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CD20-Negative Nodular Lymphocyte-Predominant Hodgkin Lymphoma

A 20-Year Consecutive Case Series From a Tertiary Cancer Center

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Context.—Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare, indolent Hodgkin lymphoma subtype with distinct clinicopathologic features and treatment paradigms. The neoplastic lymphocyte-predominant cells typically express bright CD20 and other B-cell antigens, which distinguishes them from Hodgkin/Reed-Sternberg cells of lymphocyte-rich classic Hodgkin lymphoma.

Objective.—To characterize the clinicopathologic features of CD20-negative NLPHL at a single institution.

Design.—A retrospective search for CD20-negative NLPHL in our pathology archives and medical records was conducted.

Results.—Of 486 NLPHL patients identified with CD20 available for review, 14 (2.8%) had LP cells with absent CD20 expression. Patients with prior rituximab administration (n = 7) and insufficient clinical history (n = 1) were excluded, leaving 6 patients with rituximab-naive, CD20-negative NLPHL. A broad immunohistochemical panel showed the LP cells in all cases expressed B-cell antigens, particularly Oct-2, although PAX5 and CD79a were frequently also dim. CD30, CD15, and Epstein-Barr virus-encoded small RNAs were negative in all evaluated cases. Two patients had high-risk variant immunophenotypic expression pattern D. One patient had extranodal disease, involving the spleen and bone, and was suspected to have large cell transformation. Standard NLPHL therapy was given, including local radiation and/or chemotherapy. Of 5 patients with available follow-up, 4 are alive in complete remission after therapy, and 1 is alive with relapsed disease.

Conclusions.—NLPHL can lack CD20 de novo without prior rituximab therapy. In such cases, extensive immunophenotyping helps distinguish NLPHL from lymphocyte-rich classic Hodgkin lymphoma, which differ in clinical behavior and therapy. In our series, CD20-negative NLPHL showed both classic and variant histologic patterns and the expected range of clinical behavior seen in NLPHL, including 1 case with suspected large cell transformation.

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Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare, indolent germinal-center derived B-cell lymphoma characterized by a nodular proliferation of large folded, multilobed neoplastic lymphoid cells in a background of small B lymphocytes and variable components of follicular helper and other T-cell subsets. The large neoplastic lymphoid cells, so-called lymphocyte-predominant (LP) cells or “popcorn” cells because of their cytomorphology, typically express multiple B-cell antigens, such as CD20. The main morphologic mimic of NLPHL is lymphocyte-rich classic Hodgkin lymphoma (LRCHL), which is distinguished by weak to absent B-cell antigen expression, such as CD20, consistent expression of CD30, frequent CD15 expression, and in situ hybridization staining for Epstein-Barr virus-encoded small RNAs (EBER) in approximately 40% to 50% of cases. In a European Task Force on Lymphoma project that studied large numbers of NLPHL across multiple oncological sites, 115 of 426 cases (27%) initially diagnosed as NLPHL were reclassified as LRCHL based on immunophenotype. In the same study, only 5 of 211 NLPHL cases (2%) had neoplastic LP cells that lacked CD20 expression. Currently, CD20 expression is one of the key immunophenotypic characteristics that separates NLPHL from LRCHL in the 2017 World Health Organization classification of lymphoid malignancies. Pathologic distinction between NLPHL and LRCHL is important given significant differences in disease biology and treatment paradigms. We present 6 cases of NLPHL reviewed at our institution with absent CD20 expression without prior rituximab exposure and characterize their B-cell antigen expression pattern and clinicopathologic characteristics.
ing on small B cells within the tissue. Immunohistochemistry showed appropriate internal control stains in formalin-fixed blocks when necessary. For all cases, CD20 (clone L26) was available for review. Of these patients, 14 have lymphocyte-predominant cells lacking CD20. Of the CD20-negative subset, 6 patients did not receive prior rituximab, meeting study inclusion criteria. Analysis of the CD20-positive subset shows the vast majority had strong diffuse CD20 staining, while smaller subsets had either dim or subset staining.

**METHODS**

Two cases of CD20-negative NLPHL were received on the Stanford Hematopathology Consultation service over a 1-week period and reviewed at a multidisciplinary Hodgkin lymphoma tumor board. Review of these cases prompted a retrospective search of the pathology report database at Stanford, including all cases accessioned between January 1, 1999 and December 31, 2018 with a definite diagnosis of NLPHL by World Health Organization criteria. Cases with LP cells with either (1) typical diffuse, strong CD20 expression, (2) focal or subset CD20 expression, or (3) dim or weak CD20 expression were excluded, leaving only NLPHL with completely CD20-negative LP cells. For the purposes of this study, focal or subset immunostain expression was defined as staining 10% or less of LP cells and dim or weak was defined as less expression than background B cells. Patients with incomplete medical history or prior rituximab administration were also excluded. All CD20-negative cases were reviewed for immunohistochemical variant patterns as described by Fan et al. Additional immunohistochemical stains were performed on archival unstained sections from the paraffin-embedded, formalin-fixed blocks when necessary. For all cases, CD20 (clone L26) immunohistochemistry showed appropriate internal control staining on small B cells within the tissue.

