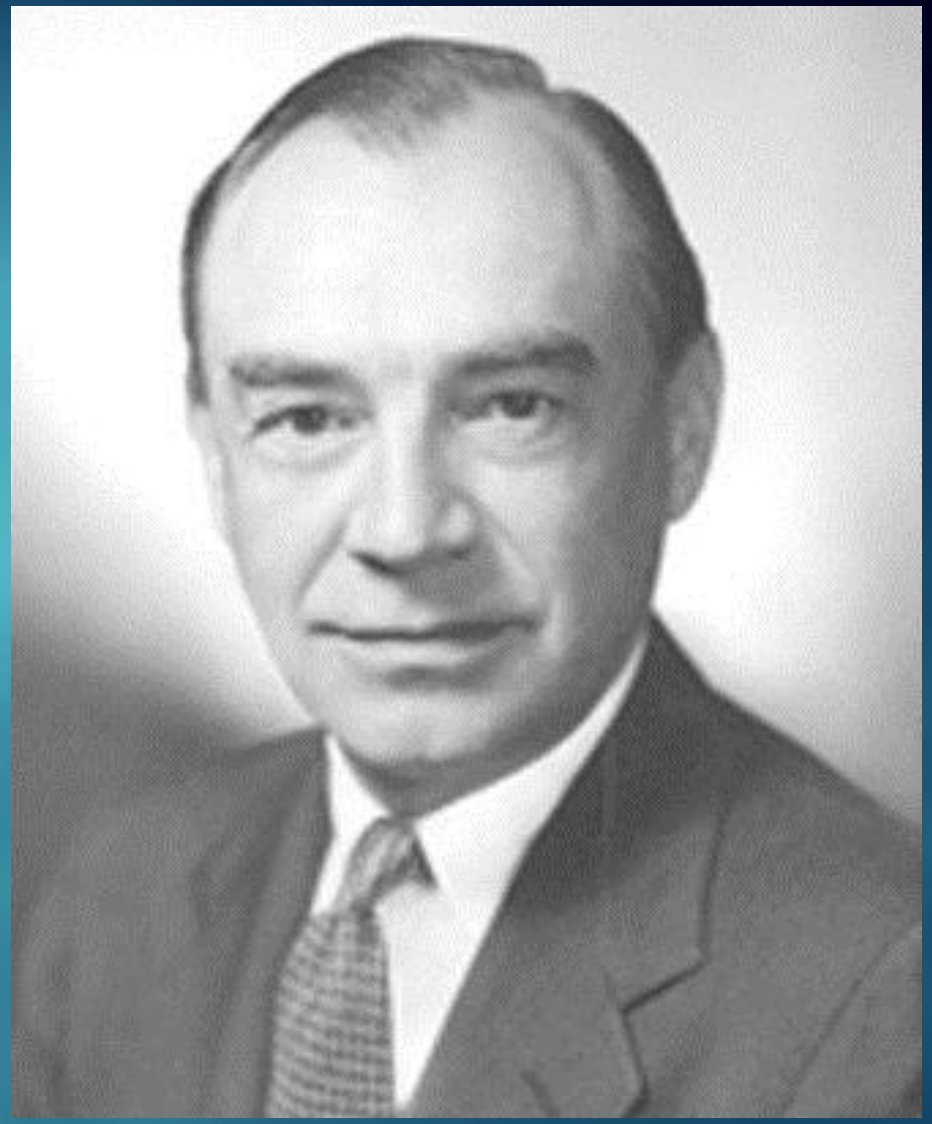
Immunohistochemical role in the diagnosis of non Hodgkin lymphoma

Lymphohaemopoietic neoplasms are complex groups of malignancy requiring accurate and precise diagnosis and prognostication for optimal treatment.

