

Prognostic value of cytophotometric analysis of DNA in lymph node aspirates from patients with non-Hodgkin's lymphoma

J Vučković, M Dubravčić, JM Matthews, SN Wickramasinghe, M Dominis, B Jakšić

Abstract

To classify cases of non-Hodgkin's lymphoma in terms of expected clinical behaviour and survival, kinetic parameters measured by cytophotometry were assessed in 62 patients between 1978 and 1987. The influence of the number of cells with increased DNA content (more than 2N) on survival was evaluated. Analyses were carried out on the small samples obtained by needle aspiration biopsy of lymph nodes before treatment, using microdensitometry and Feulgen staining. Patients whose lymphomas contained less than 6% of cells with increased DNA content had a mean survival of 81.3 months and those whose lymphomas contained 6% or more of such cells had a mean survival of 18.5 months. A significant difference in survival using the same criteria was also noticed for patients with both low grade lymphomas and those with intermediate and high grade lymphomas.

It is concluded that cytophotometric analysis of lymph node aspirates is of prognostic value in the initial assessment of non-Hodgkin's lymphoma.

Non-Hodgkin's lymphomas (NHL) are malignant lympho-proliferative disorders characterised by highly diverse clinical appearances and behaviour.¹ Over the past few decades substantial efforts have been made to determine those characteristics in individual tumours which predict clinical behaviour and response to treatment. To date, the main prognostic feature has been histological classification. Using the size and the morphological appearance of the cells, as well as the pattern of growth (nodular compared with diffuse), NHL can be divided into categories of prognostic importance.²⁻⁴ Despite ongoing efforts to refine the histological criteria, pathologists are still unable to predict the behaviour of many of these tumours and the search for additional variables continues.

The kinetic behaviour of NHL has also been studied in relation to prognosis. Various techniques have been used, including mitosis counts,⁵ morphometry,⁶ tritiated thymidine (³HTdR) labelling,⁷⁻¹⁰ and cytophotometry.¹¹ Over the past decade DNA flow cytometry has been widely used to explore the kinetic features of NHL.^{12,13} These studies have been

performed mainly on samples obtained by lymph node biopsy and not aspiration. There are only a few published papers on the cytophotometry of NHL and they have largely concerned lymph node imprints.¹¹ The aim of this study was to try to solve the problem of assessing prognosis in NHL on the basis of cytophotometric DNA measurements in lymph node aspirates.

Methods

Ninety six patients with NHL were observed over 10 years (1978-1987). Sixty two of them, 36 men and 26 women, had suitable lymph node aspirates for DNA analysis. The age of the patients ranged from 18 to 80 years (mean 56.8 years). Mean observation time was 37.6 months. All patients were studied before treatment. Clinical staging was performed according to the Ann Arbor classification.¹⁴ Seven patients were in stage I, 14 patients were in stage IIA, and the other 41 patients were in more advanced stages of disease (IIB, III, and IV). Histological classification was carried out according to the International Working Formulation.⁴ Thirty patients had low grade histology. The other 32 patients with intermediate and high grade histology were considered together as a higher grade group.

Lymph node aspirates were initially smeared and stained with May-Grünwald-Giemsa stain in the standard way. Smears were destained with trichloroacetic acid and stained again by the Feulgen method.¹⁵ Cytophotometric measurements were carried out on an integrating microdensitometer (Vickers M85). Two hundred cells (including small lymphocytes) were analysed in each smear. The resulting measurements of DNA content were given in arbitrary units (AU). The quantity of DNA in the nuclei of examined cells equalling the diploid value of DNA seen in mature small lymphocytes was designated 2N. Deviations from the 2N value were only counted as "true" deviations when they lay outside the 10% range. For example, if the value for 2N (calculated as a mean value for 20 small lymphocytes) was 30 AU then all values from 27 to 33 AU were regarded as 2N. Cells with a DNA content higher than 2N were considered to be in the proliferative phases of the cell cycle (S, G₂, M) or as aneuploid. Lymphomas in which less than 6% of the total cells in the aspirate (including the small lymphocytes) had an increased DNA amount were regarded

Health Center, "Dr Peter Vitezica" and Center for Oncology, ul. Maksima Gorkog 2, 5800 Split, Yugoslavia
J Vučković
M Dubravčić

Department of Haematology, St Mary's Hospital Medical School, London
JM Matthews,
SN Wickramasinghe

University Clinic, "Dr Ozren Novosel", Zagreb, Yugoslavia
M Dominis
B Jakšić

Correspondence to:
Dr J Vučković.

