Best Practices in Contemporary Diagnostic Immunohistochemistry

Panel Approach to Hematolymphoid Proliferations

Steven H. Swerdlow, MD
Disclosure

• My approach to this topic is solely my own.
• I make no personal claims to be an expert.
• Some of this material has been used in other presentations & will be used in others in the future.
• I have no conflicts of interest.
Learning Objectives

• To understand the **goals** of immunohistochemical panels in diagnostic hematopathology

• Know how to use a basic panel approach to help to diagnose the more common lymphoid proliferations.

• To accomplish the above without breaking anyone’s bank!
Agenda

• What are the goals of performing immunohistochemical stains in the evaluation of lymphoid proliferations?

• Review how limited IHC panels, with potential additional follow-up stains, help us recognize and classify some of the more common lymphomas.
• Not addressing the relative value of flow cytometric versus immunohistochemical studies.
• 30 minutes is a very short period of time.
• Concentrate on dealing with the most common situations we encounter, not on how to solve the deepest mysteries of hematopathology.
IHC panels as an *aid* in diagnostic decision making
Lymph node biopsy (extranodal proliferation)

Benign
- Specific/non-specific reactive proliferation

Malignant
- Lymphoma (B/T/NK)
- Other (eg metastatic carcinoma)
  
  Hodgkin lymphoma
  - Precise type

  Non-Hodgkin lymphoma
  - Precise type
WHO Classification

- B-cell neoplasms
  - Precursor B-cell neoplasm (1)
  - Mature (peripheral) B-cell neoplasms (17)
- T and NK-cell neoplasms
  - Precursor T-cell neoplasm (2)
  - Mature (peripheral) T-cell neoplasms (14)
- Hodgkin lymphoma (Hodgkin disease)
  - Nodular lymphocyte predominant
  - Classical (4)
Purpose of the IHC Stains

• Provide information that is not otherwise apparent but is of diagnostic utility – assess light chain staining in plasma cells
Double kappa/lambda Immunostain (plasma cell staining)

Hyperplastic tonsil

Gastric MALT lymphoma cells
Identify subtle neoplastic infiltrates that may otherwise be inapparent

- For example, “in situ” mantle cell or follicular lymphomas
- Use of a panel of stains can increase confidence in the interpretation of other stains, eg, CD43+ B-cells in a region where cyclin D1 staining is present.
Purpose of IHC Stains

• Internal “private” quality control
  – You think you know what you are looking at but are you correct?
  – You don’t want to end up making an unfortunate diagnostic error.
Mantle cell lymphoma
Differential Diagnosis for MCL

- Reactive hyperplasia
- B-cell CLL/small lymphocytic lymphoma
- Lymphoplasmacytic lymphoma
- Follicular lymphoma
- Marginal zone B-cell lymphoma
- Splenic marginal zone lymphoma
- Hairy cell leukemia
- B-lymphoblastic leukemia/lymphoma (B-cell ALL)
- B-cell prolymphocytic leukemia
- Diffuse large B-cell lymphoma
- Peripheral T-cell lymphoma & T-Lbic lymphoma/leukemia
So shouldn’t we just use all the stains we have available to cover all possible diagnoses?

NO!
Why use directed panels?

• More economical – indiscriminant use of IHC stains will be medically unbelievable.

• Actually can cut down on diagnostic errors – indiscriminant use of IHC sets you up to be mislead by real or apparent results you would be better off not seeing.
Panels -- two

• Major purpose evaluation of potential B-cell neoplasms
• Major purpose evaluation of potential Hodgkin lymphomas
Relative Incidence of ML

Seer data (HL vs. NHL) & NHL Classification project, Blood 89:3909
Multipurpose Panel to Deal with the Most Common B-cell Lymphomas

- CD20 – B-cells (& rare T-cell lymphomas)
- CD3 – T- & NK cells
- CD5 – T-cells & small B-cell subset
- CD43 – T-cells & small B-cell subset
- CD10 – Mostly B-cell subset (FCC)
- Bcl-6 – Mostly B-cell subset (FCC) – weak staining may not be of the same significance
- Bcl-2 – Many lymphoid cells but not many normal FCC
- Cyclin D1 – normal lymphoid cells negative
Multipurpose Panel to Deal with the Most Common B-cell Lymphomas

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How do we use the panel to...

