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The Pre-Treatment Proliferative Activity of Non-Hodgkin's Lymphoma Cells

J. H. SCARFFE and DEREK CROWTHER

Cancer Research Campaign, Department of Medical Oncology, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, United Kingdom

Abstract—*The percentage (%) of cells in S phase of the cell cycle assessed by flow cytometry from biopsies of 69 patients with non-Hodgkin's lymphoma ranged from 0.1 to 34%, with a mean of 5% and a median of 2%. There was a wide range in the percentage of cells in S phase within each histological group of the Rappaport classification, except for the diffuse lymphocytic well differentiated lymphomas in which the results were uniformly low. The pretreatment proliferative activity was higher in the 'unfavourable' than the 'favourable' prognosis histological group* (P=0.02). *No difference was found between the proliferative activity of apparently localised and generalised disease. There were significantly more complete remissions after chemotherapy in patients with stage III or IV disease, whose biopsies contained 5% or more cells in S phase* (P=0.015). *The patients whose lymphomas were found to have a high proliferative activity prior to treatment had shorter remissions than those whose lymphomas were of low proliferative activity* (P=0.04).

INTRODUCTION

LYMPHOMAS other than Hodgkin's disease form a protean group and vary greatly in histological pattern and clinical features. The histological classification of this group of diseases is a complex and controversial subject. This is reflected in the large number of classifications in current use [1–5].

All the histological classifications are highly subjective, and there is a wide variation in the clinical course of patients with histologically similar disease.

The proliferative activity of tumour cells estimated by thymidine labelling index (TLI) or from DNA histograms generated by flow cytometry show considerable variation in human tumours of similar morphology [6]. These pretreatment estimates of proliferative activity have been shown in some malignancies to help predict response to treatment [7–9] and the length of remission obtained [7, 10].

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Address correspondence to: Dr J. H. Scarffe, Department of Medical Oncology, Christie Hospital and Holt Radium Institute, Wilmslow Road, Manchester M20 9BX, United Kingdom.

The prognostic significance of pretreatment proliferative studies in non-Hodgkin's lymphoma (NHDL) is not clear. The early studies of Peckham and Cooper showed no correlation with histology or prognosis [11, 12]. In 1977 preliminary results from our group suggested that the pretreatment percentage of cells in the DNA synthetic (S) phase of the cell cycle correlated with the Rappaport classification, response to treatment, and inversely with the length of first remission [13]. In the same year Silvistrini *et al.* [14] using the Kiel histological classification [5] found that all the patients whose lymph node histology was of the high malignancy grade consistently had a high TLI, but not all the low malignancy group had a low TLI. The same group in 1978 [15] demonstrated an inverse relationship between TLI and survival in the 34 patients they studied.

In an attempt to clarify the position, pretreatment proliferation studies were performed on a larger group of patients than previously reported. The proliferation studies were correlated with the Rappaport histological classification, stage, response to treatment, length of first remission and survival. The Rappaport classification was used because it has proved a useful prognostic indicator in clinical investi-

gations [16] and has been widely accepted. There is, however, a continuing dispute over the scientific accuracy of the classification, particularly regarding the true nature of the histiocytic lymphoma which has been shown in most cases to be lymphoid in origin [17, 18].

The proliferation studies were performed by flow cytometry since NHDL patients are readily biopsied, and single cell suspensions can be made easily. The percentage of cells in the different phases of the cell cycle can be rapidly calculated for a large population of cells within 1 or 2 hr of a sample being taken.

MATERIALS AND METHODS

Patients

Specimens from 79 lymph nodes, three spleens and four infiltrated marrows from 77 adult patients (age range 17–72 yr) with NHDL were studied prior to treatment. Five patients had two lymph nodes sampled and four patients had bone marrow and lymph node samples studied. The lymph node pathology was reviewed and classified according to Rappaport [1]. For treatment purposes the patients were divided on the basis of previously reported studies into a 'favourable' prognosis histology group with a median survival greater than 5–7 yr, and an 'unfavourable' prognosis histology group with a median survival of less than two years [19]. The 'favourable' group consisted of all nodular histologies and diffuse lymphocytic well differentiated (DLWD) lymphomas. The 'unfavourable' group consisted of all the diffuse histologies except DLWD.

