**Review Article**

**Pitfalls in diagnostic hematopathology – Part II**

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**Abstract:** The overlapping features of malignant lymphomas create a diagnostic “grey zone”, and lead to the invention of “grey zone lymphomas”. There are several major grey zone lymphomas: 1) Lymphomas with overlapping features of Hodgkin lymphoma and large B-cell lymphoma; 2) Lymphomas with overlapping features of Burkitt lymphoma and diffuse large B-cell lymphoma; 3) Lymphomas with overlapping features of nodular lymphocyte-predominant Hodgkin lymphoma and T-cell/histiocyte rich large B-cell lymphoma; 4) Lymphomas with overlapping features of Hodgkin lymphoma, anaplastic large cell lymphoma (ALCL) and peripheral T-cell lymphoma (PTCL); 5) T-cell classical Hodgkin lymphoma and ALCL-HL. The second review of this series will be dedicated to discussion of the “grey zone” features of the lymphomas and how to narrow down the “grey zone” between those lymphomas.

**Key words:** DLBCL, Hodgkin lymphoma, Burkitt lymphoma, grey zone lymphoma, diagnostic pitfalls

**Introduction**

Another common pitfall in diagnostic hemato-pathology is the “grey zone” between different lymphomas. On the one hand, “grey zone” reflects our limitations in understanding the biology of malignant lymphomas. On the other hand, it indicates the complexity and overlapping features of malignant lymphomas, and the occasional arbitrary nature of our classification. Generally, related lymphomas create the “grey zone”. Despite advances in immunohistochemistry, flow cytometry, cytogenetics and molecular studies, distinctions between classical Hodgkin lymphoma (cHL) and related diseases such as primary mediastinal large B-cell lymphoma (PMLBL), nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), T-cell/histiocyte rich large B-cell lymphoma (T/HRLBL) and anaplastic large cell lymphoma (ALCL) are still difficult in many cases (*Table 1*). Previous lack of clear-cut diagnostic criteria imposed a problem for both pathologists and clinicians. Recognizing this fact, a term “grey zone lymphoma” was first introduced at a workshop held in 1998 by a group of 12 expert hematopathologists from the U.S., Canada and Europe [1]. Although more strict criteria were utilized to define the well-established entities, 11 years later, cases that have overlapping features remain pitfalls in the hematopathological diagnosis. These overlapping features are increasingly acknowledged by the World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues (hereafter referred to as “WHO Classification”) [2].

In my experience, it may not be difficult to diagnose a “grey zone lymphoma”, but it is sometimes hard to determine where to draw the line for the “grey zone”. In the second part of this review series, I will discuss the pitfalls in diagnosing “grey zone lymphoma” and review the criteria that help differentiate lymphomas with “grey zone” features.

**Classical Hodgkin lymphoma versus primary mediastinal large B-cell lymphoma – classical “grey zone lymphoma”**

Hodgkin lymphomas were classified by the WHO Classification into cHL and NLPHL. While NLPHL will be discussed in the next section, cHL is characterized by proliferation of large atypical neoplastic cells with abundant cytoplasm, large vesicular nuclei (one or multiple) and prominent eosinophilic nucleoli. The binucleated forms were often referred to as “Reed-Sternberg cells”. Currently, all the large neoplastic cells in cHL, including Reed-Sternberg (RS) cells and its
variants (Figure 1A), are collectively called Hodgkin-Reed-Sternberg (HRS) cells in memory of the three pioneers in the description and diagnosis of cHL [3-5]. Recent investigation suggests that HRS cells are bona fide B-cells that are crippled in the production of immunoglobulin [6, 7], as a result of aberrant expression of transcription regulators [8]. Because of this, HRS cells are weakly PAX5+, occasionally express CD20, and have IGH rearrangement [9]. However, cHL is usually CD30+ and CD15+, but is negative for CD45, CD3, and CD79a. Since the HRS cells secret many cytokines such as PDGF, TGF-β and IL-6 etc. [10, 11], cHL is often associated with a background of reactive lymphocytes, plasma cells, eosinophils and fibrosis (Figure 1A). The reactive background is so characteristic that some hematopathologists believe that a diagnosis of cHL cannot be made without the associated reactive background. However, similar features can also be seen in the PMLBL, which is characterized by proliferation of large atypical lymphoid cells in a background of compartmentalizing fibrosis, or delicate mesh-like sclerosis. Sometimes individual neoplastic cells are scattered in a background of fibrosis, resembling shells in the sand (Figure 1B). These fibrotic backgrounds can also be seen in DLBCL, particularly in DLBCL of retroperitoneal lymph nodes. In contrast, the cHL fibrosis is usually band-like, with neoplastic cells forming clusters surrounded by fibrotic bands. These patterns are particularly useful in evaluating needle core biopsy specimens that have limited amount of tissue and usually lack a histological architecture. Since both cHL and PMLBL are lymphomas of the mediastinum, the overlapping features of cHL and PMLBL cause a major challenge in lymphoma diagnosis.

