Eur J Cancer, Vol. 26, No. 5, pp. 635–637, 1990.
Printed in Great Britain

0277–5379/90$3.00 + 0.00

Feature Articles

DNA Flow Cytometry of Non-Hodgkin's Lymphomas

J.C. Macartney and R.S. Camplejohn

BACKGROUND

DOES DNA flow cytometry have a role in the management of non-Hodgkin's lymphoma (NHL)? We have assessed the impact of this technique on the classification, diagnosis and prognosis of NHL and some of the reasons for conflicting results.

DNA flow cytometry allows the rapid measurement of cellular DNA in terms of ploidy and proliferative activity (either S phase fraction or S + G2M fraction). Since DNA is reduplicated during the S phase, the proportion of cells in various phases of the cell cycle can be measured and aneuploid populations can be detected. Compared with other methods for assessing cell proliferation, DNA flow cytometry has the advantages of speed and representative sampling, the ability of combination with cell marker studies, and the method is less labour intensive; all this despite the high capital cost. Fresh or histologically processed tissue can be sampled. The latter is particularly useful since it allows retrospective analysis of large, well characterized series of tumours. The major disadvantages are the dilutional effect of non-tumour cells, unless simultaneous cell marker measurements are made, and insensitivity if tumour cells are present in small numbers (e.g. as in Hodgkin's disease).

Early reports documented the flow cytometry characteristics of NHL [1, 2]. Aneuploidy occurs in high and low grade NHL but is more frequent in the former. Similarly, proliferative activity is greater in high grade NHL but there is substantial overlap between grades and histological subtypes. Given the association with grade of NHL, it is hardly surprising that the level of proliferative activity also correlates with the expected clinical outcome. Several large series have now been reported: fourteen concern a mixture of high and low grade NHL [3–16] and six in which high grade NHL only are considered [17–22].

Most studies show a frequency of about 15% aneuploidy (range 7–30%) in low grade NHL and 50% aneuploidy (30–80%) in higher grades of malignancy. Outlying values are associated with smaller, less representative numbers of cases. Aneuploidy is encountered more frequently in B-cell NHL than in equivalent grades of T-cell NHL [7, 21, 23], with the exception of mycosis fungoides. The series of Braylan *et al.* [6] is unique in that it combined surface marker studies with DNA cytometry to measure specifically the neoplastic cell population without the dilutional effects of reactive lymphocytes, which accounts for the higher rate of aneuploidy (80%) in that series. Although the overall frequency of DNA aneuploidy is greater in high grade NHL, there is heterogeneity within individual histopathological subtypes and ploidy is of little value for detailed classification. Besides isolated reports there is no convincing association between stage and aneuploidy, with the possible exception of extranodal gastric NHL [24].

PROGNOSIS

Ploidy status by itself has little or no effect on prognosis in terms of either overall survival or attainment and duration of remission. There are three reports only in which aneuploidy in high grade NHL had an adverse effect on survival [8, 15, 19] and one study found the opposite [20]. The prognostic effect did not reach statistical significance in one of these series, in a second it only applied to stage I/II tumours and numbers were small. In none of these studies was the effect demonstrable after multivariate analysis.

There are greater difficulties in assessing reports of proliferative activity in NHL. Not all investigators calculate proliferative activity for aneuploid tumours, which may bias results since aneuploid tumours may be more proliferative. Results are also expressed in different ways, either S phase or S + G2M fraction and as either means or medians. In addition two or three grades may be used. Finally it should be remembered that S phase and S + G2M fractions are indices not rates, and high values do not necessarily equate to increased proliferation.

Table 1 lists the results for proliferative activity, excluding small, less representative studies or those in which no mean value was cited. Overall average S phase fractions were 3–4% for low grade and about 12% for high grade NHL. Some reports gave surprisingly high or low values suggesting that either the case mix or measurement differed significantly from other series. All investigators commented on the substantial scatter of results

Correspondence to J.C. Macartney.
J.C. Macartney and R.S. Camplejohn are at the Departments of Histopathology and Richard Dimbleby Cancer Research, UMDS, St. Thomas' Hospital, London SE1 7EH, U.K.

