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*Case report*

# Nodular sclerosing Hodgkin's disease and large cell lymphoma. Immunophenotypic characterization of a composite case

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**Summary.** Composite lymphomas have rarely been reported in Hodgkin's disease (HD), except in the lymphocyte predominance sub-type, and immunohistochemical investigations have been performed in only few cases. We describe the histological and immunophenotypical findings in a case of composite nodular sclerosing HD and high-grade, large cell non-Hodgkin's lymphoma (NHL). In our case HD and NHL cells displayed striking morphological and immunophenotypical divergence, suggesting a lack of correlation between the two neoplasms.

**Key words:** Composite lymphoma – Hodgkin's disease – Non-Hodgkin's lymphoma – Immunohistochemistry

## Introduction

The concept of "composite lymphoma" was first proposed by Custer (1954) to describe the occurrence of two divergent histological types of lymphoma within the same patient. Later this term was strictly applied to patients with two different types of malignant lymphoma within the same site (Kim and Dorfman 1974), while the term "discordant lymphoma" was used to describe the occurrence of different histological types of lymphoma at different anatomical sites.

A variable percentage (2–5%) of patients with Hodgkin's disease (HD) develop non-Hodgkin's lymphomas (NHL), mainly large cell lymphoma, although some low-grade NHLs have been described (Jacquillat et al. 1984). In several cases patients received chemotherapy for HD before the emergence of the second malignancy (Casey et al. 1990). Compositive lymphomas have been rarely reported in HD subtypes, except lymphocyte predominance (LP), and immunohistochemical investigations have been performed in only a few cases (Hansmann et al. 1989; Gonzales et al. 1991). We describe the histological and immunophenotypical findings in a case of composite nodular sclerosing HD (NS-HD) and high-grade, large-cell NHL.

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## Case report

A 37-year-old man presented in October 1989 with a 4-week history of intermittent fever and malaise. Physical examination revealed enlarged lymph nodes in the right supraclavicular fossa, but no hepatosplenomegaly. Haematological findings were: Hb 11.5 g/dl, WBC $5.3 \times 10^9$/l with 78% neutrophils, 5% eosinophils, 12% lymphocytes and 5% monocytes, platelets $234 \times 10^9$/l. The erythrocyte sedimentation rate was 94 mm/h. Urine was normal. Serum albumin was low and alpha-2 globulin elevated. Liver function tests were normal; LDH and copper levels were normal; computed tomography showed enlargement of mediastinal and abdominal lymph nodes. A bone marrow biopsy showed involvement by HD. Histological examination of the supraclavicular lymph node demonstrated NS-HD with a distinct area of monomorphous large cell NHL proliferation (Fig. 1). The patient was treated with eight cycles of pro-MACE-CytaBOM with complete remission. Re-staging in January 1990 showed no relapse and at the last follow-up (September 1991) the patient was alive without disease.

## Materials and methods

Only formalin-fixed, paraffin-embedded material was available for study. 3 μm thick paraffin sections were stained with haematoxylin and eosin (H&E), Giemsa, periodic acid-Schiff (PAS) and Gomori silver impregnation for reticulin fibres. Immunohistochemistry for a range of monoclonal antibodies and antisera (Table 1) were performed using either the streptavidin-peroxidase conjugate method (Shi et al. 1988), or the alkaline phosphatase anti-alkaline phosphatase technique (Cordell et al. 1984). Enzyme digestion was required prior to staining with Ber H2 (0.1% pronase in PBS pH 7.4, 15 min, 37° C) and MAC 387 (0.1% trypsin in 0.1% calcium chloride, pH 7.8, 15 min, 37° C). Positive controls for immunohistochemistry were provided by normal tonsils. Immunoreactivity for Ber H2 was also tested on sections of cases of NS and mixed cellularity HD. Negative controls were obtained by substituting the primary antibodies with normal mouse serum.

