Immunohistochemical classification of malignant lymphomas

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Thanks to Jan Klos
Important issues

• Distinction from non-hematopoietic neoplasms
• Distinction from reactive lesions
• Correct classification of lymphoma
• Prognosis in lymphoma

Methods

• Histology
• Immunophenotyping
  - Immunohistochemistry
  - Flow Cytometry
  - Molecular biology
Advantages and disadvantages of IHC in lymphoma

- Works on fixed tissue
- Wide spectrum of available antibodies
- Correlates antigen expression with morphology
- Available in most of pathology laboratories

- Turn-around time IHC 2-5 days
- Less sensitive than Flow Cytometry
- Usually one antigen/slide at the time assessed

Technical issues and interpretation are critical
Neoplasms of lymphatic tissue comprise approximately 5% of all human malignancies

Relative frequency of lymphoid malignancies

10 B-cell
3 Hodgkin
1 T-cell
Determination of cell lineage in lymphoma

**Blasts:**  TdT+, CD10+, CD34-/+ , CD1a+ (T-blasts)

**B-cell:**  CD20+, Pax5+, CD79a+

**Plasma cells:**  CD138+, Kappa/Lambda+, CD79a+/-

**T-cell:**  CD3+, (TCRαβ/γδ+; CD2+, CD5+, CD7+)

**NK-cell:**  CD2+, CD56+, c-CD3ξ+

**Hodgkin/Reed-Sternberg cells:**  CD15+, CD30+
Acute Lymphatic Leukaemia/
Lymphoblastic Lymphoma
Terminal deoxynucleotidyl transferase (TdT) is quite specific for lymphoid cells: Acts as DNA polymerase in B- and T-cell precursors.

- Present in cortical thymocytes and few precursor cells in bone marrow
- Positivity is strongly indicative for acute lymphoblastic leukaemia (ALL) and lymphoblastic lymphoma (LBL)
- 30% of blast crisis in chronic myeloid leukaemia (CML) are positive.
- Rare cases (1-5%) of ALL are negative
- 20% of AML M0 and 5% of M1/M2 are positive
- 30% of Blastic Plasmacytoid Dendritic Cell Neoplasm are positive
- 40% of neuroendocrine tumours may be positive
TdT in B-ALL

TdT in T-ALL

TdT in AML

TdT+ cells in Merkel cell carcinoma
**CD34** - Transmembrane glycoprotein with cell adhesion function. Normally present on 1-4% of bone marrow cells and endothelial cells. Expressed on majority of AML and B-ALL, rare on T-ALL as well as many solid tumours.

- Application: identification of **blasts** in myelodysplastic syndrome (MDS), classification of acute leukaemia, differential diagnosis of mesenchymal tumours.
B-cell lymphomas
CD20

Membrane protein, virtually specific for B-cells, involved in cell activation, proliferation and differentiation.

- Large majority of B-cell leukaemia/lymphoma
- Early stage precursor B lymphoblastic leukaemia/lymphoma may be negative
- Chronic lymphocytic leukaemia/small cell lymphoma may show a weak staining.
- Plasma cell neoplasms are as a rule CD20 negative.
  - A special type of CD20 positive myelomas account for 10-20% of the cases. These myelomas are generally characterized by a more mature plasma cell morphology, chromosomal translocation t(11;14) resulting in cyclin D1 expression, and a better prognosis.
CD20

- May be positive in some T-cell lymphomas
- Follicular dendritic cells are weakly positive
- Spindle cells in thymoma are often positive
- B-cell lymphomas negative after Rituximab therapy (transitional)
Diffuse Large B-Cell Lymphoma before treatment

Diffuse Large B-Cell Lymphoma after treatment with Rituximab
CD20 in B-ALL

CD20 in T-ALL

CD20 in NLP HL

CD20 aberrant expression in AITL
Nordic immunohistochemical Quality Control: CD20

Lymph node

B-CLL

With HIER

Without HIER
CD79a

Involved in signal transduction in B-cells.

- Back up highly specific pan-B marker
- Expressed at all stages of B-cell differentiation including ~50% of plasma cells
- Recommended for CD20 negative cases

- Few cases of B-ALL may be negative
- Both plasma cells and mature B-cells stained
- Some cases of AML t(8;21) are positive
- Expressed only in single cases of classic HL
CD79a in B-ALL

Clone HM57 CD79a

CD79a negative DLBCL

Clone HM57 CD79a
IHC – Controls and CSQI

CD79a

B-CLL – Ins.

