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Table of Contents

- Getting Your Paper Published: An Editor's Perspective
- Practical Immunohistochemistry in Hematopathology: A Review of Useful Antibodies for Diagnosis

Getting Your Paper Published: An Editor's Perspective

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The process of scientific research is only useful if novel and robust results can be communicated to others such that the stock of human knowledge increases. Consequently publication must be seen as a central part, if not the central part, of the research process. Of course there are many benefits to authors contingent on publication, ranging from kudos to enhanced employment prospects and even tenure, but it is the communication of novel information that is paramount. Many find the process relatively easy while others struggle. Some authors are well coached by their mentors. Others have not benefited from such development, and in particular for junior researchers the key steps of grant writing and paper writing can be daunting and full of frustration. In this short review I will provide a personal perspective on the issue of writing scientific papers in the biomedical arena, based on my experiences as an author, a reviewer and an editor. I shall approach this task with a series of Ten Lessons, each of which builds into a short course that will successfully develop your writing skills

Lesson 1: Develop your skills by reading

As with all skills the ability to write well can be learned. Some find it easy, others hard, but without doubt all can improve and develop their skills. As with all educational experiences there is a relationship between effort expended and rewards. A key 'educational device' in the school of scientific writing is reading! Read much and widely! Read scientific papers in front rank journals and examine closely the style and approach of authors who have succeeded. Look at the structure and clarity of language. Observe the simple structure of sentences and paragraphs. See how the well-crafted paper is concise and to the point. Notice how good authors use simple language without verbosity and flowery, overly inflated statements. There is much to be gained from time spent reading!

Lesson 2: Have something to say

There are thousands of journals and perhaps hundreds of thousands of papers published every year. It is a sad fact that the majority of papers are not cited and it is likely that many are not read by more than a handful of people. This then begs the question as to when is something worth publishing? 'The key point here is 'have something to say'! Only when you have a clear message should you begin to think about the publication process. Your message should be clear and it should be a significant addition to the literature. Consequently you need to have a good grasp of the relevant literature relating to your message, and also the techniques and methods you have used, their advantages and disadvantages, value and limitations, and the general background of the area. Why did you start, what did you do, what answer did you get and what does it mean anyway?'

This of course relates to the general paradigm of Introduction, Materials and Methods, Results and Discussion (sometimes referred to as IMRAD). The Introduction should set the scene and in a concise manner define the general background to the subject of investigation. It should not be overly long and should be in proportion to the rest of the manuscript. A useful strategy is to end this section with the question (or hypothesis) that is being posed.

The Materials and Methods of any research paper should contain enough information for the reader to understand exactly what was done. This is crucial. It may be that to save space extraneous detail might be placed in supplementary information that is only published online. This is now increasingly common but there still needs to be enough information in the paper to explain core methodologies, including patient groups. The practice of placing all Materials and Methods in supplementary material online is, in my view, unfortunate and indeed reprehensible.

The Results section should contain clear statements of all the core data and observations in a logical order. It should not contain interpretation nor materials and methods! Display items and tables should not replicate information provided in text. As with the Materials and Methods it is now common for additional data and results to be placed online and this is entirely appropriate. However, as with the Materials and Methods there should be full disclosure of the key results in the main body of the paper, again sufficient to allow the reader to understand the key data.

In the Discussion, the data should be placed in a broad context and appropriately discussed. It is common for authors to extend discussions into all sorts of tangential areas and to speculate wildly. In my view it is important to stay focused on the point of the paper. However, it should be said that it is good practice to mention and discuss potential problems and caveats of the studies reported and to consider (or at least mention) opposing or alternate views and hypotheses. A balanced paper will consider the field in the round.

A number of other elements are important in papers today. A conflict of interest (COI) statement is important. Conflicts of interest in themselves are not a bar to publication: not knowing about COI is however regrettable. A recent Commentary on this subject has been published. Statements of author contribution are increasingly seen as an important part of good writing and publication practice. Both COPE and the ICMJP have guidelines on what constitutes authorship and a

Lesson 3: Understand the structure of a scientific article

The core structure of a research article (I will not touch upon reviews here although the basic principles are common) is well established. Despite being criticized by some authorities, the basic structure was crystallized by Austin Bradford Hill in the statement: statement of contribution can be useful as this forces

authors to think about who has done what and who really should be an author. Clear statements about ethical approval and governance issues should be documented with relevant reference numbers. Other information that needs to be carefully collated will include the affiliations and up to date contact details of all authors as well as sources of funding and relevant acknowledgements and thanks. It is rare for research to be undertaken by one person and the many contributions of others should always be appropriately acknowledged: a 'thank you' goes a long way!

Lesson 4: Understand the simple rules of writing

In Lesson 1 we considered the value of reading widely. There are general rules of writing and George Orwell outlined these. Drawing from his ideas and those of Tim Albert, one can make a number of key points.

- Never use a long word where a short one will do
- If it is possible to cut a word out, then cut it out
- Keep sentence constructions simple
- Avoid one-sentence paragraphs
- Use simple punctuation

Over and above these rules I would recommend two 'tests' that assist in writing. First, the 'tell it to a friend' test. Can you explain the points you are wishing to make in your paper in simple terms to a colleague or even to a relative who is not an expert? If you are able to do this your understanding of what you wish to convey is excellent. If you cannot then are you sure you have a full grasp of the field? Second, the 'read it out loud' test. Having written your paper, and once you are very happy with it, take it to a quiet place. Perhaps in an empty room or an empty field. There slowly read it out loud to yourself! You will be amazed how something you have written and looks fine on paper, sounds awful when you read it out loud. The grammatical errors and poorly contrasted sentences will jump out as you speak them!

Finally it is important to get others to read you draft manuscripts. Do not rush to send manuscripts to journals. A few days extra with input and advice from others, from mentors and colleagues can be invaluable. If nothing else they may spot typographical errors and other small points that an author can become blind to after spending days or weeks crafting their magnum opus.

Lesson 5: How to decide where to send your paper

It is a simple fact that there exists a clear hierarchy of journals: there are those very high profile journals such as the *New England Journal of Medicine*, *The Lancet*, *Cell*, *Nature* and *Science* that command huge respect and in whose pages are often (but not invariably) carried research reports, reviews and other articles of major importance. Publishing in such high impact journals is the pinnacle of careers and inevitably few authors achieve this. There lie just below these a wide array of journals whose

impact is only slightly less and also carry major impact articles. Then within any given specialty there are specialist journals and here to there is a range of 'quality' and impact. For example, in Pathology there are currently 71 journals listed by ISI ranging from the research journal with the highest impact: *Journal of Pathology* (Impact Factor 6.446) to the *Korean Journal of Pathology* (Impact Factor 0.064) with the mean Impact Factor of this grouping being 2.25. While Impact Factor is a widely criticized parameter it does have some utility in providing a ranking of journals. By this means potential authors can make some judgment of where their work is best placed. An important lesson it is to understand the spectrum of possible journals and the bibliometric measures that are used to create a hierarchy of them.

So then how does an author (and his colleagues) decide on where to send a manuscript? From Lesson 5 you will now have an understanding of the 'league tables' of journals and as a simple rule one should aim as high as possible. High impact journals will inevitably have more exposure and weight than low impact journals. But the Impact Factor is not the only variable to consider. Authors need to ask questions about the appropriateness (or fit) of their work with the journal. They need to read the aims and scope of the journal: does the proposed manuscript 'fit' in the journal's area of coverage? Authors should look at copies of the journals that they are considering and see if they publish the kinds of work that the authors are going to report. Authors need to consider some other important issues such as the time a journal takes to undertake the review process. This can be gleaned from examining published papers and examining the dates of submission and acceptance. Some journals are quick: others notoriously slow. Even when a manuscript has been accepted there is an interval between acceptance and publication: what is this for the journals you are considering? Again there is great variation. The *Journal of Pathology* currently has a mean time to first decision of 13 days. The mean time to final decision is 22 days. From acceptance to publication online of an edited but not typeset version is just a few days. Other journals are much slower. Authors would like their work disseminated as quickly as possible and so these issues will influence the decision-making process.

The final group of issues relevant to decisions regarding publication relate to costs and the nature of access. Authors should consider the quality of printing and the quality of the journal website and its online versions. Authors should consider the costs involved: are there submission charges? Are there page charges? What is the cost of color figures? Does the journal charge for supplementary material online? Over and above this, authors should consider the pros and cons of open access journals, that is journals where the author pays a significant fee (typically in excess of \$3000) to have the paper free to all via the Internet. All of these factors will influence the final choice of destination for your manuscript.

Lesson 6: The instructions to authors and the need to worry about detail

Each journal will publish in hard copy and/ or via its website a clear set of instructions of how a manuscript should be produced and prepared for submission to that journal. While it is the case that there are differences in detail between all journals, in reality the basic principles are the same. However it is crucial that any

author reads and carefully considers all of the issues in these instructions and attends to the issues with a focus on detail. **Take for example the Instructions to Authors for the Journal of Pathology.** Here we set the instructions out in a 'checklist' based manner with a range of headings that follow the normal structure of a paper. Each heading is hyper linked to further text with explanatory detail. That detail is important and manuscripts that are submitted without attention to the various points outlined are usually returned to the authors without review. This wastes everyone's time! Moreover it sends a signal to the editor that the authors do not worry about detail. If they do not worry about detail in the submission process can the editor be sure they worry about detail in the research? It sends a very worrying signal!

