

Review

Diagnosis of Hodgkin's disease: an update on histopathological and immunophenotypical features

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Summary. The Hodgkin lymphoma (HL) is a B-cell lymphoma, as was proved by molecular studies with single-cell PCR. Histologically, it is characterized by a minority of neoplastic cells, Reed-Sternberg cells and its variants, related to a variable non-neoplastic inflammatory background. Nowadays, (WHO classification) the following types of HL are recognized: Nodular Paragranuloma and the Classical Hodgkin Lymphoma, the latter including Nodular Sclerosis, Mixed Cellularity, Lymphocyte-rich Classical Hodgkin Lymphoma and Lymphocyte Depletion. Morphology together with immunohistochemical studies allows to classify the different forms of Hodgkin lymphoma and to make a differential diagnosis with non-Hodgkin lymphomas. All classical Hodgkin lymphomas are treated similarly, and chances for remission and survival are currently good. Molecular parameters should be added to the current classification and patients could benefit from new therapeutic targets.

Key words: Hodgkin lymphoma, Reed-Sternberg cell, Morphology, Clinical features, Immunophenotype, Molecular studies

Introduction

In 1832, Thomas Hodgkin described the disease that bears his name in a manuscript entitled "On some morbid appearances of the absorbent glands and spleen". In this publication he recorded seven autopsy cases: six were personal observations in the Guy's Hospital (London) and the seventh was a contribution from Robert Carswell. The relevance of Hodgkin's observations, however, was not fully recognized until 1865, when Sir Samuel Wilks coined the term

"Hodgkin's disease" (HD) (Abbondanzo, 2003).

Later on, at the beginning of the twentieth century, Fox, an American pathologist from the University of Pennsylvania (Philadelphia) performed a histological study of those cases during a visit to Guy's Hospital. This study led him to conclude that only three out of the seven cases were actually HD and the others were classified as tuberculosis, syphilis and "systemic lymphomatosis" (Bonadonna, 2000).

Hodgkin's disease has been a paradigm in medicine. It was the first neoplastic disease defined on the basis of the histological classification of a characteristic neoplastic cell (the Reed-Sternberg cell). It was also one of the first neoplasms where the clinical evolution was correlated to the regions anatomically involved. Furthermore, it was one of the earliest neoplasms to be treated with chemotherapy and radiotherapy (Bonadonna, 2000).

HD is one of the most frequent malignant lymphomas in Western countries. It is characterized by a minority of neoplastic cells – Hodgkin's and Reed-Sternberg cells (HRS cells) and its variants – within a dominant non-neoplastic background variably composed of lymphocytes, eosinophils, histiocytes, and plasma cells. For a long time, the nature of the neoplastic cells has remained elusive and highly controversial. Now it is clear that Hodgkin's disease is a lymphoid proliferation, mostly of B-cell nature, as evidenced by immunophenotypical and molecular studies (single-cell PCR). In fact, the new WHO classification introduces the term Hodgkin lymphoma (HL), which will be used hereafter (Jaffe et al., 2001).

Based on the morphology of neoplastic cells, the immunophenotype and the composition of the inflammatory infiltrate, two biological and clinically different entities may be distinguished: nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and classical Hodgkin lymphoma (CHL). The basic recognition of these two entities, first in the REAL classification (Harris et al., 1994), and later in the WHO

classification (Jaffe et al., 2001) is based on prior classifications by Jackson and Parker (1947) and Lukes and Butler (1966). The REAL and the WHO classifications incorporated the concept of NLPHL as being similar to the nodular paragranuloma of Jackson and Parker (1947).

Thus, at present, the following types of HL are recognized: NLPHL (nodular paragranuloma) and the CHL, the latter including nodular sclerosis (NS), mixed cellularity (MC), lymphocyte rich-CHL (LR-CHL), and lymphocyte depletion (LD) (Jaffe et al., 2001).

The purpose of the present review is to provide an update for the general practising pathologist about the diagnostic criteria and subclassification of HL in the light of the current WHO classification.

NLPHL (nodular paragranuloma)

Clinical features

NLPHL (nodular paragranuloma) is a subtype of HL with a favourable prognosis, amounting only to a small percentage of the total cases of HL (around 5%). It can occur at any age, but there is a peak of incidence in the 4th decade, which contrasts with the bimodal distribution of CHL (3rd and 7th decades). Patients are typically asymptomatic, with longstanding localized peripheral lymphadenopathy. The more frequently affected lymph nodes are cervical and axillary; in contrast with CHL, mediastinal involvement is very unusual. Almost all patients with rare exceptions are in stage I or II at the moment of diagnosis (Poppema et al., 1979a; Hansmann et al., 1984; Mason et al., 1994). The disease has an indolent evolution and the prognosis is favourable despite the frequent relapses, which are usually local, and responds well to treatment. In fact, life expectancy in stage I patients is virtually the same as in the general population. Causes of death in NLPHL patients are non-Hodgkin lymphomas, other malignancies or treatment complications, but not the disease itself (Regula et al., 1988; Mason et al., 1994; Bodis et al., 1997; Karayalcin et al., 1997). Consequently, it has been questioned if additional treatment to surgical excision is needed in a patient with single lymph node involvement (Mason et al., 1994).

About 2-7% of NLPHL patients develop a non-Hodgkin lymphoma, usually a diffuse large B-cell lymphoma (DLBCL) (Karayalcin et al., 1997; Hansmann et al., 1989; Bennet et al., 1991; Wickert et al., 1995); however, this secondary DLBCL has a more indolent clinical course and a more favourable prognosis than the de novo DLBCL (Sundeen et al., 1988; Hansmann et al., 1989; Chittal et al., 1990; Grossman et al., 1991).

These clinical findings alone would be enough to make a clear-cut distinction between NLPHL and CHL, but this concept is also supported by morphological and immunophenotypical features, as discussed later (Mason et al., 1994; Pileri et al., 2002).

Morphology

Histopathologically, it has a nodular pattern of growth (Fig. 1), at least partially, and tends to efface the lymph node architecture, only preserving some reactive lymphoid follicles, which are compressed in the subcapsular zone. The neoplastic nodules are usually large and have poorly defined boundaries. Immunohistochemistry with B cell (Fig. 2) or follicular dendritic cell markers (Fig. 3) may help to recognize the nodular architecture in cases with no obvious nodularity. There are, however, many variations on this typical picture, as recognised by Fan et al. (2003) (see below in "immunophenotype"). In some cases, one may observe diffuse growth areas, but the existence of a purely diffuse variant of paragranuloma is a very controversial topic. Most cases formerly diagnosed in this fashion are actually non-Hodgkin lymphomas (T-cell/histiocyte-rich B-cell lymphomas) or lymphocyte rich classical HL (Harris et al., 1994; Jaffe et al., 2001). According to current criteria the detection of one nodule showing the typical features of NLPHL in an otherwise diffuse growth pattern is sufficient to exclude the diagnosis of T-cell/histiocyte-rich B-cell lymphoma (Jaffe et al., 2001).

The non-neoplastic population is mainly formed by small lymphocytes, frequently in a background of epithelioid cell aggregates, hence the original term of lymphocytic or histiocytic Hodgkin's disease. Intermingled with this predominant population, atypical cells with multilobated nuclei and relatively small nucleoli, with no peripheral halo, called L&H cells, can be observed. Its morphology has been compared to that of popcorn ("popcorn cells") (Fig. 4). Classical HRS cells are usually absent or very scanty. In fact, these are not required for diagnosis. Plasma cells, eosinophils and neutrophils are not usually observed either (Burns et al., 1984; Poppema, 1996).

In some cases there may be some fibrosis surrounding the nodules, reminiscent of that seen in nodular sclerosis, which has been suggested to represent a chronic tissue reaction in long-standing cases (Poppema, 1996).

A special type of lymphoid follicular hyperplasia known as progressive transformation of germinal centres is found adjacent to lymph node involvement by HL in about 20% of cases (Poppema et al., 1979b,c; Burns et al., 1984). It can also be seen in lymph nodes not affected by NLPHL from the same patient, as well as before and after the NLPHL is diagnosed. It consists of infiltration of the germinal centres by small lymphocytes from the follicular mantle, thus forming large follicles similar to NLPHL nodules. This has led to speculate that PTGC may be a precursor to NLPHL or that both may be a manifestation of a similar abnormal B-cell reaction (Poppema, 1996). In fact, there is an increase in the risk of the appearance of NLPHL in young patients with PTGC. Although the long-term risk is small, a careful follow-up of these patients is indicated (Osborne and

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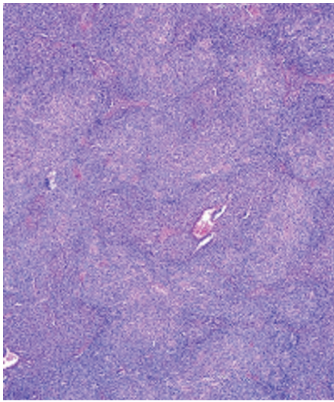