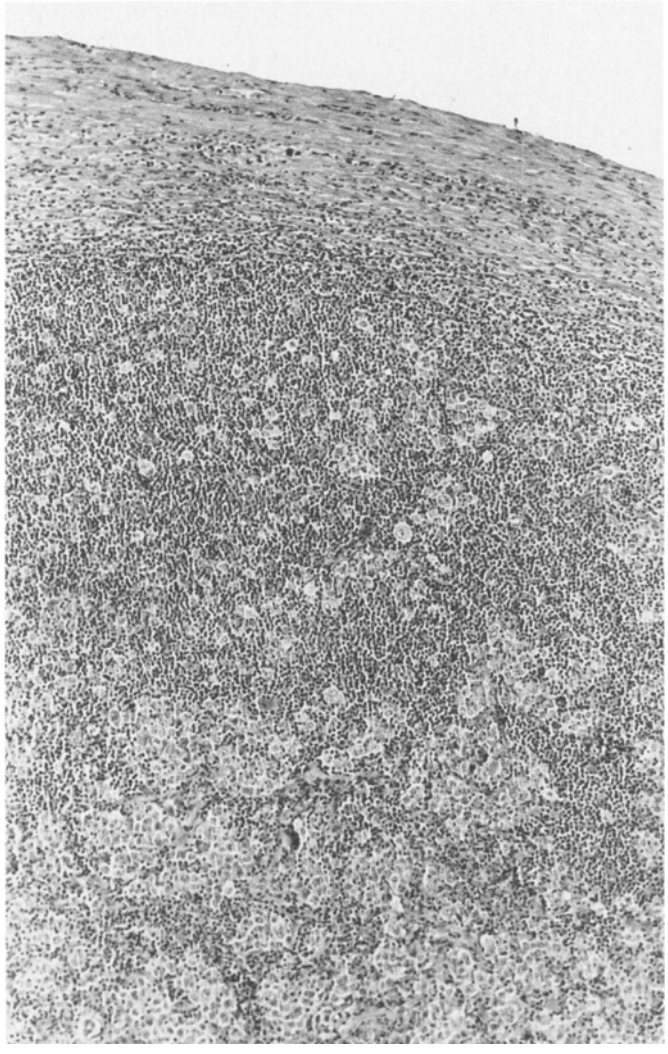

**Fig. 1.** Lymph node with an area of diffuse large cell non-Hodgkin's lymphoma (NHL), abutting on nodular sclerosing Hodgkin's disease (HD) proliferation. H&E, ×40

## Results

The histological examination of the supraclavicular lymph node showed a thickened capsule and a diffuse effacement of the normal structure by several nodules of abnormal lymphoid tissue, circumscribed by sclerotic, birefringent tissue. The nodules contained typical scattered lacunar cells, rare Reed Sternberg (RS) and Hodgkin's cells (H), admixed with small lmyphocytes and eosinophils (Fig. 2). The same section presented a distinct focal area of proliferation, composed of monomorphic large NHL cells with intermingled reactive cells (Fig. 2). These large elements showed mild to abundant pale cytoplasm, round to oval nuclei, with centrally distinct single or multiple nucleoli. Mitotic figures were frequent. A diagnosis of composite NS-HD and NHL was made.

Table 1 shows the immunohistochemical findings of HD and NHL cells. The various markers characteristic of RS cells, such as CD 15 (Tü 9) (Fig. 3), CD 30 (Ber H2), HLA-DR, HLA-DR invariant chain (CD 74), were strongly expressed on the majority of the RS and H cells. A diffuse cytoplasmic staining for the epithelial membrane antigen (EMA) was observed in scattered RS and H cells, while no positivity was evident for the leucocyte common antigen (LCA, CD 45) and for the pan B and pan T employed. In contrast, NHL cells were CD 15, CD 30, and EMA negative, expressed LCA (CD 45), L 26 (CD 20), MB 2 and LN 1 (CDw 75), displaying a B-cell related phenotype (Figs. 3, 4). Only scattered neoplastic B cells showed cytoplasmic positivity for LN 2 (CD 74). The pan-T markers Beta F1, CD 3, MT 1 (CD 43) and UCHL 1/A6 (CD 45 RO) (Berti et al. 1991), were negative on both HD and NHL cells and were only confined to some small lymphocytes intermingled among HD cells.