Patients were staged using the Ann Arbor classification for Hodgkin's disease [20]. Staging laparotomies were not performed, but intensive staging including bone marrow aspirate and trephine, percutaneous liver biopsies, cerebrospinal fluid (CSF) examinations, abdominal lymphography and computerised tomography were all used as indicated.

Patients with stage I and II disease were treated with radiotherapy at a dose of 3000 rad (30 Gy) to the involved field over 3 weeks, and in some cases adjuvant chemotherapy. Patients with stage III and IV disease were treated with combination chemotherapy in protocols of the Manchester Lymphoma Group. Chemotherapy was followed by irradiation to areas of residual disease or of bulky disease at presentation. The chemotherapy used to treat most 'favourable' prognosis histology patients was a CVP regime consisting of vincristine 1.4 mg/m^2 i.v. on day 1, cyclophosphamide 400 mg/m^2 and prednisolone 40 mg/m^2 orally on days 1–5 inclusive, every 3 weeks. The majority of patients with 'unfavourable' prognosis histology were treated with the VAP regimen consisting of vincristine 1.4 mg/m^2 i.v. weekly, adriamycin 50 mg/m^2 i.v. on alternate weeks and prednisolone 40 mg/m^2 orally daily for 6 weeks, followed by oral 'maintenance' with 6-mercaptopurine, methotrexate and cyclophosphamide for 2 yr. One patient received the VAP regimen without the adriamycin, and three patients received chlorambucil 6 mg/m^2 orally for 14 days each month. Remission status was assessed after 6 weeks of the VAP regimen, or after six courses of CVP or chlorambucil.

Remission status was defined as follows:

1. Complete remission (CR). Normal performance status associated with complete resolution of all clinical, radiological, biochemical and bone marrow biopsy evidence of disease.
2. Good partial remission (GPR). Normal performance status associated with a greater than 90% resolution of tumour mass.
3. Partial remission (PR). Improved performance status associated with less than 90% but greater than 50% resolution of tumour mass.
4. No response (NR). Patients with less than 50% resolution of tumour mass.

The length of follow up ranged from 6 to 55 months with a median of 33 months.

Methods

Twenty-eight of the lymph node specimens were obtained by surgical removal of the whole node, and 50 by fine needle aspiration biopsy of the lymph node *in situ*. Aspiration biopsy was used since the majority of patients had already had a formal lymph node biopsy before referral to the treatment centre. Lymphocyte preparations from surgically removed lymph nodes and spleens were obtained by gentle teasing, and disaggregation with forceps and scissors. The aspiration biopsies of lymph nodes and bone marrows were collected into 2 ml of heparinised medium 199 (Wellcome Laboratories). Red cell contamination in the splenic and bone marrow samples was removed by hypotonic lysis. Small clumps of cells were broken up by repeated syringing through needles of decreasing bore. The single cell suspension was then fixed in

50% methanol, treated with ribonuclease (Sigma) and stained with propridium iodide (Calbiochem) according to the method of Crissman and Steinkamp [21]. Peripheral blood lymphocytes from normal donors used as controls in estimating the DNA content of the G_1 peak were separated from heparinised blood on a Ficoll–Triosil density gradient before staining in parallel to the test preparation.

