Histopathology of Hodgkin's Lymphoma

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Abstract: In the last few years, there has been a greater understanding of the spectrum and biology of Hodgkin's lymphoma. In standard texts, Hodgkin's lymphoma is classified as 2 distinct entities, namely nodular lymphocyte predominant Hodgkin's lymphoma and classical Hodgkin's lymphoma. However, recent evidence suggests that classical Hodgkin's lymphoma is not a single disease. Although the mixed cellularity and lymphocyte-depleted subtypes may be part of a biologic continuum, the nodular sclerosis subtype has a distinct epidemiology, clinical presentation, and histology. Nodular sclerosis Hodgkin's lymphoma, particularly those cases presenting with mediastinal disease, also seems related to primary mediastinal B-cell lymphoma. As Hodgkin's lymphoma is a B-cell neoplasm, there is also a better appreciation today of cases that may be borderline with conventional B-cell lymphomas. We present an update on the histopathological features of Hodgkin's lymphoma and the immunohistochemical tools available for diagnosis in the clinical setting.

Key Words: classical Hodgkin's lymphoma, nodular lymphocyte predominant Hodgkin's lymphoma, primary mediastinal large B-cell lymphoma, gray zone lymphomas, Epstein-Barr virus, epidemiology, pathology, immunophenotyping, epidemiology

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HISTORICAL BACKGROUND

In 1832, Thomas Hodgkin first reported "On some morbid appearances of the absorbent glands and spleen" describing postmortem findings in 7 patients with enlarged lymph nodes and spleen.[1] Thirty-three years later Wilks[2] confirmed Hodgkin's findings in 15 additional patients and proposed to name the disease "Hodgkin's disease." In 1898 and 1902, the characteristic binucleated and multinucleated cells in Hodgkin's disease were independently described by Carl Sternberg and Dorothy Reed (Fig. 1). She disputed the view of Carl Sternberg, who related the abnormal cellular proliferation to tuberculosis.[3] She made a number of other significant observations, noting an early age peak in children and adolescents, and that the health of the patient was generally excellent before disease onset. She also noted the presence of anergy in patients with Hodgkin's disease.[4] However, neither Reed nor Sternberg recognized the neoplastic nature of the disease. Gall and Mallory[5] established the first modern classification system of lymphoma, based on their studies of 618 cases, which also included Hodgkin's disease. In 1944, Jackson and Parker[6] developed the first classification of Hodgkin's disease which contained 3 subtypes of Hodgkin's disease: paragranuloma, granuloma, and sarcoma (Table 1).

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The modern classification of Hodgkin's disease was introduced by Lukes and Butler.[7] Importantly, they recognized the unique features of nodular sclerosis which was former subsumed under the large group of "Hodgkin's granuloma." Recognizing the importance of the inflammatory background, they described lymphocytic and histiocytic (L&H) predominance, and lymphocyte depletion (LD) Hodgkin's lymphomas. L&H Hodgkin's could be nodular or diffuse, and LD was likewise divided into diffuse fibrosis and reticular subtypes. Mixed cellularity remained somewhat of a wastebasket category, for those cases not meeting criteria for one of the other subtypes. In the subsequent Rye conference in New York these 6 subtypes were reduced again to 4 subtypes: lymphocyte predominant, nodular sclerosis, mixed cellularity, and lymphocyte depleted.[8] This classification could be readily used by pathologists with high reproducibility, and correlated well with clinical features, and remained unaltered for almost 3 decades. Based on new phenotypic, genotypic, morphologic, and clinical findings, the Revised European American Lymphoma (REAL) classification in 1994 included Hodgkin's lymphoma as one of the lymphoid neoplasms, and distinguished between 2 major types: nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL) and classical Hodgkin's lymphoma (CHL). CHL was further classified into 4 subtypes: nodular sclerosis CHL (NSCHL), mixed cellularity CHL (MCCHL), lymphocyte-rich CHL (LRCHL), and lymphocyte-depleted CHL (LDCHL).[9] The terminology recommended in the REAL classification was incorporated into the World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues (Table 1), including the substitution of the term Hodgkin's lymphoma for Hodgkin's disease.[10,11]

The original name "Hodgkin's disease" was based on the uncertain status of the disease, infectious or neoplastic, and the uncertain cell of origin. Multiple studies have proven now the neoplastic nature of the Hodgkin's cell and also a B lymphocyte origin in nearly all cases.[12,13] Therefore, the name "Hodgkin's lymphoma" is preferred instead of the term "Hodgkin's disease."

The WHO classification is primarily based on the histopathological and immunophenotypic characteristics of the different Hodgkin's lymphoma subtypes. Findings from molecular analysis and clinical studies from the recent decades confirm the principles of the WHO classification. But at the same time, new epidemiologic and molecular findings suggest that CHL is not a single disease but consists of more than 1 entity with distinct characteristics. Moreover, the CHL subentities NSCHL and MCCHL/LDCHL together with NLPHL form 95% of Hodgkin's lymphoma. Here, we discuss the categories of Hodgkin's lymphoma focusing on NLPHL, NSCHL, and MCCHL/LDCHL.