## Discussion

We have analysed the case of a patient with NS-HD, presenting within the same lymph node a distinct focal area of proliferation composed of large monomorphous NHL cells. On paraffin sections, typical RS and lacunar cells expressed the CD 15 and the CD 30 antigens, displayed HLA-DR and the HLA-DR associated invariant chain (CD 74), but lacked LCA (CD 45) immunoreactivity. Only few H and RS cells showed a diffuse cytoplasmic staining for EMA. The large monomorphous NHL cells reacted with the antibodies LCA (CD 45) (more than 60% of cells), L 26 (CD 20), MB 2 and LM 1 (CDw 75) (see Table 1), showing a B-cell-related phenotype, and no expression of the antigens usually expressed by RS cells. Therapy was dictated by the more aggressive component of the lymphoma and the patient was treated with eight cycles of pro-MACE-CytaBOM, with good response to therapy, no relapse and complete remission 2 years after the diagnosis. Even if the problem concerning the origin and identity of the tumour cells in HD is unresolved, the most recent immunophenotypic and gene rearrangement studies suggest that H and RS cells may represent a subset, or a differentiation stage, of highly activated lymphoid cells (Stein 1987). Poppema et al. (1985) were the first to demonstrate by immunohistochemical means the possible evolution of large cell immunoblastic lymphoma from nodular paragranuloma, providing strong evidence for a biological relationship between LP-HD and large B-cell NHL. Nevertheless, morphological distinction between HD and NHL is still extremely difficult. This problem is particularly emphasized in NS-HD with aggressive histology (grade II NS; MacLennan et al. 1989), in the syncytial variant of HD (Strickler et al. 1986), presenting cohesive sheets of atypical cells and in the CD 30-positive anaplastic large cell lymphoma (ALC-L) with interstitial or band fibrosis and nodular appearance (Stein and Mason 1986). Casey et al. (1990) described a series of monomorphous lymphoma which arose in patients with HD, NS type, while we recently reported five cases of CD 30-positive ALC-L expressing the CD 15 antigen, suggesting that a progression may also occur between HD and

**Table 1.** Immunohistochemical findings

| Antibody/ source | CD/distribution | HD cells | NHL cells |
|---|---|---|---|
| LCA/D | CD 45; pan leucocyte | − | +/− |
| L 26/D | CD 20; pan B cells | − | + |
| MB 2/B | 28 kDa molecule on B cells | − | + |
| MB 1/B | CD 45 RA; pan B cells | − | − |
| LN 1/B | CDw 75; follicular centre cells and erythroid cells | − | + |
| MT 2/B | CD 45 R; B and memory T cells | − | − |
| Kappa/D | Kappa light chain | − | − |
| Lambda/D | Lambda light chain | − | − |
| Beta F 1/TCS | Beta chain of T-cell receptor | − | − |
| CD 3/D | CD 3; pan T cells | | |
| MT 1/B | CD 43; T cells, myeloid cells, monocytes and B-cell subsets | − | − |
| UCHL 1/D | CD 45 RO; T cells, myeloid cells, monocytes | − | − |
| A6/Dr. Aversa, Standsford Un. | CD 45 RO; T cells, myeloid cells monocytes | − − | − − |
| Ber H2/D | CD 30; activated T and B cells, Reed-Sternberg cells | + | − |
| LN 2/B | CD 74; HLA-DR invariant chain | + | − |
| LN 3/B | HLA-DR | + | − |
| EMA/D | Epithelial membrane antigen | +/−§ | − |
| TÜ9/B | CD 15; Hapten X | + | − |
| MAC 387/D | 12–26 kDa antigen on granulocytes and macrophages | − | − |
| LN 5/B | Macrophages and B-cell subset | − | − |
| KP 1/D | CD 68; macrophages | − | − |
| Lysozyme/D | Macrophages and myeloid cells | − | − |
| PCNA/D | Human proliferating cell nuclear antigen | − | +/− |

B, Biotest; D, Dako; TCS, T Cell Sciences; +, most/all cells positive; +/−, some cells positive; §, diffuse cytoplasmic staining; −, negative