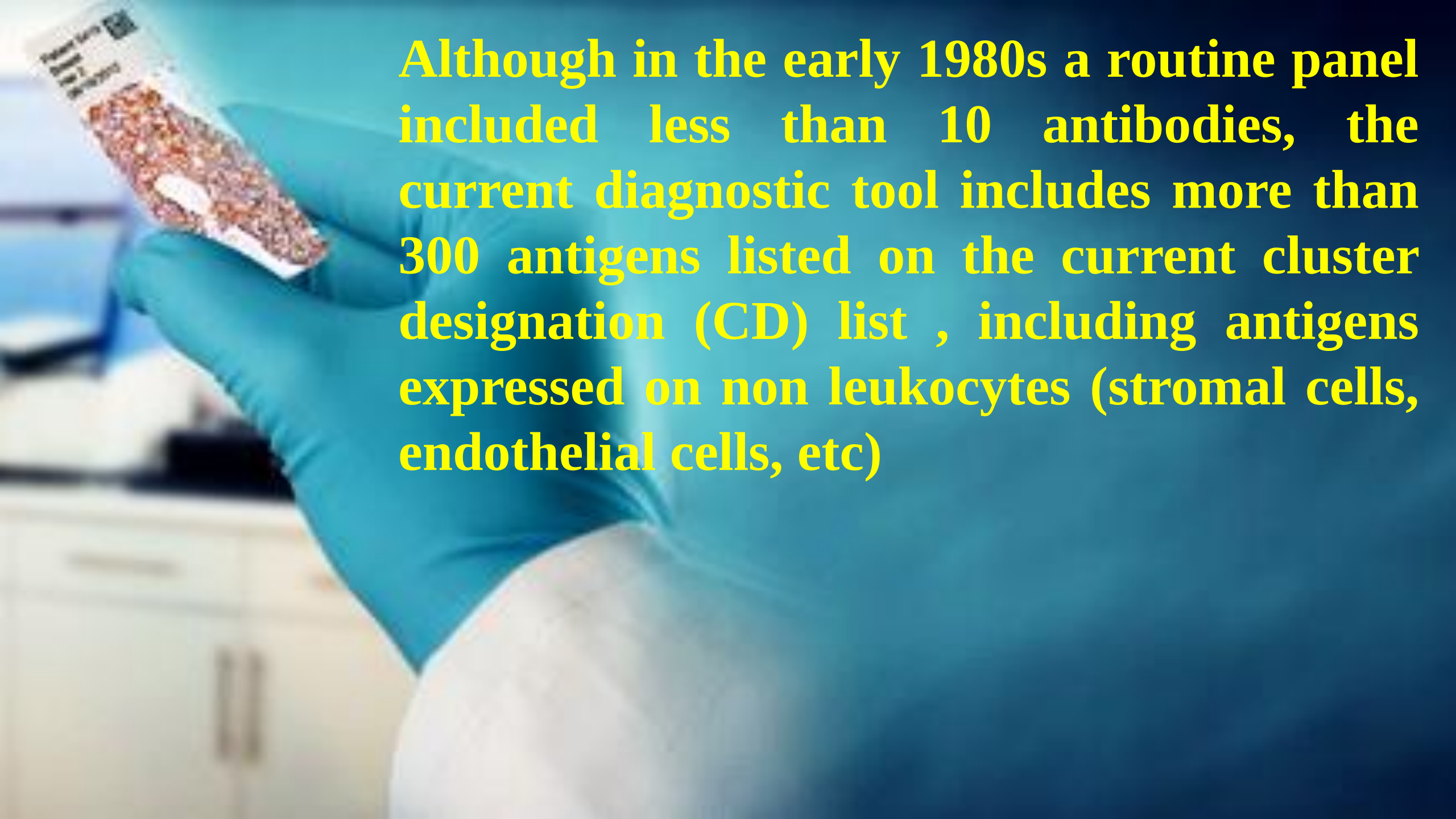
Discrepancy between initial and review diagnosis of lymphoma is common , 10-30% rate of misdiagnosis is reported.

While the morphology is still the backbone of histopathology diagnosis of lymphoma, no lymphoma nowadays is diagnosed without immunohistochemical backup.

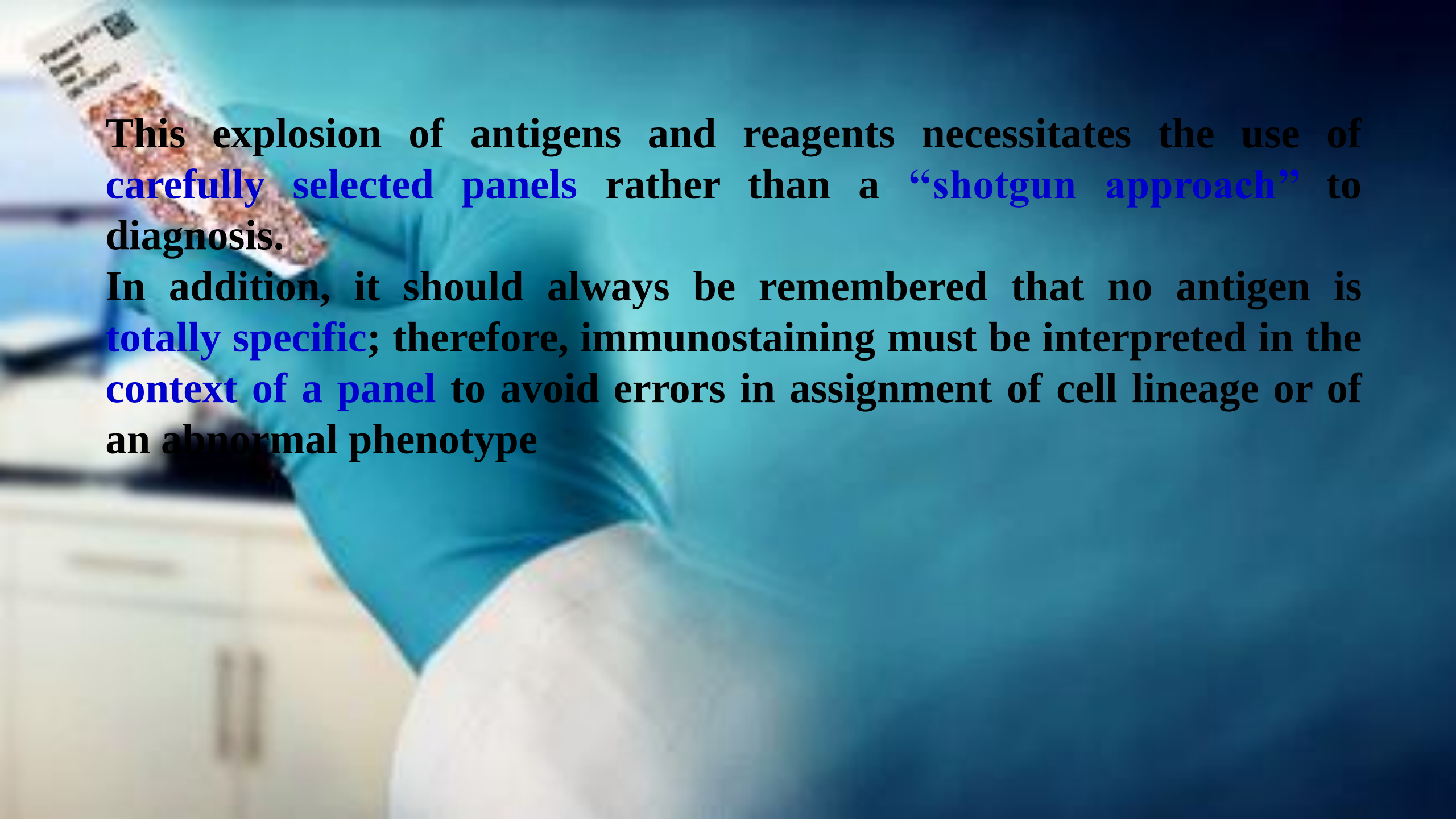
Diagnostic immunohistochemistry relies on collections of monoclonal antibodies that recognise surface, intracytoplasmic or nuclear molecules involved in cell differentiation, communication, adhesion and signalling.



