INTRODUCTION

Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) comprises approximately 5% of Hodgkin lymphomas and exhibits distinct differences in clinical, histologic and immunophenotypic features from those of classical Hodgkin lymphoma (CHL). Investigations employing microdissection techniques and single cell polymerase chain reaction assays have shown that the atypical large cells, also known as popcorn cells or lymphocyte and histiocyte ("L&H") cells in NLPHL are clonal B-cells.\(^1,2\) NLPHL is recognized as an indolent, germinal center-derived B-cell lymphoma with a predisposition for local recurrence and a low rate (3-5%) of progression to diffuse large B-cell lymphoma (DLBCL).\(^3-11\) Its occurrence can be preceded by or be closely associated with florid reactive follicular hyperplasia and progressive transformation of germinal centers although it is unclear whether these should be considered precursor lesions.\(^7,12-16\)

Clinically, NLPHL occurs in young adults (median age 35 years) and affects men in 75% of cases. Over half of the patients present with early stage disease and only a small minority demonstrates systemic symptoms. The disease is often confined to peripheral nodes in the cervical, axillary, inguinal and epitrochlear regions although other nodal sites such as mesenteric lymph nodes may also be affected. Bulky disease and mediastinal widening are rare as are the involvement of other organs including spleen, bone marrow, liver, lung and the skeleton. The disease is slowly progressive with frequent relapses but it generally remains indolent and sensitive to therapy.\(^5,10,11\) In patients presenting with early stage favorable disease the five-year overall and event-free survival approximate 95 – 100% for both the pediatric and adult age groups.\(^11\) The overall mortality of patients with NLPHL is increased in comparison to the general population due primarily to the development of secondary malignancies and cardiac failure. Thus, radiation and chemotherapy of reduced intensity and monoclonal antibody therapy (Rituximab) are preferred and have been found to be efficacious in the treatment of NLPHL.\(^10,11,17,18\)

The lymph node is usually significantly enlarged, exhibits a nodular architecture and a varying number of atypical large (L&H) cells with folded or multilobated nuclei. The background is rich in reactive lymphoid cells and histiocytes; the latter may be epithelioid and occur singly, in small clusters or form granulomas. Historically, nodular or diffuse patterns of NLPHL have been described: the nodular pattern exhibits L&H cells within nodules of small non-neoplastic B-cells and the diffuse pattern is composed of L&H cells in a diffuse infiltrate of reactive T-cells.\(^19\) The World Health Organization (WHO) requires that at least a partial nodular architecture be present for the diagnosis of NLPHL.\(^7\) However, several variant immunoarchitectural patterns have since been recognized: serpiginous/interconnected nodular pattern, nodular with prominent extra-nodular L&H cells, nodular with T-cell-rich background, diffuse “moth-eaten” with B-cell-rich background as well as a mixture of these patterns.\(^16\) The characteristic immunophenotypic profile of the large atypical cells in NLPHL in comparison to CHL is summarized in Table 1.

Table 1: Comparison of Immunohistologic Features of NLPHL and CHL

<table>
<thead>
<tr>
<th>Marker</th>
<th>NLPHL – L&amp;H cells</th>
<th>CHL – Hodgkin/RS cells</th>
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<tbody>
<tr>
<td>CD45 (LCA)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CD20</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>CD30</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>CD15</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>EBV</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>EMA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CD57+ T-cells</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
NLPHL can occur simultaneously with or progress to DLBCL and their clonal relationship has been well documented in the literature.\textsuperscript{2,8,20-25} The separation of NLPHL, particularly those with a diffuse component, from T-cell-rich B-cell lymphoma (TCRBCL) can be extremely challenging. In addition, NLPHL may also be difficult to distinguish from nodular lymphocyte-rich classical Hodgkin lymphoma (NLRCHL) and T-cell lymphomas, particularly those complicated by secondary large B-cell proliferations which are often associated with EBV.

