

Childhood lymphoma in Yorkshire

A M Davison, P A McKinney, C C Bailey, I Lewis, R A Cartwright, C O'Brien

Abstract

Aims: A histopathological review of 43 cases of childhood non-Hodgkin's lymphoma (NHL) in an attempt to identify histological variables of prognostic importance.

Method: Each case was reclassified according to the Working Formulation and an attempt made to allocate an immunophenotype using a panel of monoclonal antibodies. Results were correlated with clinical data on site and survival.

Results: Of the 43 cases, 30 were males and 13 females. There were 17 cases of lymphoblastic lymphoma, 15 cases of small non-cleaved cell lymphoma (SNCC), and four cases of large cell lymphoma. The SNCC group was subdivided into 10 cases of Burkitt's lymphoma and five cases of non-Burkitt's lymphoma. An immunophenotype was allocated in 65.1% of cases (23 B, 5 T). The SNCC cases were spread throughout the 0-16 year age range while the lymphoblastic lymphoma cases tended to occur in older children. Most mediastinal tumours were lymphoblastic lymphoma and most abdominal tumours were SNCC. Statistical analysis failed to show a significant difference in survival among histological subgroups or immunophenotypes.

Conclusion: No histological variables of prognostic importance were identified partly due to the great variation in treatment regimens, standard of supportive care, and prognosis over the period of the study (1972 to 1988).

The incidence of non-Hodgkin's lymphoma (NHL) in children under 15 years of age is about 1 in 40,000 and shows a preponderance of male cases (ratio 2.5:1).¹⁻³ These lymphomas are predominantly extranodal, high grade, diffuse and often leukaemic. Most cases arise in either the abdomen (31%), the head and neck (29%), or the mediastinum (26%)³ but disseminate early, and most (65%) are widespread at diagnosis.⁴ In contrast to adult NHL, where a multitude of subtypes are observed, 95% of all childhood NHL fall into one of three groups⁵: small non-cleaved cell (SNCC), lymphoblastic and large cell. SNCC is subdivided into Burkitt's lymphoma and non-Burkitt's lymphoma.

The prognosis in childhood NHL has improved dramatically over the past 30 years due to a combination of improved clinical

staging and supportive care, advances in histological classification⁵ and chemotherapy, and the coordination of treatment in recognised centres.² The relative prognostic importance, however, of histological subclassification and immunophenotype compared with clinical stage, for example, is controversial.^{3 6-9}

The purpose of this study was to review cases of childhood NHL, which occurred in the Yorkshire region from 1972 to 1988, using current histological criteria, to allocate an immunophenotype to each case with the aid of monoclonal antibodies reactive in paraffin wax sections, and to correlate these findings with available survival data. Unfortunately, complete data on clinical stage were not available.

Methods

One hundred and four cases of non Hodgkin's lymphoma (NHL), occurring in children aged 16 and under, between the years of 1972 and 1988, in the Yorkshire region were registered with the Yorkshire Children's Tumour Registry. Fifty of the cases were unsuitable for further study either because the material could not be traced (n = 23), the diagnosis had been made on a specimen of bone marrow (n = 13), insufficient tissue remained (n = 12) or there had been a change in diagnosis (n = 2). Freshly prepared haematoxylin and eosin stained sections (5 µm) from the 54 available cases were reviewed and sections from the most suitable block were subjected to a panel of antibodies (table 1).¹⁰⁻¹⁴

The immunogold silver staining (IGSS) method¹⁵ was used for the light chain antibodies, while the remaining four monoclonal antibodies were applied using the three stage streptavidin-biotin method.¹⁶ Negative controls were run for both methods substituting normal rabbit and mouse serum, respectively, for the primary stages of the IGSS and streptavidin-biotin methods.