Fig. 1

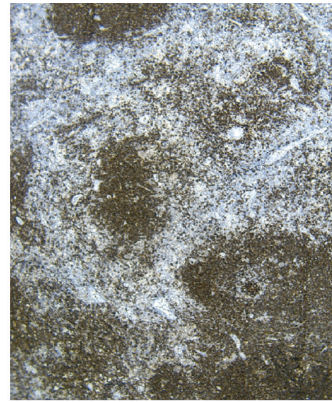


Fig. 2

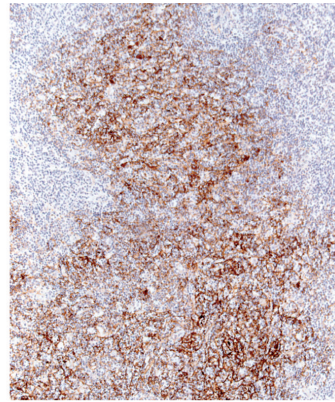


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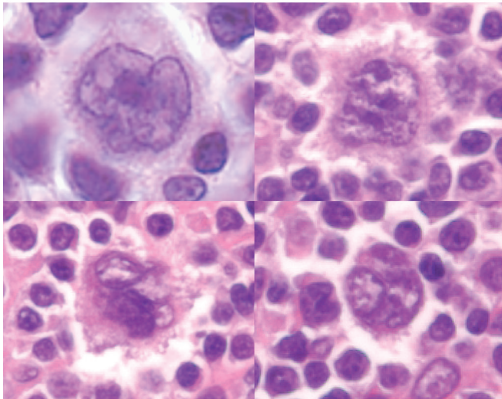


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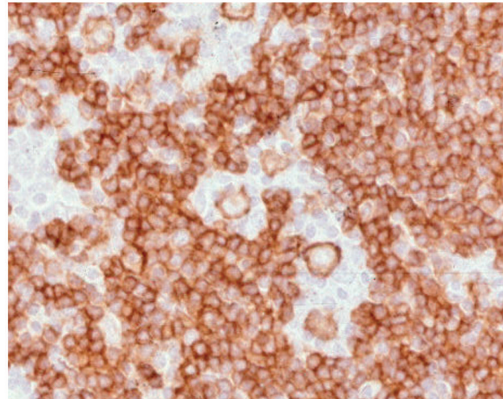


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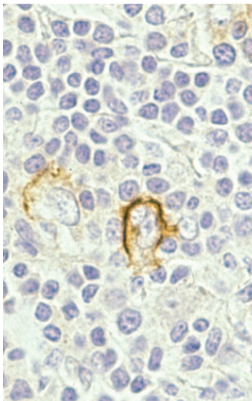


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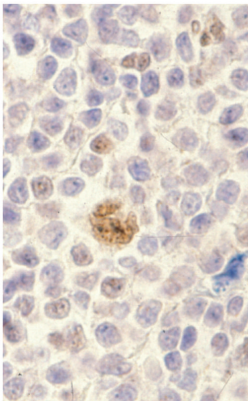


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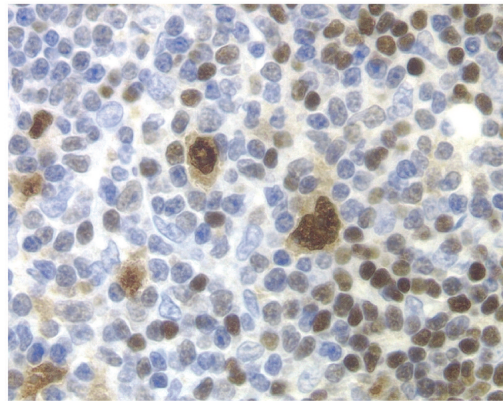


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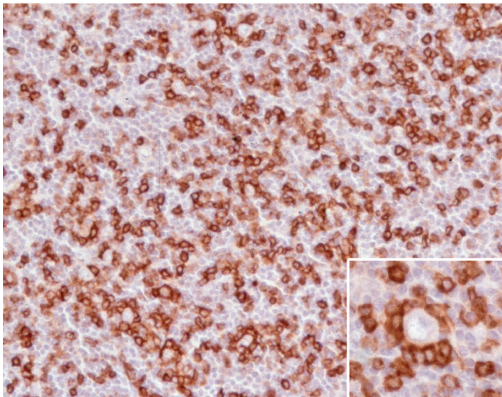


Fig. 9

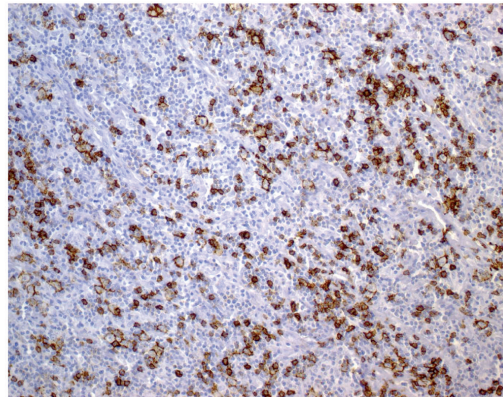


Fig. 10

Fig. 1. NLPHL: nodular pattern (HE). x 2.5

Fig. 2. NLPHL: nodular architecture highlighted by a B-cell marker (CD20). x 5

Fig. 3. NLPHL: nodular architecture highlighted by a follicular dendritic cell marker (CD21). x 5

Fig. 4. NLPHL: various appearance of L&H cells (HE). x 100

Fig. 5. NLPHL: CD20 positivity in L&H cells and background lymphocytes (note rings of small T-lymphocytes, negative for the B-cell marker, surrounding L&H cells). x 20

Fig. 6. NLPHL: L&H cells showing EMA immunoreactivity. x 20

Fig. 7. NLPHL: nuclear bcl6 immunoreactivity in L&H cell. x 20

Fig. 8. NLPHL: L&H nuclei are strongly immunostained with Oct2 antibody. x 20

Fig. 9. NLPHL: abundant CD57+ lymphocytes forming rosettes around L&H cells (inset). x 10

Fig. 10. NLPHL: immunostaining for CD20 in a diffuse area. There is a decrease in background small-B lymphocytes (compare to Fig. 2). x 10

Butler, 1984; Hansmann et al., 1990; Osborne et al., 1993).

Immunophenotype

The diagnosis of NLPHL should be confirmed by immunohistochemical studies. This is mandatory when the treatment is going to be different to that established for classical HL (Nicholas et al., 1990; Bishop et al., 1991; von Wasielewski et al., 1997b).

Neoplastic cells express leukocyte common antigen (CD45), J chains (Poppema, 1980; Stein et al., 1986) and other B-cell markers (CD20, CD79a, CDw75, CD74) and epithelial membrane antigen (EMA) (Figs. 5, 6) (Delsol et al., 1984; Pinkus and Said, 1985; Pinkus et al., 1985; Sherrod et al., 1986; Coles et al., 1988; Pinkus and Said, 1988) (Fig. 4). CD30 and CD15 are usually negative (Pinkus et al., 1985; Chittal et al., 1988; Pinkus and Said, 1988). It must be remembered that reactive lymphoid blasts may exhibit CD30 positivity; they can be distinguished from L&H cells by their morphology (no popcorn appearance). Only uncommonly L&H cells show weak expression of CD30 (Anagnostopoulos et al., 2000; Jaffe et al., 2001; Pileri et al., 2002). Latent Epstein-Barr (EBV) infection is not detected in L&H cells, either using immunohistochemical methods (latent membrane protein or LMP) or in situ hybridization (Epstein-Barr early RNA or EBER) (Weiss et al., 1991; Alkan et al., 1995).

Regarding studies of clonality in L&H cells by means of immunohistochemistry, the Isaacson group described immunoglobulin light chain restriction in most cases (Schmid et al., 1991). Immunoglobulin heavy chains may be also positive (Stein et al., 2001). Very recently, IgD positivity in L&H cells has been described in a subset of NLPHL (Prakash et al., 2006) clinically characterized by striking male predominance, younger median age and more frequent cervical lymph node involvement than IgD-negative cases. In addition, in IgD-positive cases L&H cells tend to involve the interfollicular region in a background relatively rich in T-cells.

The nuclear protein encoded by the *bcl-6* gene, associated with the normal development of the germinal centre, is expressed by the L&H cells (Fig. 7) (Falini et al., 1996, 1997; Kraus and Haley, 2000). On the contrary, *bcl-2* protein is negative (Algara et al., 1991; Alkan et al., 1995).

Other markers that have become recently of interest for the diagnosis of NLPHL –and on HL in general– are B-cell transcription factors. Four proteins have been further studied: octamer-binding transcription factor 2 (Oct2), B-cell Oct-binding protein 1 (BOB.1), B-cell specific activator (BSAP), and PU.1. Oct2 is an immunoglobulin transcription factor which, in conjunction with its coactivator BOB.1, triggers the promoter of immunoglobulin genes. Both proteins are regularly positive in NLPHL. Oct2 is particularly useful to identify L&H cells, since the strong nuclear positivity

of L&H cells, more intense than that of bystander B lymphocytes, highlights their presence and their nuclear atypia (Fig. 8) (Jaffe et al., 2001; Stein et al., 2001; Browne et al., 2003). BSAP, also termed PAX-5 (the protein encoded by the *Pax-5* gene), is also positive in L&H cells (Browne et al., 2003); PU.1 is more variable, but it is expressed in the majority of cases of NLPHL (more frequently in IgD-negative than in IgD-positive cases) (Torlakovic et al., 2001; Marafioti et al., 2004; Prakash et al., 2006).

The small lymphocytes present in the NLPHL nodules are mainly polyclonal B-lymphocytes with the characteristics of mantle zone lymphocytes (IgM+, IgD+). Intermingled with them, there are T lymphocytes ringed or forming a rosette around L&H cells. Like those of germinal centres, they are usually CD4+ CD57+ (Fig. 9) (Poppema, 1989; Kamel et al., 1993) and also coexpress *bcl-6* (Kraus and Haley, 2000). Nodules also have a prominent meshwork of follicular dendritic cells. Internodular regions show T lymphocyte predominance, as occurs in diffuse areas (Fig. 10), where the follicular dendritic cell meshwork disappears (Hansmann et al., 1991). Very recently, Fan et al. (2003) have documented six distinct immunoarchitectural patterns in NLPHL: "classic" (B-cell-rich) nodular, serpiginous/interconnected nodular, nodular with prominent extranodular L&H cells, T-cell-rich nodular, diffuse with a T-cell-rich background (T-cell-rich B-cell lymphoma [TCRBCL]-like), and a (diffuse) B-cell-rich pattern. They found the presence of a diffuse pattern (TCRBCL-like) to be associated with recurrent disease. There is also a tendency for progression to an increasingly more diffuse pattern over time (Fan et al., 2003; Abdulkader et al., 2005).

