

Usefulness of K-i (CD-30) Marker in Hodgkin's Disease

K.A. Shakoor, A. Saleh, M.S. Khanzada (Department of Pathology, BMSI, Jinnah Postgraduate Medical Centre, Karachi.)

Abstract

Objective: To identify Hodgkin's and Reed Sternberg like cells by a single Immunomarker (Ki-1; CD-30) and to highlight diagnostic specificity of these cells in Hodgkin's disease, as these cells are frequently encountered in other lymphoproliferative disorders on H&E sections.

Methodology: Seventy-nine histologically diagnosed cases of lymph node biopsies (59 cases of Hodgkin's disease, 15 cases of Non Hodgkin's lymphoma and 5 cases of non neoplastic lymphoid tissue) were subjected to Ki-1 (CD 30) immunostaining as all cases revealed Hodgkin's and/or Reed Sternberg like cells on H&E stained sections.

Results: Out of 59 cases of Hodgkin's disease, 49 (83%) showed Ki-1 (CD-30) immunoreactivity for Reed Sternberg and Hodgkin's cells. None of the 15 cases of non-Hodgkin's lymphoma (2 small lymphocyte type, 11 diffuse large cells type, 2 lymphoblastic type) showed positive Ki-1 (CD-30) immunostaining. Only one case of the remaining 5 cases of non-neoplastic lymphoid lesion (2 toxoplasmic lymphadenitis, one histiocytic necrotizing lymphadenitis, one reactive hyperplasia and one chronic non specific lymphadenitis) showed positive Ki-1 immunostaining.

Conclusion: Ki-1 (CD-30) is a reliable marker for Hodgkin's Reed Sternberg like cells in histologically proven cases of Hodgkin's disease but not for similar cells encountered in other lymphoproliferative lesions (JPMA 52:442; 2002).

Introduction

The nature of Hodgkin's disease has been the subject of intense debate for more than 115 years. The origin of Reed Sternberg cells has also been the subject of a number of conflicting reports¹⁻³. However, recent developments in molecular biology have demonstrated intimate relationship between Hodgkin's disease, LMP-1 of EBV and B-cell origin of Hodgkin's/Reed Sternberg cells (HRS-cells)⁴⁻⁷. To guide the clinician for effective treatment and a better survival of patients with Hodgkin's disease, an accurate diagnosis at an early stage is of utmost importance⁸. A diagnostic test for Hodgkin's disease would be of great clinical value because difficult diagnostic problems often arise in the pathology of lymphoma, more so in T-cell lymphoma and T-cell rich B-cell lymphoma⁹. In most cases a diagnosis can be made on a lymph node biopsy. However, sometimes especially in the early stages or when a small node has been excised, the changes are not enough for differentiation from an inflammatory reaction¹⁰.

The immunostaining technique highlights the Reed Sternberg and Hodgkin's cells and could be of diagnostic value in the differentiation from benign hyperplasia and other neoplasms¹¹. The origin of the neoplastic cells in Hodgkin's disease is still obscure. However, the establishment of permanent Hodgkin's cell lines has led to the search for tumor specific antigens and/or for membrane markers on Hodgkin's and Reed Sternberg cells which may indicate the normal equivalent cells¹².

Monoclonal antibodies that are specific for the malignant cells in Hodgkin's disease could prove useful not only in the diagnosis and therapy of Hodgkin's disease, but also in the determination of the cells of origin of Hodgkin's disease and its biological function⁹. The phenotypic expression of Hodgkin's and Reed Sternberg cells was determined by analysis with a panel of monoclonal antibodies by

immunohistochemical technique. Demonstration of several antigenic markers including T200, anti-HLA-DR, IgG, anti-leu¹⁰, Hefi-1, anti-leuMi, OKT9, Ki-1 and lack of CD45 (LCA) on the Reed Sternberg and Hodgkin's cell is well established^{2,9,11,13}. However, in view of unequivocal positivity of Ki-1 (CD-30) in cases of Anaplastic Large Cell Lymphoma (ALCL), and primary mediastinal large B-cell lymphoma (PmBL), as well as other Reed-Sternberg like cells, it is mandatory to prove the specificity of this marker in cases of Hodgkin's disease¹⁴⁻¹⁸.