Cases were classified according to the Working Formulation¹⁷ using accepted criteria (table 2).¹⁸ Subsequently, for each case the antibody staining was assessed as positive, negative, or inconclusive. A positive reaction for L26 or MB2 was interpreted as indicating a B cell immunophenotype. Allocation of a T cell immunophenotype required a positive reaction for UCHL1 and MT1 because of the well documented lineage infidelity of MT1, in particular in lymphoblastic lymphoma.¹² MT1 staining alone resulted in the classification of the immunophenotype as indeterminate or not otherwise specified (NOS).¹⁹

University
Department of
Pathology, Leeds
General Infirmary,
Leeds
A M Davison

Information and
Statistics Division,
Scottish Health
Service, Edinburgh
P A McKinney

Department of
Paediatric Oncology,
Seacroft Hospital,
Leeds
C C Bailey

Department of
Paediatric Oncology,
Seacroft Hospital,
Leeds
I Lewis

Leukaemia Research
Fund Centre for
Clinical
Epidemiology,
University of Leeds
R A Cartwright

Department of
Pathology, Morriston
Hospital, Swansea
C O'Brien

Correspondence to:
Dr A M Davison,
Department of
Histopathology, Sunderland
Royal Infirmary, New
Durham Road, Sunderland

Accepted for publication
31 July 1991

Table 1 Concentration and specificity of antibodies

Antibody	Concentration		Major specificity
	First antibody	Second antibody	
L26	1 in 100	1 in 250	B cells, Reed-Sternberg cells ¹⁰
MB2	1 in 10	1 in 100	B cells, non-lymphoid tissue ¹¹
MT1	1 in 5	1 in 100	T cells, myeloid cells, macrophages ¹²
UCHL1	1 in 20	1 in 250	T cells, myeloid cells, macrophages ¹³
κ	1 in 10000	1 in 100	κ light chain ¹⁴
λ	1 in 10000	1 in 100	λ light chain ¹⁴

Results

Of the 54 cases studied, 43 were classified as non-Hodgkin's lymphoma, 10 were undiagnosable mainly because of poor preservation, and one was reclassified as Hodgkin's disease.

Analysis of the 43 lymphoma cases (table 3) showed that by far the largest group was lymphoblastic lymphoma (17), followed by Burkitt's lymphoma (10), non-Burkitt's lymphoma (5), and large cell (4). Poor morphology meant that six cases could only be classified as high grade NHL (NOS). There was one case of a centroblastic/centrocytic lymphoma in a 13 year old male.

Table 4 gives a breakdown of the diagnoses by sex into two age groups: 0-7 and 8-16 years. Of the 10 cases of Burkitt's lymphoma each age group contains five, while all five cases of non-Burkitt's lymphoma are in the younger age group. Conversely, most of the lymphoblastic lymphoma cases (13/17) are in the older age group.

The 43 cases of lymphoma consisted of 30 males and 13 females, a ratio of about 2:1. Although the cases were divided fairly evenly throughout the 0-16 years age range, the male predominance (16/19) was most noticeable in the 0-7 years age group (table 4).

Over half of the cases (23/43) were of B cell type, 15 cases were indeterminate, and only five cases were of T cell type. An immunophenotype (B or T) was therefore allocated in 65.1% of cases.

Comparison of the allocation of an immunophenotype among the three main diagnostic groups showed that all the large cell cases were typed (all B), and most of the SNCC cases (13/15) were typed (all B), but only seven out of 17 lymphoblastic lymphoma could be typed (four T, three B).

The assessment of site of origin was based on all available clinical data and was not necessarily the site of biopsy. Most lymphoblastic lymphoma cases (12/17) arose in sites other than the mediastinum or the abdomen, with

Table 3 Review diagnosis of lymphoma cases

Diagnosis	Numbers (%)
Lymphoblastic	17 (39.5)
Burkitt's	10 (23.3)
non-Burkitt's	5 (11.6)
Large cell	4 (9.3)
Others	7 (16.3)
Total	43 (100)

only four in the former and one in the latter (table 5). In the SNCC group Burkitt's lymphoma and non-Burkitt's lymphoma most (8/15) arose in the abdomen with only one mediastinal non-Burkitt's lymphoma case. Three of the four large cell lymphoma cases were abdominal in origin.

Of the lymphomas arising in the mediastinum, most (4/6) were lymphoblastic lymphoma, while 50% (7/14) of the abdominal lymphomas were Burkitt's type.

Analysis of the data revealed no significant difference in survival time among the major histological subgroups or among the B, T, and "null" immunophenotypes. Table 6 documents individual cases by age, sex, diagnosis, immunophenotype and survival.

Of the lymphoblastic lymphoma (NOS) cases, the longest survival to date is 163 months in a 3 year old girl, but survival does not seem related to sex or age. Four lymphoblastic lymphoma cases were of T cell type. All three male patients died within one month of diagnosis and the only patient alive at the last follow up (50 months) was female and at 15 years also the eldest. Three lymphoblastic lymphoma cases were of B cell type. The longest survival to date is 149 months in a 7 year old boy and the youngest patient (2 year old boy) died one month after diagnosis.