The stained samples were analysed in a model 4800A Cytofluorograf with a model 2100 multichannel distribution analyser (Biophysics System Inc., Mahopac, N.Y.). In this instrument single cells in suspension pass through a focused argon ion laser beam (488 nm). For each cell an estimate of size was obtained by measuring the forward angle light scatter (1–19°), and of the DNA content by measuring the laser excited fluorescence of the DNA–propridium iodide complex. These two measurements were analysed simultaneously in two electronic channels and displayed as a scatter diagram. The multichannel distribution analyser was used to produce a 100 channel frequency distribution of the DNA content of at least 10,000 nucleated cells for each sample. The number of cells in each channel of the histogram was printed on a paper tape providing a permanent record from which the percentage of cells in the various phases of the cell cycle was calculated.

The number of cells in the G_1 peak and the G_2 peak plus mitosis (m) was calculated separately, and then subtracted from the total number of cells in the DNA histogram to give the percentage of cells in the S phase of the cell cycle. The number of cells in the G_1 peak was calculated by assuming that the distribution was almost symmetrical. If we assume that all the cells to the left of the centre of the peak are cells in G_1, then the number of G_1 cells contributing to the peak is twice that number, the remainder being made up of early S phase cells. However, the peak is not always absolutely symmetrical and the centre of the distribution is not always in the middle of the highest channel, which means that a correction is necessary.

If the peak channel is n, and the total number of cells in the nth channel is An, then the centre of the distribution is at a fraction F from the left of the nth channel where

$$F=\frac{A_n-A_{n-1}}{(A_n-A_{n-1})+(A_n-A_{n+1})}.$$

The sum of the cells in the peak can then be calculated from the following formula

$$\text{Sum}=2\left[\sum_{i=1}^{n-1}A_i+F.A_n\right].$$

A similar calculation was performed to calculate the sum of cells in the G_2+M peak. The DNA index was defined as the DNA content of the lymphoma cells G_1 peak relative to that of normal peripheral blood lymphocytes. Thymidine labelling and cell surface marker studies were performed as previously described. [10, 22]. Life table survival and remission duration curves were compared using log rank tests. [23].

RESULTS

Eight out of 79 lymph node samples were not evaluable in terms of proportion synthesising DNA because tumour cell polyploidy made accurate analysis of DNA content impossible in such a heterogeneous population. The percentage of cells in S phase of the cell cycle found in 66 lymph node biopsies and three splenic samples from 69 evaluable patients ranged from 0.1 to 34% with a mean of 5% and a median of 2%. Marker analysis of the three splenic samples showed that the cell suspension contained more than 90% monoclonal B cells in each case. A good correlation was found between the percentage of cells in S phase calculated from the DNA histogram obtained by flow cytometry and the TLI in samples from 18 patients in whom both were measured at the same time ($r=0.91$, $P<0.001$).

Three patients had cells aspirated from a lymph node immediately before surgical removal in order to ensure that all suspensions obtained by aspiration and by teasing a whole node gave similar results. The percentage of cells in S was 0.5, 3.1 and 5% from the aspiration biopsies and 0.8, 2.9 and 4.5% from the lymph node suspension obtained after surgical removal indicating a close correlation between the two methods. Samples from two different involved sites were examined in nine patients (Table 1). The results obtained from different sites were very similar. This statement was true when comparing lymph nodes with totally replaced bone marrow samples, and lymph nodes of different size. The percentage of cells in samples removed from two adjacent lymph nodes of 2 and 6 cm in diameter from patient 45 were 4.5 and 5.5% respectively.

Table 1. The percentage of lymphoma cells in S phase from different biopsy sites

Patient No.	Bone marrow*	Lymph node 1	Lymph node 2
3		3.7	4.0
10		0.9	0.5
17	—	1.0	0.6
45	—	4.5	5.5
52	—	2.1	2.3
4	1.5	2.0	—
7	5.6	4.7	—
29	0.7	1.0	—
39	0.2	0.5	—

*Bone marrow >90% infiltrated with lymphoma cells.

Histology

There was a wide range in the percentage of cells found in the S phase within each of the histological groups of the Rappaport classification except for the DLWD group in which the results were uniformally low (Fig 1). The percentage of cells in S phase in different histological subtypes was compared using the Mann–Whitney *U*-test and the only statistically significant difference was found between the DLWD and DLPD groups ($P=0.002$).

