# AN IMMUNOHISTOCHEMICAL ANALYSIS OF HODGKIN'S DISEASE AND ANAPLASTIC LARGE CELL LYMPHOMA USING ANTIBODIES EFFECTIVE IN PARAFFIN SECTIONS.

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Dissertation submitted in fulfilment of Part III M.Med (Anat.Path).

University of Cape Town, February, 1992.

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To Renate, for her encouragement
and support and for tolerating the many
mood swings that invariably accompany a
project of this nature.

**ACKNOWLEDGEMENTS**

I am indebted, and extremely grateful to the following persons:

Firstly, my supervisor, Pauline Close, for her encouragement, critical advice and support from the conception of the project to its completion.

Secondly, to Sumaya Cornelius, for her expert technical assistance, particularly for performing the immunohistochemistry, and assisting in the collection of material from private laboratories.

Thirdly, my father, Professor R.C. Tustin, for proof reading the manuscript and offering much-needed critical advice and encouragement, and

Fourthly, to Beverly Seymour, for her invaluable assistance in preparing the graphics presented in this dissertation.

This work is partially sponsored by the Cancer Research Trust.

I, **Richard Philip Tustin**, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other University.

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## SUMMARY

The nature of the malignant cell in Hodgkin's disease still remains a source of controversy. Heterogeneity of marker expression by these cells may reflect heterogeneity of origin, supporting the concept that Hodgkin's disease is not a single entity but rather a heterogeneous group of disorders. Morphological and immunophenotypic similarities between Hodgkin's disease and anaplastic large cell lymphoma suggest that the latter may represent the link between Hodgkin's disease and non-Hodgkin's lymphomas.

A comprehensive histological and immunohistochemical analysis of Hodgkin's disease was undertaken in order to determine (1) the lineage of the neoplastic cells, (2) the relationship between immunophenotype and histologic subtype and, (3) to compare these results with a meta-analysis of published series. Cases of anaplastic large cell lymphoma were analysed to investigate the relationship between this lymphoma and Hodgkin's disease. A panel of 13 antibodies effective in routinely processed tissue was applied to 71 cases of Hodgkin's disease, 4 cases of anaplastic large cell lymphoma and 2 cases in which the distinction between Hodgkin's disease and non-Hodgkin's lymphoma was not clear on morphological and immunohistochemical grounds.

Immunohistochemical analysis employed the streptavidin and indirect immunoperoxidase techniques. The panel of antibodies used included those directed against the CD15 antigen (LeuM1), CD30 antigen (BerH2), epithelial membrane antigen (EMA), leucocyte common antigen (LCA), as well as B-cell (L26, LN1, PanB, MB2), T-cell (CD3, TUCHL1) and histiocytic (CD68) associated antigens. The

monoclonal antibodies, CD21 and vimentin, were also included to determine the follicular dendritic cell (FDC) pattern in the lymph nodes and vimentin expression in the Reed-Sternberg cells respectively, since it has been shown recently that Reed-Sternberg cells can express this antigen.

On review of the morphological features and immunophenotype obtained in each case, 56 cases were diagnosed as Hodgkin's disease and 13 as anaplastic large cell lymphoma. The diagnosis was uncertain in 8 cases. The results showed clear phenotypic separation of nodular lymphocyte predominance Hodgkin's disease (8 cases) from other subtypes. The lymphocytic and histiocytic cells (L&H) of lymphocyte predominance, nodular Hodgkin's disease were reactive for B-cell markers in all cases. LN1 was expressed by the L&H cells in all cases and L26 in 7 cases. LCA was expressed in 7 cases, EMA in 2 cases and LeuM1 and BerH2 were negative in all cases. The immunophenotype of the tumour cells in the only case of lymphocyte predominance, diffuse did not differ greatly from the findings in lymphocyte predominance, nodular Hodgkin's disease. The tumour cells were reactive for L26 and LN1 but negative for LeuM1, BerH2 and EMA. These findings suggest that the nodular and diffuse forms are closely related.

Within the other subtypes i.e. nodular sclerosis (20 cases), mixed cellularity (16 cases), lymphocyte depleted (1 case), and Hodgkin's disease, NOS (8 cases), a B phenotype was expressed in 6 cases (4 cases of nodular sclerosis and 2 of Hodgkin's disease, NOS). No case expressed a T phenotype. LeuM1 was expressed in 57% and BerH2 in 70% of cases, with LeuM1 more consistently expressed in nodular sclerosis (70%) than in mixed cellularity Hodgkin's disease (44%).

BerH2 was more consistently expressed by the tumour cells in mixed cellularity (81%) than in nodular sclerosis Hodgkin's disease (60%).

In only 4 cases in this series was the original diagnosis that of anaplastic large cell lymphoma. Nine cases, previously diagnosed as Hodgkin's disease, were placed in this category on review, and of these 9 cases, 5 were originally diagnosed as nodular sclerosis, Hodgkin's disease. Morphologically all 13 cases of anaplastic large cell lymphoma had a distinct syncitial growth pattern. BerH2 was expressed by the tumour cells in all cases, whereas EMA expression was obtained in only 31%, and LCA in 46% of cases. LeuM1 was expressed in 31% of cases, a figure considerably higher than that obtained from the meta-analysis (10%). A T phenotype was obtained in 38% of cases and a B phenotype in 23%.

There remained a group of 8 cases in this study, in which, after review, a definitive diagnosis could not be made after careful consideration of both morphological features and immunophenotypic profile of the tumour cells. Five of these cases had a syncitial growth pattern and in one, the tumour cells were non-cohesive. The morphological characteristics favoured a diagnosis of anaplastic large cell lymphoma in most of these cases, however, the immunophenotype of the tumour cells did not always support this diagnosis. This group highlights the difficulty in distinguishing between Hodgkin's disease and anaplastic large cell lymphoma.

The findings in this study do not differ greatly from published consensus with regard to expression of CD15, CD30, and EMA by the tumour cells in Hodgkin's disease. Several differences were,

however, noted when comparing cell lineage obtained with that of the meta-analysis. Sixteen percent of cases of Hodgkin's disease in this series expressed a B phenotype with none expressing a T phenotype. The corresponding figures in the meta-analysis are 25% and 12%, respectively. Likewise, 38% of cases of anaplastic large cell lymphoma were of T-cell lineage and 23% of B-cell lineage in this study, compared to 59% and 17%, respectively, in the meta-analysis of published series.

The findings in this study support the concept that Hodgkin's disease is not a single entity. The heterogeneous immunophenotypic profile of Hodgkin's disease is also manifest by anaplastic large cell lymphoma and they cannot be distinguished by immuno-histochemical analysis alone. The morphological and immuno-phenotypic similarities between Hodgkin's disease and anaplastic large cell lymphoma suggest that anaplastic large cell lymphoma may represent the link between Hodgkin's disease and non-Hodgkin's lymphoma. Immunophenotypic profile cannot be used as the sole diagnostic discriminant and diagnosis should be based upon the immunophenotypic profile of the tumour cells in conjunction with careful morphological assessment.

## AIM

A histological and immunophenotypic analysis of Hodgkin's disease and anaplastic large cell lymphoma was undertaken:

1) to determine the lineage of the neoplastic cells;
2) to investigate the relationship between immunophenotype and histologic subtype;
3) to compare the Cape Town analysis of Hodgkin's disease and anaplastic large cell lymphoma with that of a meta-analysis of published studies.

## INTRODUCTION

Hodgkin's disease was first diagnosed over 150 years ago. The nature of the malignant cell in Hodgkin's disease, the Reed-Sternberg cell and its variants, still remains a source of controversy. It has been characterized as arising from T lymphocytes, B lymphocytes, histiocytes or interdigitating reticulum cells. The expression of the Ki-1 antigen suggests derivation from activated lymphoid cells. With the advent of newer lineage-specific monoclonal antibodies and the application of molecular biologic techniques, new information regarding the nature of the neoplastic cell in Hodgkin's disease has come to light. There is now evidence that it is not a single disease entity but a heterogeneous group of lymphomas (10,18,25,33,34).

Lymphocyte predominance, nodular subtype of Hodgkin's disease has recently been shown to be of B-cell derivation on the basis of phenotypic and morphologic findings (10,33,42). Furthermore,

recent evidence suggests that this subtype may transform into a large cell lymphoma of B-cell type (35,37,38,41,43). In a number of cases the two diseases have been found to coexist in the same lymph node, suggesting that nodular lymphocyte predominance Hodgkin's disease represents a B-cell malignancy in evolution (33,39,42,43).

Analysis of other subtypes of Hodgkin's disease has shown considerable heterogeneity, with B, T and null pheno- and genotypes represented in all subtypes (8,10,11,12,46,48).

Anaplastic large cell lymphoma (Ki-1 lymphoma) is a newly described lymphoma defined on the basis of the reactivity of the tumour cells with the Ki-1 monoclonal antibody in addition to characteristic clinical and pathological features (4,22,23,25). The fact that Ki-1 antibody also reacts with Reed-Sternberg cells raises the possibility that Ki-1 lymphoma may represent the link between non-Hodgkin's lymphoma and Hodgkin's disease(10,46).

## HODGKIN'S DISEASE

The basic criteria upon which the histological diagnosis of Hodgkin's disease is made were comprehensively set down by Luke *et al.* in 1966 and have not changed in any major respect since then (50,53). The initial diagnosis is usually made on a lymph node biopsy and the criteria apply to the disease in this location. These criteria, however, still apply when the diagnosis is made by examination of tissue from an extranodal site. In these cases the diagnosis must be made only after stringent evaluation of the histological findings (34,51).

## Histological Diagnosis

There are two basic criteria which must be met before a histological diagnosis of Hodgkin's disease can be made (34,78).

These are:

1. the unequivocal demonstration of the Reed-Sternberg cell in its classical form, and
2. the appropriate immunoreactive background.

### 1. *The Reed-Sternberg cell*

The standard description of the Reed-Sternberg cell is based on its appearance in formalin-fixed paraffin embedded sections stained with haematoxylin and eosin. It is a large cell containing a variable but generally abundant amount of cytoplasm which varies in its staining characteristics from faintly eosinophilic to dense and amphophilic. There is classically, either a bilobed nucleus or two nuclei with the heterochromatin being aggregated peripherally beneath the nuclear membrane. Each lobe or nucleus has a large nucleolus which is characteristically , but not invariably, eosinophilic. There is typically a clear zone around each nucleolus giving it an inclusion-like appearance.

Variants of the classical Reed-Sternberg cell are frequently found in most cases of Hodgkin's disease and are characteristic of certain subtypes of Hodgkin's disease. Indeed, they are of major importance in the histological subtyping of the disease and may also be of importance in the recognition of secondary lesions.

The mononuclear variant, commonly referred to as the **Hodgkin cell**, has morphological features which are similar to the Reed-Sternberg

cell, the only difference being that it contains a single unilobed nucleus. The **polylobated version** is a larger cell showing bizarre nuclear multilobation with multiple inclusion-like nucleoli. These cells shows marked variation in size and shape. The **lacunar variant**, which is characteristic of the nodular sclerosing sub-type, contains abundant clear or vacuolated cytoplasm and a small unilobed nucleus with a fine chromatin network and without a prominent nucleolus. The cytoplasmic vacuolation is probably artifactual as it occurs most commonly in formalin-fixed tissue and is not as prominent in B5-fixed tissue. The lacunar variant does not show complete sub-type fidelity: it may also be found in mixed cellularity Hodgkin's disease and thus must not be relied on entirely in the diagnosis of the nodular sclerosing subtype. Other factors must also be taken into consideration when subtyping the disease.

The **lymphocytic and histiocytic (L&H) variant** of the Reed-Sternberg cell, more commonly known as the popcorn cell, is present in all cases of lymphocyte predominance Hodgkin's disease and is particularly numerous in the nodular form. It may also be present in the other subtypes of Hodgkin's disease, but is rarely prominent (51). The cell is variable in size and has a nucleus which shows complex lobation and a fine chromatin network and contains multiple small nucleoli. Its cytoplasm is usually pale and scanty (50).

Lastly, **"mummified"** Reed-Sternberg cells are Reed-Sternberg cells which have undergone necrobiosis during which the nuclei have become pyknotic and the cytoplasm condensed and deeply eosinophilic.

2.*The immunoreactive background.*

Equally important in the diagnosis of Hodgkin's disease is the presence of an appropriate background cellular response, as the mere presence of Reed-Sternberg cells is not sufficient evidence on which to base a definitive diagnosis of Hodgkin's disease. The characteristic feature of this response is that it invariably leads to architectural distortion with loss of the normal structure of the affected tissue. The response consists of a wide range of cell types including lymphocytes, granulocytes, histiocytes, plasma cells and fibroblasts. The varying patterns of cellular response also play an important role in subtyping and therefore in assessing prognosis. The extent of the lymphocytic infiltration, for example, plays an extremely important role which is described in some detail below. Except in the nodular form of the lymphocyte predominance subtype in which B lymphocytes predominate, the lymphocytes are mainly T lymphocytes - in fact the frequent apposition of T lymphocytes to the perimeter of Reed-Sternberg cells has prompted speculation that some kind of interaction takes place between them (10,33,74).

The presence of eosinophils is of dubious prognostic significance. Neutrophil infiltration is often associated with necrosis and therefore tends to be a manifestation of rapidly advancing disease (33). Histiocytic reactions may assume several different forms. Microgranulomas, or even larger granulomas resembling those seen in tuberculosis, may be found in Hodgkin's disease.

The pattern of fibrosis plays an important role in the subtyping of Hodgkin's disease. The presence of birefringent collagen bands is

central to the recognition of the nodular sclerosis subtype of Hodgkin's disease: in other subtypes the collagen distribution is more diffuse and less well organized. Vascular proliferation is pronounced in association with the collagen bands in the nodular sclerosis subtype, but is minimal in other forms of Hodgkin's disease (33,78).

## Histological Subtyping of Hodgkin's Disease

The Rye classification adopted by the Nomenclature Committee at the Rye Conference in 1966 is still widely employed (50,53). There has been some concern regarding the concept, implicit in the Rye classification, that the lymphocyte predominance, mixed cellularity, and lymphocyte depleted subtypes represent phases in the evolution of Hodgkin's disease as recent studies suggest that this concept may require modification (33,36,39).

In the early stages of Hodgkin's disease focal involvement of a lymph node may be encountered which may be restricted to the paracortical areas between the follicles. In addition, there is often striking follicular hyperplasia; and the interfollicular involvement may be missed, leading to misinterpretation as reactive follicular hyperplasia. Diagnostic Reed-Sternberg cells and mononuclear variants are characteristically evident interspersed between the reactive follicles; this pattern of nodal involvement has been referred to as *interfollicular Hodgkin's disease*. It should not, however, be regarded as a specific subtype of Hodgkin's disease. Its importance, from a diagnostic viewpoint, lies in the fact that it may be confused with a benign lesion resulting in

unnecessary delays in the diagnosis and therapy of a potentially curable disease (50,51,54).

**Four major categories (or subtypes) are accepted:**

*1. Lymphocyte predominance Hodgkin's disease*

*2. Mixed cellularity Hodgkin's disease*

*3. Nodular sclerosis Hodgkin's disease*

*4. Lymphocyte depleted Hodgkin's disease*

*1. Lymphocyte predominance Hodgkin's disease*

In the Rye classification, lymphocyte predominance Hodgkin's disease is considered a discrete entity with either a nodular or diffuse histologic growth pattern. These two subtypes were originally classified separately, according to Lukes *et al.* in 1966 (50), as the nodular, and the diffuse lymphocytic and histiocytic subtypes of Hodgkin's disease. It is now recognized that lymphocyte predominance Hodgkin's disease includes at least two different processes (48,52), each of which has distinct morphological characteristics and exhibits distinct immunophenotypic characteristics and clinical features. These are the nodular and diffuse subtypes of lymphocyte predominance Hodgkin's disease. Although the two subtypes were amalgamated into a single type designated lymphocyte predominance Hodgkin's disease in the Rye scheme, they are still distinguished from one another by sometimes subtle, but recognizable, histological features.

Features common to both the nodular, and the diffuse subtypes are a lymphocytic and histiocytic component in the background, and the

presence of the lymphocytic and histiocytic (L&H) variant of the Reed-Sternberg cell (so-called popcorn cell). Classic Reed-Sternberg cells are difficult to find, and if numerous, the case should probably be classified as mixed cellularity Hodgkin's disease. The L&H variants are numerous in the nodular form and sparse in the diffuse form. Eosinophils, plasma cells and foci of fibrosis are scanty or absent.

The lymph node architecture is usually effaced and the infiltrate may have a nodular or diffuse pattern of growth (53). The nodular pattern may be so pronounced as to simulate under low power the appearance of a follicular lymphoma; the nodules are, however, larger and more irregular in size than those of follicular lymphoma. It is not unusual to find clearly reactive germinal centres in some parts of the node. In such instances, the appearances may closely resemble those observed in progressive transformation of germinal centres. This condition may, however, precede or accompany the development of lymphocyte predominance, nodular Hodgkin's disease (37,38,40,41).

Lymphocyte predominance, nodular type rarely evolves into other subtypes of Hodgkin's disease. If nodal recurrence takes place, the histological appearance may be similar to that found at initial diagnosis or may exhibit evolution towards a non-Hodgkin's lymphoma of large cell type (33,36,39). Chittal *et al.*, in a recent study in 1990, conclude that their data reinforces current thinking that nodular lymphocyte predominance Hodgkin's disease is a B-cell malignancy in evolution and that it is not truly representative of Hodgkin's disease in terms of biological or clinical behaviour (vide infra)(43). According to some authors, transition of nodular

lymphocyte predominance Hodgkin's disease to diffuse lymphocyte predominance Hodgkin's disease does not occur (35,41). Recent evidence has shown, however, that the immunophenotypic characteristics of the two subtypes are similar suggesting a closer relationship between the two than has being previously thought (vide infra)(70).

Poppema *et al.* were the first to suggest that the lymphocyte predominance, nodular subtype of Hodgkin's disease may arise from B-cell regions of the node and specifically from progressively transformed germinal centres (35,41). The two lesions have been reported together in the same lymph node (35,41,83). Osborne and Butler have, however, found cases of progressive transformation occurring independently of nodular lymphocyte predominance Hodgkin's disease (40). In both lymphocyte predominance, nodular Hodgkin's disease and progressive transformation relapses are common and alternate lesions may be seen in sequential biopsies (41). Nicholas *et al.* reported on one case of nodular lymphocyte predominance Hodgkin's disease which had three relapses during a ten year period; biopsies taken at the time of each relapse showed progressive transformation of germinal centres without evidence of nodular lymphocyte predominance Hodgkin's disease (20). The morphological distinction between nodular, lymphocyte predominance Hodgkin's disease and progressive transformation is dependent upon the demonstration of L&H variants of the Reed-Sternberg cell in the former disease and the absence thereof in the latter (38,40,41).

In accordance with this line of thought, it has now conclusively been shown that the L&H variant of the Reed-Sternberg cell in nodular, lymphocyte predominance Hodgkin's disease arises from a B-

cell (12,13,20,42,47). These cells have also been shown to contain J chain, a protein synthesized by B-cells whose major function is the binding of polymeric immunoglobulin to secretory components on the surface of epithelial cells. Its presence within the cell cytoplasm indicates endogenous synthesis rather than uptake from the extracellular fluid and is therefore an indicator of B-cell lineage (10,46,49,57).

In the diffuse type of lymphocyte predominance Hodgkin's disease the parenchyma is partially or completely effaced by an infiltrate consisting mainly of small lymphocytes and a large number of histiocytes. The histiocytes are randomly distributed and often form microgranulomas. The presence of L&H cells helps to distinguish this subtype from the other subtypes and from lymphocytic forms of non-Hodgkin's lymphoma. The diffuse form rarely recurs as such; progression to other subtypes is more likely to occur. Regula *et al.* reported on two cases initially diagnosed as diffuse lymphocyte predominance Hodgkin's disease (36). Both had recurrences which were interpreted as being mixed cellularity Hodgkin's disease and both died as a result of lymphocyte depleted Hodgkin's disease. The authors conclude that diffuse lymphocyte predominance Hodgkin's disease may actually represent a special subtype of mixed cellularity Hodgkin's disease. Evolution towards non-Hodgkin's lymphoma of large cell type has been reported to occur in rare cases (33).

Immunophenotypic differences between nodular and diffuse subtypes were first described by Poppema *et al.* in 1979, and were later elaborated on by Pinkus and Said (41,42) who found that L&H variants in lymphocyte predominance, nodular Hodgkin's disease are

positive for B-cell specific antigens and negative for CD15 (LEUM1) antigen. On the other hand, immunological studies on the diffuse form, while being relatively few in number, have demonstrated a phenotype quite different from that described in the nodular form (12,20,47,57,61,63). In one of these a B-cell phenotype was expressed in only 4 out of a total of 15 cases of diffuse lymphocyte predominance Hodgkin's disease (20). All 4 cases lacked CD15 expression. These findings are analogous to those obtained in nodular lymphocyte predominance Hodgkin's disease. Six of the 15 cases expressed both CD15 and CD30 antigens and were reclassified as mixed cellularity, Hodgkin's disease, while another was reclassified as interfollicular Hodgkin's disease. In another study, a similar result was experienced; only a minority of cases of diffuse lymphocyte predominance Hodgkin's disease expressed a B-cell phenotype (36). In a more recent investigation in 1991, it was found that a much higher percentage of cases expressed a B-cell phenotype (59%)(70). These results are in direct contrast to those mentioned above and suggest an immunophenotype closely resembling that seen in nodular lymphocyte predominance Hodgkin's disease.

*2. Mixed cellularity Hodgkin's disease*

Histologically, the mixed cellularity subtype has three characteristic features. Firstly, classic Reed-Sternberg cells and Hodgkin cells are numerous and are easy to find. Other Reed-Sternberg cell variants may be present but are in the minority. Secondly, lymphocytes, histiocytes, plasma cells, eosinophils and neutrophils are present in appreciable numbers. Thirdly, the reticulin pattern is tangled and disorganized and lacks the birefringent collagen bands

seen in nodular sclerosis Hodgkin's disease. Microgranulomas and focal necrosis may be present.

Difficulties do arise in defining mixed cellularity Hodgkin's disease, e.g. lymphocyte predominance with an undue prominence of classic Reed-Sternberg cells, nodular sclerosis in the cellular phase with lacunar cells but no collagen bands, and lymphocyte depleted Hodgkin's disease characterized by a predominance of pleomorphic Reed-Sternberg cells, may all be classified as mixed cellularity Hodgkin's disease.

### *3. Nodular sclerosis Hodgkin's disease*

Nodular sclerosis Hodgkin's disease is the most commonly recognized histopathological subtype of Hodgkin's disease in many studies - in the clinical trials conducted by the British National Lymphoma Investigation, 75% of the cases are classified as nodular sclerosis Hodgkin's disease (56).

Nodular sclerosis Hodgkin's disease is characterized by broad collagen bands dividing the lymph node into well-defined nodules. Pronounced vascularity may be apparent in the collagen bands. Classical Reed-Sternberg cells and lacunar cells are present in significant numbers in the nodules. The cytological composition in the nodules may be one of lymphocyte predominance, lymphocyte depletion or mixed cell type. A case should be classified as nodular sclerosis if the features described above are present irrespective of the cell types seen. Granuloma formation is not unusual and there may be extensive necrosis in some cases.

Prior to 1985, nodular sclerosis Hodgkin's disease had been subclassified into lymphocyte predominance, mixed cellularity and lymphocyte depleted types on the basis of the cellular background and the relative number of lacunar cells. In 1985 the British National Lymphoma Investigation Panel showed that the cytological composition of the nodules may have a bearing on the prognosis (54). It was demonstrated that the prognostic value of the classification could be enhanced by the subdivision of nodular sclerosis Hodgkin's disease into two categories which MacLennan *et al.* named *type 1* and *type 2*, the distinction being based upon the cellular background, the relative number of lacunar cells present and the degree of cytological atypia of the Hodgkin's cells (64). They demonstrated unequivocally that there is a large and statistically significant survival difference at 5 years between patients with *type 1* and *type 2* (82,7% and 66,1% alive at 5 years respectively).

In *type 1* the nodules show either a predominance of lymphocytes or a mixed cellularity pattern, and the prognosis is better than any other subtype apart from lymphocyte predominance Hodgkin's disease. In *type 2* more than half the nodules show lymphocyte depletion or a mixed cellularity pattern associated with marked pleomorphism of Reed-Sternberg cells. The prognosis is less favorable than the mixed cellularity subtype and only marginally better than the lymphocyte depleted subtype (56,64). MacLennan *et al.* suggest that at least 25% of the cellular nodules should show adverse cytological features, i.e. pleomorphism of Reed-Sternberg cells and lymphocyte depletion, before a case is classified as *type 2*. Borderline cases should be classified as *type 1* (64).

Nodular sclerosis Hodgkin's disease shows type fidelity and does not appear to transform into other subtypes. The histological pattern remains stable over long follow-up periods and the same histological pattern is maintained in relapse biopsies (33,52,78). More recently, there has been some discussion as to whether or not nodular sclerosis, Hodgkin's disease can evolve into anaplastic large cell lymphoma (Personal communication, Professor K. Lennert: 1991), suggesting an overlap between these two disease processes.

Two other forms of nodular sclerosis Hodgkin's disease are recognized. In the so-called *cellular phase* of nodular sclerosis there is initial expansion of interfollicular or perifollicular zones by a neoplastic infiltrate in which lacunar cells and classical Reed-Sternberg cells are present in significant numbers. While there is an absence of prominent banded sclerosis, sclerotic change is often evident in the capsule. Subsequent development of birefringent collagen bands gives rise to the typical nodular pattern of nodular sclerosis. The cellular phase is considered to be an early stage in the development of nodular sclerosis Hodgkin's disease (33,52,78).

