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Original Contribution

American Registry of Pathology Expert Opinions: Immunohistochemical evaluation of classic Hodgkin lymphoma

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ABSTRACT

The diagnosis of classic Hodgkin lymphoma requires immunohistochemical confirmation in most cases and one can argue for these studies as standard-of-care in the diagnostic workup. The authors propose a panel of studies for primary identification of CHL to include: CD3, CD20, CD15, CD30 and PAX5. When pattern discordances are identified, additional assessment is recommended. In the case of overexpression of B lineage markers by Hodgkin/Reed-Sternberg cells, or a differential diagnosis that includes large B-cell lymphoma or variants, additional markers recommended are: CD45, OCT2, BOB1, CD79a and MUM1/IRF4. If primary mediastinal large B cell lymphoma is considered in the differential diagnosis, suggested additional markers include: P63, CD23, CD45 and CD79a. When considering a differential diagnosis that includes anaplastic large cell lymphoma we suggest: ALK, CD45, pan T cell antigens (such as CD2, CD5, CD7, and CD43), and cytotoxic markers (granzyme, perforin, and TIA1). If peripheral T cell lymphoma or T cell lymphomas of follicular helper origin are considered in the differential diagnosis, the following panel is recommended: pan T cell antigens, CD4, CD8, one or more follicular dendritic cell markers, and assessment for Epstein-Barr virus (EBV) infection, preferably EBV encoded RNA (EBER) as assessed by in situ hybridization When the differential diagnosis includes nodular lymphocyte predominant Hodgkin lymphoma, recommended additional studies include OCT2, CD21 and/or CD23, PD1, and assessment for EBV infection. The authors recognize that these panels may not be adequate to completely characterize other lymphomas, but these panels will usually be sufficient to distinguish classic Hodgkin lymphoma from other lymphoma types.

1. Introduction

This is the first article in a series designated “American Registry of Pathology Expert Opinions”. In this series of articles, the goal is to provide short, focused reviews of diagnostic approaches in evaluating common or challenging issues in the routine practice of pathology. These articles reflect the views of a group of experienced practitioners in subspecialty practice, with the goal to provide practical and useful guides to a specific diagnosis or problem area. The first area chosen for this series is classic Hodgkin lymphoma (CHL), one of the more common diagnoses in lymphoma pathology.

By definition, CHL is characterized by large Hodgkin/Reed-Sternberg (HRS) cells in an appropriate cellular milieu of non-neoplastic inflammatory cells [1]. The cellular origin of the HRS cell is a germinal center B cell with abnormalities leading to a “crippled” B cell program expression. It is this underlying biology that contributes to the distinctive immunophenotype of the HRS cell, and the cellular milieu it creates, leading to the features of CHL.

In recent years, as our understanding of the underlying biology of CHL has expanded, so too have potential mimics of CHL. In classic cases, the histologic findings can be so characteristic as to raise the question as to whether any ancillary studies are needed. In view of the current state of practice, however, in essentially all cases “identifying” HRS cells is based on confirmation of their immunophenotype using immunohistochemistry or other related techniques. This is not to suggest that other techniques, such as flow cytometry or molecular

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profiling could not be used for confirming the diagnosis. Rather, in our opinion, immunohistochemistry using routinely processed tissues is convenient and has become the current "gold standard" for confirming the diagnosis of Hodgkin lymphoma in current practice.

2. Phenotype, "by the book"

As is stated in textbooks, HRS cells are CD15 positive, CD30 positive, and CD45 negative (with the mnemonic—minded practitioner noting that all of the numbers are multiples of 15). As a first approximation, this immunophenotype is quite useful for identifying HRS cells in CHL. However, to the experienced hematopathologist, there are several underlying weaknesses to this approach. First, the expression of CD15 is neither uniform in HRS cells in an individual case, nor present in all cases. Expression of CD15 can vary in individual cells of CHL, both in intensity, cellular distribution and in focality [1]. In addition, some CHL cases completely lack CD15 expression, but still have other characteristic morphologic and immunohistochemical features of CHL.