**CASE PRESENTATION**

The case selected for presentation was seen at Stanford over a period of approximately 30 years. The initial diagnosis rendered in 1975 was based on morphologic evaluation of H&E sections. Two subsequent lymph node biopsies showed no evidence of lymphoma. This case illustrates some of the complexities involved in differentiating the histologic and immunoarchitectural features of NLPHL and the overlap of these features with classical Hodgkin lymphoma, progressive transformation of germinal centers and angioimmunoblastic and peripheral T-cell lymphomas. It is hoped that the recognition of the variant immunoarchitectural patterns and progression of NLPHL will be diagnostically useful, permit better understanding of the morphologic continuum from NLPHL to DLBCL and aid in the separation of NLPHL and its progression from their morphologic mimics.

**Clinical History**

The patient is a 61-year old man who presented with a mesenteric mass. A 5.0 cm mesenteric lymph node was excised. Approximately thirty years previously the patient had undergone a supraclavicular lymph node biopsy and the diagnosis of nodular sclerosis classical Hodgkin lymphoma ("cellular phase") was rendered for which he received chemotherapy and subtotal lymphoid irradiation. The patient had undergone two subsequent lymph node biopsies at 4 and 13 years after his initial treatment. Both of those biopsies had shown reactive follicular hyperplasia with progressive transformation of germinal centers.

**Histologic Features**

The lymph node biopsies from 1975, 1979, 1988 and 2003 have been reviewed and the salient findings are described below:

**1975 – Right supraclavicular lymph node:** Sections show a moderately enlarged lymph node with a nodular architecture without dense sclerotic bands. The nodules are composed of a mixed background rich in small lymphocytes, histiocytes and plasma cells within which are variable numbers of atypical large cells. These large cells show binucleation and prominent nucleoli typical of Reed-Stenberg (RS) cells and mononuclear variants of RS cells. The morphologic findings are compatible with classical Hodgkin lymphoma. The spleen was also involved at laparotomy (Stage IIISA).

**1979 and 1988 – Right and left inguinal lymph nodes:** Sections of both inguinal lymph nodes show enlargement and involvement by an exuberant reactive follicular hyperplasia. Occasional follicles are markedly enlarged, have disrupted germinal centers and are infiltrated by small lymphocytes – a pattern typical of progressive transformation of germinal centers. These nodules did not exhibit Hodgkin/RS cells or L&H cells.

**2003 – Mesenteric lymph node:** Sections of the mesenteric lymph nodes show massive enlargement and effacement of the normal nodal architecture by a mottled diffuse and partially nodular atypical lymphoid proliferation. The large nodules are filled with an infiltrate of small lymphocytes admixed with epithelioid histiocytes and are studded with atypical large cells exhibiting an open, vesicular chromatin pattern and prominent single or multiple nucleoli. Rare large cells showed morphologic features of classical Hodgkin cells. The atypical large cells were also present outside lymphoid nodules. In the mottled areas, these atypical large cells formed clusters whilst focally there were sheets of atypical large cells with numerous mitotic figures and karyorrhectic debris.
Immunohistologic Features
No immunohistologic studies were performed on the biopsies from 1975, 1979 and 1988. At the time of the 2003 review a CD20 stain was performed on the 1975 case; the large cells were positive for CD20 indicating that the original diagnosis was most likely NLPHL and not classical Hodgkin lymphoma.

Immunohistologic features of the mesenteric lymph node biopsy from 2003 are summarized in Table 2.