Considering such a wide phenotypic characteristics of RS cells a study was designed to evaluate the immunoreactivity of Reed Sternberg cells and its variants to Ki-1 (CD-30), monoclonal antibody in paraffin sections and to specify this marker for Hodgkin's disease as compared to non Hodgkin's lymphoma and other non-neoplastic lymphoid lesions.

Material and Methods

Seventy-nine lymph node biopsies, initially diagnosed as Hodgkin's disease (9), non-Hodgkin's lymphoma (15) and non-neoplastic lymphoid hyperplasia (5) revealing Hodgkin's Reed Sternberg like cells on H&E sections were reviewed.

Hodgkin's disease and non-Hodgkin's lymphoma cases were diagnosed according to Rye classification and working formulation respectively¹⁹⁻²¹. Areas denoting Hodgkin's/Reed Sternberg like cells were demarcated for immunostaining with Ki-1 (CD-30) monoclonal antibody.

The monoclonal antibody Ki-1 (CD-30) which is commercially available as "Ber-H2" (1:20 DAKO, Denmark) and the kit labeled streptavidin-biotin (DAKO) was directly obtained from DAKO. The technique used here is based on labeled streptavidin-biotin (LSAB) method.

For immunohistochemical studies thin (3-5 μ m) paraffin sections were mounted on slides coated with poly-L-lysine. Sections were deparaffinized and dehydrated, and placed in phosphate buffered saline (PBS), pH 7.2-7.6. Enzymatic digestion of tissue sections was achieved for 10 minutes at room temperature, in a solution containing trypsin 0.1% (0.1 ml/100 ml PBS pH 7.2-7.6). Endogenous peroxidase activity was quenched by first incubating the sections for five minutes with 3% hydrogen peroxide.

Slides were then rinsed in phosphate buffered saline (PBS) and placed in staining dishes and immersed in 1-mmol/L concentration of EDTA. After micro waving for 15-20 minutes and cooling (30 minutes) the slides were rinsed in water and then PBS. The slides were then incubated with primary mouse monoclonal antibody, then biotinylated antimouse polyclonal antibody and finally with Streptavidin-peroxidase (30 minutes each). Slides were rinsed with PBS between each step. The slides were then covered with substrate-chromogen solution (3%-amino-9-ethyl-carbazole) and incubated for 5-10 minutes. Rinsed in PBS and then water and incubated with copper sulphate 0.5% for minutes, rinsed again and counterstained in hematoxylin and a cover glass applied.

Interpretation: The characteristic staining patterns consisted of a diffuse red colour within cytoplasm and cytoplasmic membrane. Nuclei and nucleoli did not react. The positive control slides were examined under light microscope for the presence of a red coloured end product at the cytoplasmic membrane level. The presence of this colour can be interpreted as a positive staining result, indicating presence of antigen against Ki-1 (CD-30) monoclonal antibody. The absence of specific staining in the negative control slides confirmed the specificity of the primary antibody.

Results

Results of Ki-1 immunoreactivity in 59 cases of Hodgkin's disease, including mixed cellularity (30 cases), nodular sclerosis (9 cases), lymphocyte depletion (11 cases) lymphocyte predominance (6

cases) and unclassified (3 cases) are summarized in table 1.

Table 1. Ki-1 immunoreactivity for Reed-Sternberg cells in different types of Hodgkin's disease.

Histologic type	No. of cases	Ki- 1 Immunoreactivity			
		Positive		Negative	
		No.	%	No.	%
Mixed cellularity	30	28	93.33	2	6.67
Nodular sclerosis	9	8	88.89	1	11.11
Lymphocyte predominance	6	3	50.00	3	50.00
Lymphocyte depletion	11	9	81.82	2	18.18
Unclassified	3	1	33.3	2	66.67
Total	59	49	83.05	10	16.95

Table 2. Comparison of immunoreactivity for Ki-1 in Hodgkin's disease, non-Hodgkin's lymphoma and non-Neoplastic Lymphoid tissue.