Secondly, CD45 is often difficult to interpret. There are differences in the reactivities of commercially available CD45 clones and we have observed variable quality results from laboratory to laboratory in our review of consultation cases. In practice, negative HRS cells that are surrounded completely by positive small lymphocytes can be challenging to assess. With a strong CD45 clone, the pathologist hopes to find HRS cells lacking membranous accentuation of CD45 reactivity or two adjacent HRS cells where the intervening cell membranes are negative. In less uniformly reactive CD45 stains, finding clear difference between non-reactive HRS cells and other admixed non-reactive cells (histiocytes, dendritic cells) can make proving negativity more challenging.

3. Initial evaluation of Hodgkin lymphoma

For the initial evaluation of classic Hodgkin lymphoma, the consensus of our group is that a panel of antibodies specific for: CD3, CD20, CD15, CD30, and PAX5 is most helpful and may be all that is necessary to provide a confident diagnosis (Fig. 1). These markers are discussed below (Tables 1 and 2).

3.1. CD3

Evaluation of T cell antibody is critical in identifying the distribution and reactivity of cells in CHL and CD3 is the most commonly used pan T cell marker. CD3 is not expected to be positive in the HRS cells of classic Hodgkin lymphoma and when present in HRS-like cells, should raise the possibility of a T cell lymphoma. There have been reports of expression of T cell antigens in about 20% of cases of CHL, most often CD4 or CD2, and uncommonly CD3 [2,3].

3.2. CD20

CD20 is the most commonly used pan B cell antigen for immunohistochemical analysis of paraffin-embedded tissue sections. Its use has been both favored and complicated by the near ubiquitous use of anti-CD20 therapies (such as rituximab) in the past decade for B cell lymphomas. CD20 expression by HRS can be identified in many cases of Hodgkin lymphoma, if adequately sensitive techniques are used [4]. However, at the level of sensitivity of immunohistochemistry, HRS cells are positive for CD20 in up to 31% of cases [5,6]. In contrast to typical B cells, HRS cells in most cases of classic Hodgkin lymphoma express CD20 weakly and individual cells are variably positive. Downregulation of CD20 expression (e.g. weak and variable) may be seen in activated states of B cells (immunoblasts), so this expression pattern in not entirely reliable to distinguish B cells from HRS cells. A subset of classic Hodgkin cases does express strong and/or uniform CD20, in our experience < 5% and perhaps as low as 1%. In cases with strong and/or uniform expression of CD20, the differential diagnosis of B cell non-Hodgkin lymphoma rather than CHL should be strongly considered.