- Support the presence of a lymphoid neoplasm?
- Help classify the lymphoid neoplasm, once it is identified?
Ways in which our IHC Panel Might Suggest the Presence of a Lymphoid Neoplasm

- Recognizable population of CD5+ B-cells
  - Small populations seen by flow cytometry in normal individuals usually not identified in paraffin sections
  - We occasionally see some expression in normal mantle zones
Features to Suggest Neoplasia

- Numerous CD10 or bcl-6+ cells outside of follicular centers
- Bcl-2+ follicular centers
  - Beware of nodules of normal bcl-2+ non-FCC or follicles with numerous T-cells
  - FC colonized by non-follicular type lymphomas can also be bcl-2+
Cyclin D1+ lymphoid cells but beware! Two cyclin D1 stains, one negative case.
Features to Suggest Neoplasia

• Numerous CD43+ B-cells
  – Useful to raise concern in CD5- B-cell proliferations
  – Beware of non-neoplastic CD43+ B-cell populations, e.g., in subepithelial portions of tonsils, in myoepithelial sialadenitis, in atypical marginal zone hyperplasias of childhood & in plasma cells

• Sheets of B-cells where there shouldn’t be sheets of B-cells
  – Most useful at extranodal sites but beware of very B-cell rich benign breast infiltrates
  – Lymph nodes may have numerous interfollicular B-cells in perisinus & medullary areas.
Why use an antibody for bcl-6?

- Highlights bcl-6+, CD10- follicles – a non-specific finding but one that can be associated with marginal zone lymphomas (follicular colonization)
- Less specific but more sensitive FCC-associated marker used in the assessment of DLBCL
Gastric MALT lymphoma
Gastric MALT lymphoma

Bcl-6

CD10
LN in patient with lacrimal gland MALT lymphoma

Bcl-2
LN in patient with lacrimal gland MALT lymphoma
LN in patient with lacrimal gland MALT lymphoma
**K & λ are not in our standard panel**

- In many (but not all laboratories) κ & λ stains fail to reveal specific surface Ig staining (as is present on most B-cell lymphomas).
  - A major strength of FCIPS
  - Still useful in evaluating proliferations with plasmacytoid or overt plasma cells
  - Tend to use in r/o MZL situations
  - Sometimes is helpful in other circumstances even in labs that are not κ & λ experts
  - Light chain class restriction not an absolute indicator of neoplasia!
Immunophenotype - B-cell Lymphomas
Beware of exceptions to these “rules”!

CD20+

CD5+
- Cyclin D1-
  - Variable
    - DLBCL
- Cyclin D1+
- CD10-
- CD10+

CD5-
- CD10-
- CD10+
- Bcl-6-
- Bcl-6+

- FL
- MCL
- DLBCL
- MZL/MALT
- LPL
- DLBCL
Modifying the Panel for Non-Small Cell Hematopoietic/Lymphoid Neoplasms

- Can eliminate the CD43
- Bcl-2 and bcl-6 still can be useful but, in most circumstances, not as critical as in potential small B-cell lymphomas.
  – We still use them.
Major Purposes of Panel for Large Cell, Blastoid, etc. Type Hematopoietic/Lymphoid Proliferations

• Establish cell of origin (a critical 1\textsuperscript{st} step) or make you realize you need to broaden your differential diagnosis & potentially use additional stains
  – Important just to show a large cell neoplasm is CD20+
May help identify the neoplastic cells as in this T-cell/histiocyte rich DLBCL

CD20
Help classify an aggressive B-cell lymphoma – together with histopathology, clinical & other ancillary studies

- Picks up blastoid MCL
- Helps to diagnose Burkitt lymphoma (Ki-67 also helpful here if no cytogenetic/FISH studies) – not always easy
Remember FISH studies can be also be performed using paraffin embedded material
What is the role of IHC panels in prognostication of DLBCL?

• Some individual IHC stains may be of prognostic importance at least in subsets of cases, eg, bcl-2 expression is often considered an adverse prognostic indicator.
Proposed IHC algorithms used to identify the reportedly more aggressive non-GC type DLBCL that was identified and most recently popularized by gene profiling studies.

NEJM 348:1777, 2003
Hans, et al, 2004

CD10  \rightarrow  \text{GC type}  \\
\downarrow \quad \downarrow + \\
BCL-6  \rightarrow  \text{Non-GC type}

MUM-1  \uparrow \rightarrow  \text{GC type}  \\
\uparrow +
Problems with Prognostication

• As therapies change, prognostic indicators change.
  – Work with your clinicians

• No uniformly agreed upon clinically relevant algorithm for dealing with the DLBCL, but some may still be useful

• Whereas some IHC stains are fairly uniform in their appearance & interpretation, others are not (technical factors, subjective interpretation, different criteria for positivity).
• IHC prognostic markers in DLBCL
  – Extreme variation between laboratories for many stains (bcl-6, MUM-1 & Ki-67) & moderate variation between pathologists even when same stains were reviewed
  – But use of the an algorithm almost like I illustrated led to “substantial agreement” (after cases that could not be scored were eliminated & using one set of stains)
May need other markers if a non-hematolymphoid neoplasm is in the differential or if there is any chance of a lymphoblastic or myeloid neoplasm