Of the 10 Burkitt's lymphoma cases four were alive at the last follow up (longest survival 114 months) including both the girls. One of the five non-Burkitt's lymphoma cases (7 year old girl) accounted for the longest survival (179 months) of any of the 43 cases in the study. The two longest surviving cases in this category (53 and 179 months) were also the only two female patients in this group. Survival of SNCC cases does not seem related to age.

Of the four large cell lymphomas, the longest survival (159 months) is the only female case and the shortest survival (less than one month) is in the youngest patient (3 years).

Discussion

Of the 43 cases of lymphoma reviewed in this study, the largest single category was lympho-

Table 2 Criteria for diagnosis of childhood NHL¹⁸

Criteria	Lymphoblastic	Burkitt's	non-Burkitt's	Large cell
Nuclear size*	Smaller	Same	Same	Larger
Nuclear shape	Convolutated	Regular	Irregular	Variable
Chromatin pattern	Fine	Clumped	Clumped	Clumped
Nucleoli	Inconspicuous	Prominent	Prominent	Variable
Cytoplasm	Scanty	Moderate	Moderate	Variable
Cytoplasmic vacuoles	Inconspicuous	Prominent	Prominent	Inconspicuous
Mitoses	++	++	++	Variable
"Starry sky"	+	++	++	Variable

*Relative to the size of a macrophage.

Table 4 Diagnosis by age group, and sex

Age group (years)	Sex	Diagnosis					Total
		Lymphoblastic	Burkitt's	non-Burkitt's	Large cell	Others	
0-7	M	3	5	3	1	4	16
	F	1	0	2	0	0	3
8-16	M	8	3	0	2	1	14
	F	5	2	0	1	2	10
Total		17	10	5	4	7	43

Table 5 Site of lymphomas

Site	Lymphoblastic	Burkitt's	non-Burkitt's	Large cell	Others	Total
Abdomen	1	7	1	3	2	14
Mediastinum	4	0	1	0	1	6
Others	12	3	3	1	4	23
Total	17	10	5	4	7	43

phoblastic (39.5%), followed by SNCC (34.9%), and large cell (9.3%). These figures do not differ widely from those of previous studies^{3,18} which place SNCC as the largest group (39-50%), have fewer lymphoblastic cases (28-34%), and slightly more large cell cases (14-26%).

We are aware that the material examined in this study may not be entirely representative of population based data because of the high proportion of the total cases which were unsuitable for investigation. In particular, there is a suspicion that the number of mediastinal lymphoblastic lymphoma cases may be an underestimate because the diagnosis was often made on examination of a pleural aspirate or bone marrow specimen, neither of which provided suitable material for this study.

Criteria for histological typing¹⁸ (table 2) seem to be reproducible and in most cases allow lymphoblastic lymphoma to be differentiated from large cell and SNCC types. Particularly useful criteria are nuclear size, shape, chromatin pattern and prominence of nucleoli. Lymphoblastic lymphoma (fig 1) have small convoluted nuclei with a fine chromatin pattern and inconspicuous nucleoli. Large cell lymphomas (fig 2) have large nuclei with clumped chromatin and often have prominent nucleoli. SNCC lymphomas (fig 3 and 4) have medium sized nuclei with clumped chromatin and prominent nucleoli. Further division of SNCC into Burkitt's lymphoma and non-Burkitt's lymphoma types can be difficult, and indeed debate exists as to whether or not it is a useful exercise.^{6,7,18} The division is made on an assessment of whether the nuclei have a regular (Burkitt's lymphoma) or an irregular (non-Burkitt's lymphoma) shape.

The sex ratio of 2:1 (males:females) in our series compares favourably with previous studies,^{2,3} although the male predominance (16/19) in the 0-7 years age group is a striking feature which does not seem to have been reported previously.

The peak incidence of childhood NHL is said to occur between the ages of 7 and 11 years and is considered quite rare (4.8% of all cases) below the age of 3 years.²⁰ Our study, however, found a fairly even distribution of cases throughout the 0 to 16 years age range (table 4) and a greater number of cases (9.3%) below the age of 3 years.

Although the cases of Burkitt's lymphoma were evenly distributed across the 0-16 years age range (table 4), all the non-Burkitt's lymphoma cases fell into the younger age group, and most of the lymphoblastic lymphoma cases occurred in older children, 13 out of the 17 occurring in the 8-16 years age group.

