Nodular Lymphocyte Predominant Hodgkin Lymphoma
An Immunophenotypic Reappraisal Based on a Single-Institution Experience
Patricia Uherova, MD, Riccardo Valdez, MD, Charles W. Ross, MD, Bertram Schnitzer, MD, and William G. Finn, MD

Key Words: Nodular lymphocyte predominant Hodgkin lymphoma; Immunohistochemistry

Abstract
In our experience, certain commonly cited immunophenotypic features of nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) are not encountered in day-to-day practice. We reviewed 60 cases of NLPHL (18 women, 42 men; median age, 34 years) to discern immunophenotypic features from a large, single-institution cohort. All cases contained lymphocytic and histiocytic (L&H) cells. These cells expressed CD20 in 98% (59/60), CD79a (usually faint) in 87% (27/31), CD30 in 7% (4/59), epithelial membrane antigen in 21% (12/56), bcl-2 in 5% (2/41), and bcl-6 in 83% (30/36) of cases. CD10 was negative in all 36 cases studied; 100% of cases (55/55) demonstrated CD3+ rosettes. Although CD57+ T cells were common within the background infiltrate, CD57+ rosettes were seen in only 48% of cases (15/31) and were rare when encountered. Based on these patterns, we conclude that bcl-2 and bcl-6 may be useful additions to the immunophenotypic analysis of NLPHL, but that the diagnostic usefulness of epithelial membrane antigen and CD57 rosettes may have been overemphasized in previous reports.

Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is a unique entity in the category of Hodgkin lymphoma. It is an indolent neoplasm that originates from germinal center–derived B cells. Characteristic histopathologic features include the presence of large nodular structures composed of predominantly small lymphocytes and histiocytes, absence of follicles with well-defined germinal centers, and the presence of neoplastic Reed-Sternberg cell variants with multilobated, folded nuclei called “popcorn” cells or “lymphocytic and histiocytic” (L&H) cells. The morphologic features of NLPHL may overlap with other types of lymphoma, including lymphocyte-rich classical Hodgkin lymphoma or T cell–rich large B-cell lymphoma, and, thus, the diagnosis of NLPHL needs to be confirmed by immunohistochemical analysis.

Numerous studies have characterized the immunophenotypic features of NLPHL. The neoplastic L&H cells generally express B cell–related antigens, such as CD20, CD79a, and J chain. In contrast with Hodgkin cells, L&H cells lack CD15 and almost never express CD30. They have been shown to frequently express epithelial membrane antigen (EMA) as well. In contrast with classical Hodgkin lymphoma, L&H cells lack CD15 and almost never express CD30.

Many of the small lymphocytes within the nodules are mature nonneoplastic B cells. Similar to the mantle zone B lymphocytes, these cells express surface IgM and IgD. The B-cell nodules also contain a follicular dendritic cell meshwork that can be highlighted by anti-CD21 antibody. The background T lymphocytes within the nodules are present in variable numbers, with a predominance of small T cells in rare cases. In addition to helper phenotype, many of these
cells have been reported to express CD57 and to form rosettes around L&H cells. The CD57+ rosetting pattern often is considered a common and distinct feature of NLPHL.

In our experience, some of the commonly cited immunophenotypic features of NLPHL are not generally encountered in day-to-day practice. Furthermore, the expression patterns of antigens only recently available for analysis in paraffin sections (such as CD10 and bcl-6) are not widely reported. Therefore, we reviewed our experience with NLPHL to discern the common immunophenotypic features from a large, single-institution cohort.

Materials and Methods

Cases

A search of our surgical pathology archives revealed 72 cases of NLPHL during a 10-year period. Of these 72 cases, 60 were available for additional review. Clinical data at diagnosis, including age, sex, site of involvement, and stage of the disease, were obtained from medical records.