*Table 1. Proliferative activity (%) and grade in NHL**

Ref.	Method	Grade		
		Low	Intermediate	High
4	S	2.3 (0.1–11)	—	6.5 (0.1–34)
5	S	2.2 (0.8)	12.1 (4.9)	22.6 (11.1)
9	S	4.8	—	11.5
10	S	1.0 (0.5–10)	4.0 (0.4–35)	27.0 (4.6–56)
14	S	12.0 (6.2)	—	16.0 (7.1)
15	S	4.3 (0.6–14.4)	—	11.7 (2–32)
16	S	4.4 (1.4–16.3)	—	13.0 (3.9–35.2)
18	S	—	—	10.0 (3–46)
22	S	—	12.5	9.0 (0.5–32)
8	S + G2M	4.7	—	26.2
12	S + G2M	15.7	—	24.24
17†	S + G2M	—	—	14.1 (1.7–48.5)
20†	S + G2M	—	—	18.8†
22	S + G2M	—	—	14.0 (2.51)

*Single figures in parentheses are S.D., otherwise range shown.
†Calculated from data on G0/G1.

both within and between grades and histological subtypes of NHL. Although several workers have attempted to derive values for proliferative activity that discriminate between grades of NHL or between reactive hyperplasia and lymphoma [9, 25], in general this has not been fruitful. The best results come from Barlogie's group [8] where a multiparameter approach was adopted and grade correctly predicted in just over two-thirds of cases. The relation between immunophenotyping and proliferative activity is confused. Some series found lower proliferative activity in T-cell NHL [11, 21] but this was not so in other reports [7, 23, 26]. There are no convincing reports of a direct relation between proliferative activity and stage, symptoms or tumour bulk.

Although the kinetic heterogeneity of NHL limits the use of proliferative activity for diagnostic purposes, the same heterogeneity may make this measurement of prognostic value. Within a single subtype of NHL those cases with highest proliferative activity may be the most aggressive, which raises the possibility that treatment can be selected to suit individual tumours. If there is an effect on prognosis, we also need to know whether this is independent of other prognostic variables and whether the effect is stronger or weaker than these factors. In particular the effect of grade must be accounted for, otherwise DNA flow cytometry becomes little more than an expensive exercise in tumour grading.

There are three studies that document a worse prognosis for those cases with low grade follicular NHL with high proliferative activity (S phase fraction above 5%), either in terms of overall survival [16, 27] or increased likelihood of transformation to high grade lymphoma [11]. In the most recent study B symptoms and high proliferative activity were the strongest predictors of survival after multivariate analysis [16].

The situation in high grade NHL is more complex (Table 2). It should be noted that treatment schedules were not standard in some series, follow-up times differed and different variables were tested in multivariate analyses. Nevertheless, there is a strong suggestion that high proliferative activity adversely affects survival, at least in the short term, although the relation with long-term survival may be smaller. The effects on remission induction are not obvious.

*Table 2. Prognosis and proliferative activity in high grade lymphomas**

Ref.	Method	Cut-off (%)	Prognostic end-point	Comments
17	S + G2M	20	Survival and remission	Low proliferative activity most significant factor associated with long survival; no effect on achievement of remission; univariate and multivariate analysis
18	S	19	Survival	High proliferative activity most significant factor associated with poor survival; univariate and multivariate analysis
19	S + G2M	10–18	Survival	Intermediate proliferative activity associated with poor survival; univariate and multivariate analysis
20	S + G2M	20	Survival and remission	Low proliferative activity associated with longer survival and higher remission rate; univariate analysis
21	S + G2M	22	Survival and remission	Low proliferative activity associated with longer survival; no effect on remission induction; univariate analysis
22	S and S + G2M	—	Survival and remission	Low proliferative activity associated with increased remission induction but no effect on overall survival, univariate analysis; no prognostic effect of proliferative activity, multivariate analysis
8	S + G2M	15	Survival and remission	High proliferative activity associated with remission but shorter survival; univariate and multivariate analysis
14	S	12	Survival	No effect on overall survival; univariate analysis
15	S	16	Survival	No effect on survival; univariate analysis
16	S	16	Survival	Low proliferative activity associated with longer survival; univariate and multivariate analysis

*17–20 report either wholly or in part on diffuse large cell lymphomas. 21 and 22 contained mixed high grade NHL. 8 and 14–16 were mixed high and low grade NHL, but results in the table are for high grade NHL only.