Where do authors make the biggest mistakes?

Without question the biggest errors come from (1) manuscripts, figures and tables in the wrong file format (if it says a Word file do not send a PDF), (2) incorrect font and text formats (double spaced means double spaced, not single spaced; no line numbering means no line numbering etc), (3) incorrect format of references in the text or in the bibliography, and (4) incorrect resolution for figures and other display items. With regard to the latter sadly it is the case that many authors do not realize that what looks excellent on a computer screen (resolution 72 dpi) is wholly inadequate for publication purposes, where 300 dpi are often needed. Furthermore the dimensions of an image are important. Regularly one sees figures being submitted which are 300 dpi and seemingly fine, but they are perhaps only 10 by 5 mm: when expanded to fit a full column (86mm wide) or full page (176 mm wide) the resolution falls to unacceptable levels. It seems odd that authors invest considerable time in generating data but fall at the final hurdle when preparing their figures. Again attention to detail and the meticulous following of instructions to authors is crucial to success in publication.

Lesson 7: Understanding the steps after manuscript submission

Your manuscript has been written and after careful proofreading and worrying about all the issues in the instructions to authors, you and your co-authors are happy. Today nearly all submissions are via some online manuscript handling system. Before you begin this process make sure you have an electronic folder with all the correct files present, in the correct file format and with sensible (preferably unique) file names. Calling your main manuscript file 'manuscript.doc' is not smart since it is not unique. Use something in naming file that is unlikely to be confused, perhaps including the first author name, a key word, and maybe the date. Perhaps Bloggs_VEGF_hepatoma_October_2010.doc, for example. Make sure you have the final version available of each relevant file, not draft versions. Make sure track changes and comments are turned off. As with Lesson 6, worry about the detail! Most journals will want the email and relevant other contact details of all authors, so have these to hand. Again such information will be found in the instructions to authors of any reputable journal. Some journals may ask for suggestions of reviewers. If so ensure you have some sensible suggestions to make and you can sometimes suggest

reviewers that you do not want to be involved: I would suggest you indicate why you have non-preferred reviewers. When suggesting reviewers avoid colleagues in your own institution or those who have obvious conflict of interest.

After successful submission all you can do is wait. The editorial team will review the manuscript and it is increasingly common for a proportion of manuscripts to be returned to authors un-reviewed (for the *Journal of Pathology* this is about 30% of manuscripts). This is usually because the manuscript is felt by the editors to be not in the scope of the journal or to in some way not be likely to have a good chance of succeeding in the re-view process. Hopefully this will be done in a few days and saves everyone time. Do not be dejected, but recognize the editor has probably done you a service, hastening your submission to a more appropriate journal.

After peer review, it is extraordinarily rare for a manuscript to be accepted without change: in 6 years as an Editor-in-Chief, I have only accepted two such articles! In the *Journal of Pathology* less than 25% of submitted manuscripts are ultimately accepted. A proportion of those go through one or more rounds of major revision while the rest go through one or two rounds of minor revision. Different journals have slightly different policies but for the *Journal of Pathology* minor revision usually means there is a need for some significant redrafting or re-working of the manuscript without the need for additional experimentation. In contrast major revision usually entails the need for additional data, experiments or control studies.

After ultimate acceptance of your manuscript, it is usual for the editor to undertake some degree of editing of the text. A 'copy-editor'; whose role is to ensure that the manuscript conforms to house style, may carry out further text editing. At that point it moves to the typesetter who creates (now by electronic means) a final version. At this point a PDF proof will be sent to the corresponding author: it is essential that they respond to the questions posed by the typesetter (usually issues of confirmation of key detail) as soon as possible. This is the last opportunity for any minor changes to be made! At some time after that the manuscript will be published online and in a print version.

Lesson 8: Understand what editors like

Editors are simple people! They like authors to follow the instructions to authors and this is a huge step in winning over an editor. Many would be astonished by how common it is for authors to completely fail to comply with key issues in the instructions to authors (see Lesson 7). Over and above this editors like manuscripts that have a good 'fit' with the journal's aims and scope and address a clear research question. A message or story that is important is desirable if not essential, and concise clear writing is important. The title should be short, informative and to the point and the abstract should be clear and comprehensive but without unnecessary material. The display items and tables should be clear and of good quality and the legends appropriate.

Lesson 9: Be aware of what editors do not like!

The corollary of Lesson 8 is that there is a range of things that editors really do not like! Papers that do not fit with the journal, that are unoriginal, that are overly long or in any other way do not comply with the instructions to authors are the bane of the editor's life. It may seem overly picky to some, but it is crucial to comply with formatting instructions to be found in the instructions to authors, whether they be in relation to font size, spacing, page numbers, line numbering, abbreviations, reference format and style. In addition, editors do not like manuscripts that contain poor science. For example, studies based on flawed or incorrect assumptions or that use inappropriate methodologies are problematic. In addition, poor experimental controls will compromise a potentially important study as will poor images and data presentation. Careful scrutiny of these issues by colleagues and co-authors is better than having a manuscript returned without review! Attention to detail can avoid these pitfalls (see Lesson 7).

Lesson 10: Do not give up and understand the peer review process

Authors need to understand that most journals only accept a fraction of the submitted material. However if one is persistent and recognizes that there is a hierarchy of journals one can get your work published. A recent study of papers rejected from the *Journal of Pathology* revealed that most did get published eventually and almost invariably in a lower impact factor journal [9]. That is to say manuscripts tend to settle at a level in relation to their perceived quality (of course authors tend to perceive their work as being world class and one has to become objective of one's efforts).

A crucial element of the publication process is the comments of reviewers and editors. The entire peer review process is intended to improve manuscripts and the quality of the scientific record. Certainly reviewers are fallible and as human beings can be influenced by external factors. However, on receipt of reviewers comments examine them with care. Even if you profoundly disagree with one or more comments, look carefully at your manuscript. Is there a possibility that the reviewer has important observations relevant to your work? Even if your paper is rejected from that particular journal you should examine your manuscript in the light of the comments. It may lead you to undertake more experiments (or make more observations). In addition at the very least it may lead you to redraft your manuscript. Use the reviewers' comments! They are intended to help.

Final comment

The 10 lessons in this short course will, I hope, guide authors (and especially tyros) through some of the key steps in scientific writing. But some further words might be useful. It is crucial that you read, and know, the literature that relates to your area. What you try and publish should be influenced by what others have published: be guided by that. As indicated by Lesson 1 it is important to read and read: by reading you will see good practice and hopefully develop your skills: and

as with any skill practice leads to improvement, even if perfection is rarely achieved!

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Practical Immunohistochemistry in Hematopathology: A Review of Useful Antibodies for Diagnosis

Lu, Ji, Chang, Karen L.

Abstract

This review article offers some useful panels of immunohistochemical stains and discusses their use in determining a hematopathology diagnosis. As a comprehensive review of the vast array of hematolymphoid malignancies is beyond the scope of this study, the suggestions are based on broad morphologic categories such as follicular proliferations, paracortical expansions, diffuse small-cell infiltrates, diffuse large-cell infiltrates, and Hodgkin-like infiltrates. The review article also discusses the most common hematolymphoid malignancies and their immunohistochemical profiles, and how to use immunophenotyping to differentiate them from other entities. Common diagnostic pitfalls and misconceptions about certain antibodies will also be discussed. New antibodies, such as SOX11, will also be explored in the context of specific disease entities for which they may be of use.

Many of the hematolymphoid disorders are derived from the same cell types in different stages of maturation and their morphologic differences may be subtle. With experience, one may arrive at the correct diagnosis based on the hematoxylin and eosin (H&E)-stained sections alone. This may be quite challenging, particularly if one does not see these specimens frequently. A panel of immunohistochemical stains may help to determine lineage, subclassify tumors, and screen for common morphologic mimics. The purpose of this review article is to suggest some useful panels of immunostains and to discuss their use in arriving at the final diagnosis. As a comprehensive review of the vast array of hematolymphoid malignancies is beyond the scope of this study, the suggestions are based on broad morphologic categories such as follicular proliferations, paracortical expansions, diffuse small-cell infiltrates, diffuse large-cell infiltrates, and Hodgkin-like infiltrates. These panels, which serve as a starting point to think about a vast differential diagnosis, require alteration for each individual case, based on the morphology. The authors stress that morphology is more diagnostic than immunophenotype, but where discrepant, the pathologist may seek assistance from a specially trained hematopathologist. The first part of this review article offers suggested antibody panels and how to use them to narrow the differential diagnosis. The second section is a

discussion of the most common hematolymphoid malignancies, their immunohistochemical profiles, and how to use immunophenotyping to differentiate them from other entities. Common diagnostic pitfalls and misconceptions about certain antibodies will also be discussed. New antibodies, such as SOX11, will also be explored in the context of specific disease entities for which they may be of use.