Albert Coons
American pathologist and immunologist



Although in the early 1980s a routine panel included less than 10 antibodies, the current diagnostic tool includes more than 300 antigens listed on the current cluster designation (CD) list , including antigens expressed on non leukocytes (stromal cells, endothelial cells, etc)



This explosion of antigens and reagents necessitates the use of carefully selected panels rather than a “shotgun approach” to diagnosis.

In addition, it should always be remembered that no antigen is totally specific; therefore, immunostaining must be interpreted in the context of a panel to avoid errors in assignment of cell lineage or of an abnormal phenotype

A recommended basic panel

Based on morphology

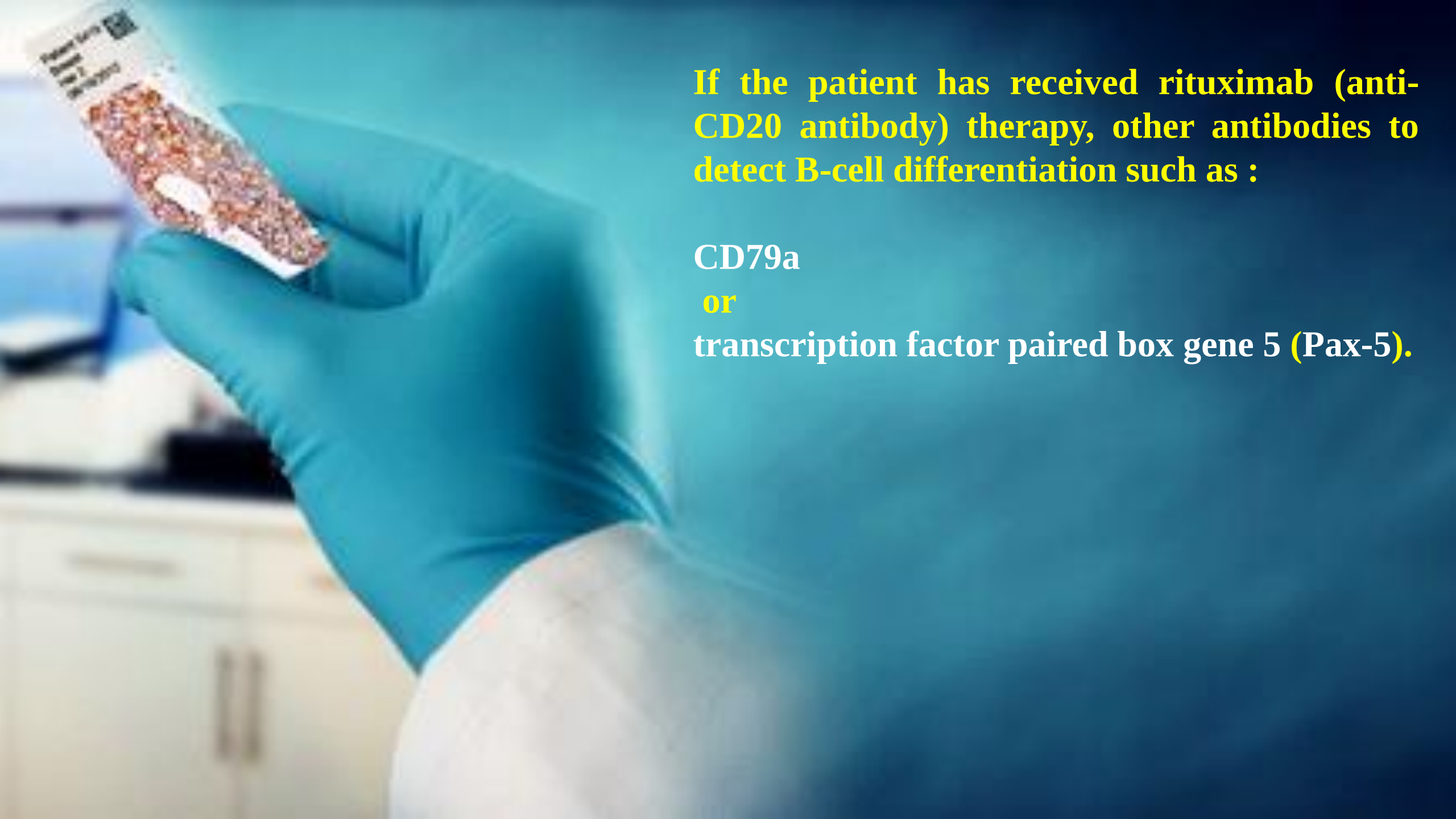
The beginning IHC panel includes antibodies against