CLASSICAL HODGKIN'S LYMPHOMA

Although NLPHL has been identified as an entity distinct from CHL,[14] there is also a greater appreciation today for the differences between NSCHL and the other subtypes, mainly MCCHL/LDCHL and LRCHL.[15] NSCHL affects young adults, is associated with mediastinal involvement, and requires an intact immune system for its development. In addition to histologic differences, its cytokine milieu and background lymphocyte population differ from other

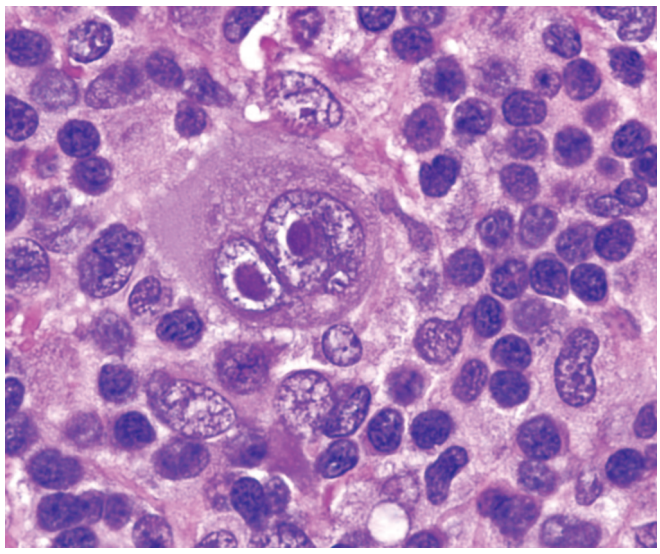

FIGURE 1. Classic Reed-Sternberg cell with owl-eye inclusion-like nucleoli. The nucleoli are approximately the same diameter as the background lymphocytes (H&E).

TABLE 1. Evolution of Hodgkin's Lymphoma Classification

Jackson/Parker, 1944	Lukes/Butler, 1966	Rye Conference, 1966	REAL/WHO, 1994/2008
Paragranuloma	L&H, nodular	LP	NLPHL
	L&H, diffuse		LRCHL
Granuloma	NS	NS	NSCHL
	MC	MC	MCCHL
Sarcoma	LD, diffuse fibrosis	LD	LDCHL
	LD, reticular		

REAL indicates Revised European American Lymphoma; NS, nodular sclerosis; MC, mixed cellularity.

subtypes of CHL. The presence of an overlap with primary mediastinal large B-cell lymphoma (PMLBCL) suggests a thymic origin for mediastinal NSCHL. MCCHL and LDCHL represent a spectrum, sharing many features related to incidence, pattern of spread, and association with immunodeficiency.[15] LRCHL, the most recently defined subtype, is perhaps the least well understood, with respect to its epidemiology and cellular origins (Table 2). Epstein-Barr viral (EBV) sequences are found in 20% to 90% of cases of CHL. The incidence of EBV-positivity varies with the age at presentation, the histologic subtype, and epidemiological factors such as geographic distribution or underlying immunodeficiency.[16] EBV-positive CHL is seen most often in the very young or the very old. There has been considerable speculation regarding the variations in the rate of EBV positivity. Can EBV play a hit and run role, or is there another virus linked to CHL pathogenesis?[17–19]

NODULAR SCLEROSIS CLASSICAL HODGKIN'S LYMPHOMA

Representing approximately 70% of all CHL, and perhaps an even higher proportion in developed countries, NSCHL is the most frequent subtype and its incidence has continued to rise over the past decades.[20] This entity stands apart from other forms of CHL and NLPHL. NSCHL is more frequent in resource-rich areas and the patient's high socioeconomic status is considered as 1 risk factor.[21,22] The disease affects primarily young adults and is less often observed in the elderly.[23] Interestingly, in contrast to all other forms of CHL and NLPHL, NSCHL demonstrates a female predominance. Association with EBV is rarely reported.[24] Compared with MCCHL and LDCHL, NSCHL shows a different risk factor pattern, suggesting that this entity may not have the same etiology as the other CHL types.[25,26] Gene expression profiling studies have supported the unique aspects of NSCHL, and in addition have identified signatures correlating with the generally good clinical outcome.[27] Prominently expressed genes include those involved in apoptotic induction and cell signaling.