Tonsil – Ins.
Tonsil – Opt.
## IHC – Controls and CSQI

### Table 1. Abs and assessment marks for CD79a, run 29

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1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody, pAb: polyclonal antibody.
PAX-5

B-cell specific antigen (BSAP), transcription factor highly specific for B-cells.

- Present from early stages of differentiation to mature B-cells but not in plasma cells.
- Highly sensitive and specific nuclear back up marker of B-cells
- Recommended for CD20 negative cases

Positive also in classic HL (weak) and majority of B-ALL

- Very rare positivity in T-ALL and frequent in AML t(8;21)
- Positivity reported in high % of neuroendocrine carcinomas, mixed mesodermal tumour and metanephric carcinoma
PAX-5 in B-ALL

PAX-5 positive B-cells in T-ALL

PAX-5 in DLBCL

PAX-5 - weaker positivity in classic Hodgkin Lymphoma
PAX-5 reactivity in Small Cell Carcinoma and Merkel Cell Carcinoma

PAX-5+ in Small Cell Carcinoma metastasis in liver

PAX-5+ in Merkel Cell Carcinoma

Synaptophysin
BCL-2

Anti-apoptotic protein. Present on nuclear, mitochondrial membranes and SER.

- Present on many cells including mature B- and T-lymphocytes but
downregulated in germinal centre cells
- Application in differential diagnosis of follicular lymphoma vs. reactive hyperplasia

- Positive in ~95-100% of follicular lymphoma grade I/II but only in 70-80% grade III.
- Majority of primary cutaneous follicular lymphoma are negative because of lack of t(14;18)
Bcl-2 in Follicular lymphoma

Bcl-2 in primary cutaneous Follicular lymphoma

Bcl-2 in B-ALL

Bcl-2 in MCL

BCL-2 negative staining in Burkitt lymphoma
Transcription regulating nuclear phosphoprotein. Expressed in nuclei of B-cells in germinal centres and subpopulation of T-cells.

- Expressed in all **follicular lymphomas** including primary cutaneous type, Burkitt lymphoma, mediastinal large B-cell lymphoma (LBCL), 40-50% of diffuse large B-cell lymphoma, some cases of high grade marginal zone lymphoma (MZL), but not in other types.
- NLP HL is often positive but classic HL is negative
- Does not differentiate between follicular lymphoma and hyperplasia
- Positivity in T-cells may be confusing
Bcl-6 in follicular lymphoma

Bcl-6 in DLBCL
CD10 (CALLA)

Membrane bound endopeptidase.

- Expressed on **stem cells** in BM and hematolymphoid cells, immature B-cells and follicular centre (FC) cells
- Used for classification of B-cell neoplasia: positive in >90% **B-ALL**, Burkitt lymphoma, FC lymphoma, many DLBCL but rare in other types of B-cell lymphomas
- Useful in diagnosing angioimmunoblastic T-cell lymphoma (AITL)

- 20% of T-ALL are also positive
- Exceptional positivity in AML
- Positive on neutrophils, many epithelial and mesenchymal tumours
CD10+ in B-ALL

CD10+ in Follicular Lymphoma

CD10+ T-cells in AITL

CD10+ blastoid MCL
**CYCLIN D1**

Cell cycle protein expressed in G1/S phase.
- Present in virtually all **mantle cell lymphomas** (MCL)
- Present in Plasma Cell Myelomas with t(11:14)
- Endothelial cells may serve as internal positive control

- Weak reactivity in Hairy Cell Leukaemia
- Weak/moderate positivity in proliferation centres in B-CLL
- Present also in non-lymphoid solid tumours
Cyclin D1 in MCL

Cyclin D1 in follicular lymphoma

Cyclin D1 in plasma cell myeloma

Cyclin D1 in parathyroid adenoma
Antibody Selection in Immunohistochemical Detection of Cyclin D1 in Mantle Cell Lymphoma

Emina Torlakovic, MD, PhD, Søren Nielsen, HT, and Mogens Vyberg, MD
Ki-67

Nucleoprotein present G1-M phase of cell cycle but not in G0.

- Positive cells represent proliferating cell fraction (Ki-67+ fraction) often correlating with prognosis
- Valuable for classification of lymphoma into: low grade (<20%) and high grade (> 40%) of Ki-67+
- Indicates blastic transformation in low grade lymphomas
- Ki-67+ closely to 100% cells indicates Burkitt lymphoma, ALL or Burkitt-like DLBCL.

- Only rough estimates of Ki-67 are in practical use
Ki-67 in T-ALL

Ki-67 in immunoblastic DLBCL

Ki-67 in B-CLL; lymph node

Ki-67 in blastic MCL; skin
Light kappa or lambda chain (but never both!) present in all immunoglobulin molecules.