In another variant of nodular sclerosis, the so-called *syncitial variant*, there is a clustering of lacunar cells, particularly around areas of necrosis. They form sheets and cohesive nests often simulating the appearance of a metastatic carcinoma, melanoma, non-Hodgkin's lymphoma (and, more specifically, anaplastic large cell lymphoma) or thymoma. More characteristic features of nodular sclerosis Hodgkin's disease, such as collagen bands, are focally present in all cases. Immunohistochemical stains, such as LeuM1 (anti-granulocyte marker), Ki-1 (marker of

lymphoid activation), LCA (leucocyte common antigen, a marker of all leucocytes), S100 (a neural marker, positive in melanocytes) and the keratin stains, are of assistance in determining the correct diagnosis (4,6,11,15,19,25,59) - the absence of keratin expression in the large atypical cells excludes thymomas and carcinomas whereas expression of LeuM1 and Ki-1 favours nodular sclerosis Hodgkin's disease. LCA expression serves to distinguish non-Hodgkin's lymphoma from Hodgkin's disease. The tumour cells in Hodgkin's disease do not typically express LCA. LCA expression, together with Ki-1 antigen expression in the absence of LeuM1 positivity, suggests a diagnosis of anaplastic large cell lymphoma (vide infra). Lack of immunostaining for S100 protein usually permits exclusion of malignant melanoma. The morphologic and immunophenotypic characteristics of this variant suggest that it may represent evolution of Hodgkin's disease into anaplastic large cell lymphoma (Personal communication - Professor K. Lennert: 1991).

*4. Lymphocyte depleted Hodgkin's disease*

This group includes two morphologically different subtypes, designated as *diffuse fibrosis* and *reticular* in the Rye classification. In the *diffuse fibrosis* subtype, pleomorphic Reed-Sternberg cells are set in a background of heavy deposition of collagen fibres which are almost devoid of lymphocytes and other cells. The *reticular* subtype is characterized by the presence of a large number of pleomorphic Reed-Sternberg cells, many of which have a bizarre configuration, among atypical mononuclear cells and other elements. Foci of necrosis are more common than in other subtypes.

This subtype needs to be distinguished from non-Hodgkin's lymphoma of large cell type and from the syncitial variant of nodular sclerosis Hodgkin's disease.

## Immunohistochemistry of Hodgkin's Disease

In the last decade a large number of immunocytochemical markers have been applied to cases of Hodgkin's disease. Included in these are the conventional lymphocyte markers, such as those directed at the leucocyte common antigen, immunological markers directed at the monocyte/macrophage system, antigranulocyte markers, and antibodies directed against antigens which are considered to be indicative of lymphocyte activation. These markers have not proven to be as helpful in the investigation of Hodgkin's disease as they have been in non-Hodgkin's lymphoma (2). The results have tended to be variable and inconsistent, and are often difficult to interpret. Factors which may play a role in the interpretation of results, and which may lead to incorrect or misleading results, include differences in tissue fixation, whether or not trypsin digestion was employed, the particular method employed and the type, sensitivity and specificity of the antibody used in the study (1).

Reed-Sternberg cells consistently fail to express **leucocyte common antigen (CD45)**, the L&H variant in lymphocyte predominance, nodular subtype, being a notable exception (4,6,12,20,42). In the series reported by Hall *et al.*, the Reed-Sternberg cells in only 8% of cases of nodular sclerosis, mixed cellularity, and lymphocyte depleted Hodgkin's disease were positive for leucocyte common antigen (LCA), while, in sharp contrast, all their cases of nodular, lym-

phocyte predominance Hodgkin's disease were strongly positive (4). Dorfman *et al.* and Pinkus and Said obtained similar results in their two series (42,63). In none of their cases of nodular sclerosis and of mixed cellularity Hodgkin's disease did the Reed-Sternberg cells express LCA, while the Reed-Sternberg cells and L&H cells in all their cases of nodular, lymphocyte predominance expressed the antigen. Specific studies confined to investigating cases of lymphocyte predominance, nodular subtype have confirmed these findings (20,36,70). Published immunophenotypic studies of diffuse, lymphocyte predominance Hodgkin's disease report conflicting results (vide infra). One of these has shown the neoplastic cells to have a phenotype similar to that seen in nodular, lymphocyte predominance Hodgkin's disease (i.e. LCA positive), while in another, the cells failed to express LCA and have a phenotype similar to that seen in nodular sclerosis and mixed cellularity Hodgkin's disease (20,70).

B- and T-cell markers have been negative in some studies (13,61) whilst others have demonstrated the presence of B- and T-cell associated antigens (see discussion below) (8,10,11, 12,14,18,23,46). The L&H variant in lymphocyte predominance, nodular subtype, consistently expresses B-cell antigens (20,46).

In paraffin sections, the cytoplasm of Reed-Sternberg cells gives strongly positive reactions with **immunoglobulin antibodies:** The pattern is polytypic in character suggesting that Reed-Sternberg cells take up the immunoglobulins by a process of absorption - in fact, in frozen section material on which highly sensitive immunostaining techniques are used, Reed-Sternberg cells do not stain positively for kappa and lambda chains. This is also true

for those cases in which there is a strong staining in paraffin embedded tissue, which indicates either that positive staining for immunoglobulins in paraffin tissue is probably artifactual or that the cytoplasmic immunoglobulins are inaccessible in frozen section. In most cases, the staining in paraffin sections probably represents passive uptake of immunoglobulin during formol fixation, i.e. it does not represent true synthesis of immunoglobulin by the Reed-Sternberg cell. It has now been shown that Reed-Sternberg cells lack mRNA for immunoglobulin synthesis in the majority of cases (46,49).

With the exception of those in lymphocyte predominance Hodgkin's disease, Reed-Sternberg cells do not possess **cytoplasmic J chain**, the presence of which is highly characteristic of B-cell lineage (vide supra)(46,49). Stein *et al.* demonstrated J chain in the cytoplasm of Reed-Sternberg cells and L&H cells in 20 out of 32 cases of nodular, lymphocyte predominance Hodgkin's disease (57). The Reed-Sternberg cells in 54 cases of nodular sclerosis, mixed cellularity and lymphocyte depleted Hodgkin's disease did not contain J chain. Only 1 case out of a total of 11 cases of diffuse, lymphocyte predominance Hodgkin's disease contained J chain.

Reed-Sternberg cells constantly lack markers directed towards the **monocyte/macrophage** system (4,6,46). Occasional reports of Reed-Sternberg cells and their variants expressing monocyte/macrophage/histiocyte markers have been published (48). The consensus of opinion is, however, that the Reed-Sternberg cell does not express these antigens in the majority of cases.

Antibodies to **epithelial membrane antigen (EMA)** give a pattern of reactivity for Reed-Sternberg cells similar to that for leucocyte common antigen and only the L&H variant in lymphocyte predominance subtype stains positively with any regularity. Jackson *et al.* in his series of 82 cases of non-lymphocyte predominance Hodgkin's disease found only 2 cases in which the Reed-Sternberg cells expressed EMA, while all 4 cases of the lymphocyte predominance, nodular subtype expressed EMA on their Reed-Sternberg cells and L&H variants (17). These results have subsequently been verified by several other studies (12,13,20).

Antibodies directed against **granulocyte markers** and, more specifically, the **CD15** antibodies directed against an oligosaccharide called hapten X, react positively with Reed-Sternberg cells and their variants in most cases belonging to the nodular sclerosis, mixed cellularity and the lymphocyte depleted subtypes, but not as a rule in the lymphocyte predominance, nodular subtype (4,6,11,12,13,14,16,17,20,42,57,58,59). In the diffuse form of the lymphocyte predominance subtype results have been equivocal (17,57).

CD15 antibodies were in the past used extensively in the diagnosis of Hodgkin's disease. However, not all Reed-Sternberg cells are recognized, and it is acknowledged that CD15 also stains cells in non-Hodgkin's lymphoma, including Reed-Sternberg-like cells (4,6,11,12,58,63). In a review of published literature in 1987, Hall *et al.* found the sensitivity and specificity in detecting cases of Hodgkin's disease, using CD15 antibodies, to be only 80% and 80,6% respectively (17). CD15 immunostaining can thus not be regarded as a sensitive or specific marker of Hodgkin's disease.

In a subsequent study, it was shown that the prognosis of Hodgkin's disease is more favorable in CD15 antigen positive cases and that this marker may be useful as a prognostic factor in Hodgkin's disease (47).

Reactions to antibodies directed against the **S100 antigen**, an antigen strongly expressed in interdigitating reticulum cells, are consistently negative in Reed-Sternberg cells and their variants (14,46). In a series of 23 cases, Kornstein *et al.*, found that none of the Reed-Sternberg cells expressed S100 antigen (58).

In 1990 two papers were published documenting the reactivity of Reed-Sternberg cells with antibodies against **vimentin** (30,31). Vimentin is the most primitive intermediate filament and is the main intermediate filament in haemopoietic cells. Expression is highly variable within the different lineages of these cells. It is expressed in monocytes, macrophages and interdigitating reticulum cells while it disappears completely during the maturation of erythrocytes and megakaryocytes. In B-lymphocytes it is expressed only in the immature stage, while in T-lymphocytes it is retained through all stages of development up to and including the mature cell.

In one paper, it was reported that Reed-Sternberg cells and Hodgkin's cells were strongly positive for vimentin in 45 of 63 cases of Hodgkin's disease (31). The authors indicated that Hodgkin's disease could be divided into three groups on the basis of different patterns of antigen expression by Reed-Sternberg cells and Hodgkin's cells. The first group could be interpreted as cases in which Reed-Sternberg cells and Hodgkin's cells originated from

germinal center B-cells - they expressed L26 and LN1 (and often EMA) with absence of vimentin and LeuM1 expression. LN1 is expressed by germinal B lymphocytes and L26 by mature B lymphocytes. Seven of the 10 cases which expressed this pattern belonged to the lymphocyte predominance, nodular subtype. The second group (5 cases) expressed L26 and vimentin and did not express LN1 and EMA. It was suggested that this group represented immature B-cell Hodgkin's disease. The third group was the largest, with the Reed-Sternberg cells and Hodgkin's cells expressing vimentin and/or LeuM1 in 48 cases. L26, LN1 and EMA were not expressed in these cases. None of these 48 cases expressed S100 positivity - the strong positivity for vimentin coupled with LeuM1 positivity suggests, in the authors view, monocytic or histiocytic origin of these cells. This paper highlights once again the heterogeneity within Hodgkin's disease (31).

In the second study, 38 cases were examined and stained for vimentin (30). In only thirteen of the cases did the Reed-Sternberg cells express vimentin. All of the positive cases belonged to the nodular sclerosis subtype. The Reed-Sternberg cells in 56,5% of cases of nodular sclerosis subtype expressed vimentin in the study. The significance of these findings with respect to the origin of the Reed-Sternberg cell is unclear.

Monoclonal antibodies to vimentin appear to be of limited value in establishing the diagnosis of non-Hodgkin's lymphoma. Giorno *et al.*, in his series of 30 cases of non-Hodgkin's lymphomas, found that only 11 of them expressed vimentin in the malignant cells, and there was no correlation between vimentin immunoreactivity and the histologic type of lymphoma (60). More studies need to be

undertaken to assess the importance of vimentin staining of the Reed-Sternberg cell in Hodgkin's disease. This is one of the aims of the investigation reported in this dissertation.

Reed-Sternberg cells commonly express antigens that are considered to be **markers of lymphocyte activation.** These include **class 2 histocompatibility antigens** and receptors for **interleukin** 2 and **transferrin** (61,66). The antibody **Ki-1** also identifies lymphoid cells during the activation process (66) and is directed against the **CD30** antigen. A closely related antibody effective in paraffin sections and called **BerH2** has been used in numerous studies of Hodgkin's disease.

Chittal *et al.* in 1988 showed a clear phenotypic separation of nodular, lymphocyte predominance Hodgkin's disease from the other subtypes - the neoplastic cells in nodular, lymphocyte predominance Hodgkin's disease were all negative for BerH2, whereas the Reed-Sternberg cells in a proportion of the other subtypes were positive (12). This was confirmed again in a more recent investigation in 1990 aimed specifically at cases of lymphocyte predominance, nodular Hodgkin's disease in which it was found that the neoplastic cells were uniformly negative for BerH2 antibody (43). Several other workers have confirmed this finding in lymphocyte predominance, nodular Hodgkin's disease (4,6,13,20,46,36). In a study by Nicholas *et al.* it was reported that 6 cases out of a total of 57 cases of nodular, lymphocyte predominance Hodgkin's disease expressed the Ki-1 antigen although, subsequently, several of these cases were later reclassified as belonging to the mixed cellularity group of Hodgkin's disease (20). Bishop *et al.* studied a series of 17 cases of diffuse, lymphocyte predominance Hodgkin's

disease but found none which were positive for BerH2 (70). Numerous other studies have confirmed expression of Ki-1 antigen by Reed-Sternberg cells in all the other subtypes of Hodgkin's disease, i.e. nodular sclerosis, mixed cellularity and lymphocyte depleted subtypes of Hodgkin's disease (4,6,12,13,23,25,29,46). The various subtypes of Hodgkin's disease are therefore heterogeneous in their expression of Ki-1 antigen.

In 1985 Stein *et al.* described a new type of non-Hodgkin's lymphoma, which they termed the anaplastic large cell lymphoma or Ki-1 lymphoma, in which the tumour cells also react with the CD30 antibody (66). Stein *et al.* have suggested that there may be a close cellular relationship between Hodgkin's disease and this lymphoma. There is evidence that the Reed-Sternberg cells in Hodgkin's disease release lymphokines and other substances that account for the characteristic heterogeneous reactive cellular infiltrate occurring in this condition. It is possible that, in contrast, the neoplastic cells in Ki-1 positive large cell lymphoma are non-secretory and hence attract relatively few reactive cells. Whether or not anaplastic large cell lymphoma represents the link between Hodgkin's disease and non-Hodgkin's lymphoma is a matter still under debate at present (10,66).

It appears that there are immunophenotypic differences in the neoplastic cells among different cases of **nodular sclerosis Hodgkin's disease.** LeuM1, which, as mentioned above, is only positive in 70 to 80% of all cases of Hodgkin's disease (17), also appears to vary in expression in the nodular sclerosis subtype (12). Variability of antigen expression associated with markers of B and T-cells has also been described (29,48). In one investigation using immuno-

electronmicroscopy, T-cell antigen expression was found in 6 of 20 cases (29). Similar heterogeneity with respect to both T- and B-cell markers was found in other investigations (48). In addition, several reports of monocyte/macrophage/histiocyte antigen expression in nodular sclerosis Hodgkin's disease leave unanswered the question of whether there is a subset of cases that have this phenotype (48,80). Gene rearrangement studies have shown that cases of nodular sclerosis Hodgkin's disease are frequently associated with rearranged immunoglobulin genes (vide infra). Thus, it appears that among cases of nodular sclerosis Hodgkin's disease there is no specific genotype or no constant immunophenotype. It seems, therefore, that although nodular sclerosis Hodgkin's disease is considered to be a discrete entity according to the Rye classification, it is, in fact, heterogeneous in its histological, immunophenotypic and genotypic characteristics.

The immunophenotypic characteristics of the tumor cells in **lymphocyte predominance Hodgkin's disease** have been alluded to in the discussion of this subtype above. The Reed-Sternberg cells and L&H variants thereof are, in the **nodular** subtype, characteristically positive for B-cell specific antigens and are negative for LeuM1, EMA and CD30 markers. Conflicting reports regarding the immunophenotype of L&H variants in **diffuse**, lymphocyte predominance Hodgkin's disease have been encountered. The published consensus of opinion has it that the phenotype of the L&H cells conforms more closely to the phenotype in nodular sclerosis and mixed cellularity Hodgkin's disease. Bishop *et al.* have, however, found the opposite in their series, i.e. B-cell positivity in 59% of their cases, with LeuM1 and EMA antigen expression in a minority of cases. CD30

markers were negative in all their cases (70). These results do not differ greatly from the findings in nodular lymphocyte predominance Hodgkin's disease. Hansman *et al.*, in a series of 20 patients with mixed nodular/diffuse lymphocyte predominance Hodgkin's disease and diffuse lymphocyte predominance Hodgkin's disease, reported a similar experience - positivity with L26, a B-cell marker in 14/20 of the cases and negativity of LeuM1 in all but one of them. (75). These findings suggest that the tumour cells in nodular, nodular/diffuse, and diffuse lymphocyte predominance Hodgkin's disease are B-cell derived and closely related, and provides justification for the grouping of these subtypes into one subgroup of Hodgkin's disease, i.e. lymphocyte predominance Hodgkin's disease. Diffuse lymphocyte predominance Hodgkin's disease and nodular lymphocyte predominance Hodgkin's disease may thus represent variants of the same disease entity. Nicholas *et al.*, on the other hand, in their series of 15 cases of diffuse lymphocyte predominance Hodgkin's disease, found that only a minority of cases with a diffuse architecture (4/15) expressed a B-cell phenotype (20). Seven of their cases, however, contained classic Reed-Sternberg cells which expressed CD15 and CD30 antigens - all 7 were reclassified as mixed cellularity or interfollicular Hodgkin's disease. Some workers argue for a three-fold subdivision of lymphocyte predominance Hodgkin's disease (57,70) with nodular and diffuse forms and, in addition, a third subtype of mixed lymphocyte predominance Hodgkin's disease in which the atypical cells are of classic type. The nodular and diffuse types are thought to be closely related, both with clearly discernible L&H type cells. The mixed form of lymphocyte predominance Hodgkin's disease is thought to be closely related to mixed cellularity Hodgkin's

disease, i.e. CD15/CD30 positivity of Reed-Sternberg cells, from which it differs only in the predominance of lymphocytes among its background cells. Whether this subdivision will find a place in future classifications still remains to be seen.

When lymphocyte predominance Hodgkin's disease is defined morphologically (as is done in most studies) there is an overlap in its phenotype with other forms of Hodgkin's disease. This suggests that morphology is an imprecise discriminant, and that morphologically defined lymphocyte predominance Hodgkin's disease may be impure, consisting as it apparently does of a mixture of B-cell derived disease and "conventional" Hodgkin's disease which happens to have a lymphocyte predominance appearance. This raises the questions as to whether lymphocyte predominance Hodgkin's disease should be redefined in phenotypic terms, whereby the atypical cells are B-cell marker positive and CD15 and CD30 negative and to whether morphology or immunophenotype should form the primary diagnostic criterion for the definition of lymphocyte predominance Hodgkin's disease.

In the **mixed cellularity** and **lymphocyte depleted** forms of Hodgkin's disease, the Reed-Sternberg cells and their variants are positive for CD15, CD30 markers and negative for LCA and EMA in the majority of cases (4,13,33). This pattern of staining is similar to that seen in nodular sclerosis Hodgkin's disease. In only a minority of cases are the neoplastic cells negative for CD15 and CD30 markers (12). Markers for B- and T-cell antigens are negative in the majority of studies published (4,11,12,13,14). Cibull *et al.*, using a marker for CD3 antigen (specific for T-cells), showed that a proportion of Reed-Sternberg cells and their variants in a series

of cases of nodular sclerosis, mixed cellularity, and lymphocyte depleted Hodgkin's disease expressed this antigen, thus suggesting a T-cell origin in these cases (18). Expression of T-cell antigens by Reed-Sternberg cells was found in nodular sclerosis, mixed cellularity but not in lymphocyte predominance Hodgkin's disease by Kadin *et al.* (29). Likewise, studies using L26 antibodies (specific for B-cells), have shown that Reed-Sternberg cells and their variants express this marker in a minority of cases (4,6,8). Norton *et al.*, in a series of 27 cases, found 6 cases of mixed cellularity Hodgkin's disease which expressed L26 (8). Thus it seems that while most cases are negative for B- and T-cell markers in mixed cellularity, and lymphocyte depleted Hodgkin's disease, positive cases have been described, and as in the nodular sclerosis Hodgkin's disease described above, these subtypes also appear to be heterogeneous in their immunophenotypic characteristics.

In conclusion, it is apparent that there is no single immuno-histochemical finding which can be regarded as pathognomonic of Hodgkin's disease. However, two fairly distinct immuno-histochemical profiles for the Reed-Sternberg cell and its variants can be constructed. In all but the lymphocyte predominance subtypes, the Reed-Sternberg cell and its variants react positively with CD15 and CD30 antibodies, and weakly or not at all with EMA and leucocyte common antigens. In addition, J chain cannot be demonstrated in the cytoplasm. In the lymphocyte predominance, nodular subtype a pattern is seen that is almost the complete reverse: CD15 and CD30 negativity, leucocyte common antigen and EMA positivity and the presence of J chain in the cytoplasm. Furthermore, the L&H variants are usually positive for B-cell markers. Lymphocyte pre-

dominance Hodgkin's disease appears to be morphologically, immunophenotypically and clinically a separate entity, i.e. a B-cell non-Hodgkin's lymphoma. Within the remaining subtypes, while most are of null phenotype, i.e. do not express T- or B-cell markers, cases of both T and B lineage do occur. It must, however, always be borne in mind that variability in staining pattern may be observed in a number of cases of all subtypes of Hodgkin's disease.

## Gene rearrangement techniques

Similar inconsistencies have been encountered when gene re-arrangement techniques have been applied to the study of Hodgkin's disease (55,68,72,73). The low density of tumour cells makes it difficult to identify genetic abnormalities and, even if abnormalities are found, it is difficult to be certain whether they relate to the tumour cells or to reactive lymphoid cells (55). B and T lymphocytes undergo rearrangements of immunoglobulin and T-cell receptor genes early in their differentiation pathways. Heavy chain genes apparently rearrange earlier than light chain genes in B-cell development, and exclusive rearrangements of heavy chain genes are frequently found among pre-B-cell tumours. In T-cells, three genes undergo somatic rearrangement, but the order in which this occurs, and the specificity of rearrangement in determining the lineage of a cell are not well established. Griesser *et al.* showed that rearrangement of T-cell receptor beta chain occurs before the T-cell receptor gamma chain gene, and that rearrangement of the gamma chain locus by itself is a poor indicator of cell lineage (69).

Rearrangements are random so that the structure of the resulting gene varies widely from cell to cell. In B- and T-cell malignancies, clonal expansion of a single cell results in all members of the clone having the same rearrangement. In addition, the genes that are rearranged in the tumour cells give some indication as to lineage of the cell. Clonal rearrangements of immunoglobulin genes have been detected in several cases mainly of the nodular sclerosis subtype. These rearrangements are located mainly in the light chain genes but clonal rearrangements of both light and heavy chains have also been found (55,77). These results suggest a B-cell origin since light chain rearrangements are confined to cells of B-cell lineage. Clonal rearrangement of the T-cell receptor genes have been found in the absence of clonal immunoglobulin gene rearrangement suggesting a T-cell derivation of the Reed-Sternberg cell (69). Weiss *et al.*, in a series of 8 cases found no rearrangements of T-cell receptor genes (55). Roth *et al.* in a series of 18 cases found T-cell receptor beta chain gene rearrangement in one case of mixed cellularity Hodgkin's disease (71). No immunoglobulin gene rearrangements were detected).

In a review article on 139 pooled cases reported in the literature, Anastasi *et al.* found that only 45 of them demonstrated gene rearrangements (48). In the majority of cases there were no rearrangements of either the immunoglobulin gene or the T-cell receptor gene. This can be interpreted as indicating either absence of monoclonal B-cells or T-cells, or that there were insufficient numbers of such cells present to allow for adequate detection by the methods used. Rearranged heavy and light chain genes were present in 16 cases, and rearranged immunoglobulin light chain

genes alone in 4. Rearrangements of the T-cell receptor beta chain genes or beta and gamma chain genes were present in 10 cases, whereas rearranged T-cell receptor gamma chain genes alone were detected in the remaining 15 cases.

In correlating these findings with histological subtypes, it would appear that cases of nodular sclerosis Hodgkin's disease are frequently associated with rearranged immunoglobulin genes, but even this trend is not consistent. 5 of 6 cases of nodular sclerosis Hodgkin's disease, in one reported series, had rearrangements of T-cell receptor genes (72). Thus, it appears that among the cases of nodular sclerosis Hodgkin's disease there is no specific genotype. There is even less data available concerning the mixed cellularity, and lymphocyte depleted subtypes of Hodgkin's disease that can be used as a basis for speculation.

In summary, while the results of gene rearrangement studies are inconclusive and confusing, they do suggest a B- and/or T-cell origin of the Reed-Sternberg cell in a proportion of cases of Hodgkin's disease.

## ANAPLASTIC LARGE CELL LYMPHOMA

Anaplastic large cell lymphoma (Ki-1 lymphoma) was first recognized as an entity in 1982 and described in detail in 1985 (66). It is composed entirely of anaplastic large cells reactive with the monoclonal antibody, Ki-1 (later designated CD30), which was first raised against a Hodgkin's disease cell line. Retrospective analyses of cases diagnosed as histiocytic malignancies and large cell lymphoma showed that the neoplastic cells in some of these tumours expressed the Ki-1 antigen and, furthermore, shared certain distinct morphological features (24). Immunophenotyping of the neoplastic cells has shown most to be of T-cell phenotype with the remaining being of B-cell or null-cell phenotype (vide infra) (21,22,24).

Two cytomorphologically distinct groups of anaplastic large cell lymphoma have been delineated (21). Clinically, the two groups of lymphomas differed with respect to stage of disease, frequency of bone marrow involvement and median survival rate. In the first group, the lymphomas are composed predominantly of large pleomorphic cells with moderate grey-blue or deep blue-staining cytoplasm. A poorly demarcated paranuclear halo is often visible. Multinucleated giant cells are frequently observed, some of which display Reed-Sternberg-like features. The nuclei contain one or, less frequently, more than one large, prominent nucleolus. Wreath-like nuclei are found in all these cases and embryo-like nuclei are a regular feature.

In the second group, the lymphomas have a relatively monomorphic appearance due to less variability of cell size and less pronounced

nuclear pleomorphism. The cytoplasmic basophilia is moderate or intense in the majority of cases. Paranuclear halos are rarely detected. Multinucleated giant cells and Reed-Sternberg-like cells are rare. Large nucleoli are not a prominent feature, the nuclei more often containing multiple delicate nucleoli. Wreathlike and embryo-like nuclei are not a constant feature.