### Table 2: Immunohistologic Features of the Mesenteric Lymph Node Biopsy

<table>
<thead>
<tr>
<th>Immunostain</th>
<th>L&amp;H cells</th>
<th>Description</th>
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<tbody>
<tr>
<td>CD20</td>
<td>Positive</td>
<td>Nodular areas: nodules rich in B-cells with scattered large atypical cells within and outside the nodules Mottled areas: scattered large atypical cells surrounded by CD20-negative small cells Diffuse areas: sheets of large atypical cells</td>
</tr>
<tr>
<td>CD3</td>
<td>Negative</td>
<td>Nodular areas: few cells within nodules Mottled areas: majority of small cells and particularly those surrounding large atypical cells (ringing or rosetting pattern) Diffuse areas: majority of small T-cells</td>
</tr>
<tr>
<td>CD30</td>
<td>Negative</td>
<td>Occasional scattered cells</td>
</tr>
<tr>
<td>CD15</td>
<td>Negative</td>
<td>Occasional scattered cells</td>
</tr>
<tr>
<td>CD57</td>
<td>Negative</td>
<td>Highlight cells ringing large atypical cells</td>
</tr>
<tr>
<td>EMA</td>
<td>Positive</td>
<td>Highlight large atypical cells (similar distribution to CD20)</td>
</tr>
<tr>
<td>PAX5</td>
<td>Positive</td>
<td>Highlight large atypical cells (similar distribution to CD20)</td>
</tr>
<tr>
<td>EBV in situ</td>
<td>Negative</td>
<td>Highlight small lymphoid cells in nodular and diffuse areas and large atypical cells</td>
</tr>
<tr>
<td>CD45</td>
<td>Positive</td>
<td>Nodular areas: intact follicular dendritic cell (FDC) meshworks Mottled areas: disruption or lack of FDC meshworks Diffuse areas: scattered FDCs only</td>
</tr>
<tr>
<td>CD21</td>
<td>Negative</td>
<td>Nodular areas: intact follicular dendritic cell (FDC) meshworks Mottled areas: disruption or lack of FDC meshworks Diffuse areas: scattered FDCs only</td>
</tr>
</tbody>
</table>

Differential Diagnosis
- Progressive transformation of germinal centers
- Nodular lymphocyte-rich classical Hodgkin lymphoma
- Angioimmunoblastic T cell lymphoma
- Peripheral T-cell lymphoma complicated by a proliferation of large B-cells (B cell-rich T cell lymphoma)
- Nodular lymphocyte predominant Hodgkin lymphoma with progression to T cell-rich diffuse large B-cell lymphoma

Final Diagnosis
Nodular lymphocyte predominant Hodgkin lymphoma with progression to T cell-rich diffuse large B-cell lymphoma

Clinical Follow-Up
The initial diagnosis of classical Hodgkin lymphoma was based on the histologic findings on the right supraclavicular lymph node biopsy as well as the clinical presentation. The patient had multiple sites of involvement including the spleen; an intravenous pyelogram had also shown displacement of the right kidney from presumed para-aortic disease. He was treated with MOPP chemotherapy and subtotal lymphoid irradiation and was without disease for approximately 30 years, although he was at risk for developing a secondary malignancy, particularly a non-Hodgkin lymphoma from his prior subtotal lymphoid irradiation. At presentation in 2003, he was diagnosed with NLPHL with progression to TCRBCL. He had high risk disease based on the International Prognostic Index (IPI) and was treated with
RCHOP followed by consolidative high dose chemotherapy with stem cell transplantation. He developed cardiomyopathy as a complication (probably due to subtotal irradiation as well as transplantation) but has remained in complete remission since 2003.

DISCUSSION

The diagnosis of NLPHL can be made challenging by several factors: its intricate immunoarchitectural patterns, its co-existence with florid follicular hyperplasia and progressive transformation of germinal centers, its progression to T-cell rich B-cell lymphoma and diffuse large B-cell lymphoma both of which may be focal and subtle, and its overlap with the histologic and immunophenotypic characteristics of classical Hodgkin and T-cell lymphomas. The morphologic and immunophenotypic features of each entity listed in the differential diagnosis are discussed below in the context of the index case.