HELPFUL IMMUNOHISTOCHEMICAL PANELS

Immunohistochemical panels are selected based on the morphologic examination. Recognition of more specific features on the H&E stained sections will result in more tailored and targeted immunohistochemical panels. For this section, we will assume that only very general architectural patterns are present, so the panels will be very broad and encompass many entities in the differential. Then, depending on the staining profile of these initial panels, one can generate a list of differential diagnoses and refer to section II for more detailed discussion of each disease. Alternatively, if the morphology is pathognomonic, one may choose to bypass the initial panel and skip straight to confirmatory stains for that tumor.

General Stains

Most lymph nodes and even extranodal lymphoid tissue consistently show some degree of reactive changes. Certain patterns of reactive hyperplasia may suggest an underlying etiology. The morphologic differentiation of these reactive conditions is beyond the scope of this review. Perhaps, more important than determining the etiology of a reactive lymphoid hyperplasia is to exclude a lymphoma. In lymph nodes, it is helpful to note that most cases of reactive hyperplasia show a combination of follicular, paracortical, and/or sinus hyperplasia, although one pattern may predominate. When hyperplasia of a single component is seen to the exclusion of the others, one should be suspicious of a neoplastic process.

Several stains compose the backbone of most of the panels listed below. Most of the stains are aimed at B-cell lesions, which comprise the majority of lymphomas. These include CD20, CD3, bcl-2, and CD43. CD30 may also be added to screen for Hodgkin lymphoma (HL). One should understand the normal staining pattern of these markers in benign/reactive lymphoid tissues. CD20 should highlight localization of B-cells mainly within follicles. Primary follicles express bcl-2, but lack CD10 and bcl-6. Secondary follicles contain a germinal center and a mantle zone. The germinal center cells express CD10 and bcl-6 but lack bcl-2. The mantle zone is composed of similar cells as the primary follicle. Scattered B-cells, including large immunoblasts, may be present in the paracortical region. Immunoblasts show weak, variable CD30 positivity, which characteristically varies in intensity from cell to cell. Both paracortical B-cells and immunoblasts may be increased in reactive conditions but are always outnumbered by the paracortical T-cells. Diffuse sheets of CD20-positive cells outside follicles should raise suspicion for B-cell lymphoma. The CD3 immunostain highlights T-cell localization predominantly to the paracortical region, with only scattered T-cells inside germinal centers. CD43 should always be performed in parallel with CD3 and CD20 to assess the distribution of staining. In most reactive conditions, the CD43 stain should parallel that of CD3, with no co-localization on CD20-positive cells. The bcl-2 staining pattern also parallels that of CD3 with additional staining in primary follicles and mantle

zones. CD5 is another helpful marker for B-cell and T-cell malignancies. However, most B-lymphomas that express CD5, such as chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and mantle cell lymphoma (MCL), are also positive for CD43. Therefore, CD5 is not included in the “general” panel but still helpful under certain conditions detailed below.

Follicular Pattern

This pattern refers to a proliferation of many nodules of lymphocytes, which resemble follicles. The main differential is between reactive follicular hyperplasia and follicular lymphoma (FL). Multiple well-described morphologic features are helpful in making this distinction, but one finding alone is not sufficient. When faced with ambiguous cases, a panel of immunohistochemical stains may be helpful. Most cases can be clarified using the basic panel of CD20, CD3, bcl-2, CD43, and/or CD5, with the addition of CD10 and/or bcl-6. Kappa and [lambda] immunostains are helpful in a minority of cases; flow cytometry has greater sensitivity for light chain evaluation. Reactive germinal center B-cells stain for CD10 and bcl-6, but not bcl-2. Germinal centers that are bcl-2 positive are almost always lymphoma. However, 3 caveats exist. First, only germinal centers should be assessed, because normal primary follicles and mantle zones are bcl-2 positive. Co-localization of CD10, bcl-6, and bcl-2 positivity is helpful as CD10 and bcl-6 are not expressed in primary follicles or mantle zone B-cells. Second, scattered bcl-2-positive cells within germinal centers likely represent normal follicular helper T-cells and should not be interpreted as neoplastic. The exception is when increased, scattered bcl-2-positive neoplastic B-cells inside the germinal center show stronger staining than the adjacent bcl-2-positive T-cells or the mantle cells. This finding is suggestive of FL-in-situ. Lastly, lack of bcl-2 staining does not exclude a diagnosis of FL, especially in the pediatric population, in which up to two-thirds of cases are bcl-2 negative. Bcl-2 is not specific for FL and does not distinguish it from other small B-cell lymphomas that may colonize follicles, such as MCL or less commonly marginal zone lymphoma (MZL). CD43 positivity with lack of CD10 expression is uncharacteristic for FL and raises the possibility of MCL or MZL. Coexpression of CD5 on the B-cells is suggestive of MCL or CLL/SLL. Ki-67 may be helpful, showing a higher proliferation rate in reactive germinal centers and often lower numbers of positive nuclei in FL. CD20 is useful in establishing B-lineage and assessment of diffuse versus follicular architecture. One may also see a vaguely nodular pattern composed of larger and more spaced-out nodules than normally seen in FL, composed of a mixture of B and T-cells. Consideration should be given to nodular lymphocyte-predominant HL (NLPHL) and careful histologic examination for lymphocyte-predominant cells (LP cells, formerly L&H cells) and additional stains are warranted.

Paracortical Hyperplasia Pattern

The paracortical region is the space between the follicles and is usually composed of T-cells with a lesser degree of admixed B-cells, including large immunoblasts, dendritic cells, macrophages, plasma cells, and/or eosinophils and plasmacytoid dendritic cells. Predominant reactive hyperplasia of the paracortical region is also relatively common but more difficult to distinguish from the multitude of lymphomas, both B- and T-cells, which mimic it. Importantly, reactive paracortical hyperplasia is almost always accompanied by follicular and/or sinus hyperplasia. In some instances, such as with acute

infectious mononucleosis, the paracortical hyperplasia may overshadow the follicular hyperplasia, although reactive follicles should still be evident. Lymphomas may also preferentially involve the paracortical region with some residual reactive follicles still present. The tumors that mimic reactive paracortical hyperplasia are usually those with a mixed infiltrate. The main neoplastic entities to consider include T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL), classical HL (cHL), and NLPHL, and T-cell lymphomas, including peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS), and angioimmunoblastic T-cell lymphoma.

For all of the entities, the initial panel of immunostains is similar and should include CD20, CD3, bcl-2, CD43, CD30, and Ki-67. In a child, Epstein-Barr virus (EBV) stains should also be added to exclude acute infectious mononucleosis. Some small B-cell lymphomas may preferentially involve the paracortical region and spare the follicles; these are easily recognized as sheets of CD20-positive B-cells. As the paracortical region comprises predominantly T-cells admixed with other cell types, involvement by T-cell lymphoma may be harder to diagnosis. Expansion of the CD3-positive areas to push the CD20-positive B-cells to the periphery of the lymph node indicates effacement of the architecture and is highly suggestive of lymphoma. Loss of bcl-2 expression is also suggestive of T-cell lymphoma. Assessment of bcl-2 expression on B-cells is not as helpful in this setting as they are difficult to separate from the paracortical T-cells. CD43 expression is expected to follow CD3 and is not generally lost in T-cell lymphoma. Ki-67 is normally low in the paracortical region whereas PTCL generally has an elevated proliferative rate. The CD20 stain may emphasize a vaguely nodular architecture not evident on the H&E, raising the possibility of NLPHL. Rare, scattered, large, atypical cells with uniformly strong CD20 expression is concerning for THRLBCL.

The CD30 staining pattern deserves special attention. In lesions resembling reactive paracortical hyperplasia, CD30 is used to screen for cHL. In cHL, the Hodgkin cells show a membrane and/or paranuclear/Golgi staining pattern that is of fairly uniform intensity from cell to cell. Diffuse cytoplasmic staining alone should not be considered positive. The pitfall is that reactive paracortical immunoblasts, seen in reactive paracortical hyperplasia and even some T-cell lymphomas, may also be CD30 positive. In contrast to the Hodgkin cells, CD30 staining in immunoblasts is usually weaker and show variability in intensity from cell to cell. Cases with concurrent follicular hyperplasia, no good Hodgkin or Reed Sternberg cells, and variable CD30 staining do not require further workup for cHL. Others may require additional stains.