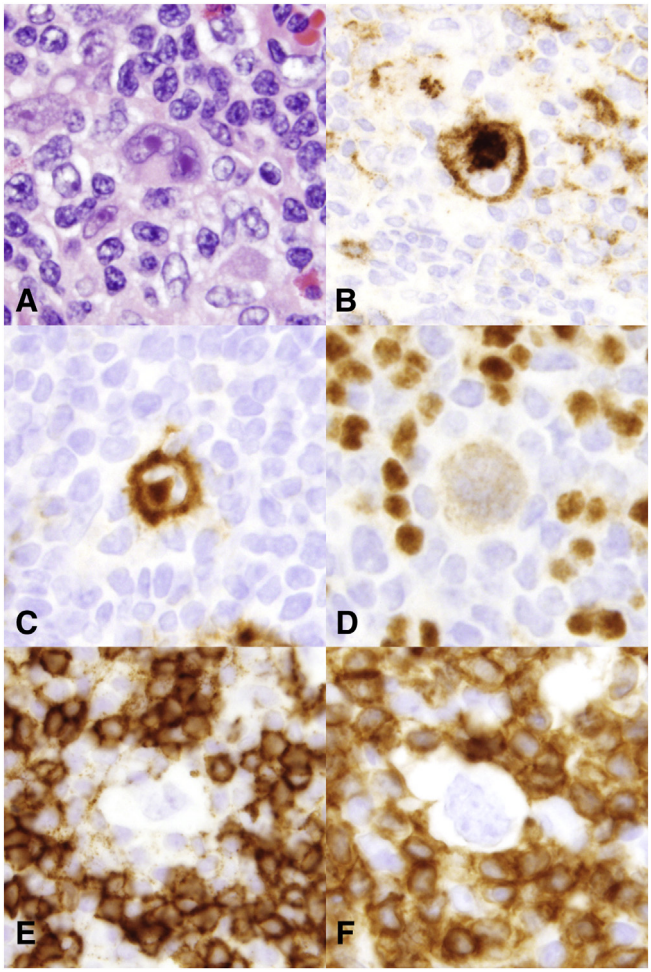

Fig. 1. Images from classic Hodgkin lymphoma, A. H&E, B. CD15, C. CD30, D. PAX5 (note decreased intensity compared to background B cells), E. CD20, F. CD3.

3.3. CD15

CD15 expression can be helpful in confirming the diagnosis of CHL. When present, HRS cells show prominent staining for CD15 with membranous expression and paranuclear/Golgi accentuation. Along with other markers such as CD30, CD15 reactivity strongly supports a CHL diagnosis. However, it should be noted that it is common for CD15 expression to be variably or focally expressed by HRS cells. In addition, CD15 may be absent in up to one third of otherwise typical cases of CHL [1]. Therefore, the lack of CD15, by itself, does not exclude a CHL diagnosis.

When interpreting CD15 expression a few potential pitfalls should be considered. CD15 expression is more reliable in B5-fixed tissues than formalin-fixed tissues, even with antigen retrieval. In addition, CD15 is not specific for HRS cells and can be expressed by non-neoplastic histiocytes, granulocytes, various types of carcinoma, and a subset of T cell lymphomas, including those with CD30 expression [7]. Although subtle, when present in T-cell lymphomas, the CD15 positivity tends to be more diffuse, granular and cytoplasmic than the expression seen in CHL [8]. Importantly, histiocytes within lymphomas often show some granular positivity for CD15 and these cells must be distinguished from HRS cells.

Table 1
Reported immunoreactivity of markers used for diagnosis of classic Hodgkin lymphoma.