NSCHL also has distinct clinical features. In 80% of patients with NSCHL the mediastinum is involved and 50% of patients with NSCHL present with bulky disease. Involvement of extralymphatic organs or bone marrow is not common. B symptoms occur in approximately 40% of all patients. At time of diagnosis more than 50% of patients have stage II disease.[28,29]

Histopathology of NSCHL

NSCHL differs from other subtypes in terms of the growth pattern and the characteristics of the neoplastic cells. The characteristic Hodgkin/Reed Sternberg (HRS) cell is termed a lacunar cell, because the cytoplasmic membrane is often retracted in formalin-fixed tissues (Fig. 2). Compared with classical HRS cells, lacunar cells have smaller nuclei, less prominent nucleoli, and more abundant cytoplasm. Classic HRS cells can also be found but usually are rare in NSCHL. Lacunar cells are found in cellular nodules, containing variable numbers of small lymphocytes. Neutrophils and eosinophils may be abundant, sometimes forming microabscesses within the nodules. The nodules are surrounded by dense collagen bands poor in fibroblasts. HRS cells express interleukin (IL-)13 and fibroblasts from NSCHL type are positive for the IL-13 receptor.[30] The production of transforming growth factor beta by the neoplastic cells also has been implicated in the fibrotic reaction.[31] The fibrosis often begins in the lymph node capsule, with fibrosis invaginating into the lymph node along vascular septa. In contrast to MCCHL

TABLE 2. Major Categories of Hodgkin's Lymphoma

	NSCHL	MCCHL and LDCHL	NLPHL
Risk factors			
Socioeconomic status	High	Low	No risk factors
HIV infection	Negative	Positive	
Gender predominance	Female	Male	Male
Age	Young adults	Children or elderly	Young adults
EBV infection	Negative	Positive	Negative
Lymphoid tissue involved	Mediastinal, cervical and axial lymph nodes	Generalized disease, lymph nodes and bone marrow	Peripheral and mesenteric lymph nodes, no mediastinal involvement

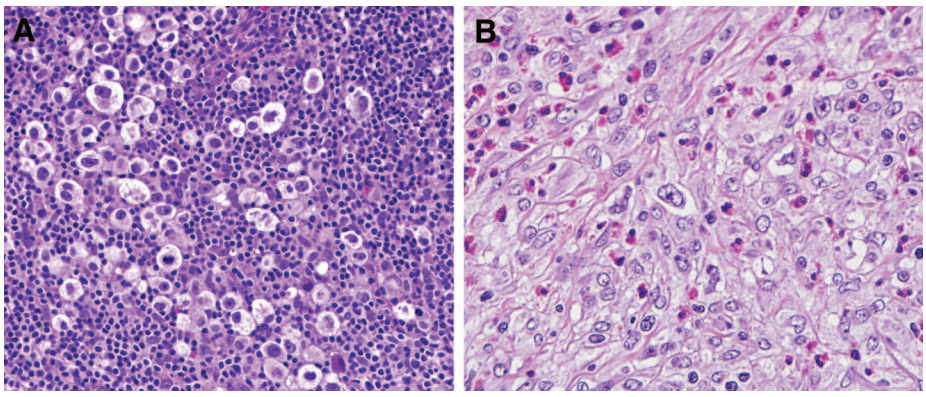

FIGURE 2. Nodular sclerosis Hodgkin's lymphoma. A, HRS cells have features of lacunar cells, and cluster within the nodular aggregates (H&E). B, This case is an example of the fibrohistiocytic variant of NSCHL, grade II. Normal lymphocytes are relatively sparse, and histiocytes and eosinophils are abundant (H&E).

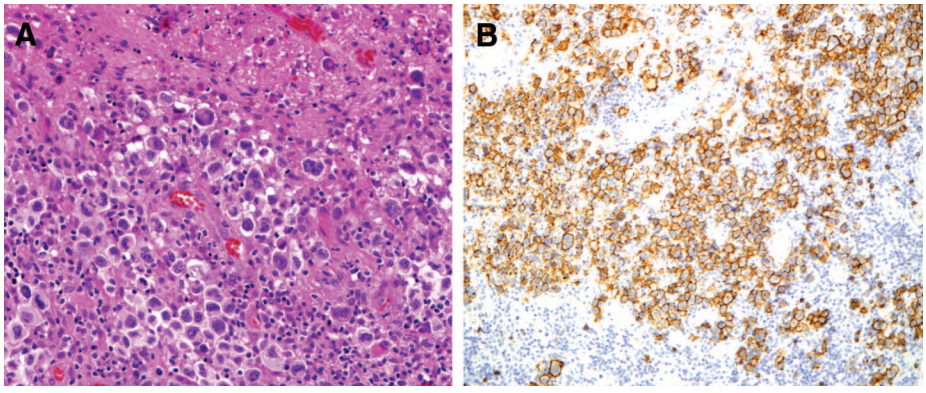

FIGURE 3. B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin's lymphoma. A, In this example of a "gray zone" lymphoma, the neoplastic cells resemble lacunar cells and are palisaded around an area of necrosis (H&E). B, Immunohistiochemical studies showed that the neoplastic cells were strongly CD20-positive and CD15-negative (not shown). This phenotype is atypical for CHL (CD20 immunostain).

and LDCHL, B cells form a higher proportion of the lymphocytic background in NSCHL.[32] In some cases with focal involvement, atypical cells are found within B-cell follicles, consistent with an origin from germinal center B cells.[33,34]