Diagnosis	No. of cases	Ki- 1 Positivity
Hodgkin's disease	59	49 (83.05)
Non-Hodgkin's lymphoma	15	0
Non-neoplastic lymphoid tissue	5	1*

*Suspected case of Hodgkin's disease lymphocyte depletion type

Out of 59 cases of Hodgkin's disease, 49 (83%) cases showed positive staining with Ki-1 monoclonal antibody. Most of the cases of mixed cellularity, nodular sclerosis and lymphocyte depletion showed positive staining. Only three, out of six cases of lymphocyte predominance showed positive staining. This was most likely due to the fact that classic RS cells are positive while L and H cells are negative

for CD30 (Figures 1 and 2).

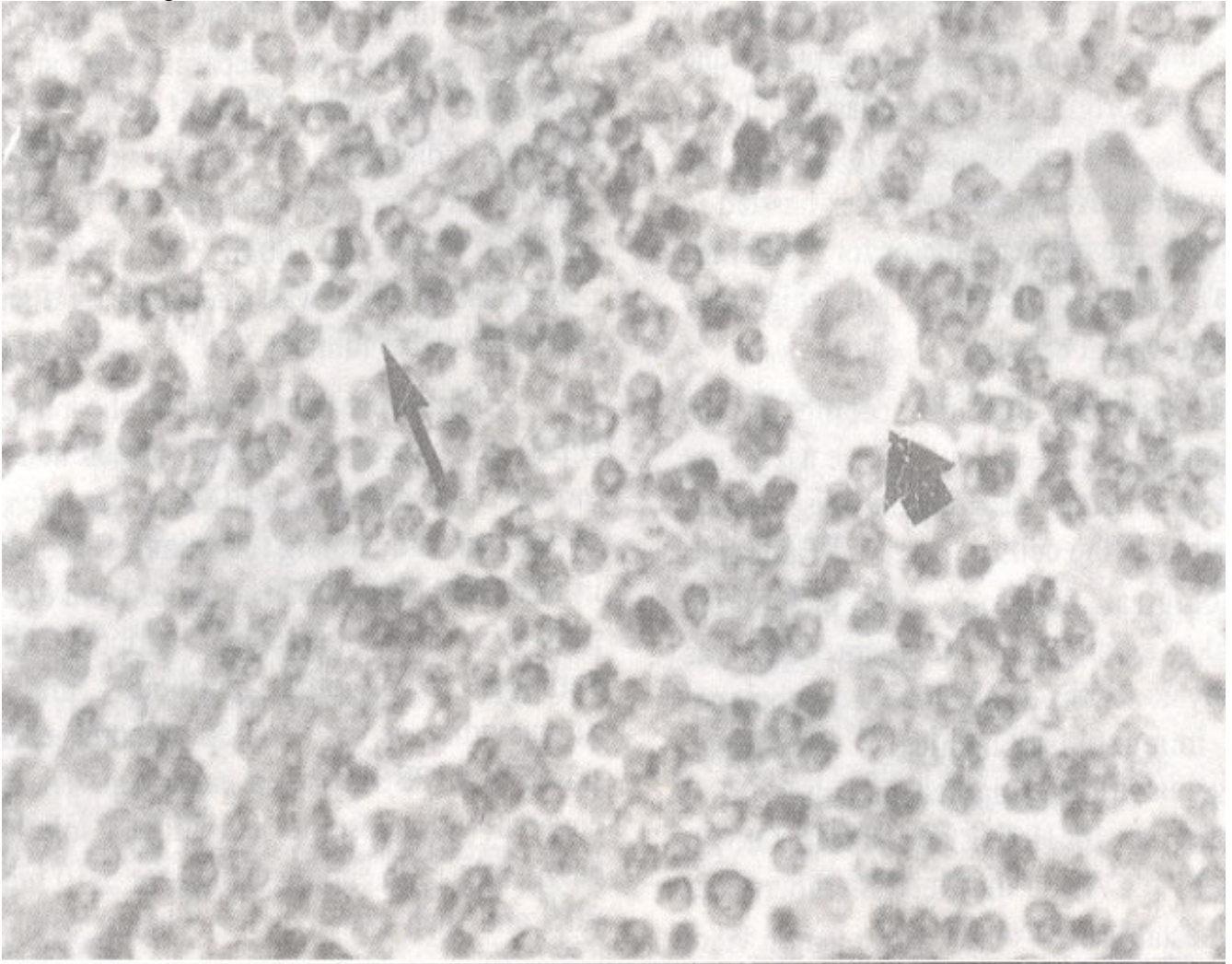


Figure 1. Hodgkin's disease lymphocyte predominance type showing intensive infiltration by mature lymphocytes (thin arrow) with few indistinct mostly mononuclear variant of Reed-Sternberg cells (thick arrow) (H&E x 200).

