CONCISE REPORT

Independent Prognostic Significance of a Nuclear Proliferation Antigen in Diffuse Large Cell Lymphomas as Determined by the Monoclonal Antibody Ki-67

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To assess the prognostic significance of the growth fraction in diffuse large cell lymphoma (DLCL), we studied 105 DLCL patients with the monoclonal antibody Ki-67 applied to frozen tissue sections. Ki-67 detects a nuclear antigen associated with cell proliferation not found in resting cells. Ki-67 findings and other clinical prognostic factors were correlated with outcome using univariate and multivariate analyses in the proportional hazards model. High proliferative activity, defined as nuclear Ki-67 expression in >60% of malignant cells (Ki-67 > 60), was found to be a strong predictor of poor survival among these patients (P = .003, log-rank). The 19 patients with Ki-67 > 60% had a median survival of 8 months compared with a median survival of 39 months for the 86 patients with Ki-67 ≤ 60%. Examination of pretreatment clinical variables indicated the patient groups were similar with regard to age, sex, stage, B symptoms, tumor bulk, and lactate dehydrogenase (LDH). Both patient groups received comparable curative intent therapy and showed comparable complete response rate predating treatment differences as modifying outcome. Multivariate analysis indicated Ki-67 is an independent predictor of survival (multivariate P = .006). Further statistical analysis using only B-cell DLCL patients treated with CHOP (63 patients) indicated that Ki-67 > 60 retained strong prediction of poor outcome (P = .002, log-rank) among this homogeneous group. We conclude that high proliferative activity (Ki-67 > 60) is an independent factor allowing laboratory prediction of probable poor outcome of DLCL.

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From a Clinical Perspective, the treatment of diffuse large cell lymphoma (DLCL) is at once a major success and an ongoing dilemma.1-4 It is a therapeutic success because approximately one-third of the patients are cured with chemotherapy; it’s a dilemma because the other two-thirds are not.5-8 The challenge for physicians treating individual patients is to predict which of these outcomes is likely.9 Knowing which patient might be initially unresponsive to therapy or subject to relapse might allow exploration of newer therapeutic options which could be tailored to these patients. To this end, clinical prognostic factors have been employed to predict outcome in DLCL patients.1,3,4,6 Laboratory prediction of outcome has included identification of lactate dehydrogenase (LDH) as a prognostic factor.9 Growth fraction as determined by flow cytometry DNA content assessment6,7 and radioactive labeling using tritiated thymidine8 have both been of predictive value. By these means high proliferative states in lymphoma have been associated with poor prognosis, although not always at the level of statistical significance.9

To obviate the more arduous, costly, and sometimes more indirect assessments of flow cytometry and radioactive labeling, we have sought to determine the proliferation status of DLCL by using the monoclonal antibody Ki-67 which detects a nuclear antigen associated with proliferation on tissue sections.10 Recent preliminary study without statistical adjustment for clinical prognostic factors has found a variable relationship of Ki-67 to outcome among non-Hodgkin’s lymphomas.11,12 This study investigates Ki-67 in a single morphologic entity, DLCL, with statistical adjustment for clinical prognostic factors, seeking to establish the relationship of growth fraction determination to clinical factors and outcome.

MATERIALS AND METHODS

Patient population/selection. From 1978 to 1987, 115 consecutive patients with the initial histologic diagnosis of DLCL13 were evaluated at the Arizona Cancer Center. Our analyses exclude ten patients whose tissue specimens were inadequate for immunologic study, leaving 105 evaluable patients.

Tissue section assessment of Ki-67 and phenotype. We used a previously described immunohistochemical method14 employing primary mouse anti-human monoclonal antibodies (see below), biotin conjugated second stage, avidin-horseradish peroxidase, and the chromagen diaminobenzidine tetrahydrochloride (DAB) as a detection agent on snap-frozen tissue sections. We employed the monoclonal antibody Ki-67 (Dakopatts, Copenhagen) to detect nuclear proliferation antigen and the following panels to detect B and T cell antigens, respectively: B cell panel; Bl(CD20), B4 (CD19) (Coulter Immunology, Hialeah, FL), and Leu 16 (CD20) (Becton Dickinson, Mountain View, CA); and T cell panel; Leu 1 (CD5), Leu 4 (CD3), Leu 5 (CD2), and Leu 9 (CD7) (Becton Dickinson). Negative controls employed 2% bovine serum albumin (BSA) (Gamma Biologicals, Houston) in phosphate buffered saline (PBS).