Antibody	Reported reactivity	Reference
CD30	> 95% (92–100)	Browne 2003 [5], Nam-Cha 2009 [6], Hoeller 2010 [15], Liang 2018 [20]
PAX5	> 95% (80–98)	Browne 2003 [5], Nam-Cha 2009 [6], Liang 2018 [20]
Fascin	> 95% (95–100)	Pinkus 1997 [28], Bakshi 2007 [29]
PAX8	94%	Liang 2018 [20]
CD40	94%	Kim 2003 [30]
CD95	91%	Kim 2003 [30]
MUM1/IRF4	90% (67–100)	Nam-Cha 2009 [6], Hoeller 2010 [15], Valsami 2007 [31]
PD-l-L1	90% (65–100)	Menter 2016 [32], Sakakibara 2018 [33]
BCL2	65% (60–72)	Liang 2018 [20], Rassidakis 2002 [34]
Cyclin A	87%	Bai 2004 [35]
Cyclin B1	84%	Bai 2004 [35]
Cyclin E	79–80%	Hoeller 2010 [15], Bai 2004 [35]
Cyclin D2	72%	Bai 2004 [35]
Ki67	65%	Bai 2004 [35]
P16	68–81%	Liang 2018 [20], Bai 2004 [35]
CD15	65% (26–90)	Browne 2003 [5], Nam-Cha 2009 [6], Hoeller 2010 [15], Liang 2018 [20]
P53	50% (35–60)	Liang 2018 [20], Bai 2004 [35], Elenitoba-Johnson 1996 [36]
RB	40%	Bai 2004 [35]
Cyclin D3	39%	Bai 2004 [35]
P27	33%	Bai 2004 [35]
EBER	20% (28–36)	Nam-Cha 2009 [6], Liang 2018 [20], Lee 2014 [37]
EBV-LMP1	19–36%	Nam-Cha 2009 [6], Hoeller 2010 [15], Lee 2014 [37]
CD20	25% (7–44)	Browne 2003 [5], Nam-Cha 2009 [6], Hoeller 2010 [15], Liang 2018 [20], Rassidakis 2002 [38]
OCT2	20% (13–56)	Browne 2003 [5], Nam-Cha 2009 [6], Hoeller 2010 [15]
CD22	15–19%	Browne 2003 [5]
BOB.1	15% (6–62)	Browne 2003 [5], Nam-Cha 2009 [6], Hoeller 2010 [15], Valsami 2007 [31]
CD21	12–20%	Asano 2011 [2]
CD79A	10% (6–11)	Browne 2003 [5], Hoeller 2010 [15], Valsami 2007 [31]
MAL	10%	Copie-Bergman 2002 [39]
CD19	10%	Masir 2006 [40]
TIA1	10% (6–25)	Liang 2018 [20], Krenacs 1997 [41], Cazals-Hatem 2001 [42]
EMA	9–19%	Liang 2018 [20], Cazals-Hatem 2001 [42]
Granzyme B	3–32%	Asano 2011 [2], Liang 2018 [20]
BCL6	5% (0 − 31)	Nam-Cha 2009 [6], Liang 2018 [20]
CD45	5% (0–14)	Liang 2018 [20], Cazals-Hatem 2001 [42]
CD2	4–8%	Tzankov 2005 [3], Liang 2018 [20]
CD43	4% (0 − 21)	Liang 2018 [20], Cazals-Hatem 2001 [42]
P63	3–4%	Hoeller 2010 [15], Liang 2018 [20]
Cyclin D1	2%	Bai 2004 [35], Cho 2017 [43]
CD23	1.3–10%	Nam-Cha 2009 [6]
CD3	1–6%	Asano 2011 [2], Tzankov 2005 [3], Liang 2018 [20]
CD45RO	1–5%	Tzankov 2005 [3]
GCET1	0–21%	Nam-Cha 2009 [6]
CD4	0–12%	Asano 2011 [2], Tzankov 2005 [3]; Liang 2018 [20]
CD8	0% (0–6)	Asano 2011 [2], Tzankov 2005 [3], Liang 2018 [20]
CD7	0–3%	Tzankov 2005 [3], Liang 2018 [20]
CD5	0% (0.4–3)	Tzankov 2005 [3], Liang 2018 [20]
ALK1	0%	Liang 2018 [20]
CD10	0%	Liang 2018 [20]
CD117	0%	Rassidakis 2004 [44]
Perforin	0%	Krenacs 1997 [41]

3.4. CD30

Identification of CD30 expression in HRS cells is a cornerstone of diagnosis of CHL. The staining pattern should include strong membranous expression and Golgi accentuation. This should be present in virtually all HRS cells.

While it may be possible to diagnose CHL with a lack of CD30 expression, this circumstance is so rare that it should give pause. In these cases, additional immunohistochemical staining and possibly consultation should be considered. Importantly, CD30 reactivity is compromised in B5-fixed tissue and is one possible circumstance when CD30 may appear to be weak of negative in CHL.

3.5. PAX5

PAX5 is expressed in 80–98% of cases of CHL, and confirms the B cell nature of HRS cells [5,6]. PAX5 should be seen in all HRS cells, and is usually uniform in expression. The expression of PAX5 is characteristically nuclear and dim in HRS cells, in contrast to normal B cells which have much stronger nuclear staining. Either uniform strong expression of PAX5 or a complete lack of PAX5 should raise the possibility of other diagnoses.

When expected results with the primary panel are not met, mimickers of CHL should be excluded. Potential mimickers include, but are not limited to, diffuse large B-cell lymphoma (DLBCL) not otherwise specified (NOS) and DLBCL subtypes, in particular, primary mediastinal large B-cell lymphoma, T-cell/histiocyte-rich large B-cell lymphoma, and EBV-positive DLBCL, B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and CHL, also known as gray zone lymphoma (GZL); anaplastic large cell lymphoma (ALCL); T-cell lymphomas; and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). We recommend a customized, extended panel for each mimicker of CHL.

4. What additional stains should be performed in cases of CHL with an atypical immunophenotype raising the differential diagnosis of diffuse large B cell lymphoma and T-cell/histiocyte-rich large B cell lymphoma?