Progressive transformation of germinal centers (PTGC) \(^{12-14,26-33}\)

- Follicular structures several times larger than typically seen in reactive follicular hyperplasia
- Follicles composed of mantle zone B-cells with significant numbers of CD4 T-cells that often co-express CD57
- Variably sized clusters of residual germinal center cells without the presence of the L&H cells of NLPHL
- Typically occurs in isolated follicles in a background of reactive follicular hyperplasia although PTGC may be more frequent in young males
- Most frequently presents as an incidental finding (about 5% of reactive lymph nodes) may precede, occur simultaneously or follow a diagnosis of NLPHL

| Table 3: Comparison of Histologic and Immunohistologic Features of PTGC and NLPHL |
|---|---|---|
| **PTGC** | **NLPHL** |
| Low power | High power | Single large follicle | Multiple large nodules (mass lesion) |
| (can be highlighted by CD10, BCL6 or Ki-67) | Rare germinal centers in the nodules in 15% of cases |
| CD20, CD79a, or PAX5 | No L&H/"popcorn" cells | L&H/"popcorn" cells |
| CD3 and CD57 | No ringing of L&H cells but occasional macrophages may be ringed by T-cells | Ringing of L&H/"popcorn" cells by T-cells |

The index case showed multiple enlarged B-cell rich nodules studded with L&H cells highlighted by CD20 and ringed by CD57+ and CD3+ T-cells. In addition, there were mottled and diffuse areas with CD20+ large cells in a background rich in small T-cells. These features do not support PTGC as a diagnostic consideration. However, the two prior inguinal lymph node biopsies excised in 1979 and 1988 showed reactive follicular hyperplasia with features typical of progressive transformation of germinal centers.

Nodular Lymphocyte Rich Classical Hodgkin Lymphoma (NLRCHL) \(^6,7,34\)

- Comprise 5% of Hodgkin lymphoma (approximately the same frequency as NLPHL) with a higher median age and a male predominance (also similar to NLPHL)
- Nodular or less commonly diffuse infiltrate of small lymphocytes with an absence of eosinophils and neutrophils
- May have regressed germinal centers within nodules
- Small lymphocytes within nodules are mantle-zone lymphocytes (IgM+IgD+)
• A relatively uniform population of binucleate classic Reed-Sternberg cells and their mononuclear counterparts both of which usually have prominent eosinophilic nucleoli similar to other subtypes of CHL
• Immunophenotype of the atypical large cells is similar to other subtypes of CHL: they express CD30, are usually also positive for CD15 and PAX5 with a subset expressing CD20. They lack expression of CD45 (LCA) and T-cell associated markers
• Up to 40% of CHL have EBV-positive Hodgkin cells

The low power architecture of a lymph node involved by NLPHL and NLRCHL demonstrate several similarities that include a nodular architecture usually devoid of sclerotic bands (typical of nodular sclerosis Hodgkin lymphoma), eosinophils and neutrophils. The cell composition of the nodules is predominated by small B cells and scattered atypical large cells. The Hodgkin/RS cells however differ from L&H cells in their immunophenotype (please see Table 1 for comparison of immunohistologic features of NLPHL and CHL). The index case showed CD20+ and CD45+ large atypical cells morphologically typical of L&H cells. These cells were ringed by CD57+ CD3+ T-cells and lacked staining for CD30 and CD15. In addition, the mottled and diffuse areas with increased small T-cells and CD20+ large cells (indicative of progression), is not compatible with a diagnosis of classical Hodgkin lymphoma.

**Angioimmunoblastic T-cell Lymphoma (AITL)**

• Effacement of lymph node architecture by a diffuse or paracortical expansion of immunoblasts, vascular proliferation and admixed eosinophils and plasma cells
• Expansion of extrafollicular CD21-positive FDC meshworks
• CXCL13+ and CD10+ T-cells away from follicles
• Clonal T cell receptor gene rearrangements
• Associated with EBV in 50% of cases
• Clonal B-cell proliferations found in 35% of patients