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Fraction of cells with increased DNA content (DNA > 2N) in relation to histological subtypes of NHL

Histology*	No of patients	Median for number of DNA > 2N cells (%)
Low grade		
Lymphocytic	7 (11.3)	1.5
Lymphoplasmacytic/plasmacytoid/†	9 (14.5)	3.5
Follicular SCC and M (CB-CC)‡	10 (16.0)	5.8
Other low grade	4 (6.5)	1.0
Intermediate and high grade		
Diffuse SCC and M (CC, CB-CC)	5 (8.1)	6.5
Large non-cleaved (CB)	4 (6.5)	19.0
Immunoblastic	9 (14.5)	13.0
Large cell unclassified	9 (14.5)	12.0
Lymphoblastic	3 (4.9)	6.5
Other high grade	2 (3.2)	

*According to International Working Formulation; SCC—small cleaved cell lymphoma; M—mixed cell lymphoma.

†Included as a separate category, not as a part of lymphocytic lymphoma.

‡CC—Centrocytic; CB—Centroblastic.

as low DNA (L-DNA). Other patients (6% or more cells with increased DNA amount) were regarded as high DNA (H-DNA). Thus the L-DNA group comprised hypoproliferative cases and the H-DNA group hyperproliferative and aneuploid cases.

Statistical analysis was performed with an SAS software system.¹⁶ Life tables were calculated by the Kaplan-Meier method and compared using the Wilcoxon test. The Spearman rank correlation coefficient was also used.

Results

The fraction of cells with DNA content of more than 2N varied from 0.5% to 90%. Thirty two patients had less than 6% of such cells and so they belonged to the L-DNA group and 30 patients fell into the H-DNA group. The table shows the histological classifications and the median value for the number of cells with increased DNA content in each histological group. Mean actuarial survival for all patients was 55.1 months (95% confidence interval (CI) = 44.5-65.7) and median survival was 50 months. A significant negative correlation was shown to exist between the fraction of cells with DNA content of more than 2N and survival ($r = 0.51$, $p = 0.0001$). Mean survival for the low grade group was 71.5 (CI 57.3-85.8, median 98) months and for the high grade group 28.1 (CI 20.8-35.5, median 22) months (fig 1). The difference in survival between the two groups was significant ($p = 0.012$). Mean survival for patients with L-DNA lymphomas was 81.3 (CI 69.5-93.1, median 98) months, and for those with H-DNA 18.5 (CI 14.7-22.3, median 19) months (fig 2). The difference was highly significant ($p = 0.0001$). In the low grade group 23 patients also belonged to the L-DNA group and their mean survival was 85 (CI 71.4-99.5, median 100) months while mean survival for the other seven patients (low grade and H-DNA) was 23.3 (CI 15.3-31.2, median 20) months (fig 3). This

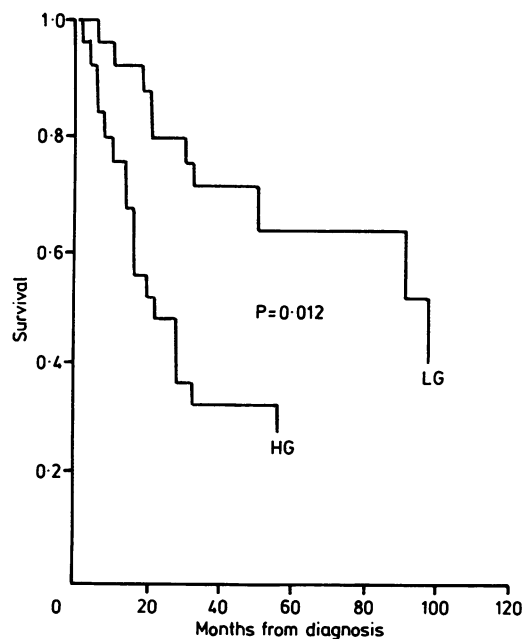


Figure 1 Survival of NHL according to the degree of malignancy; LG = low grade; HG = high and intermediate grade.