Samples from 43 patients whose lymph node pathology was considered to be of the 'unfavourable' prognosis histological types had significantly more cells in S phase than samples from 26 patients with 'favourable' prognosis histology. (Table 2, $P=0.021$).

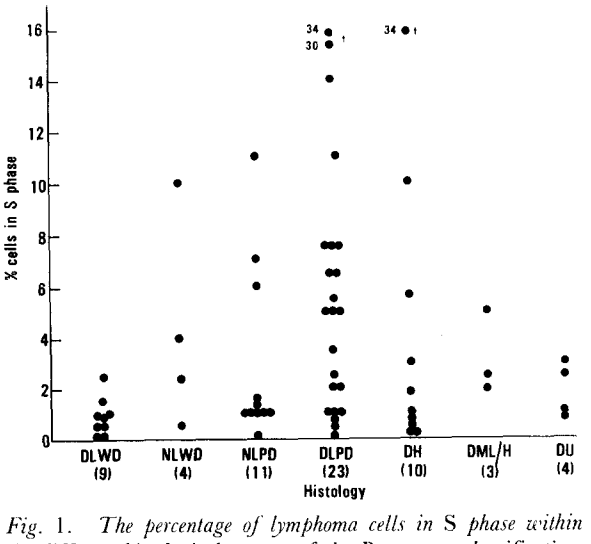

Fig. 1. The percentage of lymphoma cells in S phase within the different histological groups of the Rappaport classification. Nodular lymphocytic well differentiated (NLWD), diffuse lymphocytic well differentiated (DLWD), nodular mixed lymphocytic/histiocytic (NML/H), diffuse mixed lymphocytic/histocytic (DML/H), nodular lymphocytic poorly differentiated (NLPD), diffuse lymphocytic poorly differentiated (DLPD), nodular histiocytic (NH), diffuse histiocytic (DH), diffuse undifferentiated (DU).

Table 2. Comparison of the percentage of lymphoma cells in S phase from patients with 'favourable' and 'unfavourable' prognosis lymph node histology

Histology	Favourable prognosis	Unfavourable prognosis
No. studied	26	43
Range	0.1–11	0.1–34
Mean	2.35	6.5
Median	1	3

Mann–Whitney $U=745$, $Z=2.31$, $P=0.021$.

Ploidy

The flow cytometer was routinely calibrated with fluorescent beads or normal peripheral blood white cells. It became evident that although the majority of patients had a diploid or near diploid G_1 peak DNA content, there was some variation. The DNA content of the G_1 peak was carefully controlled with peripheral blood lymphocytes from normal donors stained in parallel with the lymphoma samples from 17 patients. The DNA content of the lymphoma G_1 cells relative to that of the normal donor peripheral blood lymphocytes was expressed as a DNA index (Table 3). The three normal lymph

Table 3. DNA index of biopsies from patients with NHDL

Patient No.	Source	Histology	DNA index*	DNA Content
68	Lymph Node	DML/H	0.7	Hypodiploid
64	Spleen	NLPD	0.74	Hypodiploid
54	Lymph Node	NLPD	0.79	Hypodiploid
58	Spleen	DLPD	0.87	Hypodiploid
57	Lymph Node	DLPD	0.91	Hypodiploid
40	Lymph Node	NLWD	0.97	Diploid
37	Lymph Node	NH	1.0	Diploid
3	Lymph Node	DLWD	1.0	Diploid
10	Lymph Node	DLWD	1.0	Diploid
27	Lymph Node	DLPD	1.0	Diploid
9	Lymph Node	DML/H	1.03	Diploid
14	Lymph Node	DLPD	1.13	Hyperdiploid
59	Lymph Node	DLWD	1.21	Hyperdiploid
47	Lymph Node	NLWD	1.22	Hyperdiploid
19	Lymph Node	DLPD	1.25	Hyperdiploid
16	Lymph Node	DH	1.25	Hyperdiploid
4	Lymph Node	DLPD	1.27	Hyperdiploid