To distinguish these two entities, several features (Table 2) are useful: 1) cHL usually have classical RS cells; while neoplastic cells in PMLBL resemble RS cells; 2) cHL fibrosis is usually band-like and surrounding the aggregates of neoplastic cells; while fibrosis in PMLBL is compartmentalizing and mesh-like; 3) CD45 and CD79a are positive in PMLBL and negative in cHL; 4) PAX5 is weakly positive in cHL, but strongly positive in PMLBL; 5) CD15 is usually negative in PMLBL; only rarely, large B-cell lymphoma could express CD15 [13]. MAL is usually expressed in PMLBL [14], but not in cHL. When MAL is indeed expressed,

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**Table 1. Common lymphomas with overlapping or “grey zone” features**

<table>
<thead>
<tr>
<th>Classical Hodgkin lymphoma</th>
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<tbody>
<tr>
<td>Primary mediastinal large B-cell lymphoma</td>
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<tr>
<td>Burkitt lymphoma</td>
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<tr>
<td>Diffuse large B-cell lymphoma</td>
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<tr>
<td>Nodular lymphocyte predominant Hodgkin lymphoma</td>
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<tr>
<td>T-cell/histiocyte rich large B-cell lymphoma</td>
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<tr>
<td>Classical Hodgkin lymphoma</td>
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<tr>
<td>ALK-negative anaplastic cell lymphoma (ALCL)</td>
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<tr>
<td>Peripheral T-cell lymphoma</td>
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<tr>
<td>Classical Hodgkin lymphoma</td>
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<tr>
<td>T-cell classical Hodgkin lymphoma</td>
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<tr>
<td>ALCL-like Hodgkin lymphoma</td>
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**Figure 1.** Comparison of nodular sclerosis cHL (A) and PMLBL (B). (A) One large RS cell (indicated by an arrow) is surrounded by bands of fibrosis in a background of small lymphocytes, eosinophils and plasma cells. (B) Scattered large RS-like cells (arrow heads) are in a background of delicate fibrosis, reactive small lymphocytes and histiocytes. Eosinophils are rare to absent.
Pitfalls in diagnostic hematopathology

Table 2. Features to differentiate classical Hodgkin lymphoma (cHL) and Primary mediastinal large B-cell lymphoma (PMLBL)

<table>
<thead>
<tr>
<th>Features</th>
<th>cHL</th>
<th>PMLBL</th>
</tr>
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<tbody>
<tr>
<td><strong>Morphologic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neoplastic cells</td>
<td>Hodgkin-Reed-Sternberg (HRS) cells</td>
<td>HRS-like cells</td>
</tr>
<tr>
<td>Background</td>
<td>Reactive small lymphocytes, eosinophils and plasma cells</td>
<td>Reactive small T-cells and B-cells; rare eosinophils</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Band-like; nodular</td>
<td>Mesh-like; compartmentalizing</td>
</tr>
<tr>
<td><strong>Immunophenotypic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD15</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>CD20</td>
<td>-/+</td>
<td>+</td>
</tr>
<tr>
<td>CD30</td>
<td>+</td>
<td>weak +</td>
</tr>
<tr>
<td>CD45</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD79a</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>BCL6</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>MAL</td>
<td>-/+</td>
<td>+</td>
</tr>
<tr>
<td>PAX5</td>
<td>weak +</td>
<td>+</td>
</tr>
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</table>

the cHL is usually associated with an adverse prognosis [15], and may be transforming into large B-cell lymphoma. Although recent work showed that these two lymphomas had similarities in molecular signature [16], the clinical management differs [17, 18].

Simply, when the large neoplastic cells are CD45-negative, diagnosis favors cHL. When the large neoplastic cells are CD45+/CD79+, but are negative for CD15, diagnosis favors large B-cell lymphoma. When lymphoma cells express CD15, CD20, CD30, CD45, CD79a, PAX5 and/or MAL, they represent a “grey zone lymphoma”. The “grey zone lymphoma” is currently defined by the 2008 WHO Classification as “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL” [2]. It is reasonable to believe that “grey zone lymphoma” and lymphocyte-depleted cHL may be the intermediate between these two lymphomas. The cHL to DLBCL transformation may represent an example of lymphoma evolution (Figure 2). Currently, “grey zone lymphoma” is managed as DLBCL by using REPOCH regimen [19].