**RESULTS**

**CD20 Reactivity in NLPHL**

Of 492 NLPHL cases identified, 486 (98.6%) had CD20 immunohistochemical expression available. Of these, 14 (2.8%) NLPHL lacked CD20 expression, 12 (2.4%) had dim CD20, 15 (3.1%) had focal or subset CD20, and 1 (0.2%) had equivocal CD20 expression. The remaining 444 (90.0%) NLPHL had LP cells with bright uniform CD20 expression. Of the CD20-negative NLPHL subset, 1 case was excluded because of incomplete clinical history (as we could not exclude prior exposure to rituximab) and 7 cases were excluded because of documented prior rituximab therapy. Ultimately, 6 rituximab-naive, CD20-negative NLPHL cases (1.2% of all NLPHL) met inclusion criteria for the current study (Figure 1).

**B-Cell Transcription Program in CD20-negative NLPHL**

All 6 cases included in this study had LP cells that (1) lacked CD20 immunohistochemical expression on surgical material performed for initial diagnosis, and (2) did not have a prior history of rituximab administration after careful review of the medical record (ie, rituximab naive). LP cells in these patients expressed other B-cell markers, such as PAX5, CD79a, Bob.1, and Oct-2, with varying intensities. Other B-cell antigens were frequently also uncharacteristically dim; in particular, CD79a expression was subset, dim, or equivocal in all 5 cases tested, and PAX5 expression was dim in 5 of 6 tested cases. By contrast, Oct-2 staining was uniformly positive and not subset or dim in all 5 cases tested. Immunohistochemical stains for CD30 and CD15 and in situ hybridization for EBER were negative in LP cells in all 6 cases. The complete LP cell immunophenotype, including multiple B-cell markers, for all patients is outlined in Table 1. Two of 6 cases (33%) showed immunohistochemical variant pattern D (T-cell–rich nodular pattern) as described by Fan et al.

Fine needle aspiration biopsy (FNAB) was performed for patient 1 before surgical sampling. None of the other patients had prior FNAB. Based on the absence of CD20 and presence of CD30 on large cells, the diagnosis rendered for patient 1 by FNAB was “atypical CD30-positive lymphoid infiltrate suspicious for classic Hodgkin lymphoma.” Flow cytometry did not show any monoclonal B-cell population and a gate on monocytes and other large mononuclear cells showed no evidence of an abnormal population. Follow-up surgical material revealed a nodular proliferation of large lymphocyte-predominant cells that in some areas showed Hodgkin/Reed-Sternberg-like cytomorphology (Figure 2, A and B). Despite complete negativity for CD20 (Figure 2, C), full immunophenotyping of the LP cells showed expression of multiple B-cell markers (Figure 3, A through D). The absence of CD30, CD15 (not pictured), and EBER in LP cells confirmed the diagnosis of NLPHL (Figure 3, E and F). Of note, CD30 stained numerous scattered immunoblasts with intermediate to small variable cell size and a paracortical distribution.

**Clinical Features**

Medical records were available for review in all 6 patients. All patients had rituximab-naïve NLPHL and complete treatment history. One patient had clinical features highly suspicious for large cell transformation and extranodal involvement, including hypermetabolic splenic and bone lesions on positron emission tomography imaging. Of 5 patients with available follow-up, 4 patients remain in complete remission after chemotherapy and/or radiotherapy. Patient 1 had new minimally hypermetabolic bone lesions 2 years after 2 years of complete remission, which on fine needle aspiration and core biopsy showed recurrent disease most consistent with NLPHL.

The clinical characteristics of the patients are summarized in Table 2.