B-cell
T-cell
CD45RB, CD15, and CD30 if large dysplastic cells are seen.
light chains (if numerous plasma cells are present)

B-Cell Markers

If there is alteration of B-cell areas, CD20 is the most widely used pan–B-cell marker.

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) may be weakly positive or occasionally negative for CD20 by IHC.



If the patient has received rituximab (anti-CD20 antibody) therapy, other antibodies to detect B-cell differentiation such as :

CD79a
or
transcription factor paired box gene 5 (Pax-5).

antibodies against CD5, CD10, CD23, CD43, B-cell lymphoma 2 protein (BCL-2), and B-cell lymphoma 6 protein (BCL-6) would also be useful initially to further characterize the neoplasm.

SMALL B-CELL NEOPLASMS

The Role of CD5 and CD10 in the classification of small B-cell neoplasms
CD10 and BCL-6 protein expression in FL.
The use of BCL-2 expression in the evaluation of small B-cell Proliferation
The use of FDC markers in the evaluation of small B-cell Proliferation
The use of Proliferation markers in the evaluation of small B-cell Proliferation

The Role of CD5 and CD10 in the classification of small B-cell neoplasms

Neoplasms that express CD5 and lack CD10 (CD5+ CD10−) predominantly include:

CD5+ CD10−

Mantle cell lymphoma (MCL) and Small lymphocytic lymphoma (CLL/SLL) .

(CD5- CD10+) phenotype is seen in:

Follicular Lymphoma (FL)

CD10 expression has been reported in approximately 2% to 6% of MZLs.
Rare cases of blastoid variant of MCL may lack CD5 and express CD10.

(CD5- CD10-) phenotype is most often present in :

Marginal zone lymphoma (MZL)

A small but significant number (4%–25% of cases) of MCLs lack detectable CD5 expression using either flow cytometry or IHC.

The neoplasms with dual expression of CD5 and CD10 (CD5+ CD10+) are very uncommon representing less than 1% of B-cell neoplasms.

This immunophenotype has been described in

1% of FLs

4% to10% of MCLs

less than 1% of CLL/SLLs.

The Use of CD23 in Subcategorizing CD5 + Small B-Cell Neoplasms

Immunohistochemistry using CD23 is helpful in the differential diagnosis of the CD5 small B-cell group neoplasms CLL/SLL and MCL.

CD23 is expressed in CLL/SLL (82%–95%).

while 3% to 13% of MCLs can be CD23 +.

Cyclin D1 expression would confirm the diagnosis of MCL.

Is Cyclin D1 Expression Specific for MCL

Lack of cyclin D1 expression has been reported in approximately 10% to 25% of MCLs .

The lack of cyclin D1 expression by IHC may be attributed in part to technical factors, particularly the types of antibodies used to detect the antigen. Recent studies have demonstrated the efficacy of rabbit monoclonal antibodies that have increased sensitivity without significant loss of specificity.

Cyclin D1 is normally expressed in scattered large nuclei of histiocytes, endothelial cells, fibroblasts, and rare normal mantle cells

Cyclin D1 is expressed in MCL nuclei, and cytoplasmic expression alone is nonspecific

Although cyclin D1 expression strongly supports MCL, other lymphoid neoplasms also overexpress cyclin D1 like CLL/SLLs , splenic MZL.

