Papers

Expression of Ki-67 nuclear antigen in B and T cell lymphoproliferative disorders

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Abstract

Aims: To determine whether the proliferation rates of tumour cells may relate to prognosis and reflect disease activity.

Methods: Blood mononuclear cells from 155 patients with B cell (n = 120) or T cell (n = 35) chronic lymphoproliferative disorders were tested with the monoclonal antibody Ki-67 by indirect immunoperoxidase or immunoalkaline phosphatase techniques. B cell diseases included chronic lymphocytic leukaemia (CLL), CLL in prolymphocytic transformation (CLL/PL), prolymphocytic leukaemia (B-PLL) and non-Hodgkin's lymphoma (B-NHL) in leukaemic phase. The T cell diseases comprised large granular lymphocyte (LGL) leukaemia, T-PLL, and T-NHL.

Results: These showed significantly higher proportions of Ki-67 positive cells in T cell (11.2%) than in B cell (2.9%) disorders (p < 0.001). The highest values were found in NHL of both B and T cell types, particularly when low grade disease transformed to high grade. The lowest percentages of Ki-67 positive cells were found in CLL (1.4%) and LGL leukaemia (1.7%); intermediate values were seen in B PLL (3.3%) and T PLL (5.8%).

Conclusions: There is a positive correlation between prognosis and proliferation rates in chronic B and T cell lymphoproliferative disorders. Estimation of Ki-67 in circulating leukaemic cells could be used to determine prognosis in low grade malignancies.

Chronic lymphoproliferative disorders comprise a heterogeneous group of diseases resulting from the clonal proliferation of mature B and T lymphoid cells. Although they may present problems of differential diagnosis, they have distinct clinical, morphological, and evolutionary features. Previous studies on cell proliferation in these diseases have been done using several methods,¹⁻⁵ but it is not yet known whether kinetic studies on peripheral blood cells reflect the growth fraction properties of the tumour. Studies of cell proliferation rates with the monoclonal antibody Ki-67 have mainly been applied to lymph nodes of non-Hodgkin's lymphoma (NHL). However, few studies have been performed on peripheral blood⁶⁻¹⁰ and diseases such as prolymphocytic

leukaemia and some NHL in leukaemic phase have not been investigated.

The purpose of this study was to investigate the proliferation rate of a group of B and T mature lymphoproliferative disorders using the monoclonal antibody Ki-67 on the circulating cells by means of immunocytochemistry. We also compared the results obtained with immunoperoxidase and alkaline phosphatase anti-alkaline phosphatase (APAAP) and investigated if reactivity with Ki-67 is a feature of some of these disorders.

Methods

A series of 155 patients with mature B and T cell malignancies were studied. The diagnosis was based on clinical features, cell morphology, immunophenotype, and in some cases by histological and ultrastructural analysis.

Peripheral blood mononuclear cell suspensions were obtained by density gradient cell separation using Lymphoprep (Nycomed, Oslo, Norway) and cytopsin slides were prepared at a concentration of 2×10^6 cells/ml (Cytopsin II, Shandon Products Ltd, UK). Immunophenotyping was carried out on cell suspension by indirect immunofluorescence using a panel of monoclonal antibodies against B and T cell antigens and fluorescein conjugated (FITC) goat anti-mouse immunoglobulins F(ab)₂ fragment (Cappel, West Chester, England) as a second layer and analysed on a FACScan flow cytometer (Becton-Dickinson, Mountain View, California).

Determination of growth fraction was performed on cytopsin using the monoclonal antibody Ki-67 (Dako, High Wycombe, England) using immunoperoxidase and alkaline phosphatase anti-alkaline phosphatase (APAAP) techniques. Briefly, cytopsin wrapped in foil paper and stored at -20°C were thawed and fixed in pure acetone for 10 minutes. The cells were incubated with the monoclonal antibody Ki-67, followed by sequential 30 minute incubations with peroxidase labelled rabbit anti-mouse immunoglobulins and swine anti-rabbit immunoglobulins (Dako, High Wycombe). The developing solution contained 30 mg of 3,3'-diaminobenzidine (Sigma, Poole) and hydrogen peroxidase in phosphate buffered saline (PBS). The slides were counterstained with haematoxylin-S (BDH) and mounted with DPX (BDH). For the APAAP method, rabbit anti-mouse immunoglobulins and APAAP complexes (Dako,

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Table 1 Expression of Ki-67 in B cell diseases

Disease	No of cases	% of positive cells			Mean (SEM) of all cases	Mean (SEM) of positive cases
		0	0.2-2.0	> 2.0		
CLL	70	22	29	19	1.4 (0.2)	2.0 (0.2)
CLL/PL	11	5	2	5	1.8 (0.6)	3.3 (0.7)
B-PLL	9	5	1	3	3.3 (2.0)	7.4 (3.4)
B-NHL	29	7	11	11	6.3 (2.2)	8.3 (2.8)
IBT*	1	0	0	0	17.8%	

*Immunoblastic transformation: patient with Richter's syndrome and peripheral blood disease with large cells (fig 1).