- Specific marker of B-cells expressed first on membrane and later in cytoplasm of cells maturing towards plasma cells
- 80% of B-cell lymphomas are monoclonal by flow cytometry
- Values $\kappa:\lambda > 8:1$ and $1:4 = \text{monoclonality in IHC material}$
- Positive in cytoplasm of many lymphomas (FL, MCL, MZL) and all immunoblastic, plasmablastic lymphomas and myelomas

- Interpretation of $\kappa/\lambda$ staining together is mandatory!
- Difficult to demonstrate membrane staining in paraffin sections
- High rate of background staining
DLBCL

Kappa

Lambda

Plasma cell myeloma; Dutcher bodies; bone marrow

Kappa

Lambda
Plasma cell myeloma bone marrow

Kappa

Lambda

Burkitt lymphoma

Kappa

Lambda
Inappropriate antibody dilution – Ig light chains

IgK: Dako pAb A0191

~1:300  ~1:3,000  ~1:30,000
Inappropriate antibody dilution – Ig light chains

239 IgK tests, 12 Abs:
- 12% optimal
- **Dako pAb A0191:**
  - 17% optimal
  + TRS/Ci 3.000-16.000:
  - 29% optimal
- **All other Abs:**
  - 0% optimal
CD138 (Syndecan-1)

Transmembrane adhesion molecule important for cell growth, differentiation and adhesion

- Expressed in various epithelial cells, early pre-B lymphocytes and plasma cells
- Sensitive marker of plasma cells, plasmacytoma and plasmacytic differentiation together with Ig kappa/lambda
- Positive staining identifies poor prognostic (activated) subtype of DLBCL

- Positivity in early precursor B-ALL and many carcinomas
CD138 on normal plasma cells in bone marrow

CD138 in plasma cell myeloma

CD138+ metastatic lobular carcinoma in bone marrow

CD138

CKHMW
MUM-1/IFR4

Nucleoprotein regulating development and maturation of B-cells to plasma cells.

- Present late in B-cell development after CD10 and bcl-6
- Positive staining in plasma cells and their proliferations, lymphoplasmacytic lymphoma (LPL), DLBCL of activated phenotype, classic Hodgkin lymphoma, some T-cell lymphomas
- Positive staining identifies poor prognostic (activated) subtype of DLBCL
- High % of positive staining in melanocytic tumours and clear cell sarcoma is reported
Germinal center cell phenotype of DLBCL

Activated B-cell phenotype of DLBCL

CD10

BCL-6

MUM1
T-/NK- lymphoma markers
T-cell & NK-cell lymphomas

- Rare neoplasms
- More frequent in Asia
- Related to HTLV-1 infection (7% life time risk)
- Symptoms are often related hypercalcaemia (ATLL) and hemophagocytic syndromes
- Prognosis and response to therapy is poorer than in other lymphomas
- Broad morphologic spectrum with overlapping features
- Abnormal antigen expression or loss of reactivity for one or more typical T-cell antigens are immunohistochemical hallmarks
- Diagnosis depends on correlation of clinical picture, morphology, immunophenotype, cytogenetics and molecular findings
CD3

Transmembrane polypeptide; functions as a part of TCR complex specific for T-cells and expressed in the cytoplasm of NK-cells.

- Membranous expression specific for T-cells
- Expressed in ~90% of T- and majority of NK-cell lymphomas

- Difficult to distinguish membrane and cytoplasmic (T-cells) from purely cytoplasmic staining (NK-cells and immature T-cells)
- Some T-cell lymphomas are negative (e.g. anaplastic large cell lymphoma - ALCL)
c-CD3 in T-ALL

CD3 rich background in classic Hodgkin lymphoma

c-CD3 in PTCL (peripheral T-cell)

c-CD3 in cutaneous ALCL
CD5

Transmembrane glycoprotein expressed on T-cells and subpopulation of B-cells (weaker) - used as fairly specific pan-T-cell marker.

• Expression lost in some T-cell lymphomas
• Negative in NK-cell lymphomas
• Among B-cell lymphomas CD5 is characteristic for B-CLL and MCL, but 10% of DLBCL cases are also positive.
• Other B-cell lymphomas are usually negative.
• Some non-haematopoietic tumours including thymic carcinomas are positive
CD5 in T-ALL

Loss of CD5 in cutaneous ALCL

Loss of CD5 and retained CD7 expression in Hepatosplenic T-cell lymphoma
CD5 in B-CLL

CD5 in DLBCL
ALK

Growth hormone (pleiotropin) receptor. Present only in a cytoplasm of a few cells in CNS.