Antigen receptor genes

Single-cell PCR studies have demonstrated that L&H cells bear monoclonal rearrangement of immunoglobulin genes (Braeuninger et al., 1997; Marafioti et al., 1997; Ohno et al., 1997), but conventional PCR studies with whole tissue DNA more often give rise to a polyclonal pattern (Linden et al., 1988; Said et al., 1991; Angel et al., 1993; Pan et al., 1996; Manzanal et al., 1997).

Differential diagnosis

PTGC

This is a reactive lesion formed by large lymphoid nodules mainly composed of small mantle zone lymphocytes and scattered germinal centre cells. PTGC nodules are usually well limited and separated by typical reactive follicles. However, in NLPHL the lymph node architecture is generally not conserved and nodules are arranged in a tighter fashion, thus conferring them a certain degree of "molding". Irregular nodules, poorly circumscribed nodules with blurred diffuse areas can be

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observed. True L&H cells are only observed in LPHL. In PTGC large B cells are centroblasts, which may show some similarity with L&H cells, but are usually not surrounded by the frequent rings of bcl6+ and CD57+ T-lymphocytes typical of NLPHL (Burns et al., 1984; Kraus and Haley, 2000).

LR-CHL

As we will describe below, LR-CHL frequently exhibits a nodular pattern of growth, and sometimes a proportion of the neoplastic cells, immersed in a lymphocytic background, may resemble L&H cells. The difficulty of the differentiation from NLPHL and the clinical relevance of such a distinction make immunophenotypical studies necessary. Neoplastic cells exhibit the same immunohistochemical profile as in other subtypes of CHL. They usually express CD30, CD15, MUM1/IRF4 (see below) and, in about 50% of the cases, LMP-EBV. T-cell rosettes around the neoplastic cells are not in general CD57+ (Anagnostopoulos et al., 2000; Jaffe et al., 2001; Marafioti et al., 2004).

T-cell/histiocyte-rich B-cell lymphoma

This variety of large B-cell lymphoma may pose serious problems regarding the differential diagnosis with NLPHL, because the nodularity and the B-cell content may be partially or totally lost in some cases of NLPHL. TCHRBCL is a diffuse lymphoma formed by large atypical B cells –which may simulate L&H cells, HRS cells, centroblasts and immunoblasts– scattered within an abundant, reactive population formed by histiocytes and small T lymphocytes. In contrast to NLPHL, patients with TCHRBCL usually present with advanced stages and the disease has a bad prognosis. The immunophenotype of TCHRBCL and NLPHL is essentially the same for the neoplastic cells, as they typically express CD20, EMA and bcl-6. Nevertheless, there are some recently described differential findings: neoplastic cells of TCHRBCL are leukocyte-specific phosphoprotein (LSP1)-positive and PU.1 negative, whereas the L&H cells of NLPHL are mostly LSP1-negative, with variable PU.1 expression (Marafioti et al., 2003, 2004; Prakash et al., 2006). Fortunately, there are additional differences related to the background population. In TCHRBCL there are almost no small B-lymphocytes, and aggregates of follicular dendritic cells are not found. Usually, only scattered CD57+ cells are detected, they do not form rings around neoplastic cells and do not coexpress bcl6. Background T-lymphocytes are essentially CD8/TIA1-positive cells. The detection of monoclonal rearrangement of immunoglobulin genes by conventional PCR in whole tissue DNA is more in favour of a diagnosis of TCHRBCL, especially when a non-nested PCR is performed (Chittal et al., 1991; Delabie et al., 1992; Kamel et al., 1993; De Jong et al., 1996; McBride et al., 1996; Fleming et al., 1998;

Rüdiger et al., 1998; Kraus and Haley, 2000; Fraga et al., 2002; Boudova et al., 2003) (Table 1).

Classical HL

Clinical features

Classical HL approximately comprises 95% of HL cases. It shows a bimodal age distribution, with a peak at 15-35 years of age and a second peak in late life. In order of frequency, it usually involves cervical lymph nodes, followed by mediastinal, axillary and paraaortic regions. Primary extranodal involvement is rare. About half of the patients are low stage (I/II). Bone marrow involvement is uncommon (5%). Systemic symptoms are present in 25-40% of the patients (Jaffe et al., 2001; Pileri et al., 2002).

Classical HL includes NSHL, MCHL, LRCHL, and LDHL. At present, with the advent of modern therapy, the histological subtype has lost significance as prognostic factor, and stage and systemic symptoms are much more important in this respect (Josting et al., 2000).

Histological subtypes

NSHL is the most frequent form of HL, comprising 80% of cases. It was typically described at the clinical level in young women with mediastinal bulks. Nowadays, we know that – although it usually affects young people – it may appear at any age and may show peripheral lymphadenopathy and multiple locations. However, mediastinal supradiaphragmatic involvement

Table 1. Differential features between HL and TCHRBCL.

Diagnostic criteria	TCHRBCL	LPHL	CHL
CD45 expression	+	+	-
CD30 expression	-/w	-/w	+
CD15 expression	-	-	+/-
CD79a expression	+/-	+/-	-/+ Variable
CD20 expression	+	+	-/+ Variable
BOB.1 expression	+	+	-/w
Oct2 expression	+s	+s	-/w
EMA expression	+/-	+/-	Rare
PU.1 expression	-	+/-	-
PAX 5 expression	+	+	Weakly +
MUM1 expression	-/+	-/+	+
EBV	-	-	+ Variable
Ig gene rearrangement	monoclonal	polyclonal	polyclonal
Bcl-6+/CD57+ rosettes	-	+	-
Amount of TIA-1+ cells	Very high	Low	High
Amount of CD20+ small lymphocytes	Very low	High	Low
FDC	-	+	+
Stage	III / IV	I / II	I / III
Bone marrow involvement	+/-	-	-

FDC, follicular dendritic cells; W, Weakly positive in some instances; (s) strongly positive; See text for details and references.

is predominant. Most patients present with stage II disease (Colby et al., 1982; Jaffe et al., 2001; Pileri et al., 2002).

Histologically, NSHL is characterised by nodular growth pattern, collagen bands and lacunar cells (Fig. 11). The nodularity is usually obvious, but sometimes may be very focal. The fibrosis is typically made up of collagen bands poor in fibroblasts and has been classically described as birefractive under polarised light. It is usually accompanied by thickening of the capsule. Lacunar cells are characteristic of NSHL. They have an abundant, slightly eosinophilic cytoplasm that appears to be empty (lacuna) in formalin-fixed biopsies. They tend to have multilobated nuclei with smaller nucleoli than classical HRS cells, but considerable morphological variation exists. Classical HRS cells can also be found, but they are usually rare (Figs. 12, 13). The background is composed of lymphocytes, histiocytes, plasma cell, eosinophils, and neutrophils (Jaffe et al., 2001; Pileri et al., 2002).

Some morphological variations on this general picture have been underlined. The label "cellular phase" of nodular sclerosis has been applied to cases with lacunar cells and overt tendency to nodule formation but without collagen band deposition. Also, a "syncytial variant" has been described in which neoplastic cells form large aggregates that may contain necrotic foci. A more aggressive behaviour has been advocated for this variant, because of their frequent association with bulky disease and high stage (Colby et al., 1982; Strickler et al., 1986; Ben-Yehuda Salz et al., 1990).

The British National Lymphoma Investigation (BNLI) has developed a two-grade system for NSHL that is included in the WHO classification. Although not necessary for routine clinical purposes, its use is advisable in order to test its supposed -and controversial- prognostic value in larger series and protocol studies (Jaffe et al., 2001; Pileri et al., 2002). Briefly, grade 2 is characterised by increased numbers of HRS cells (sheets of cells filling a 40x hpf) in at least 25% of the nodules (Bennet et al., 1983; d'Amore et al., 1992; MacLennan et al., 1992; Ferry et al., 1993; Hess et al., 1994; van Spronsen et al., 1997).

MCHL is the second most frequent subtype of CHL cases (about 15%). Peripheral lymph nodes are frequently affected and mediastinal involvement is rare. There is a tendency to involve the spleen and abdominal lymph nodes. Patients often present with disease in stage III or IV (Jaffe et al., 2001).

Morphologically, there is a diffuse histological pattern. The HRS cells are the classical bi- or multinucleated cells with large nucleoli and perinuclear haloes. The cellular background is the same as in NS with the exception of the prominent fibrosis and the thickening of the capsule; nevertheless, some interstitial fibrosis may be seen (Jaffe et al., 2001). It is not rare to find a marked epithelioid reaction with granuloma-like clustering (Patsouris et al., 1989).

MCHL may focally involve the interfollicular areas of lymph nodes and be accompanied by follicular

hyperplasia or regressed germinal centres. This histological variant must be kept in mind to avoid overlooking or confusion with Castleman's disease (Doggett et al., 1983; Maheswaran et al., 1991). This interfollicular pattern occurs very rarely in large B cell lymphomas.

LR-CHL comprises about 4% of all HL cases. Its clinicopathological profile differs from the other subtypes of HL, although it is closer to that of NLPHL. It shows a higher prevalence in middle age males (over 50 years) and most patients present with stages I or II. There is a tendency to subdiaphragmatic nodal involvement and only rarely mediastinal involvement or bulky disease are present. After the treatment, the survival is similar to NLPHL. Late relapses are more common than in the other types of CHL and do not behave aggressively, although the prognosis is not as good as in NLPHL relapses (von Wasielewski et al., 1997a; Diehl et al., 1999; Anagnostopoulos et al., 2000).