difference was highly significant ($p = 0.0005$). In the high grade group nine patients also belonged to L-DNA group and their mean survival was 47.2 (CI 33.8-60.7, median could not be calculated) months which is significantly different from the survival (mean 16.8 CI 12.2-21.4, median 15 months) found in the other 23 patients of the same histological group ($p = 0.046$) (fig 4).

Discussion

The principal characteristics of our group of patients did not differ from those of other groups described elsewhere in terms of their mean age, slight male predominance, and clin-

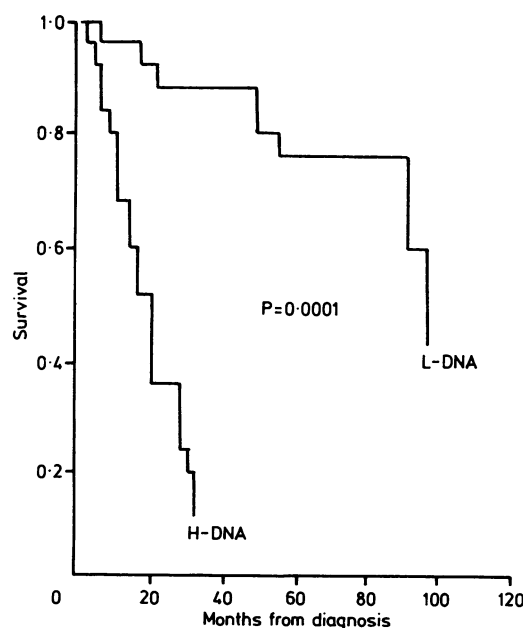
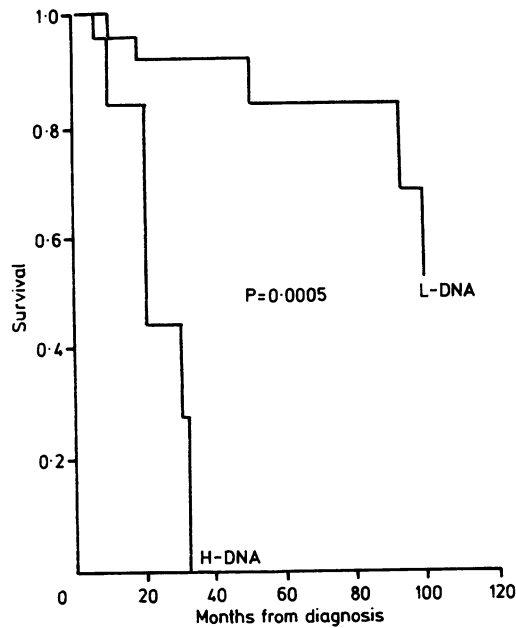


Figure 2 Survival of NHL according to DNA content. L-DNA, NHL with less than 6% of cells having increased DNA content; H-DNA, NHL with 6% or more of such cells.

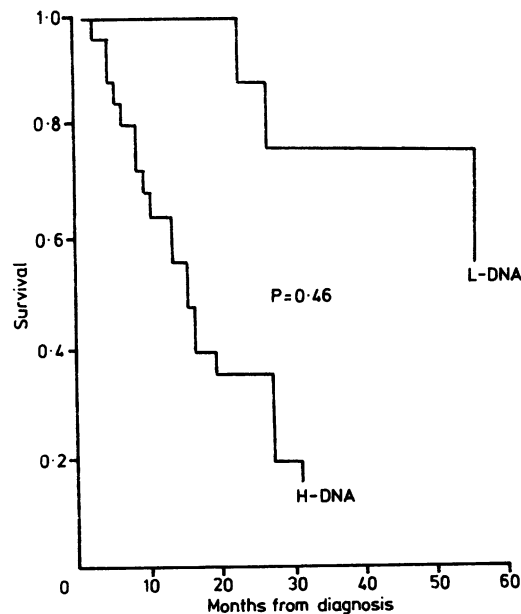
Figure 3 Survival of low grade NHL according to DNA content.