- Expressed in ALK+ ALCL and its morphological variants and in rare cases of ALK+ DLBCL
- Found also in 50-70% of inflammatory myofibroblastic tumour and few solid tumours

ALK-1 t(2;5) 80% of cases
ALK-1 t(1;2), t(2;3), Inv2 - 15% of cases
Hodgkin lymphoma markers
CD30

Non specific, membrane bound activation marker.

- Present in activated immunoblasts, HRS cells in classic HL, ALCL, lymphomatoid papulosis, many PTCL, some DLBCL
- NLPHL is usually negative
- Seen also in virus infected cells and plasma cells
- Some non-lymphoid tumours positive (embryonal carcinoma)

- Only membrane and Golgi staining accepted as positive
- Cytoplasm staining only – uncertain significance
- Expressed on immunoblasts in reactive conditions
CD30 in reactive germinal center

CD30 in ALCL

CD30 classic Hodgkin Lymphoma

CD30 in PTCL
CD15

Haematopoietic differentiation antigen of carbohydrate nature

• Positive on mature granulocytes (strong), myeloid and monocytic cells (also leukemic), many carcinomas and CMV infected glandular cells.
• Used to differentiate classic HL from the most of its mimics and subtyping of acute leukaemia.

• Mediastinal LBCL occasionally positive
• 20% of classic HL and all NLPHL are negative
• ALK1- ALCL is positive in cytoplasm
• 15% of ALL co-express CD15 in minority of cells
• Sometimes weak staining
CD15+ in Granulocytes

CD15+ in classic Hodgkin

CD15+ in AML - bone marrow

CD15+ in ALK-1 negative ALCL
Carb-3 Is the Superior Anti-CD15 Monoclonal Antibody for Immunohistochemistry

Rasmus Rohe, MD, Søren Nielsen, HT, and Mogens Vyberg, MD
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Immunohistochemical classification of malignant lymphomas

Prof. Mogens Vyberg
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Thanks to Jan Klos
Supplementary material
Aberrant expression of T-cell antigens in Classical Hodgkin Lymphoma

5% of classical Hodgkin’s lymphoma express T-cell markers

T-cell marker expression, clonal immunoglobulin heavy chain- (IgH) and T-cell receptor (TCR) gene rearrangement in Hodgkin’s and Reed–Sternberg cells of classical Hodgkin’s lymphoma.

A Tzankov et al. T-cell markers in Hodgkin’s lymphoma
Modern Pathology (2005)18,1542–49
- **Granzyme B, TIA-1 and Perforin** are the most common cytotoxic proteins present on CD8+ T-cells and NK-cells.
- Granular staining pattern - Identify cytotoxic phenotype of T/NK-cells.

| Granzyme B in extranodal NK/T-cell lymphoma nasal type | Granzyme B in LGL - intravascular growth |
**OCT-2** (Octamer transcription factor-2) nucleoprotein.

**BOB.1** (B-cell Octamer binding protein 1)- nuclei and cytoplasm.

- Both are present in all Ig producing B-cells cells.
- Both (80%) or one (20%) are absent in HRS cells in classic HL
- Both are positive in all types of DLBCL and

**BOB.1 in DLBCL**

**OCT2 in DLBCL**
Other useful antibodies
Membrane bound receptor (C3d, EBV receptor). Present on dendritic cells, mantle zone and marginal zone B-lymphocytes, but not on activated B-cells.

• Used to demonstrate **FDC network** in lymphomas: FL, MZL, NLPHL, AITL

• Positive also in dendritic cell tumours, littoral cell haemangioma of the spleen and some cases of endemic Burkitt lymphoma
CD21 in Angioimmunoblastic lymphoma
**LMP-1/EBER for detecting of EBV infection**

**LMP-1** (latent membrane protein 1) gives positive staining in minority of cases in cytoplasm of HRS cells in classic HL, AITL, PTLPD, extranodal NK/T-cell lymphoma nasal type, infectious mononucleosis (not all latency patterns are positive).

**EBER** based on in situ hybridisation is much more sensitive

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**HHV-8**

Human Herpes Virus 8 latent nuclear antigen

Positive cytoplasmic staining:
- Primary effusion lymphoma
- Multicentric Castleman’s disease

**LMP-1+ in classic Hodgkin Lymphoma**
Cytokeratin staining - application in lymphoma

- Identification of lymphoepithelial lesions
- Identification of lymphomas with aberrant CK expression

**PANCK in mantle cell lymphoma**

**PANCK in MALT Lymphoma**
Aberrant antigens in lymphoma

PANCK (CK8/18)+ in PBL

P63+ DLBCL