Light Microscopic and Immunohistochemical Analysis

Tissue samples were fixed with 10% formalin, B-5 fixative, or both and processed routinely for paraffin embedding. Sections of paraffin-embedded tissue (3-5 µm thick) were stained with H&E. Immunohistochemical stains were performed in all cases on the deparaffinized sections of formalin- and/or B-5-fixed tissue. Sections were pretreated before immunostaining depending on the specific antibody and stained on the Ventana ES automated stainer using the avidin-biotin complex method (Ventana Medical Systems, Tucson, AZ). All reagents placed on the Ventana instrument were purchased from Ventana, except for primary antibodies. Anti–bcl-6 staining was done on the DAKO Autostainer using DAKO LSAB+ Kit (DAKO, Carpinteria, CA). Immunohistochemical stains performed at the time of the initial diagnosis included anti–CD3 epsilon, CD15, CD20, CD30, CD45, CD57, and EMA. Additional stains for bcl-2, bcl-6, CD10, and CD79a were performed in available cases.

For each case, CD57+ cells and numbers of rosettes formed by these cells were evaluated in 3 randomly selected nodules. Cases were divided into the following groups: based on the average number of CD57+ cells per nodule, few (50 or fewer cells per nodule) or many (>50 cells per nodule); and based on the number of rosettes per nodule, absent (0 per nodule), few (1-4 per nodule), or many (5 or more per nodule) CD57+ rosettes. Immunohistologic findings were interpreted by consensus of all authors.

Results

Clinical and Morphologic Findings

Clinical and morphologic findings are summarized in Table 2. Patients included 18 women and 42 men with a median age of 34 years (range, 5-78 years). Lymphoma involved cervical lymph nodes in 18, axillary lymph nodes in 15, inguinal lymph nodes in 21 cases, abdominal lymph nodes in 2 cases, and retroperitoneal or pelvic lymph nodes in 1 case each. The site of involvement was unknown in 2 cases. Clinical information about the stage of the disease was available for only 11 cases. Eight patients had low-stage disease (stage I or II), and 3 patients had high-stage disease (stage III or IV).

Histologic findings included effacement of the lymph node architecture by large, back-to-back nodules, occasionally with a discrete rim of benign uninvolved tissue, in 58 of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Immunohistochemical Stains</th>
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<tbody>
<tr>
<td>Antigen</td>
<td>Antibody (Clone)</td>
</tr>
<tr>
<td>CD3 epsilon</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>CD10</td>
<td>56C6</td>
</tr>
<tr>
<td>CD15</td>
<td>MMA</td>
</tr>
<tr>
<td>CD20</td>
<td>L26</td>
</tr>
<tr>
<td>CD30</td>
<td>BerH2</td>
</tr>
<tr>
<td>CD45</td>
<td>2B11, PD7/26/16</td>
</tr>
<tr>
<td>CD57</td>
<td>HNK-1</td>
</tr>
<tr>
<td>CD79a</td>
<td>JCB117</td>
</tr>
<tr>
<td>Epithelial membrane antigen</td>
<td>E29</td>
</tr>
<tr>
<td>bcl-2</td>
<td>100</td>
</tr>
<tr>
<td>bcl-6</td>
<td>PG-B6p</td>
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</table>

* Sections were microwaved (MW) in a 10-mol/L concentration of citrate buffer, pH 6, for 10 or 15 minutes; microwaved in a 0.25-mol/L tris(hydroxymethyl)aminomethane concentration/0.1-mol/L EDTA concentration buffer (T-EDTA), pH 9; or treated for 20 minutes at 95°C (water bath) with DAKO Target Retrieval solution, high pH.
60 cases. The remaining 2 cases exhibited a nodular growth pattern with diffuse areas occupying less than 50% of the examined tissue. The neoplastic nodules were composed of small cells, with scattered neoplastic cells exhibiting characteristic L&H morphologic features with folded and lobated nuclei. Well-defined germinal centers were not identified within the infiltrate.