In a study published in 1989, Chan *et al.*, also described two cell types in these lymphomas (22). Their type 1 cells correspond well with those present in the second group described above, the only difference being that these cells were described as having an angulated border which imparted a squamoid appearance to them. Their description of the type 2 cells correspond well with those in the first group described above.

Partial infiltration of lymph nodes occurs in 40 to 50% of cases of anaplastic large cell lymphoma, with total obliteration of the nodal architecture occurring in the remainder. The pattern of involvement is distinctive in that the T-zones or paracortical areas are preferentially involved with sparing of follicles in the majority of cases. The cells are often cohesive, imparting a trabecular pattern to the involved areas. The sinuses are frequently infiltrated by the lymphoma cells. Fibrous thickening of the capsule, and distinct fibrous bands within the node which subdivide it into nodules, are frequently found. The large lymphoma cells are often intermingled with lymphocytes, histiocytes, plasma cells and/or eosinophils (22,24). This background, together with the presence of occasional Reed-Sternberg-like cells, may cause confusion resulting in an incorrect diagnosis of Hodgkin's disease, particularly the syncitial variant

of nodular sclerosing Hodgkin's disease. As mentioned above, this form of nodular sclerosis Hodgkin's disease may represent a stage in the evolution of Hodgkin's disease to anaplastic large cell lymphoma. Immunologic studies may be useful in determining the correct diagnosis (vide infra) (24).

Anaplastic large cell lymphoma most commonly presents with peripheral lymphadenopathy. Extranodal disease at presentation was documented in 40% of 41 cases in one investigation, either in combination with lymphadenopathy or strictly confined to extranodal tissues (21). The skin represents the preferential site of primary extranodal Ki-1 lymphoma (21,24,32). Bone marrow involvement was documented in 30% of cases in the abovementioned study, and occurred exclusively in patients over 40 years of age. Primary extranodal involvement of the bone and lung have not, as yet been reported.

Anaplastic large cell lymphoma can mimic a variety of tumours. In a recent case report a dermal tumour presenting on the leg of a 45 year old male patient was described which, on histological examination, showed a myxoid stroma reminiscent of myxoid malignant fibrous histiocytoma. Subsequent lymph node biopsy revealed a well developed storiform pattern also reminiscent of a malignant fibrous histiocytoma. Immunohistochemistry, however, confirmed the lymphoid nature of the tumour (LCA, Ki-1 and T-cell marker positivity of the tumour cells) (45).

A unique chromosomal abnormality, i.e. t(2;5)(p23;q35) has recently been demonstrated in 2 cases (28). Further karyotypic analysis of a greater number of cases is required to determine the importance

of this finding. Ultrastructural studies performed on 14 cases of anaplastic large cell lymphoma demonstrated numerous surface microvillous projections in 3 of them (44). These are indistinguishable from those present in microvillous (anemone, filiform) lymphomas. Histologically, microvillous lymphomas and anaplastic large cell lymphomas, have certain features in common; Sinus involvement is frequently present in the former tumour, although the growth pattern is mostly diffuse in nature, the tumour cells not often forming syncitial sheets. Immunologically, these tumors do not express CD30, are EMA negative and a high percentage (86% in this study) are of B-cell phenotype whereas anaplastic large cell lymphomas express CD30, are frequently EMA positive, and the majority are of T-cell phenotype (vide infra). Microvillous lymphomas should be considered in the differential diagnosis of anaplastic large cell lymphoma, particularly in those with a sinus growth pattern. Immunophenotypic analysis plays an important role in distinguishing between these two tumours.

**Immunohistochemistry of anaplastic large cell lymphoma**

The Ki-1 monoclonal antibody was originally raised in 1982 against a Hodgkin's disease-derived cell line. It reacts with both Hodgkin cells, of all histological types, and an otherwise undefined population of cells in the perifollicular areas of normal lymphoid tissues. At the Third International Workshop and Conference on Human Leucocyte Differentiation Antigens in 1986, the Ki-1 antibody was evaluated and included in a new cluster of differentiation i.e. CD30. The antigen with which it reacted is considered to be lymphocyte activation-associated. Another monoclonal antibody, BerH2,

which was prepared in 1989, has the additional capability of being able to recognize a different formaldehyde-resistant epitope, facilitating the demonstration of the CD30 antigen in routinely processed histological material (25).

In 1985 it was shown that 2-7% of non-Hodgkin's lymphomas are Ki-1 positive and that these fall into two groups (66). In the first group, Ki-1 is expressed in a variable proportion of tumour cells. This first group encompasses cases of mycosis fungoides, pleomorphic T-cell lymphoma, T-immunoblastic lymphoma and Lennert's lymphoma.

In the second group, nearly all the tumour cells express Ki-1. This group encompasses the anaplastic large cell lymphomas (Ki-1 lymphoma). Furthermore, Tavares De Castro and Stein, found that, immunologically, anaplastic large cell lymphomas can be subdivided into 4 groups on the basis of expression of B- or T-cell markers as follows (10): (1) 60% express T-cell markers; (2) 14% exclusively B markers; (3) 20% express both T and B markers; and (4) 6% have neither B nor T markers. All the cases in this study expressed other markers of lymphoid activation, i.e. HLA-DR and interleukin-2 receptor.

In two studies, using antibodies effective in paraffin sections, it was found that all cases of this type of tumour stained positively for the CD30 antigen but were negative for LeuM1 (24). In one of the studies all cases stained positively for LCA, whereas in the other only 54% were positive (21). In the latter study the neoplastic cells expressed EMA in 58% of the cases. In the former study a T-cell phenotype was demonstrated in 72% of the cases and

only one case showed equivocal staining for B-cell antibodies, while in the latter study, 68% were classified as T-cell lymphomas, and 10% as B-cell lymphomas, but the remaining 22% could not be assigned to a certain lineage and were designated as anaplastic large cell lymphomas of null cell type. Falini *et al.*, in a large series of 165 cases demonstrated a 38% negativity rate of the tumor cells for CD45 antigen (LCA) and a 70% positivity rate for EMA. (81).

In accordance with the above findings Hansmann *et al.*, in a study of 11 cases published in 1991, demonstrated reactivity in the tumour cells in all 11 cases for CD30. Of their 11 cases, however, the tumour cells in 2 cases were positive for LeuM1 (82). Dorfman *et al.* also showed LeuM1 positivity in 4 cases of anaplastic large cell lymphoma (63).

In conclusion, anaplastic large cell lymphomas represent an immunologically heterogeneous category of lymphoid neoplasms derived from activated lymphoid cells, most of which appear to be of T-cell phenotype. Expression of LCA, LeuM1 and EMA is variable, a fact which should be borne in mind when making the diagnosis of anaplastic large cell lymphoma.

### Gene rearrangement studies

An investigation of large cell lymphomas which express the CD30 antigen confirmed the previous phenotypic evidence that these tumours are of heterogeneous cell lineage (vide supra); 16 of 30 cases demonstrated T-cell receptor beta gene rearrangement and 6 immunoglobulin gene rearrangement (27). Of the 8 cases in which

neither immunoglobulin nor T-cell beta gene rearrangement could be demonstrated, phenotypic evidence suggested a T-cell lymphoma in 2 cases and a B-cell lymphoma in 4. Disparity, therefore, occurs in some cases between phenotypic and genotypic evidence of T or B clonality in this group of lymphomas.

**Hodgkin's disease verses anaplastic large cell lymphoma**

Hodgkin's disease and anaplastic large cell lymphoma have many striking histological similarities. In the latter tumour many of the cells resemble Hodgkin cells while others resemble Reed-Sternberg cells, and both tumours express markers of lymphocyte activation. Furthermore, some cases of Hodgkin's disease evolve into Ki-1 positive anaplastic large cell lymphoma and the two entities can coexist in the same lymph node or in different lymph nodes of the same patient (10,Personal communication: Professor K. Lennert).

Problems may arise in differentiating between Hodgkin's disease, particularly the syncitial variant of the nodular sclerosing type, and anaplastic large cell lymphoma. The combined application of LeuM1, LCA and EMA to these cases can be very helpful. Negative staining for LeuM1 and positive staining for LCA and/or EMA strongly suggest the diagnosis of anaplastic large cell lymphoma, although LeuM1 positivity has been reported to occur in anaplastic large cell lymphoma (vide supra). Reed-Sternberg cells are positive for LeuM1 and negative for LCA in the majority of cases (11,12,13,16,17,33). The lymphocyte predominance type of Hodgkin's disease is unlikely to be confused histologically with anaplastic large cell lymphoma. It should also be borne in mind that, as

opposed to anaplastic large cell lymphoma, Hodgkin's disease rarely involves the skin (24). In a comprehensive review of 465 cases of Hodgkin's disease only 3,4% (16 cases) had histologically verified cutaneous involvement (65), compared to 15% in anaplastic large cell lymphoma (32).

Tavares De Castro and Stein (1988) have, after review of all the evidence, come to the conclusion that Hodgkin's disease and anaplastic large cell lymphoma are related entities and that they result from the neoplastic transformation of activated lymphocytes of either B or T-cell origin (10). The main difference between the two disease processes is that, in Hodgkin's disease, there is a prominent reactive component which is absent in the other tumour. This difference can be explained by the production of lymphokines by the neoplastic cells of Hodgkin's disease, a property not shared by the neoplastic cells in anaplastic large cell lymphoma. Cases in which nodular sclerosis Hodgkin's disease has apparently evolved into anaplastic large cell lymphoma can be explained by diminution of lymphokine production with tumour progression.

The similarity between Hodgkin's disease and anaplastic large cell lymphoma may have important therapeutic implications. Intensive chemotherapy regimens used in the treatment of anaplastic large cell lymphomas, and which yield high cure rates in this disease, may be of value in the treatment of advanced Hodgkin's disease.

## MATERIAL AND METHODS

### Tissue analysed

The material for this study was obtained from the surgical pathology records of the Department of Anatomical Pathology, University of Cape Town and most of the cases emanated from Groote Schuur Hospital. In addition, those cases referred from private laboratories in and around Cape Town in which suitable material was still available, were also included. The period selected was 1985, and 1987 to 1990, and all cases diagnosed in this 5 year period were included in the study. Those cases acquired during 1986 were excluded from the study, as a sufficient number of cases was obtained in the above 5 year period. The tissue analysed was fixed in 10% formol saline or B5, followed by routine processing and paraffin-wax embedding. All were stained with haematoxylin and eosin and reassessed morphologically before immunohistochemical analysis was performed. The number of cases investigated and their original diagnoses are outlined in Figures 1 and 2.

The majority of cases were lymph node biopsies, with two biopsies of epidural tissue included. Initial reassessment was based on morphology as seen in haematoxylin and eosin stained sections using histologic criteria as set forth by Lukes and Butler and subsequently modified at the Rye conference. Cases of nodular sclerosis Hodgkin's disease were further subdivided into types 1 and 2 on the basis of lymphocyte depletion and cellular pleomorphism, as previously described by MacLennan *et al.* (vide supra) (56,64).

**Immunohistochemical analysis**

All cases in the study were subjected to immunohistochemical analysis using a panel of 13 different antibodies which are detailed, together with their cluster of differentiation (CD), molecular weight, specificity, source and pattern of staining in Table 1 and Table 2. All the antibodies used are monoclonal antibodies, with the exception of CD3, a polyclonal antibody. The antibody against the intermediate filament, vimentin, was also included in the panel since it has been shown that Reed-Sternberg cells can express this antigen (vide supra) (30,31). The antibodies in the panel were selected on the basis of those used in numerous previously published studies in which monoclonal antibody panels were employed in attempts to determine which antigens were expressed by the Reed-Sternberg cell and its variants in Hodgkin's disease (4,11,12,13,61,75), as well as those used in several studies in each of which only one antibody was assessed (8,15,17,18,25,26,29,58,63,66,67,76). All B-cell positive tumours underwent further investigation for expression of surface and cytoplasmic immunoglobulins using polyclonal antibodies (Dakopatts) to kappa and lambda light chains, and IgG, IgM and IgA heavy chains.

Methods employed in this study for labelling of antigens included the two and three stage indirect immunoperoxidase technique (85) and the 3-stage peroxidase-conjugated streptavidin procedure (a modification of the avidin-biotin complex method of Hsu *et al.*) (84). A peroxidase-antiperoxidase method was used for the detection of cytoplasmic immunoglobulins and the streptavidin technique for the detection of surface immunoglobulins (85).

In the initial stages of the study only the indirect peroxidase procedures were applied. Subsequently, however, only the

streptavidin technique was employed, as this is a more sensitive method for the labelling of antigens in histological specimens (1,84). For the CD3 antibody, the streptavidin procedure was applied for the entire study.

| ANTIBODY | SOURCE | PATTERN OF STAINING |
|---|---|---|
| DakoM1* | DAKOPATTS | Paranuclear, variable membrane staining. |
| BerH2 | DAKOPATTS | Membrane and paranuclear staining. |
| EMA | DAKOPATTS | Membrane and variable paranuclear staining. |
| LCA | DAKOPATTS | Membrane staining. |
| TUCHL1 | DAKOPATTS | Membrane staining. |
| PanB | DAKOPATTS | Membrane staining. |
| CD3 | DAKOPATTS | Perinuclear cytoplasmic or horseshoe-like distribution around periphery of cytoplasm and variable membrane staining. |
| L26 | DAKOPATTS | Membrane staining. |
| LN1 | ICN | Membrane and paranuclear, occasional diffuse cytoplasmic staining. |
| MB2 | DAKOPATTS | Cytoplasmic staining. |
| CD68 | DAKOPATTS | Membrane and cytoplasmic staining. |
| CD21 | DAKOPATTS | Membrane staining. |
| Vimentin | DAKOPATTS | Diffuse cytoplasmic or membrane, variable paranuclear or crescent shaped area along nuclear membrane. |

Table 1. Pattern of antibody staining and source of antibody (5,79).
LCA = Leucocyte common antigen. EMA = Epithelial membrane antigen.
*DakoM1 and LeuM1 have a similar specificity - LeuM1 is the better known antibody and is more frequently referred to in the literature (33, 78). Hence, all further reference to DakoM1 in this study will be as LeuM1.

| ANTIBODY | ANTIGEN | MW | MAIN CELLULAR DISTRIBUTION |
|---|---|---|---|
| LeuM1 | CD15 | 105-200 | Granulocytes, Reed-Sternberg cells, epithelium, dendritic reticulum cells. |
| BerH2 | CD30 | 105-120 | Activated lymphoid cells, Hodgkin and Reed-Sternberg cells, malignant cells in anaplastic large cell lymphoma, embryonal carcinoma, macrophages. |
| EMA | | 34,49 | Plasma cells, epithelium, Reed-Sternberg cells in lymphocyte predominance, nodular Hodgkin's disease, some non-Hodgkin's lymphomas. |
| LCA | CD45 | 190-220 | Leucocyte restricted, occasional histiocytes. |
| TUCHL1 | CD45RO | 185 | Mainly T-cells, B-cell subset, monocytes, macrophages. |
| PanB | CD45R | 220,205 | B-cells, occasional T-cells. |
| CD3 | CD3 | 19,21,26 | T-cells, Purkinje cells in cerebellum. |
| L26 | CD20 | 33 | Mature B-cells with loss of reactivity at plasma cell stage. |
| LN1 | CDw75 | 53 | Germinal centre B-cells, T cell subset, macrophages. |
| MB2 | | 28 | B-cells, macrophages. |
| CD68 | CD68 | 110 | Monocytes/macrophages, granulocytes. |
| CD21 | CD21 | 145 | Mature B-cells, dendritic reticulum cells |
| Vimentin | | 57 | Cells of mesenchymal origin, monocytes/macrophages, immature B lymphocytes, all T lymphocytes, endothelial cells , variable expression in Reed-Sternberg cells. |

Table 3. Monoclonal antibodies used in this study (3,7,79,44).
CD = Cluster of differentiation, when applicable. MW = Molecular weight in kiloDalton. LCA = Leucocyte common antigen. EMA = Epithelial membrane antigen.

The initial steps in the labelling procedure were the same for all methods. All sections were first deparaffinized and then rehydrated in a series of alcohols (absolute to 70% alcohol). Endogenous peroxidase activity was blocked with 1% hydrogen peroxide in methanol for 15 minutes.

This was followed, in the streptavidin procedure, by predigestion with 0,1% trypsin freshly made up in 0,1% calcium chloride solution at $37^0$ C for 3-8 minutes. Sections were then blocked with normal rabbit or swine serum (depending on the species that produced the secondary antibody) for 10 minutes. In the case of primary monoclonal mouse antibodies, the secondary antibody is produced in the rabbit, and for polyclonal rabbit antibodies in swine. Excess serum was tapped off, and this was followed by incubation with primary antibody at room temperature for 30 minutes. Between all subsequent steps, the slides were rinsed in phosphate buffered saline. Incubation with biotinylated secondary antibody (rabbit antimouse for primary monoclonal mouse, and swine antirabbit for primary polyclonal rabbit antibodies) followed for 30 minutes. After rinsing, the slides were incubated with peroxidase-conjugated streptavidin for 30 minutes. Substrate, 3,3 diaminobenzidine tetrahydrochloride (DAB), was then applied for 5-7 minutes to allow the peroxidase reaction to take place. The slides were then rinsed in water, counterstained with Mayer's haematoxylin, blued with Scott's solution, dehydrated in ascending (graded) alcohol solutions, cleared in xylene and mounted for microscopy.

In the three stage indirect immunoperoxidase method, predigestion with trypsin was performed only on those sections used for labelling with BerH2, LeuM1, LCA, CD21 and CD68. This step was ex-

cluded when labelling with MB2, L26, UCHL1, LN1 and PanB. The two stage method was only used for labelling with EMA and vimentin, and excluded a predigestion step. Steps in these two methods included incubation in normal rabbit serum for 10 minutes followed by incubation in primary antibody for 1 hour in the three stage method and 2 hours in the two stage method (stage 1). In both methods, the slides were incubated in peroxidase-conjugated rabbit antimouse antibody for 30 minutes (stage 2). This was followed, in the three stage method, by incubation in peroxidase-conjugated swine antirabbit antibody for 30 minutes (stage 3), a step excluded in the two stage method. Substrate (DAB) was then applied for 5-7 minutes. All steps in these methods were preceded by rinsing in phosphate buffered saline and/or water. Subsequent counterstaining, blueing and mounting were performed as mentioned above in the streptavidin procedure.

The peroxidase-antiperoxidase (PAP) method used for labelling the heavy and light chain components of immunoglobulins was basically similar to that described for the three stage method, except for a few minor modifications. Enzyme digestion with trypsin was performed in all cases for all antibodies (mu, gamma, alpha, kappa, lambda). Normal swine serum was used to eliminate non-specific background staining. After incubation with primary antibody, the slides were incubated with swine antirabbit antibody followed by incubation with peroxidase-antiperoxidase complex for 30 minutes. Prior to each step, the slides were rinsed with phosphate buffered saline for 20 minutes, as opposed to a brief rinse in the other methods. The latter steps of substrate addition, counterstaining,

blueing and mounting were the same as those used in the other methods.

Negative controls were included in all cases, and were obtained by omission of the primary antibody. Positive external controls were not employed. Instead, positive internal controls were assessed in all slides. Intra- and extravascular granulocytes were used as internal positive controls for LeuM1, plasma cells for EMA and BerH2, B lymphocytes for the B-cell markers L26, LN1, PanB and MB2, T lymphocytes for T-cell markers TUCHL1 and CD3, histiocytes for CD68, endothelial cells for vimentin, and follicular dendritic cells for CD21.

Cases were assessed for reactivity based on the pattern of staining and the percentage of positive cells present. Five categories were determined: 0-19%, 20-39%, 40-59%, 60-79% and 80-99, and 100% when all cells showed positive labelling. Depending on the staining pattern with T-cell, B-cell and histiocytic specific markers, cases were divided into those of T-cell, B-cell, null cell or histiocytic lineage. The background immunoreactive cellular response was also assessed with regard to the number of T or B lymphocytes present and categorized into 3 groups - those with a predominantly T-cell, B-cell or a mixed cellular background. The number of histiocytes present in the background was also arbitrarily assessed and grouped into 3 categories, i.e. those with few, moderate or numerous histiocytes.

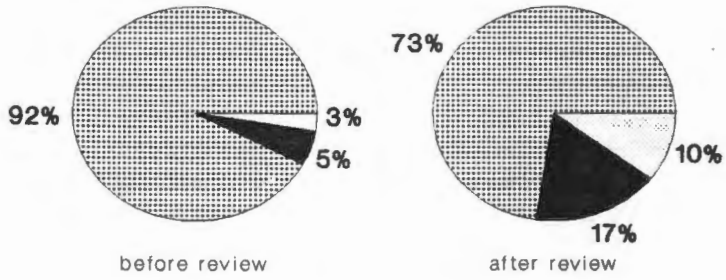
## RESULTS

Of the 77 cases in the study, 71 were originally diagnosed as Hodgkin's disease, 4 as anaplastic large cell lymphoma, and 2 were placed in the category Hodgkin's disease/Non-Hodgkin's lymphoma in which the distinction between Hodgkin's disease and non-Hodgkin's lymphoma was not possible on morphological and immunohistochemical grounds. On review, 56 of the cases were diagnosed as Hodgkin's disease, 13 as anaplastic large cell lymphoma, and 8 were placed in the Hodgkin's disease/non-Hodgkin's lymphoma category (see Figure 1).

### HODGKIN'S DISEASE

Of the 71 cases originally diagnosed as Hodgkin's disease, 32 were of nodular sclerosis, 13 mixed cellularity, 11 lymphocyte predominance, 2 interfollicular and 8 lymphocyte depleted Hodgkin's disease. 5 cases were not further classifiable on morphological and immunohistochemical grounds (Figure 2).

The 56 cases diagnosed as such, after review, comprised 20 cases of nodular sclerosis, 16 of mixed cellularity, 9 lymphocyte predominance, 2 interfollicular, and 1 lymphocyte depleted Hodgkin's disease. 8 cases were not further classifiable. These diagnoses are categorized in greater detail in Table 3.



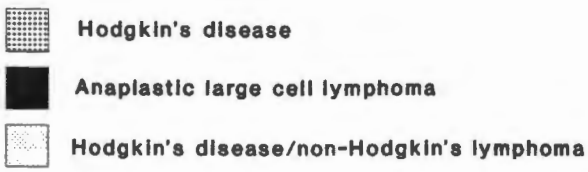




**Figure 1: Distribution of cases as a percentage of the total number of cases before and after review.**

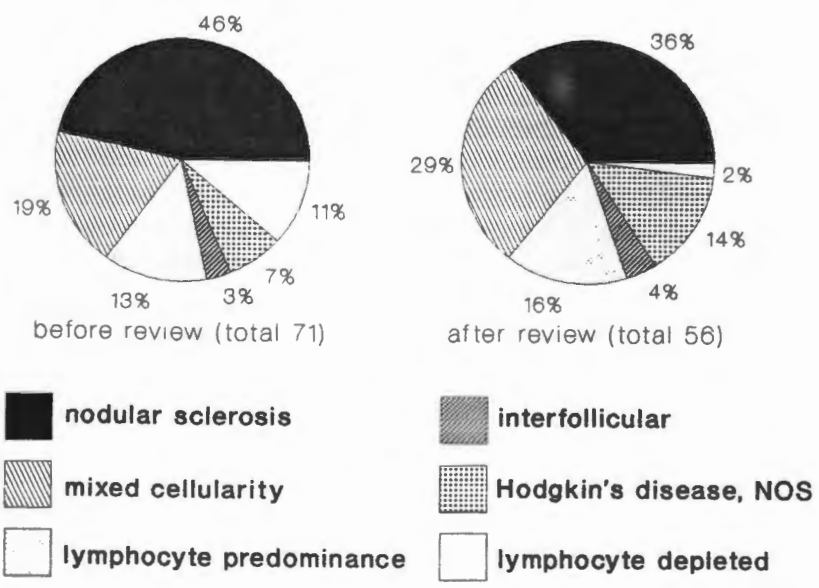



**Figure 2: Distribution of cases of Hodgkin's disease as a percentage of the total number of cases before and after review.**

| SUBTYPE | NUMBER OF CASES |
|---|---|
| Nodular sclerosis, type 1 | 15 |
| Nodular sclerosis, type 2 | 5 |
| Mixed cellularity | 16 |
| Lymphocyte depleted | 1 |
| Lymphocyte predominance - nodular | 8 |
| Lymphocyte predominance - diffuse | 1 |
| Interfollicular Hodgkin's disease | 2 |
| Hodgkin's disease - N.O.S | 8 |
| TOTAL | 56 |

Table 3: Diagnoses of the 56 cases of Hodgkin's disease after review.
N.O.S = not otherwise specified.

## NODULAR SCLEROSIS, HODGKIN'S DISEASE

Twenty cases of nodular sclerosis Hodgkin's disease, types 1 and 2, were diagnosed as such on review (Figs. 3 and 4). No cases of syncitial variant or cellular phase of nodular sclerosis Hodgkin's disease were diagnosed during the course of the study. Seventy percent (14 of 20) of cases of nodular sclerosis, Hodgkin's disease types 1 and 2, expressed the CD15 antigen, as recognized by LeuM1 antibody (Table 4). The pattern of staining for LeuM1 was paranuclear in 87% of the 14 cases, and in half of them less than 40% of the Reed-Sternberg cells and variants expressed the antigen. One case showed cytoplasmic staining in less than 20% of the cells (Table 6).

Similar results to the LeuM1 staining were obtained with the antibody which recognizes the CD30 antigen, BerH2 - 60% (12 of 20 cases) expressed the CD30 antigen. The pattern of staining for BerH2 was paranuclear, with or without cytoplasmic membrane stain-

ing, in all the positive cases. Positivity was obtained in more than 40% of the cells in 11 of the 12 positive cases (Table 7).