Burkitt lymphoma versus diffuse large B-cell lymphoma

Burkitt lymphoma (BL) is a well established pathologic and clinical entity, which is featured by the characteristic “starry-sky” histology, monotonous medium-sized cytology with vacuolated basophilic cytoplasm and multiple small nucleoli, extremely high proliferation index and presence of c-MYC rearrangement [20]. Due to its germinal center origin, the cells are CD10+ and BCL6+, and negative for BCL2. Morphologic variants that have prominent nucleoli and c-MYC rearrangement exist. Historically, they were called “Burkitt-like” or “Atypical Burkitt” lymphoma because of their atypical morphologic features and similar aggressive clinical behavior as classical Burkitt lymphoma [21]. Most of the DLBCL originate from germinal center B-cells, and thus...
they have a similar immunophenotype as BL. In contrast, approximately 30% of DLBCL have a t(14;18)(q32;q21) chromosomal abnormality and typically no c-MYC rearrangement. Morphologically, DLBCL cells are large and pleomorphic, and they frequently also have a high proliferation index. It is generally not difficult to differentiate classical BL from DLBCL. However, due to their similar origin, overlaps between atypical BL and DLBCL do exist and impose a challenge for the hematopathologists.

Lymphomas with overlapping features of DLBCL and BL have long been recognized [22]. Lymphomas with DLBCL morphology and c-MYC rearrangement were identified and diagnosed as DLBCL with c-MYC rearrangement [23]. DLBCL with both t(14;18)(q32;q21) and c-MYC rearrangement were found to have a more aggressive clinical course than DLBCL without c-MYC rearrangement [24]. A group of aggressive B-cell lymphomas with atypical morphology (atypical for either BL or DLBCL) have been identified [25], which could have BCL2 expression, lower (than classical BL) proliferation rate and presence of c-MYC rearrangement. These lymphomas could also have “starry-sky” histology, extremely high proliferation rate, and absence of c-MYC rearrangement. Clinically, these lymphomas behaved aggressively and responded favorably to high-intensive chemotherapy for BL. The atypical morphology of these lymphomas is determined by their genetic abnormalities, such as the coexistence of t(14;18)(q32;q21) and c-MYC rearrangement, trisomy 8, or other yet unidentified abnormalities that have a similar effect as c-MYC (Figure 3). In 2005, an American group and a European group independently identified the BL molecular signature in both classical BL and some DLBCL [26, 27]. Based on accumulating information of these lymphomas, the 2008 WHO Classification listed them as a separate entity [28]. It is generally agreed that these lymphomas should be managed aggressively as BL.

Since c-MYC rearrangement is a hallmark of BL, detection of c-MYC rearrangement is required for the diagnosis of BL. Although t(2;8)(p12;q24), t(8;14)(q24;q32) and t(8;22)(q24;q11) could cover up to 90% of the c-MYC rearrangements, other c-MYC rearrangement was also detected [29]. Whether c-MYC rearrangement is specific for BL remains controversial. Besides BL, c-MYC rearrangement has been identified in chronic lymphocytic leukemia [30], follicular lymphoma [31], and DLBCL [32]. Presence of c-MYC rearrangement is often associated with an aggressive clinical course and poor prognosis in all those lymphomas.

**Nodular lymphocyte predominant Hodgkin lymphoma versus T-cell/histiocyte rich large B-cell lymphoma**

It is currently believed that progressively transformed germinal center (PTGC), NLPHL and T/HRLBL are related and represent different stages of a progressive disease [33, 34]. PTGC is a benign disease, whereas NLPHL is a mature B-cell lymphoma with indolent clinical course. T/HRLBL has an aggressive clinical course and should be treated as DLBCL. Because of this clinical implication, distinguishing NLPHL from T/HRLBL is critically important.

NLPHL is limited to lymph nodes of a young patient, while T/HRLBL often involves the extranodal sites, such as bone and soft tissue, in addition to lymph node. Several arbitrary features were proposed to differentiate NLPHL and T/HRLBL (Table 3). It is generally believed that NLPHL is almost always associated with a background of reactive small B-cells. Previous studies used <10% of the background small B-cells to favor T/HRLBL. In my experience, classical T/HRLBL has hardly any small B-cells in the background.
Pitfalls in diagnostic hematopathology

Table 3. Features to distinguish nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and T-cell/histiocyte rich large B-cell lymphoma (T/HRLBL)