*DNA index

$$= \frac{\text{DNA content of NHDL } G_1 \text{ cells}}{\text{DNA content of normal donor lymphocyte } G_1 \text{ cells}}.$$

nodes studied had a diploid DNA content, with DNA indices of 1, 0.96 and 1.03. The variation of the DNA index above and below 1 represented a difference of only one channel between the control lymphocytes and the lymph node specimens on the multichannel analyser. Of the 17 NHDL patients studied, six patients had a hyperdiploid and five a hypodiploid DNA content. Unfortunately chromosome studies were performed on only two patients. Patient 57 with only a small change in DNA index from normal had a karyotype of 45XX. Patient 54 showed four different abnormalities with metaphases containing 45, 43, 41 and 38XY chromosomes. The relative difference in DNA content between the lymphoma and normal cells measured using flow cytometry indicated that the predominant clone was probably 38XY.

Stage

There was no statistically significant difference between the percentage of cells in S phase of samples obtained from 11 patients with stages I/II disease and from 32 patients with stages III/IV diseases all of whom had histology of 'unfavourable' prognosis (Table 4).

Table 4. Comparison of the percentage of lymphoma cells in S phase between patients with stage I/II *and stage* III/IV NHDL *of 'unfavourable' prognosis lymph node histology*

	Stage I and II	Stage III and IV
No. studied	11	32
Range	0.5–34	0.1–34
Mean	9.9	5.4
Median	5	2.5
Mann–Whitney	$U=229.5$,	$Z=1.44$, $P=0.136$.

Response to treatment

Since all 13 patients with stage I/II disease achieved a complete remission, no correlation between proliferative activity and response could be made for these stages.

Three of the 56 patients with stage III/IV disease received total body irradiation as primary treatment leaving 53 patients evaluable for response to chemotherapy. Twenty-six patients received the CVP regimen, 23 the VAP regimen, three oral chlorambucil and one vincristine and prednisolone. The patients were divided into a high proliferative activity group with 5% or more cells in the S phase. There were significantly more complete remissions ($P=0.015$) and responses (CR+GPR) in the high proliferative activity group (Table 5, $P=0.034$). Only three of the 23 patients with favourable prognosis histology had high proliferative activity and two of these achieved a complete remission, and one good partial remission (Table 6). When the 30 stage III/IV patients with histology of 'unfavourable' type were analysed separately, there were significantly more complete remissions in the group with high proliferative activity (Table 7, $P\simeq0.04$). The subgroup treated with the VAP regimen only, gave similar results, but just failed to reach statistical significance (Table 8, $P=0.06$).

Table 5. The effect of pretreatment proliferative activity on the response to chemotherapy in 53 patients with stage* III/IV NHDL

S phase (%)	Total	Complete remission No. (%)	Good partial remission	Partial response or no response
Low (0.1–4.9)	38	10 (26)	13	15
High (≥5)	15	10 (67)	4	1
	53	20 (38)	17 (32%)	16

*26 Patients received the CVP regimen.
23 Patients received the VAP regimen.
3 Patients received oral chlorambucil.
1 Patient received the VP regimen.

For complete remission $\chi^2_1=5.83$, $P=0.015$.
For response (CR+GPR) Fishers exact test $P=0.034$.

Table 6. The effect of pretreatment proliferative activity on the response to chemotherapy of 23 patients with stage* III/IV *non-Hodgkin's lymphoma with 'favourable' prognosis lymph node histology*

S Phase (%)	Total	Complete remission No. (%)	Good partial remission No. (%)	Partial remission or no response
Low (0.1–4.9)	20	6	5	9
High (≥5)	3	2	1	0
	23	8 (35)	6 (26)	9

*20 Patients received the CVP regimen and 3 oral chlorambucil.