Classical Hodgkin Lymphoma-like Pattern

Classical Hodgkin lymphoma characteristically shows lymph node effacement by rare Hodgkin cells in a cellular milieu composed of small T-cells, histiocytes, eosinophils, plasma cells, and neutrophils. The most common nodular sclerosis subtype also shows broad fibrous bands that radiate from a thickened capsule and entrap nodules of infiltrate. Occasionally, cHL may only involve the paracortical regions, the so-called interfollicular pattern, and is most often seen with mixed cellularity subtype. Conversely, in some reactive conditions, especially acute infectious mononucleosis, immunoblast numbers may be greatly increased, clustered, and even show bilobated and multinucleated forms resembling Hodgkin cells. When cHL is suspected, the initial panel of immunohistochemical stains should include CD20, CD3, CD15, CD30, and CD45. If

available, PAX-5 is also a helpful addition to this panel. In addition to consistent and uniform CD30 positivity, Hodgkin cells are also usually positive for CD15 (85%) and PAX-5 (90%), with a subset positive for CD20 (20% to 50%), and are consistently negative for CD45. In contrast, reactive immunoblasts are consistently negative for CD15 and variably positive for CD20, CD45, and PAX-5. Keep in mind that CD15 is positive in granulocytes, which are small and multilobated. These should not be confused with Hodgkin or Reed Sternberg cells. CD15 may show variable positivity between different Hodgkin cells of the same case. PAX-5 is a nuclear stain and evaluation should be performed at high power as it shows characteristic weak positivity in cHL. Evaluation of CD45 is often difficult because of the abundant admixed T-cells that often surround Hodgkin cells. To be considered a positive stain, the large cells should show circumferential membranous staining that is equal or stronger when compared with the surrounding lymphocytes / Cases where CD30 is positive and CD15 is negative pose the most difficulties in diagnosis and may require further workup to exclude reactive paracortical hyperplasia, anaplastic large cell lymphoma (ALCL), other T-cell lymphomas, and certain types of diffuse large B-cell lymphoma (DLBCL).

Diffuse Small Cell Infiltrate

B-cell lymphomas may be separated by the predominant size of the neoplastic cells. Using a histiocyte or endothelial cell nucleus as reference, small cells are smaller than an endothelial cell nucleus, intermediate cells are about the same size, and large cell are larger. Most of these entities are easily identified as malignant based on the morphology alone. A monomorphic proliferation is usually evident on low power, with at least focal effacement of the lymph node architecture. One should keep in mind that most of these malignancies are actually composed of a range of cell sizes, which is more obvious on high power. However, patternless sheets of large cells are usually indicative of large cell transformation. The following is a proposed diagnostic approach for classifying so-called small-cell or low-grade B-cell lymphomas based on immunohistochemistry.

For lymphomas composed primarily of small cells, a basic panel should include CD20, CD3, CD5, CD10, CD43, and bcl-2. Cyclin-D1 (bcl-1) and CD23 may also be added to the initial panel, along with [kappa] and [lambda], especially if plasmacytoid features are present. The pattern of CD20 staining is important to confirm the initial morphologic impression of a B-cell malignancy by showing an expanded B-cell population. The CD20 stain may also be used to highlight architectural findings that may not be obvious on the H&E-stained sections, such as nodularity in FL. CD10 positivity, along with lack of CD5 and CD43 expression, is typical of FL. CLL/SLL is usually positive for CD5, CD23, and CD43. MCL is also positive for CD5 and CD43, with cyclin-D1 expression. Bcl-2 positivity is seen in most small B-cell lymphomas and is helpful for distinguishing MZL from reactive monocytoid B-cell proliferations in the lymph node. Although much less common, T-cell lymphomas such as PTCL, NOS can also comprise a diffuse small cell infiltrate. T-cell lymphomas will generally show expression of CD3 and CD43 with variable loss of bcl-2 and CD5.

Diffuse Large Cell Infiltrate

For an infiltrate composed of patternless sheets of large hematolymphoid cells, DLBCL is the most common entity and usually the diagnosis is straightforward. However, there is

a broad differential diagnosis that needs to be considered, including carcinoma, melanoma, reactive paracortical hyperplasia, HL, and other non-Hodgkin hematolymphoid neoplasms. The latter includes lymphoblastic lymphoma (LBL), Burkitt lymphoma, blastic MCL, FL, acute myeloid leukemia, T-cell lymphomas, and precursor plasmacytoid dendritic cell neoplasm. Although this list may seem daunting, an initial panel of paraffin immunohistochemistry should help to sort out most of this list with relative ease. The distinction from carcinoma and melanoma is readily made using pancytokeratin and S100. Assuming a hematolymphoid disorder has already been established, a helpful panel may include CD20, CD3, CD30, cyclin D1, terminal deoxynucleotidyl transferase (TdT), CD43, and Ki-67. If morphologic features are highly suggestive of DLBCL, then bcl-2, bcl-6, CD10, and MUM1 may be performed initially to save time. The straightforward case of DLBCL will be positive for CD20, with a Ki-67 that is elevated but less than 80%, and negative for CD3, CD30, cyclin D1, TdT, and CD43. Positivity for any of the latter markers does not exclude DLBCL but rather acts as a screening test for the other entities in the differential diagnosis and should warrant additional workup. The following is a brief list of positive stains and the entities for which they should raise suspicion: CD3—T-cell lymphomas including ALCL and T-LBL; CD30—ALCL, cHL, and reactive paracortical hyperplasia; cyclin D1—blastic MCL; TdT—LBL, acute myeloid leukemia, and precursor plasmacytoid dendritic cell neoplasm; CD43—Burkitt lymphoma, T-cell lymphomas, acute myeloid leukemia, and LBL; and Ki-67 approaching 100%—Burkitt lymphoma. A few special considerations should be noted. First, in a child with tonsillar or cervical lymphadenopathy, be extremely wary of diagnosing DLBCL and always perform EBV studies to exclude acute infectious mononucleosis. Second, remember that low-grade B-cell lymphomas may have scattered large cells and groups of large cells in proliferation centers. Only use this panel if large cells are present in patternless sheets to avoid misdiagnosing a case of low-grade B-cell lymphoma as DLBCL. Third, rare cases may show immunoblastic morphology but lack all of the above markers, such as in plasmablastic lymphomas or plasmablastic plasmacytoma. Primary effusion lymphomas also typically lack pan-B-cell markers, but the clinical setting should help with this diagnosis. A very rare type of large-cell lymphoma called anaplastic lymphoma kinase (ALK)-positive large B-cell lymphoma is negative for pan-B-cell markers and CD30. CD138, [kappa], [lambda], and ALK1 immunostains along with EBV-encoded RNA (EBER) in-situ hybridization may be helpful in these null phenotype cases.

COMMON HEMATOLYMPHOID NEOPLASMS

Small B-cell Lymphomas

Follicular Lymphoma

FL is a lymphoma composed of centrocytes and centroblasts with at least a focal follicular pattern. The follicles are usually equally sized and crowded; they may or may not have a mantle zone and they lack polarization. Centrocytes are usually small cells with irregular/cleaved nuclei, condensed chromatin, and inconspicuous nucleoli. Centroblasts are large cells with round/noncleaved nuclei, vesicular chromatin, and 1 or

more peripheral nucleoli. FL is positive for all pan-B-cell markers including CD20, CD79a, PAX-5, and the B-cell transcription factors BOB.1 and OCT-2. The germinal center markers CD10 and bcl-6 are positive in most cases of FL., and aberrant coexpression of CD5, CD23, or CD43 is rare. Bcl-2 is positive in most cases, with the exception of grade 3, which can be bcl-2-negative in up to 25% of cases. Although grading is based on morphology, assessment of diffuse architecture is best performed on immunohistochemical stained sections, such as CD20. Note that CD10 may be negative in the interfollicular portion of FL. As discussed earlier, one may find FL-in-situ, characterized by bcl-2-positive neoplastic B-cells inside the germinal center that show stronger staining than the adjacent bcl-2-positive T-cells or the mantle cells.

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

CLL/SLL involving lymph nodes shows a vaguely nodular appearance because of the presence of proliferation centers containing prolymphocytes and paraimmunoblasts or immunoblasts. In extranodal sites or small biopsies, the subtle nodular architecture may not be appreciated. CLL/SLL also expresses pan-B-cell markers although the reactivity for CD20 is usually weak. Most cases are positive for CD5, CD23, CD43, and bcl-2, and are negative for CD10 and bcl-1 (cyclin D1). The CD23 positivity and bcl-1 negativity is useful in distinguishing CLL/SLL from MCL. Rare cases of CLL/SLL may be CD23 negative and even bcl-1 positive. In difficult cases, the distinction rests with morphologic identification of proliferation centers, which excludes MCL. Identification of the t(11;14) translocation, although earlier reported in CLL/SLL, is now thought to exclude this diagnosis. Positivity for CD5 and CD23 helps in making the distinction from MZL. However, rare cases of MZL may be positive for CD5 and even CD23, therefore, morphologic examination is still most important, with rare cases necessitating flow cytometry or cytogenetics. ZAP-70 is positive in about half of the cases and correlates with poorer prognosis, although evaluation is difficult in paraffin sections. Immunoglobulin studies in paraffin sections are usually negative unless the cells exhibit some plasmacytoid features. Ki-67 usually shows low proliferation in diffuse areas and high rates in proliferation centers. MUM1 is also positive in proliferation centers and may be useful.