Fifty percent of both type 1 and type 2 cases (10 of 20) were both LeuM1 and BerH2 positive, 25% were LeuM1 positive and BerH2 negative, 10% were LeuM1 negative and BerH2 positive, and 15% were both LeuM1 and BerH2 negative.

Vimentin was detected in 13 cases (10 type 1 and 3 type 2). Staining was perinuclear and/or cytoplasmic in 92% of cases (12 of the 13 cases). Positivity was obtained in more than 40% of cells in the positive cases.

LCA was expressed weakly (<20% of cells) in a paranuclear distribution in one case (type 1) and more strongly (40-60% of cells) on the cytoplasmic membrane in a second case. Both cases failed to express a B or T-cell lineage. EMA was not detected in any of the cases.

A B-cell phenotype was obtained in 4 cases (all type 1) (Table 4). The tumour cells in all 4 cases expressed only one B-cell marker - L26 in 2 cases and LN1 in the remaining two cases. Staining was paranuclear and membranous for L26 and paranuclear for LN1. CD45 (LCA) was not detected in any of the cases. LeuM1 was detected in 2 cases and BerH2 in 3. LeuM1 and BerH2 positivity together with L26 or LN1 positivity was obtained in 2 cases. Of these 4 cases, 2 expressed vimentin. Surface and cytoplasmic immunoglobulin markers were negative in all 4 cases.

The immunoreactive background consisted predominantly of T lymphocytes in 80% of cases of nodular sclerosis Hodgkin's disease, with an equal number of both B- and T-cells present in the remaining

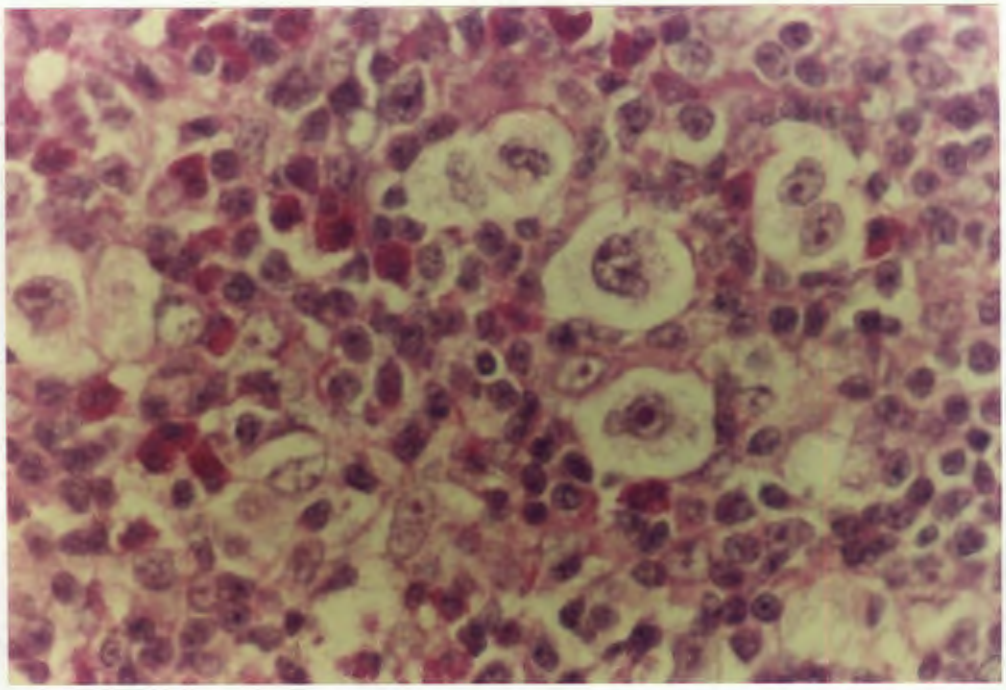


Figure 3: Nodular sclerosis Hodgkin's disease, type 1 with prominent lacunar cells. (H + E, 400×).

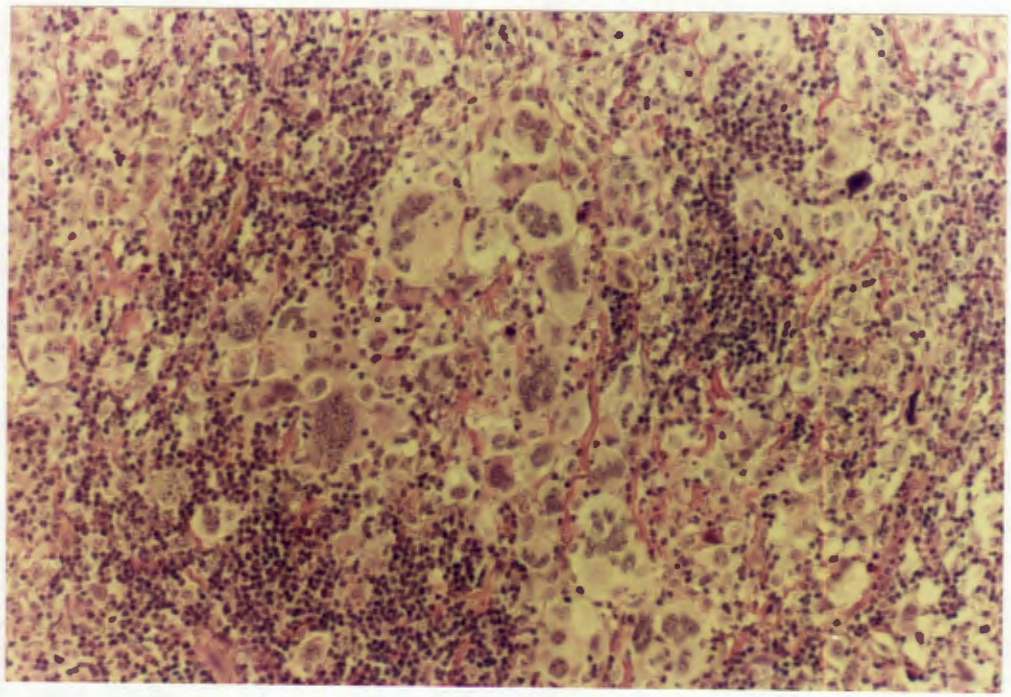


Figure 4: Nodular sclerosis Hodgkin's disease, type 2. Note the pleomorphic tumour cells and scarcity of lymphocytes in the background. (H + E, 100×).

| SUBTYPE | TOTAL | LEUM1 | BERH2 | EMA | VIM. | T CELL | B CELL | NULL CELL |
|---|---|---|---|---|---|---|---|---|
| NS,1 | 15 | 12 | 10 | 0 | 10 | 0 | 4 | 11 |
| NS,2 | 5 | 2 | 2 | 0 | 3 | 0 | 0 | 5 |
| MC | 16 | 7 | 13 | 0 | 9 | 0 | 0 | 16 |
| LP,NOD. | 8 | 0 | 0 | 2 | 1 | 0 | 8 | 0 |
| LP,DIF. | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| LD | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| IF | 2 | 1 | 2 | 1 | 1 | 0 | 0 | 2 |
| HD,N.O.S. | 8 | 2 | 5 | 0 | 4 | 0 | 3 | 5 |
| TOTAL | 56 | 24 | 33 | 3 | 28 | 0 | 16 | 40 |

Table 4: Hodgkin's disease: Analysis of immunohistochemical results and immunophenotype of Reed-Sternberg cells and variants.
T-cell = T phenotype, B-cell = B phenotype and Null cell = null phenotype. NS,1 = nodular sclerosis, type 1. NS,2 = nodular sclerosis, type 2. MC = mixed cellularity. LP,NOD. = lymphocyte predominance, nodular. LP,DIF. = lymphocyte predominance, diffuse. LD = lymphocyte depleted. IF = interfollicular. HD,N.O.S. = Hodgkin's disease, not further classifiable.

| SUBTYPE | TOTAL | LCA | L26 | PANB | LN1 | MB2 | TUCHL1 | CD3 |
|---|---|---|---|---|---|---|---|---|
| NS,1 | 15 | 1 | 2 | 0 | 2 | 0 | 0 | 0 |
| NS,2 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| MC | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LP,NOD. | 8 | 7 | 7 | 2 | 8 | 4 | 0 | 0 |
| LP,DIF. | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| LD | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| IF | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| HD,N.O.S. | 8 | 1 | 3 | 0 | 1 | 1 | 0 | 0 |
| TOTAL | 56 | 11 | 13 | 2 | 12 | 5 | 0 | 0 |

Table 5: Expression of Leucocyte common antigen (LCA) and immunophenotypic profile in Hodgkin's disease.
The abbreviations as to subtype are the same as those used in Table 4.

cases. Eleven (55%) of cases had numerous histiocytes in the background, while 7 (35%) had a moderate number, and in 2 only a few histiocytes were present. Follicular dendritic cell (FDC) networks were present in 50% of cases (8 of 15 type 1 and 2 of 5 type 2) (Table 6).

## MIXED CELLULARITY HODGKIN'S DISEASE

In sharp contrast to the results obtained in the nodular sclerosis subtype, only 44% (7 of 16 cases) of the cases expressed the CD15 antigen (Figs. 5 and 6) whereas the CD30 antigen (BerH2) was detected in 81% of cases. The pattern of staining of the Reed-Sternberg cells and Hodgkin's cells was similar to that described in nodular sclerosis Hodgkin's disease. Over 40% of the tumour cells expressed CD15 in all cases. The percentage of cells expressing CD30 was slightly higher (>60%) (Tables 7 and 8). In only one case did the tumour cells fail to express both CD15 and CD30 antigens whereas 5 cases were both LeuM1 and BerH2 positive, 2 were LeuM1 positive/BerH2 negative and, surprisingly, 8 cases were LeuM1 negative/BerH2 positive.

Positivity for vimentin was obtained in 56% (9 of 16 cases) of cases. The pattern of staining was again perinuclear and/or cytoplasmic in all positive cases. In 5 of the 9 cases over 80% of the tumour cells expressed vimentin. All cases lacked reactivity with LCA, EMA and all T- and B-cell markers.

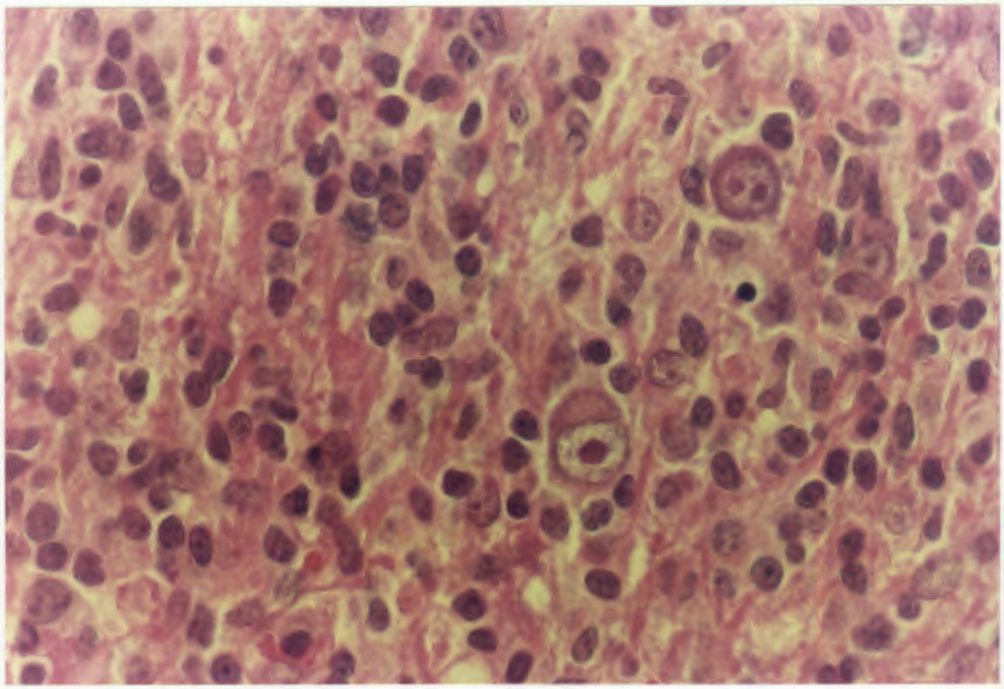


Figure 5: Mixed cellularity Hodgkin's disease with a typical Reed-Sternberg cell and Hodgkin cell. Note the inclusion-like nucleolus in the Hodgkin cell. (H + E, 400 ).

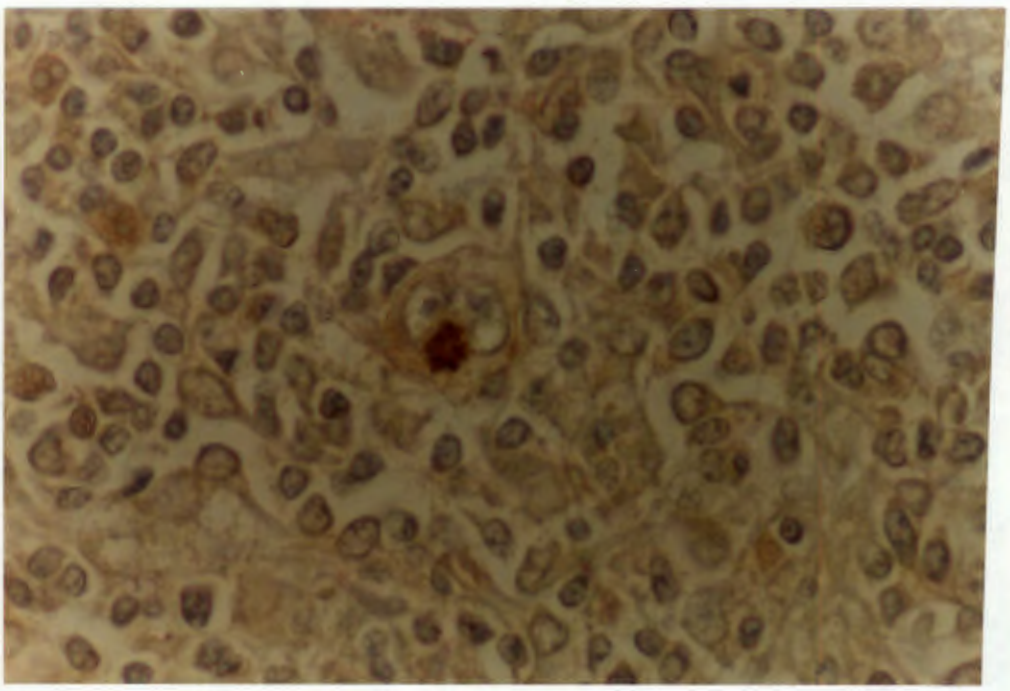


Figure 6: Expression of LeuM1 by the tumour cells in a case of mixed cellularity Hodgkin's disease. (400 ).

| SUBTYPE | TOTAL | HISTIOCYTES | | | BACKGROUND | | |
|---|---|---|---|---|---|---|---|
| | | NUM. | MOD. | FEW. | T-CELL | B-CELL | MIX. |
| NS,1 | 15 | 7 | 6 | 2 | 12 | 0 | 3 |
| NS,2 | 5 | 4 | 1 | 0 | 4 | 0 | 1 |
| MC | 16 | 10 | 6 | 0 | 15 | 1 | 0 |
| LP,NOD. | 8 | 0 | 7 | 1 | 2 | 2 | 4 |
| LP,DIF. | 1 | 0 | 1 | 0 | 1 | 0 | 0 |
| LD | 1 | 0 | 1 | 0 | 1 | 0 | 0 |
| IF | 2 | 0 | 2 | 0 | 2 | 0 | 0 |
| HD,N.O.S | 8 | 4 | 2 | 2 | 6 | 0 | 2 |
| ALCL | 13 | 7 | 6 | 0 | 7 | 2 | 4 |

Table 6: Analysis of immunoreactive background in all cases including anaplastic large cell lymphoma. Num. = numerous, Mod. = moderate and few = few histiocytes in the background. Mix. = an equal number of T and B-cells in the background. ALCL = anaplastic large cell lymphoma. The abbreviations as to subtype are the same as those used in Table 4.

| SUBTYPE | % of cells BerH2 positive | | | | | Total BerH2 Positive. |
|---|---|---|---|---|---|---|
| | < 20% | 20-40% | 40-60% | 60-80% | 80-100% | |
| NS,1 | 0 | 1 | 2 | 3 | 4 | 10/15 |
| NS,2 | 0 | 0 | 0 | 0 | 2 | 2/5 |
| MC | 0 | 4 | 0 | 3 | 6 | 13/16 |
| LD | 0 | 0 | 0 | 0 | 1 | 1/1 |
| IF | 0 | 0 | 1 | 0 | 1 | 2/2 |
| HD,N.O.S. | 0 | 0 | 2 | 1 | 2 | 5/8 |
| ALCL | 0 | 0 | 0 | 2 | 11 | 13/13 |

Table 7: Analysis of percentage of cells positive for BerH2 (CD30) in all subtypes of Hodgkin's disease and anaplastic large cell lymphoma excepting lymphocyte predominant, nodular and diffuse subtypes as none was positive for BerH2. ALCL = anaplastic large cell lymphoma. The abbreviations as to subtype are the same as those used in Table 4.

| SUBTYPE | % of cells LeuM1 positive | | | | | Total LeuM1 positive |
|---|---|---|---|---|---|---|
| | < 20% | 20-40% | 40-60% | 60-80% | 80-100% | |
| NS,1 | 4 | 2 | 2 | 1 | 3 | 12/15 |
| NS,2 | 1 | 0 | 0 | 1 | 0 | 2/5 |
| MC | 0 | 0 | 2 | 1 | 4 | 7/16 |
| LD | 0 | 0 | 0 | 0 | 0 | 0/1 |
| IF | 0 | 1 | 0 | 0 | 0 | 1/2 |
| HD,N.O.S. | 1 | 0 | 1 | 0 | 0 | 2/8 |
| ALCL | 1 | 0 | 0 | 1 | 2 | 4/13 |

Table 8: Analysis of percentage of cells positive for LeuM1 in all subtypes of Hodgkin's disease and anaplastic large cell lymphoma excepting lymphocyte predominance Hodgkin's disease as none was positive.
The abbreviations as to subtype are the same as those used in Table 4.

FDC networks were seen in 5 (42%) of the 12 cases in which they were sought. The immunoreactive background in the overwhelming majority of cases (15 of 16 cases, or 94%) consisted of T lymphocytes (Table 6). In only one case did B-cells predominate. Histiocytes were numerous in 63% of cases with a moderate amount present in the remaining 37%.

## LYMPHOCYTE PREDOMINANCE, NODULAR AND DIFFUSE HODGKIN'S DISEASE

The L&H cells in all cases of both the nodular and diffuse subtypes (Figs. 7 and 8) failed to react with LeuM1 and BerH2 antibodies (Table 4).

The tumour cells in only 2 (25%) of the 8 cases of lymphocyte predominance, nodular Hodgkin's disease expressed EMA - in both cases the pattern of staining was cytoplasmic and was strongly expressed

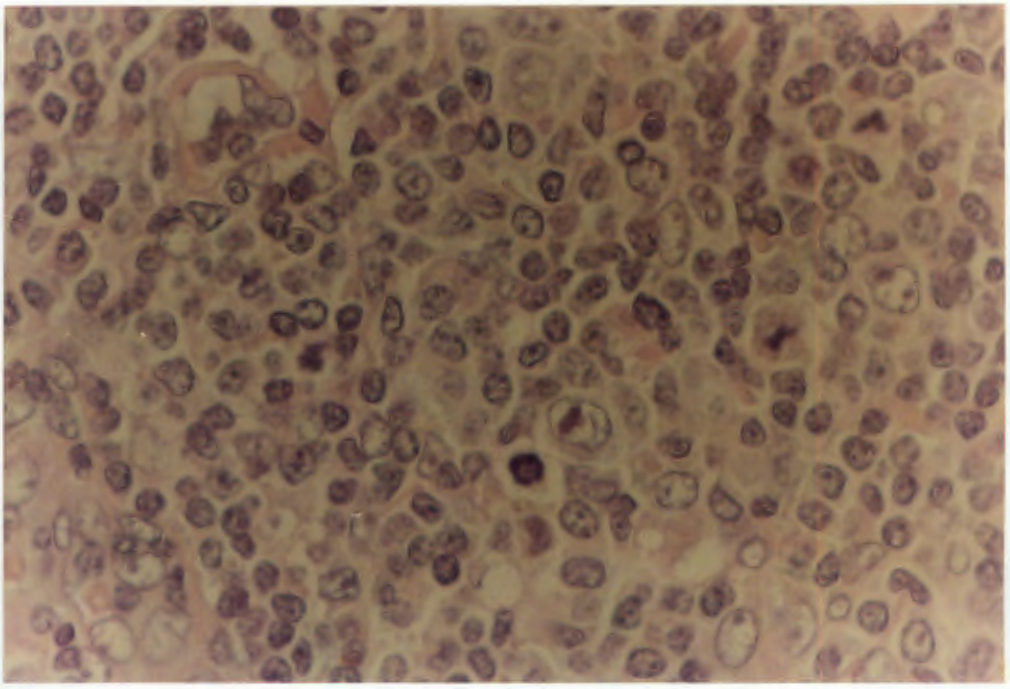


Figure 7: Lymphocytic and histiocytic variant (L&H) of a Reed-Sternberg cell in a case of nodular lymphocyte predominance Hodgkin's disease. (H + E, 400×).

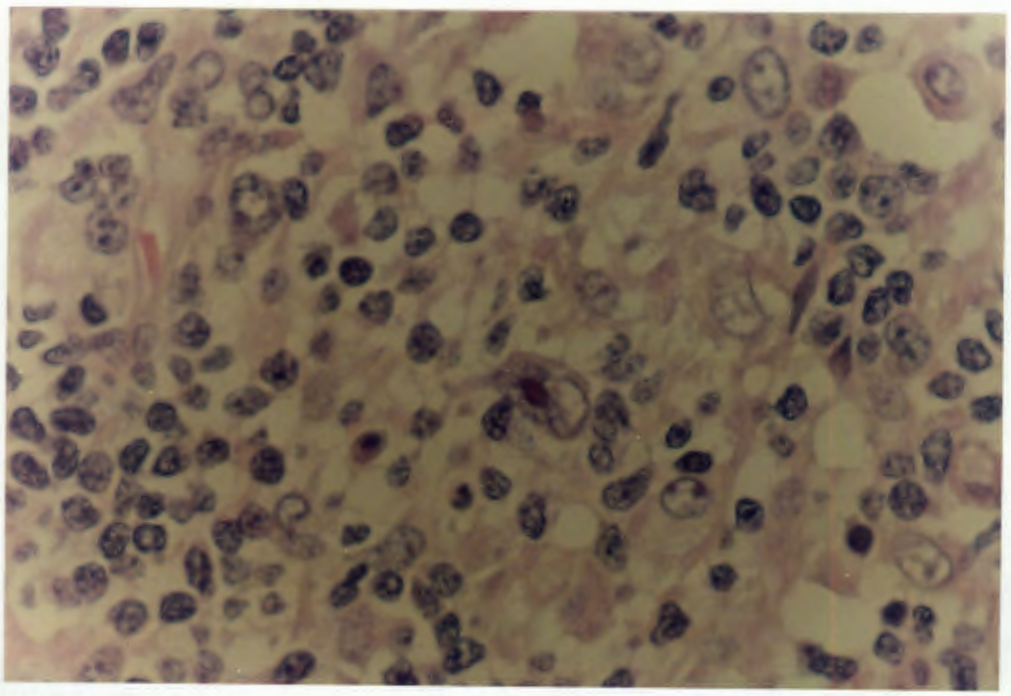


Figure 8: Lymphocyte predominance Hodgkin's disease, diffuse subtype. A tumour cell in the only case of this subtype in the study. (H + E, 400×).

(>80% of cells) in one case. EMA was detected in only 20-40% of tumour cells in the second case.

LCA (CD45) was detected in 7 (88%) of the 8 cases of lymphocyte predominance, nodular Hodgkin's disease in a membranous (6 cases) and a membranous and paranuclear (1 case) distribution. In 5 cases more than 40% of the L&H cells expressed the antigen. The single case of lymphocyte predominance, diffuse Hodgkin's disease failed to express LCA.

Of the B-cell markers L26, was expressed by the tumour cells in 7 of the 8 cases and LN1 in all 8 cases (Figs. 9 and 10). The pattern of staining for both these antibodies was paranuclear and/or membranous in all positive cases. The overwhelming majority (>80%) of L&H cells in the positive cases expressed these antigens. The tumour cells in the lymphocyte predominance, diffuse case expressed both L26 and LN1 in a similar pattern and distribution to that described above. MB2 was expressed in 4 (50%) of the 8 cases of lymphocyte predominance, nodular Hodgkin's disease, and PanB in 2 (25%). The tumour cells in the lymphocyte predominance, diffuse case failed to express these antigens. These antigens were strongly expressed in all positive cases (>60% of L&H cells positive). The pattern of staining was cytoplasmic for MB2, and paranuclear and/or membranous for PanB.

All 4 B-cell markers were positive in 1 case of lymphocyte predominance, nodular Hodgkin's disease. In 3 cases, 3 markers (L26, LN1, MB2) were positive, and in 4 cases, 2 markers were positive (3 cases for L26 and LN1, and 1 case for LN1 and MB2).