- Markers of immaturity: TdT, CD34, CD1a, (if considering T-LB), CD117 (not completely restricted to blasts)
- Myeloid/monocytic-associated markers: MPO, lysozyme, neutrophil elastase, CD117 (on some ALL), CD68 (PGM1), CD163, CD14
No time to discuss the approach to T-cell lymphomas.
Dealing with Suspected Hodgkin Lymphoma

Education by the Experts
Hodgkin Lymphoma According to the WHO

- Nodular lymphocyte predominant Hodgkin lymphoma
  - vs PTGC, T/HR DLBCL, small B-cell lymphomas, cHL, other
- Classical Hodgkin lymphoma (4 types)
  - vs NHL (gray zone cases), florid reactive hyperplasias (eg, mono), NLPHL
Panel for evaluating possible HL (expand if necessary depending on ddx & level of complexity)

- CD20, CD3
- CD15, CD30
- LCA (CD45) – controversial
- EMA
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Darkest red is the most positive.
“...it is advisable to include EMA in the first line panel and to ask for ALK staining in EMA+, CD15- lesions with morphologic features suggestive of NSHL.” Vassallo, et al, AJCP, 2006

ALK+ ALCL mimicking NSHL – 10 cases
Supplemental Panels

• Troubles diagnosing NLPHL
  – Pick out L&H cells: J chain (but may be weak or negative), bcl-6
  – Demonstrate nodularity: CD21 (FDC)
  – Identify characteristic (although not invariable background population): CD57 (numerous & if lucky rosettes)
Troubles Diagnosing cHL

- MUM-1: identifies classical R-S cells in almost all cHL
- PAX5 (pan-B-cell): weak + in most R-S cells
- Oct2 & Bob.1: one or both should be negative in classical R-S cells; however, especially with LRCHL, exceptions may occur
Other Panels

- Multiple myeloma evaluations: CD138, kappa, lambda, cyclin D1
- T-cell lymphomas: pan-T-cell & major T-cell subset markers, NK-associated markers, cytotoxic markers, CD10 & CD21, EBV
- Total unknowns or cases with conflicting data
Summary

• Seen how the use of a relatively limited panel of IHC stains can help deal with many of the more common hematopathologic diagnoses.
• Some cases will require additional stains & there will be some situations requiring fewer.
• All of the above must be put into the context of the histopathologic studies and sometimes additional ancillary studies as well.
(Imitation) Pearls of Pathology

• Establish a differential diagnosis based on clinical & morphologic evaluation prior to ordering additional ancillary studies on paraffin embedded tissues – don’t order stains blindly and don’t order them wildly.

• Interpret the stains with your differential diagnosis in mind – don’t interpret the stains blindly (1+, 2+, 3+) and don’t interpret them wildly (know the implications of your observations).

• Use panels of IHC stains and be sure that everything adds up – discrepant staining patterns may be an important clue to a specific diagnosis, to problems with a given stain or problems with your proposed diagnosis.
References

• See the handout but if you use nothing else
Thank you for participating!

Please complete the course evaluation before you leave.
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- My approach to this topic is solely my own.
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Learning Objectives
- To understand the goals of immunohistochemical panels in diagnostic hematopathology
- Know how to use a basic panel approach to help to diagnose the more common lymphoid proliferations
- To accomplish the above without breaking anyone’s bank!

Agenda
- What are the goals of performing immunohistochemical stains in the evaluation of lymphoid proliferations?
- Review how limited immunohistochemical panels, with potential additional follow-up stains, help us recognize and classify some of the more common lymphomas.

Other general comments about this presentation
- We will not be addressing the relative value of flow cytometric versus paraffin section immunophenotypic studies. We personally use flow cytometric studies, when appropriate, if we receive fresh tissue. Paraffin section immunostains are used when we receive tissue already fixed (or already embedded) and when the flow cytometric studies fail to provide the phenotypic data needed to make a confident and precise diagnosis.
- The limited length of this presentation precludes a comprehensive review of all the panels of immunostains that need to be utilized by hematopathologists in order to evaluate hematopathologic specimens. It also precludes a discussion of all the strengths, problems and pitfalls with the two major panels being presented.
- We will concentrate on dealing with the most common situations we encounter, not on how to solve the deepest mysteries of hematopathology.
- This handout cannot be used to determine whether a specific immunostain or set of immunostains might be inappropriate in a given case. Each case has unique features and raises different issues in the eyes of even very well-trained pathologists.
We use paraffin section immunohistochemical panels as an aid in making a series of diagnostic decisions when we evaluate lymph node biopsies or hematopoietic/lymphoid infiltrates at extranodal sites. The following chart illustrates the basic decisions that we attempt to make:

Whereas in the past, we would need to have a lengthy discussion as to what classification especially of the non-Hodgkin lymphomas to use as we made our diagnostic decisions, it is clear in 2007 that we should use the 2001 WHO classification. The classification, which is outlined below, is being revised with a new monograph tentatively scheduled to come out in 2008.