Some morphologic variations of NSCHL exist. The "syncytial variant" of NSCHL is characterized by cohesive sheets of lacunar cells within the nodules. In the "cellular phase" of NSCHL, a nodular growth pattern is present, but concentric fibrous bands are not formed.[35,36] As NSCHL is the most common subtype of CHL in Western countries, and yet differs in its clinical behavior, there have been attempts to grade NSCHL based on the proportion of neoplastic cells. The term "lymphocyte-depleted" (LD) form of NSCHL was coined to describe those cases having a high proportion of HRS cells, often with prominent necrosis.[37] Patients with advanced stage LD-NSCHL had a poorer prognosis and a higher relapse rate than NSCHL without lymphocytic depletion. Interestingly, the adverse prognostic impact of LD-NSCHL was not observed in patients with low-stage disease treated with radiation therapy.[38] A 2-grade system for classification of NSCHL was developed by the British National Lymphoma Investigation.[39,40] This grading of NSCHL was based on the cellularity of the nodules, the quantity of sclerosis, and the amount and atypia of neoplastic cells. NSCHL was considered grade II if 1 of the 3 following features was fulfilled within the neoplastic nodules: (A) more than 25% with a high proportion of tumor cells and necrosis, (B) more than 80% show a fibrotic or fibrohistiocytic composition, or (C) more than 25% contain numerous large bizarre or anaplastic cells. In the absence of these characteristics, NSCHL was considered grade I. The clinical relevance of grading of NSCHL has not been confirmed in all studies and has remained controversial.[41–43] As therapy has improved, the prognostic significance of grade has diminished.[43] Interestingly, grade seems most relevant in those patients who relapse, with shortened survival seen in those patients relapsing with grade II disease.[42] Variations on the British National Lymphoma Investigation grading system also have been proposed.[44] Grading has not been required by the WHO classification and is considered optional for clinical practice.[43]

A close relationship of NSCHL to PMLBCL and a possible origin from a thymic B cell has been described in recent studies.[45–47] Both entities share several features. The low-affinity immunoglobulin (Ig)E receptor CD23, known to be expressed on thymic B cells and PMLBCL, also is expressed in some cases of NSCHL. A lack of immunoglobulin (Ig) expression and HLA class I antigens is observed in both[48]; and *MAL*, a gene that encodes a protein associated with lipid rafts in T cells and epithelial cells, is expressed in both PMLBCL and some cases of NSCHL with mediastinal involvement.[49,50] Other subtypes of CHL do not express this gene.

The transitional morphology and phenotype of mediastinal gray zone lymphomas (MGZLs) provide further proof of a close relationship between PMLBCL and NSCHL.[47,11] The 2008 WHO classification recognizes a subset of cases in which a distinction between PMLBCL and NSCHL is not possible (Fig. 3). These cases may be designated as "B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin's lymphoma." Most but not all of these cases present with mediastinal disease.

Cases with features of MGZLs were most likely included in the past in the category of "Hodgkin's-like" or "Hodgkin's-related" anaplastic large cell lymphoma (ALCL).[51] Because of the strong CD30 expression in both entities, early studies suggested a relationship to ALCL. Today, it is appreciated that they share no biologic relationship, as CHL is of B cell origin and ALCL is of T-cell origin.[52] The optimal therapy for MGZLs is not yet determined.[53,54] However, both chemotherapy and radiation seem required for long-term disease-free survival.[55]

MIXED CELLULARITY AND LYMPHOCYTE-DEPLETED CLASSICAL HODGKIN'S LYMPHOMA

MCCHL is the second most frequent subtype of all CHL with a frequency between 10% and 20%, whereas LDCHL is the rarest subtype (<5%) in Western countries.[11] In most developing countries, MCCHL and LDCHL are the predominant CHL subtypes.[56–58] Both entities have overlapping epidemiological, clinical, and biologic features, which clearly differ from NSCHL. In contrast to NSCHL, low-socioeconomic status seems to be a risk factor for MCCHL and LDCHL.[21] Human immunodeficiency virus (HIV) infection is an additional relevant risk factor for the development of MCCHL or LDCHL, not only in developing countries but also in Western countries.[59–62] A recent study showed that the incidence of Hodgkin's lymphoma is increasing relative to B-immunoblastic lymphomas among HIV-positive patients, as highly active retroviral therapy improves immune function in this cohort.[61]