Immunohistochemical Stains

Immunophenotypic characteristics of L&H cells are summarized in **Table 3**. These neoplastic cells strongly expressed CD20 in 59 (98%) of 60 cases. Staining for CD79a also was identified in the majority of the cases evaluated (27/31); however, it was predominantly weak. L&H cells were CD45+ and CD15– in all cases. Staining for EMA was observed in only 12 (21%) of 56 cases. Weak membrane staining for CD30 was detected in a small subset of L&H cells in rare cases of NLPHL (4/59 [7%]). In addition, CD30 highlighted large lymphoid cells located predominantly outside B-cell nodules (presumably immunoblasts) in 9 cases. Contrary to the weak expression detected in L&H cells, immunoblasts were strongly CD30+.

L&H cells showed nuclear bcl-6 staining in 30 (83%) of 36 cases (including only rare positive cells in 5 of 30 cases). CD10 expression was absent in all 36 cases studied, and only 5% (2/41) showed bcl-2 expression within the L&H cells.

Immunohistologic features of the reactive background infiltrate are given in **Table 4**. In all cases, we identified nodules composed of small CD20+ B lymphocytes intermingled with aggregates of CD3+ T cells rosetting around L&H cells and giving the nodules a moth-eaten appearance. L&H cells were surrounded by easily identifiable CD3+ T-cell rosettes in 54 (98%) of 55 cases. Less frequent CD3+ rosettes were observed in the 1 remaining case.

The majority of cases (23/31 [74%]) had high numbers of CD57+ cells scattered throughout the nodules singly, in small clusters, or in rosettes surrounding the neoplastic L&H cells. Few CD57+ cells (50 or fewer per nodule) were observed in only 8 cases (26%). Regarding rosettes, in more than half of the cases (16/31 [52%]) CD57+ rosettes were not identified at all. Easily identifiable CD57+ rosettes in numbers comparable to the number of CD3+ rosettes were present in only 2 (6%) of 31 cases.

**Discussion**

Although the diagnosis of NLPHL might be suggested on morphologic grounds alone, the distinction from other types of Hodgkin disease and non-Hodgkin lymphoma (NHL) can be difficult. The need for immunohistochemical confirmation was demonstrated during the early 1990s by a retrospective study of NLPHL cases diagnosed by morphologic criteria. Of the 34 cases with nodular architecture, 11 had an atypical phenotype and were reclassified as classical Hodgkin disease (HD). Similar results were obtained from a large multinational study conducted under the auspices of the European Task Force on Lymphoma. Of 248 cases diagnosed by morphologic examination as NLPHL, 73 showed a
Table 3
Summary of Immunophenotypic Findings for Lymphocytic and Histiocytic Cells

<table>
<thead>
<tr>
<th>Antibody</th>
<th>No. (%) of Positive Cases</th>
</tr>
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<tbody>
<tr>
<td>CD20 Strong</td>
<td>59/60 (98)</td>
</tr>
<tr>
<td>Weak</td>
<td>1/60 (2)</td>
</tr>
<tr>
<td>CD79a, weak</td>
<td>27/31 (87)</td>
</tr>
<tr>
<td>CD30</td>
<td>4/59 (7)</td>
</tr>
<tr>
<td>CD45</td>
<td>41/41 (100)</td>
</tr>
<tr>
<td>CD15</td>
<td>0/60 (0)</td>
</tr>
<tr>
<td>Epithelial membrane antigen</td>
<td>12/56 (21)</td>
</tr>
<tr>
<td>bcl-2</td>
<td>2/41 (5)</td>
</tr>
<tr>
<td>bcl-6</td>
<td>30/36 (83)</td>
</tr>
<tr>
<td>CD10</td>
<td>0/36 (0)</td>
</tr>
</tbody>
</table>