**WHO Classification -- outline**

- B-cell neoplasms
  - Precursor B-cell neoplasm (1)
  - Mature (peripheral) B-cell neoplasms (17)
- T and NK-cell neoplasms
  - Precursor T-cell neoplasm (2)
  - Mature (peripheral) T-cell neoplasms (14)
- Hodgkin lymphoma (Hodgkin disease)
  - Nodular lymphocyte predominant
  - Classical (4)

**Purpose of performing immunohistochemical stains**

One of the major purposes of using immunohistochemical stains is to provide information that is not otherwise apparent but is of diagnostic utility. In other words, these stains may demonstrate findings that are either not at all apparent morphologically or draw our attention to possibly very subtle morphologic features that otherwise might be missed. Some of the latter may only be
apparent when confirmed by the immunohistochemical studies. Examples of this would include use of anti-kappa and anti-lambda stains to assess whether plasma cell populations are light chain class restricted, or using a panel of stains to identify “early” or “in situ” involvement of lymph nodes by mantle cell or follicular lymphomas.2,3 While some of these cases can be recognized morphologically, especially in retrospect, others cannot be.

Another important purpose of these stains is as an internal “private” quality control. This is much more cost effective than sending every diagnostic lymph node biopsy to a consultant (something that each pathologist should decide for themselves based on their confidence level) and certainly more cost effective than making an error that could potentially result in irreparable damage to a patient. Even if one thinks the routine stained sections are obvious, there will be cases where even the most sophisticated experts may be fooled. The differential diagnosis for example for mantle cell lymphomas includes reactive lymph nodes, indolent lymphomas, aggressive lymphomas and even acute leukemias (see below).4 In spite of this long list and the potential for a whole host of diagnostic errors, the diagnosis can usually be made with grade confidence with the help of immunophenotypic studies. Furthermore, whereas cyclin D1 staining used to be quite problematic due to frequent distracting non-specific cytoplasmic staining and weak specific nuclear staining, better antibodies are now available.5

**Differential diagnosis for MCL**

- Reactive hyperplasia
- B-cell CLL/small lymphocytic lymphoma
- Lymphoplasmacytic lymphoma
- Follicular lymphoma
- Marginal zone B-cell lymphoma
- Splenic marginal zone lymphoma
- Hairy cell leukemia
- B-lymphoblastic leukemia/lymphoma (B-cell ALL)
- B-cell prolymphocytic leukemia
- Diffuse large B-cell lymphoma
- Peripheral T-cell lymphoma & precursor T-lymphoblastic lymphoma/leukemia

**So shouldn’t we just use all the stains we have available to cover all possible diagnoses? Why use directed panels?**

- Directed panels of immunohistochemical stains are more economical than using every antibody available in one’s laboratory. Indiscriminant use of immunohistochemical stains only encourages those who wish to restrict reimbursement for medically very believable pathologic evaluations.
- While it might not seem readily apparent, directed panels can actually cut down on diagnostic errors. Indiscriminant use of immunohistochemical stains can mislead pathologists when staining patterns are seen and misinterpreted,
based either on an insufficient knowledge base, an unusual immunoreactivity, or an artifactual positive or negative staining pattern.

**Immunohistochemical panels in diagnostic hematopathology**

Two immunohistochemical panels will be presented along with ancillary immunohistochemical stains that may be added in selected circumstances. The bulk of our discussion will cover a panel whose major purpose is to evaluate potential B-cell neoplasms but which can also provide important clues as to the presence of a T-cell (CD3+, CD5+), natural killer cell (e.g., CD3+, CD5- proliferation) or even Hodgkin lymphoma. There will not be time to explain its use in those circumstances. The second panel is one to use in cases of suspected Hodgkin lymphoma. Once again, additional immunohistochemical stains that can be added to the basic panel in more problematic situations will also be covered. These two panels can be used as part of the workup for probably at least ¾ of malignant lymphomas in the United States given the marked predominance of B-cell lymphomas and the significant proportion of Hodgkin lymphomas compared to the T-cell and certainly natural killer cell neoplasms.6,7

**Multipurpose panel to deal with the most common B-cell lymphomas**8,9

- CD20 – present on B-cells, a small subset of T-cells and rare T-cell lymphomas.
- CD3 – the paraffin section immunohistochemical stain detects both T-cells and natural killer cells.
- CD5 – present on T-cells, a small B-cell subset and selected B-cell lymphomas.10,11
- CD43 – present on T-cells, a small B-cell subset, selected B-cell lymphomas and myeloid cells.12 Some would be less enthusiastic about the inclusion of CD43 in a panel such as this.
- CD10 – identifies most follicular/germinal center B-cells, a subset of lymphoblasts, a small subset of mature T-cells, neutrophils and some epithelial cells.13-15
- Bcl-6 – identifies most follicular/germinal center B-cells, a small T-cell subset and some other cells.16 Weak staining may not have the same implications as stronger staining. Less critical than CD10 but still can be very useful.
- Bcl-2 – Many lymphoid cells but not many normal follicular/germinal center B-cells positive.16,17
- Cyclin D1 – normal lymphoid cells negative.4,16

We now need to answer the following two questions:

1. How do we use the panel to support the presence of a lymphoid neoplasm?
2. How do we use the panel to help classify a lymphoid neoplasm, once it is identified?

