Proliferation in non-Hodgkin's lymphoma: a comparison of Ki-67 staining on fine needle aspiration and cryostat sections

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Abstract

Assessment of the growth fraction of non-Hodgkin's lymphomas may provide useful additional prognostic information to that obtained with conventional histological criteria. The monoclonal antibody Ki-67 has been reported to provide such information immunocytochemically in tissue biopsy specimens from lymphoma as well as other tumours. This study was undertaken to assess whether this approach could be extended to fine needle aspiration (FNA) biopsy specimens which are becoming increasingly important in the diagnosis of lymphoma. In 21 cases of non-Hodgkin's lymphoma the rate of tumour proliferation estimated by Ki-67 immunostaining of FNA material, obtained from surgically removed specimens, was compared with that obtained on tissue biopsy. The correlation between both preparations was excellent, indicating that FNA biopsy material is suitable for the immunocytochemical assessment of the growth fraction of non-Hodgkin's lymphoma.

Histological criteria for subtyping non-Hodgkin's lymphoma are an important factor in planning treatment regimens and for predicting clinical outcome.¹ Such classifications predict the overall clinical features of a group of patients with the same type of tumour, but the prognostic value for an individual patient is limited by wide variation in individual behaviour of lymphomas within each subgroup. This is particularly true for a number of tumours of low grade histological type but which follow a rapidly progressive clinical course. If such tumours could be identified earlier their outcome could be modified by using treatment better suited to a high grade tumour. This might be achieved by measuring the growth fraction of a tumour as this is believed to be an important factor determining response to treatment and survival.²

Previous studies of the proliferation in non-Hodgkin's lymphoma using autoradiography,³ flow cytometry,⁴ tritiated thymidine incorporation⁵ and transferrin receptor detection⁶ have shown a good correlation between the rate of proliferation and other indicators of therapeutic outcome or survival. These techniques have not been widely introduced into pathological practice, either because they are

too time consuming (autoradiography) or inaccurate (anti-transferrin receptor antibody).

Ki-67 is a monoclonal antibody which recognises a nuclear associated antigen expressed in all phases of the cell cycle except Go.⁷ Measurements of proliferation made with Ki-67 correlate well with other more conventional methods of assessing cell proliferation—for example, tritiated thymidine and bromodeoxyuridine incorporation.⁸ It has been shown to be of value in assessing the proliferative rate of many tumours such as those of the breast,⁹ lung,¹⁰ cervix,¹¹ and lymphoma¹²⁻¹⁴ by immunostaining cryostat sections of surgically excised biopsy specimens. For lymphoma, in particular, it has been shown that patients with histologically low grade non-Hodgkin's lymphoma and high proliferation rates have a worse clinical course than patients with low proliferation rates.^{13,15}

Fine needle aspiration biopsy is increasingly being used for the diagnosis of lymphoproliferative disorders¹⁶ and has been shown to be suitable for immunocytochemical analysis.¹⁷⁻²⁰ If immunocytochemical measurement of proliferation is confirmed as an important variable in the management of lymphoma then it will be necessary to perform such measurements on fine needle aspiration material. The aim of the present study was therefore to assess the suitability of fine needle aspiration biopsy specimens, obtained from fresh surgically resected specimens, for Ki-67 immunostaining and to compare results achieved with those on cryostat sections from the same tumours.

Methods

Eighteen lymph nodes and three spleens from patients with non-Hodgkin's lymphomas were resected surgically and received fresh in the laboratory. Material was aspirated from these using a 21 gauge fine needle and a 10 ml plastic syringe held in a lightweight aluminium holder (Cameco, Sweden). Smear preparations were stained by both Papanicolaou and Giemsa methods. Whenever possible, a third air dried smear was prepared for immunostaining. The remaining material was suspended in 5 ml of tissue culture fluid (RPMI, Gibco) by drawing fluid into the syringe, and after disconnecting the needle (to minimise trauma to the cells), expelling it back into an appropriate tube. Twelve cytopspins were prepared (Shandon cytopsin 2; 550 rpm for five minutes) using about 250 µl of the cell suspension for each

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Table 1 Monoclonal antibodies used in this study

Monoclonal antibody	Specificity	Source
Ki-67	Nuclear associated antigen	Dako
3D4 (CD3)	T cells	Dako
4KB128 (CD22)	B cells	Dako
4KB5 (CD45RA)*	B cell associated	Dako
UCHLI (CD45RO)*	T cell associated	Dako

*Antibodies used on paraffin wax embedded, formalin fixed, tissue sections.