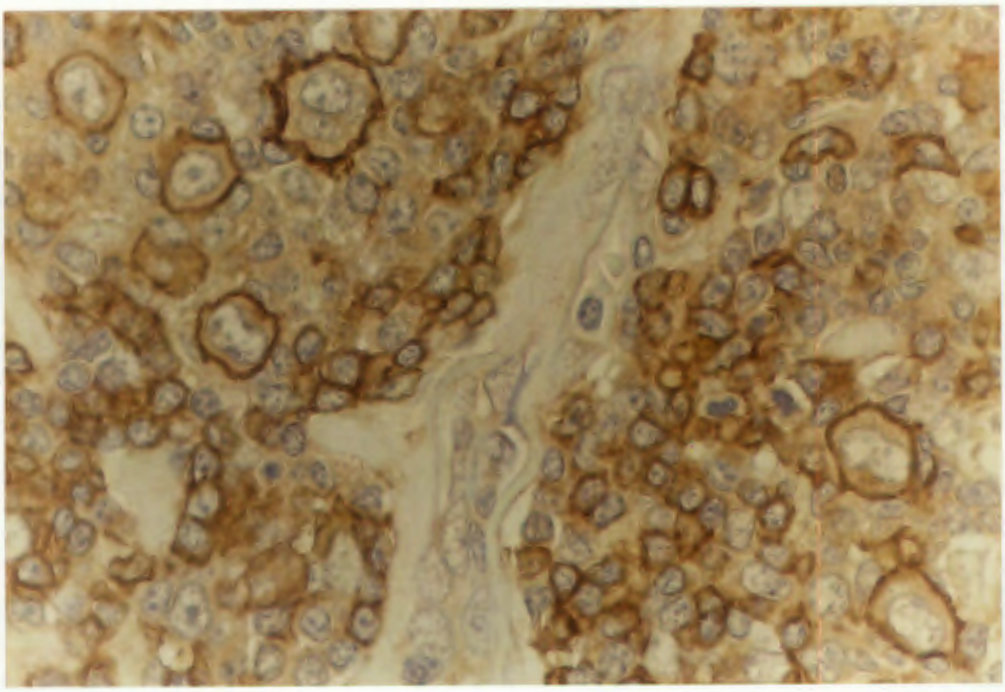


Figure 9: Expression of L26 by the L&H cells in the same case shown in figure 7. (400X).

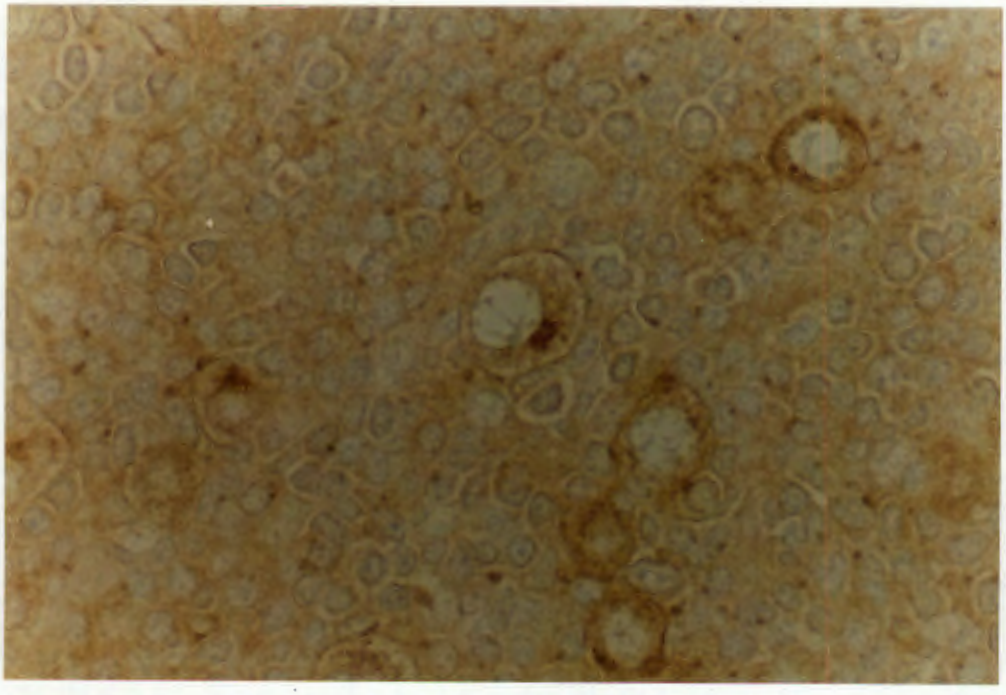


Figure 10: Expression of LN1 by the tumour cells in one of the other cases of nodular lymphocyte predominance Hodgkin's disease. (400X).

Antibodies directed against surface and cytoplasmic immunoglobulin markers were negative in all cases. In one case strong non-specific cytoplasmic staining for kappa light chain was evident in the tumour cells.

T-cell markers were negative in all cases.

Vimentin positivity was obtained in the tumour cells in only one case of nodular lymphocyte predominance Hodgkin's disease. This occurred in a perinuclear distribution in less than 20% of the cells.

FDC networks were a prominent feature in 8 (89%) of the 9 cases of lymphocyte predominance Hodgkin's disease including the diffuse case. Equivocal staining was obtained in the remaining nodular case.

The immunoreactive background in lymphocyte predominance, nodular Hodgkin's disease consisted of a mixture of both T and B lymphocytes in 50% of the cases. Of the remaining 4 cases, 2 had a background consisting predominantly of B-cells and 2 of T-cells. In the diffuse case, T-cells predominated (Table 6). Histiocytes were moderate in number in 7 of the 8 cases but were few in number in the remaining case. They were numerous in the single representative of diffuse lymphocyte predominance Hodgkin's disease in the series.

## LYMPHOCYTE DEPLETED HODGKIN'S DISEASE

Only one case was encountered in the series under study (Figs. 11 and 12). BerH2 was strongly expressed in over 80% of the tumour

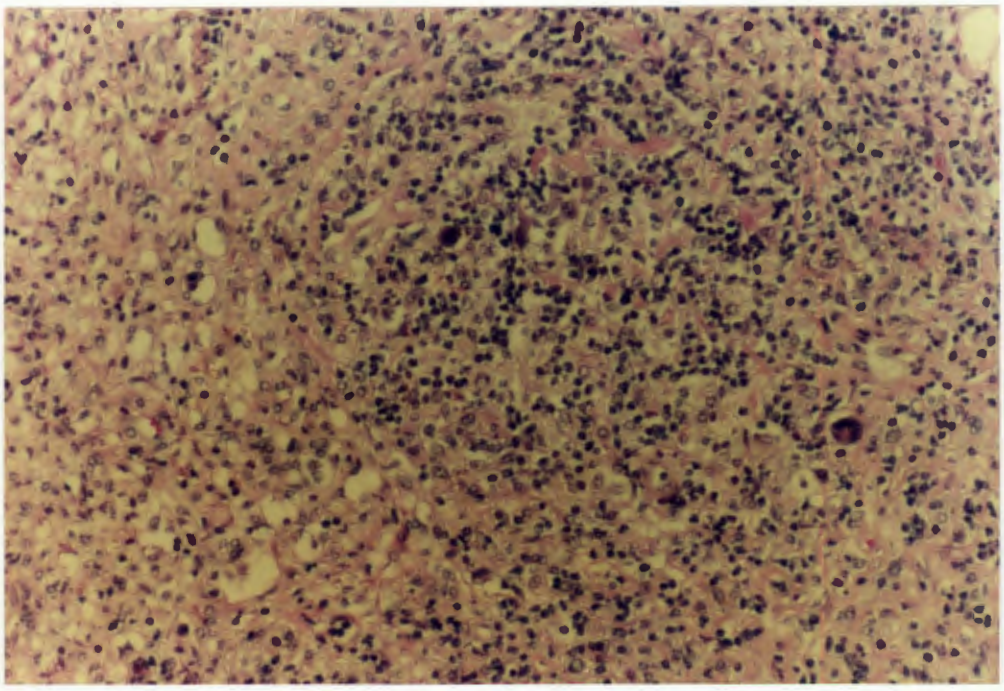


Figure 11: A low power view of lymphocyte depleted, diffuse fibrosis Hodgkin's disease. Note the heavy deposition of collagen fibres in the background. (H + E, 100X).

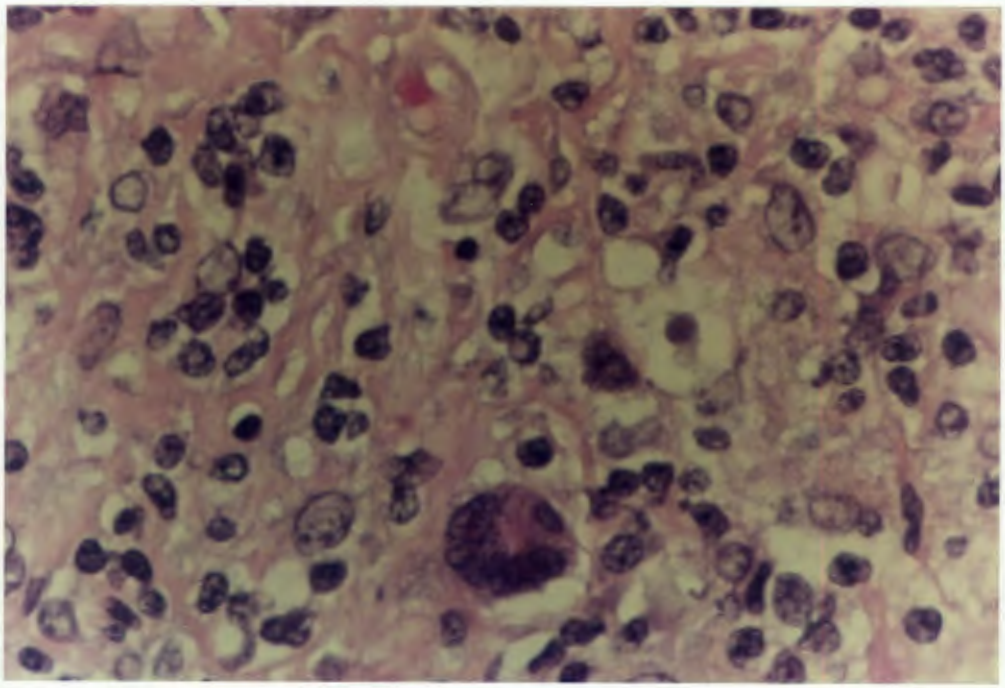


Figure 12: High power view of the same case shown in figure 11 to demonstrate the pleomorphic tumour cells surrounded by collagen fibres. (H + E, 400X).

cells (Table 6). The tumour cells failed to express all other antigens (LeuM1, EMA, Vimentin, LCA and all T and B-cell markers) (Tables 4 and 5). FDC networks were absent. T lymphocytes were the dominant cell in the background which also included a moderate number of histiocytes.

## INTERFOLLICULAR HODGKIN'S DISEASE

Of the 2 cases originally diagnosed as interfollicular Hodgkin's disease, the diagnosis remained the same in one, and was changed,

in the second case to Hodgkin's disease, N.O.S. Two cases were diagnosed as interfollicular Hodgkin's disease on review (Table 3), one of which had originally been diagnosed as mixed cellularity Hodgkin's disease. LeuM1 was not expressed by the tumour cells in one of the cases - the tumour cells, however, expressed BerH2 strongly in more than 80% of the cells in this case. Cytoplasmic EMA positivity was also observed in this case as was vimentin positivity. The tumour cells in the second case expressed both LeuM1 and BerH2. They, however, failed to express EMA and vimentin. LCA and all B- and T-cell markers were negative in both cases. The immunoreactive background in both cases was similar and consisted of T lymphocytes intermixed with numerous histiocytes. FDC networks were prominent in both cases.

## HODGKIN'S DISEASE, NOT FURTHER CLASSIFIABLE (N.O.S.)

In 8 cases a diagnosis of Hodgkin's disease could be made, however, for various reasons given below, no further subtyping was possible. Of the 5 cases originally placed in this group, the diagnosis remained the same in 3 of them. The remaining 2 cases were placed in the mixed cellularity subtype and the Hodgkin's disease/Non-

Hodgkin's disease category after review. The immunohistochemical results for the individual cases are tabulated in Table 9.

| CASE | LEUM1 | BERH2 | EMA | LCA | L26 | LN1 | MB2 | CD3 | TUCHL |
|---|---|---|---|---|---|---|---|---|---|
| 1. | + | + | - | - | + | - | + | - | - |
| 2. | - | - | - | - | + | + | nd | - | - |
| 3. | - | + | - | - | - | - | - | - | - |
| 4. | - | + | - | - | - | - | - | - | - |
| 5. | + | - | - | - | - | - | - | - | - |
| 6. | - | + | - | - | - | - | - | - | - |
| 7. | - | + | - | - | - | - | - | - | - |
| 8. | - | - | - | + | + | - | - | - | - |

Table 9: Immunohistochemical analysis of cases of Hodgkin's disease in the unclassifiable group. (+) = positive, (-) = negative, nd = not done.

Three (nos 1,2 and 8) of the cases in this category were of B phenotype and in only one these was BerH2 and LeuM1 positive (case 1). This case was a biopsy from an extradural tumour and showed extensive central necrosis. Reed-Sternberg cells were present. L26 was weakly expressed in a small percentage of tumour cells in this case whereas MB2 was positive in a much higher percentage of them, while LeuM1 was weakly expressed in <20% and BerH2 in 40-60% of the tumour cells (Tables 7, 8). LCA was negative and vimentin was positive. On immunophenotypic and morphological characteristics this case was more in keeping with Hodgkin's disease than non-Hodgkin's lymphoma.

Of the remaining two cases of B phenotype, one (no. 2) had previously been diagnosed as nodular sclerosis Hodgkin's disease and had the typical nodular pattern seen in nodular sclerosis Hodgkin's disease. However, numerous atypical cells with a monomorphic appearance were evident scattered amongst the typical

Reed-Sternberg cells - these cells were not unlike those seen in non-Hodgkin's lymphoma. They were, however, not typical of those seen in anaplastic large cell lymphoma and failed to express BerH2. They, like the Reed-Sternberg cells, also expressed L26 and LN1. The tumour cells failed to express LeuM1, EMA and vimentin.

The third case of B phenotype had previously been diagnosed as lymphocyte depleted Hodgkin's disease. On review, it was decided that the morphological features were not typical of this subtype, or any of the other subtypes for that matter, which is the reason why it has been placed in this group. The tumour cells failed to express LeuM1, BerH2, EMA and vimentin and but more than 80% of them strongly expressed both LCA and L26.

In all three of the above cases, the Reed-Sternberg cells and variants thereof failed to express surface or cytoplasmic immunoglobulin markers.

The remaining 5 cases in this category did not show the morphological or immunohistochemical features typical of any specific subtype of Hodgkin's disease. Cases 3, 4 and 6 had a similar phenotype and were BerH2 positive and LeuM1 and EMA negative, but only case 6 expressed vimentin. All 3 were of null phenotype, failing to express CD45 or related antigens. Morphologically, case 3 appeared to be evolving from interfollicular Hodgkin's disease into nodular sclerosis Hodgkin's disease. Case 4 had features not unlike those seen in mixed cellularity Hodgkin's disease, except that few lymphocytes were present in the background - it was thought that this case may represent the lymphocyte depleted end of the spectrum of mixed cellularity Hodgkin's

disease. As far as case 6 is concerned, no definitive conclusion could be reached as to wether its morphological features represented mixed cellularity or lymphocyte depleted Hodgkin's disease. Immunohistochemical findings did not aid in further delineating the subtype. All of the 3 cases had an immunoreactive background consisting predominantly of T lymphocytes admixed with moderate to numerous histiocytes.

The lymph node specimen in case 5 of this unclassified group of Hodgkin's disease showed features in keeping with interfollicular Hodgkin's disease and with those of angiofollicular lymphoid hyperplasia (Castleman's disease), hyaline vascular subtype, evident in the surrounding lymphoid tissue. Numerous sheets of plasma cells were also present in the interfollicular areas, a feature described in the hyaline vascular subtype. Reed-Sternberg cells were inconspicuous but were more easily identifiable on staining with LeuM1, which they expressed in a typical paranuclear pattern. No other immunohistochemical markers were expressed by these cells.

The last case (no.7) in this group showed only partial involvement of the lymph node specimen by the neoplastic process. Further subtyping was not possible. Typical Reed-Sternberg cells and variants thereof were present and expressed both BerH2 and vimentin in typical pattern. All other markers, including LeuM1, were negative.

## ANAPLASTIC LARGE CELL LYMPHOMA

Morphologically, on haemotoxylin and eosin stained sections, all 13 cases of anaplastic large cell lymphoma in the study had a distinct syncitial growth pattern (Figure 13). In 2 cases, permeation of the

subcapsular sinus was prominent. Reed-Sternberg-like cells were present in all cases (Figure 14). Four of the 13 cases showed dense capsular sclerosis with, in addition, broad collagen bands separating the lymphoid tissue into well-defined nodules - features not unlike those seen in nodular sclerosis Hodgkin's disease. The quality of the fibrosis, however, was different to that seen in nodular sclerosis Hodgkin's disease (vide supra), with a looser appearance and extension into the cellular areas so that the nodules were less defined than in Hodgkin's disease. The immunohistochemical results of individual cases are listed in Table 10.

| CASE | BERH2 | LEUM1 | EMA | LCA | L26 | LN1 | MB2 | CD3 | TUCHL |
|---|---|---|---|---|---|---|---|---|---|
| 1. | + | - | - | + | - | - | - | + | + |
| 2. | + | + | - | +/- | - | - | - | + | - |
| 3. | + | - | + | + | - | + | + | +/- | + |
| 4. | + | + | - | + | - | - | + | + | +/- |
| 5. | + | - | - | +/- | - | - | - | + | +/- |
| 6. | + | - | - | - | + | - | - | - | - |
| 7. | + | - | + | + | + | - | + | - | - |
| 8. | + | + | - | - | + | - | - | - | - |
| 9. | + | - | - | + | - | - | - | - | - |
| 10. | + | - | - | - | - | - | - | - | - |
| 11. | + | - | + | + | - | - | - | - | - |
| 12. | + | - | + | - | - | - | - | - | - |
| 13. | + | + | - | +/- | - | - | - | - | - |
| TOTAL | 13 | 4 | 4 | 6 | 3 | 1 | 3 | 4 | 2 |

Table 10: Immunohistochemical results of all anaplastic large cell lymphomas in the study.
(+) = positive, (-) = negative, (+/-) = equivocal.

In only 4 cases in our series was the original diagnosis that of anaplastic large cell lymphoma and these diagnoses were made in 1990. Three of the remaining 9 cases had previously been diagnosed as lymphocyte depleted Hodgkin's disease - 1 showed diffuse effacement of the nodal architecture by fibrous tissue and was diagnosed as diffuse fibrosis subtype and another had features in

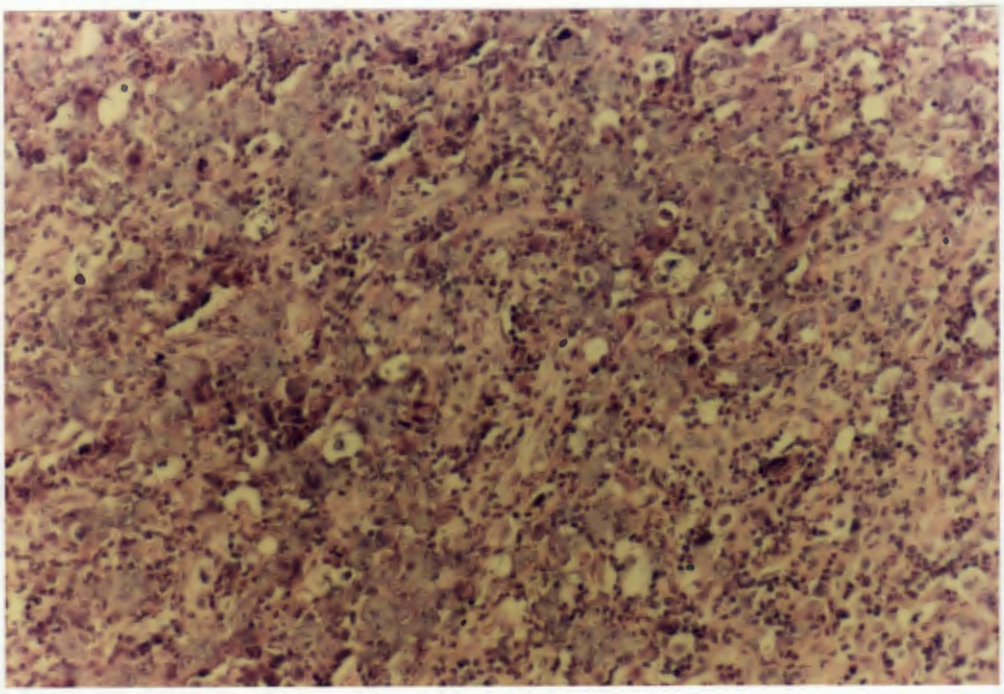


Figure 13: Anaplastic large cell lymphoma. Note the syncitial growth pattern of the tumour cells and extension of the fibrous tissue into the cellular areas. (H + E, 100X).

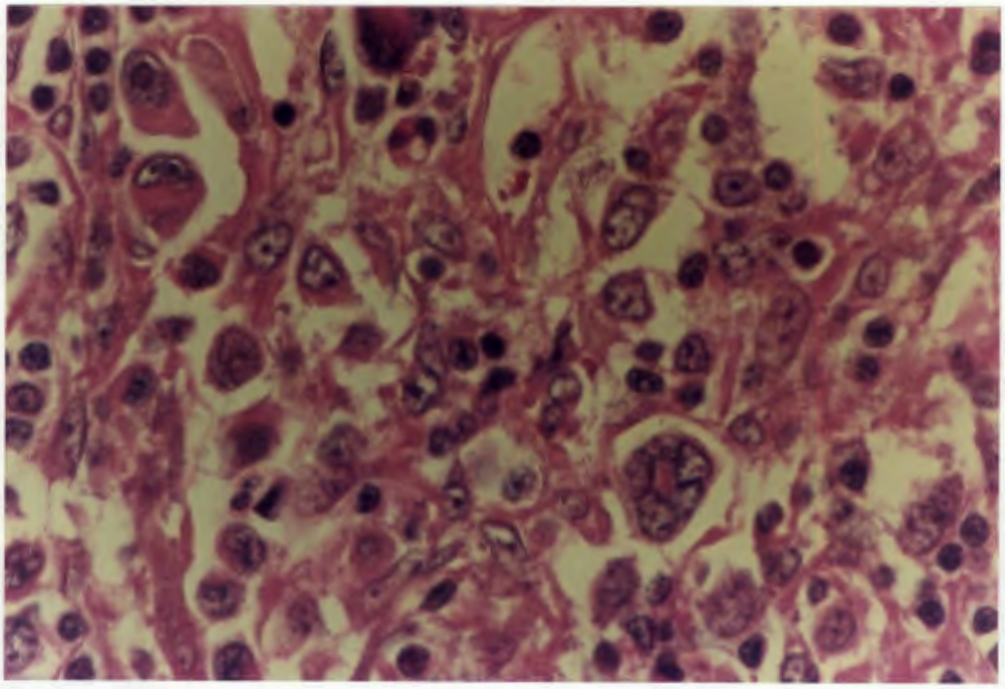


Figure 14: Anaplastic large cell lymphoma demonstrating wreath-like and Reed-Sternberg-like cells. (H + E, 400X).

keeping with the reticular subtype and was treated as such. Of the remaining 6 of these 9 cases, 5 had originally been diagnosed as nodular sclerosis Hodgkin's disease (1 syncitial variant). A definitive primary diagnosis was not made in the remaining case - the differential diagnosis lay between Hodgkin's disease and non-Hodgkin's lymphoma. The foregoing 9 cases were rediagnosed as anaplastic large cell lymphoma on the basis of both their morphological features and immunohistochemical results discussed below.

The tumour cells in all 13 cases expressed the CD30 antigen (BerH2) (Figure 15). In 85% (11 of the 13 cases) 80-100% of the tumour cells expressed the antigen and in all cases the pattern of staining was paranuclear and/or membranous (Table 7). Positive staining for LeuM1 was obtained in 4 of the 13 cases. In only 1 case were less than 20% of the tumour cells positive - expression occurred in more than 60% of tumour cells in the remaining cases (Table 8). The pattern of staining was the same as that for BerH2, i.e. paranuclear and/or membranous.

EMA positivity was obtained in only 4 (31%) of 13 cases (Figure 16). Six cases (46%) showed reactivity with LCA and 3 showed equivocal staining (Figure 17). Of the 6 positive cases 4, in addition, also expressed other T- or B-cell markers. All 3 equivocal cases also expressed one or more T- or B-cell markers.

Five cases (38%) expressed antigens suggesting a T phenotype, and 3 cases (23%) a B phenotype. The antibody TUCHL1 was positive in 2 cases of T phenotype, equivocal in 2 cases and negative in 1 case. CD3 was expressed in 4 cases and equivocal in 1. Both CD3 and TUCHL1 were positive in only 1 case.

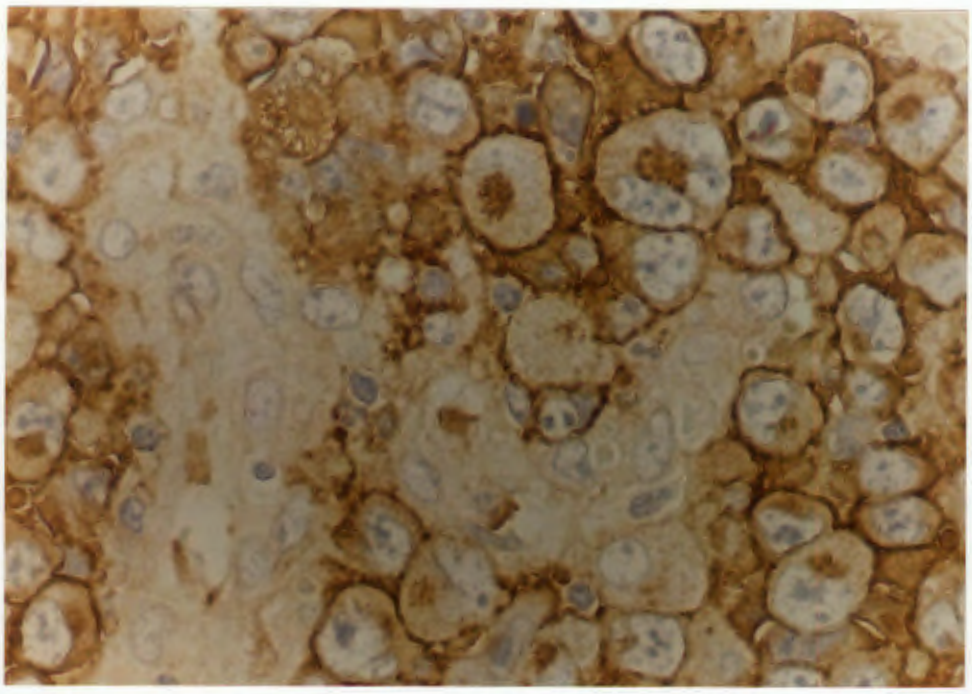


Figure 15: Expression of BerH2 in a membrane and paranuclear distribution by the tumour cells in anaplastic large cell lymphoma. (400X).