In contrast to the gender distribution in NSCHL, MCCHL and LDCHL are more common in men than in women. In addition, MCCHL has a bimodal age peak, presenting in both pediatric and elderly patients. LDCHL is primarily a disease of older individuals and associated with greater underlying immune compromise.[63] EBV infection of neoplastic cells is frequently detected in both MCCHL and LDCHL and distinguishes these subtypes from both NSCHL and NLPHL, which are generally EBV negative.[18] In addition to their epidemiologic and pathogenic features, MCCHL and LDCHL also have distinct clinical characteristics. Typically, MCCHL and LDCHL do not involve the thymus gland or mediastinum, which are preferentially involved in NSCHL. In contrast, peripheral lymph nodes and bone marrow are common sites of involvement of MCCHL and LDCHL. B symptoms are frequent. In the past, the histologic subtype was considered a major factor impacting prognosis.[64] Since the development of highly effective therapies, even for patients with advanced stage disease, other factors such as comorbidities including HIV-infection are more important prognostic factors than histologic classification.[65] Nonetheless, histologic subclassification remains relevant, as LDCHL and MCCHL have a worse prognosis compared with other subtypes of CHL.[66]

As NSCHL is subdivided into 2 grades, according to the proportion of tumor cells, MCCHL and LDCHL also may be viewed as 2 grades of a single disease entity.[15] The grading reflects both the proportion of normal lymphocytes within the background and the degree of underlying immunodeficiency in the patient. Subsuming MCCHL and LDCHL into a single entity supports the concept of a 3 disease hypothesis of Hodgkin's lymphoma as already suggested by MacMahon[67] in 1966 (Table 2).

Histopathology of MCCHL and LDCHL

Morphologically, MCCHL usually shows obliteration of the lymph node architecture. In cases of partial involvement the infiltrate is paracortical, with residual hyperplastic or regressed lymphoid follicles. In contrast to NSCHL, fibrosis if present is fine, fibrillar, and disorganized. Importantly, HRS cells are typical in appearance without lacunar or popcorn variants. As suggested by its name, the background in MCCHL comprises a mixture of different inflammatory cell types including lymphocytes, plasma cells, histiocytes, eosinophils, and neutrophils. The composition of the background can vary from patient to patient. In some cases, a granulomatous reaction may be prominent and may obscure the diagnostic cells.[68]

LDCHL shows a highly variable appearance but is characterized by 1 common feature: relative predominance of HRS cells in relation to the depleted background lymphocytes. Two main patterns of LDCHL were initially described in the Lukes-Butler[69] classification: a reticular/sarcomatous pattern and a diffuse fibrosis pattern. The reticular or sarcomatous variant was characterized by large numbers of HRS cells. This variant is rarely diagnosed today, and many such cases in the past were probably pleomorphic lymphomas of either B or T lineage.[65] Even today the distinction from a pleomorphic B-cell lymphoma may be difficult; such cases are sometimes termed "gray lymphomas," or in the 2008 WHO classification, "B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin's lymphoma."[70,11] These tumors often contain Reed-Sternberg-like cells, but differ phenotypically, in that CD20 is more often expressed, and CD15 is negative. Diffuse large B-cell lymphoma of the elderly also may resemble CHL. The neoplastic cells are EBV positive, and show a broad spectrum of cytologic features, including HRS-like cells.[71,72]

The diffuse fibrosis variant is defined as HRS cells embedded in a background of diffuse fibrosis. Fibroblasts can either be increased in number or even absent; recognition of diagnostic HRS cells may be difficult in these cases. Other cases of LDCHL contain abundant histiocytes but relatively few reactive lymphocytes (Fig. 4). This histologic picture may be seen in HIV-associated CHL. LDCHL should be distinguished from NSCHL, grade II, which may contain abundant HRS cells. It is likely that early series contained examples of NSCHL, grade II, miscategorized as LDCHL. LDCHL is the rarest subtype of Hodgkin's lymphoma, and the proportion of cases diagnosed as such has decreased in recent years because of introduction of immunophenotypic and molecular studies and the exclusion of morphologically similar lymphomas.

LYMPHOCYTE-RICH CLASSICAL HODGKIN'S LYMPHOMA

The most recently identified subtype LRCHL has a rate of approximately 5% of all CHL. In the past, this subtype was often misinterpreted as NLPHL, but with immunophenotypic studies was shown to be a variant of CHL.[9,73] Initially, there was speculation that this might be a variant of NSCHL in "early phase," but in patients with multiple biopsies, the histologic features remained unchanged. The clinical characteristics of LRCHL clearly differ from NSCHL. Patients with LRCHL are predominantly of male gender. The median age is higher than NSCHL and NLPHL.[74,75] Most patients

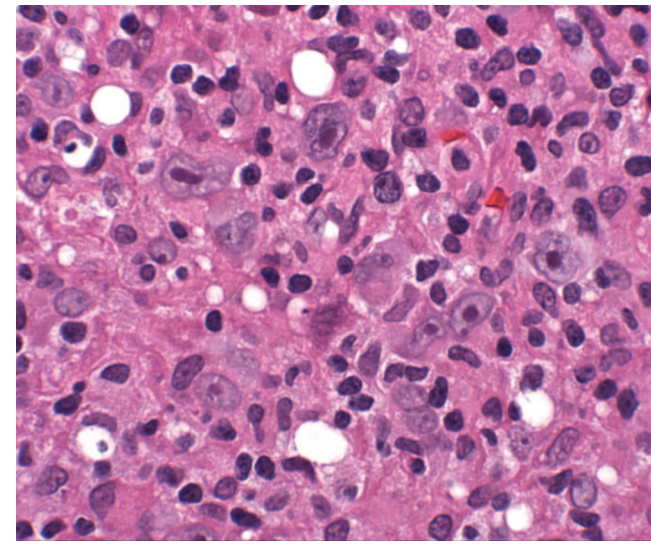

FIGURE 4. Lymphocyte-depleted CHL. This uncommon subtype presented in this 90-year-old man with generalized lymphadenopathy. HRS-cells are abundant and the background contains numerous histiocytes (H&E).