Table 2 Subtypes of lymphoma used in this study

<i>B cell immunophenotype:</i>		
Chronic lymphocytic leukaemia (CLL)		3 cases
Hairy cell leukaemia (HCL)		3 cases
Centroblastic/centrocytic (cc/cb)		7 cases
Centroblastic		5 cases
<i>T cell immunophenotype:</i>		
Angioimmunoblastic lymphoma (AIL)		2 cases
Pleomorphic large cell lymphoma (PLC)		1 case

well. These were air dried for two to 18 hours, after which one was stained by the Giemsa method to check the number and preservation of the cells. The remainder were wrapped back to back in aluminium foil and stored at -20°C until required for immunostaining.

Representative blocks were taken from each lymph node, snap frozen in liquid nitrogen, and stored at a temperature below -70°C . Cryostat sections for immunostaining were prepared as required. The remaining tissue was fixed in formalin, routinely processed, and paraffin wax sections stained with haematoxylin and eosin.

Details of Ki-67 and other monoclonal antibodies used for lymphoma phenotyping are detailed in table 1.

Immunocytochemical staining was performed using the alkaline phosphatase anti-alkaline phosphatase technique (APAAP), as described previously.²¹

For the first 10 cases in this study, Ki-67 staining was assessed by counting the number of positively labelled cells in five randomly selected areas of 2 mm^2 as described previously.¹¹ This permitted the cases to be categorised into three grades as follows: grade

1, $<10\%$ of tumour cells staining with Ki-67; grade II, $10\text{--}30\%$ of tumour cells staining with Ki-67; grade III, $>30\%$ of tumour cells staining with Ki-67.

On blind review of these 10 cases the same grade was ascribed to each by direct visual inspection, which is quicker and more convenient. The proliferation grades for the remaining 11 cases were therefore established using this means alone.

Lymphomas were classified according to the modified Kiel classification²² from the histological appearances of the paraffin wax embedded sections combined with the immunocytochemical results on cryostat sections (table 2).

Results

The results of Ki-67 staining are given in table 3, with typical examples illustrated in fig 1. Two cases of follicular lymphoma (centroblastic/centrocytic) had to be eliminated from the study because of cytoplasmic staining in the cytospin preparations which made assessment of nuclear staining impossible. Cytospin and smear preparations gave the same proliferative grade in each of the six smear cases for which both type of preparation was available.

There was complete agreement in proliferation grade between cytological and histological preparations in 16 of the 19 cases studied. In the remaining three cases, all follicular lymphomas (centroblastic/centrocytic) in the fine needle aspiration biopsy specimen gave a lower proliferative grade than in the cryostat sections.

The correlation between histological grade (low and high) and Ki-67 proliferation grade was good. All high grade lymphomas had Ki-67 grades of II or III; no low grade lymphoma was grade III.

Discussion

This study shows that cytological preparations, either in the form of conventional smears or cytopins, are suitable for estimating the proliferation rate of most non-Hodgkin's lymphomas using monoclonal antibody Ki-67. The results obtained from such preparations are comparable with those obtained using cryostat sections. This is in contrast to an earlier study using fine needle aspiration material from surgically resected lung carcinomas²³ which found a poor correlation between cytological and histological preparations. The reason for this difference may lie in the nature of the lesion being sampled. For fine needle aspiration biopsy specimens, Ki-67 staining is most easily assessed on preparations of loosely aggregated cells as in lymphoma rather than preparations containing clumps of tumour cells, such as is seen in many carcinomas.

Cytological preparations from three follicular lymphomas showed a lower Ki-67 grade than the tissue sections. This finding is in keeping with the study by Schrape *et al*,¹⁵ who found that analysis of cell suspensions of follicular lymphomas (obtained by passing the material through a wire mesh) gave a lower estimate of the proliferation rate of the lym-

Table 3 Comparison of Ki-67 staining of non-Hodgkin's lymphomas using cytological preparations and cryostat tissue sections

Case No	Histological type	Smear/cytospin	Cryostat	Immunophenotype
1	CLL	Grade I	Grade I	B cell
2	CLL	Grade I	Grade I	B cell
3	CLL	Grade II	Grade II	B cell
4	HCL	Grade I	Grade I	B cell
5	HCL	Grade I	Grade I	B cell
6	HCL	Grade I	Grade I	B cell
7	cb/cc	Grade I	Grade II	B cell
8	cb/cc	Grade I	Grade II	B cell
9	cb/cc	Grade I	Grade II	B cell
10	cb/cc	Grade I	Grade I	B cell
11	cb/cc	Grade I	Grade I	B cell
12	cb/cc	*****cytoplasmic staining*****		
13	cb/cc	*****cytoplasmic staining*****		
14	Centroblastic	Grade II	Grade II	B cell
15	Centroblastic	Grade II	Grade II	B cell
16	Centroblastic	Grade III	Grade III	B cell
17	Centroblastic	Grade III	Grade III	B cell
18	Centroblastic	Grade II	Grade II	B cell
19	PLC	Grade II	Grade II	T cell
20	AIL	Grade II	Grade II	T cell
21	AIL	Grade I	Grade I	T cell