ical staging.^{3,17,18} Some differences in prevalences of individual histological types might be attributable to disagreements among observers in histological interpretation and variations in the geographic distribution of NHL types.^{19,20}

Fine needle aspiration cytology of lymph nodes has recently gained wide acceptance as an accurate method of diagnosing and classifying NHL.^{21,22} It is also very useful for the selection of a representative lymph node for surgical biopsy, for the diagnosis of recurrent NHL, for staging the extent of disease, for the diagnosis of cases in which tissue biopsy is not possible, and as a variable for treatment follow up.²³ Kinetic studies using ³H-thymidine uptake (³HTdR) in lymphoma cells obtained through fine needle aspiration biopsy have indicated a significant correlation between labelling index (LI) and survival.⁹ A high LI was associated with a poor prognosis. There

Figure 4 Survival of high grade NHL according to DNA content.



has been no such study using cytophotometry.

Kinetic techniques using histological samples have been widely used and lead to similar conclusions. It has been shown that a low mitotic index indicates longer survival in patients with low grade NHL.⁵ These results agree with our observation of the different behaviour pattern of low grade NHL which depends on DNA analysis. The LI is known to be a reliable variable for the prognosis of NHL.

In a series of 88 adult patients with NHL analysed by Costa *et al* the prognostic importance of the LI seemed to be superior to the Rappaport classification.⁹ Del Bino *et al* grouped 123 adult cases of NHL into fast proliferating and slow proliferating tumours with a discrimination point for the LI of 4.1%.¹⁰ Six year survival was 66% for slow and 18% for fast proliferating NHL. This difference in survival was highly significant ($p < 0.0001$) and very similar to the difference in survival between the L-DNA and H-DNA groups found in our study. The same authors showed a significant difference in survival dependent on the LI within the low grade lymphoma group according to the Kiel classification, and the low and intermediate grades of the Working Formulation, which was also consistent with our findings. The labelling index correlates with the S phase obtained by flow cytometric analysis.²⁴

By using flow cytometry, Lenner *et al* found that independent prognostic variables in NHL were clinical stage, S phase fraction ("cut-off" point 4%), and age of patient while significance was not achieved for histology and systemic signs of disease.²⁵ Levine *et al* set the discrimination point for "S" between slow and fast proliferative lymphomas at 10%.²⁶ They found that the median survival for slow proliferative NHL was over 52 months and for fast proliferative NHL 39.2 months, and concluded that there seemed to be a relation between tumour proliferative state, therapeutic response, and survival. Numerous studies deal with the kinetics of high grade lymphomas, and their conclusions are mainly consistent with ours. Wooldridge *et al* analysed 52 diffuse large cell and diffuse mixed NHL and set the discrimination point for "S" at 20%.²⁷ They found a positive effect for slow proliferative states on achievement of complete remission and on survival. The predicted two year actuarial survival for slow proliferative lymphomas was 68% and for high proliferative lymphomas only 10% ($p = 0.01$). Bauer *et al*, in a group of 50 diffuse large cell lymphomas with the same discrimination point for "S", found that high proliferative activity was the most important negative prognostic factor for survival.²⁸ Low proliferative activity was connected with a higher rate of complete remission, but this was not significant.

It can be concluded that cytophotometric analysis of cellular DNA content, performed on material obtained by aspiration biopsy of lymph nodes, is of substantial value in assessing the prognosis of NHL. Low proliferative

lymphomas can be determined by this method and definite prognostic characteristics can be attributed. In the H-DNA cases, although there were definite prognostic characteristics for the whole group, we could not decide with certainty whether they were really hyperproliferative or aneuploid. To resolve that problem cytophotometric analysis of a higher number of cells should be done or a flow cytometer could be used. An alternative approach is a careful mitosis count or 3HTdR labelling in such cases. The difference in survival of patients with NHL in each histological group, according to cytophotometric DNA analysis, indicates that this method could, at the very least, be a valuable complementary technique to histology in the initial assessment of NHL.

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