High Wycombe) were used as second and third layers. The reaction was developed in a medium containing naphthol As-MX phosphate (Sigma), levamisole, dimethylformamide, and Fast-Red Salt TR (Sigma, Poole). Subsequently, the slides were counterstained with haematoxylin-S (BDH) and mounted with glycerol (Dako). The percentage of Ki-67 positive cells was estimated by light microscopy (oil immersion) by counting 500 cells in each sample. Only those cells with nuclear staining were considered positive.

The statistical analysis was performed on a Macintosh microcomputer with a Stat View 512+ software program.

Results

Immunophenotyping disclosed 120 patients with B lymphoid disease: 70 with chronic lymphocytic leukaemia (CLL), one with CLL in immunoblastic transformation, 11 with CLL with increased numbers of prolymphocytes (CLL/PL), nine with prolymphocytic leukaemia (B-PLL) and 29 with non-Hodgkin's lymphoma (B-NHL) in leukaemic phase. The remaining 35 were T cell disorders: 10 large granular lymphocyte LGL leukaemia,

two T cell leukaemia in immunoblastic transformation, 13 T-PLL and 10 T-NHL in leukaemic phase.

The results of Ki-67 expression were compared by both methods in circulating cells from 64 patients with B-CLL. The number of cases with Ki-67 positive cells and the mean of percentage of the Ki-67 positive cells were slightly higher with immunoperoxidase than with APAAP, but this difference was not significant. The immunoperoxidase method showed, overall, a better morphological definition of the positive cells than APAAP. Because of this, we subsequently evaluated the reactivity of Ki-67 in all the haematological malignancies using immunoperoxidase.

Results in the patients with B cell diseases are shown in table 1. The highest proliferation rates were observed in a patient with a previous diagnosis of CLL who developed Richter's syndrome (fig 1) and in cases of B-NHL; CLL cases had low mean values. Intermediate values were observed in CLL/PL and B-PLL. The B-NHL group comprised five patients with intermediate (mantle zone) lymphoma, six patients with high grade (large cell) NHL, and 18 with low grade malignancy, all in leukaemic phase. The highest percentages of Ki-67 positive cells were observed in patients with high grade malignancy (range 0.8-57%). These results agree with those of similar studies carried out on tissue sections.¹¹⁻¹³ The highest value for Ki-67 in NHL group was seen in a lymphoplasmacytic lymphoma which transformed to large cell lymphoma (fig 2).

Among T cell diseases the highest expression of Ki-67 was observed in two patients with mature T cell leukaemia in immunoblastic transformation and in T-NHL. The latter group comprised one patient with peripheral T cell lymphoma, five patients with Sézary syndrome and four with adult T cell leukaemia/lymphoma. These patients had high Ki67 values: 15.4%, 540%, and 6.5-60%, respectively. The lowest values were observed in patients with LGL leukaemia. Patients with T-PLL had intermediate values between these two groups (table 2).

Overall, the results in the cases of T cell disease were higher than those in B cell diseases; this difference was significant (table 3). It should be noted that within each group of roughly equivalent diseases, such as CLL and LGL leukaemia, B and T PLL, and B and T NHL, those with a T cell phenotype always expressed higher Ki-67 values (fig 3).

Discussion

There was a highly significant difference in the proportion of circulating Ki-67 positive cells between the two main groups of B and T mature lymphoproliferative disorders. Our findings also show that there is a range of proliferation rates detected on circulating cells in each group of disorders which may correlate with prognosis.

Several approaches have been used to estimate proliferation rates in different human tumours. Usually they are estimated from the

Figure 1 Nuclear expression of Ki-67 in the large cells of a patient with B cell CLL in transformation (immunoperoxidase).