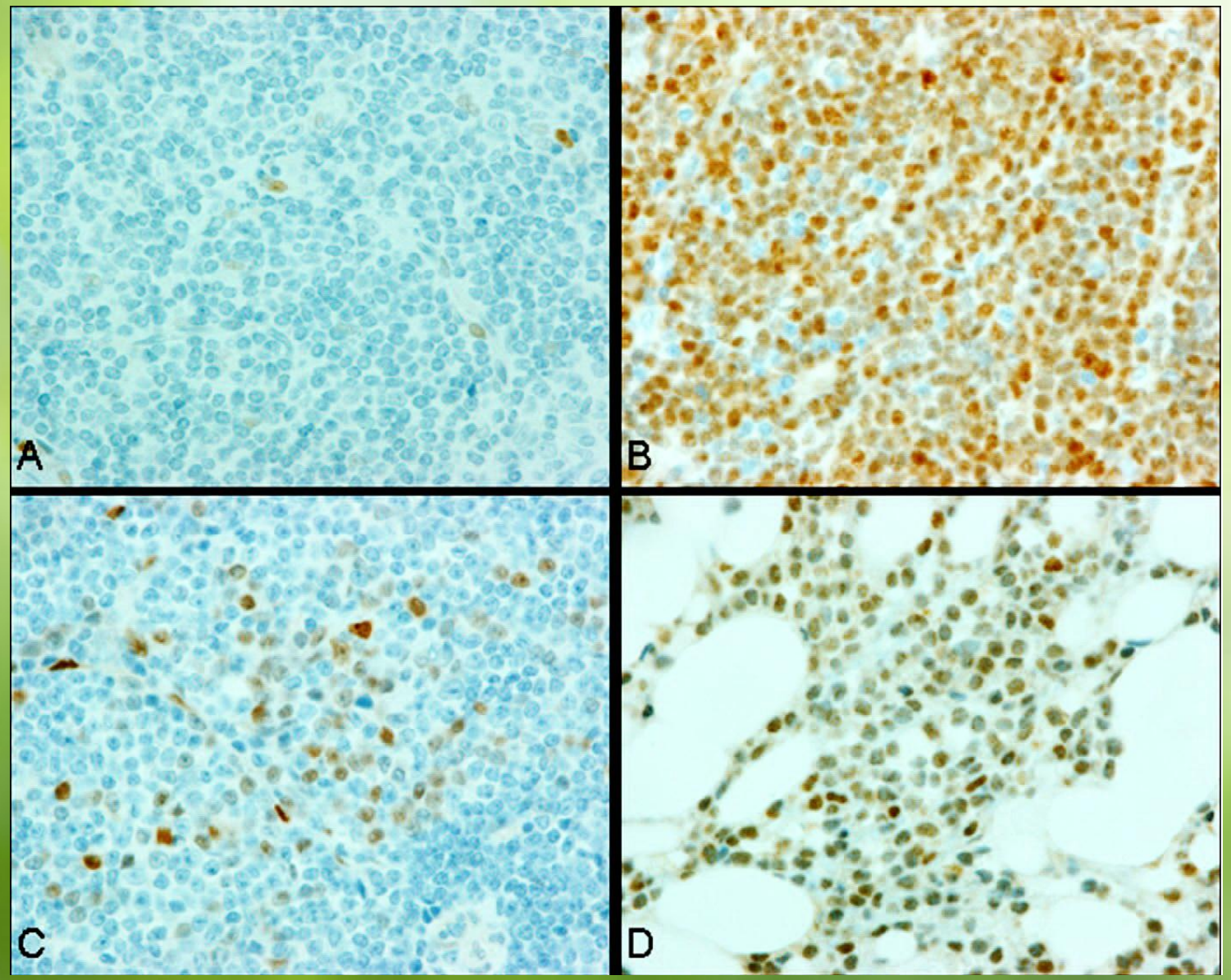
Cyclin D1 expression is a defining feature of mantle cell lymphoma (MCL) but can be present in other small B-cell neoplasms.

A,Cyclin D1 expression is present in scattered, reactive endothelial, histiocyte, and stromal cell nuclei in a benign tonsil.

B, Strong nuclear expression of cyclin D1 is seen in MCL.

C, Cyclin D1 is expressed in the nuclei of immunoblasts in the proliferation centers of chronic lymphocytic leukemia/small lymphocytic lymphoma.

D, Cyclin D1 is also weakly expressed in the nuclei of hairy cell leukemia.



CD10 and BCL-6 Protein Expression in FL.

CD10 and BCL-6 are expressed in normal and neoplastic follicle centers .

The presence of CD10 lymphocytes and groups of BCL-6–positive small lymphocytes outside of follicles strongly supports a neoplastic proliferation .

A few isolated BCL-6–positive cells may normally be present in the interfollicular areas of lymph nodes (predominantly T cells)
but large numbers of interfollicular BCL-6–positive cells would be supportive of FL .
Approximately 10% to 30% of FLs lack expression of CD10 by IHC, and BCL-6 may be helpful in identifying a follicular origin.

The Use of BCL-2 Expression in the Evaluation of Small B-Cell Proliferation

BCL-2 is an antiapoptotic molecule normally expressed in resting B cells of the normal mantle zone and primary follicles.

BCL-2 is down-regulated in normal germinal center B cells .

Immunostaining for BCL-2 is most useful in distinguishing reactive follicular hyperplasia from FL.

B cells in reactive follicle centers lack BCL-2 expression, whereas , approximately 85% to 90% of FLs are BCL-2 positive.