The histological picture of LR-CHL consists of HRS cells -mainly classical or lacunar, although sometimes a proportion may resemble L&H cells- scattered in a background rich in small lymphocytes and devoid of neutrophils and eosinophils. The architecture may be diffuse, but most commonly is nodular; therefore, the differential diagnosis must be made mainly with NLPHL. The nodular variant of LR-CHL, formerly known as "follicular" HL (Ashton-key et al., 1995), contains follicles with regressed germinal centres and neoplastic cells within the mantle zones or at the junction with interfollicular regions (Anagnostopoulos et al., 2000).

LDHL is the less frequent form (1%). Immunohistochemical studies have probably made this form almost disappear because the majority of the cases diagnosed in the past were actually anaplastic or pleomorphic large cell lymphomas. Other cases may represent lymphocyte-depleted variants of NSHL (Jaffe et al., 2001). Histologically, LDHL is a diffuse form of CHL with a relative predominance of HRS cells to the background lymphocytes. Two patterns may be recognised. One resembles MCHL, but with more abundant HRS cells; sometimes the HRS cells exhibit a sarcomatous morphology. The other pattern is characterized by few HRS cells in background of diffuse fibrosis, with or without fibroblasts. It should be noted that when a focal nodular sclerosing pattern is present, the case should be diagnosed as NSHL (Jaffe et al., 2001). LDHL is more often diagnosed in HIV-seropositive patients and in developing countries. The median age of patients is not very different to the other subtypes if current criteria for diagnosis are applied. Patients show systemic symptoms, lymphadenopathy - more often abdominal- and hepatosplenomegaly, and there is usually bone marrow involvement at diagnosis. Accordingly, advanced stage disease is frequent (about 70%). This is the keypoint in relation to prognosis, since it has been shown that, with modern treatments, the prognosis of LDHL is comparable to the other subtypes of CHL in the same stage (Kant et al., 1986).

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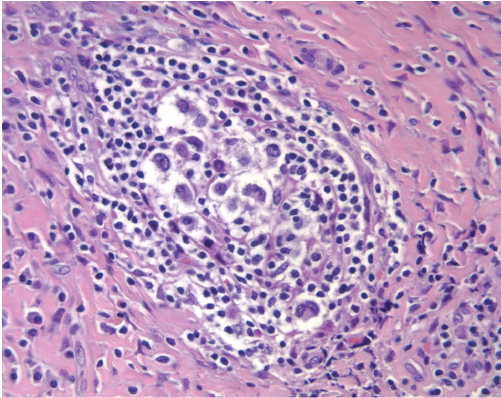


Fig. 11

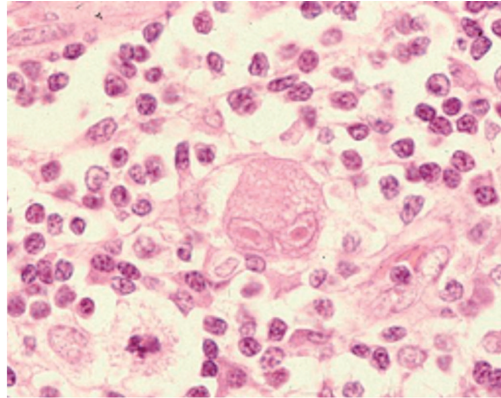


Fig. 12

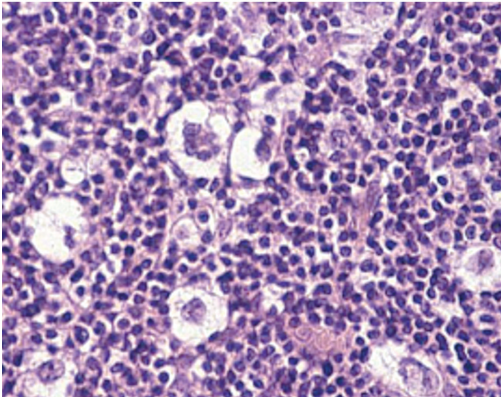


Fig. 13

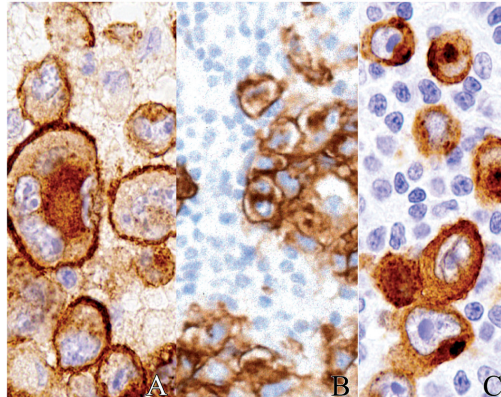


Fig. 14

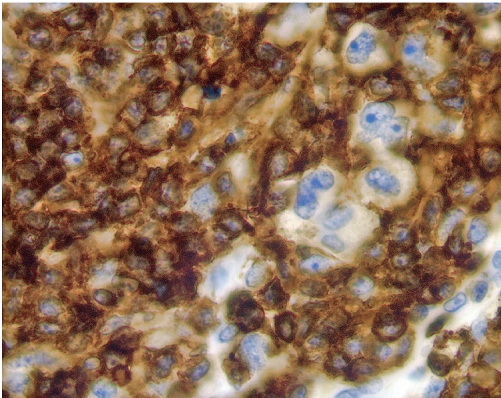


Fig. 15

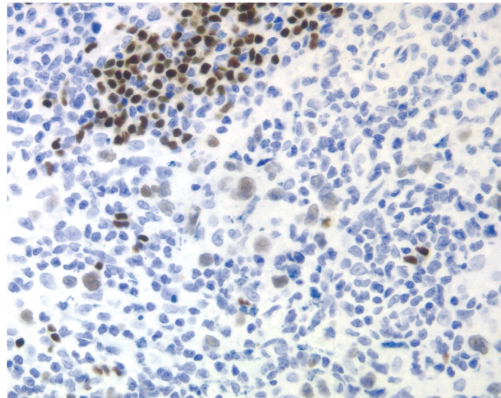


Fig. 16

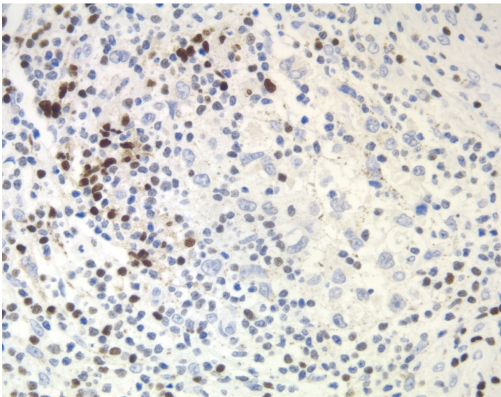


Fig. 17

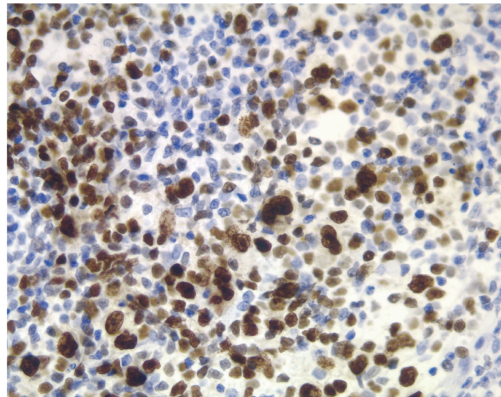


Fig. 18

Fig. 11. NSHL: small nodule, composed of lacunar cells and inflammatory background, surrounded by sclerosis, x 20

Fig. 12. CHL: diagnostic HRS cell (HE). x 100

Fig. 13. NSHL: lacunar cells (formalin-fixed material, HE). x 20

Fig. 14. CHL: A, B and C, HRS cells exhibiting immunoreactivity for CD30, CD15 and EBV (LMP1), respectively. x 100

Fig. 15. CHL: CD45 stains strongly background lymphocytes, whereas HRS cells are negative. The membranes of the neoplastic cells are not highlighted by immunostaining; this is more evident where the lymphocytic rimming is not complete. x 40

Fig. 16. CHL: BSAP/PAX5 is only weakly positive in the nuclei of some HRS cells; in contrast, the nuclei of the small B-lymphocytes are strongly positive. x 20

Fig. 17. CHL: Oct2 immunostaining displaying positivity in the background population, but not in the neoplastic cells. x 20

Fig. 18. CHL: HRS cells are positive for MUM1/IRF4. x 20

Immunophenotype

Immunophenotypically, HRS cells (lacunar or classical) express CD30 (Stein et al., 1985) and CD15 in membrane and Golgi (Fig. 14), and are negative for CD45 (Fig. 15). In approximately 30% of CHL cases, HRS cells express CD20, and more rarely, about 10% of cases, CD79a; these immunostainings are characteristically heterogeneous (von Wasielewski et al., 1997a; Jaffe et al., 2001; Tzankov et al., 2003). With respect to B-cell transcription factors, HRS cells are usually positive for BSAP/PAX-5, but Oct2 and/or BOB.1 expressions are typically absent or very weak (Figs. 16, 17). It must be underlined that BSAP/PAX-5 nuclear expression by HRS cells is weaker than in small B-lymphocytes; a strong expression in large neoplastic cells is more typical of B-cell lymphoma (Jaffe et al., 2001; Stein et al., 2001; Browne et al., 2003; Garcia-Cosio et al., 2004). Bcl-6 nuclear protein is variably expressed in HRS cells of about 40% of CHL cases (Carbone et al., 1998). MUM1/IRF4 (Multiple Myeloma-1/Interferon Regulatory Factor-4), a marker for late centrocytes and plasma cells, is positive in virtually all cases of CHL (Fig. 18), whereas it is negative or weakly expressed in a proportion of L&H cells in NLPHL (Falini et al., 2000; Carbone et al., 2002; Buettner et al., 2005).