PLC: pleomorphic large cell
AIL: angioimmunoblastic lymphoma

Figure 1 Comparison of Ki-67 staining between a case of hairy cell leukaemia, showing a low proliferation rate (grade I) on both smear (a) and cryostat (b) preparations and a case of centroblastic non-Hodgkin's lymphoma, displaying a high proliferation rate (grade III) in (c) cytospin and (d) cryostat preparations. Arrowheads indicate positively stained nuclei. All preparations counterstained with haematoxylin.

phoma than that obtained using cryostat sections. The way in which sampling may affect the assessment of Ki-67 staining is illustrated diagrammatically in fig 2. Fine needle aspira-

tion will sample both the follicular areas of the lymphoma (with a relatively high Ki-67 count) and the interfollicular areas containing non-neoplastic reactive lymphoid cells (with a relatively low Ki-67 count), giving an average value of proliferation for all the lymphoid cells within the specimen. Cryostat sections allow the observer to choose areas of neoplasia producing a Ki-67 count which has not been diluted by non-proliferating cells in the interfollicular areas. Hall *et al*¹³ assessed the number of proliferating cells in cryostat sections of non-Hodgkin's lymphomas using a method which corrected for the number of non-tumour cells within the lymphoma.¹³ Even though this was not done in the present study, however, the values chosen for our grading system—that is, grade I <10%; grade II 10–30%; grade III >30%—are comparable with those obtained by Hall and other workers. Hall *et al* regarded a value of >20% Ki-67 positive cells as high grade¹³; Schrape *et al* found that most of their histologically high grade lymphomas had Ki-67 values of >25%¹⁵; and Gerdes *et al* concluded that high grade lymphomas had a Ki-67 count of >26%. It should be borne in mind, however, that some types of non-Hodgkin's lymphoma, such as angioimmunoblastic lymphadenopathy may contain a large reactive

Figure 2 Schematic representation of distribution of Ki-67 positive cells within a cb/cc follicular lymphoma. Region A represents the cells sampled by FNA while region B shows a typical area used for Ki67 assessment on cryostat sections. These sampling differences account for the lower Ki67 grade obtained using FNA material compared with the grade obtained using cryostat sections of follicular cc/cb lymphoma.

element, producing an underestimation of the lymphoma growth fraction. Thus while most non-Hodgkin's lymphomas should lend themselves to assessment of proliferative state using this technique, there may be a minority which, owing to their large reactive component, will not.

Two cases in this study had to be discarded because of cytoplasmic staining with Ki-67. This has been reported in previous studies using epithelial tissues,^{11 23} though its explanation remains unclear. The fact that it is seen more commonly in cytological than histological preparations of lymphoma suggests that it may be artefactual rather than a cross reaction.

Immunohistological analysis of fine needle aspiration material has been used previously to assess proliferation in lymphomas. Oertel *et al* used a monoclonal antibody against transferrin receptor (OKT9) and found a correlation between histological grade and the degree of OKT9 staining.²⁰ Its value in clinical practice is doubtful, however, because it also stains macrophages, activated lymphoid cells, and dendritic reticulum cells^{15 24 25} which can produce misleading results in individual cases. Ki-67, on the other hand, gives a measure of the tumour growth fraction in most cases, which is not obscured by surrounding reactive cells because they are usually not proliferating.

In conclusion, using material aspirated from surgically resected specimens, the present study has shown that monoclonal antibody Ki-67 is suitable for use with fine needle aspiration biopsy specimens in the assessment of growth fraction in non-Hodgkin's lymphoma. Although biopsy tissue is likely to remain the mainstay of lymphoma diagnosis, fine needle aspiration investigation is likely to increase as a simple investigation for excluding non-malignant conditions and for those patients in whom a biopsy is inappropriate. It will be important, then, to determine the importance of Ki-67 in lymphoma fine needle aspiration specimens to know whether the information is of value to clinical management. In addition, fine needle aspiration may become an auxiliary investigation for monitoring the course of a lymphoma during treatment. For example, it could be envisaged that serial Ki-67 measurements of low grade non-Hodgkin's lymphomas might detect a progression to high grade type before it becomes clinically manifest, so that optimal treatment could be undertaken.

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