Table 4
Summary of Immunophenotypic Findings for Reactive Background Infiltrate

<table>
<thead>
<tr>
<th>Finding</th>
<th>No. (%) of Affected Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodules composed of B cells</td>
<td>60/60 (100)</td>
</tr>
<tr>
<td>CD3+ T-cell rosettes*</td>
<td></td>
</tr>
<tr>
<td>Frequent (&gt;5 per nodule)</td>
<td>54/55 (98)</td>
</tr>
<tr>
<td>Few (1-4 per nodule)</td>
<td>1/55 (2)</td>
</tr>
<tr>
<td>Absent</td>
<td>0/55 (0)</td>
</tr>
<tr>
<td>CD57+ T-cell rosettes</td>
<td></td>
</tr>
<tr>
<td>Frequent (&gt;5 per nodule)</td>
<td>2/31 (6)</td>
</tr>
<tr>
<td>Few (1-4 per nodule)</td>
<td>13/31 (42)</td>
</tr>
<tr>
<td>Absent</td>
<td>16/31 (52)</td>
</tr>
<tr>
<td>CD57+ T cells</td>
<td></td>
</tr>
<tr>
<td>Few (&lt;50 per nodule)</td>
<td>8/31 (26)</td>
</tr>
<tr>
<td>Many (&gt;50 per nodule)</td>
<td>23/31 (74)</td>
</tr>
</tbody>
</table>

* Reactivity for CD3 not assessable in additional 3 cases.

Image 2
A. Weak membrane expression of CD30 identified in “popcorn” lymphocytic and histiocytic (L&H) cells (×40). B. Nuclear bcl-2 expression in popcorn L&H cells and in numerous small lymphocytes within the nodules (×40). C. Cytoplasmic bcl-6 positivity in popcorn L&H cells and in numerous small lymphocytes within the nodules (×40). D. Immunostaining for CD57 highlights numerous CD57+ lymphocytes and rare CD57+ rosettes (×40).
classical Hodgkin phenotype and were reclassified as classical HD. In addition, 9 of 40 cases with an initial diagnosis of classical HD were reclassified as NLPHL after review of the immunostains. As the initial morphologic diagnoses were made by a panel of experienced hematopathologists, the relatively high percentage of misdiagnosed cases further illustrates the necessity of immunohistochemical analysis in the differential diagnosis of NLPHL.

In the present study, we reviewed 60 cases of NLPHL diagnosed in our institution during a 10-year period. Basic immunohistochemical stains were performed in all cases at the time of the initial diagnosis, and the diagnosis of NLPHL was confirmed by a consensus of all authors. None of the cases was reclassified (12 of 72 cases retrieved from our database were not available for review).

The neoplastic L&H cells strongly expressed CD20, with weak staining seen in only 1 case. Similar to the findings published by the European Task Force on Lymphoma, CD79a was less sensitive than CD20 in establishing the B-cell phenotype of L&H cells. In all cases negative for CD79a, CD20 highlighted the diagnostic L&H cells. Although staining with anti-CD79a may be useful for confirming the B-cell phenotype in difficult or controversial cases, it is unnecessary to include this antibody in a first-tier immunohistochemical panel.

The expression of EMA by L&H cells was reported initially by Delsol et al and then by others. In these studies, cytoplasmic EMA staining was detected in approximately 60% of cases. EMA expression by neoplastic cells correlated with expression of J chain and has been considered a helpful immunophenotypic finding in the diagnosis of NLPHL. However, in the present study, the expression of EMA was observed in only 21% of cases (12/56). Findings similar to our results were reported by Nguyen et al, who found a low frequency of EMA expression by L&H cells and concluded that EMA staining might be of limited value in the distinction of progressive transformation of germinal centers and NLPHL. All the reviewed studies used the same antibody (EMA, clone E29, DAKO); however, it is possible that differences in pretreatment and detection techniques resulted in the different sensitivities of the immunostaining for L&H cells. A lower frequency of EMA-positive cases also might reflect a different spectrum of NLPHL cases included in the studies.

Weak membrane staining for CD30 was detected in a small subset of L&H cells in rare cases of NLPHL, as previously published. In addition, CD30 highlighted immunoblasts that were located predominantly outside B-cell nodules. Contrary to the weak expression occasionally detected in L&H cells, immunoblasts were strongly CD30+. CD30 expression in reactive immunoblasts is a potential pitfall in CD30− cases because these cells may be mistaken for classical Hodgkin cells.