In almost 50% of the CHL cases, HRS cells express the latent membrane protein (LMP-1), encoded by EBV, which may be useful for the differential diagnosis with non-Hodgkin lymphomas and NLPHL (Fig. 14). The frequency of EBV latent infection is higher in MCHL and LR-CHL than in NSHL. Most HIV+ cases are EBV infected (Desol et al., 1992; Brousset et al., 1993, 1994; Bellas et al., 1996).

The expression of T cell antigens in HRS cells detected by immunohistochemistry is rare, although there are some cases (Falini et al., 1987; Dallenbach and Stein, 1989). Single-cell PCR in these cases usually demonstrates monoclonal immunoglobulin gene rearrangement; monoclonal T-cell receptor gene rearrangement is exceptional (Seitz et al., 2000).

Antigen receptor genes

Single-cell PCR demonstrates monoclonal IgH gene rearrangement in more than 98% of the cases of CHL; only exceptional cases have been described bearing monoclonal T-cell receptor gene rearrangement (Küppers et al., 1994; Brauninger et al., 1999; Hummel et al., 1999; Küppers et al., 1999; Marafioti et al., 2000; Seitz et al., 2000). Nevertheless, as in NLPHL, conventional PCR studies with whole tissue DNA more often give rise to a polyclonal pattern (Manzanal et al., 1997; Jaffe et al., 2001).

Differential diagnosis

An important differential diagnosis of classical HL

is large B cell lymphoma, particularly TCHRBCL. It can be distinguished from classical HL by the immunophenotypic profile of the neoplastic cells: in TCHRBCL they are CD30-, CD15-, CD45+ and EMA+. The presence of a monoclonal IgH gene rearrangement by conventional PCR also favours TCHRBCL (Chittal et al., 1991; Delabie et al., 1992; Kamel et al., 1993; McBride et al., 1996; Fleming et al., 1998; Rüdiger et al., 1998; Kraus and Haley, 2000; Fraga et al., 2002; Boudová et al., 2003) (Table 1).

The primary mediastinal (thymic) large B cell lymphoma (PMLBCL) may pose differential diagnosis with classical HL because of the presence of HRS-like cells and a sclerotic background. Tumour cells of PMLBCL strongly express CD45, CD20, and CD79a. Not uncommonly, they are CD30 positive; however, they lack CD15 and EBV latent infection. Additional markers more in favour of PMLBCL are CD23 and MAL protein; the latter is expressed in about 75% of PMLBCL, but it can also be present in a minority of HL cases. In fact, there is a certain degree of overlap and some tumors –fortunately few– lie in a “gray zone” between both entities (Chadburn and Frizzera, 1999; Copie-Bergman et al., 1999; Traverse-Glehen et al., 2005; Garcia et al., 2005).

The LDHL may show a sarcomatoid appearance, but major difficulties may arise to differentiate it from anaplastic large cell lymphomas (ALCL) on morphological grounds. However, ALCL usually shows some evidence of cytotoxic/T- cell phenotype ALK expression, and monoclonally rearranged TCR genes; B-cell markers, CD15 and EBV are characteristics of CHL. Other cases of LDHL that show diffuse fibrosis can be, in fact, NSHL rich in tumor cells (Benharroch et al., 1998; Falini et al., 1998; Jaffe et al., 2001).

Diagnosis of extranodal involvement

Primary diagnosis of HL at extranodal locations requires the presence of diagnostic cells with typical phenotype in the proper inflammatory context. In the more common situation, that is, the diagnosis of bone marrow involvement for staging purposes in a patient with previous diagnosis of HL, it is enough to see mononuclear HL cells in an appropriate cellular background. Classically, the presence of atypical “histiocytes” or “reticulum cells” or the presence of necrosis or fibrosis with appropriate inflammatory cells has been regarded as suggestive, but not diagnostic, of HL (Lukes, 1971; Rappaport et al., 1971). However, most of these cases can now be interpreted as certain infiltration with the help of immunohistochemistry; if doubt persists, a contralateral biopsy is warranted (Franco et al., 2004).

Conclusion

The diagnosis of HL is still based on the presence of Reed-Sternberg cells in an appropriate cellular

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background. Accurate diagnosis and classification require immunohistochemical studies in many cases. NLPHL has a highly favourable prognosis compared to CHL, and therefore a different therapeutic approach can be applied, making the distinction between both types of HL essential. All classical HL subtypes are treated similarly, and chances for remission and survival are currently good.

New molecular and biological parameters should be added to the current classification, in order to enable the identification of patients who need a more aggressive therapy and who could benefit from new therapeutic targets.

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References

- Abbondanzo S.L. (2003). Thomas Hodgkin. *Ann. Diagn. Pathol.* 7, 333-334.
- Abdulkader I., Fraga M., González-Quintela A., Caparrini A., Bello J.L., Galvan C., Varo E. and Forteza J. (2005). Prolonged survival after liver transplantation for Hodgkin's disease-induced fulminant liver failure. *Hepatogastroenterology* 52, 217-219.
- Algara P., Martínez P., Sánchez L., Villuendas R., Orradre J.L., Oliva H. and Piris M.A. (1991). Lymphocyte predominance Hodgkin's disease (nodular paragranuloma). A Bcl-2 negative germinal centre lymphoma. *Histopathology*, 19, 69-75.
- Alkan S., Ross C.W., Hanson C.A. and Schnitzer B. (1995). Epstein-Barr virus and Bcl-2 protein overexpression are not detected in the neoplastic cells of nodular lymphocyte predominance Hodgkin's disease. *Mod. Pathol.* 8, 544-547.
- Anagnostopoulos I., Hansmann M.L., Franssila K., Harris M., Harris N.L., Jaffe E.S., Han J., van Krieken J.M., Poppema S., Marafioti T., Franklin J., Sextro M., Diehl V. and Stein H. (2000). European Task Force on Lymphoma project on lymphocyte predominance Hodgkin's disease: histologic and immunohistologic analysis of submitted cases reveals 2 types of Hodgkin's disease with a nodular growth pattern and abundant lymphocytes. *Blood* 96, 1889-1899.
- Angel C.A., Pringle J.H., Naylor J., West K.P. and Lauder I. (1993a). Analysis of antigen receptor genes in Hodgkin's disease. *J. Clin. Pathol.* 46, 337-340.
- Angel C.A., Pringle J.H., Primrose L. and Lauder I. (1993b). Detection of immunoglobulin heavy chain rearrangements in Hodgkin's disease using PCR. *J. Clin. Pathol.* 46, 940-942.
- Ashton-Key M., Thorpe P.A., Allen J.P. and Isaacson P.G. (1995). Follicular Hodgkin's disease. *Am. J. Surg. Pathol.* 19, 1294-1299.
- Bellas C., Santon A., Manzanal A., Campo E., Martín C., Acevedo A., Varona C., Forteza J., Morente M. and Montalban C. (1996). Pathological, immunological, and molecular features of Hodgkin's disease associated with HIV infection. Comparison with ordinary Hodgkin's disease. *Am. J. Surg. Pathol.* 20, 1520-1524.
- Benharroch D., Meguerian-Bedoyan Z., Lamant L., Amin C., Brugieres L., Terrier-Lacombe M.J., Haralambieva E., Pulford K., Pileri S., Morris S.W., Mason D.Y. and Delsol G. (1998). ALK-positive lymphoma: a single disease with a broad spectrum of morphology. *Blood* 91, 2076-2084.
- Bennett M.H., MacLennan K.A., Easterling M.J., Vaughan Hudson B., Jelliffe A.M. and Vaughan Hudson G. (1983). The prognostic significance of cellular subtypes in nodular sclerosing Hodgkin's disease: an analysis of 271 non-laparotomised cases (BNLI report no. 22). *Clin. Radiol.* 34, 497-501.
- Bennett M.H., MacLennan K.A., Vaughan Hudson G. and Vaughan Hudson B. (1991). Non-Hodgkin's lymphoma arising in patients treated for Hodgkin's disease in the BNLI: a 20-year experience. *Ann. Oncol.* 2 (Suppl 2), S83-S92.
- Ben-Yehuda-Salz D., Ben-Yehuda A., Polliack A., Ron N. and Okon E. (1990). Syncytial variant of nodular sclerosing Hodgkin's disease. A new clinicopathologic entity. *Cancer* 65, 1167-1172.
- Bishop P.W., Harris M., Smith A.P. and Elsam K.J. (1991). Immunophenotypic study of lymphocyte predominance Hodgkin's disease. *Histopathology* 18, 19-24.
- Bodis S., Kraus M.D., Pinkus G., Silver B., Kadin M.E., Canellos G.P., Shulman L.N., Tarbell N.J. and Mauch P.M. (1997). Clinical presentation and outcome in lymphocyte-predominant Hodgkin's disease. *J. Clin. Oncol.* 15, 3060-3066.
- Bonadonna G. (2000). Historical review of Hodgkin's disease. *Br. J. Haematol.* 110, 504-511.
- Boudova L., Torlakovic E., Delabie J., Reimer P., Pfistner B., Wiedenmann S., Diehl V., Müller-Hermelink H.K. and Rudiger T. (2003). Nodular lymphocyte-predominant Hodgkin lymphoma with nodules resembling T-cell/histiocyte-rich B-cell lymphoma: differential diagnosis between nodular lymphocyte-predominant Hodgkin lymphoma and T-cell/histiocyte-rich B-cell lymphoma. *Blood* 102, 3753-3758.
- Braeuninger A., Kuppers R., Strickler J., Wacker H., Rajewski K. and Hansmann M.L. (1997). Hodgkin and Reed-Sternberg cells in lymphocyte predominant Hodgkin's disease represent clonal populations of germinal center-derived tumor B-cells. *Proc. Natl. Acad. Sci. USA* 94, 9337-9342.
- Brauninger A., Hansmann M.L., Strickler J.G., Dummer R., Burg G., Rajewski K., Kuppers R. (1999). Identification of common germinal-center B-cell precursors in two patients with both Hodgkin's disease and non-Hodgkin's lymphoma. *N. Engl. J. Med.* 340, 1239-1247.
- Brousset P., Schlaifer D., Meggetto F., Bachmann E., Rothenberger S., Pris J., Delsol G. and Knecht H. (1994). Persistence of the same viral strain in early and late relapses of Epstein-Barr virus-associated Hodgkin's disease. *Blood* 84, 2447-2451.
- Brousset P., Vassallo J., Knecht H., Delsol G., Lamant L. and Odermatt B.F. (1993). Detection of Epstein-Barr Virus in Hodgkin's disease. *Appl. Immunohistochem.* 1, 213-219.
- Browne P., Petrosyan K., Hernandez A. and Chan J.A. (2003). The B-cell transcription factors BSAP, Oct2, and BOB.1 and the Pan-B-cell markers CD20, CD22, and CD79a are useful in the differential diagnosis of classic Hodgkin lymphoma. *Am. J. Clin. Pathol.* 120, 767-777.
- Buettner M., Greiner A., Avramidou A., Jäck H.M. and Niedobitek G. (2005). Evidence of abortive plasma cell differentiation in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma. *Hematol. Oncol.* 23, 127-132.
- Burns B.F., Colby T.V. and Dorfman R.F. (1984). Differential diagnostic features of nodular L & H Hodgkin's disease, including progressive transformation of germinal centers. *Am. J. Surg. Pathol.* 8, 253-261.
- Carbone A., Ghoghini A., Gaidano G., Franceschi S., Capello D., Drexler