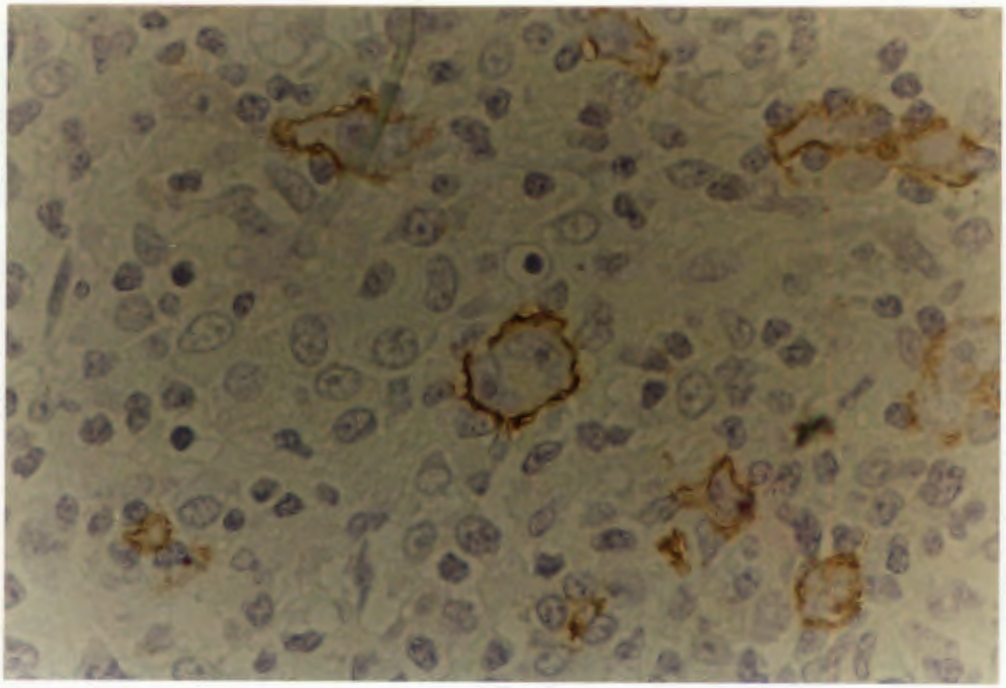


Figure 16: EMA expression by the tumour cells in anaplastic large cell lymphoma. Positivity was obtained in only 31% of cases in this study. (400X).

Of the 3 cases of B phenotype, 2 cases only expressed L26 (in one case in less than 20% of tumour cells) and one case expressed the antigens recognized by L26, PanB and MB2 antibodies in a large percentage of the tumour cells.

Cross reactivity of antibodies occurred in two cases of T-cell anaplastic large cell lymphoma. In both cases, the antibody MB2 cross reacted with the tumour cells. LN1 also cross reacted with the tumour cells in one case.

Vimentin was expressed in 7 (54%) of the 13 cases. In all of these cases a high percentage of tumour cells were reactive.

FDC networks were present in 3 cases (1 T phenotype and 2 null phenotype). With the exception of the 2 cases of null phenotype, T lymphocytes were the predominant cell in the background. B lymphocytes were the dominant cell in the 2 cases of null phenotype. Histiocytes were numerous in all cases.

## HODGKIN'S DISEASE/NON-HODGKIN'S LYMPHOMA

In 8 of the 77 cases, the distinction between Hodgkin's disease and non-Hodgkin's lymphoma could not be made. These cases were placed in a separate category. The original diagnosis in these cases was either that of Hodgkin's disease or anaplastic large cell lymphoma. In 5 cases in this group, the differential diagnosis, upon review, lay between anaplastic large cell lymphoma and syncitial variant of nodular sclerosis Hodgkin's disease (Figs. 18 and 19), and in 1 case between anaplastic large cell lymphoma and mixed cellularity Hodgkin's disease (Figure 20). The morphological features suggested a diagnosis of anaplastic large cell lymphoma in most of

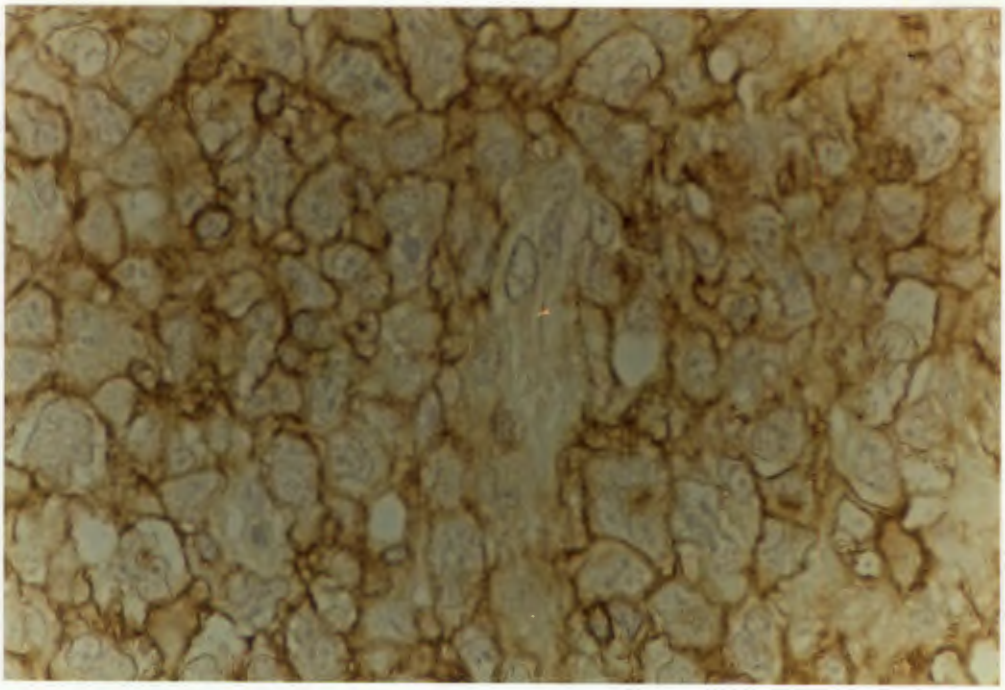


Figure 17: Reactivity with LCA was obtained in only 46% of cases of anaplastic large cell lymphoma in this study. The syncitial growth pattern of the tumour is readily apparent in this field. (400X).

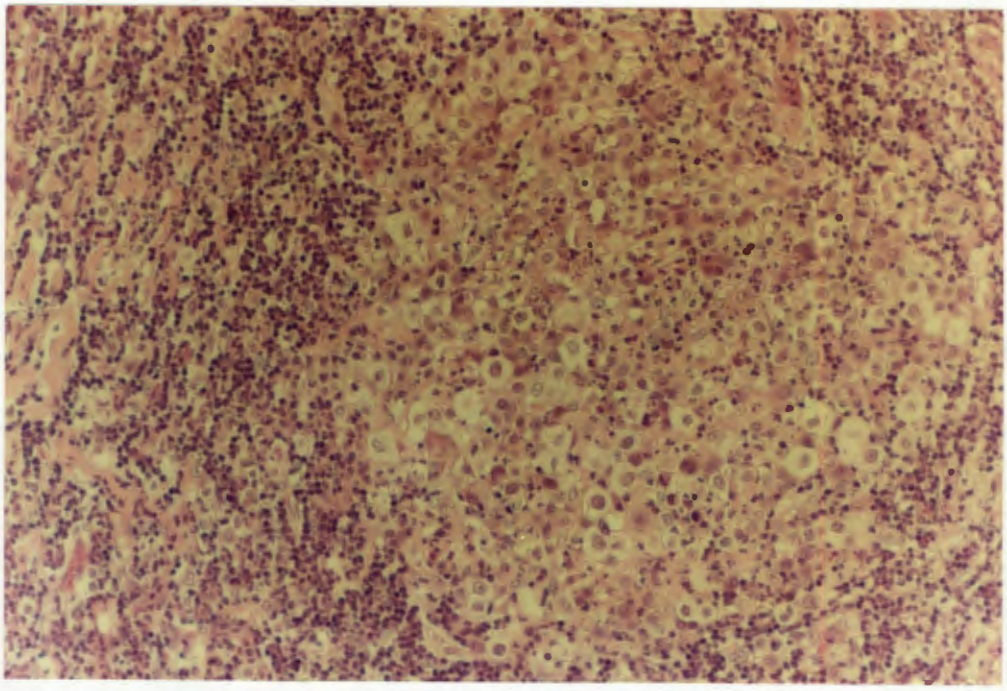


Figure 18: One of the cases from the Hodgkin's disease/non-Hodgkin's lymphoma group. Note the prominent nodular sclerosis and the syncitial sheets of tumour cells. (H + E, 100X).

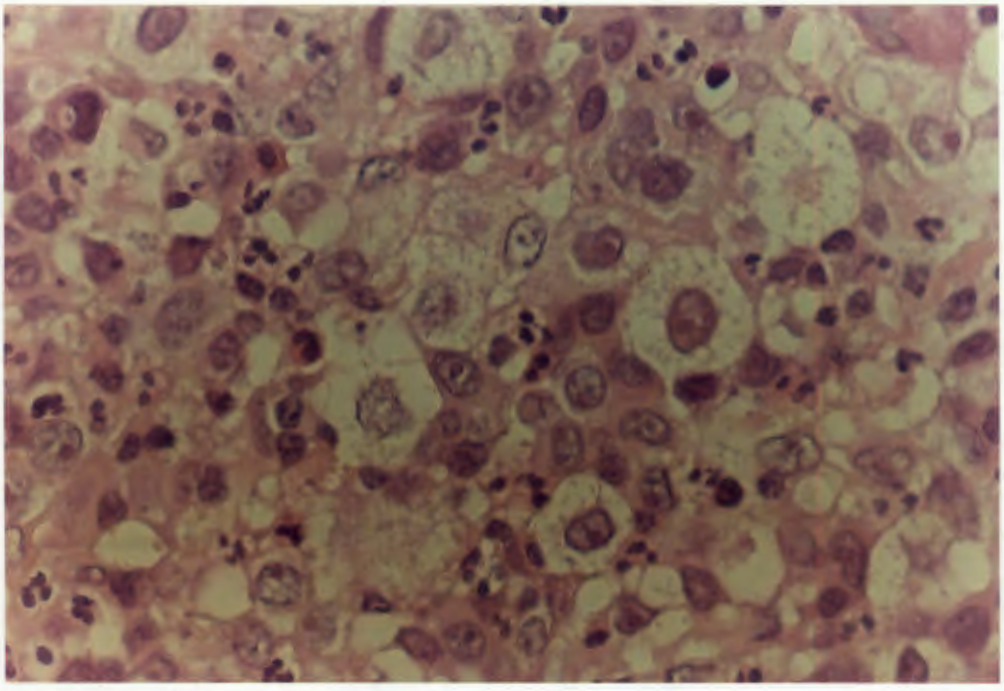


Figure 19: A high power view of the syncitial sheets of tumour cells in the same case shown in figure 18. (H + E, 400X).

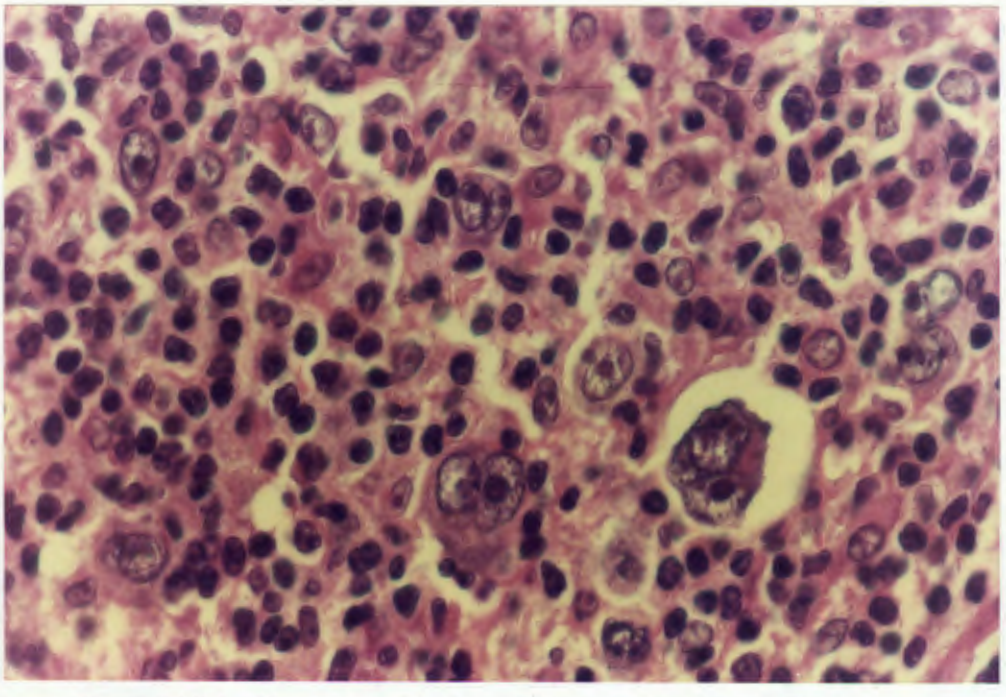


Figure 20: Hodgkin's disease/non-Hodgkin's lymphoma. The only case in which a syncitial growth pattern was not present. Note the Reed-Sternberg-like cells. (H + E, 400X).

these cases, but immunohistochemical results did not always support this diagnosis. Immunohistochemical staining was suboptimal in 1 case. The eighth case had initially been diagnosed as lymphocyte predominant Hodgkin's disease. EMA, LCA, L26, LN1 and MB2 were all strongly expressed by the tumour cells, which failed to express LeuM1 and BerH2. Numerous epithelioid histiocytes and a predominance of T lymphocytes were present in the background. No FDC networks could be demonstrated. On reappraisal of the cytological features it was decided that the features were more in keeping with a T-cell rich B-cell lymphoma.

The immunohistochemical results of seven of the cases are tabulated in Table 11. The case with suboptimal immunohistochemistry (case 6 in Table 11) had morphological features characteristic of anaplastic large cell lymphoma, but despite repeated attempts BerH2 was always negative. Expression of CD30 antigen is a prerequisite for the diagnosis of anaplastic large cell lymphoma. The tumour cells expressed LCA and vimentin in this case. All other markers were negative.

| CASE | LEUM1 | BERH2 | EMA | LCA | L26 | LN1 | MB2 | CD3 | TUCHL |
|---|---|---|---|---|---|---|---|---|---|
| 1. | + | + | +/- | - | + | - | + | - | - |
| 2. | - | + | - | + | - | - | - | - | - |
| 3. | + | + | - | - | - | - | - | - | - |
| 4. | + | + | - | - | - | - | - | - | - |
| 5. | - | +/- | - | - | - | - | - | - | - |
| 6. | - | - | - | + | - | - | - | - | - |
| 7. | - | - | - | + | - | - | - | - | - |

Table 11: Analysis of immunohistochemical results in cases with a syncitial growth pattern in which a definitive diagnosis could not be made. Case 1 showed morphological features more in keeping with mixed cellularity Hodgkin's disease.
(+) = positive, (-) = negative and (+/-) = equivocal staining.

Cases 3 and 4 expressed both LeuM1 and BerH2 - LeuM1 in only 20-40% of the tumour cells and BerH2 in more than 60% of the tumour cells. In both cases, vimentin was also expressed by the tumour cells, LCA, EMA and B- and T-cell markers were negative, and a distinct syncitial growth pattern was present although case 4 showed only partial involvement of the lymph node making further morphological assessment difficult. Eosinophils were numerous in the involved area. In case 1 the tumour cells were not cohesive. Reed-Sternberg-like cells were prominent as were mononuclear Hodgkin-like cells. Immunohistochemically the tumour cells also expressed LeuM1, and BerH2 and vimentin, and, in addition, L26 and MB2 were also positive. LCA was negative. EMA was equivocal. The immunoreactive background consisted predominantly of T lymphocytes and numerous histiocytes. Morphologically and immunohistochemically there was an overlap between mixed cellularity Hodgkin's disease and anaplastic large cell lymphoma.

The 3 remaining cases in this group (nos. 2, 5 and 7) were all LeuM1 negative. In case 2, the tumour cells expressed BerH2 and LCA in 80-100% of the tumour cells. B- and T-cell markers were negative. Vimentin was positive. Cohesive, syncitial sheets of tumour cells were a prominent feature in this case, and the question was raised as to whether or not this case represented evolution of nodular sclerosis Hodgkin's disease into anaplastic large cell lymphoma. Equivocal staining for BerH2 was obtained in case 5, and all other markers were negative, including vimentin. This node was also only partially involved with a large focus of necrosis evident in the area of involvement. Case 7 was both LeuM1 and BerH2 negative. The tumour cells did express LCA and vimentin.

All other markers were negative. A syncitial growth pattern was clearly evident with a predominance of B-cells in the background. Histiocytes were sparse.

## DISCUSSION

Despite numerous immunological studies of Hodgkin's disease, the histogenesis of the Reed-Sternberg cell and its variants remains uncertain. Morphological criteria still play an extremely important role in the diagnosis of the disease although in recent years immunophenotyping of Reed-Sternberg and Hodgkin's cells has made a significant contribution to its diagnosis and subtyping. Immunophenotyping also plays an important role in the diagnosis of anaplastic large cell lymphoma - expression of CD30 antigen by the tumour cells is a prerequisite for the diagnosis of this lymphoma. It has been repeatedly suggested in the past that expression of the CD15 antigen by the Reed-Sternberg cell and variants, as recognized by the antibody LeuM1, may be used as a marker of Hodgkin's disease. Critical analysis, however, indicates that this is neither a sensitive nor a specific marker of Hodgkin's disease (16). The findings in the study reported here are consistent with this view. Only 57% of cases of nodular sclerosis, mixed cellularity and lymphocyte depleted Hodgkin's disease in this study expressed the CD15 antigen, compared to 69% of cases in a meta-analysis of published series (Table 12). If one analyses the various subtypes separately, then LeuM1 was more consistently expressed in nodular sclerosis Hodgkin's disease (70%) than in mixed cellularity Hodgkin's disease (44%); the corresponding figures in pooled published data are 70% and 68% respectively (4,12,13,17,18,29,47,70,76), the latter percentage being considerably higher than that seen in our series (Table 12, Figure 21).

Expression of CD30 antigen in this study compared favorably with that seen in published literature (Table 12). The CD30 antigen, as recognized by BerH2, was more consistently expressed by the tumour cells in mixed cellularity Hodgkin's disease (81%) than in nodular sclerosis Hodgkin's disease (60%), and was expressed in the only case of lymphocyte depleted Hodgkin's disease. The corresponding figures in the meta-analysis are 73% and 75% respectively. If one looks at the group as a whole, then 70% positivity was obtained for BerH2 in this study compared to 75% in published series (Figure 22).

Interestingly, in 50% of cases of nodular sclerosis Hodgkin's disease, the tumour cells were both LeuM1 and BerH2 positive as compared to only 31% of cases of mixed cellularity Hodgkin's disease. Fifty percent of cases of mixed cellularity Hodgkin's disease expressed CD30 antigen but were LeuM1 negative compared to only 9% in nodular sclerosis Hodgkin's disease. Twenty seven percent of cases of nodular sclerosis Hodgkin's disease were LeuM1 positive and BerH2 negative compared to 13% in mixed cellularity Hodgkin's disease.

Epithelial membrane antigen expression (EMA) by the tumour cells was consistently negative in all subtypes of Hodgkin's disease (excluding lymphocyte predominance Hodgkin's disease) in this study. Most published series report a few cases of EMA expression by the Reed-Sternberg cells and variants in all subtypes (Table 12).

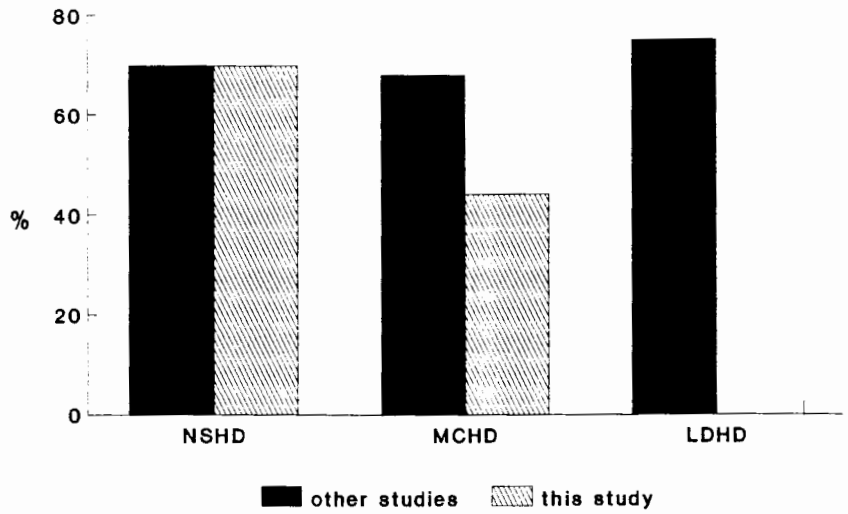



Figure 21:Percentage of LeuM $_1$ positive cases in the various subtypes of HD (excluding lymphocyte predominance HD) compared to the results obtained in other studies (see table 12).

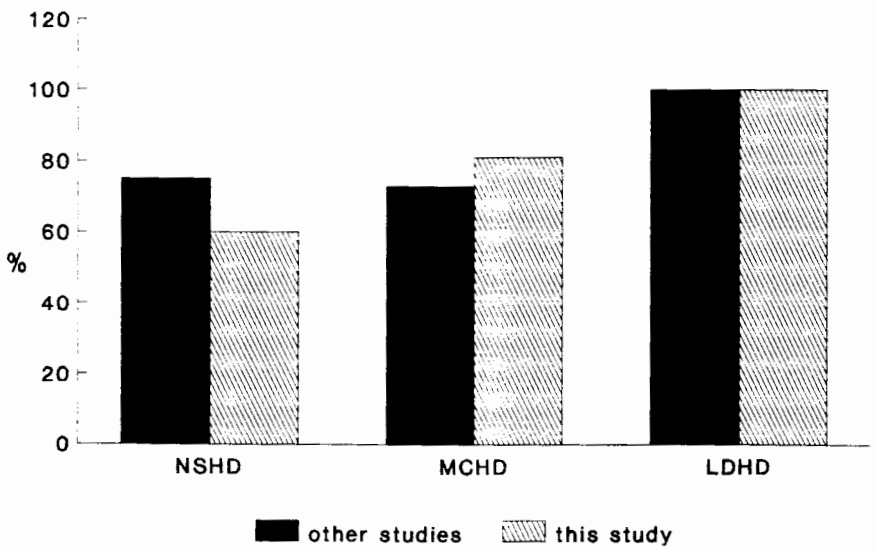



Figure 22:Percentage of BerH$_2$ positive cases in the various subtypes of HD (excluding lymphocyte predominance HD) compared to the results obtained in other studies (see table 12).

| REFERENCE | LEUM1 | BERH2 | EMA | LCA | T CELL | B CELL |
|---|---|---|---|---|---|---|
| NODULAR SCLEROSIS: | | | | | | |
| Hall *et al.*[4] | 3/15 | 5/15 | 1/15 | 0/15 | 0/15 | 3/15 |
| Chittal *et al.*[12] | 16/16 | 14/15 | 5/16 | 2/13 | 0/16 | 3/16 |
| Angel *et al.*[13] | 19/20 | 20/20 | 3/20 | | 0/20 | 8/20 |
| Bishop *et al.*[70] | 5/5 | 1/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| Norton *et al.*[76] | 11/12 | | | | | |
| Jack *et al.*[17] | 21/33 | | 1/33 | | | |
| Kadin *et al.*[29] | 5/6 | 6/6 | | 0/20 | 6/20 | |
| Cibull *et al.*[18] | | | | | 13/41 | |
| Petrella *et al.*[47] | 48/77 | | | | | |
| Total | 128/184 | 46/61 | 10/89 | 2/53 | 19/117 | 14/56 |
| **This study** | **14/20** | **12/20** | **0/20** | **2/20** | **0/20** | **4/20** |
| MIXED CELLULARITY: | | | | | | |
| Hall *et al.*[4] | 6/15 | 15/15 | 2/15 | 3/5 | 0/15 | 6/15 |
| Chittal *et al.*[12] | 29/35 | 32/34 | 7/33 | 6/26 | 0/35 | 4/35 |
| Angel *et al.*[13] | 5/9 | 9/9 | 5/9 | | 0/9 | 5/9 |
| Bishop *et al.*[70] | 4/8 | 5/8 | 0/8 | 0/8 | 0/8 | 0/8 |
| Norton *et al.*[76] | 12/14 | | | | | |
| Jack *et al.*[17] | 16/20 | | 1/20 | | | |
| Kadin *et al.*[29] | 1/2 | 2/2 | | 0/2 | 2/2 | |
| Cibull *et al.*[18] | | | | | 4/11 | |
| Petrella *et al.*[47] | 8/17 | | | | | |
| Total | 81/120 | 63/86 | 15/85 | 9/41 | 6/80 | 15/67 |
| **This study** | **7/16** | **13/16** | **0/16** | **0/16** | **0/16** | **0/16** |
| LYMPHOCYTE DEPLETED: | | | | | | |
| Hall *et al.*[4] | 2/5 | 5/5 | 1/5 | 0/5 | 0/5 | 2/5 |
| Angel *et al.*[13] | 1/1 | 1/1 | 1/1 | | 0/1 | 1/1 |
| Norton *et al.*[76] | 2/2 | | | | | |
| Jack *et al.*[17] | 4/4 | | 0/4 | | | |
| Total | 9/12 | 6/6 | 2/10 | 0/5 | 0/6 | 3/6 |
| **This study** | **0/1** | **1/1** | **0/1** | **0/1** | **0/1** | **0/1** |
| GRAND TOTAL | 218/316 | 115/153 | 27/184 | 11/99 | 25/203 | 32/129 |
| **THIS STUDY** | **21/37** | **26/37** | **0/37** | **1/37** | **0/37** | **4/37** |

Table 12 : Published immunophenotype of Reed-Sternberg cells and variants in the various subtypes of Hodgkin's disease (excluding lymphocyte predominance Hodgkin's disease) compared to this study. Note that in the published series a variety of B-cell and T-cell markers were used: the results of the published series have been aggregated. Grand Total = sum of all positive cases in the various subtypes represented in the table.