The syncytial variant of nodular sclerosis CHL is characterized by sheets of Hodgkin (lacunar) cells, frequent coagulative necrosis, and, in some cases, sinusoidal involvement by Hodgkin cells. This neoplasm is a well-known mimicker of DLBCL, NOS. Mixed cellularity CHL is characterized by HRS cells in a variable background with small lymphocytes, histiocytes, plasma cells, and eosinophils, which can resemble EBV-positive DLBCL or T-cell/histiocyte-rich large B-cell lymphoma. The diffuse variant of lymphocyte-rich CHL shares morphologic features with T-cell/histiocyte-rich large B-cell lymphoma – scattered large neoplastic cells in the background of numerous small lymphocytes and occasional histiocytes. Lymphocyte-depleted CHL is characterized by relatively increased HRS cells, which are often pleomorphic or multinucleated, in a background milieu composed of histiocytes and relatively few small lymphocytes or eosinophils. Lymphocyte-depleted CHL can mimic DLBCL, NOS or EBV-positive DLBCL since EBER expression is frequent in lymphocyte-depleted CHL.

When DLBCL needs to be excluded, the following antibody panel is helpful: CD45, CD79a, MUM1/IRF4, OCT2, BOB1 and BCL6. Lymphoma cells in DLBCL are CD15−, CD20+ (> 95% of cases), CD30−/+ (20%), CD45+ (> 95%), CD79a+ (~90%), PAX5+ (> 95%), MUM1/IRF4+/− (~60%), OCT2+ (> 95%), BOB1+ (> 95%), and BCL6−/+ (~60%) [5,9]. In comparison, the HRS cells of CHL are CD15+ (~70% of cases), CD20−/+ (~20%), CD30+ (> 95%), CD45− (> 95%), CD79a−/+ (5–10%), PAX5 dim+ (> 95%), MUM1/IRF4+ (> 95%), OCT2−/+, BOB1−/+ (~30%) and BCL6 [2,5,10].

MUM1/IRF4 is a very sensitive marker for HRS cells of CHL and, if

Table 2
Summary of immunohistochemical evaluation and expected results in classic Hodgkin lymphoma versus important differential diagnoses.

Evaluation	Stains (expected results in CHL)	Comments
Initial evaluation	CD15 (+), CD30 (+), PAX5 (+, weak-moderate intensity), CD20 (− or weak-variable), CD3 (negative)	
Versus DLBCL/TCHRLBCL	CD45 (−), OCT2 (−), BOB1 (−), CD79a (−), MUM1 (+)	
Versus PMLBCL	CD23 (−, very rarely weak and variable), CD45 (−), P63 (−), CD79a (−)	EBER and CD15 provide high specificity for CHL compared to PMLBCL
Versus ALCL	ALK (−), CD45 (−), pan T cell antigen* (−), cytotoxic markers* (−)	(Pan T: (CD2, CD5, CD7, CD43); cytotoxic markers (perforin, granzyme, TIA-1)
Versus PTCL/AITL	Pan T cell antigen (−), CD4 (−), CD8 (−), EBV (+ in 40%), FDC marker (−)	FDC markers (CD21, CD23, CD35, D2-40)
Versus NLPHL	CD21/CD23* (−), OCT2 (−/+), PD1* (*), EBV (−)	* Evaluation of FDC networks and composition of nodules, which may be present in LR-CHL as well as NLPHL

ALCL – anaplastic large cell lymphoma; AITL – angioimmunoblastic T cell lymphoma; CHL - classic Hodgkin lymphoma; DLBCL – diffuse large B cell lymphoma; FDC – follicular dendritic cell; NLPHL – nodular lymphocyte predominant Hodgkin lymphoma; PMLBCL – primary mediastinal large B cell lymphoma; PTCL – peripheral T cell lymphoma; TCHRLBCL – T cell/histiocyte-rich large B cell lymphoma.