Hodgkin's disease: histopathology and immunophenotype

- H.G., Falini B. and Dalla-Favera R. (1998). Expression status of BCL-6 and syndecan-1 identifies distinct histogenetic subtypes of Hodgkin's disease. *Blood* 92, 2220-2228.
- Carbone A., Gloghini A., Aldinucci D., Gattei V., Dalla-Favera R. and Gaidano G. (2002). Expression pattern of MUM1/IRF4 in the spectrum of pathology of Hodgkin's disease. *Br. J. Haematol.* 117, 366-372.
- Chadburn A. and Frizzera G. (1999). Mediastinal large B-cell lymphoma vs classic Hodgkin lymphoma. *Am. J. Clin. Pathol.* 112, 155-158.
- Chittal S.M., Caveriviere P., Schwarting R., Gerdes J., Al Saati T., Rigal-Huguet F., Stein H. and Delsol G. (1988). Monoclonal antibodies in the diagnosis of Hodgkin's disease. The search for a rational panel. *Am. J. Surg. Pathol.* 1, 274-285.
- Chittal S., Al Saati T. and Delsol G. (1997). Epithelial membrane antigen in hematology neoplasms. A review. *Appl. Immunohistochem.* 5, 203-215.
- Chittal S.M., Alard C., Rossi J.F., al Saati T., Le Tourneau A., Diebold J. and Delsol G. (1990). Further phenotypic evidence that nodular, lymphocyte-predominant Hodgkin's disease is a large B-cell lymphoma in evolution. *Am. J. Surg. Pathol.* 14, 1024-1035.
- Chittal S.M., Brousset P., Voigt J.J. and Delsol G. (1991). Large B-cell lymphoma rich in T-cells and simulating Hodgkin's disease. *Histopathology* 19, 211-220.
- Colby T.V., Hoppe R.T. and Warnke R.A. (1982). Hodgkin's disease: a clinicopathologic study of 659 cases. *Cancer* 49, 1848-1858.
- Coles F.B., Cartun R.W. and Pastuszak W.T. (1988). Hodgkin's disease, lymphocyte-predominant type: immunoreactivity with B-cell antibodies. *Mod Pathol.* 1, 274-285.
- Copie-Bergman C., Gaulard P., Maoche-Chretien L., Briere J., Haioun C., Alonso M.A., Romeo P.H. and Leroy K. (1999). The MAL gene is expressed in primary mediastinal large B-cell lymphoma. *Blood* 94, 3567-3575.
- Dallenbach F.E. and Stein H. (1989). Expression of T-cell-receptor beta chain in Reed-Sternberg cells. *Lancet* 2, 828-830.
- d'Amore E.S., Lee C.K., Aeppli D.M., Levitt S.H. and Frizzera G. (1992). Lack of prognostic value of histopathologic parameters in Hodgkin's disease, nodular sclerosis type. A study of 123 patients with limited stage disease who had undergone laparotomy and were treated with radiation therapy. *Arch. Pathol. Lab. Med.* 116, 856-861.
- De Jong D., Van Gorp J., Sie-Go D. and Van Heerde P. (1996). T-cell rich b-cell non-hodgkin's lymphoma: a progressed form of follicle centre cell lymphoma and lymphocyte predominance hodgkin's disease. *Histopathology* 28, 15-24.
- Delabie J., Vandenbergh E., Kennes C., Verhoef G., Foschini M.P., Stul M., Cassiman J.J. and De Wolf-Peeters C. (1992). Histiocyte-rich B-cell lymphoma. A distinct clinicopathologic entity possibly related to lymphocyte predominant Hodgkin's disease, paragranuloma subtype. *Am. J. Surg. Pathol.* 16, 37-48.
- Delsol G., Brousset P., Chittal S. and Rigal-Huguet F. (1992). Correlation of the expression of Epstein-Barr virus latent membrane protein and in situ hybridization with biotinylated BamHI-W probes in Hodgkin's disease. *Am. J. Pathol.* 140, 247-253.
- Delsol G., Gatter K.C., Stein H., Erber W.N., Pulford K.A., Zinne K. and Mason D.Y. (1984). Human lymphoid cells express epithelial membrane antigen. Implications for diagnosis of human neoplasms. *Lancet* 2, 1124-1129.
- Diehl V., Sextro M., Franklin J., Hansmann M.L., Harris N., Jaffe E., Poppema S., Harris M., Franssila K., van Krieken J., Marafioti T., Anagnostopoulos I. and Stein H. (1999). Clinical presentation, course, and prognostic factors in lymphocyte-predominant Hodgkin's disease and lymphocyte-rich classical Hodgkin's disease: report from the European Task Force on Lymphoma Project on Lymphocyte-Predominant Hodgkin's Disease. *J. Clin. Oncol.* 17, 776-783.
- Doggett R.S., Colby T.V. and Dorfman R.F. (1983). Interfollicular Hodgkin's disease. *Am. J. Surg. Pathol.* 7, 145-149.
- Emmerich F., Meiser M., Hummel M., Demel G., Foss H.D., Jundt F., Mathas S., Krappmann D., Scheiderei C., Stein H. and Dorken B. (1999). Overexpression of I kappa B alpha without inhibition of NF-kappaB activity and mutations in the I kappa B alpha gene in Reed-Sternberg cells. *Blood* 94, 3129-3134.
- Falini B., Stein H., Pileri S., Canino S., Farabbi R., Martelli M.F., Grignani F., Fagioli M. Minelli O. and Ciani C. (1987). Expression of lymphoid-associated antigens on Hodgkin's and Reed-Sternberg cells of Hodgkin's disease. An immunocytochemical study on lymph node cytopins using monoclonal antibodies. *Histopathology* 12, 1229-1242.
- Falini B., Bigerna B., Fizzotti M., Pulford K., Pileri S.A., Delsol G., Carbone A., Paulli M., Magrini U., Menestrina F., Giardini R., Pilotti S., Mezzelani A., Ugolini B., Billi M., Pucciarini A., Pacini R., Pellicci P.G. and Flenghi L. (1998). ALK expression defines a distinct group of T/null lymphomas ("ALK lymphomas") with a wide morphological spectrum. *Am. J. Pathol.* 153, 875-886.
- Falini B., Bigerna B., Pasqualucci L., Fizzotti M., Martelli M.F., Pileri S., Pinto A., Carbone A., Venturi S., Pacini R., Cattoretti G., Pescarmona E., Lo Coco F., Pellicci P.G., Anagnostopoulos I., Dalla-Favera R. and Flenghi L. (1996). Distinctive expression pattern of the BCL-6 protein in nodular lymphocyte predominance Hodgkin's disease. *Blood* 87, 465-471.
- Falini B., Fizzotti M., Pileri S., Liso A., Pasqualucci L. and Flenghi L. (1997). Bcl-6 protein expression in normal and neoplastic lymphoid tissues. *Ann. Oncol.* 8 (Suppl 2), 101-104.
- Falini B., Fizzotti M., Pucciarini A., Bigerna B., Marafioti T., Gambacorta M., Pacini R., Alunni C., Natali-Tanci L., Ugolini B., Sebastiani C., Cattoretti G., Pileri S., Dalla-Favera R. and Stein H. (2000). A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells. *Blood* 95, 2084-2092.
- Fan Z., Natkunam Y., Bair E., Tibshirani R. and Warnke R.A. (2003). Characterization of variant patterns of nodular lymphocyte predominant hodgkin lymphoma with immunohistologic and clinical correlation. *Am. J. Surg. Pathol.* 27, 1346-1356.
- Ferry J.A., Linggood R.M., Convery K.M., Efird J.T., Eliseo R. and Harris N.L. (1993). Hodgkin disease, nodular sclerosis type. Implications of histologic subclassification. *Cancer* 71, 457-463.
- Fleming M.D., Shahsafai A. and Dorfman D.M. (1998). Absence of dendritic reticulum cell staining is helpful for distinguishing T-cell-rich-B-cell lymphoma from lymphocyte predominance Hodgkin's disease. *Appl. Immunohistochem.* 6, 16-22.
- Fraga M., Garcia-Rivero A., Sánchez-Verde L., Forteza J. and Piris M.A. (2002). T-cell /histiocyte-rich large B-cell lymphoma is a disseminated aggressive neoplasm. Differential diagnosis with Hodgkin's lymphoma. *Histopathology* 41, 1-14.
- Franco V., Tripodo C., Rizzo A., Stella M. and Florena A.M. (2004). Bone marrow biopsy in Hodgkin's lymphoma. *Eur. J. Haematol.* 73, 149-155.
- Garcia J.F., Mollejo M., Fraga M., Forteza J., Muniesa J.A., Perez-Guillermo M., Perez-Seoane C., Rivera T., Ortega P., Piris M.A.