Mantle Cell Lymphoma

MCL usually shows expression of CD5 and CD43, but with slightly less frequency than CLL/SLL. CD10 and CD23 are usually negative, which is helpful in the distinction from FL and CLL/SLL, respectively. Cyclin D1 (bcl-1) expression is seen in almost all cases and is associated with the t(11;14)(q13;32) translocation between the genes CCND1 (BCL-1) and Ig heavy chain. Cyclin D1 staining is nuclear and characteristically varies in intensity among the tumor cells in a given lymphoma. One should be careful to recognize positive nuclear staining in endothelial cells, which serves as an internal control. Cyclin D1-negative cases may show overexpression of cyclin D2 or D3. Rare cases may lack expression of the cyclin proteins and may also lack the t(11;14)(q13;q32) translocation, and the diagnosis of MCL should be made only if all other features are classic. A recently described nuclear staining antibody, SOX11, shows similar sensitivity as cyclin D1 in

small studies of MCL. In fact, 1 cyclin D1-negative MCL has been reported to be SOX11 positive. Other lymphoid neoplasms that may also show cyclin-D1 positivity include plasma cell neoplasms (up to 25% of cases), hairy cell leukemia (HCL; up to 50% of cases but usually only a subset of cells), and CLL/SLL (rare cases showing weak staining in proliferation centers). Of these, only the latter shows morphologic similarities. MCL does not contain admixed large cells or proliferation centers, a helpful feature in distinguishing this from the other common small cell lymphomas such as FL, MZL, and CLL/SLL. The blastoid and pleomorphic variants of MCL contain large cells, but all the neoplastic cells are large, and in the blastoid variant, they are uniform in appearance, resembling a LBL. Both of these variants are also positive for cyclin D1, helping to make the distinction from other entities such as LBL, DLBCL, and Burkitt lymphoma.

Marginal Zone B-cell Lymphoma

MZL is separated into extranodal, nodal, and splenic types, all of which show similar immunophenotyping. All express CD20 and are usually negative for CD5, CD10, CD23, and cyclin D1. Expression of CD43 is present in one-third of cases. Plasmacytic differentiation is often present and quite striking, usually with demonstrable CD138 expression and light chain restriction. The lack of CD5 and CD10 help to differentiate MZL from CLL/SLL, MCL and FL with relative ease in most cases. A more difficult distinction is between MZL and reactive proliferations such as reactive monocytoid B-cell hyperplasia in lymph nodes or severe chronic gastritis. The finding of monotypic immunoglobulin light-chain restriction is diagnostic of malignancy, but this is usually only found in paraffin sections when plasmacytoid features are present. In lymph nodes, bcl-2 positivity in a monocytoid B-cell population is diagnostic of malignancy. Bcl-2 positivity in small biopsies of extranodal sites may be very difficult to interpret and should not be used as the sole indicator of malignancy. Aggregates of bcl-2-positive B-cells in a stomach biopsy may represent primary follicles or reactive mantle or marginal zone B-cells. Reactive marginal zone hyperplasia of the spleen, abdominal lymph nodes, or ileal mucosa may express bcl-2 in or near the follicles. In extranodal sites such as the stomach, CD43 positivity in the B cells may be the most easily identified feature of malignancy. Another indicator of malignancy is the presence of sheets of CD20-positive cells without an associated, compartmentalized T-cell population. Many cases of extranodal MZL will not show any of the above-mentioned immunohistochemical criteria for malignancy. One must rely on morphologic features and if necessary, gene rearrangement studies.

Lymphoplasmacytic Lymphoma

Lymphoplasmacytic lymphoma (LPL) is often associated with Waldenström macroglobulinemia (WM), defined in the 2008 World Health Organization (WHO) Classification scheme as any concentration of IgM monoclonal gammopathy combined with bone marrow involvement by LPL. LPL most commonly but not always produces an IgM paraprotein. LPL is characterized morphologically by an infiltrate composed of small lymphocytes, plasmacytoid lymphocytes, and plasma cells. One sees immunohistochemical expression of pan-B-cell markers and frequently CD43, usually

with no expression of CD5, CD10, CD23, or cyclin D1. As with most lymphomas with plasmacytic differentiation, monotypic light-chain expression is usually detectable in paraffin sections. The mature plasma cells are CD138 positive. IgM paraprotein is not unique to LPL; WM-like clinical presentations may be seen in other lymphomas including CLL/SLL and splenic MZL. LPL usually does not stain for CD5 or CD10, a feature that offers distinction from most other small B-cell lymphomas. However, differentiation from MZL may be difficult because of similar immunophenotypes and only subtle morphologic differences, such as the monocytoid appearance and plasma cell compartmentalization of MZL. Sometimes, the diagnosis of “small B-cell lymphoma with plasmacytic differentiation,” as recommended by the WHO, may be necessary. The presence of a spectrum of cells with lymphoid and plasmacytoid features, along with CD20 expression, helps to distinguish LPL/WM from pure plasma cell neoplasms.

Plasma Cell Neoplasms

This contains a pure population of plasma cells that usually appear mature but occasionally show atypical or plasmablastic/immunoblastic cytology. Plasma cell neoplasms consistently express CD138, and frequently express CD79a, MUM-1, and CD43. Aberrant expression of CD56 and CD117 is common in neoplastic but not reactive plasma cells. There is usually no expression of CD45, CD20, or PAX-5. Monotypic light-chain restriction is demonstrable in paraffin sections, usually with expression of IgG and IgA. Cyclin D1 expression is seen in approximately one-quarter to one-third of cases and correlates with the presence of the t(11;14) translocation. SOX11 protein expression is not seen, even in plasma cell neoplasms with this translocation. The differential diagnosis includes reactive plasmacytosis, Castleman disease, and small B-cell lymphomas that contain a prominent plasma cell infiltrate such as LPL, MZL, and CLL/SLL. The initial panel should include CD138, [kappa], [lambda], CD43, CD45, CD20, and CD3. Monotypic light-chain restriction differentiates a plasma cell neoplasm from reactive plasmacytosis. If the light chain stains are noncontributory, CD56 may be helpful. The plasma cells in Castleman disease may show [lambda] restriction (but not [kappa]); human herpesvirus 8 (HHV-8) positivity may be helpful if there is clinical suspicion. The lack of a CD20-positive/CD45-positive infiltrate helps to distinguish a plasma cell neoplasm from small B-cell lymphomas that may contain plasmacytoid differentiation or plasma cell infiltrates. Plasmablastic plasma cell neoplasms cannot be distinguished from plasmablastic lymphoma by morphology or immunohistochemistry and requires clinical correlation. CD138 expression is not specific for plasma cell neoplasms and may be seen in several nonhematolymphoid malignancies such as carcinoma, sarcoma, and melanoma. In cases with plasmablastic morphology and CD138 positivity, one must also establish a hematolymphoid origin with a panel that may include CD45, CD79a, CD43, [kappa], [lambda], CD20, CD3, CD30, pancytokeratin, and S100. CD43 is fairly specific for hematolymphoid malignancies (both lymphoid and myeloid), and has only rarely been reported in other tumors. In contrast, MUM-1, although present in plasma cell neoplasms and other lymphomas, is also expressed in melanoma.

Hairy Cell Leukemia

Hairy cell leukemia (HCL) is an indolent B-cell neoplasm composed of cells with oval nuclei, abundant cytoplasm, and “hairy” cytoplasmic projections, which impart a “fried-eggs” appearance in histologic sections. The disease primarily involves the bone marrow and spleen; occasional liver, lymph node, and skin involvement are seen. Bone marrow involvement shows a characteristic interstitial pattern that is distinctive from other small B-cell neoplasms. However, its histologic appearance in the lymph node and spleen overlaps with MZL. A helpful panel of immunostains may include CD20, CD3, CD43, annexin A1, DBA.44, tartrate-resistant acid phosphatase (TRAP), bcl-1, CD5, and/or CD23. HCL is generally positive for CD20, CD45, annexin A1, DBA.44, TRAP, and bcl-2. A subset of cells show weak expression of bcl-1 in some cases. Although DBA.44 and TRAP are sensitive markers of HCL, they both may be positive in some cases of MZL. SOX11 staining correlates with bcl-1-positive cases. The cells are negative for CD5, CD23, and CD43. Bcl-1 is not a very sensitive marker for HCL but is consistently negative in MZL. Perhaps, the most sensitive and specific marker is annexin A1, which shows cytoplasmic staining in almost all HCL cases and is negative in all other B-cell lymphomas, including MZL.