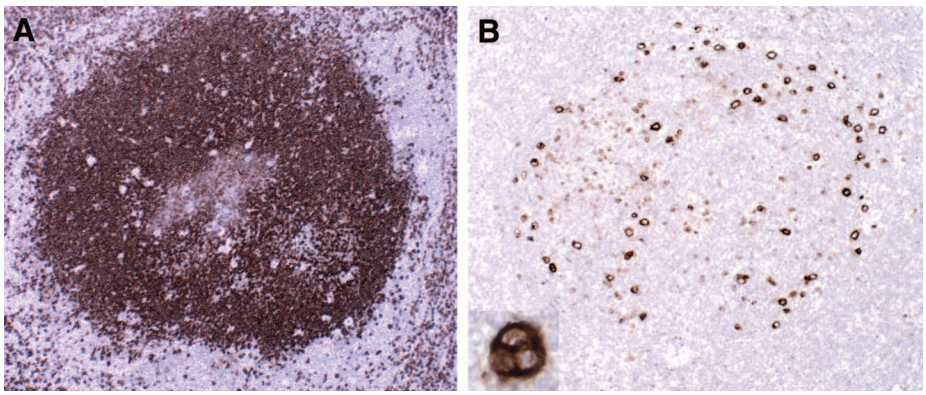

FIGURE 5. Lymphocyte-rich CHL. A, HRS cells are found within expanded follicles, mainly at the periphery in the mantle and marginal zone (CD20 immunostain). B, CD30 highlights the HRS cells at the periphery of a B-cell follicle. Inset shows a CD30-positive Reed-Sternberg cell at high power.

present with peripheral lymphadenopathy, usually stage I or stage II. In contrast to NSCHL, mediastinal involvement is rare. B symptoms are usually absent. The tumor cells are usually EBV negative.[76] The prognosis is very good with event-free and overall survival (OS) of 97% at 30 months.

Histopathology of LRCHL

Most cases of what is now recognized as LRCHL have a nodular growth pattern[73]and were first referred to as follicular Hodgkin's disease.[77] The lymphoid follicles are typically regressed, with the neoplastic cells localized to the far mantle and marginal zones of the follicles (Fig. 5). The individual HRS cells are rosetted by T-cells, so that in immunohistochemical stains the expanded marginal zones have a moth eaten appearance. Other inflammatory cells, including eosinophils and plasma cells, are generally sparse. Cytologically, the HRS cells have the phenotype of CHL; however, their cytologic features are often intermediate between those of lymphocyte predominant (LP) cells and classical HRS cells. They tend to have smaller nucleoli, and less cellular atypia than HRS cells in other forms of CHL. Thus, it is not surprising that these cases were often misdiagnosed as NLPHL before the routine use of immunophenotyping.[73]

A less number of cases of LRCHL have a diffuse growth pattern, with classical HRS cells in a background of mainly small lymphocytes. Cases with these features were probably described first by Lennert and Mohri, who considered them a lymphocyte predominant type of MCCHL.[78,9] The clinical features of this rare variant are not delineated.

NODULAR LYMPHOCYTE PREDOMINANT HODGKIN'S LYMPHOMA

About 5% of all Hodgkin's lymphomas are classified as NLPHL. This subtype differs from CHL clinically, epidemiologically, and with regard to its immunophenotype and genetics (Table 2).[14] NLPHL is more common in men than in women (3:1).[74] Patients are often young adults between the age of 30 to 50 years, and most often present with peripheral lymphadenopathy without B symptoms. NLPHL is the only subtype in which mesenteric lymph node involvement may be seen. The mediastinum is spared. Association with EBV is rarely seen.[79] The prognosis of NLPHL is good, even in patients who are followed without treatment.[66] Paradoxically, late relapses are not uncommon in NLPHL, but even after relapse the prognosis is good.[80,74]

Histopathology of NLPHL

NLPHL arises in the follicular environment, and nearly always has a follicular or nodular growth pattern, although over time the process may become diffuse. NLPHL lacks HRS cells. The neoplastic cell of NLPHL was originally termed the L&H cell, after the original description of this form of HL by Lukes et al[7] as "lymphocytic and histiocytic predominance." These cells have also been referred to as "popcorn" cells, but the WHO classification of 2008 recommended the use of the term "LP cell."[11] In comparison with classical HRS cells, the nucleoli of the LP cells are smaller, multiple, and basophilic. A variety of patterns have been described, but expansion of the follicular structure is nearly always seen.[81]