The single-cell analysis of immunoglobulin genes has revealed that the neoplastic L&H cells are derived from germinal center B cells. Reliable markers reflecting germinal center phenotype, such as CD10 and bcl-6, have only recently been available for analysis in paraffin sections. The bcl-6 protein is a zinc finger transcriptional repressor encoded by the bcl-6 proto-oncogene and is implicated in the pathogenesis of diffuse large B-cell lymphoma. The bcl-6 protein is expressed by germinal center B cells and related lymphomas. CD10 is a membrane-associated neutral endopeptidase known as enkephalinase, which is expressed by a wide variety of normal human tissues, including reactive and neoplastic follicle center cells. Overexpression of bcl-2 protein has been detected in the majority of follicle center cell lymphomas.

In our study, a high proportion of NLPHL cases displayed bcl-6 reactivity in the absence of bcl-2 and CD10, thus confirming the findings of previous studies. Staining for CD10 does not have great usefulness in the immunohistochemical panel for NLPHL: the absence of CD10 expression also was reported in classical HD, and diffuse large B-cell lymphomas exhibit a variety of staining patterns, including bcl-6 positivity and CD10 negativity. Falini et al described strong nuclear expression of bcl-6 by the majority (>75%) of neoplastic cells in all NLPHL cases and the absence of bcl-6 staining in more than 70% of classical HD cases. In contrast, bcl-2 expression is absent in almost all NLPHL cases, but a relatively high frequency (>60%) of bcl-2 expression was reported in classical HD. Thus, the different patterns of expression (bcl-6+/bcl-2− more likely seen in NLPHL; bcl-6−/bcl-2+ in classical HD) might be useful in the differential diagnosis of difficult and controversial cases.

The CD57+ rosetting pattern often is cited as a common distinctive feature of NLPHL. In the present study, CD3+ rosettes were seen in all cases studied, but CD57+ rosettes were few when encountered, and these few rosettes were seen in fewer than half of the cases. The finding of a lower frequency of rosettes than previously reported might be attributed to the methodologic differences between laboratories (eg, a commonly used automated system with a polyspecific secondary antibody in our laboratory vs the isotype-specific secondary antibody described in some previous studies). Like our results, some of the published studies on NLPHL showed absence of CD57+ rosettes in a majority of cases, but with many CD57+ lymphocytes found within nodular structures.

The present study focused on the analysis of immunohistochemical markers that have achieved common use within diagnostic surgical pathology laboratories. It is...
important to note, however, that several more recent studies have identified novel markers that may prove useful for the diagnosis and differential diagnosis of NLPHL. Many of these studies have focused on transcription factors integrally involved in B-cell antigen expression and immunoglobulin gene expression. For example, the transcription factors Oct2, BOB.1, and PU.1 seem to be expressed more often in NLPHL and B-cell NHL than in classical HD, indicating their potential future diagnostic usefulness. In contrast, the B-cell–specific activator protein (BSAP) does not seem to reliably distinguish NLPHL from classical HD and B-cell NHL, since it is expressed in all of these lymphomas. The overall trend in these recent studies seems to concur with the findings of numerous other studies documenting a general lack of B-cell antigen expression in the neoplastic cells of classical HD, in contrast with the more overt B-cell phenotype generally expressed by the L&H cells of NLPHL. The general application of these novel markers to the routine diagnosis of NLHPL and other lymphomas will require additional study.

In our experience, the basic panel of antibodies including anti-CD3, CD15, CD20, CD30, and CD45 may be sufficient to confirm the characteristic L&H immunophenotype in most cases of NLPHL. CD30 expression in reactive immunoblasts is a potential pitfall in CD30– cases. Our data also showed that anti-CD79a was less sensitive than anti-CD20 for establishing the B-cell phenotype of L&H cells, and, thus, anti-CD79a does not need to be included in a first-tier immunohistochemical panel. Staining for bcl-2 and bcl-6 proteins might be a potential addition to the immunophenotypic analysis of NLPHL in difficult and controversial cases. The usefulness of EMA and CD57+ rosettes may have been overemphasized in previous reports.

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References