Large B-cell Lymphomas

Diffuse Large B-cell Lymphoma

This is a heterogeneous group of lymphomas with multiple variants and subtypes that have been divided based on morphologic, immunohistochemical, genetic, and clinical features. The morphologic variants include centroblastic, immunoblastic, and anaplastic. The molecular and immunohistochemical subgroups include germinal center B-cell-like (GCB) and activated or nongerminal center B-cell-like (ABC). With the use of rituximab therapy, the clinical significance of these variants is no longer clear. These variants are currently all combined into the category of DLBCL, NOS (DLBCL, NOS) under the current WHO classification. These lymphomas are positive for CD20, CD79a, and PAX-5 as well as the B-cell transcription markers BOB.1 and OCT-2, but stains other than CD20 are usually only needed in morphologically difficult cases. If a diagnosis of DLBCL, NOS is suspected by the initial panel, the addition of bcl-2, bcl-6, CD10, and MUM1 may be considered. For patients above 50 years of age, EBV-latent membrane protein 1 (LMP1) may be added under certain circumstances to exclude EBV-positive DLBCL of the elderly. DLBCL, NOS is positive for CD10 in approximately half of cases; bcl-6 is positive in approximately three-quarters; MUM-1 is positive in approximately one-half; bcl-2 is positive in approximately one-half; and some cases may also be positive for CD5, CD23, and CD43. Ki-67 expression varies from 30% to 100%. Some cases, especially the anaplastic variant of DLBCL, are also positive for CD30. These cases are negative for ALK protein and T-cell markers. Using immunohistochemistry to divide DLBCL into GCB and ABC groups is optional. The GCB type is positive for CD10 (>30% of cells) and/or bcl-6, but negative for MUM-1. The ABC type is typically negative for CD10 and positive for bcl-6 and MUM-1.

The distinction between DLBCL and Burkitt lymphoma may be very difficult. Burkitt lymphoma is suspected if one sees a monomorphic appearance with a starry-sky pattern,

or if Ki-67 approaches 100% and the cells are positive for CD10 and bcl-6. Bcl-2 negativity, seen in approximately two-thirds of Burkitt lymphomas, and CD43 positivity, seen in approximately 50% of Burkitt lymphomas and only 10% of DLBCL, also raises suspicion for Burkitt lymphoma. Fluorescence in situ hybridization studies are recommended when Burkitt lymphoma is highly suspected, with the caveat that 10% of DLBCL also contain the c-myc translocation, although usually in a background of complex karyotype.

In the presence of CD30 positivity in DLBCL, the other considerations are ALCL and cHL. In most cases of DLBCL, strong CD20 expression in sheets of large cells is seen, with only focal variable positivity for CD30.. These cases are not uncommon and can be comfortably diagnosed as DLBCL without further workup. Difficult cases include those with less large cells and more inflammatory background and fibrosis, such as the case with primary mediastinal large cell lymphoma. A helpful panel to distinguish these cases from cHL may include CD15, CD45, CD79a, BOB.1, OCT-2, and PAX-5.. Usually, not all of these markers are necessary to make the distinction. Consistently strong CD20 membrane positivity of all the malignant cells favors DLBCL, whereas variable intensity staining in a subset of cells favors cHL. CD15 is a very helpful marker, because it is positive in cHL and negative in DLBCL. CD45, CD79a, BOB.1, and OCT-2 are usually negative in cHL and positive in DLBCL. The intensity of PAX-5 (weak in cHL and strong in DLBCL) may also be helpful.

The 2008 WHO classification divides DLBCL into 4 subtypes and 8 distinct disease entities a few of which involve the lymph nodes and may show a clinical presentation similar to DLBCL, NOS. For some, the distinction from DLBCL, NOS requires morphology and immunohistochemistry. These include THRLBCL, EBV-positive DLBCL of the elderly, and ALK-positive large B-cell lymphoma.

T-cell/Histiocyte-rich Large B-cell Lymphoma

This is a subtype of DLBCL in which the neoplastic B-cells are scarce compared with the inflammatory infiltrate. The requirement of less than 10% neoplastic cells has been removed in the 2008 WHO and replaced by a more descriptive definition with scattered, singly dispersed large B-cells that do not aggregate or form sheets. As the name suggests, the background is composed of mostly of T-cells and histiocytes. Scattered plasma cells and eosinophils are also present. The immunohistochemical profile of the large cells is similar to DLBCL, NOS. The cells show expression of pan-B-cell markers, are usually negative for CD30 and CD15, and invariably negative for EBV. These help to distinguish this entity from cHL, acute infectious mononucleosis, and other EBV-associated neoplasms such as lymphomatoid granulomatosis and EBV-positive DLBCL of the elderly. The distinction with PTCL requires recognition of morphologic atypia in the small and the large cells, with a confirmatory T-cell panel if suspicion arises. The most difficult entity in the differential diagnosis is NLPHL, the discussion of which is presented in the NLPHL section.

EBV-positive DLBCL of the Elderly

This occurs in patients who are above 50 years of age and have no other causes for immunosuppression other than aging. The disease initially involves lymph nodes and commonly involves extranodal sites such as skin, lung, tonsil, and stomach. Morphologically, 2 forms have been described. One is histologically indistinguishable from DLBCL, NOS and the differential diagnosis is similar. The other resembles cHL, with a polymorphous infiltrate and scattered Hodgkin-like cells. The immunohistochemical profile of both forms is similar to DLBCL, NOS, including variable CD30 expression, with the exception of EBV positivity, which is definitional. However, EBV positivity is not completely specific: lymphomatoid granulomatosis, plasmablastic lymphoma, primary effusion lymphoma, and DLBCL associated with chronic inflammation are all EBV positive and show CD20 staining in the large neoplastic cells. Clinical information and other immunostains will help to distinguish among these entities. EBV stains are advised for DLBCL if the patient is above 50 years of age, necrosis is present, the cells show pleomorphism and/or Reed Sternberg-like cells, or extranodal involvement is present. The differential with HL is discussed above and in the cHL section later in this review.

ALK-positive Large B-cell Lymphoma

This represents a small subset of B-cell lymphomas that morphologically resemble immunoblastic DLBCL, with a sinusoidal growth pattern. Immunohistochemistry shows a granular and dot-like cytoplasmic ALK protein reactivity, which indicates a variant translocation. These tumors are negative for pan-B-cell markers such as CD20, negative for CD30, only weakly positive for CD45, and often show aberrant expression of CD4 and CD57. The tumors are also positive for plasma cell-associated markers CD138, epithelial membrane antigen (EMA), and MUM1, and often show light-chain restriction. Proof of B-lineage may require gene rearrangement studies. The morphologic differential diagnosis includes ALK-positive ALCL and plasmablastic lymphoma. ALCL can be easily excluded by its positive CD30 stain, which is negative in ALK-positive large B-cell lymphoma. ALK positivity excludes plasmablastic lymphoma.

DLBCL-related, Clinically Distinct Large B-cell Lymphomas

These have characteristic presentation and sites of involvement, which usually hint at the diagnosis. These include primary DLBCL of the central nervous system, primary cutaneous DLBCL, leg type, primary mediastinal large B-cell lymphoma, intravascular large B-cell lymphoma, DLBCL associated with chronic inflammation, lymphomatoid granulomatosis, plasmablastic lymphoma, large B-cell lymphoma arising in HHV-8-associated multicentric Castleman disease, and primary effusion lymphoma. Most of these show similar immunophenotype as DLBCL, NOS and are primarily defined by the clinical presentation. A few with distinct immunophenotypes are discussed below.

Primary Cutaneous DLBCL, Leg Type

This is a primary cutaneous B-cell lymphoma that preferentially affects the lower legs of elderly women and shows monomorphic sheets of centroblasts and immunoblasts. Any primary cutaneous B-cell lymphoma with this morphology should fall under this category, even if not present on the leg. These tumors express pan-B-cell antigens, including CD20, as well as bcl-2, bcl-6, MUM-1, and FOX-P1, and are usually negative for CD10. The main differential is with primary cutaneous follicle center lymphoma and distinction can be made with recognition of a follicular architecture and using a typical DLBCL panel containing bcl-2, bcl-6, CD10, and MUM-1. Both are bcl-6 positive, but primary cutaneous DLBCL, leg type usually shows expression of bcl-2, MUM-1, and FOX-P1 whereas primary cutaneous follicle center lymphoma is usually negative for these markers.

DLBCL Associated With Chronic Inflammation

This encompasses pyothorax-associated lymphoma and DLBCL arising after other long-standing chronic inflammation such as chronic osteomyelitis, metallic implants, or chronic skin ulcers. Pyothorax-associated lymphoma, unlike primary effusion lymphoma, presents with a tumor mass. Its phenotype is similar to DLBCL, with some cases showing plasmacytic differentiation and aberrant T-cell antigen expression. EBV-LMP and EBV in-situ hybridization are usually positive.

Lymphomatoid Granulomatosis

This is an EBV-driven, extranodal angiocentric and angiodestructive large cell lymphoma with admixed reactive T-cells. The large cells are EBV-positive and express CD20, variably express CD30, and do not express CD15. Although EBV-LMP may be positive, EBER in situ hybridization is the standard and used for grading, which is based on the amount of EBER-positive large cells in relation to the reactive mixed inflammatory background. Diffuse sheets of large cells without the admixed reactive infiltrate are not considered lymphomatoid granulomatosis and should be classified under other clinicopathologic entities such as DLBCL, NOS or EBV-positive DLBCL of the elderly. Distinction from the other main angiodestructive lymphoma (extranodal natural killer/T-cell lymphoma, nasal type) can be made from the CD20 positivity. Lower-grade lesions with fewer large cells and CD30 positivity may need to be distinguished from cHL with a similar strategy outlined above .