Table 7. The effect of pretreatment proliferative activity on the response to chemotherapy of 30 patients with stage* III/IV NHDL *of 'unfavourable' prognosis lymph node histology*

S Phase (%)	Total	Complete remission No. (%)	Good partial remission No. (%)	Partial remission or no response
Low (0.1–4.9)	18	4 (22)	8 (44)	6
High (≥5)	12	8 (67)	3 (25)	1
	30	12 (40)	11 (37)	7

*23 Patients received the VAP regimen.
6 Patients received the CVP regimen.
1 Patient received the VP regimen.
For complete remission $\chi^2_1 = 4.22$, $P \simeq 0.04$.

Table 8. The effect of pretreatment proliferative activity on the response to chemotherapy with the VAP *regimen of 23 patients with stage* III/IV NHDL *of 'unfavourable' prognosis lymph node histology*

S Phase (%)	Total	Complete remission No. (%)	Good partial remission No. (%)	Partial remission or no response
Low (0.1–4.9)	14	4 (28)	5	5
High (≥5)	9	7 (78)	2	0
	23	11 (48)	7 (30)	5

For complete remission Fishers exact test $P = 0.06$.

Length of first remission

Four of the 13 stage I/II patients were treated with radiotherapy and chemotherapy, and remain in remission after at least 18 months follow-up. Nine patients received radiotherapy alone as primary treatment. Two of these patients had a localised relapse outside the irradiated field, and after restaging were again treated with radiotherapy. The length of remission after eleven radiotherapy treatments in the nine patients tended to be shorter in those patients with a high pretreatment proliferative activity (Table 9).

Four patients with stage III/IV disease were converted from good partial remission to complete remission with additional chemotherapy and/or radiotherapy. There were therefore 24 (45%) of the 53 stage III/IV NHDL patients to assess for length of first remission. The 10 patients whose pretreatment lymphoma cells showed a high proliferative activity had shorter first remissions than those whose lymphoma cells showed low pretreatment proliferative activity (Fig. 2). The proliferation studies were not of prognostic significance for length of first remission in the 7 patients with 'favourable' prognosis histology, but were for the 17 patients with histology of 'unfavourable' prognosis (Fig. 3). The length of first remission was significantly shorter in the group with the higher proliferative activity ($P = 0.04$). There was an equal spread of patients with diffuse histiocytic and DLDP histology between the two groups.

Table 9. The effect of pretreatment proliferative activity on remission length of patients with stage I/II NHDL *treated with involved field radiotherapy only*

Patient	Pathology	S Phase (%)	Remission length (months)
31	PDDL	0.5	21
33	DH	1.0	38+
52	PDDL	2.0	9
52*	PDDL	2.0	27
26	DU	2.5	34+
37	NH	3.5	4
69	PDDL	7.5	2
69*	PDDL	7.5	6
50	WDNL	10.0	12
55	PDDL	11.0	3
50	PDDL	34.0	3

*Localised relapse in same patient retreated with radiotherapy.

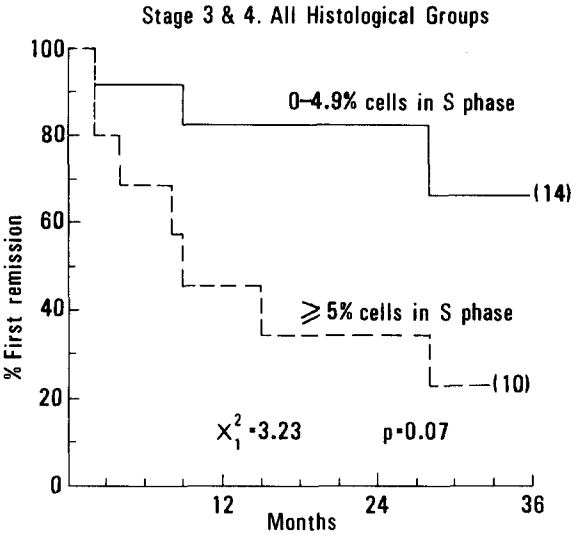

Fig. 2. The effect of pretreatment proliferative activity on the length of complete remission of 24 patients with stage III/IV *NHDL.*