Hodgkin's disease: histopathology and immunophenotype

- (2005). Large B-cell lymphoma with Hodgkin's features. *Histopathology* 47, 101-110.
- Garcia-Cosio M., Santon A., Martin P., Camarasa N., Montalban C., Garcia J.F. and Bellas C. (2004). Analysis of transcription factor OCT.1, OCT.2 and BOB.1 expression using tissue arrays in classical Hodgkin's lymphoma. *Mod. Pathol.* 17, 1531-1538.
- Grossman D.M., Hanson C.A. and Schnitzer B. (1991). Simultaneous lymphocyte predominant Hodgkin's disease and large-cell lymphoma. *Am. J. Surg. Pathol.* 15, 668-676.
- Hansmann M.L., Zwingers T., Boske A., Loffler H. and Lennert K. (1984). Clinical features of nodular paraganuloma (Hodgkin's disease, lymphocyte predominance type, nodular). *J. Cancer Res. Clin. Oncol.* 108, 321-330.
- Hansmann M.L., Fellbaum C., Hui P.K. and Zwingers T. (1988). Correlation of content of B cells and Leu7-positive cells with subtype and stage in lymphocyte predominance type Hodgkin's disease. *J. Cancer Res. Clin. Oncol.* 119, 405-410.
- Hansmann M.L., Stein H., Fellbaum C., Hui P.K., Parwaresch M.R. and Lennert K. (1989). Nodular paraganuloma can transform into high-grade malignant lymphoma of B type. *Hum. Pathol.* 20, 1169-1175.
- Hansmann M.L., Fellbaum C., Hui P.K. and Moubayed P. (1990). Progressive transformation of germinal centers with and without association to Hodgkin's disease. *Am. J. Clin. Pathol.* 93, 219-226.
- Hansmann M.L., Stein H., Dallenbach F. and Fellbaum C. (1991). Diffuse lymphocyte-predominant Hodgkin's disease (diffuse paraganuloma). A variant of the B-cell-derived nodular type. *Am. J. Pathol.* 138, 29-36.
- Harris N.L., Jaffe E.S., Stein H., Banks P.M., Chan J.K., Cleary M.L., Delsol G., De Wolf- Peeters C., Falini B. and Gatter K.C. (1994). A revised european-american classification of lymphoid neoplasms: A proposal from the International lymphoma study group. *Blood* 5, 1361-1392.
- Hess J.L., Bodis S., Pinkus G., Silver B. and Mauch P. (1994). Histopathologic grading of nodular sclerosis Hodgkin's disease. Lack of prognostic significance in 254 surgically staged patients. *Cancer* 74, 708-714.
- Hummel M., Marafioti T. and Stein H. (1999). Clonality of Reed-Sternberg cells in Hodgkin's disease. *N. Engl. J. Med.* 340, 394-395.
- Jackson H. and Parker F. (1947). *Hodgkin's disease and allied disorders.* Oxford University Press. New York.
- Jaffe E., Lee Harris N., Stein H. and Vardiman J.W. (2001). *World Health Organization Classification of Tumours. Pathology and genetics of tumors of haematopoietic and lymphoid tissues.* IARC Press. Lyon. pp 238-253.
- Josting A., Wolf J. and Diehl V. (2000). Hodgkin disease: prognostic factors and treatment strategies. *Curr. Opin. Oncol.* 12, 403-411.
- Kamel O.W., Gelb A.B., Shibuya R.B. and Warnke R.A. (1993). Leu 7 (CD57) reactivity distinguishes nodular lymphocyte predominance Hodgkin's disease from nodular sclerosing Hodgkin's disease, T-cell-rich B-cell lymphoma and follicular lymphoma. *Am. J. Pathol.* 142, 541-546.
- Kant J.A., Hubbard S.M., Longo D.L., Simon R.M., DeVita V.T. and Jaffe E.S. (1986). The pathologic and clinical heterogeneity of lymphocyte-depleted Hodgkin's disease. *J. Clin. Oncol.* 4, 284-294.
- Karayalcin G., Behm F.G., Gieser P.W., Kung F., Weiner M., Tebbi C.K., Ferree C., Marcus R., Constine L., Mendenhall N.P., Chauvenet A. and Murphy S.B. (1997). Lymphocyte predominant Hodgkin's disease: clinico-pathologic features and results of treatment. The Pediatric Oncology Group experience. *Med. Pediat. Oncol.* 29, 519-525.
- Kraus M.D. and Haley J. (2000). Lymphocyte predominance Hodgkin's disease: the use of bcl-6 and CD57 in diagnosis and differential diagnosis. *Am. J. Surg. Pathol.* 24, 1068-1078.
- Kuppers R., Klein U., Hansmann M.L. and Rajewsky K. (1999). Cellular origin of human B-cell lymphomas. *N. Engl. J. Med.* 341, 1520-1529.
- Küppers R., Rajewsky K., Zhao M., Simons G., Laumann R., Fisher R. and Hansmann M.L. (1994). Hodgkin and Reed-Sternberg cell picked from histological sections show clonal immunoglobulin rearrangements and appear to be derived from B cells at various stages of development. *Proc. Natl. Acad. Sci. USA* 91, 10962-10966.
- Linden M.D., Fishleder A.J., Katzin W.E. and Tubbs R.R. (1988). Absence of B-cell or T-cell clonal expansion in nodular, lymphocyte predominant Hodgkin's disease. *Hum. Pathol.* 19, 591-594.
- Lukes R.J. (1971). Criteria for involvement of lymph node, bone marrow, spleen and liver in Hodgkin's disease. *Cancer Res.* 31, 1755-1767.
- Lukes R.J. and Butler J.J. (1966). The pathology and nomenclature of Hodgkin's disease. *Cancer Res.* 26, 1063-1081.
- MacLennan K.A., Bennett M.H., Vaughan Hudson B. and Vaughan Hudson G. (1992). Diagnosis and grading of nodular sclerosing Hodgkin's disease: a study of 2190 patients. *Int. Rev. Exp. Pathol.* 33, 27-51.
- Maheswaran P.R., Ramsay A.D., Norton A.J. and Roche W.R. (1991). Hodgkin's disease presenting with the histological features of Castleman's disease. *Histopathology* 18, 249-253.
- Manzanal A.I., Santon A., Acevedo A., Aguilera B., Oliva H. and Bellas C. (1997). Molecular analysis of the IgH gene in 212 cases of Hodgkin's disease. Correlation of IgH clonality with the histologic and the immunohistochemical features. *Mod. Pathol.* 10, 679-685.
- Marafioti T., Hummel M., Anagnostopoulos I., Foss H.D., Falini B., Delsol G., Isaacson P.G., Pileri S. and Stein H. (1997). Origin of nodular lymphocyte-predominant Hodgkin's disease from a clonal expansion of highly mutated germinal-center B cells. *N. Engl. J. Med.* 337, 453-458.
- Marafioti T., Hummel M., Foss H.D., Laumen H., Korbjuhn P., Anagnostopoulos I., Lammert H., Demel G., Theil J., Wirth T. and Stein H. (2000). Hodgkin and reed-sternberg cells represent an expansion of a single clone originating from a germinal center B-cell with functional immunoglobulin gene rearrangements but defective immunoglobulin transcription. *Blood* 95, 1443-1450.
- Marafioti T., Jabri L., Pulfor K., Brousset P., Mason D.Y. and Delsol G. (2003). Leucocyte-specific protein (LSP1) in malignant lymphoma and Hodgkin's disease. *Br. J. Haematol.* 120, 671-678.
- Marafioti T., Mancini C., Ascani S., Sabattini E., Zinzani P.L., Pozzobon M., Pulford K., Falini B., Jaffe E.S., Müller-Hermelink H.-K., Mason D.Y. and Pileri S.A. (2004). Leukocyte-specific phosphoprotein-1 and PU.1: two useful markers for distinguishing T-cell-rich lymphoma from lymphocyte-predominant Hodgkin's disease. *Haematologica* 89, 957-964.
- Mason Y.D., Banks P.M., Chan J., Cleary M.L., Delsol G., de Wolf Peeters C., Falini B., Gatter K., Grogan T.M., Harris N.L., Isaacson P.G., Jaffe E.S., Knowles D.M., Müller-Hermelink H.K., Pileri S., Raffkiaer E., Stein H. and Warnke R. (1994). Nodular lymphocyte predominance Hodgkin's disease. A distinct clinicopathological entity. *Am. J. Surg. Pathol.* 18, 526-530.
- McBride J.A., Rodríguez J., Luthra R., Ordóñez N.G., Cabanillas F. and Pugh W.C. (1996). T-cell-rich B large-cell lymphoma simulating lymphocyte-rich Hodgkin's disease. *Am. J. Surg. Pathol.* 20, 193-