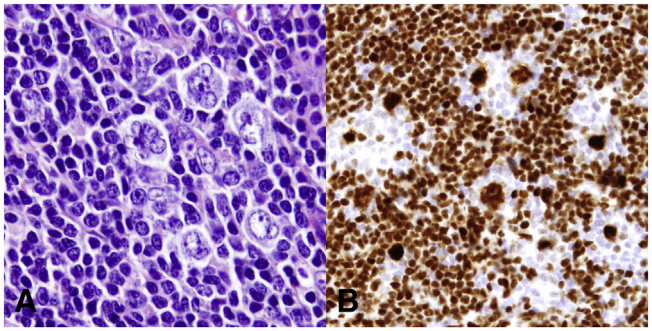

Fig. 2. Images of nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). A. "LP" cells of NLPHL, B. OCT2 staining of NLPHL which stains many background B cells, but clearly highlights the "LP" cells from the background.

negative, the diagnosis of CHL needs to be reconsidered [10]. Alternatively, when CD45 expression in HRS-like cells is present, the diagnosis is unlikely to be CHL [11]. As noted above, a caveat is that interpretation for CD45 can be difficult. Expression of BOB1 is reported in HRS cells of > 50% of CHL cases, whereas OCT2 expression can occur but is less frequent in CHL [5,12]. Importantly, expression of both BOB1 and OCT2 is rare in CHL [13].

5. What additional stains should be performed when the differential diagnosis includes primary mediastinal large B cell lymphoma?

The distinction between CHL involving the mediastinum and primary mediastinal large cell lymphoma can be challenging, but very important because these entities receive different first-line therapies and have different outcomes. This challenge is largely imposed by the significant morphologic similarities; both entities share the presence of large neoplastic cells in a fibrotic or sclerotic stroma and their immunophenotypes are overlap. Difficulties often arise when evaluating only limited core biopsy materials for microscopic assessment of mediastinal masses. In such cases, a sensitive and specific adjunctive diagnostic marker panel is essential.

Immunophenotypically, primary mediastinal large B cell lymphomas are positive for CD20, CD23, CD45 and CD79A as well as CD30. However, in contrast to CHL, CD30 is less intensely and variably expressed in the large cells. In CHL, CD20 and CD79A are characteristically expressed only in a small subset of cases and cells. CD15 can be detected in a sizable percentage of CHL cases and is rarely observed in PMBCL. Both CD15 and EBV expression, either EBER or latent membrane protein type 1 (LMP1) provide high sensitivity for CHL in this differential diagnostic context. CD23 and P63 are particularly useful markers in the differential diagnosis as well, with high positive predictive value of 98%, and 96% for primary mediastinal large B cell lymphomas and very rare in CHL [14]. The expert group agreed that an optimal panel composed of CD23, CD79A, CD45, P63, and testing for EBV infection would help address CHL versus primary mediastinal large B cell lymphoma, in addition to the primary panel [15]. It is worth mentioning that in some cases with scarce large cells in a background of lymphoid cells and fibrosis, morphologic evaluation of the architecture as well as interpretation of CD45 immunostaining can be problematic.

6. What additional stains should be performed when the differential diagnosis includes nodular lymphocyte predominant Hodgkin lymphoma?

Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL), unlike CHL, has a much better developed B-cell program and is defined as neoplastic lymphocyte predominant (LP) cells in a nodular, nodular and diffuse, or predominantly diffuse background of small lymphocytes, epithelioid histiocytes, macrophages, and in nodular areas, networks of follicular dendritic cells (Fig. 2). Six patterns of NLPHL have been described by Fan and colleagues that have prognostic importance, with patterns C-F having a higher frequency or relapse, advanced stage disease, and a worse prognosis [16,17].

Essentially, NLPHL is a neoplasm of the germinal center and therefore LP cells express germinal center B-cell markers and the background cells consistent with a germinal center microenvironment. The LP cells are positive for pan B-cell antigens (CD20 strongly), CD45 (~90–95% of cases), CD79A, OCT2, BOB1, BCL6, Ki67, EMA (~50%), and IgD (up to 25% of cases), and negative for pan T-cell antigens, CD10, CD15, CD30 and EBV infection. It should be noted that CD15 is rarely positive in LP cells of recurrent NLPHL, CD30 can be weakly expressed by LP cells in 5–10% of cases, and EBV is positive in 3–5% of cases.