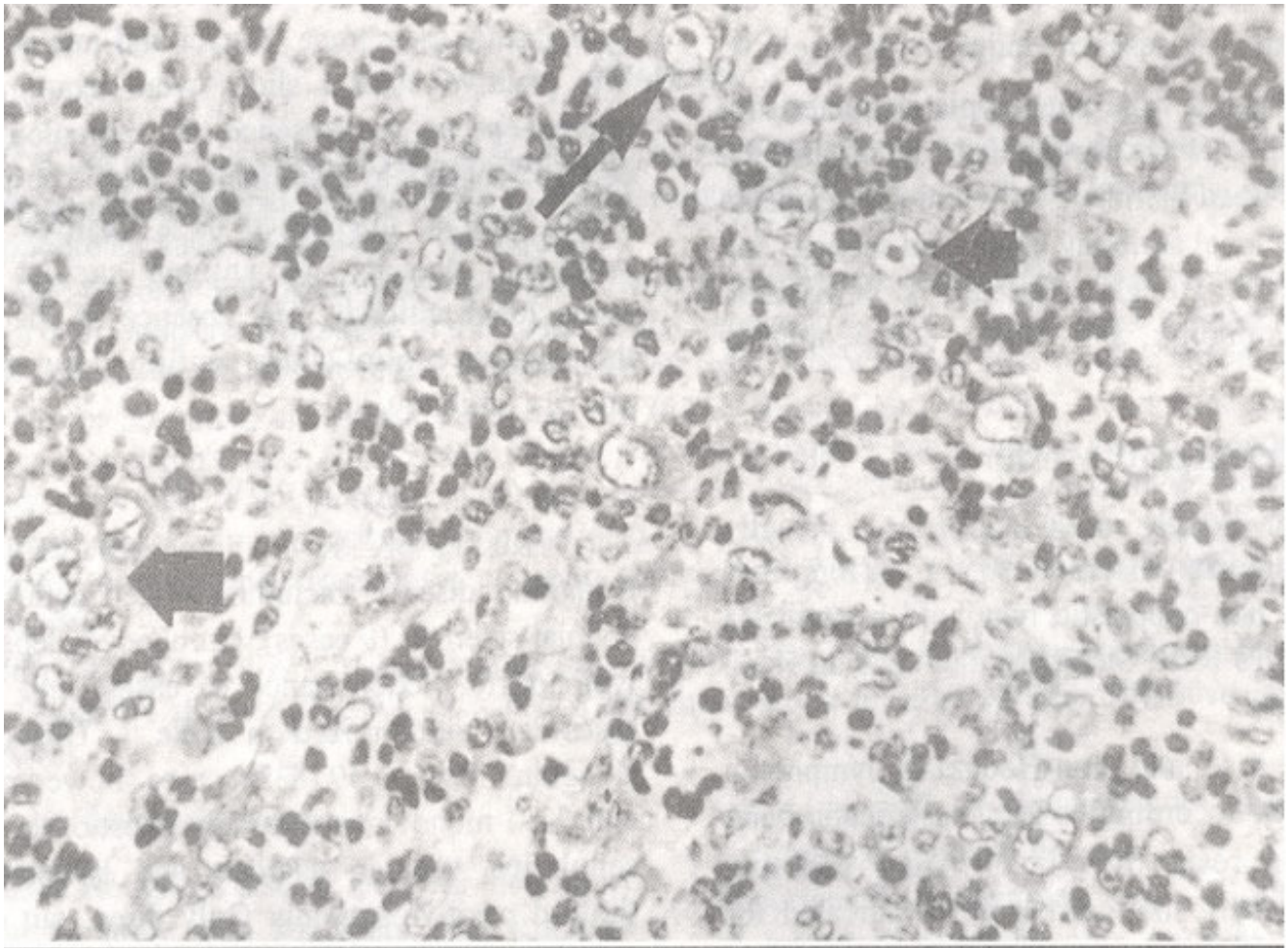


Figure 2. Hodgkin's disease lymphocyte predominance type. Note the Ki-1 immunoreactivity highlight the mononuclear Hodgkin's cells (thin arrow) and Reed-Sternberg cells (thick arrow) while lymphocytes are non-reactive [immunoperoxidase technique labelled streptavidin biotin (LSAB) method x 200].

In most of the cases characteristic staining pattern consisted of a diffuse red color within cytoplasm and cytoplasmic membrane. Nuclei and nucleoli did not react.

Hodgkin's disease noted staining reaction also in lymphocytes of few lymph nodes revealing partial involvement of the node. However, the morphological differentiation from Reed Sternberg cells was distinct. Fresh cases, which were fixed in buffered formalin for a period of about 24 hours, displayed good results.

In some cases, which were diagnosed clearly by hematoxylin and eosin staining, Reed Sternberg cells disappeared on deeper sectioning resulting in negative staining. It is worth noting that reactive histiocytes with or without phagocytes did not show any immunostaining response. Fifteen cases of non-Hodgkin's lymphoma including diffuse small lymphocytic cell low grade (2 specimens), diffuse large and small-cleaved intermediate grade and small non-cleaved high grade (11 and 2 specimens) respectively displayed negative response to Ki-1 immunostaining.

Of the five cases of non-neoplastic lymphoid tissue including Toxoplasmosis lymphadenitis (2 specimens), histiocytic necrotizing lymphadenitis (one specimen) and reactive hyperplasia (one specimen), only the last one revealed Ki-1 positivity in bizarre spindle cells similar to fibroblasts, Diagnosis of Hodgkin's disease lymphocyte depletion type was proposed in this case.

Discussion

There is much controversy concerning the origin and nature of the neoplastic cells (Reed Sternberg and Hodgkin's cells) in Hodgkin's disease. However, establishment of tumour-specific antigens and/or membrane markers on Reed Sternberg and Hodgkin's cells does help in the diagnosis of these cells. Hodgkin's and Reed Sternberg cells specific antigens were identified after reactivity against L428 cell line which proved to be selectively reactive with Hodgkin's and RS cells^{22,23}.