Plasmablastic Lymphoma

This is associated with immunodeficiency states such as human immunodeficiency virus and advanced age. The cells may resemble immunoblasts or show more immature plasmacytic differentiation. The immunophenotype is similar to plasma cell neoplasms, but CD56 expression is less common. EBV-LMP is usually negative but EBV in-situ hybridization is usually positive. HHV-8 is negative.

Large B-cell Lymphoma Arising in HHV-8-associated Multicentric Castleman Disease

This is recognized by sheets of plasmablasts, which efface the normal nodal architecture, in a background of multicentric Castleman disease. Most of these patients are human immunodeficiency virus-positive. These cells are positive for HHV-8 latent nuclear antigen 1, a nuclear stain, and show IgM heavy chain and [lambda] light chain restriction. EBV is usually negative.

Primary Effusion Lymphoma

This usually presents as a serous effusion without an associated tumor mass. The effusion contains cells that cytologically resemble plasmablastic lymphoma. Immunophenotypically, CD45, CD138, and EMA are usually positive, but CD20, CD79a, and PAX-5 are negative. EBV-LMP is usually negative, but EBV may be shown by in situ hybridization. Similar to plasmablastic lymphoma and normal plasma cells, BOB.1 and OCT-2 are positive. HHV-8 is invariably positive.

Lymphoblastic Lymphoma/Leukemia

This is a monomorphous proliferation of small to intermediate-sized cells with scant cytoplasm, fine chromatin, and inconspicuous nucleoli. B- and T-acute lymphoblastic leukemias (ALL)/LBL have different clinical presentations but similar morphology. Both are consistently positive for TdT, CD43, and CD99, and frequently positive for CD34. CD45 is present on most but not all cases. Both may also express myeloid markers such as CD33, but they never express myeloperoxidase. Flow cytometry and cytochemistry may help to establish the lineage of a blastic neoplasm. With paraffin immunohistochemistry, PAX-5 is the most sensitive and specific marker to assign B-lineage. Most B-ALL/LBL will also be positive for CD10, CD79a, and BOB.1, whereas CD20 and OCT-2 are often negative. No expression of T-cell antigens is seen. T-ALL/LBL usually shows expression of CD3 (cytoplasmic) and CD7, although only CD3 is considered lineage specific. The remaining T-cell antigens CD1a, CD2, CD4, CD5, and CD8 are variably expressed. Coexpression of CD4 and CD8 or lack of both may be seen. A panel containing TdT, CD43, CD3, PAX-5, CD79a, CD10, myeloperoxidase, CD20, bcl-1, and Ki-67 is helpful to assign lineage and to exclude morphologic mimics such as acute myeloid leukemia (myeloperoxidase positive), blastic MCL (bcl-1 positive and TdT negative), and Burkitt lymphoma (CD20 positive, TdT negative, and Ki-67 approaching 100%). TdT-positive neoplasms should be stained for myeloperoxidase because some myeloid lineage leukemias, particularly those with t(8;21), may express TdT and/or PAX-5. CD79a and CD10 expression may sometimes be seen in T-ALL/LBL.

Burkitt Lymphoma

This shows a monomorphous proliferation of intermediate-sized cells with moderate amounts of basophilic cytoplasm, vesicular chromatin, and 1 or more medium nucleoli. Admixed tingible body macrophages impart a starry-sky appearance, whereas admixed reactive inflammatory cells are sparse. Burkitt lymphoma shows consistent expression of pan-B-cell markers CD20, CD79a, PAX-5, BOB.1, and OCT-2, as well as CD10 and bcl-

6. Bcl-2 is usually negative but may be weakly positive in up to one-third of cases. CD43 coexpression is common. The Ki-67 stain shows nuclear positivity in nearly 100% of cells. Burkitt lymphoma does not stain for TdT, myeloperoxidase, CD117, CD34, CD5, or cyclin D1. These help to distinguish it from most other monomorphous medium-sized cell infiltrates. Differentiation from DLBCL is discussed in the DLBCL, NOS section.

Hodgkin Lymphoma

Classical Hodgkin Lymphoma

This is a B-lineage lymphoma derived from germinal center B-cells. The diagnosis relies on identification of classic Reed-Sternberg cells and/or so-called Hodgkin cells, which are basically uninucleated Reed-Sternberg cells. The neoplastic cells often represent less than 10% of the infiltrate, which comprises a mixture of reactive T-cells, histiocytes, eosinophils, neutrophils, and plasma cells. The neoplastic cells are consistently positive for CD30, usually positive for CD15, weakly positive for PAX-5, variably positive for CD20, and negative for CD45 and CD3. Characteristics and pitfalls of evaluating these first-line stains are discussed in Section I. In addition, Hodgkin cells are positive for MUM-1 and bcl-2, and negative for CD79a, BOB.1, OCT-2, CD43, CD138, and T-lineage markers. EBV-LMP is positive in one-third of cases and is strongly associated with children and the elderly, and with mixed cellularity and lymphocyte depletion subtypes. Bcl-6 is also positive in one-third of cases. The background reactive inflammatory infiltrate usually shows abundant CD3-positive and CD45-positive T-cells, with the exception of the lymphocyte-rich subtype, which shows abundant CD20-positive B-cells, and the lymphocyte depletion subtype, in which lymphocytes are replaced by histiocytes and other inflammatory cells.

When the morphology is classic and CD15 is positive, the diagnosis is usually straightforward. Difficulties arise because CD15 is negative in 15% of cHLs and almost all entities within the differential diagnosis are also CD15 negative. In cases that are also CD20 negative, especially when abundant Hodgkin cells are present and form sheets, one must exclude ALCL, which lacks CD45 and CD3 expression in 33% and 60% of cases, respectively. The addition of PAX-5, EBV-LMP, CD43, ALK, and cytotoxic markers, such as TIA-1, granzyme B, and/or perforin, should clarify most cases. ALCLs are consistently negative for PAX-5 and EBV, and most ALCL cases are positive for CD43 and cytotoxic markers. In CD30-positive/CD15-negative tumors that are also CD20-positive, one should consider the diagnosis of THRLBCL, which is CD30 positive in 5% of cases and universally CD20 positive. This entity is also consistently positive for CD45, but assessment of CD45 expression is not always straightforward, given the diffuse T-cell infiltrate. The addition of BOB-1 and OCT-2 are helpful in these cases. These transcription factors are expressed in almost all DLBCLs and only 10% of cHLs. Reactive paracortical hyperplasia is usually easily distinguished from cHL because of its accompanying follicular hyperplasia. A common mistake is to focus only on the high-power appearance of the reactive immunoblasts, which may resemble Hodgkin cells because of prominent nucleoli and variable CD30 positivity. Occasionally, the mixed cellularity subtype of cHL may show an interfollicular pattern. The combined features of

CD15 negativity, variable CD45 positivity, and variable CD30 positivity can usually lead one to make a diagnosis of reactive paracortical hyperplasia and not interfollicular HL. The lymphocyte-rich subtype of cHL may show a nodular pattern of B-cells surrounding Hodgkin cells, which may closely resemble NLPHL (see below).

Nodular Lymphocyte Predominant Hodgkin Lymphoma

As the name implies, NLPHL shows a nodular or nodular and diffuse infiltrate of reactive B-cells, sometimes with increased epithelioid histiocytes. The diagnosis relies on identification of LP cells (formerly known as L&H cells), which are characterized by large multilobated cells with vesicular chromatin and one or more peripherally located nucleoli. Nodularity is not always evident on the H&E-stained section, but the CD20 immunostain should highlight the characteristic nodular pattern, because the majority of the internodular areas are composed of T-cells. The nodules are larger and more widely spaced than cases of FL but still show a meshwork of CD21-positive/CD23-positive/CD35-positive follicular dendritic cells. The LP cells are CD30 negative, CD15 negative, and consistently CD20 and CD45 positive. They are also usually positive for CD79a, PAX-5 (moderate staining, in contrast to weak or faint staining of cHL Reed Sternberg cells), BOB.1, OCT-2, and bcl-6. Bcl-2 is positive in one-third of cases. The LP cells are negative for MUM1, CD43, and T-cell markers. In addition to a large number of B-cells, the nodules also contain increased numbers of T-cells, which have a follicular helper phenotype (positive for CD57, PD1, and bcl-6). The authors prefer using the CD57 and PD1 stains because they are negative in LP cells and will highlight the T-cells by characteristically encircling LP cells. Therefore, if NLPHL is suspected, the immunohistochemical panel should include CD20, CD3, CD45, CD30, CD15, CD57, and PD1. If the nodules are still not evident on the CD20 stain, addition of CD21, CD23, or CD35 is helpful to bring out the nodularity.