Ways in which our IHC panel might suggest the presence of a lymphoid neoplasm

- **Identify areas where there are clearly CD5+ B-cells based on a comparison of the CD3, CD5 and CD20 stains.** Whereas small populations of CD5+ B-cells are routinely seen by flow cytometry in normal individuals, recognizable areas where CD5+ B-cells aggregate are usually not identified in paraffin sections. We do occasionally see some expression in normal mantle zones.

- **Identify numerous CD10+ or bcl-6+ cells outside of follicular centers.** Scattered positive cells of B-cell or T-cell origin can be seen, in addition to CD10+ neutrophils normally. Sometimes one even sees some scattered CD10+ lymphoblasts. It should also be kept in mind that not all neoplastic CD10 or bcl-6+ cells outside of follicles are necessarily related to follicular center cells. Other lymphomas can also express these antigens such as some lymphoblastic lymphomas and some T-cell lymphomas.

- **Identify bcl-2+ follicular centers, since most normal follicular center cells (FCC) are bcl-2 negative.** One does need to beware of “positive” nodules of normal bcl-2+ non-FCC B-cells (e.g., primary follicles or mantles cut tangentially) or follicles with numerous T-cells. One must also remember that follicles colonized by non-follicular type lymphomas can also be bcl-2+.

- **Identify numerous CD43+ B-cells in a fashion analogous to identifying CD5+ B-cells.** CD43 will help identify a distinct but overlapping population of normal and neoplastic B-cells compared to CD5 and, thus providing additional information since sheets of CD43+ B-cells are not seen in most non-neoplastic circumstances. However, one must be aware of situations where paraffin section immunohistochemical stains will reveal non-neoplastic CD43+ B-cell populations, e.g., in subepithelial portions of tonsils, in myoepithelial sialadenitis, in atypical marginal zone hyperplasias of childhood and in some plasma cells.

- **Finding sheets of B-cells where there shouldn't be sheets of B-cells.** This is most useful at extranodal sites but you must beware of very B-cell rich benign breast infiltrates. Lymph nodes may have also have numerous interfollicular B-cells in perisinus and medullary areas. Especially when one has a section cut tangential to the lymph node capsule, one may see sheets of extrafollicular B-cells in a reactive lymph node.

Why include an antibody for bcl-6 in the panel since we already have one follicular/germinal center associated antibody that detects CD10?

The answer to this question relates to the observation that some follicular centers may be CD10 negative. This is particularly the case in some marginal zone lymphomas where bcl-6+, CD10- follicles may reflect follicular colonization.
Whether the bcl-6+ cells are a part of the neoplasm can sometimes be difficult to determine (luckily, it is often not critical to make this assessment). As will be discussed below, bcl-6 is a less specific but more sensitive FCC-associated marker also used in the assessment of diffuse large B-cell lymphomas (and is also present in some T-cell lymphomas such as about half of anaplastic large cell lymphomas and in precursor T-lymphoblastic lymphomas).  

Why aren't stains for kappa and lambda included in the basic IHC panel being discussed?

The main reason is that in many (but not all) laboratories, including our own, \( \kappa \) and \( \lambda \) stains fail to reveal specific surface immunoglobulin staining in most cases. Most B-cell lymphomas have surface rather than significant amounts of cytoplasmic immunoglobulin expression. Detection of surface kappa and lambda expression is one of the major strengths of flow cytometric immunophenotypic studies. Nevertheless, paraffin section immunohistochemical stains for kappa, lambda and sometimes the immunoglobulin heavy chains are useful in evaluating potential lymphoid or plasmacytic neoplasms, especially if there is plasmacytoid differentiation or overt plasma cells. We tend to add kappa and lambda stains to our panel in evaluating potential marginal zone lymphomas since they often fail to have an “aberrant” B-cell phenotype and a moderate number (based on the site and type) may have plasmacytic differentiation. Remember also, however, that light chain class restriction is not an absolute indicator of neoplasia!

