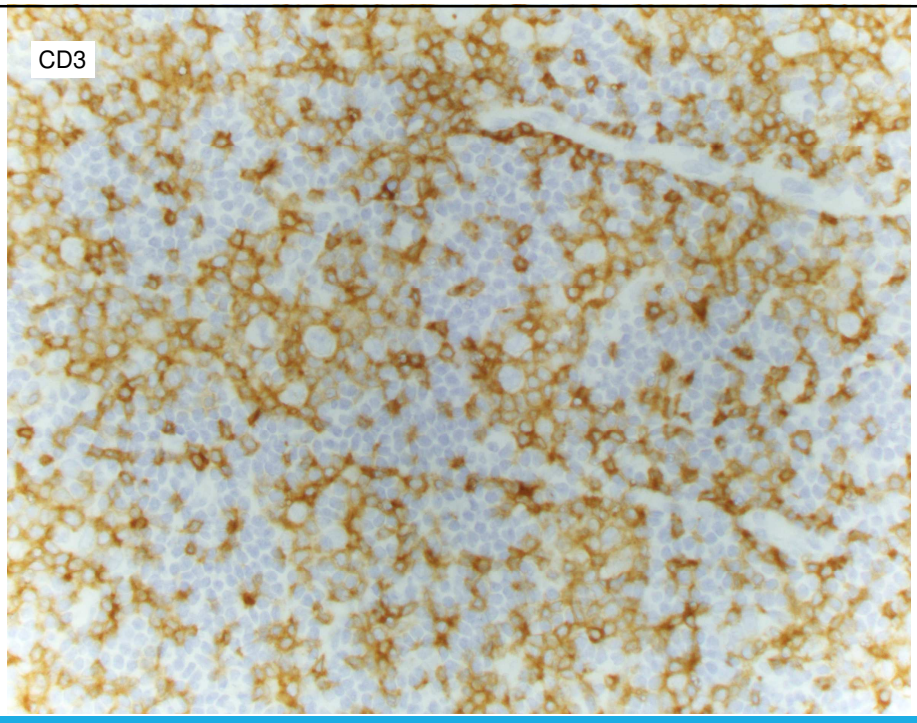
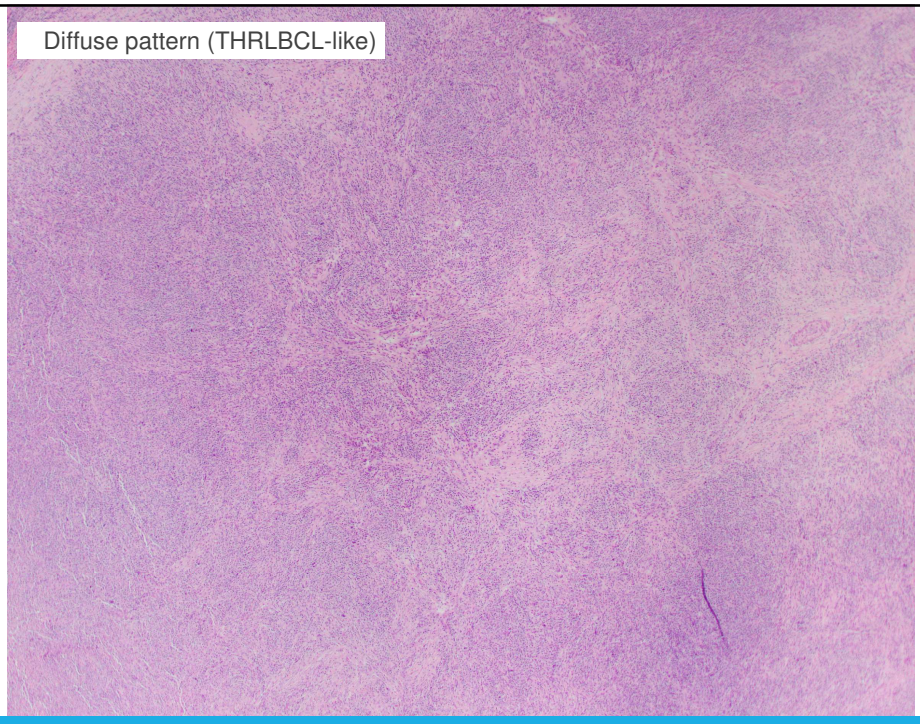
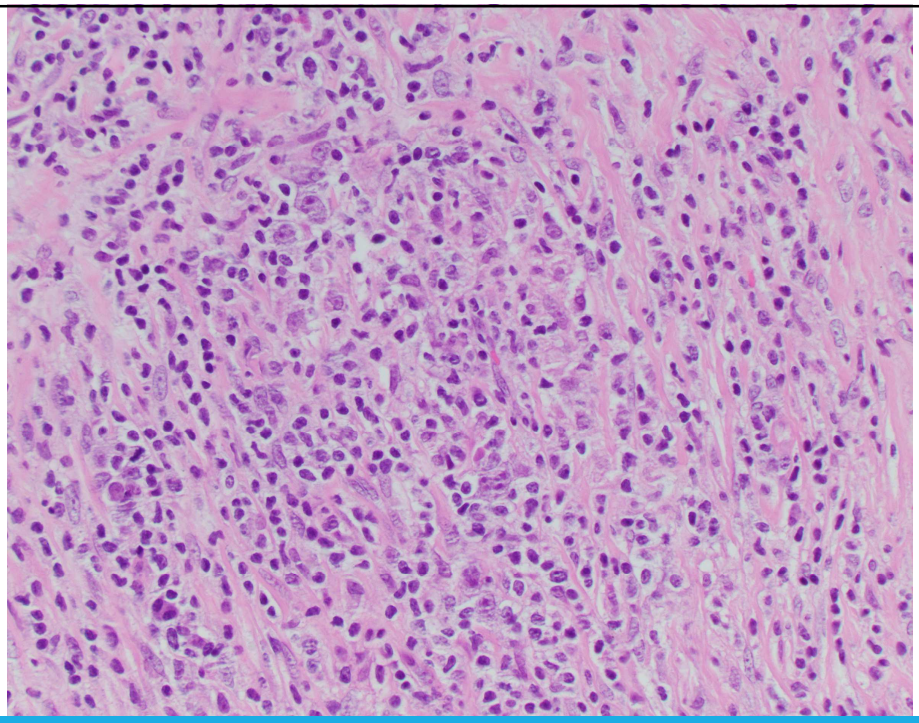

CD3





Atypical cells positive for PAX5, CD20, OCT2, EMA IgD; negative for CD30
Vaguely nodular with large cells associated with CD21+ FDC meshworks

Hodgkin lymphoma: summary

Diagnosis of both classic Hodgkin lymphoma and nodular lymphocyte predominant Hodgkin lymphoma depends on morphology and immunohistochemisty

- Adequate tissue needed for diagnosis
- Panels of IHC; flow typically not helpful
- Knowledge of clinical context

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