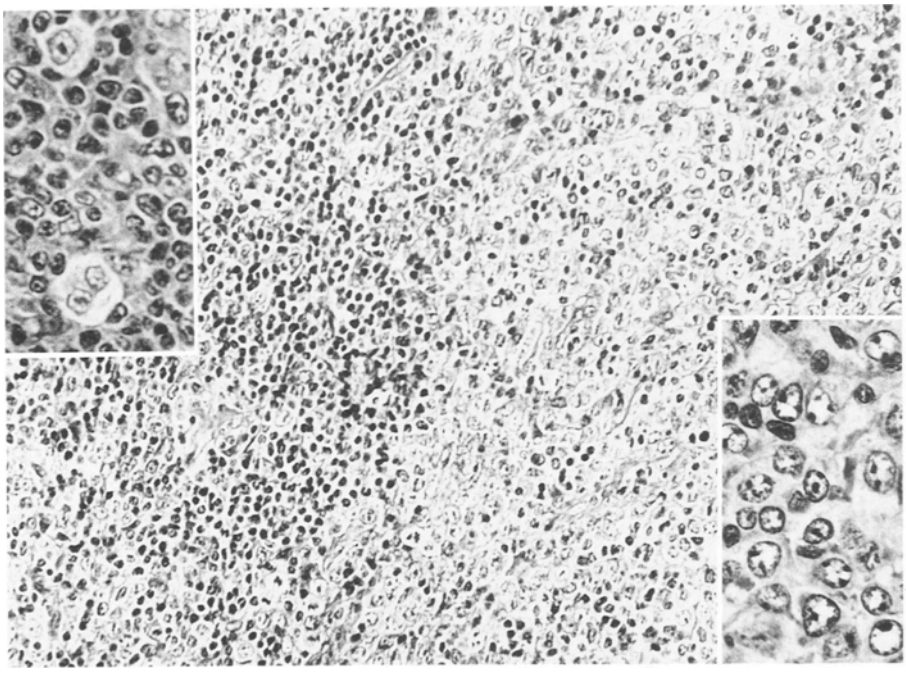

**Fig. 2.** Border area between HD and NHL. *Upper inset* with Hodgkin and RS cells surrounded by small lymphocytes. *Lower inset* with the monomorphous proliferation of large NHL cells. H&E, ×63; *insets:* H&E, ×250

CD 30-positive ALC-L (Rosso et al. 1990). At present, it is not clear whether large cell lymphoma arising in HD represents another neoplasm, or may be considered a transformation of HD to higher-grade neoplasm. The first possibility is supported by the morphological and phenotypic heterogeneity of this second growth. Confirmation of the second hypothesis requires the development of a specific marker for the neoplastic cells of HD,