<table>
<thead>
<tr>
<th>Features</th>
<th>NLPHL</th>
<th>T/HRLBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Architecture</td>
<td>Nodular</td>
<td>Diffuse</td>
</tr>
<tr>
<td>Neoplastic cells</td>
<td>&quot;Popcorn&quot;-like L&amp;H cells</td>
<td>Centroblastic, immunoblastic or RS-like</td>
</tr>
<tr>
<td>Background</td>
<td>Mixed populations of small T- and B-cells; rosette small T-cells; aggregate small B-cells; nodular follicular dendritic cell meshwork</td>
<td>Scattered small T-cells and numerous reactive histiocytes; rare small B-cells</td>
</tr>
<tr>
<td>Immunophenotypic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neoplastic cells</td>
<td>CD20+/CD45+/CD79a-/BCL2-</td>
<td>CD20+/CD45+/CD79a+/BCL2+</td>
</tr>
<tr>
<td>Background cells</td>
<td>CD3+/CD57+ (rosette T-cells); CD21+ (follicular dendritic cells)</td>
<td>CD3+ (small T-cells); CD68+</td>
</tr>
</tbody>
</table>

background. Background small lymphocytes are almost entirely CD3+ T-cells. Reactive histiocytes can be prominent (Figure 4). Some believe that vague nodularity favors NLPHL; however nodular large cell transformation has been reported [35]. In these cases, “NLPHL with focal large cell transformation” is preferred for the diagnosis.

It is rare that NLPHL involves bone marrow [36]. When marrow or other organs are involved, it is usually diagnosed as T/HRLBL [37]. NLPHL may be regarded as an “in situ” T/HRLBL – when it goes to an extranodal site, it becomes T/HRLBL. Even if there are rare cases of true NLPHL involving bone marrow, it should be managed as DLBCL or T/HRLBL [38].

Classical Hodgkin lymphoma versus anaplastic large cell lymphoma versus peripheral T-cell lymphoma

ALCL is another CD30+ lymphoma that should be differentiated from cHL [39]. Morphologically, ALCL cells can resemble HRS cells and often show “hall-mark” cells. ALK-positivity distinguishes ALK+ ALCL from cHL [40, 41]. However, differentiating ALK-negative ALCL from cHL can be a challenge. Since cHL is of a B-cell origin [6, 7], it is often weakly positive for PAX5. Conversely, ALK-negative ALCL is a T-cell lymphoma, and is almost never positive for PAX5. When peripheral T-cell lymphoma (PTCL) expresses CD30, it becomes another differential entity in this complicated “trio”. As in ALK-negative ALCL, PTCL is also negative for PAX5. A consensus is still lacking among experts on the definition of ALK-negative ALCL [42]. Some believe that ALK-negative ALCL should be included in PTCL, unspecified. Others argue

that ALK-negative ALCL has distinctive morphological features. A recent large study from the International Peripheral T-cell Lymphoma Project suggested that ALK-negative ALCL was clinically and immunophenotypically different from PTCL [43]. This study also showed that patients with ALK-negative ALCL had a superior failure free survival (FFS) and overall survival (OS) rate than those with PTCL (36% vs 20%; and 49% vs 32%, respectively).

Classical Hodgkin lymphoma, B-cell or T-cell origin?

T-cell cHL has been reported [44, 45]. The neoplastic cells in those reported cases were even weakly positive for PAX5 [45], a B-cell specific activator protein (BSAP). There was an
ongoing debate over the existence of a “T-cell cHL”. A recent study analyzed 259 cases of cHL and identified that 5% of the HRS cells expressed at least one T-cell markers [46], indicating that T-cell antigens could be aberrantly expressed in HRS cells. The REAL classification also proposed an ALCL-like HL (ALCL-HL) as a provisional entity [47] based on its RS-like morphology and CD30+/CD15+ immunophenotype. However, some PTCL can also be CD30+/CD15+ [48]. Although a randomized clinical trial concluded that ALCL-HL had an equal response to regimens for high-grade lymphomas and regimens for cHL [49], microarray studies showed that this lymphoma clustered with T-cell lymphomas rather than cHL [50], suggesting that it is a bona fide T-cell lymphoma. Although some pathologists believe T-cell cHL does exist [51], almost all the microdissected single cell analyses of cHL pointed to a B-cell origin [52, 53]. Currently, “T-cell cHL” is not recognized by the WHO Classification [2].

Summary

In summary, overlapping features are common between malignant lymphomas, reflecting a continuum of lymphocyte development and lymphoma evolution. Awareness of these features will help us to deal with atypical lymphomas with a prepared mind. Recognizing the challenges in diagnosis of lymphomas with “grey zone” features will increase our confidence in committing to an accurate diagnosis.

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Pitfalls in diagnostic hematopathology


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