- 201.
- Nicholas D.S., Harris S. and Wright D.H. (1990). Lymphocyte predominance Hodgkin's disease. An immunohistochemical study. *Histopathology* 16, 157-165.
- Ohno T., Stribley J.A., Wu G., Hinrichs S.H., Weisenburger D.D. and Chan W.C. (1997). Clonality in nodular lymphocyte-predominant Hodgkin's disease. *N. Engl. J. Med.* 337, 459-465.
- Osborne B.M. and Butler J.J. (1984). Clinical implications of progressive transformation of germinal centers. *Am. J. Surg. Pathol.* 8, 725-733.
- Osborne B.M., Butler J.J. and Gresik M.V. (1993). Progressive transformation of germinal centers: comparison of 23 pediatric patients to the adult population. *Mod. Pathol.* 5, 135-140.
- Pan L.X., Diss T.C., Peng H.Z., Norton A.J. and Isaacson P.G. (1996). Nodular lymphocyte predominance Hodgkin's disease: a monoclonal or polyclonal B-cell disorder? *Blood* 87, 2428-2434.
- Patsouris E., Noel H. and Lennert K. (1989). Cytohistologic and immunohistochemical findings in Hodgkin's disease, mixed cellularity type, with a high content of epithelioid cells. *Am. J. Surg. Pathol.* 13, 1014-1022.
- Pinkus G. and Said J. (1985). Hodgkin's disease, lymphocyte predominance type, nodular--a distinct entity? Unique staining profile for L&H variants of Reed-Sternberg cells defined by monoclonal antibodies to leukocyte common antigen, granulocyte-specific antigen, and B-cell-specific antigen. *Am. J. Pathol.* 116, 1-6.
- Pinkus G. and Said J. (1988). Hodgkin's disease, lymphocyte predominance type, nodular--further evidence for a B cell derivation. L & H variants of Reed-Sternberg cells express L26, a pan B cell marker. *Am. J. Pathol.* 133, 211-217.
- Pinkus G.S., Thomas P. and Said J.W. (1985). Leu-M1--a marker for Reed-Sternberg cells in Hodgkin's disease. An immunoperoxidase study of paraffin-embedded tissues. *Am. J. Pathol.* 119,
- Pileri S.A., Ascani S., Leoncini L., Sabattini E., Zinzani P.L., Piccaluga P.P., Pileri A. Jr., Giunti M., Falini B., Bolis G.B. and Stein H. (2002). Hodgkin's lymphoma: the pathologist's viewpoint. *J. Clin. Pathol.* 55, 162-176.
- Poppema S. (1980). The diversity of the immunohistological staining pattern of Sternberg-Reed cells. *J. Histochem. Cytochem.* 20, 788-791.
- Poppema S. (1989). The nature of the lymphocytes surrounding Reed-Sternberg cells in nodular lymphocyte predominance and in other types of Hodgkin's disease. *Am. J. Pathol.* 135, 351-357.
- Poppema S. (1996). Nodular lymphocyte predominance type of Hodgkin's disease. In: *Pathology of lymph nodes*. Weiss L.M. Churchill-Livingstone Press. New York. pp 215-228.
- Poppema S., Kaiserling E. and Lennert K. (1979a). Epidemiology of nodular paraganuloma (Hodgkin's disease with lymphocytic predominance, nodular). *J. Cancer. Res. Clin. Oncol.* 95, 57-63.
- Poppema S., Kaiserling E. and Lennert K. (1979b). Hodgkin's disease with lymphocytic predominance, nodular type (nodular paraganuloma) and progressively transformed germinal centres. A cytohistological study. *Histopathology* 3, 295-308.
- Poppema S., Kaiserling E. and Lennert K. (1979c). Nodular paraganuloma and progressively transformed germinal centers. Ultrastructural and immunohistologic findings. *Virchows Arch.* 31, 211-225.
- Prakash S., Fountaine T., Raffeld M., Jaffe E.S. and Pittaluga S. (2006). IgD positive L&H cells identify a unique subset of nodular lymphocyte predominant Hodgkin lymphoma. *Am. J. Surg. Pathol.* 30, 585-592.
- Rappaport H., Berard C.W., Butler J.J., Dorfman R.F., Lukes R.J. and Thomas L.B. (1971). Report on the committee on histopathological criteria contributing to staging in Hodgkin's disease. *Cancer Res.* 31, 1864-1865.
- Regula D.P., Hoppe R.T. and Weiss L.M. (1988). Nodular and diffuse types of lymphocyte predominance Hodgkin's disease. *N. Engl. J. Med.* 318, 214-219.
- Rüdiger T., Ott G., Ott M.M., Müller-Deubert S.M. and Müller-Hermelink H.K. (1998). Differential diagnosis between classic Hodgkin's lymphoma, T-cell-rich B-cell lymphoma and paraganuloma by paraffin immunohistochemistry. *Am. J. Surg. Pathol.* 22, 1184-1191.
- Said H., Sasoon A., Shintaku I., Kurtin P. and Pinkus G. (1991). Absence of bcl-2 major breakpoint region and Jh gene rearrangement in lymphocyte predominant Hodgkin's disease. Results of Southern blot analysis and polymerase chain reaction study. *Am. J. Pathol.* 138, 261-264.
- Schmid C., Sargent C. and Isaacson P. (1991). L and H cells of nodular lymphocyte predominant Hodgkin's disease show immunoglobulin light-chain restriction. *Am. J. Pathol.* 139, 1281-1289.
- Seitz V., Hummel M., Marafioti T., Anagnostopoulos I., Assaf C. and Stein H. (2000). Detection of clonal T-cell receptor gamma-chain gene rearrangements in Reed-Sternberg cells of classic Hodgkin disease. *Blood* 95, 3020-3024.
- Sherrod A.E., Felder B., Levy N., Epstein A., Marder R., Lukes R.J. and Taylor C.R. (1986). Immunohistologic identification of phenotypic antigens associated with Hodgkin and Reed-Sternberg cells. *Cancer.* 57, 2135-2140.
- Stein H., Hansmann M.L., Lennert K., Brandtzaeg P., Gatter K.C. and Mason D.Y. (1986). Reed-Sternberg and Hodgkin cells in lymphocyte-predominant Hodgkin's disease of nodular subtype contain J chain. *Am. J. Clin. Pathol.* 86, 292-297.
- Stein H., Mason D.Y., Gerdes J., O'Connor N., Wainscoat J., Pallesen G., Gatter K., Falini B., Delsol G., Lemke H., Schwarting R. and Lennert K. (1985). The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. *Blood* 66, 848-858.
- Stein H., Marafioti T., Foss H.D., Laumen H., Hummel M., Anagnostopoulos I., Wirth T., Demel G. and Falini B. (2001). Down-regulation of BOB.1/OBF.1 and Oct2 in classical Hodgkin disease but not in lymphocyte predominant Hodgkin disease correlates with immunoglobulin transcription. *Blood* 97, 496-501.
- Strickler J.G., Michie S.A., Warnke R.A. and Dorfman R.F. (1986). The "syncytial variant" of nodular sclerosing Hodgkin's disease. *Am. J. Surg. Pathol.* 10, 470-477.
- Sundeen J.T., Cossman J. and Jaffe E.S. (1988). Lymphocyte predominant Hodgkin's disease nodular subtype with coexistent "large cell lymphoma". Histological progression or composite malignancy? *Am. J. Surg. Pathol.* 12, 599-606.
- Torlakovic E., Tierens A., Dang H.D. and Delabie J. (2001). The transcription factor PU.1, necessary for B-cell development is expressed in lymphocyte predominance, but not classical Hodgkin's disease. *Am. J. Pathol.* 159, 1807-1814.
- Traverse-Glehen A., Pittaluga S., Gaulard P., Sorbara L., Alonso M.A., Raffeld M. and Jaffe E.S. (2005). Mediastinal gray zone lymphoma: the missing link between classic Hodgkin's lymphoma and mediastinal large B-cell lymphoma. *Am. J. Surg. Pathol.* 29, 1411-1421.

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- Tzankov A., Zimpfer A., Pehrs A.C., Lugli A., Went P., Maurer R., Pileri S. and Dirhofer S. (2003). Expression of B-cell markers in classical Hodgkin lymphoma: a tissue microarray analysis of 330 cases. *Mod. Pathol.* 16, 1141-1147.
- van Spronsen D.J., Vrints L.W., Hofstra G., Crommelin M.A., Coebergh J.W. and Breed W.P. (1997). Disappearance of prognostic significance of histopathological grading of nodular sclerosing Hodgkin's disease for unselected patients, 1972-92. *Br. J. Haematol.* 96, 322-327.
- von Wasielewski R., Mengel M., Fischer R., Hansmann M.L., Hubner K., Franklin J., Tesch H., Paulus U., Werner M., Diehl V. and Georgii A. (1997a). Classical Hodgkin's disease. Clinical impact of the immunophenotype. *Am. J. Pathol.* 151, 1123-1130.
- von Wasielewski R., Werner M., Fischer R., Hansmann M.L., Hubner K., Hasenclever D., Franklin J., Sextro M., Diehl V. and Georgii A. (1997b). Lymphocyte-predominant Hodgkin's disease. An immunohistochemical analysis of 208 reviewed Hodgkin's disease cases from the German Hodgkin Study Group. *Am. J. Pathol.* 150, 793-803.
- Weiss L., Chen Y., Liu X. and Shibata D. (1991). Epstein-Barr virus and Hodgkin's disease: a correlative in situ hybridization and polymerase chain reaction study. *Am. J. Pathol.* 139, 1259-1265.
- Wickert R.S., Weisenburger D.D., Tierens A., Greiner T.C. and Chan W.C. (1995). Clonal relationship between lymphocytic predominance Hodgkin's disease and concurrent or subsequent large-cell lymphoma of B lineage. *Blood* 86, 2312-2320.

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