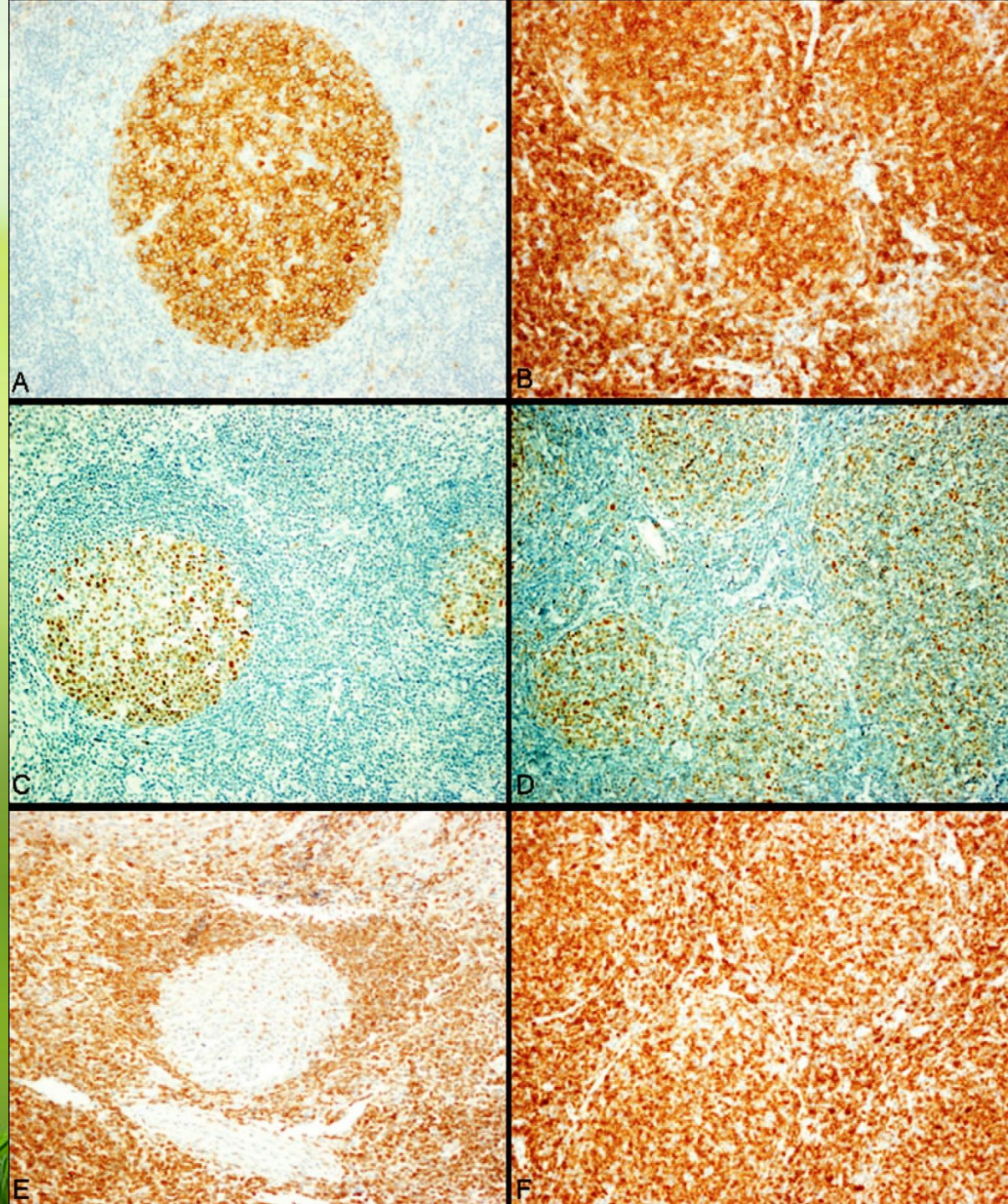
however, some FLs have a mutation in the *BCL2* gene leading to a false negative result on IHC .

A and B, CD10 is expressed in germinal center cells in both reactive and neoplastic follicles; however, in FL (B) CD10 expression can be seen in cells invading the interfollicular area.

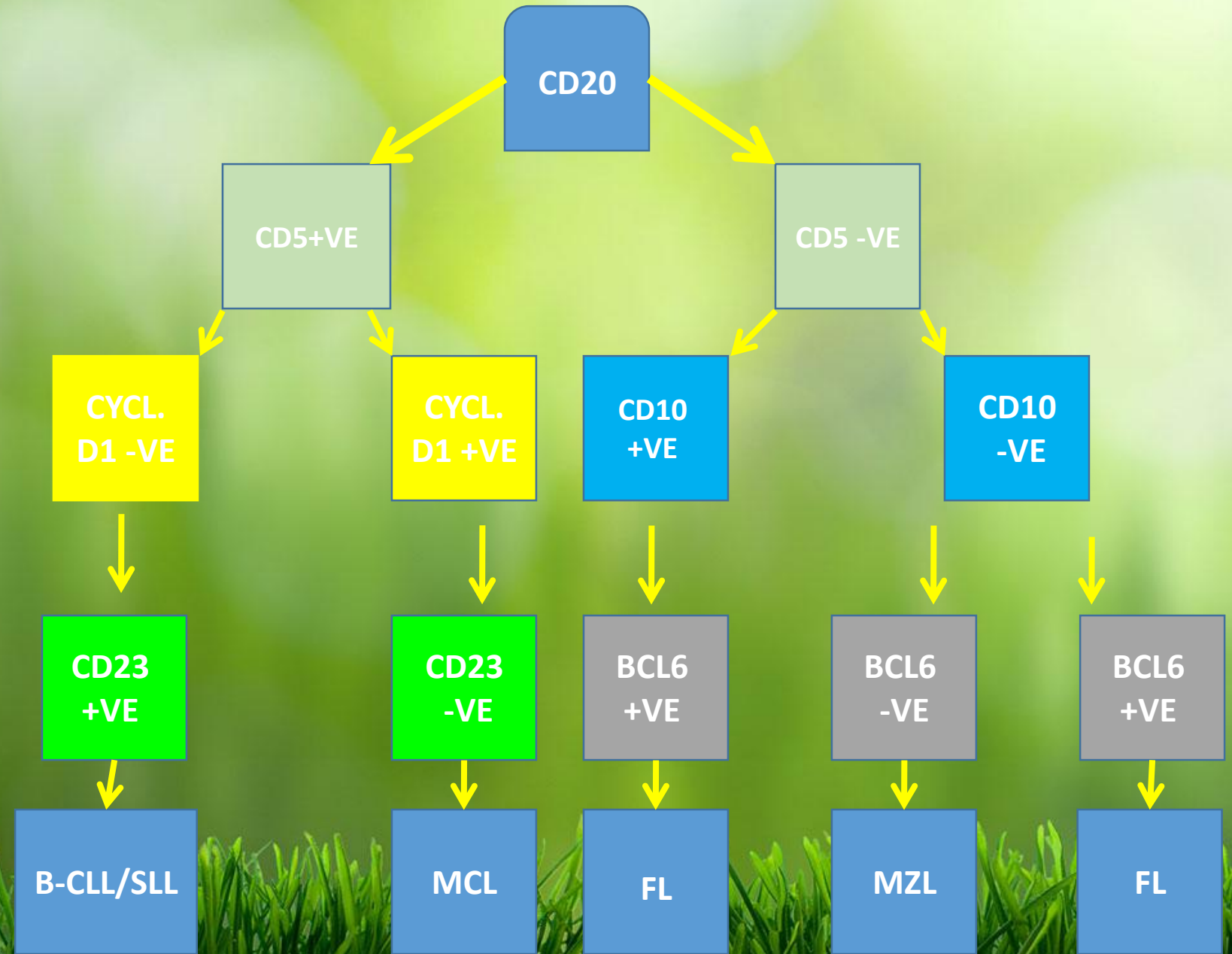
C and D, BCL-6 is expressed predominantly in large cells in the reactive and neoplastic germinal centers with only rare positive large nuclei in the interfollicular area in the reactive process (C). BCL-6 is weakly expressed in FL (D) in the cleaved cells invading the interfollicular area.