No fixed pattern appears to emerge from these findings in nodular sclerosis, mixed cellularity and lymphocyte depleted Hodgkin's disease. While LeuM1 and BerH2 may be of assistance in diagnosing Hodgkin's disease, they cannot be relied upon. A negative finding does not exclude a diagnosis of Hodgkin's disease. More importantly, a positive result obtained with either LeuM1 or BerH2 does not necessarily confirm the diagnosis as one of Hodgkin's disease. Morphological criteria still play an extremely important role in assessing any case even though morphological distinction between Hodgkin's disease and anaplastic large cell lymphoma is not always clear cut.

In this series of 13 cases of anaplastic large cell lymphoma, the tumour cells in all cases expressed CD30 antigen in a large percentage of the cells. In addition, 4 of the 13 cases (31%) expressed the CD15 antigen. In a review of published literature of anaplastic large cell lymphomas only 10% of cases were LeuM1 positive - considerably lower than that seen in our study (Table 14). In addition, in most series in which positive results were obtained, only a small percentage of the tumour cells expressed CD15 - in this study a larger percentage of tumour cells (>60%) expressed this antigen in 3 of the 4 positive cases (Table 8). In these cases, morphological and cytological features were of help in making a final diagnosis, although these features were not always helpful in distinguishing Hodgkin's disease from anaplastic large cell lymphoma.

Considerable overlap exists between the morphological features of Hodgkin's disease and anaplastic large cell lymphoma (vide supra). This was borne out by several cases in this study in which a

definitive diagnosis could not be made despite careful morphological assessment and adequate immunophenotyping in these cases. In six cases with a distinct syncitial growth pattern, a definitive diagnosis could not be made (Table 11). The differential diagnosis in these cases included the syncitial variant of nodular sclerosis Hodgkin's disease and anaplastic large cell lymphoma. One of these cases, originally diagnosed as nodular sclerosis Hodgkin's disease, appeared to be evolving into anaplastic large cell lymphoma. An additional 5 cases were originally diagnosed as nodular sclerosis Hodgkin's disease - these were included in the study as cases of Hodgkin's disease. However, after reappraisal of the morphological features and immunohistochemical results, it was decided to change the diagnosis to anaplastic large cell lymphoma. Of these 5 cases, one was previously diagnosed as syncitial variant of nodular sclerosis Hodgkin's disease. Furthermore, not one diagnosis of syncitial variant of nodular sclerosis Hodgkin's disease was made in this study. This raises the question as to the very existence of this subtype of nodular sclerosis Hodgkin's disease and one may ask the question: Does this variant represent (1) nodular sclerosis Hodgkin's disease, (2) nodular sclerosis Hodgkin's disease evolving into anaplastic large cell lymphoma, or (3) anaplastic large cell lymphoma with morphological features mimicking nodular sclerosis Hodgkin's disease? Immunophenotyping of the 5 cases in which a definitive diagnosis of nodular sclerosis Hodgkin's disease or anaplastic large cell lymphoma could not be reached, led to further confusion as the tumour cells in 3 cases expressed BerH2 only and in 2 cases both LeuM1 and BerH2. LeuM1 was not expressed on its own in any case. Evolution of nodular sclerosis Hodgkin's disease into anaplastic large cell lymphoma does occur (Personal communica-

tion - Professor K. Lennert) and it is possible that these cases were doing just that. Clinical data, such as presenting features, response to treatment and length of survival, may be of aid in helping to answer this question. Further studies correlating clinical findings with pathological features in such cases are essential to enable us to understand the disease process and make the correct diagnosis. The answer to this question has important therapeutic implications - more intensive chemotherapy regimens are used in the treatment of anaplastic large cell lymphoma as compared to Hodgkin's disease. Implementation of appropriate treatment can be started timeously at initial diagnosis.

Of the 37 cases of nodular sclerosis, mixed cellularity and lymphocyte depleted Hodgkin's disease, only 4 cases (11%) in this study were of B phenotype and all belonged to the nodular sclerosis subtype (type 1). If one includes the cases from the unclassifiable group, then 15,6% were of B phenotype. No staining was seen in any case with antibodies that recognize antigens expressed by T lymphocytes. If we compare our data to those in the literature, it becomes apparent that our findings are similar to those in the meta-analysis (Table 12, Figs. 23 and 24) in which 25% of cases of nodular sclerosis Hodgkin's disease are of B phenotype and only 16% are of T phenotype. In mixed cellularity Hodgkin's disease, the percentages are 22% and 8% for B and T phenotype, respectively, while for lymphocyte depleted Hodgkin's disease the corresponding figures are 50% and 0%. No cases with a T-cell phenotype have been reported. Two of the cases of nodular sclerosis Hodgkin's disease in this study expressed CD45 antigen (LCA) - in both cases all B-

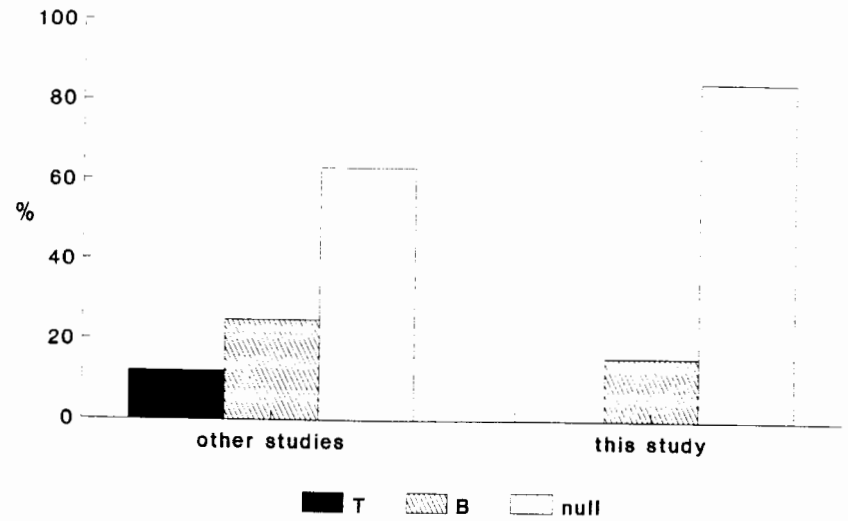



Figure 23: Percentage of pooled cases of HD with a T, B or null phenotype, including unclassifiable cases but excluding lymphocyte predominance Hodgkin's disease,compared to published series.

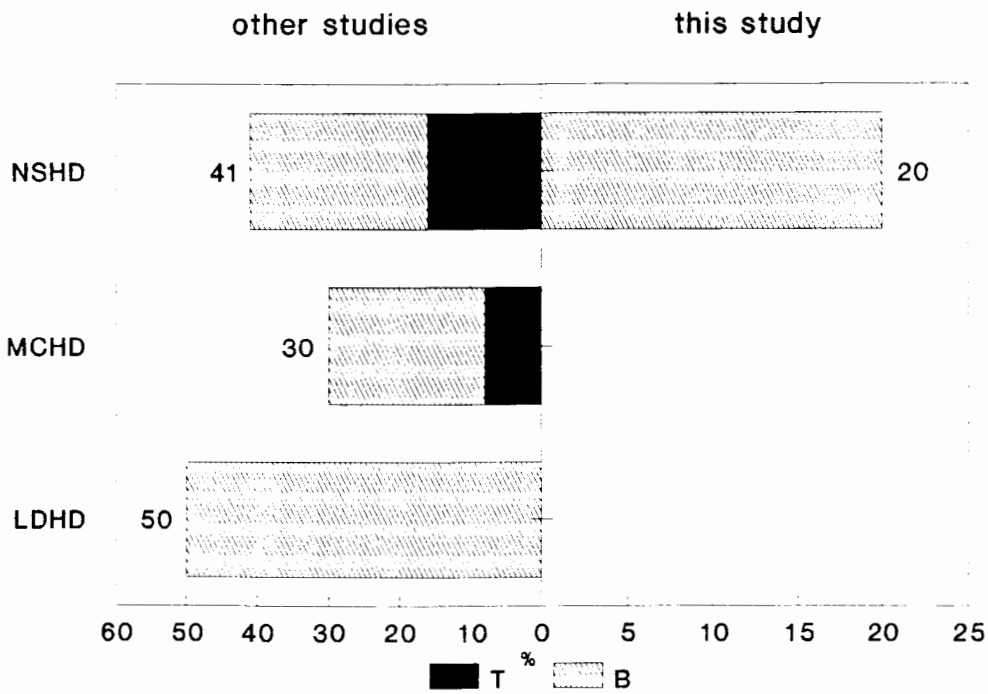



Figure 24:Percentage of cases with a T or B phenotype in nodular sclerosis, mixed cellularity and lymphocyte depleted Hodgkin's disease compared to published studies.

cell markers were negative. Most reports indicate that only a minority of cases show CD45 immuno-reactivity (2 out of 53 in published data). The results obtained in the present study are very similar to those achieved in the studies by Chittal *et al.*(4) and Hall *et al.* (12). The available evidence therefore suggests that some, but not all, cases of Hodgkin's disease may be of B-cell origin. Immunoglobulin heavy and/or light chain restriction could not be detected in any of our cases with a B phenotype - in one case non-specific uptake of immunoglobulin heavy and light chains by Reed-Sternberg cells was detected, a feature well described in Hodgkin's disease and which may be attributed to formol induced damage to the cell membrane resulting in passive uptake of the immunoglobulin chains. Immunoglobulin gene rearrangements have been described to occur in some cases of Hodgkin's disease (vide supra) further supporting the view that some cases are of B-cell origin.

In the overwhelming majority of the cases of nodular sclerosis mixed cellularity and lymphocyte depleted Hodgkin's disease encountered in this study, the immunoreactive background consisted predominantly of T lymphocytes admixed with moderate to numerous histiocytes (Table 6), a feature well documented in Hodgkin's disease.

In this review of 77 cases, a diagnosis of lymphocyte depleted Hodgkin's disease was made in only one case. This is consistent with the findings obtained in most other studies; the diagnosis is seldom made (Table 12). Several cases originally diagnosed as lymphocyte depleted Hodgkin's disease were placed in other categories after reassessment - in fact, 2 cases were reclassified as

anaplastic large cell lymphoma. In both cases, the BerH2 antibody was not available at the time of initial diagnosis. Most cases showing diffuse fibrous replacement of the lymph node were placed in the category of mixed cellularity Hodgkin's disease (lymphocyte depleted end of the spectrum). The immunophenotype of the cells in our one case were similar to that described in studies elsewhere (Table 12).

Our findings in the lymphocyte predominance subtype of Hodgkin's disease show only minor differences in the immunophenotype of the L&H cells as compared to the published consensus (Table 13). The negativity of the L&H cells with LeuM1 and BerH2 antibodies is in accordance with reports of most other investigators. EMA expression, however, was found to be present in only 25% (2 of 8 cases) of cases in our study - these findings are consistent with those in some reported studies (4,70,20) but are discordant with other reported studies (12,43,17,57,17). The consensus of opinion is, however, that the L&H cells in most cases of lymphocyte predominance Hodgkin's disease do express EMA - 44% of cases reported in the literature expressed EMA, but, the incidence rose to as much as 71% in some studies, if it was assessed separately (12).

The L&H cells in all cases of both diffuse and nodular subtypes of lymphocyte predominance Hodgkin's disease expressed one or more B-cell antigens, as recognized by the antibodies L26, LN1, PanB and MB2 in this study, suggesting a B-cell origin of the tumour cells. Seventy-nine percent of pooled reported cases of nodular lymphocyte predominance Hodgkin's disease in the literature were of B phenotype (Table 11). Several independent studies, however, also

| REFERENCE | LEUM1 | BERH2 | EMA | LCA | T CELL | B CELL |
|---|---|---|---|---|---|---|
| LP,NODULAR: | | | | | | |
| Hall *et al.*[4] | 0/5 | 0/5 | 1/5 | 5/5 | 0/5 | 5/5 |
| Chittal *et al.*[12] | 2/14 | 1/13 | 10/14 | 6/14 | 0/14 | 14/14 |
| Angel *et al.*[13] | 1/2 | 1/1 | 0/2 | | 0/2 | 2/2 |
| Norton *et al.*[76] | 0/4 | | | | | |
| Bishop *et al.*[70] | 5/20 | 4/20 | 4/20 | 4/20 | 0/20 | 13/20 |
| Nicholas *et al.*[20] | 0/21 | 3/21 | 6/21 | | 0/21 | 21/21 |
| Chittal *et al.*[43] | 0/5 | 1/5 | 5/5 | 5/5 | 0/5 | 5/5 |
| Cibull *et al.*[18] | | | | | 3/9 | |
| Jack *et al.*[17] | 1/7 | | 4/7 | | | |
| Pinkus *et al.*[42] | 0/4 | | | 4/4 | | 2/2 |
| Stein *et al.*[57] | 4/32 | | 8/13 | | | 20/32 |
| Dorfman *et al.*[63] | 0/5 | | | 5/5 | | |
| Regula *et al.*[36] | 0/2 | | | 0/2 | | |
| Hansmann *et al.*[75] | 0/10 | 4/10 | | | 0/10 | 6/10 |
| Petrella *et al.*[47] | 4/6 | | | | | |
| Total | 17/137 | 14/75 | 38/87 | 29/55 | 3/86 | 88/111 |
| This study | 0/8 | 0/8 | 2/8 | 7/8 | 0/8 | 8/8 |
| LP,DIFFUSE: | | | | | | |
| Hall *et al.*[4] | 2/3 | 3/3 | 2/3 | 0/3 | 0/3 | 0/3 |
| Chittal *et al.*[12] | 0/4 | 2/4 | 2/4 | 3/4 | 0/4 | 4/4 |
| Bishop *et al.*[70] | 4/17 | 0/17 | 2/17 | 1/17 | 0/17 | 10/17 |
| Nicholas *et al.*[20] | 7/15 | 8/15 | 3/15 | | 0/15 | 4/15 |
| Stein *et al.*[57] | 2/11 | | 0/2 | | | 1/11 |
| Jack *et al.*[17] | 1/1 | | 0/1 | | | |
| Dorfman *et al.*[63] | 2/2 | | | 0/2 | | |
| Regula *et al.*[36] | 0/2 | | | 1/2 | | |
| Hansmann *et al.*[75] | 1/10 | 2/10 | | | 0/10 | 8/10 |
| Petrella *et al.*[47] | 2/5 | | | | | |
| Total | 21/70 | 7/49 | 9/42 | 5/28 | 0/49 | 27/50 |
| This study | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 | 1/1 |

Table 13: Published immunophenotype of the malignant cells in lymphocyte predominance Hodgkin's disease compared with this study. Note that in the published series a variety of B cell markers were used: the results of the published series have been aggregated.

reported a 100% positive rate of the L&H cells (12,13,20). The tumour cells, in this study, expressed more than two B-cell markers in all cases, with L26 and LN1 being the antibodies most

consistently positive. In only 1 case were all B markers positive. PanB was the least sensitive, being expressed by the tumour cells in only 1 case. No case of lymphocyte predominance, nodular Hodgkin's disease expressed T markers in this study. In only one reported series (18) did the tumour cells express a T phenotype on marking with the T-cell specific antibody, CD3.

Of interest were the presence of sharply defined FDC networks in 7 of the 8 cases of lymphocyte predominance, nodular Hodgkin's disease. These findings are in keeping with the findings of Alavaikko *et al.* (86) and Hansmann *et al.* (75). Of the 8 cases only 2 had an immunoreactive background consisting predominantly of B lymphocytes. This is in direct contrast to the published findings of Abdulaziz *et al.* (11) in which all 8 cases had a predominance of B-cells in the background. In an additional 2 cases in this study, the background consisted predominantly of T lymphocytes and in the remaining 4 cases of an equal number of both T and B lymphocytes. In these cases, B-cells dominated in the nodules with T-cells being more prominent in the internodular area. These findings are in accord with those of Nicholas *et al.* (20) and Bishop *et al.* (70). The question arises as to whether the nature of the background cells remains static during the course of the disease process or whether it represents a dynamic population of cells constantly changing during the course of the disease. The hypothesis that lymphokine production by the tumour cells may play an important role in determining the immunoreactive response awaits confirmation. A proportion of cases of nodular lymphocyte predominance Hodgkin's disease undergoes progression, after a period, to a

diffuse large cell non-Hodgkin's lymphoma; indeed, the two diseases may coexist in the same lymph node (vide supra). None of the cases in this study showed features to suggest such progression.

The diagnosis of diffuse lymphocyte predominance Hodgkin's disease is rarely made. Until recently, knowledge of the immunophenotype of this subtype of Hodgkin's disease was based on a small number of cases (Table 11). Several studies in the past indicated a low rate of positivity of the tumour cells for B-cell markers, the tumour cells being more commonly positive for CD15 and CD30 markers (4,57). In these respects, the immunophenotype more closely resembled that seen in types of Hodgkin's disease other than nodular lymphocyte predominance. In more recently published series in 1990 and 1991, the immunophenotype of the tumour cells more closely resembled that seen in nodular lymphocyte predominance Hodgkin's disease, i.e. CD15/CD30 negative and B-cell marker positive (70,75).

Some investigators now argue for a three-fold subdivision of lymphocyte predominance Hodgkin's disease: nodular, and diffuse and a third subtype, the so-called mixed lymphocyte predominance Hodgkin's disease in which classic Reed-Sternberg cells expressing CD15 and CD30 antigens are present. The nodular and diffuse forms are thought to be closely related both with clearly discernible L&H cells which express a B phenotype (70). Only one case of diffuse lymphocyte predominance Hodgkin's disease was assessed in this study. Classical Reed-Sternberg cells were not prominent, although present, and the tumour cells expressed neither CD15 nor CD30 antigens. The B-cell markers, L26 and LN1, were expressed by the tumour cells. These results do not differ greatly from the find-

ings in nodular lymphocyte predominance Hodgkin's disease, and are in accord with several recently published series (vide supra). In the study of Bishop *et al.*(70), 59% of their cases were of B phenotype compared to 80% in that of Hansmann *et al.* (75). L26 was the antibody used in both studies. Twenty-four percent of cases in Bishop and co-workers study expressed CD15 and none expressed CD30. The corresponding figures from the study of Hansmann and co-workers were 10 and 20%, respectively. These findings suggest that the tumour cells in nodular and diffuse lymphocyte predominance Hodgkin's disease are B-cell derived and closely related, suggesting a continuum of one disease process.

Additional findings in the single case of diffuse lymphocyte predominance Hodgkin's disease in this study were a predominantly T-cell background and the presence of ill-defined FDC networks, both features well described in the diffuse subtype (4,20,70,75). Unfortunately, due to the paucity of cases of lymphocyte predominance, diffuse Hodgkin's disease in this study, the hypothesis that the nodular and diffuse forms are indeed closely related cannot be supported or refuted. It is, however, contended that there is strong evidence to suggest that cases with a diffuse growth pattern in which the Reed-Sternberg cells and L&H cells express CD15 and CD30 and B-cell antigen expression is absent should probably be placed in the mixed cellularity category of Hodgkin's disease.

These findings highlight the difficulty in diagnosing diffuse lymphocyte predominance Hodgkin's disease using morphological criteria alone. Further work is needed to establish the relationship between morphology and immunophenotype, with a view to establishing

a definitive pathological definition of diffuse lymphocyte predominance Hodgkin's disease.

Interfollicular Hodgkin's disease is uncommon. Criteria for the diagnosis of interfollicular Hodgkin's disease include background reactive follicular hyperplasia with an admixture of cells comprising varying proportions of diagnostic Reed-Sternberg cells and variants thereof, eosinophils, plasma cells and T and B lymphocytes interspersed in the interfollicular zones. Two cases in this study fulfilled these criteria. In only 1 case did the tumour cells express CD15 and then only in a small percentage of the tumour cells, whereas CD30 was expressed by a large percentage of the tumour cells in both cases. B- or T-cell markers were not expressed in either case. These findings are in accord with those found in nodular sclerosis and mixed cellularity Hodgkin's disease in this and other studies. Interfollicular Hodgkin's disease represents an unusual pattern of focal involvement of lymph nodes by Hodgkin's disease - the importance of this pattern lies in it being misinterpreted as a reactive lymph node resulting in unnecessary delays in diagnosis and therapy. As mentioned above, this morphologic variant is not included as a distinct histologic subtype in either the Lukes and Butler or Rye classifications of Hodgkin's disease. Lukes emphasized that lymph nodes with Hodgkin's disease lacking the typical features of lymphocyte predominance, nodular sclerosis or the lymphocyte depleted subtypes should be placed in the mixed cellularity category - Doggett *et al.* have, however, encountered a number of cases in which other nodes removed at the same time showed characteristic features of nodular sclerosis Hodgkin's disease (54). This strongly suggests that

interfollicular Hodgkin's disease represents an early stage in the development of one of the other subtypes of Hodgkin's disease in a lymph node. It does not appear to differ significantly in its clinical behaviour from other subtypes of Hodgkin's disease and has no prognostic significance (51).

The group of Hodgkin's disease, not further classifiable (N.O.S.), in this study represent a miscellaneous group of cases in which, for a number of reasons, the specific subtype could not be accurately determined on morphologic and/or immunophenotypic grounds. All cases met the requirements for Hodgkin's disease, i.e. presence of classic Reed-Sternberg cells and appropriate immunoreactive response. In one case, the biopsy was that of an extradural tumour in which necrosis was a prominent feature. The tumour cells expressed CD15, CD30 and were of B phenotype - an immunophenotype consistent with that seen in nodular sclerosis Hodgkin's disease in this and other studies. However, sclerosis was not a prominent feature - this may be attributed to the fact that the biopsy was from soft tissue and not from a lymph node, and it highlights the difficulty in diagnosing Hodgkin's disease at extranodal sites. Three cases showed hypocellular lymph nodes effaced by sclerosis - the features were not typical of those seen in lymphocyte depleted Hodgkin's disease and probably represent the lymphocyte depleted end of the mixed cellularity subtype of Hodgkin's disease. In only one case was the immunophenotype not in accord with that seen in mixed cellularity Hodgkin's disease - both LeuM1 and BerH2 were negative, an uncommon finding in mixed cellularity Hodgkin's disease (only 1 of 16 cases of mixed cellularity Hodgkin's disease were negative for both antibodies in

this study). Of the remaining 4 cases, 1 showed only partial involvement of a lymph node at one pole. No distinguishing morphological features were present to enable further subtyping. Their features were not typical of interfollicular Hodgkin's disease. Another case showed features of interfollicular Hodgkin's disease evolving into nodular sclerosis Hodgkin's disease - early capsular fibrosis was present with a large number of Reed-Sternberg cells and Hodgkin's cells being present in the interfollicular zone. The seventh case was an unusual case with features of interfollicular Hodgkin's disease and angiofollicular lymphoid hyperplasia in the same node. The tumour cells typically expressed CD15. The last case in the group was originally diagnosed as nodular sclerosis Hodgkin's disease. Sclerosis with vague nodule formation was evident in the node. Classic Reed-Sternberg cells and an appropriate immunoreactive background were present, but the mononuclear variants were not of the lacunar type seen in nodular sclerosis Hodgkin's disease. Instead, many of these cells had more than one nucleolus and these were not typically eosinophilic and inclusion-like. The tumour cells did not express CD15, CD30 or EMA antigens and were of B phenotype, reactive for both L26 and LN1 thus raising the possibility of non-Hodgkin's B-cell lymphoma. In addition, the growth pattern and immunophenotype were not in keeping with that seen in anaplastic large cell lymphoma. Clinicopathological correlation may be of help in cases of this nature.

The above cases all serve to highlight the difficulty in subtyping Hodgkin's disease, even though the diagnosis of the disease can often be made using both morphological criteria and immunophenotyping of tumour cells. Immunophenotyping is of help in distinguish-

ing lymphocyte predominance Hodgkin's disease from the other subtypes of Hodgkin's disease, but it is of little help in differentiating between nodular sclerosis, mixed cellularity and lymphocyte depleted Hodgkin's disease. Morphological criteria play a more important role here.