**Immunophenotype - B-cell lymphomas**

*Beware of exceptions to these “rules”!*

![Immunophenotype Diagram]

All of the antigens in this table are detectable using IHC stains except for FMC-7. We do not use CD23 stains very often in paraffin sections but they may be useful in selected cases. DLBCL-
Large cell, blastoid and other transformed-appearing neoplasms

Much of the discussion up to this point and the examples chosen have focused on the small B-cell lymphomas. When one is faced with evaluating a large cell, blastoid or other transformed cell proliferation, the issues are often somewhat different. Oftentimes, whether or not you have a neoplasm is more readily apparent, and determining what type of neoplasm, including whether it even is of lymphoid origin, is the major decision that has to be made. In these circumstances, CD43 can be eliminated from the panel if you want as it will not be very helpful in classifying a lymphoid neoplasm (although on occasion it may provide useful information even in these type of cases, such as when in the absence in CD3 and CD20 expression it may make you consider the possibility of a myeloid sarcoma). Bcl-2 and bcl-6 still can be useful but, in most circumstances, not as critical as in potential small B-cell lymphomas. We do use them in these circumstances as they do provide useful information that we use in our diagnostic evaluation.

The basic IHC panel being discussed can provide a large amount of diagnostic information in the evaluation of these larger cell/blastoid proliferations. It will often establish the basic cell of origin (B-cell versus T-cell), which is a critical first step in evaluating these type of cases or make you realize you need to broaden your differential diagnosis and potentially use additional stains. Although we now take it for granted, remember it is important even just to show that a large cell neoplasm expresses CD20 given the important role of anti-CD20 antibodies in the therapy of many B-cell lymphomas. In other cases, the CD20 antibody may highlight that although you might have been focused on the numerous small cells present in a lymphoid proliferation, there are large, sometimes atypical-appearing, CD20+ cells raising the possibility of a T-cell/histiocyte rich diffuse large B-cell lymphoma or one of the other lymphomas in the differential diagnosis for that entity. Remembering the algorithm illustrated above for the phenotypic categorization of B-cell lymphomas, the panel will help in the diagnosis of blastoid mantle cell lymphomas and Burkitt lymphomas. Currently there is a lot of interest in refining the criteria for what should be considered a Burkitt lymphoma and what is better considered a diffuse large B-cell lymphoma or a “high grade” B-cell lymphoma, not otherwise specified. Use of the MIB-1 Ki-67 antibody is a useful addition to the panel when a Burkitt lymphoma is being considered especially if no cytogenetic data is available (looking for virtual 100% positivity). Remember, however, that cytogenetic FISH studies can also be performed using paraffin embedded material to look for C-MYC and other translocations. Also remember that while Burkitt lymphomas are expected to have C-MYC translocations, the finding is not a specific one.
What is the role of IHC panels in prognostication of diffuse large B-cell lymphomas?

In addition to their diagnostic role, there is great interest in the use of immunohistochemical stains for prognostic purposes in the lymphomas, in spite of the great challenges this poses. Specifically, the demonstration by gene profiling studies of a germinal center and one or more non-germinal center types of diffuse large B-cell lymphomas (not really a new idea), together with the apparent adverse prognostic implications of a non-germinal center type gene profile has led to renewed interest in subdividing this heterogeneous and large category of lymphomas. While gene profiling remains in the experimental realm, attempts to use IHC as a surrogate have been published. The results have not been at all uniform, however, one proposed algorithm that has been used in a number of different studies, is reported to correlate with gene profiling results and to be of prognostic utility. This algorithm classifies a diffuse large B-cell lymphoma as being of germinal center type if it is either CD10+ or CD10- but bcl-6+ and MUM-1-. All others using this panel of three antibodies (two of which are in our standard panel and one of which needs to be performed as a supplemental stain) are considered to be of non-germinal center type.

While some clinicians may be interested in knowing this information for their patients, there are many problems with the use of immunohistochemical and other prognostic indicators. First, as therapies change, so can the prognostic indicators so that what used to be important not longer is, or vice versa. Many still quoted studies relate to patients treated in the era before the widespread use of anti-CD20 antibodies, for example. It is therefore important to work closely with your clinicians to know what they are interested in. Second, there is no uniformly agreed upon clinically relevant algorithm for dealing with the diffuse large B-cell lymphomas. This, however, does not mean that all are useless. Third, whereas some immunohistochemical stains are fairly uniform in their appearance and interpretation such as CD20, others are not due to technical factors, subjective interpretation and different criteria for what should be called positive. A recent multi-institutional study from de Jong, et al (in press, Journal of Clinical Oncology) has clearly made this point. They demonstrated extreme variation between laboratories for many stains (bcl-6, MUM-1 & Ki-67) and moderate variation between pathologists even when same stains were reviewed. However, use of an only minimally modified algorithm derived from the one I illustrated led to “substantial agreement” in how the cases were categorized (after cases that could not be scored usually because of the lack of an internal positive control were eliminated and using one set of stains).