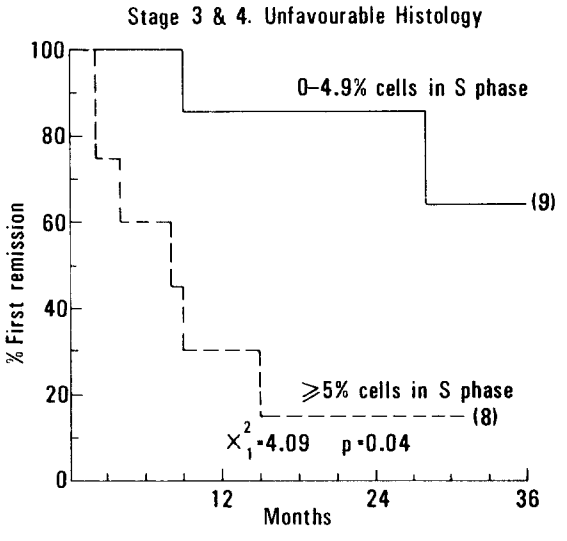

Fig. 3. The effect of pretreatment proliferative activity on the length of remission of 17 patients with stage III/IV NHDL of 'unfavourable' prognosis lymph node histology.

Survival

The median survival for the 13 stage I or II patients has not been reached, with 71% alive at 3 yr. The median survival for the 53 stage III and IV patients was 35 months. The median survival for the 23 patients with favourable prognosis histology has not been reached with 70% of the patients alive at 3 yr. The median survival of 30 patients with unfavourable prognosis histology was only 20 months, but 53% of the patients who attained a complete remission were alive at 3 yr. There was no statistically significant difference in survival between the groups with high and low proliferative activity when all stage III/IV patients were considered. There was, however, a trend for patients with high proliferative activity to have a higher proportion of patients surviving during the first fifteen months of follow up. This was most noticeable in the patients with 'unfavourable' prognosis histology.

DISCUSSION

Aspiration biopsy proved to be a simple method of collecting NHDL cells for flow cytometry studies in patients who had already undergone a formal lymph node biopsy at the referring hospital. The percentage of cells in S phase measured using flow cytometry correlated closely with the TLI using autoradiography. The range of results obtained was similar to previous TLI studies [11, 12, 14, 15, 24] and the one study using flow cytometry [25]. A close correlation was also demonstrated between the percentage of cells in S phase in cell suspensions obtained from different sites of involvement.

In eight patients gross polyploidy of the tumour with two or three separate populations detected made quantitative assessment of the percentage of cells in different phases of the growth cycle impossible. The suspicion that the DNA content of many of the other patients varied from normal was confirmed in 11 of the 17th carefully controlled specimens. The variation from normal was not great, however, and in one case the difference was confirmed on cytogenetic examination to be due to the deletion of only one chromosome. Previous studies using a microdensitometric technique have shown that NHDL are predominantly diploid [11, 12, 14, 15]. In a recent review, 23 of 26 lymph nodes studied from patients with diffuse histiocytic lymphoma and 22 of 27 with DLPD lymphoma using conventional cytogenetic techniques were found to have a modal chromosome number between 42 and 55 [26]. Our data using flow cytometry are consistent with these findings.

Flow cytometry enables minor changes in ploidy to be rapidly and accurately assessed. The predominant clones in a heterogeneous population can be rapidly assessed numerically using an assay involving several thousand cells, and the technique has an advantage over conventional chromosome studies which would be laborious in this respect. The two techniques may be used in conjunction and exploited to provide information on the proportion of cells within a heterogenous tumour cell population of different ploidy.