The background small lymphocytes of NLPHL are a mixture of B-cells and T-cells with B-cells numerous in patterns A, B, and F. These B-cells have a mantle zone immunophenotype and are positive for pan B-cell antigens (CD20 weaker than LP cells), CD23, CD45, CD79A, OCT2 (weaker than LP cells) and IgD, and negative for EMA and BCL6. Many of the T-cells in NLPHL have a T-follicular helper immunophenotype and are positive for associated markers such as PD1/CD279, CD10, CD57, and BCL6 to name a few. These T-cells often form rosettes around the LP cells. In nodular areas, many follicular dendritic cells are present that are positive for CD21, CD23, and/or CD35.

We suggest that the following panel of antibodies be used (in addition to the basic panel) when NLPHL is considered in the differential diagnosis with CHL: OCT2, CD21 and/or CD23, PD1/CD279, and EBV, either EBER or LMP1. The differential diagnosis between CHL and NLPHL will likely be influenced by the pattern of NLPHL. The nodular variant of lymphocyte-rich classical Hodgkin lymphoma can

morphologically almost exactly mimic NLPHL, patterns A-C, and immunohistochemical analysis is essential for this differential diagnosis. Mixed cellularity CHL might raise the differential diagnosis with pattern E NLPHL, particularly if the CHL lacks granulocytes.

We also have observed rare cases of NLPHL, usually following multiple recurrences, where the neoplastic cells are numerous, sclerosis is abundant, and foci of necrosis may be present suggesting a differential diagnosis with nodular sclerosis CHL.

Unlike CHL, the LP cells of NLPHL will be CD20+, CD45+, OCT2+ (strong), PAX5+ and BCL6+ and negative for T-cell antigens and usually CD15, CD30, and EBV infection. The nodular areas will have many CD21+ or CD23+ follicular dendritic cells and the small B-cells will have a mantle zone immunophenotype as stated above.

7. What additional stains should be performed when the differential diagnosis includes anaplastic large cell lymphoma?

Although CHL and ALCL have different cells of origin and distinctive biological and clinical features, these neoplasms may show similar morphological and immunophenotypic features [18-21]. Architecturally, CHL with syncytial growth pattern may mimic cohesive sheet-like growth of ALCL, and sometimes ALCL may show a nodular/sclerotic growth pattern. In particular, the lymphohistiocytic variant of ALCL with few large CD30-positive cells and a lymphohistiocytic background can resemble CHL. Cytologically, hallmark cells of ALCL may mimic Hodgkin cells. By definition, both tumors strongly express CD30, but often show limited expression of pan B-cell and T-cell markers such as CD20 (CHL) or CD3 (ALCL), respectively. Therefore, differential diagnosis may require a broader panel of markers. In addition to the initial marker panel, we recommend the use of ALK, CD45, pan T cell antigens (e.g. CD2, CD5, CD7, and/or CD43), and cytotoxic markers such as TIA1, granzyme B, and/or perforin.

Expression of the B-cell lineage marker PAX5 strongly supports a diagnosis of CHL. On the other hand, expression of ALK oncogene which is present in 60–80% of all ALCL cases is considered diagnostic. Other IHC markers that may be helpful in differential diagnosis include CD15 expression and evidence of EBV infection, both being more common in CHL. Expression of T-cell associated markers or cytotoxic markers support a diagnosis of ALCL. However, it must be noted that a subset of cases of CHL may express T-cell lineage markers, and very rarely ALCL cases may express CD15 and PAX5 [18-21]. The expression of T cell markers in CHL is usually limited to only a subset of the HRS cells. No single marker, with the exception of ALK expression supporting ALCL, is sufficient in this differential diagnosis. During a work-up for CHL, if ALCL is being considered in the differential diagnosis, a broader panel of markers including multiple T-cell lineage markers should be considered.