Limits of our panel

It is recognized that the panel being discussed will be insufficient especially in some of these large cell/blastoid neoplasms. Based on a review of the initial panel, or sometimes simply after review of the routine histologic sections, one
may need to add other markers, for example, if a non-hematolymphoid neoplasm is in the differential or if there is any chance of a lymphoblastic or myeloid neoplasm. The latter possibilities should always be considered. Remember that precursor B-lymphoblastic lymphomas can present without leukemia and that they can be CD20 positive so a high index of suspicion is important. Many of the additional panels will be influenced by the degree of suspicion for one of these other entities and the results of other immunohistochemical stains. Some markers of immaturity that can be used to help support the present of true blasts include TdT (lymphoblasts, some acute myeloid leukemias), CD34 (not on all blasts and not lineage-associated), CD1a (on a subset of T-lymphoblasts) and CD117 (on some myeloblasts and somewhat more mature myeloid forms as well as expressed by some lymphoblastic neoplasms). Some myeloid/monocytic-associated markers include myeloperoxidase, lysozyme, neutrophil elastase, CD117 (also on some lymphoblastic neoplasms, mast cells), CD68 (PGM1 is the more monocyte-associated form), CD163 (monocytic cells) and CD14 (monocytic cells).

Don’t forget about the T-cell lymphomas – just no time to review in this discussion (see potential panel of stains below).

Dealing with suspected Hodgkin lymphoma

Hodgkin lymphoma according to the WHO

- Nodular lymphocyte predominant Hodgkin lymphoma
  - The differential diagnosis includes progressive transformation of germinal centers, T-cell/histiocyte rich large B-cell lymphoma, small B-cell lymphomas, classical Hodgkin lymphoma, and other entities.
- Classical Hodgkin lymphoma (4 types – lymphocyte rich, nodular sclerosis, mixed cellularity and lymphocyte depleted)
  - The differential diagnosis includes large B-cell lymphomas (including gray zone cases where a definitive categorization cannot be rendered), florid reactive hyperplasias (e.g., mononucleosis), nodular lymphocyte predominant Hodgkin lymphoma and other entities.

Panel for evaluating possible HL (expand if necessary depending on differential diagnosis and level of complexity)

- CD20, CD3 (basic B-cell and T-cell associated markers)
- CD15, CD30 (markers of classical Reed-Sternberg cells but not specific)
- LCA (CD45) – controversial as to its utility due to some individuals’ feelings that it is impossible to interpret due to the numerous small lymphocytes present in these cases (we still like it)
- EMA (used to help identify anaplastic large cell lymphomas and a subset of nodular lymphocyte predominant Hodgkin lymphoma cases)
Hodgkin lymphoma panel - interpretation

<table>
<thead>
<tr>
<th></th>
<th>cHL</th>
<th>NLP HL</th>
<th>B-NHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20</td>
<td>Some cases positive, usually variable staining</td>
<td>Positive</td>
<td>Most positive</td>
</tr>
<tr>
<td>CD3</td>
<td>Rarely RS cells positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>CD15</td>
<td>Many cases positive</td>
<td>Negative</td>
<td>Most cases negative</td>
</tr>
<tr>
<td>CD30</td>
<td>Positive</td>
<td>Usually negative</td>
<td>Often negative</td>
</tr>
<tr>
<td>LCA</td>
<td>Negative</td>
<td>Positive</td>
<td>Usually positive</td>
</tr>
<tr>
<td>EMA</td>
<td>Only rarely positive</td>
<td>Some positive</td>
<td>Usually negative</td>
</tr>
</tbody>
</table>

Staining patterns refer to the neoplastic cells.

Why include EMA in the panel – is anaplastic large cell lymphoma really in the differential diagnosis of nodular sclerosis Hodgkin lymphoma (yes, the WHO did eliminate the Hodgkin type of ALCL)? We were recently reminded again that in fact some cases of ALCL can resemble nodular sclerosis classical Hodgkin lymphoma. These authors concluded that “…it is advisable to include EMA in the first line panel and to ask for ALK staining in EMA+, CD15- lesions with morphologic features suggestive of NSHL.”

Supplemental panels for cases of suspected Hodgkin lymphoma

In some cases the standard panel will be insufficient to make a confident diagnosis for a variety of different reasons so that supplemental panels of additional stains may be worthwhile.

- If troubles diagnosing nodular lymphocyte predominant Hodgkin lymphoma (NLP HL), one might try the following:
  - To help pick out the neoplastic L&H cells: J chain (but may be weak or negative, also stains plasma cells), bcl-6 (of course, not specific)
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- To demonstrate nodularity in order to support the diagnosis of NLPHL over a T-cell/histiocyte rich large B-cell lymphoma: CD21 (to identify follicular dendritic cells)
- To identify the characteristic, although not invariable, background population of “germinatal center type” T-cells: CD57 (looking for numerous positive cells and, if lucky, rosettes around the putative neoplastic cells)

- If troubles diagnosing classical Hodgkin lymphoma (cHL)
  - To help identify classical R-S cells in almost all cHL: MUM-1
  - To help show B-cell nature of neoplastic cells (and help show not an anaplastic large cell lymphoma): PAX5 (pan-B-cell) which is weakly positive in most R-S cells
  - To help distinguish cHL from B-cell NHL or NLPHL: Oct2 & Bob.1 -- one or both should be negative in classical R-S cells; however, especially with lymphocyte rich classical HL, exceptions may occur.