RECOMMENDATIONS

Given that more than one hundred articles on DNA flow cytometry have been published and that details of about 2500 patients have been analysed, the lack of definitive conclusions, particularly about the prognosis of high grade NHL, are disappointing. The reasons lie in the large number of confounding variables which make comparisons between studies difficult. These can be loosely grouped into pathological, clinical and technical factors.

Different histological classifications are used and there are differences in the way subtypes are grouped for subsequent data analysis. There is a high probability of lack of diagnostic reproducibility in the histopathological classifications. Series differ as to whether the effects of staging, tumour bulk and other prognostic variables are taken into account. Patients are not treated uniformly. The flow cytometric characteristics of previously treated and newly diagnosed cases may differ. Modern therapy may have a major impact on prognosis and partly nullify the effects of prognostic variables, as has occurred in Hodgkin's disease.

The methods of analysing cytometric data are not always comparable. Criteria for diagnosing aneuploidy, whether or not to measure proliferative activity in the presence of aneuploidy and how to measure proliferative activity are variable. Even when the same measurement is made, it should not be assumed that commercially available software gives similar results to either other programs or to manual methods of analysis. The overall quality of cytometry in different reports is variable, as judged by the mean coefficient of variation for tracings. There may be differences between the results obtained from paraffin-embedded and fresh tissue, although in our hands this effect is small. Finally the methods of data analysis and the end-points for measuring prognosis are different; and series differ as to whether or not to treat measurements of proliferative activity as a continuous variable.

Overall, flow cytometric measurements of proliferative activity do have a prognostic effect in NHL. Nearly all the difficulties we have described above can be resolved and there is an urgent need for further studies on carefully defined groups of cases. The results of DNA flow cytometry might also be usefully compared with measurements of Ki-67 and other proliferative antigens. It would be an advance if all workers agreed to: (1) use the working formulation for lymphoma classification, despite its shortcomings; (2) include immunophenotyping data for working formulation categories that comprise disparate biological entities; (3) analyse separately proliferative activity for diploid and aneuploid tumours; (4) include data on other accepted prognostic variables; (5) analyse the effects on both overall survival and remission; (6) include details of treatment; and (7) use both univariate and multivariate analysis. The quickest way to produce results on adequate numbers of patients would be for different centres to pool their results.