<table>
<thead>
<tr>
<th></th>
<th>AITL</th>
<th>NLPHL</th>
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<tr>
<td><strong>Low power</strong></td>
<td>Nodular or diffuse paracortical proliferation</td>
<td>Multiple large nodules (mass lesion)</td>
</tr>
<tr>
<td><strong>High power</strong></td>
<td>Immunoblastic proliferation associated with prominent vasculature and admixed eosinophils and plasma cells</td>
<td>Nodules rich in small B-cells</td>
</tr>
<tr>
<td>CD20, CD79a, or PAX5</td>
<td>No L&amp;H/&quot;popcorn&quot; cells An associated proliferation of large B-cell may be present singly, in small clusters or as sheets of B-cells</td>
<td>L&amp;H/&quot;popcorn&quot; cells Progression to T-cell rich or conventional diffuse large B-cell lymphoma may exhibit scattered or sheets-like proliferation of large B-cells</td>
</tr>
<tr>
<td><strong>CD3</strong></td>
<td>Predominance of T-cells</td>
<td>Paucity of T-cells within nodules although with increased diffuse areas the is a concomitant increase in reactive T-cells in the background</td>
</tr>
<tr>
<td><strong>CD21</strong></td>
<td>Extrafollicular expansions of follicular dendritic cell meshworks</td>
<td>Typically intact FDC meshworks are confined to follicles although with progression to DLBCL disrupted and absence of meshworks may be present</td>
</tr>
<tr>
<td><strong>EBV</strong></td>
<td>EBV-associated B-cell proliferations in up to 50% of cases</td>
<td>Negative (very rare positive cases have been reported)</td>
</tr>
</tbody>
</table>

The nodular proliferation with mottled and diffuse areas seen in the index case may be simulated by AITL, particularly an AITL complicated by a large B-cell proliferation. Both exhibit areas with scattered or clustered atypical large B-cells. However, the paracortical immunoblastic T-cell proliferation admixed with a mixed inflammatory background (eosinophils and plasma cells) together with the prominent vascular proliferation and extrafollicular dendritic cells are not characteristic of NLPHL. The lack of
immunostaining for CXCL13 and CD10 on extrafollicular T-cells also exclude AITL as a diagnostic possibility. In addition, T-cell receptor gene rearrangement studies may be used to confirm a clonal T-cell proliferation in cases of AITL. In cases of AITL complicated by a secondary large B-cell proliferation, EBV in situ hybridization studies typically highlight the atypical large B-cells. Clonal studies for the B-cell antigen receptor (IgH VDJ gene rearrangements) may show a polyclonal, oligoclonal or clonal pattern. EBV expression is extremely rare in cases of NLPHL.

Peripheral T-cell lymphoma complicated by a proliferation of large B-cells (B cell-rich T cell lymphoma)

- Effacement of lymph node architecture by a diffuse or paracortical expansion of atypical T-cells admixed with eosinophils and plasma cells
- T-cells may have clear cytoplasm and exhibit prominent vasculature
- Clonal T cell receptor gene rearrangements
- Associated with EBV in 57% of cases
- Clonal B-cell proliferations found in 35% of patients

This is a relatively newly recognized entity in which a peripheral T-cell lymphoma is found in close association with an EBV-positive large B-cell proliferation. Similar to AITL it may simulate NLPHL particular one that shows progression to TCRBCL. However, in contrast to AITL no extrafollicular dendritic cells are present. Immunophenotypic and molecular studies (see Table 4), similar to those employed in the separation of AITL from NLPHL (with the exceptions of CD21, CXCL13 and CD10 to demonstrate extrafollicular dendritic and germinal center T-cells) are useful to distinguish this entity from NLPHL with progression to TCRBCL.