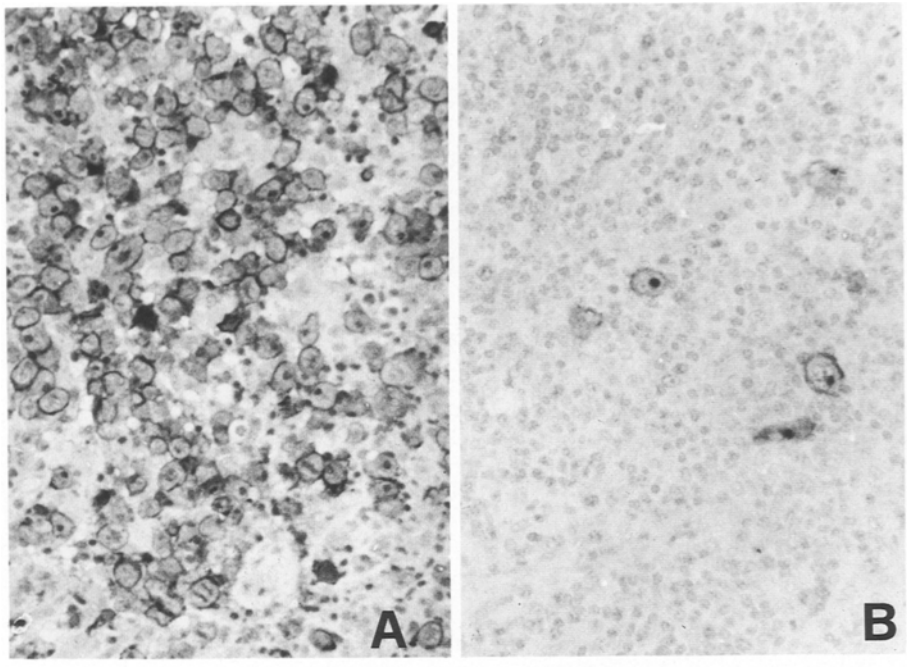


**Fig. 3.** **A** NHL cells stained with the monoclonal antibody LN1 (Cdw 75). **B** HD tissue with scattered neoplastic cells expressing the CD 15 antigen. SP and APAAP method, ×250

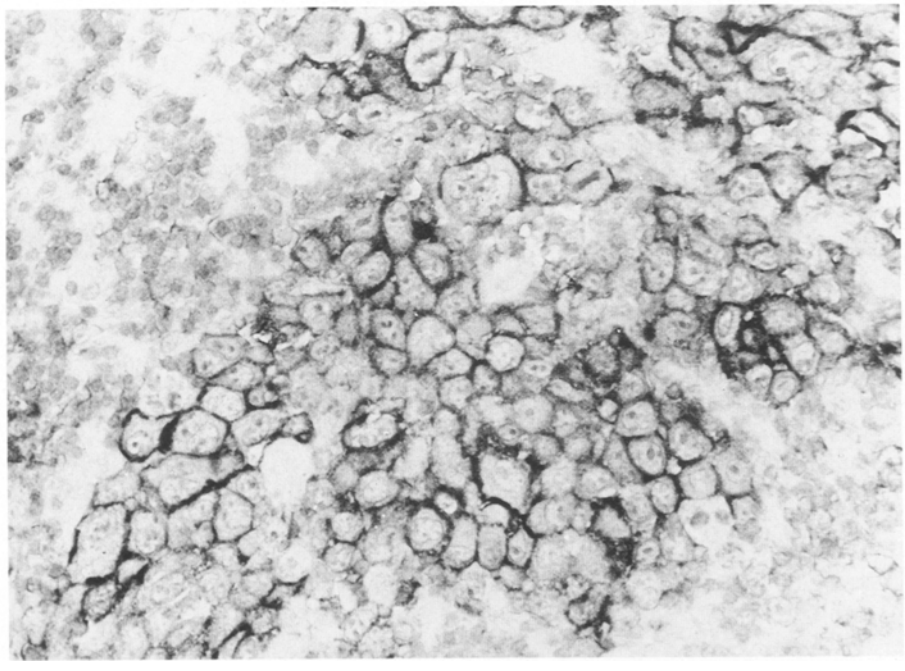

**Fig. 4.** Membrane staining of the NHL cells with the monoclonal antibody L 26 (CD 20). APAAP method, ×350

since the CD 15 and the CD 30 (Ki-1) antigens are also expressed in a variable percentage of NHL (Sheibani et al. 1986; Wieczorek et al. 1986; Piris et al. 1990). Similarly, the EMA positivity, which might be considered quite unusual in NS-HD, has been already described in this subtype of HD (Hall et al. 1988; Chittal et al. 1988). Moreover, a recent study (Schmid et al. 1991) demonstrated that in 48 of 55 (87%) of HD (NS and mixed cellularity) H and RS cells were reactive with at least one B-cell marker in frozen or paraffin sections (CD 19, CD 20, CD 22, CDw 75 and MB 2). Although these findings could still leave open the possibility that we are seeing the evolution of a single neoplastic process, in this case we observed a striking morphological and immunophenotypical divergence between H, RS and NHL cells, the last displaying the LCA (CD 45) and several antigens mainly related to B-cell lineage. This might suggest a lack of correlation between HD and the simultaneously occurring high-grade monomorphous B-NHL. Therefore, definitive evidence of a possible close relationship between HD and neoplastic B lymphocytes requires further investigations on similar additional cases and genetic analysis of the different components of the tumour.

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