Of the 43 cases, 65.1% were allocated an immunophenotype, most of which were B cell type (23/43), while only five cases were T cell type. All but one of these childhood lymphomas was classified as high grade, and it is well recognised, especially in B cell lym-

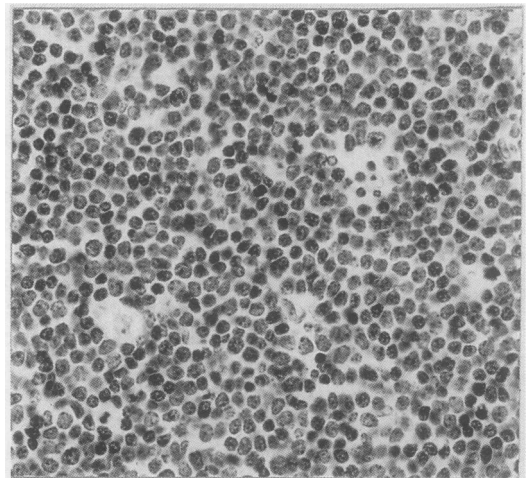


Figure 1

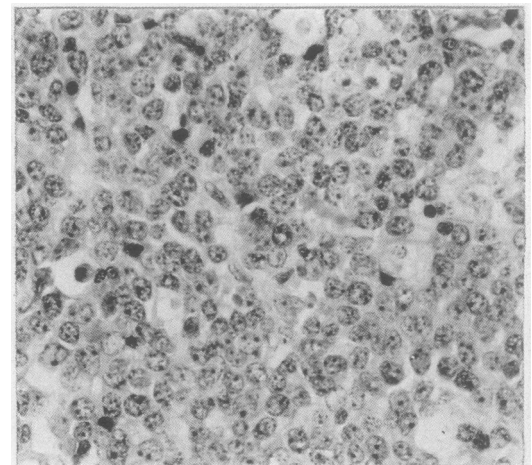


Figure 2

Figures 1 and 2 Figure 1 is a case of lymphoblastic lymphoma with small convoluted nuclei and inconspicuous nucleoli. By comparison, figure 2 is a large cell lymphoma with large nuclei and prominent nucleoli.

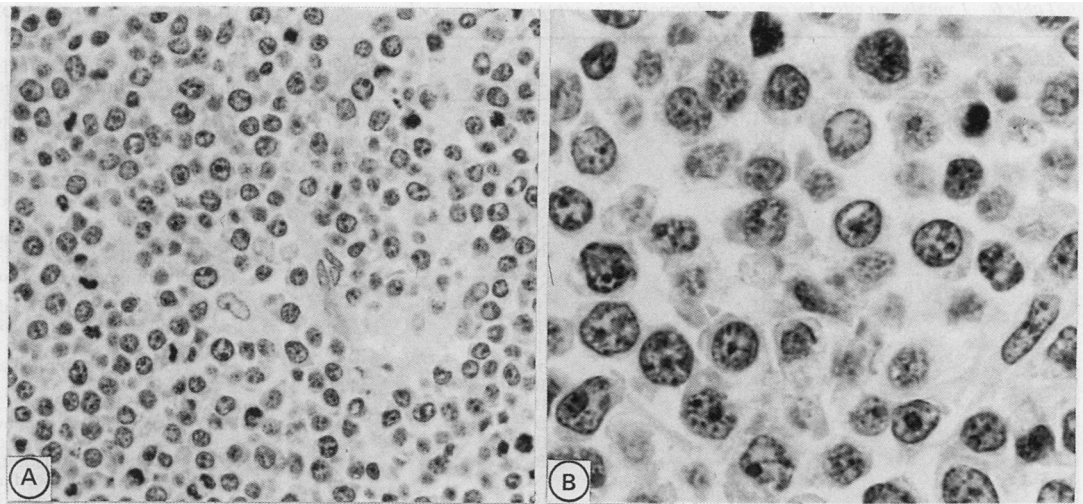


Figure 3 (A, B) This case of Burkitt's lymphoma shows medium sized nuclei with a clumped chromatin pattern and little nuclear pleomorphism.