E, BCL-2 is expressed in T cells in reactive follicles and in the interfollicular area and in the normal mantle zone lymphocytes.

F, In contrast, virtually all of the neoplastic cells in the follicle center in low-grade FL are BCL-2 positive as shown here



PHENTYPIC ALGORITHM OF MAIN MATURE B – CELL LYMPHOMA



FDC and Proliferation Markers.

FL, MCL, and CLL/SLL may have a marginal zone growth pattern resembling MZL, particularly in extranodal sites such as the spleen.

In addition to CD5, CD10, CD43, and cyclin D1, we may use immunostains for FDC and MIB-1 may be useful in this distinction.

Immunostaining for CD21, CD23, or CD35 highlights FDC making it possible to delineate follicle structures and assess their morphology.

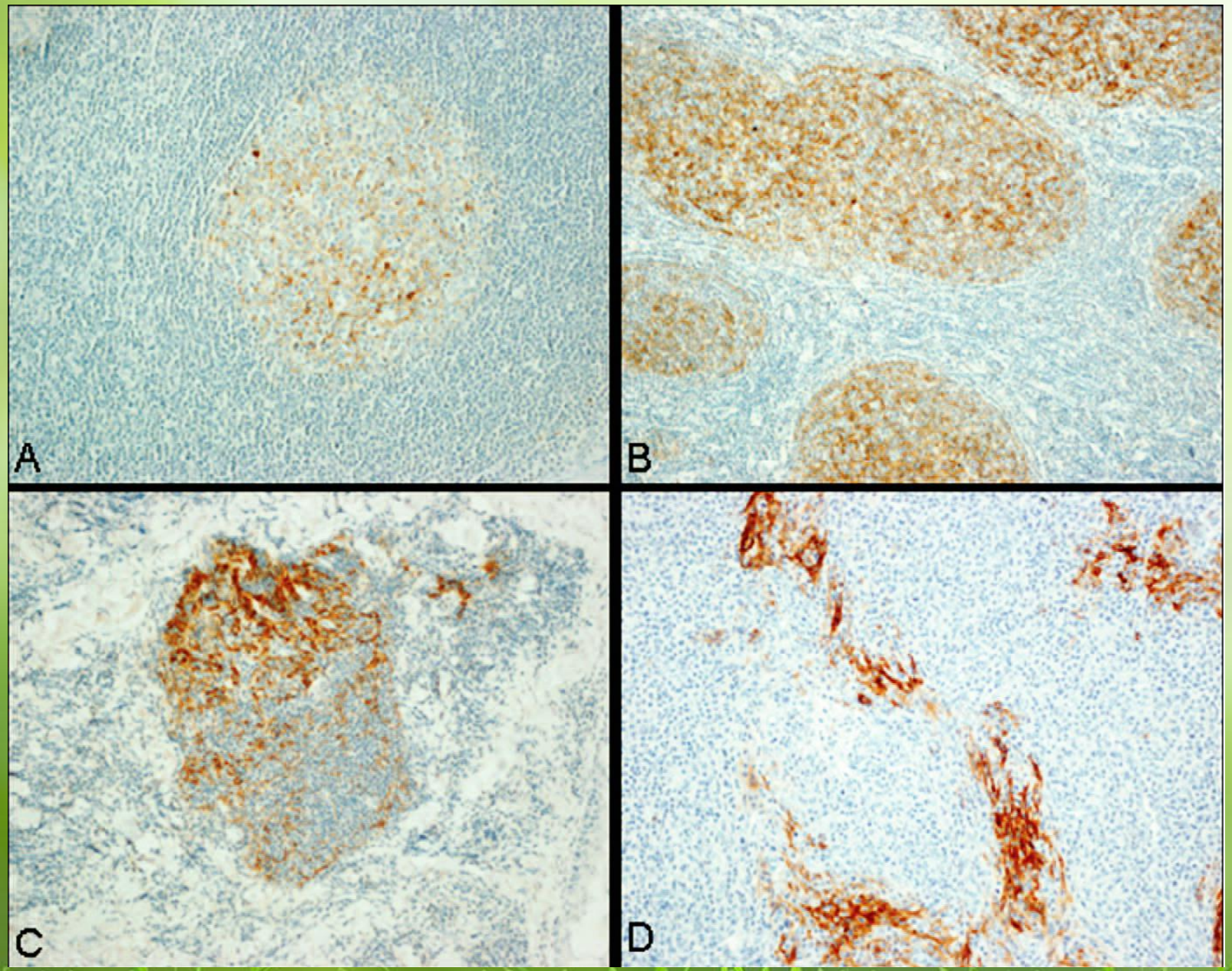
Staining for follicular dendritic cell (FDC) demonstrates the presence and architectural features of B-cell follicles and is helpful in lymphoma diagnosis.