**DISCUSSION**

We show that the LP cells of NLPHL can completely lack immunohistochemically detectable CD20, which may occur de novo without any history of rituximab administration. Many also show dimmer expression of other B-cell markers, particularly CD79a and PAX5; in this small case series, Oct-2 was uniformly expressed and all cases were negative for CD30. Admixed CD30-positive immunoblasts can increase the difficulty of the differential diagnosis with classic Hodgkin lymphoma, indicating the importance of intact
tissue architecture and a broad B-cell immunohistochemical panel to rule out LRCHL. In our archival review, a significant number of NLPHL cases had LP cells with either dim CD20 (n = 12), subset CD20 (n = 15), or equivocal CD20 (n = 1) expression. These cases with varying levels or distribution of CD20 expression represent 5.8% of all NLPHL reviewed in this study and further emphasize the need for larger surgical sampling in select cases. Positive immunostaining for PAX5, CD79a, Bob.1, and Oct-2 indicate an intact B-cell transcriptional program and favor the diagnosis of NLPHL over LRCHL in cases with overlapping features; dim staining of multiple B-cell markers, including PAX5 and CD79a, can occur, such that a broad immunohistochemical panel is indicated. Concomitant lack of CD20 and CD79a in NLPHL was previously described by the European Task Force on Lymphoma.4 We do not recommend the use of CD19 immunohistochemistry in the panel to distinguish NLPHL and LRCHL because CD19 is frequently downregulated in various B-cell neoplasms.9

The CD20-negative NLPHL subgroup described could reflect a biological continuum between NLPHL and classic Hodgkin lymphoma (CHL), as has been previously suggested.10 However, none of the patients in this series had a prior history of CHL or relapse with CHL on biopsy. Phenotypic overlap between CHL and NLPHL has been previously described, as in a well-documented series of NLPHL expressing CD15 or in 1 pediatric case series of NLPHL expressing CD30.11,12 It is notable that 1 patient in our case series had prominent CD30-positive immunoblasts, which were mistaken as neoplastic cells on small-volume sampling by FNAB. None of our cases had LP cells that expressed CD30, CD15, or EBER, although NLPHL expressing 1 of these markers has been well documented.13 It is unknown whether cases with gray zone immunophenotypes between NLPHL and CHL may identify a clinicopathologically distinct subgroup of patients for whom optimal therapy has not been established. NLPHL and CHL are closely related, yet distinct by gene expression profiling.14 Of note, 1 well-described patient presented with concurrent NLPHL and CHL that shared the same immunoglobulin gene rearrangement.15 A recent microdissection-based analysis of CHL showed JAK/STAT pathway mutations in the majority of cases.16 Genomic studies of such gray zone cases between NLPHL and CHL may help shed light on this disease spectrum. These rare archival cases may be accessible by microdissection or novel flow sorting techniques.17 Digital droplet polymerase chain reaction (PCR)-based next-generation sequencing techniques on plasma may make prospective studies of this spectrum feasible.18

NLPHL typically presents with early-stage disease with peripheral adenopathy and indolent clinical behavior, and this pattern was represented in our case series, with the majority of patients presenting with stage I-IIA disease. Two of 6 patients (33%) in our series showed high-risk variant pattern D, which is associated with a poorer prognosis and higher risk of large cell transformation.19 However, the full spectrum of NLPHL outcomes was represented; 1 patient with high-risk and aggressive features, including immunohistoarchitectural variant pattern, had extranodal involvement and was clinically suspected to have large cell transformation based on positron emission tomography imaging. Extranodal involvement in the bone or bone marrow is very rare in NLPHL.20-22

### Table 1. Immunophenotypic Characteristics of Neoplastic Lymphocyte-Predominant Cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>CD20</th>
<th>CD79a</th>
<th>CD30</th>
<th>CD15</th>
<th>CD5</th>
<th>PAX5</th>
<th>CD19</th>
<th>Oct-2</th>
<th>Bob.1</th>
<th>CD45RB (LCA)</th>
<th>BCL6</th>
<th>CD30</th>
<th>CD15</th>
<th>EBER</th>
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<tr>
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<td>Dim positive</td>
<td>Positive</td>
<td>Dim variable positive</td>
<td>Dim positive</td>
<td>Subset dim positive</td>
<td>Dim positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Subset dim positive</td>
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<td>No data</td>
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</tr>
<tr>
<td>2</td>
<td>No data</td>
<td>Subset dim positive</td>
<td>Dim positive</td>
<td>Positive</td>
<td>Dim variable positive</td>
<td>Dim positive</td>
<td>Subset dim positive</td>
<td>Dim positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Subset dim positive</td>
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<td>No data</td>
<td>No data</td>
</tr>
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<td>No data</td>
<td>No data</td>
<td>Dim positive</td>
<td>Positive</td>
<td>Dim variable positive</td>
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<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
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<td>No data</td>
<td>No data</td>
<td>No data</td>
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<td>No data</td>
<td>No data</td>
<td>Dim positive</td>
<td>Positive</td>
<td>Dim variable positive</td>
<td>Dim to negative</td>
<td>Positive</td>
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<td>Dim positive</td>
<td>Positive</td>
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<tr>
<td>6</td>
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<td>Dim positive</td>
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<td>Negative</td>
<td>Negative</td>
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</tr>
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</table>