phomas, that typing is less predictable in high grade cases.¹⁹

The most useful reagents for the identification of B cell lymphomas in paraffin wax sections are L26, MB2, and immunoglobulin stains, but as many as 10% and 12% respectively of B cell lymphomas may stain with MT1 and UCHL1.¹⁹ Similarly, although about 80% of T cell lymphomas stain with MT1 and 85% with UCHL1, occasional cases can stain with MB2 (12%) or L26 (6%).¹⁹ At the start of our study UCHL1 was regarded as the most reliable antiserum for phenotyping T cell lymphomas in paraffin wax sections.²¹

In lymphoblastic lymphoma the allocation of a phenotype can be particularly difficult. Only seven (4T and 3B) of our 17 cases could be typed. L26 is particularly useful in identifying B cell lymphoblastic lymphoma but can be negative in some cases.¹⁰ UCHL1 is also useful because although it may occasionally be negative in some T cell lymphoma, it does not stain B cell lymphoblastic lymphoma.¹³ MB2 and MT1 cannot be relied on on their own as they both stain B and T cell lymphoblastic lymphoma.¹²

Most (90%) lymphoblastic lymphoma cases are said to have an immature T cell phenotype and although MT1 may stain many of these cases, UCHL1 seems to stain fewer²² and may

not stain those with an early thymic phenotype.²³ This may partly explain our relatively low number of T cell lymphoblastic lymphoma cases. The finding of three cases of B cell lymphoblastic lymphoma in what was (on haematoxylin and eosin staining) expected to be a predominantly T cell group, highlights the need for immunophenotyping in all cases.

Most SNCC (13/15) cases were typed and all were B cell. Previous studies have reported that all Burkitt's lymphoma and most non-Burkitt's lymphoma express B cell markers, while some do not type, and some may express T cell markers.^{24,25}

All the large cell lymphomas in this series were of B cell type. A previous study has shown that most are of B cell type (50–60%), a few (5–15%) are T cell type, and the remainder are "null".²⁵

Previous studies have reported a good correlation between histological subtype and site of origin with SNCC occurring predominantly in the abdomen, lymphoblastic lymphoma in the mediastinum, and large cell lymphoma at any site.³ Most of our SNCC cases (8/15) arose in the abdomen, but only four of our lymphoblastic lymphoma cases originated in the mediastinum (table 5). As there were only six mediastinal lymphomas in the study, it may be more accurate to say that a mediastinal

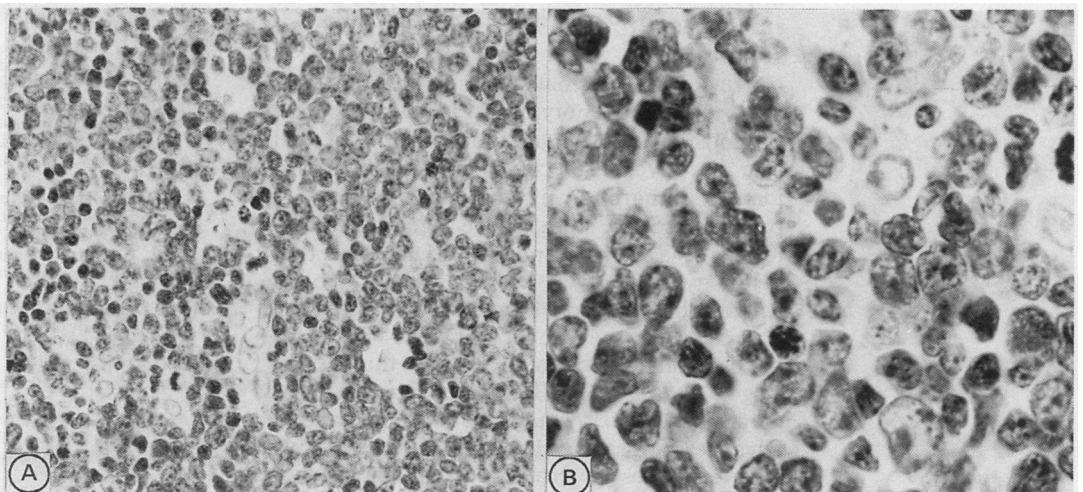


Figure 4 (A, B) This case of non-Burkitt's lymphoma shows medium sized nuclei with a clumped chromatin pattern and more nuclear pleomorphism than the Burkitt's lymphoma in figure 3.