Nodular Lymphocyte Predominant Hodgkin Lymphoma with progression to T-cell rich diffuse large B-cell lymphoma (NLPHL with progression to TCRBCL)

Traditionally, NLPHL has been described as having a nodular growth pattern with or without a diffuse component. In a study of 137 biopsies from 118 patients we previously described six distinct immunoarchitectural patterns of NLPHL (Table 5). A combination of two or more patterns was more common than a pure pattern. The presence of a diffuse pattern was more common in patients with recurrent disease and tended to be associated with progression to an increasingly more diffuse pattern over time. Sequential biopsies also showed that those with increased extranodal L&H cells were more likely to progress to TCRBCL. Small germinal centers and prominent sclerosis, two features previously associated with CHL, were found in 15% and 20% of NLPHL cases respectively, emphasizing that these features cannot be reliably used to distinguish NLPHL from CHL. The recognition of these immunoarchitectural patterns as features of NLPHL is important for its accurate diagnosis and for separation of NLPHL from CHL and TCRBCL.

Table 5: Immunoarchitectural Patterns of NLPHL

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Architecture</th>
<th>L&amp;H cells</th>
<th>FDC meshworks</th>
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<tbody>
<tr>
<td>1. &quot;Classical&quot; nodular pattern, B-cell-rich</td>
<td>Nodules with predominance of small B-cells</td>
<td>Largely confined to nodules and ringed by CD57+ T-cells</td>
<td>Prominent FDC meshworks</td>
</tr>
<tr>
<td>2. Serpiginous/interconnected nodular pattern</td>
<td>Misshapen nodules rich in small B-cells</td>
<td>Largely confined to nodules and ringed by CD57+ T-cells</td>
<td>Associated with FDC meshworks</td>
</tr>
<tr>
<td>3. Nodular with prominent extra-nodal L&amp;H cells</td>
<td>Background rich in reactive T-cells</td>
<td>Extend outside nodules and lack ringing</td>
<td>Lack FDC meshworks</td>
</tr>
<tr>
<td>4. Nodular with T-cell-rich background</td>
<td>Nodules with increased T-cells</td>
<td>Confined to nodules and ringed by CD57+ T-cells</td>
<td>Associated with FDC meshworks</td>
</tr>
<tr>
<td>5. Diffuse pattern (T-cell-rich B-cell)</td>
<td>Indistinguishable from TCRBCL (requires nodular)</td>
<td>Lack ringing</td>
<td>Lack FDC meshworks</td>
</tr>
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</table>


The immunoarchitectural pattern in the index case reflects a combination of the classical nodular pattern in some areas whilst other areas show a diffuse TCRBCL-like as well as a mottled or "moth-eaten" pattern. The extent of the nodular areas was variable and represented 10 - 80% in different sections examined. Typical L&H cells were highlighted by the immunostain for CD20 and were present within and outside nodules. They were surrounded by CD57+ T-cells in the nodular areas where intact FDC meshworks were present (CD21 immunostain). However, in the mottled areas FDC meshworks were disrupted, the background had increased numbers of T-cells and the L&H cells lacked ringing by CD57+ T-cells. The presence of a diffuse pattern together with L&H cells outside nodules were found to be associated with progression of disease in our prior study: these features were evident in this case. The lack of staining of the atypical cells for CD30 and CD15 together with the areas of TCRBCL-like pattern made the diagnosis of CHL unlike lymphoma-like component elsewhere for diagnosis). The immunoarchitectural features of this case captures the morphologic continuum between NLPHL and DLBCL and illustrates the complexity and overlap it shares with CHL and TCRBCL on one hand and with AITL and PTCL complicated by large B-cell proliferations on the other. Additionally, the presentation with NLPHL in this patient was preceded by PTGC on two prior lymph node excisions and occurred as a therapy-related secondary malignancy approximately 30-years after the diagnosis and treatment for presumed classical Hodgkin lymphoma.

TAKE HOME LESSONS

- If the lymph node architecture shows mixed nodular and diffuse areas look for the presence and the distribution of atypical large cells and the company they keep
- In an indolent lymphoma, look for an aggressive component
- A relatively short panel of immunohistologic markers (CD20, CD30, CD15, PAX5, CD45, EBV…) can be employed successfully to distinguish subtypes of Hodgkin lymphoma
- A follicular dendritic cell marker (e.g. CD21) can be very useful to highlight a nodular architecture
REFERENCES