Schwab et al (1982)¹² was able to produce 5 hybridoma antibodies after immunization of BALB/C mice with L-428 cells. The resultant monoclonal antibodies were tested by using the immunoperoxidase staining technique on frozen sections of the biopsy tissue infiltrated with Hodgkin's disease. One of the hybridoma antibodies reacted with Reed-Sternberg cells and variants but not with other cells in the biopsy material. This clone was designated Ki-1. The demonstration of Hodgkin's disease associated antigen, Ki-1 or CD-30 in the Reed Sternberg cells and its variants i.e. lacunar mononucleated/multilobated types by means of the immunohistochemical technique has been previously reported by Schwab et al, (1982)¹², Stein et al, (1985)²⁴ and Chan et al, (1995)²⁵. In the present study by using immunoperoxidase labeled streptavidin biotin (LSAB) method for demonstration of Ki-1 marker in Reed Sternberg cells and its variants immunostaining positivity was seen in 49 (83%) out of 59 cases in paraffin embedded tissues. The positive cases included all histological categories (Table 1). However on cryostat sections, Schwab et al (1982)¹² and Stein et al (1985)²⁴, demonstrated 100% positivity in their study of 14 and 35 cases respectively including all histological types of Hodgkin's disease.

Later Chan et al, (1995)²⁵ mentioned that Ki-1 marker was positive in all cases of mixed cellularity, nodular sclerosis, lymphocyte depletion and lymphocyte-rich classical Hodgkin's disease, while lymphocyte predominance revealed negative immunoreactivity for Ki-1 antigen in paraffin embedded sections. However, Shahab et al, (1997)²² demonstrated +2 reaction with Ki-1 (CD-30) in only one out of seven cases of recurrent post-BMT Hodgkin's disease in paraffin sections.