1. Braylan RC, Fowlkes BJ, Jaffe ES *et al.* Cell volumes and DNA distributions of normal and neoplastic human lymphoid cells. *Cancer* 1978, **41**, 201–209.
2. Diamond LW, Braylan RC. Flow analysis of DNA content and cell size in non-Hodgkin's lymphoma. *Cancer Res* 1980, **40**, 708–712.
3. Costa A. Mazzini G, Bino GD, Silvestrini R. DNA content and kinetic characteristics of non-Hodgkin's lymphoma: determined by flow cytometry and autoradiography. *Cytometry* 1981, **2**, 185–188.
4. Scarffe JH, Crowther D. The pre-treatment proliferative activity of non-Hodgkin's lymphoma cells. *Eur J Cancer* 1981, **17**, 99–108.
5. Diamond LW, Nathwani BN, Rappaport H. Flow cytometry in the diagnosis and classification of malignant lymphoma and leukaemia. *Cancer* 1982, **50**, 1122–1135.
6. Braylan RC, Benson NA, Nourse VA. Cellular DNA of human neoplastic B-cells measured by flow cytometry. *Cancer Res* 1984, **44**, 5010–5016.
7. Shackney SE, Levine AM, Fisher RI *et al.* The biology of tumour growth in the non-Hodgkin's lymphomas. A dual parameter flow cytometry study of 220 cases. *J Clin Invest* 1984, **73**, 1201–1214.
8. Srigley J, Barlogie B, Butler JJ *et al.* Heterogeneity of non-Hodgkin's lymphoma probed by nucleic acid cytometry. *Blood* 1985, **65**, 1090–1096.
9. Christensson B, Tribukait B, Linder I-L, Ullman B, Biberfeld P. Cell proliferation and DNA content in non-Hodgkin's lymphoma. Flow cytometry in relation to lymphoma classification. *Cancer* 1986, **58**, 1295–1304.
10. Juneja SK, Cooper IA, Hodgson GS *et al.* DNA ploidy and cytokinetics of non-Hodgkin's lymphoma. *J Clin Pathol* 1986, **39**, 987–992.
11. Macartney JC, Camplejohn RS, Alder J, Stone MG, Powell G. Prognostic importance of DNA flow cytometry in non-Hodgkin's lymphoma. *J Clin Pathol* 1986, **39**, 542–546.
12. Morgan DR, Williamson JMS, Quirke P *et al.* DNA content and prognosis of non-Hodgkin's lymphoma. *Br J Cancer* 1986, **54**, 643–649.
13. Lenner, P. Roos G, Johansson H, Lindh J, Dige U. Non-Hodgkin's lymphoma. Multivariate analysis of prognostic factors including fraction of S phase cells. *Acta Oncol* 1987, **26**, 179–183.
14. Cavalli C, Donova M, Gobbi PG *et al.* Ploidy and proliferative activity measurements by flow cytometry in non-Hodgkin's lymphomas. Do speculative aspects prevail over clinical ones? *Eur J Cancer Clin Oncol* 1989, **25**, 1755–1763.
15. Lehtinen T, Aine R, Lehtinen M *et al.* Flow cytometric DNA analysis of 199 histologically favourable or unfavourable non-Hodgkin's lymphomas. *J Pathol* 1989, **157**, 27–36.
16. Rehn S, Glimelius B, Strang P, Sundstrom C, Tribukait B. Prognostic significance of flow cytometry studies in B-cell non-Hodgkin's lymphoma. *Hematol Oncol* 1990, **8**, 1–12.
17. Bauer KD, Merkel DE, Winter JN *et al.* Prognostic implications of ploidy and proliferative activity in diffuse large cell lymphomas. *Cancer Res* 1986, **46**, 3173–3178.
18. Young GAR, Hedley DW, Rugg CA, Iland HJ. The prognostic significance of proliferative activity in poor histology non-Hodgkin's lymphoma: a flow cytometry study using archival material. *Eur J Cancer Clin Oncol* 1987, **23**, 1497–1504.
19. McLaughlin P, Osborne HM, Johnston D *et al.* Nucleic acid cytometry in large cell lymphoma. *Cancer Res* 1988, **48**, 6614–6619.
20. Wooldridge TN, Grierson HL, Weisenburger DD *et al.* Association of DNA content and proliferative activity with clinical outcome in patients with diffuse mixed cell and large cell non-Hodgkin's lymphoma. *Cancer Res* 1988, **48**, 6608–6613.
21. O'Brien CJ, Holgate C, Quirke P *et al.* Correlation of morphology, immunophenotype, and flow cytometry with remission induction and survival in high grade non-Hodgkin's lymphoma. *J Pathol* 1989, **158**, 31–39.
22. Cowan RA, Harris M, Jones M, Crowther D. DNA content in high and intermediate grade non-Hodgkin's lymphoma—prognostic significance and clinicopathological correlations. *Br J Cancer* 1989, **60**, 904–910.
23. Wain SL, Braylan RC, Borowitz MJ. Correlation of monoclonal antibody phenotyping and cellular DNA content in non-Hodgkin's lymphoma. The Southeastern Cancer Study Group experience. *Cancer* 1987, **60**, 2403–2411.
24. Joensuu H, Soderestrom K-O, Klemi JP, Eerola E. Nuclear DNA content and its prognostic value in lymphoma of the stomach. *Cancer* 1987, **60**, 3042–3048.
25. Griffin NR, Howard MR, Quirke P *et al.* Prognostic indicators in centroblastic–centrocytic lymphoma. *J Clin Pathol* 1988, **41**, 866–870.
26. Joensuu H, Klemi PJ, Eerola E. Diagnostic value of DNA flow cytometry combined with fine needle aspiration biopsy in lymphomas. *J Pathol* 1988, **154**, 237–245.
27. Egerter DA, Said JM, Epling S, Lee S. DNA content of T-cell lymphomas. A flow cytometric analysis. *Am J Pathol* 1988, **130**, 326–334.