A, Normal FDC in a reactive follicle in the tonsil.

B, Preserved "tight" FDC meshwork in low-grade follicular lymphoma.

C, Expanded and somewhat disrupted FDC meshwork in a follicle that is colonized by a marginal zone lymphoma.

D, Markedly expanded and disrupted FDC meshwork extending beyond the follicle and surrounding vessels, a characteristic feature of angioimmunoblastic T-cell lymphoma



Proliferation marker MIB-1 (Ki-67) is useful in differentiating FL from reactive follicular hyperplasia and from other small B-cell lymphomas.

Normal germinal centers have a high proliferation rate ; in contrast, a decreased proliferation rate within the germinal center is seen in low-grade FL .

T-Cell Markers

If there is expansion of interfollicular areas and a T-cell lymphoma is a consideration, CD3 is the most commonly used pan–T-cell antigen.

However , CD3 may be lost in some T-cell neoplasms, particularly anaplastic large cell lymphoma (ALCL).

antibodies against CD2, CD4, CD5, CD7, and CD8 would identify the subset distribution.

T cells are composed of CD4 (helper T-cell) and CD8 (suppressor, cytotoxic T-cell) subsets. Antibodies to detect these antigens are included in the basic panel to determine if there is an abnormal T-cell distribution (normal CD4/CD8 ratio 1.5–3:1).

Most reactive processes show a predominance of CD4 T cells.

A neoplastic T-cell or NK-cell process should be considered if a marked predominance of CD8 T cells is present, particularly at extranodal sites.

CD5 is a also a pan–T-cell antigen and is not expressed on NK cells.

CD5, however, is not lineage specific.
CD5 is expressed in
CLL/SLL
MCL
less frequently in small subsets of other B-cell neoplasms.
So , If CD5 is expressed in B-cell areas (usually weaker than in T cells), additional studies should be performed to determine the type of small B-cell neoplasm.

Antigens for Characterization of atypical large cell proliferations

if atypical large cells are present, antibodies against CD15, CD30, and CD45RB (leukocyte common antigen) should be added to the panel to rule out HL or ALCL or an epithelial neoplasm.

One should remember that CD30 is an activation antigen and commonly expressed on large cells at the periphery of B-cell follicles and in the interfollicular areas. Also, reactive processes, such as infectious mononucleosis, can have numerous large atypical, dysplastic CD30 cells.

The lack of CD15 expression does not exclude classical HL (CHL) as approximately 25% to 40% of cases may be negative or have focal expression of this marker.

CD30 and ALK-1 expression indicate an ALK-positive ALCL; absence of ALK can be seen in ALK-negative

Expression of CD45RB rules out an epithelial tumor, but very weak or absent CD45RB can be seen in tumors with plasma cell differentiation, precursor lymphoid and myeloid tumors, and ALCL (in 20%– 40% of cases).

CD15 and rarely CD30 (particularly in embryonal carcinoma) are expressed in epithelial tumors.

Distinguishing HL From NHL

Most cases of HL are readily distinguished from NHL based on the presence of a minor population of diagnostic RS cells, a characteristic predominant reactive background, and the typical immunophenotype of the RS cells.

The presence of numerous RS cells or atypical morphologic or immunophenotypic features in some HL suggests the possibility of NHL.

Most important confusion is with NLPHL

Important distinguishing features include the presence of a partly nodular pattern with FDC meshwork and CD4 T cells ringing the L&H cells in NLPHL. and the predominance of background cytotoxic CD8, lack of FDC meshwork, and scanty of small B cells in NHL

CONCLUSIONS

Immunophenotyping, although indispensable in the diagnosis and classification of hematopoietic and lymphoid neoplasms, has to be used cautiously with knowledge of the antibodies used. No antigen is totally lineage or lymphoma specific, and for this reason, immunostaining must be performed in the context of a panel. Each lymphoid neoplasm has a characteristic immunophenotype, but a potential pitfall is the small number of otherwise typical cases that can express phenotypic markers of other neoplasms or lack their characteristic markers. In addition, familiarity with the diagnostic criteria and differential diagnosis of each lymphoid tumor and ultimately correlation with morphology, ancillary molecular genetic and cytogenetic/ FISH studies, and clinical history