Table 6 Lymphoma cases by age, sex, diagnosis, phenotype and survival

Age (years)	Sex	Diagnosis	Phenotype	Survival (months)
12	M	LBL	NOS	25
3	F	LBL	NOS	163
8	F	LBL	NOS	135
14	M	LBL	NOS	139
8	M	LBL	NOS	126
12	M	LBL	NOS	13
1	M	LBL	NOS	0
15	M	LBL	NOS	0
9	F	LBL	NOS	19
8	F	LBL	NOS	0
9	M	LBL	T	0
13	M	LBL	T	0
15	F	LBL	T	50
12	M	LBL	T	0
2	M	LBL	B	1
7	M	LBL	B	149
13	F	LBL	B	94
2	M	Burkitt's	B	0
12	M	Burkitt's	B	1
8	F	Burkitt's	NOS	114
4	M	Burkitt's	B	30
13	M	Burkitt's	B	0
4	M	Burkitt's	B	70
6	M	Burkitt's	B	3
10	F	Burkitt's	B	45
10	M	Burkitt's	B	0
4	M	Burkitt's	B	0
6	M	non-Burkitt's	B	3
6	F	non-Burkitt's	NOS	53
7	F	non-Burkitt's	B	179
2 months	M	non-Burkitt's	B	18
3	M	non-Burkitt's	B	24
9	F	Large cell	B	159
10	M	Large cell	B	69
3	M	Large cell	B	0
11	M	Large cell	B	73
13	M	Centroblastic/centrocytic	B	28
10	F	NHL	NOS	7
3	M	NHL	NOS	0
3	M	NHL	NOS	16
4	M	NHL	B	167
3	M	NHL	B	35
12	F	NHL	T	52

lymphoma is more likely to be a lymphoblastic lymphoma rather than that most of these cases arise in the mediastinum. Similarly, eight out of 14 abdominal cases were SNCC, and therefore abdominal lymphoma is more likely to be of SNCC than any other type.

We found no significant difference in median survival time between the major histological subgroups or between the B, T, and "null" immunophenotypes. This is not surprising, principally because of the relatively small number of cases in our study, but also because of the great variation in histological classification,⁵ treatment regimens,² and standard of supportive care and prognosis over the past 20 years. In particular, the five year survival rate for childhood NHL in the United Kingdom has improved dramatically over the period of our study from 22% in 1972 to 39% in 1978 and 70% in 1984.²⁶

Previous studies have asserted that age, sex, and immunophenotype are not important prognostic factors,²⁻⁴ and indeed as the survival rate improves it becomes more difficult to identify subgroups of patients with a poor prognosis. Some studies have argued that clinical stage is the most important factor and that histology is relatively unimportant.^{3,8} Others⁹ suggest that distinguishing lymphoblastic lymphoma from the other groups is the main histological decision to be made, or that only in stages 3 and 4 is histology important.^{3,18} Pathologists, however would probably assert that advances in histological classification still outstrip advances in treatment and that further improvement in the survival figures will only come about by identifying additional subtypes

that would benefit from a different therapeutic approach.

Leeds Candlelighters and Leeds West Special Trustees are thanked for financial support of the Yorkshire Children's Tumour Registry and the Leukaemia Research Fund for facilitating data processing. We are particularly grateful to Mrs Helen Lilley, data manager for the Registry, for help with data collection.

We are also indebted to the consultants of the pathology departments in Yorkshire for their cooperation in providing material for the study.

Technical assistance was provided by Mr P Jackson, Mrs C Estlin, and Mr S Toms. Finally, thanks are due to Miss J I Hamblin for typing the manuscript.