The expression of CD15 and CD30 antigens by the tumour cells in anaplastic large cell lymphoma have been discussed (vide supra). Results obtained in this study with LCA (CD45) are in accord with those in the meta-analysis of published series (Table 14). Forty-six percent of the cases in this study expressed CD45 compared to 49% in pooled series. Variable expression of LCA in anaplastic large cell lymphoma has been well described. Falini *et al.* showed that 54% of their LCA negative cases reacted with antibodies against T- and B-cell markers (81).

| REFERENCE | LEUM1 | BERH2 | EMA | LCA | T CELL | B CELL |
|---|---|---|---|---|---|---|
| ANAPLASTIC Ki-1 LYMPHOMA: | | | | | | |
| Hall *et al.*[14] | 3/5 | 5/5 | 4/5 | 3/5 | 3/5 | 0/5 |
| Hansmann *et al.*[80] | 2/11 | 11/11 | | | 5/11 | 4/11 |
| Piris *et al.*[26] | 4/15 | 12/14 | 3/15 | 5/15 | 4/15 | 6/15 |
| Agnarsson *et al.*[24] | 0/19 | 18/18 | | | 13/18 | 1/18 |
| Chott *et al.*[21] | 0/41 | 41/41 | 23/41 | 22/41 | 28/41 | 4/41 |
| Total | 9/91 | 87/89 | 30/61 | 30/61 | 53/90 | 15/90 |
| This study | 4/13 | 13/13 | 4/13 | 6/13 | 5/13 | 3/13 |

Table 14: Published imunophenotype of the malignant cells in anaplastic large cell lymphoma compared with those in this study. The results of the published series have been aggregated.

In this study, 40% of LCA negative cases expressed reactivity for one or more T- or B-cell markers. These findings stress the impor-

tance of doing T- and B-cell markers in all cases of anaplastic large cell lymphoma even if the tumour cells fail to express CD45. The percentage of cases with a T phenotype in this study (38%) was less than that in published series (59%) while the percentage of cases with a B phenotype was greater (23%) than that in published data (17%) (Figure 25). Of the B-cell markers L26 was most consistently positive followed by LN1 and MB2. CD3 was the more reliable T-cell marker. EMA expression in this study was also less than that obtained in published series - 31% versus 49%.

Anaplastic large cell lymphoma can mimic anaplastic non-lymphoid malignancies both morphologically and phenotypically. The presence of CD45 negative/EMA positive tumour cells may lead to confusion and an incorrect diagnosis being made unless markers against other T- and B-cell antigens are combined with LCA, BerH2 and EMA in determining the phenotype of the tumour cells. As mentioned earlier, anaplastic large cell lymphoma can also mimic nodular sclerosis Hodgkin's disease morphologically with fibrous capsular thickening and distinct fibrous bands subdividing the lymph node into nodules. Thirty-one percent of the cases in this study had this pattern, and over half the cases in a study published in 1988 had a similar pattern (24). In 1 of our cases the pattern of fibrosis was more diffuse. All cases in our study had a syncitial growth pattern, and in 2 of them the tumour infiltrate was sinusoidal. Cytological features in the cases in this study were similar to those described in the literature with striking nuclear pleomorphism observed in most cases. The background immunoreactive response has not been well described in anaplastic large cell lymphoma - in 54% of the cases in this study T lymphocytes were the dominant cell, and in 15% (2 cases) B-cells were the dominant cell.

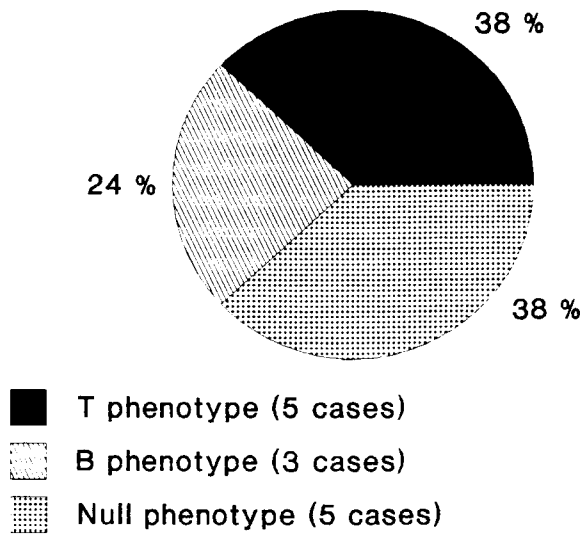



Figure 25: Percentage of cases of anaplastic large cell lymphoma with a B, T or null phenotype (total = 13).

In the remaining 31%, T- and B-cells were present in equal number. Histiocytes were present in a moderate number in all cases.

In summary, anaplastic large cell lymphomas represent a morphologically and immunophenotypically heterogeneous group of T and B-cell lymphomas which can often not be distinguished easily from Hodgkin's disease, particularly the syncitial variant of nodular sclerosis Hodgkin's disease, on morphologic or phenotypic features alone. Positivity of the tumour cells for LeuM1 in a small percentage of cases may lead to the erroneous diagnosis of Hodgkin's disease. Clinicopathological correlation may be of assistance in cases such as this, and may aid the pathologist in coming to a definitive diagnosis. The presence of skin lesions in a patient with lymphadenopathy strongly favours a diagnosis of anaplastic large cell lymphoma, as skin lesions are rare in patients with Hodgkin's disease (vide supra). Extranodal disease at presentation occurs in up to 40% of patients with anaplastic large cell lymphoma (21). The diagnosis of anaplastic large cell lymphoma in the incorrect clinical setting has certain clinical and prognostic implications for the patient, just as the incorrect diagnosis of Hodgkin's disease may lead to a delay in implementing the correct therapy which results in an increase in mortality. Several cases in this study underscored this difficulty in diagnosis, particularly those with a syncitial growth pattern alluded to earlier in the discussion. They were grouped together in the category Hodgkin's disease/Non-Hodgkin's lymphoma. Immunohistochemical results were misleading in many of the cases and morphologically all cases had features commonly seen in both Hodgkin's disease and anaplastic large cell lymphoma. Features which caused

the most problems were those of syncitial sheets of tumour cells and nodular sclerosis. In one case, the tumour cells were non-cohesive and unattached, and Reed-Sternberg-like cells were numerous. The mononuclear tumour cells cytologically resembled those seen in anaplastic large cell lymphoma. Immunophenotypically, the tumour cells expressed CD15 and CD30 antigens strongly in a large percentage of the cells and were reactive for two B-cell markers, notably L26 and LN1, and staining with EMA was equivocal. Cases of anaplastic large cell lymphoma with a non-cohesive growth pattern have been described in the literature (24). Immunohistochemically, a diagnosis of mixed cellularity Hodgkin's disease was favoured for this case, but morphologically a diagnosis of anaplastic large cell lymphoma was preferred. The B phenotype of the tumour cells does not favour one diagnosis over another as 22% of cases of mixed cellularity Hodgkin's disease in published series are of B phenotype compared to only 16% in anaplastic large cell lymphoma.

In 3 cases, a diagnosis of anaplastic large cell lymphoma on morphological grounds was favoured, but immunohistochemistry was suboptimal and BerH2 remained negative despite several attempts with different trypsinization times.

Reactivity of Reed-Sternberg cells and mononuclear variants thereof with antibodies against the intermediate filament, vimentin, warrants separate discussion as the results of few investigations have been published on the reactivity of these cells with this antibody. Results in published series as compared to this study are tabulated in Table 15.

| SUBTYPE | OUR STUDY | TAMARU *et al.*[31] | CARBONE *et al.*[30] |
|---|---|---|---|
| NS | 13/20 (65) | 12/15 (80) | 13/23 (57%) |
| MC | 9/16 (56) | 24/32 (75) | 0/7 |
| LP | 1/9 (11) | 4/11 (36) | 0/4 |
| LD | 0/1 | 5/5 (100) | 0/4 |
| IF | 1/2 (50) | - | - |
| HD,UNCL. | 4/8 (50) | - | - |
| TOTAL | 28/56 (50) | 45/63 (71) | 13/38 (34) |

Table 15: Vimentin reactivity in Reed-Sternberg cells and Hodgkin's cells in this study as compared with results in 2 published series. (%).

Vimentin was expressed in the Reed-Sternberg cells and variants in 50% of cases of Hodgkin's disease in this study and was expressed in cases from all subtypes (Figs. 26 and 27). The incidence was less than that reported by Tamaru *et al.* (71%) but more than that by Carbone *et al.* (34%). In the latter study (30), only Reed-Sternberg cells in nodular sclerosis Hodgkin's disease were reactive for vimentin, whereas in this study and that by Tamaru *et al.* (31) the cells in all subtypes were reactive. In addition, Carbone *et al.* found no differences in antigen expression on Reed-Sternberg cells between vimentin positive and negative cases within the nodular sclerosis subtype. This is in direct contrast to the findings published by Tamaru *et al.* and is also reflected in this study. Differences in antigen expression in 4 cases of nodular sclerosis Hodgkin's disease were found in this study, the results of which are tabulated in Table 16. Of these 4 cases, one was positive for L26 (Figs. 28 and 29), vimentin and LeuM1 and negative

for LN1 - a similar phenotype was obtained in one case of nodular sclerosis Hodgkin's disease in the series by Tamaru *et al.* Phenotypic expression of these antigens in this combination comprises one of the groups of three into which Tamaru *et al.* subdivides Hodgkin's disease on the basis of vimentin expression (vide supra) and has being interpreted as the tumour cells arising from immature B-cells as vimentin is expressed only in the immature stages of B lymphocytes. Only one other case from our unclassifiable group of Hodgkin's disease had a similar phenotype. None of our cases of lymphocyte predominance Hodgkin's disease expressed this phenotype as compared to 4 cases in the series of Tamaru and co-workers.

The second of their groups is characterized by expression of L26 and LN1 in the absence of vimentin and LeuM1. These cases are interpreted as arising from germinal center B-cells since LN1 is known to be expressed by germinal B lymphocytes. Eight cases in this study expressed this phenotype - 7 lymphocyte predominance and 1 unclassifiable case of Hodgkin's disease, compared to 10 cases in Tamaru and co-workers series (7 of these 10 cases being lymphocyte predominance Hodgkin's disease).

The third group is the largest one and comprises all the cases in which the Reed-Sternberg cell and variants express vimentin and/or LeuM1 in the absence of L26, LN1 and EMA. According to Tamaru *et al.*, this phenotypic expression suggests an origin from monocytes and/or histiocytes, as vimentin is expressed strongly in these cells. This, coupled with LeuM1 positivity, strongly favours an origin from these cells. Fourty-two cases in our study compared to 48 in Tamaru and co-workers' series expressed this phenotype.

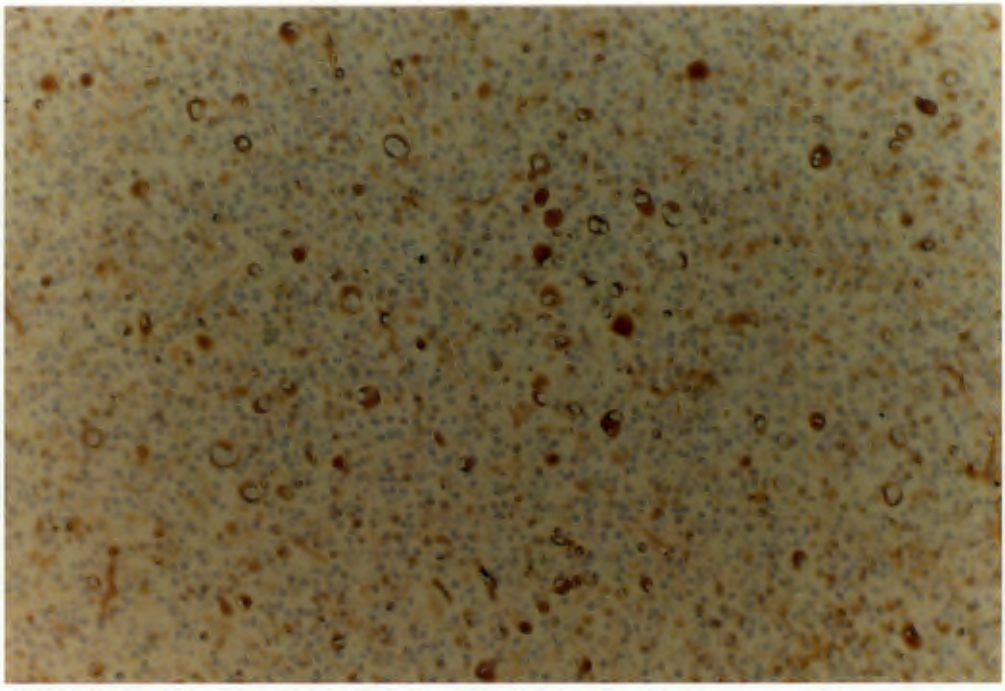


Figure 26: Vimentin. A low power view of vimentin expression by the tumour cells in mixed cellularity Hodgkin's disease. Many of the tumour cells are reactive. (100×).

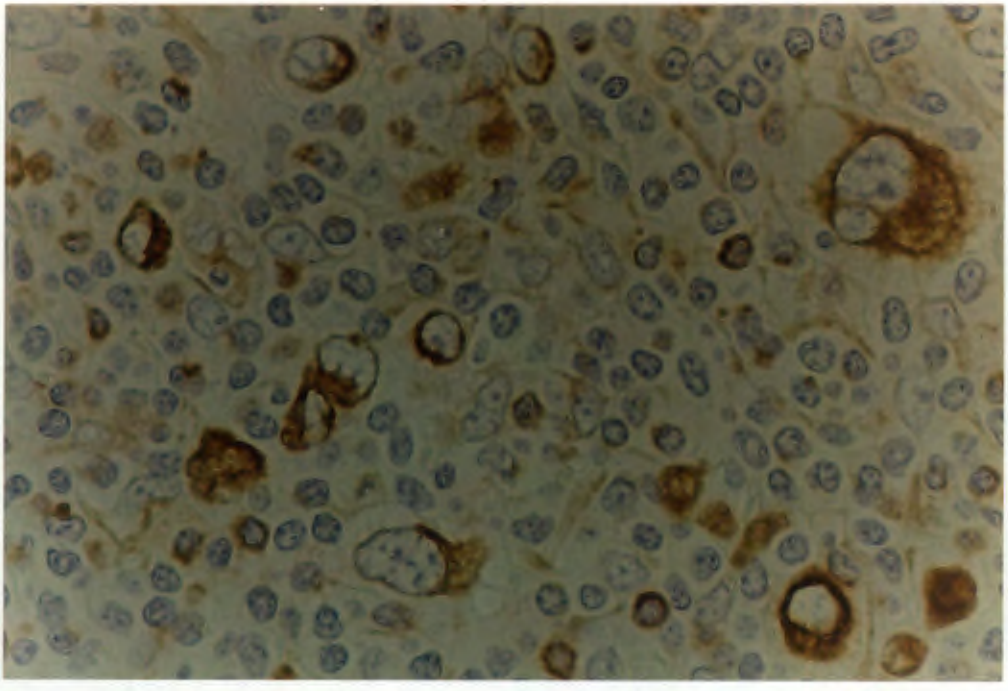


Figure 27: Vimentin. Same case as in figure 26 demonstrating reactivity of the tumour cells at higher power. (400×).

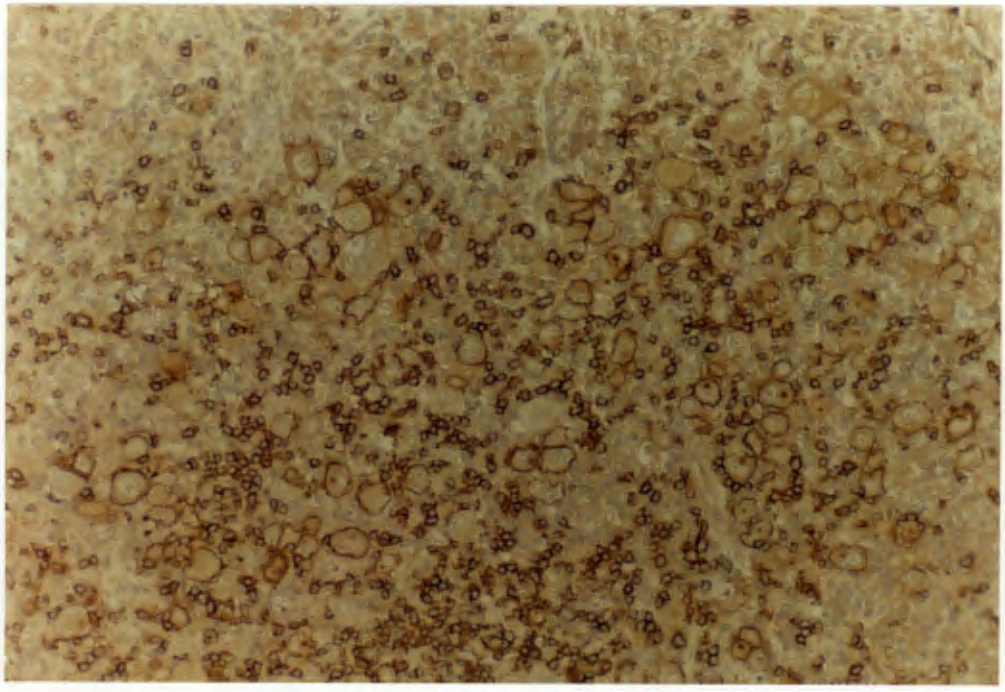


Figure 28: Nodular sclerosis Hodgkin's disease. Expression of L26 by a large number of Reed-Sternberg cells and variants. (100X).

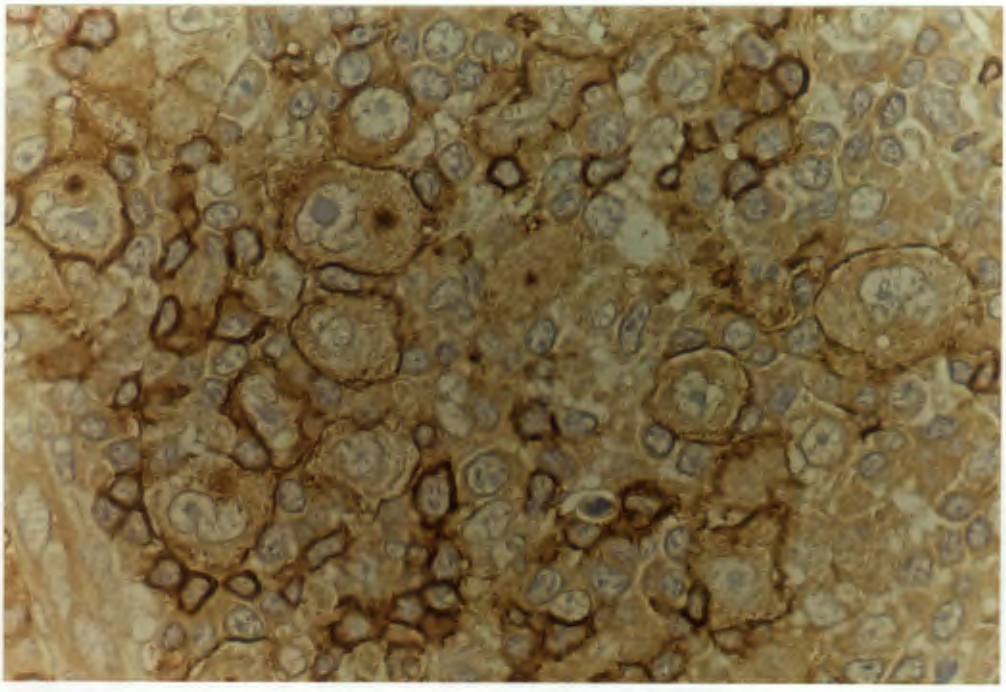


Figure 29: High power view of same case as in figure 28 demonstrating paranuclear and membrane staining of the tumour cells. (400X).

The 3 other cases of nodular sclerosis Hodgkin's disease expressed a variable phenotype. All expressed either L26 or LN1 with or without vimentin and/or LeuM1 expression (Table 16).

| CASE | LEUM1 | VIM. | L26 | LN1 |
|---|---|---|---|---|
| 1. | + | + | + | - |
| 2. | - | - | - | + |
| 3. | + | + | - | + |
| 4. | + | - | + | - |

Table 16: Expression of LeuM1 and Vimentin in 4 cases of B phenotype in this study.

One case was LN1 positive and failed to react with vimentin and LeuM1 - the significance of this finding is unclear. Another expressed LN1 together with LeuM1 and vimentin which suggests a possible germinal center origin, although these cells do not normally express vimentin. The last case in this group expressed L26 and LeuM1 but failed to react with vimentin. If the tumour cells in this case had originated from immature B lymphocytes, one would have expected that vimentin would have been expressed by the Reed-Sternberg cells. The findings, therefore are not entirely in accord with those of Tamaru *et al*.

Division of Hodgkin's disease into 3 groups on the basis of phenotypic expression of the Reed-Sternberg cells and variants thereof may have its merits, but, there does appear to be a fourth group in which the Reed-Sternberg cells do not follow a fixed pattern of antigen expression. No conclusion can be drawn as to the cell of origin of the tumour cells in this group. This again highlights the diversity of immunophenotypic expression of the Reed-Sternberg

cell and its variants in Hodgkin's disease. Differences in antigen expression, even in cases from the same subtype, were a notable finding in this series and were in direct contrast to those found by Carbone *et al*. While not conclusive the results of this study do suggest a divergent origin of these cells and go further to reinforce the concept that Hodgkin's disease represents a heterogeneous group of diseases and is not a single disease entity.

Interestingly, the tumour cells in 7 of the 13 cases of anaplastic large cell lymphoma expressed vimentin. Of the 3 cases of B phenotype, 2 expressed vimentin, and 3 of the 5 cases of T phenotype reacted with vimentin. No study has been published in which vimentin expression in anaplastic large cell lymphoma was specifically investigated, but several studies in which vimentin expression in non-Hodgkin's lymphoma was determined have been published (31,60). Vimentin expression in both T- and B-cell non-Hodgkin's lymphomas occurs frequently, and there appears to be no preferential association of vimentin immunoreactivity with the immunophenotype or histopathologic category in these lymphomas. On the basis of these studies, expression of vimentin in non-Hodgkin's lymphoma appears to be of limited usefulness in establishing the diagnosis of non-Hodgkin's lymphoma.

Monocyte/macrophage antigens, as recognized by the CD68 antibody in this study, were negative in the tumour cells from all subtypes of Hodgkin's disease and anaplastic large cell lymphoma. These results are in accord with those in published data (4,6,46). In most cases reactive histiocytes in the background stained up strongly positive and served as an outstanding internal control.

Analysis of the antibodies recognizing T- and B-cell antigens in this study showed that of the 21 cases of B phenotype (Hodgkin's disease and anaplastic large cell lymphoma), the antibody most frequently positive was L26 in 86% of cases. This was followed by LN1 in 67%, MB2 in 48% and PanB in only 14% of cases. All 4 antibodies were positive in only 1 (5%) case, 3 antibodies in 24% and 2 antibodies in 38% of cases. Thirty-three percent of cases expressed only one antibody - L26 was the most frequent single positive antibody (71% of these cases), followed by LN1 in 29%. Of the T-cell markers CD3 was expressed in 80% of cases with a T phenotype and TUCHL in 40%. Equivocal staining was obtained in 1 case with CD3 and in 2 cases with TUCHL. Both these antibodies were unquestionably positive in only 1 case. The results of this study show that the most reliable markers recognizing B-cells are L26 and LN1, with L26 being the more reliable of the two while the CD3 antibody is more reliable for the recognition of cells of T-cell lineage. LN1 has been proven to be a not very specific marker of B-cells as cross-reactivity with a subset of T-cells is known to occur (Tables 2 and 8), whereas L26 has being shown to be a specific and sensitive marker of B-cells only (8).

Importantly, the CD45 antigen, as recognized by LCA, was expressed in only 48% of cases with a B phenotype and in 60% of cases with a T phenotype. Negativity, therefore for LCA does not necessarily exclude a B- or T-cell lineage of the tumour cells in both Hodgkin's disease and anaplastic large cell lymphoma. These findings indicate that it is important to include, simultaneously, at least one T-cell and one B-cell marker in any antibody panel.

It was not possible to demonstrate heavy or light chain immunoglobulin restriction in any case of B phenotype in this study. Non-specific uptake was seen in 2 cases.

In conclusion, there is widespread agreement that Reed-Sternberg cells and mononuclear variants thereof in nodular sclerosis, mixed cellularity, and lymphocyte depleted subtypes of Hodgkin's disease exhibit an immunophenotype which for most, but not all, cases may be summarized as CD15 positive, CD30 positive, CD45 negative and EMA negative. A B or T phenotype has been reported in several studies, although in this study only a B phenotype was obtained in a minority of cases. No one antigen is uniformly expressed by the Hodgkin's cell population in any case.

The results of this study showed clear phenotypic separation of nodular lymphocyte predominance Hodgkin's disease from other subtypes. The L&H cells were reactive for B-cell markers (all cases) and LCA (most cases) but negative for EMA (most cases), LeuM1 and BerH2. Recent studies have indicated that diffuse lymphocyte predominance Hodgkin's disease has immunophenotypic features identical to that seen in nodular lymphocyte predominance Hodgkin's disease providing more evidence for a relationship between these two subtypes (57,70,75).

The heterogeneity of marker expression in Hodgkin's disease may reflect heterogeneity of origin of the tumour cells between cases supporting the concept that Hodgkin's disease is not a single disease entity but rather a heterogeneous group of disorders. Variation in vimentin expression by the Reed-Sternberg and mononuclear cells further supports this concept. Morphological and

immunophenotypic similarities between anaplastic large cell lymphoma and Hodgkin's disease suggest both these neoplasms result from the neoplastic transformation of activated lymphocytes of either B- or T-cell origin and that anaplastic large cell lymphoma may represent the link between Hodgkin's disease and the non-Hodgkin's lymphomas.

The currently available antibodies that are effective in conventionally fixed and processed material are valuable for determining the immunophenotype of the tumour cells in both Hodgkin's disease and anaplastic large cell lymphoma. They do not, however, provide a clear-cut method for distinguishing between these two tumours. Immunophenotypic profile should not be used as the sole diagnostic discriminant. Diagnosis should be based upon both immunophenotypic profile of the tumour cells as well as careful morphological assessment of each case.

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