The reported relationship between the proliferative characteristics and histological classification of NHDL has varied from study to study. The early TLI studies of Peckham and Cooper [11, 12] failed to show any correlation with the Rappaport classification. Braylan *et al.* [25] reported a general correlation between the 'clinical aggressiveness' of the disease and the size of the S phase population, but no details of treatment and response were given. Silvistrini *et al.* [14] using the Kiel classification found that while all tumours of high grade malignancy consistently had high TLIs, not all tumours of low grade malignancy had low TLIs. In the present study the percentage of cells in S varied greatly within each histological subgroup of the Rappaport classification except the DLWD group in which the percentage of cells in S was uniformly low. This consistently low percen-

tage of cells in S in the DLWD group was partially responsible for the significant difference between the 'favourable' and 'unfavourable' prognostic groups ($P=0.021$).

The response rate of patients with stage III/IV disease to chemotherapy was higher in those patients whose lymphoma cells showed a high proliferative activity before treatment. This difference was most marked in the group with histology of unfavourable prognosis. The importance of obtaining a complete remission in the 'unfavourable' prognosis histology group was reflected in the survival data. Despite the short remissions in the high proliferative activity group, the survival of these patients over the first 15 months was greater than those in the low proliferative activity group because of the higher complete remission rate.

Silvistrini [15], however, found a clear difference in survival between lymphomas of different proliferative activity. Sixteen of the 17 patients with a TLI below 5% survived at least 21 months whereas all but three of the 17 patients with a TLI greater than 5% died within 20 months. There is no information given on the differential response, or length of first remission.

The 23 patients with 'unfavourable' histology NHDL treated with the VAP regimen form part of a study of 89 patients treated by the Manchester Lymphoma Group, which has been reported separately [27]. In this series of 89 patients the overall survival was related to histological subgroup with a median survival for DLPD lymphoma of only 18 months compared with more than 4 yr in the case of DH lymphoma. The relapse-free survival for patients with DH lymphoma achieving a complete remission was longer than that for patients with DLPD ($P=0.048$). Of 22 patients with DH lymphoma who achieved a complete remission only five had died of their disease and an actuarial relapse-free survival of 72% at 4 yr was reported. Patients with DPDL lymphoma showed a continuing relapse pattern as has been shown by others. Further studies are required to determine the relationship between response to chemotherapy and the proliferative activity within each histological subgroup since the numbers in this series were small.

The prognostic significance of pretreatment proliferative activity was less clear in the favourable histology group for three main reasons. First, the number of patients studied was small. There were only three patients with stage III/IV disease of favourable prognosis histology available for prognostic evaluation with high proliferative activity. Secondly, sampling errors were likely to be higher in the nodular lymphomas since it has been shown by immunofluorescent studies that the nodules contain monoclonal B cells, but they are surrounded by a cuff of polyclonal B cells, and morphologically normal T cells may also be present within the nodule [28]. Thirdly, the division of lymphomas into high and low proliferative activity groups at 5% cells in S may have been too high to distinguish subgroups within the favourable prognosis histology group. No patient with a DLWD lymphoma had more than 2.5% cells in S phase. Proliferative characteristics may nevertheless be important, and a recent retrospective study of patients with DLWD lymphoma has shown that patients whose tumour has a high mitotic rate in excess of 30 or more mitoses per 20 high power fields have considerably poorer survival [29].

The findings in the present study suggest that pretreatment cell proliferation studies are of prognostic importance, and may be of help in improving treatment, particularly in NHDL patients with pathology of unfavourable prognostic type. Aspiration biopsy and flow cytometry has been shown to be a rapid, convenient and objective method of obtaining these data. Flow cytometry with multiparameter analysis allows measurement of other cell characteristics (such as cell size and RNA content) to be performed at the same time as the proliferation studies. Hopefully these studies, together with the development of an improved histological classification, will lead to a better selection of patients, and more appropriate treatment regimens for patients with this varied group of diseases.

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