Progressive transformation of germinal centers (PTGC) is observed in some cases of NLPHL.[82] However, when diagnosed independently, progressive transformation of germinal centers has a low incidence of progression to NLPHL.[83] NLPHL may progress to diffuse large B-cell lymphoma (DLBCL) in about 5% of cases, and in such cases DLBCL and NLPHL are often composite in the same lymph node mass.[84,85] The clinical significance of this form of progression is indeterminate, but some patients still maintain an excellent prognosis.[84,86] Clonal identity between the NLPHL and DLBCL has been shown in most cases studied at the molecular level.[87,88] Another type of histologic progression is to a process histologically indistinguishable from T-cell/ histiocyte-rich large B-cell lymphoma.[89] In these cases, the prognosis is often poor, with advanced stage disease and bone marrow involvement. However, cases in which a nodular pattern is maintained, even if T-cell rich, have a better outcome.[90]

IMMUNOHISTOCHEMISTRY OF HODGKIN'S LYMPHOMA

The diagnosis of Hodgkin's lymphoma is primarily based on the recognition of the typical tumor cells, either HRS cells or LP cells, in the appropriate environment. In addition, the character of the inflammatory background and the surrounding stroma as well as established immunophenotypic and molecular markers help to classify the disease into the various tumor subtypes. A panel of markers is used for immunophenotyping of Hodgkin's lymphoma including B-cell surface markers, transcription factors, and EBV-associated proteins (Table 3).

In nearly all cases of CHL, HRS cells are positive for CD30, a glycoprotein belonging to the tumor necrosis factor receptor superfamily.[91,92] In addition, the majority of HRS cells (85%) also express CD15, the Lewis x carbohydrate adhesion molecule.[93,94] Even though both LP cells and HRS cells are genotypically of B-cell lineage, CD20 and CD79a are only expressed by a minority of CHL cases (10%–40% $CD20^+$).[12,95,96] The wide variation in the proportion of cells expressing CD20 in CHL may be related to changes in antigen retrieval techniques, with a higher proportion of CD20-positive cases seen in recent years. However, overall the B-cell program is lost in most cases of CHL.[96] As noted above, the HRS cells of CHL are positive for EBV most often in MCCHL and

TABLE 3. Immunohistochemical Features of Hodgkin's Lymphoma

	LP Cells NLPHL	HRS Cells CHL
Nonlineage antigens		
CD45	+	−
CD30	−	+
CD15	−	+/−
B cell-associated antigens		
CD20	+	−/+
CD79a	+	−/+
J chain	+/−	−
IgD	+/−	−
B cell-related transcription factors		
BOB.1	+	−/+
OCT 2	+	−/+
PU.1	+	−
PAX5	+	+ (weak)
EBV detection		
LMP-1	−	+/−*
EBER	−	+/−*

*Often positive in MCCHL/LDCHL, usually negative in NSCHL.
+indicates positive in all cases; +/−, positive in majority of cases; −/+, positive in minority of cases; −, negative in all cases.

LDCHL. EBV is expressed with a latency II phenotype and can be detected with immunostains for LMP-1 and EBV in situ hybridization with the EBER probe.[97] Galectin-1 is expressed in CHL but not in NLPHL and seems responsible for mediation of suppression of EBV-specific T-cell immunity.[98]

The phenotype in NLPHL differs in nearly all respects from that of CHL. LP cells are usually negative for both CD15 and CD30.[14] The B-cell markers CD20 and CD79a are positive in nearly all cases of NLPHL. LP cells may express Ig and polypeptides of the light chain fraction (J chain) and Ig are detectable in the majority of cases of NLPHL.[99,100] In addition, the LP cells in a subset of cases of NLPHL express IgD; the IgD-positive cases are most frequent in young males, and often contain nodules relatively rich in T-lymphocytes (Fig. 6).[101] The common leukocyte surface antigen CD45 is detected on LP cells but not on HRS cells.

The B cell-associated transcription factors Pax5, Oct-2, BOB.1, and PU.1 again underscore the B-cell lineage origin of LP cells and HRS cells but also show specific expression patterns. Pax5, also known as B cell-specific activator protein, is crucial in B-cell lineage commitment and is expressed by LP cells and HRS cells.[102] The other transcription regulating proteins, Oct-2 and BOB.1 that are involved in germinal center formation and Ig production, are expressed by LP cells, but are often negative in CHL.[103] Oct-2 is strongly expressed in normal germinal center cells and more weakly in other B-cell populations. It is also highly expressed in LP cells, and immunostains for Oct-2 are diagnostically useful in cases in which LP cells are sparse (Fig. 6). The unique regulatory protein PU.1 required in the generation of lymphoid and myeloid cells is positive in LP cells but negative in CHL and also in T-cell/histiocyte-rich large B-cell lymphoma (Table 3).[104]