Other IHC panels for other purposes not being discussed today

- Multiple myeloma evaluations: CD138, kappa, lambda, cyclin D1
- Possible T-cell lymphomas: pan-T-cell & major T-cell subset markers, NK-associated markers, cytotoxic markers, CD10, CD21, EBV
- Total unknowns or cases with conflicting data: these are the cases that will require the greatest number of immunostains. They may not be uncommon especially in consultation practices.

Summary

We have seen how the use of a relatively limited panel of IHC stains can help deal with many of the more common hematopathologic diagnoses. Nevertheless, some cases will require additional stains and there will be some situations requiring fewer. The complexity of the cases you are dealing with will affect the number of immunostains you need to perform. IHC stains always must be put into the context of the histopathologic studies and sometimes additional ancillary studies as well.

(Imitation) Pearls of Pathology

- Establish a differential diagnosis based on clinical and morphologic evaluation prior to ordering additional ancillary studies on paraffin embedded tissues – don’t order stains blindly and don’t order them wildly.
- Interpret the stains with your differential diagnosis in mind – don’t interpret the stains blindly (simply with a long list of antibodies and 1+, 2+, 3+ designations) and don’t interpret them wildly (know the implications of your observations).
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- Use panels of IHC stains and be sure that everything adds up – discrepant staining patterns may be an important clue to a specific diagnosis, to problems with a given stain or problems with your proposed diagnosis.

Supplemental table with listing of diagnostically useful antigens detectable using paraffin-reactive antibodies

<table>
<thead>
<tr>
<th>B-cells</th>
<th>CD20, CD79a, CD22, PAX5 (BSAP), CD45RA (4KB5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markers used to define B-cell subsets &amp;/or classify B-cell neoplasms</td>
<td>CD10, bcl-6, CD23, bcl-2, CD5, CD43, cyclin D1, MUM1/IRF4</td>
</tr>
<tr>
<td>T-cells</td>
<td>CD2, CD3, CD5, CD7</td>
</tr>
<tr>
<td>Other T-cell associated markers (less specific, on some B-cells, myeloid/macrophages)</td>
<td>CD45RO, CD43</td>
</tr>
<tr>
<td>T-cell subsets</td>
<td>CD4, CD8, TIA-1, CD56, CD57, granzyme-B, beta-F1</td>
</tr>
<tr>
<td>NK-associated</td>
<td>CD56, CD57, others</td>
</tr>
<tr>
<td>Hodgkin’s Lymphoma-associated</td>
<td>CD15 &amp; CD30 (classical), J chain (NLPHL), absent/minimal Oct-2 &amp;/or Bob.1</td>
</tr>
<tr>
<td>Other markers used in lymphoma diagnosis</td>
<td>CD45 (LCA), CD30, EMA, ALK-1, clusterin, (bcl-10), Ki-67</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>CD138, κ, λ, IgG, IgM, IgA, IgD</td>
</tr>
<tr>
<td>Follicular dendritic cells</td>
<td>CD21, CD23</td>
</tr>
<tr>
<td>Other dendritic cells</td>
<td>CD1a (LC), S100 (LC &amp; IDC), CD123 (plasmacytoid)</td>
</tr>
<tr>
<td>Immature/precursor cells</td>
<td>CD34, CD99 (not specific), TdT, CD1 (thymocytes), CD117 (mostly myeloid)</td>
</tr>
<tr>
<td>Myeloid/monocytic</td>
<td>Lysozyme, myeloperoxidase, neutrophil elastase, CD117, CD68, CD163, CD14, glycoporphin (RBC),</td>
</tr>
<tr>
<td>Mast cells</td>
<td>Tryptase, CD117</td>
</tr>
<tr>
<td>Viral markers</td>
<td>EBV - LMP-1; HHV-8 (KSHV); other</td>
</tr>
</tbody>
</table>

LC – Langerhans cells, IDC- interdigitating dendritic cells, RBC – red blood cells, mega – megakaryocytes

References

15. Cook JR, Craig FE, Swerdlow SH. Benign CD10-positive T cells in reactive lymphoid proliferations and B-cell lymphomas. Mod Pathol. 2003;16:879-885
17. Utz GL, Swerdlow SH. Distinction of follicular hyperplasia from follicular lymphoma in B5-fixed tissues: comparison of MT2 and bcl-2 antibodies [see comments]. Human Pathology. 1993;24:1155-1158