In the present study undoubtedly, however, Ki-1 marker was negative in 10 (17%) cases. This was expected, as part of this study was based on paraffin-embedded sections (retrospective). Fixation often renders a tissue unreactive immunohistochemically, this effect being more pronounced with the fixatives, which give good histological preservation²⁶. However, for the detection of Ki-1 marker in Reed Sternberg cells and its variants, in paraffin sections, the tissues had to be optimally fixed with buffered formalin for 24 hours at 4°C. This rendered efficacious results noted clearly in the prospective cases of the present study fixed in buffered formalin. Fixation with formal saline for long duration produces much more difficulties to demonstrate the marker²⁶. In this proved to be superior to paraffin sections. Therefore Ki-1 antigen could be demonstrated easily by using frozen sections²⁴. Some difficulties were encountered in the identification of individual cells in serial sections, as in these cases the immunostaining was negative. It should be emphasized that cases with a lymphocyte predominance which show easily recognizable Reed Sternberg and/or Hodgkin's cells were Ki-1 positive, while those cases which revealed difficulty in recognizing Hodgkin's and Reed.

Sternberg cells appeared negative. Chan et al, (1995)²⁵ divided lymphocyte predominance into two types on the basis of morphology and immunostaining. First type was Ki-1 antigen negative, while second type was Ki-1 antigen positive lymphocyte-rich classical Hodgkin's disease. The findings in the present study are more or less identical to this observation.

In the present series, 15 cases of non-Hodgkin's lymphoma and 4 cases of non-neoplastic lymphoid hyperplasia did not show reactivity to Ki-1 marker although all cases revealed atypical histiocytes and/or Reed Sternberg like cells on H&E sections. One case diagnosed on H&E as chronic non-specific lymphadenitis when subjected to Ki-1 immunostaining revealed large bizarre spindle cells with positive immunoreactivity. In our opinion this was a case of Hodgkin's disease lymphocyte depletion type. However, for want of adequate evidence this case was not included in Ki-1 positive

series.

The reactivity to Ki-1 (CD-30) noted within lymphocytes of some cases in this study appears to be identical to that reported by Schwab et al. (1982)¹² and Stein et al.(1985)²⁴. The staining in these cells, however, clearly differed from that observed in Reed Sternberg cells. It was observed earlier that T-lymphocyte may be the normal precursor of the Reed Sternberg cell⁹. This may be demonstrated only in minority of cases while majority of Hodgkin's - Reed Sternberg cells (HRS cells) represent monoclonal outgrowth of late germinal center B-cells that have lost their capacity to express IgG (Stein, 1997)²⁷. Ki-1 reacted with a small subset of transformed lymphoid cells around the margin of activated non-neoplastic germinal center. Ki-1 positivity has also been demonstrated in large cell anaplastic lymphoma (ALCL) and diffuse large cell lymphoma (DLCL), which were not encountered in this study. DLCL, however, has been differentiated from Hodgkin's disease on the basis of immunophenotype and molecular genetics¹³⁻¹⁶

The expression of Ki-1 antigen on Reed Sternberg cells and its variants in 83% of cases including all histological types, and negative reaction in all cases of NHL and reactive lymphadenitis in the present study demonstrates that this antigen may be used as a reliable marker of Hodgkin's disease. The diagnostic value of Ki-1 monoclonal antibody is not reduced by the occurrence of Ki-1 antigen on normal cells i.e. lymphocytes, because these cells are easily distinguishable Reed Sternberg and Hodgkin's cells by morphological criteria¹². Needless to say that several other markers eg: CD 15, CD3, CD20, CD45,CD43, LMP-1, EMA, BNH, BNH-9, TiA1 and ALK1 have a diagnostic value in the final confirmation of HD²⁷ However, considerable caution is to be exercised while encountering Reed-Sternberg-like cells on NHL and reactive lymphadenitis on H&E stained sections. It is mandatory to subject these cases to Ki-1 immunostaining to avoid erroneous diagnosis; the more so as several variants of NHL have reacted strongly to this immunomarker.

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