Figure 2 Peripheral blood cells of a patient with a low grade B cell NHL in leukaemic phase, now in transformation, to a large cell lymphoma showing expression of Ki-67 (immunoperoxidase).

number of tumour cells in mitosis and entering DNA synthesis by measuring the uptake of radioactively labelled DNA precursors.^{2 14} More recently, the monoclonal antibody Ki-67 has been used to assess the growth fraction. This reagent detects a nuclear antigen present on chromosomes in all phases of mitosis and within the nucleolus of proliferating cells in all phases of the cell cycle but not in resting cells.^{15 16}

B-CLL is a disease with a variable pattern of survival with the best prognostic factors being disease staging,¹⁷ age, white cell count and sex.¹⁸ In general this disease showed the lowest proliferation rates. These results are similar to those of other cell kinetic studies performed on peripheral blood cells.⁶ CLL/PL has features intermediate between those of CLL and B-PLL,¹⁹ and is associated with disease progression and short survival.²⁰ This group had slightly higher proliferation rates than CLL and this may correlate with clinical behaviour. In the groups of mature B cell leukaemias

B-PLL has the poorest prognosis and is usually unresponsive to treatments which are effective in CLL.²¹ In B-PLL we found higher Ki-67 values than in CLL and CLL/PL.

B-NHL with leukaemic signs are associated with a variable clinical course and these had higher Ki-67 values than CLL, CLL/PL, and B-PLL. Previous studies on histological sections have shown a strong correlation between the proportion of Ki-67 positive cells and histological grade in NHL.^{11 13} Other studies have shown, however, that there is often a wide range of proliferation rates within each histological subtype, including high rates in low grade lymphomas.^{13 14 22} In series of low grade NHL Hall *et al* found a relatively high expression of Ki-67 in patients with a shorter survival than those with low Ki-67 expression.¹³ We observed the highest expression of Ki-67 in patients with B-NHL with low grade disease in transformation to high grade NHL. Our study suggests that the determination of Ki-67 in peripheral blood can be useful for detecting and monitoring patients with low grade disease who have a progressive leukaemic phase and who may be undergoing transformation to high grade disease.

Within the various types of mature T cell leukaemias LGL leukaemia often has a benign clinical course, whereas other types have a relatively aggressive course.²³ Thus the low Ki-67 expression observed in LGL leukaemia seems to correlate with a good prognosis, unlike T-PLL, a distinct disease entity with a median survival of six months.²⁴ The expression of Ki-67 in this group has not been

Table 2 Expression of Ki-67 in T cell diseases

Disease	No of cases	% of positive cells			Mean (SEM) of all cases	Mean (SEM) of positive cases
		0	0.2-2.0	> 2.0		
LGL	10	4	2	4	1.7 (0.6)	2.9 (0.6)
T-PLL	13	2	0	11	5.8 (1.4)	6.9 (1.5)
T-NHL	10	0	0	10	21.2 (5.3)	21.2 (5.3)
IBT*	2	0	0	2	44.3 (6.7)	44.3 (6.7)

*Immunoblastic transformation of low grade T cell leukaemia.

Table 3 Comparison of Ki-67 expression in B and T cell disorders

Group	No of cases	No of positive cases	Mean (SEM) of all cases	Mean (SEM) of positive cases
B	120	81	2.9 (0.6)	4.3 (0.8)
T	35	29	11.2 (2.5)	13.6 (2.8)

p < 0.001.

Figure 3 Comparison between B and T lymphoproliferative disorders. Mean and SEM values of Ki-67 positive cells.

reported previously and our study suggests that the high Ki-67 values correlate with the poor prognosis of T-PLL. The high proportion of Ki-67 positive cells found in all but one of the patients with adult T cell leukaemia/lymphoma confirms the findings of other studies on peripheral blood^{8,10} and also correlates with the poor outlook of this disease which has a median survival of five to seven months. Although some of the patients with Sézary syndrome also had high Ki-67 values, because this disease has a variable course further studies are needed to correlate this finding with prognosis.

Overall, more Ki-67 positive cells were found in T cell than B cell disorders, again correlating with the worse prognosis of patients with T cell leukaemia or T-NHL in leukaemic phase, except LGL leukaemia. The determination of Ki-67 expression in circulating cells seems to be an informative and simple tool for assessing disease activity. And it might predict the clinical course in low grade disorders such as CLL and may allow sequential studies in peripheral blood samples. Our results are similar to findings in acute lymphoblastic leukaemia which also showed a correlation between DNA labelling index, prognosis, and cell lineage,^{25,26} with T-ALL having a worse prognosis than common ALL.

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