8. What additional stains should be performed when the differential diagnosis includes peripheral T cell lymphoma/ angioimmunoblastic T cell lymphoma/T cell lymphoma with follicular helper phenotype?

T-cell non-Hodgkin lymphomas (T-NHL) present diagnostic challenges, in part reflecting their broad morphologic variation and including significant morphologic overlap with CHL [22]. With some exceptions, including systemic ALCL harboring rearrangements of *ALK* described above, specific karyotypic and molecular subsets of T-NHLs are infrequent. As such, accurate diagnosis hinges on careful histologic and IHC evaluation [1,23]. Cells virtually indistinguishable from HRS cells can be identified in peripheral T-cell lymphomas (PTCL), most notably in cases of angioimmunoblastic T-cell lymphoma (AITL). In AITL, the HRS-like cells represent EBV-positive proliferations driven by the neoplastic cells of T-follicular helper cell (TfH) origin, and can be identified in a cellular milieu reminiscent of mixed cellularity or lymphocyte-rich CHL [24-26]. Variants of PTCL with a TfH derivation can similarly harbor HRS cells negative for EBV [27]. Conversely, T-cell antigen expression is well appreciated in HRS of CHL, expanding the differential diagnosis of otherwise archetypal cases [21].

While a thorough work-up of suspected T-cell proliferations can prevent misdiagnosis, the breadth of IHC, FISH, and molecular studies may not be universally available to everyone in practice. However, if PTCL remains in the differential diagnosis after primary work-up, we recommend including a pan-T-cell panel (CD2, CD5, CD7, and CD43), CD4/CD8, TfH markers (e.g. CD10, BCL6, PD1, CKCL13, and others), EBV (EBER-ISH), and a follicular dendritic cell (FDC) stain (CD21 or CD23). As expression of two TfH markers on a CD4-positive T-cell infiltrate is considered sufficient to establish lineage by many investigators, and most pathology laboratories carry CD10, BCL6, and PD1, TfH lineage can be shown in many cases in daily practice. Expansions of the FDC meshworks are a consistent feature in AITL, but not in CHL, and loss or variability in T-cell antigen expression can be helpful diagnostic feature in PTCL.

9. Other observations in evaluation of classic Hodgkin lymphoma:

Cytologic diagnosis of CHL can be performed, but as suggested previously, the presence of close mimics with comparable cytologic findings presents diagnostic difficulties. The use of immunohistochemical stains on cell block/cytospin specimens is recommended using the panels previously suggested.

Diagnosis of CHL in bone marrow can be challenging. In patients known to have CHL, identification of CD30 positive cells in an appropriate milieu can confirm the diagnosis of CHL in staging bone marrow specimens. However, as a primary diagnosis, the criteria for CHL diagnosis are more stringent and are the same as applied in nodal sites. Thus, the workup would include using the initial panel suggested. Some caveats in bone marrow to be considered are that some markers are also expressed resident bone marrow cells, thereby complicating interpretation, such as: CD15 in granulocytes; MUM1/IRF4 in plasma cells; and CD79A in B cells and plasma cells.

It should also be noted that there is a category of diseases that may have Hodgkin-like features in association with EBV associated lymphoproliferative disorders. These lesions commonly arise in the clinical context of immunosuppression and include post-transplant and medication-related (such as methotrexate, anti-TNF medications) lymphoproliferative disorders. When these underlying etiologies are present, Hodgkin or Hodgkin-like disorders may be present and can share immunophenotypic findings with CHL.

10. Conclusion

The diagnosis of CHL relies on identification of HRS cells in an appropriate cellular milieu and usually requires immunohistochemical confirmation. We suggest a basic panel of six antibodies as a starting point for confirming a diagnosis: CD3, CD20, CD15, CD30, and PAX5. If the results are unclear or unusual or for specific differential diagnoses, we suggest additional stains be added to confirm the diagnosis. The panels suggested will be helpful in the differential diagnosis of CHL, but may not be sufficient to completely characterize other lymphoma types. In cases with highly atypical or discordant immunophenotypes, the diagnosis of CHL needs to be made with caution with additional consultation from colleagues with hematopathology expertise if needed.

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