The clinical significance of alterations in the usual phenotype in CHL has been examined in several studies.[95,105,106] A study from the German Hodgkin Study Group found that the absence of CD15 expression and expression of CD20 by the HRS cells were both negative prognostic features.[105] Portlock et al[106] also confirmed an adverse prognostic significance for CD20-positive CHL, with decrease in time to treatment failure and OS in CD20+ cases. However, another study found CD20 to be a positive prognostic factor for both failure-free survival (FFS) and OS.[95] Notably, the clinical impact was lost in patients treated in the more modern era, after 1981. Other immunophenotypic aberrancies also have been observed in CHL. For example, a subset of CHL cases show aberrant T-cell antigen expression, without evidence of T-cell gene rearrangement.[107] CD2, CD3, and CD4 are the most commonly expressed antigens on the surface of the HRS cells.

Although most cases that show a T-cell immunophenotype are also of B-cell origin on molecular analysis,[108] a T-cell origin was suggested in 3 reported cases, based on the presence of T cell receptor gene rearrangements.[109,110] However, conclusive evidence for a T-cell form of CHL is lacking. For one, at the time the 2 reports were published, it was not appreciated that peripheral T-cell lymphomas could express both CD30 and CD15, and mimic CHL at both the phenotypic and morphologic levels.[111] Additionally, cases of pleomorphic T-cell lymphomas after primary cutaneous ALCL, mycosis fungoides, and lymphomatoid papulosis may closely simulate CHL.[112] Much of the biology that we understand regarding CHL is related to its derivation from rescued germinal center B cells.[13] Conceptually, suggesting that the same disease entity may be of T-cell derivation runs counter to the view that lineage is a primary factor in defining disease entities.[9]

The inflammatory background also differs in CHL and NLPHL. NLPHL arises within the follicular environment, and early in the course of disease abundant B cells, generally IgD-positive are present.[113] The individual LP cells are rosetted by T-cells that have the phenotype of intrafollicular T-cells, expressing CD57 and PD-1.[114,115] In contrast, HRS cells in CHL generally are found in a

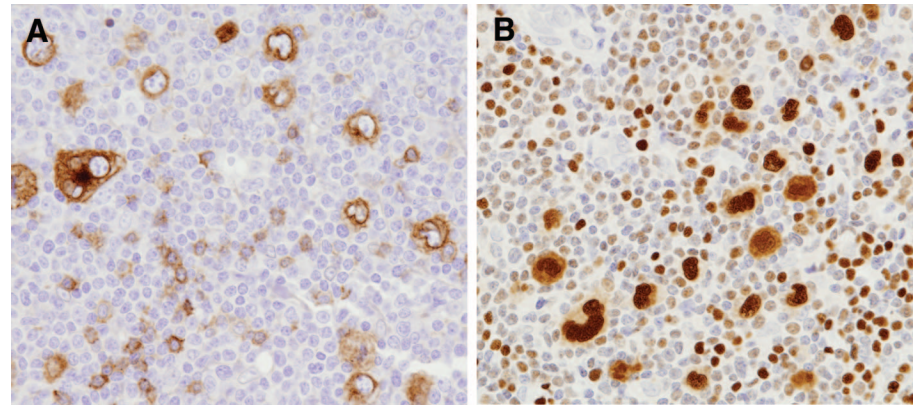

FIGURE 6. NLPHL. A, The LP cells in this case were positive for IgD, a finding most frequent in young males (IgD immunostain). B, LP cells stain intensely with OCT-2, which is useful in diagnosis when the atypical cells are sparse. Note the lobulated nuclear contours (OCT-2 immunostain).

T-cell rich background. The rosetting cells are CD4-positive T-cells that express the costimulatory molecule CD28.[116] The cytokine milieu in CHL may lead to the generation of regulatory T (Treg) cells, positive for CD4, CD25, and CCR4, which may be associated with immune escape.[117,118]

CONCLUSION

Many years ago MacMahon[67] speculated on the heterogeneity of Hodgkin's disease based on epidemiological observations. He hypothesized that Hodgkin's disease comprised 3 separate disease entities, with differing etiologies. Further studies have supported his hypothesis, and in many respects NLPHL, NSCHL, and MCCHL/LDCHL are distinctive. All cases of Hodgkin's lymphoma are unified by the fact that neoplastic cells are in the minority, and the majority of cells present in the tumor are reactive. However, the various forms of Hodgkin's lymphoma differ in the character of the neoplastic cells and in the inflammatory background. Additional differences in clinical presentation and epidemiology underscored in this review help to define these disease entities. Although it is now accepted that the neoplastic cells are of B-cell lineage, many questions remain concern the etiology of Hodgkin's lymphoma, the downregulation of the B-cell program in CHL, and the basis for the heterogeneity observed clinically and pathologically.

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