Abbreviations: EBER, Epstein-Barr virus-encoded small RNAs; LCA, leukocyte common antigen.
The diffuse variant (pattern E per Fan et al)8 of NLPHL can be very difficult to distinguish from T-cell, histiocyte-rich large B-cell lymphoma (THRLBCL). While none of the CD20-negative NLPHL described in our series showed a diffuse immunoarchitectural pattern, 1 case of NLPHL with focal CD20 expression identified during our archival search showed patterns E and F on initial biopsy. A subsequent excision specimen showed T-cell, histiocyte-rich large B-cell lymphoma–like transformation of NLPHL. This case highlights the potential biologic continuum between NLPHL and T-cell, histiocyte-rich large B-cell lymphoma, which has been previously described.14,23 Recent microdissection-based studies showed the 2 entities shared mutations, especially in variant pattern NLPHL.24,25

Regarding treatment, the role of rituximab for patients with CD20-negative NLPHL is unclear. In patients with absent CD20 expression in LP cells, rituximab monotherapy may still be clinically active as seen in many patients from this series, possibly because of the prominent B-cell–rich microenvironment. Rituximab may also provide some benefit in patients with equivocal CD20 expression. However, the standard of care for most patients with early stage NLPHL is radiotherapy alone, with rituximab typically reserved for the relapsed/refractory setting or in combination with chemotherapy for patients with advanced stage disease or large cell transformation.26–29

CONCLUSIONS

In conclusion, we show that the LP cells of NLPHL may lack CD20 expression, which may occur de novo or following prior treatment with rituximab. A biopsy that includes sufficient intact architecture to reveal the characteristic pattern of NLPHL and enables distinction of high-risk variant patterns is especially critical in cases with an atypical CD20-negative immunophenotype that may be misdiagnosed as CHL. A broad B-cell immunohistochemical panel, including PAX5, CD79a, Bob.1, and Oct-2, is indicated in these cases to distinguish between NLPHL and LRCHL, which have significant differences in clinical behavior and treatment.
Figure 3. Lymphocyte-predominant (LP) cell immunophenotype, patient 1. The LP cells express subset dim CD79a (A), subset dim PAX5 (B), diffuse moderate BOB.1 (C), and diffuse strong OCT-2 (D). CD30 is negative on LP cells (E). In situ hybridization for Epstein-Barr virus-encoded small RNAs (EBER) is negative in the LP cells and stains small lymphocytes, suggesting EBV reactivation (F). CD15 is negative (not pictured). LP cells are highlighted by arrowheads (CD79a, PAX5, BOB.1, OCT-2, CD30, EBER, all original magnifications ×200 [A–F]).
All patients have rituximab-naïve nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) and show the expected spectrum of NLPHL disease with a nodular growth pattern and abundant lymphocytes. Histopathologic analysis of submitted cases reveals 2 types of Hodgkin lymphoma: a classical type and a nodular lymphocyte-predominant type. The authors would like to thank Bijayee Shrestha MD, PhD (El Camino Hospital, Mountain View, California) for contributing a case to this series.

References


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Table 2. Clinical Characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Patterns</th>
<th>Biopsy Site</th>
<th>Stage</th>
<th>Extramedullary Involvement</th>
<th>Large Cell Transformation</th>
<th>Treatment and Disease Status at Last Follow-up</th>
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</thead>
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<tr>
<td>1</td>
<td>67</td>
<td>M</td>
<td>Pattern D</td>
<td>Right cervical lymph node</td>
<td>IVA S</td>
<td>Spleen, bone</td>
<td>Yes, clinically</td>
<td>6 cycles of R-CHOP with ongoing CR</td>
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<td>F</td>
<td>Classic</td>
<td>Retroperitoneal lymph node</td>
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<td>No</td>
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<td>Pattern D</td>
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<td>No</td>
<td>30 Gy ISRT with ongoing CR</td>
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<td>F</td>
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<td>No</td>
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<td>F</td>
<td>Classic</td>
<td>Left parotid lymph node</td>
<td>IA</td>
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<td>No</td>
<td>Local radiation, CR with no recurrences after more than 5 years of follow-up</td>
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<tr>
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<td>M</td>
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</tbody>
</table>

Abbreviations: CR, complete remission; Gy, gray; ISRT, involved site radiotherapy; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone.

All patients have rituximab-naïve nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) and show the expected spectrum of NLPHL clinical behavior.

* Patterns refers to the immunomorphologic patterns of NLPHL described by Fan et al.5 Classic immunomorphologic patterns include patterns A and B. Patients 1 and 3 showed variant immunomorphologic pattern D (T-cell–rich nodular), which has been associated with a worse prognosis and higher risk of large cell transformation compared with classic patterns per Hartmann et al.79