- Cutler SJ, Young JL, eds. Third national cancer survey: incidence data. *Nat Cancer Inst Monogr* 1975;41:100.
- Smith SD, Rubin CM, Hokvath A, Nachman J. Non-Hodgkin's lymphoma in children. *Semin Oncol* 1990;17:113-9.
- Murphy SB, Fairclough DL, Hutchison RE, Berard CW. Non-Hodgkin's lymphomas of childhood: an analysis of the histology, staging and response to treatment of 338 cases at a single institution. *J Clin Oncol* 1989;7:186-93.
- Murphy SB. Childhood non-Hodgkin's lymphoma. *N Engl J Med* 1978;299:1446-8.
- Vannier JP. Childhood non-Hodgkin's lymphomas: clinical and therapeutic aspects. *Biomed Pharmacother* 1988;42:263-70.
- Hutchison RE, Murphy SB, Fairclough DL, et al. Diffuse small noncleaved cell lymphoma in children. Burkitt's versus non-Burkitt's types. *Cancer* 1989;64:23-8.
- Wilson JF, Kjeldsberg CR, Sposto R, et al. The pathology of non-Hodgkin's lymphoma of childhood: II. Reproducibility and relevance of the histologic classification of "undifferentiated" lymphomas (Burkitt's versus non-Burkitt's) *Hum Pathol* 1987;18:1008-14.
- Wollner N, Birchenal JH, Lieberman PH, et al. Non-Hodgkin's lymphoma in children: A comparative study of two modalities of therapy. *Cancer* 1976;37:123-34.
- Anderson JR, Wilson JF, Jenkin RPT, et al. Childhood non-Hodgkin's lymphoma: The results of a randomized therapeutic trial comparing a four-drug regimen (COMP) with a ten-drug regimen (LSA₂-L₂). *N Engl J Med* 1983;308:559-65.
- Norton AJ, Isaacson PG. Monoclonal antibody L26: an antibody that is reactive with normal and neoplastic B lymphocytes in routinely fixed and paraffin wax embedded tissues. *J Clin Pathol* 1987;40:1405-12.
- Hall PA, D'Ardenne AJ, Butler MG, et al. New marker of B lymphocytes, MB2: comparison with other lymphocyte subset markers active in conventionally processed tissue sections. *J Clin Pathol* 1987;40:151-6.
- Poppema S, Hollema H, Visser L, et al. Monoclonal antibodies (MT1, MT2, MB1, MB2, MB3) reactive with leukocyte subsets in paraffin-embedded tissue sections. *Am J Pathol* 1987;127:418-29.
- Norton AJ, Ramsay AD, Smith SH, et al. Monoclonal antibody (UCHL1) that recognizes normal and neoplastic T cells in routinely fixed tissues. *J Clin Pathol* 1986;39:399-405.
- Holgate CS, Jackson P, Lauder I, et al. Surface membrane staining of immunoglobulins in paraffin sections of non-Hodgkin's lymphoma using immunogold-silver staining technique. *J Clin Pathol* 1983;36:742-6.
- Holgate CS, Jackson P, Cowen PN, et al. Immunogold-silver staining: a new method of immunostaining with enhanced sensitivity. *J Histochem Cytochem* 1983;31:938-44.
- Hsu S-M, Raine L. Protein A, avidin and biotin in immunohistochemistry. *J Histochem Cytochem* 1981;29:1349-53.
- Stansfeld AG. High grade malignant lymphomas In: Stansfeld AG, ed. *Lymph node biopsy interpretation*. Edinburgh: Churchill Livingstone, 1985:277-99.
- Wilson JF, Jenkin RDT, Anderson JR, et al. Studies on the pathology of non-Hodgkin's lymphoma of childhood. 1. The role of routine histopathology as a prognostic factor. A report from the children's Cancer Study Group. *Cancer* 1984;53:1695-704.
- Norton AJ, Isaacson PG. Lymphoma phenotyping in formalin-fixed and paraffin wax-embedded tissues: II. Profiles of reactivity in the various tumour types. *Histopathology* 1989;14:557-79.
- Hutchison RE, Ching-hon P, Murphy SB, et al. Non-Hodgkin's lymphoma in children younger than 3 years. *Cancer* 1988;62:1371-3.
- Norton AJ, Isaacson PG. Lymphoma phenotyping in formalin-fixed and paraffin wax-embedded tissues. I Range of antibodies and staining patterns. *Histopathology* 1989;14:437-46.
- Jaffe E. The role of immunophenotypic markers in the classification of non-Hodgkin's lymphomas. *Semin Oncol* 1990;17:11-9.
- Stein H, Lennert K, Feller AL, Mason DY. Immunohistological analysis of human lymphoma: correlation of histological and immunological categories. *Cancer Res* 1984;44:98-102.
- Mann RB, Jaffe ES, Braylan RC, et al. Nonendemic Burkitt's lymphoma: a B-cell tumor related to germinal centers. *N Engl J Med* 1976;295:685-91.
- Berard CW, Jaffe ES, Braylan RC, et al. Immunological aspects and pathology of the malignant lymphomas. *Cancer* 1978;42:911-21.
- Stiller CA, Bunch KJ. Trends in survival for childhood cancer in Britain diagnosed 1971-85. *Br J Cancer* 1990;62:806-15.