In practice, CD30 may be weakly positive in LP cells in up to one-third of cases. CD15 may also be positive in a smaller percentage of cases. In ambiguous cases of cHL versus NLPHL, the addition of MUM-1, BOB.1, and OCT-2 can usually help. cHL is positive for MUM-1 and negative for BOB.1 and OCT-2. NLPHL shows the opposite pattern, negative for MUM-1 and positive for BOB.1 and OCT-2. If these stains are unavailable, bcl-6 and EBV-LMP may be helpful. Approximately 40% of cHLs are EBV-LMP positive and most are bcl-6 negative, whereas LP cells are consistently EBV-LMP negative and bcl-6 positive. Once the nodular architecture is appreciated, reactive paracortical hyperplasia is generally not considered in the differential, but another reactive pattern, progressive transformation of germinal centers, may be considered. This is also a fairly easy distinction, because progressive transformation occurs in a background of follicular hyperplasia, with large nodules intermixed between reactive follicles, whereas NLPHL shows effacement of the nodal architecture and is usually separate from areas containing residual reactive follicles. The most difficult differential diagnosis is often with THRLBCL. The LP cells and neoplastic cells in THRLBCL may show identical immunophenotypes, therefore, the distinction rests in the identification of B-cell nodules and CD57-positive/PD-1-positive cells encircling LP cells. This may be extremely

difficult to identify, especially because longstanding NLPHL cases tend to contain more diffuse areas, more T-cells, and less CD57-positive cells than early NLPHL lesions.

T-cell Lymphomas

Peripheral T-cell Lymphoma, Not Otherwise Specified

This may show a variety of morphologies, which range from lesions that resemble reactive paracortical hyperplasia to those composed of abundant large atypical cells. Usually, but not always, the nodal architecture is effaced and there is no accompanying reactive follicular hyperplasia. T-cell lymphomas, in general, are rare and the initial immunohistochemical panel usually does not differ from that of B-cell panels, with inclusion of CD20, CD3, bcl-2, CD30, and CD43. Lack of morphologic and immunophenotypic features of B-cell lymphoma or HL would raise suspicion for a T-cell lymphoma. Unfortunately, there is no equivalent of immunoglobulin light-chain restriction that provides definitive and direct evidence of a neoplastic clone. Rather, evaluation depends on assessment of loss of pan-T-cell markers and/or bcl-2. A general panel for T-cell lymphoma includes the pan-T-cell markers CD2, CD3, CD5, CD7, along with bcl-2, Ki-67, CD30, and CD20. CD4 and CD8 may be added, but the CD4:CD8 ratio is not similar to the $[\kappa]:[\lambda]$ ratio for determining malignancy. PTCL, NOS shows a mature T-cell phenotype and should express at least one of the pan-T-cell markers and should not express CD1a, TdT, or B-lineage markers such as CD20, CD79a, PAX-5, BOB.1, or OCT-2. There is often loss of expression of one or more of the pan-T-cell markers, sometimes with aberrant loss of bcl-2; either one provides strong evidence for a T-cell malignancy. Most cases are composed of CD4+/CD8- T-helper cells. The Ki-67 is usually elevated, an unusual finding in the paracortical region of benign lymph nodes. Scattered CD30-positive reactive immunoblasts may be seen, but these should be scattered, with variable strength staining. EBV infection is common but likely represents infected reactive B-cells. In some cases, antigen expression is not lost and one must rely on T-cell receptor gene rearrangement studies to confirm the morphologic impression.

Angioimmunoblastic T-cell Lymphoma

This is probably derived from follicular helper T-cells. This entity is characterized by a polymorphous proliferation of intrafollicular T-helper cells that characteristically show clear cytoplasm, especially surrounding prominent high endothelial venules, and an irregular proliferation of follicular dendritic networks, some encasing high endothelial venules. Varying amounts of admixed histiocytes, eosinophils, and plasma cells may be present. Unlike PTCL, NOS, the neoplastic cells do not frequently lose pan-T-cell antigens. However, there is a characteristic immunohistochemical profile of these intrafollicular T-helper cells that is very helpful in the diagnosis. The diagnostic panel should include the usual T-cell markers CD2, CD3, CD5, CD7, and bcl-2, CD20, markers for intrafollicular T-helper cells bcl-6, CD10, CXCL13, and/or PD-1, and a marker for follicular dendritic cells CD21, CD23, or CD35. One should expect to see the majority of cells positive for bcl-6, CD10, CXCL13, or PD-1 and expanded, irregular follicular dendritic networks surrounding high endothelial venules, highlighted by CD21, CD23, or

CD35. Ki-67 is usually not increased. Incidentally, although not necessary for the diagnosis, many cases show EBV positivity in the admixed non-neoplastic B-cells.

Anaplastic Large Cell Lymphoma

ALCL encompasses several T-cell lymphomas that have distinct clinical presentations but may have overlapping morphologic features. Immunohistochemically, all ALCL have in common the distinct feature of diffuse strong CD30 expression.. In addition to CD30, other markers of lymphocyte activation are also consistently positive, including CD25, CD71, and human leukocyte antigen-DR. In addition, most ALCL cases show expression of 1 or more of the pan-T-cell markers, CD43, and at least one of the cytotoxic markers TIA-1, granzyme B, or perforin. CD45 is positive in two-thirds of cases. Some cases show a null-phenotype, with loss of all specific T-cell markers. Demonstration of CD43 and cytotoxic markers in these cases is helpful to confirm the diagnosis. Most cases are CD4 positive. CD15 is occasionally positive, as is CD68, although the more histiocyte-specific marker, CD163, is negative.

Primary cutaneous ALCL (C-ALCL), in addition to the above immunohistochemical profile, expresses cutaneous lymphocyte antigen (CLA) in most cases, whereas primary nodal ALCL does not express CLA. However, some cases of nodal ALCL with secondary skin involvement have also been reported to be CLA positive, therefore, this antibody is not likely to be as helpful as clinical correlation for making the distinction between primary cutaneous and nodal forms. In addition, nodal ALCL is ALK positive in approximately 60% to 85% of cases, with either a nuclear, cytoplasmic, or nuclear and cytoplasmic distribution, depending on the type of translocation. The most common t(2;5)/nucleophosmin-ALK translocation is associated with nuclear and cytoplasmic staining. ALK-positive ALCL cases are also usually EMA positive.

In contrast to other T-cell lymphomas and DLBCL, all of which may show patchy, variable intensity CD30 positivity, ALCL shows sheets of strong intensity CD30-positive cells . As a result of this, the differential diagnosis is limited to acute infectious mononucleosis and cHL, especially the syncytial variant of the nodular sclerosis subtype, which may form sheets of Hodgkin cells that are CD30 positive. This differential is confounded by the fact that ALCL may lose expression of CD3, be negative for CD45, and even occasionally be positive for CD15. Therefore, in suspected cases of cHL in which sheets of large cells are seen, it is a good idea to exclude ALCL with a panel of stains that include PAX-5, EBV-LMP, ALK, CD43, and cytotoxic markers TIA-1, granzyme B, and/or perforin. PAX-5 and EBV positivity supports cHL, whereas expression of the latter stains is not seen in cHL but favors ALCL. Staining for EBV is also helpful in a young patient where one should be wary of acute infectious mononucleosis, which can occasionally form sheets of large CD30-positive immunoblasts. These sheets are usually focal and do not efface the entire nodal architecture. EBV is not associated with ALCL unless it is immunodeficiency associated, such as posttransplant lymphoproliferative disorder. Other T-cell lymphomas may transform into ALCL and be indistinguishable by morphologic and immunophenotype. Correlation with clinical setting and previous pathology is necessary to make this

diagnosis. Lastly, close attention to the clinical presentation is also prudent to help exclude adult T-cell leukemia/lymphoma, which may express CD30, CD25, and T-lineage markers.

Histiocytic and Dendritic Neoplasms

This group of neoplasms, including histiocytic sarcoma, Langerhans cell histiocytosis, interdigitating dendritic cell sarcoma, and follicular dendritic cell sarcoma, is extremely rare. Many can be classified using a panel that includes CD163, Langerin, CD21, CD35, and S100. In short, CD163 is positive in histiocytic sarcoma and Langerhans cell histiocytosis; Langerin in Langerhans cell histiocytosis only; CD21 and CD35 in follicular dendritic cell sarcoma only; and S100 without the other markers in interdigitating dendritic cell sarcoma.. Langerin, a glycoprotein crucial to the formation of Birbeck granules, is a highly sensitive and specific marker for Langerhans cells, with CD1a being a comparable alternative. CD163 has been shown to be a more specific histiocytic marker than CD68 or lysozyme. A combination of these latter stains may be used in adjunct if CD163 is not available.

Blastic Plasmacytoid Dendritic Cell Neoplasm

This entity, formerly blastic natural killer-cell lymphoma, this is a rare neoplasm that morphologically resembles monomorphic blastic proliferations such as LBL, acute myeloid leukemia, blastic MCL, and Burkitt lymphoma. Skin involvement is almost universal, and bone marrow and lymph node involvements are also commonly seen. These tumors express the plasmacytoid dendritic marker CD123, as well as CD4, CD43, CD45, CD56, and CD68. TdT is often positive, but myeloperoxidase and B- and T-cell antigens are usually not expressed. Tumors with CD43 and TdT expression include acute myeloid leukemia and LBL. Positivity for CD123 should confirm the diagnosis of blastic plasmacytoid dendritic cell neoplasm.

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