Ki-67 was quantified by determining the number of positive large cells expressing nuclear Ki-67 (brown coloration cells) among the total number of large cells (blue coloration cells counterstained with methylene blue) within high power (40 × objective) microscopic fields. Field selection sought areas of highest Ki-67 expression evident by lower power scanning. Typically the total cell count (denominator) exceeded 1,000 cells (Median: 1,564 cells; range:

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703-3,718 cells). The cell counts were done without knowledge of clinical outcome.

**Clinical and demographic data.** After all sites of disease were measured and recorded, patients were staged according to the Ann Arbor staging system. Each patient had a complete blood count, chemistry panel, bone marrow aspirate and biopsy, chest radiograph, and either an abdominal computed tomographic scan or lymphangiogram. Treatment consisted of potentially curative Adriamycin-containing combination chemotherapy in 84% of patients as previously detailed including the CHOP regimen in 55%, CHOP plus irradiation in 15%, m-BACOD in 4%, ProMACE-CytaBOM in 6%, and MACOP-B in 5%. Sixteen percent of patients received palliative treatment including CVP (cyclophosphamide, vincristine, and prednisone) in 4%, irradiation alone in 4%, surgery followed by irradiation in 2%, and no therapy in 5%. We defined complete remission (CR) as the disappearance of all disease that was present at the initiation of therapy. Survival was calculated from the date of diagnosis to the date of death or last contact. All patients are in the survival analysis regardless of treatment type or cause of death.

**Statistical consideration.** Comparisons between patient groups (ie, Ki-67 ≤ 60 v >60) were based on a chi-square test for categorical data and a Wilcoxon rank-sum test for continuous data. Survival curves were estimated by the Kaplan and Meier method, and comparisons between curves were based on the log-rank and Gehan's generalized Wilcoxon tests. Cox's proportional hazards model was used to adjust the simultaneous effect of different prognostic factors on survival. All tests were two-tailed.

**RESULTS**

Ki-67 expression in our 105 DLCL patients had a substantial range (3% to 91%) with a mean of 39.7 (SD = 20.4). Association of Ki-67 with clinical outcome established a statistically significant shortened survival with Ki-67 > 60% (P = .003, log-rank). The 19 patients with Ki-67 > 60% had a median survival of 8 months compared with 39 months for the 86 patients ≤60% (Fig 1). The 60% cutpoint was chosen with regard to assessing the effect of high proliferation activity and adequate sample size. The predictive value of Ki-67 was also evident using a cutpoint of 70% (P = .04 log-rank); however, the small sample (nine patients) with Ki-67 > 70% precluded further analyses. Cutpoints below 60% diminished the capability to focus on high proliferation status. As indicated in Table 1, evaluation of pretreatment clinical variables revealed the patient groups were similar with respect to age, stage, B symptoms, tumor bulk, and LDH. Although the differences were not statistically significant, there were minor differences in the two groups with regard to the incidence of extranodal disease and B v T cell phenotype. We used the proportional hazards model to assess the prognostic importance of Ki-67 on survival after adjusting for these differences. As shown by this multivariate analysis, adjusting for phenotype and extranodal disease had little effect on the relationship of Ki-67 with survival (multivariate P = .006). Further statistical analysis using only B cell DLCL patients treated with CHOP (63 patients) indicated that Ki-67 > 60 retained strong prediction of poor outcome (P = .002, log-rank) among this homogeneous group. The B cell DLCL CHOP treated patients with Ki-67 > 60 had a median survival of 8.8 months compared to 44.9 months for the B cell DLCL with Ki-67 < 60. Thus, Ki-67 > 60% appears to be an independent predictor of survival. Furthermore, as indicated in Table 1, both groups had comparable curative treatment and similar response rate obviating survival differences due to variable treatment. Of our 105 patients, 83 had a B cell phenotype, 20 had a T cell phenotype, one a biphenotypic phenotype, and one "null" phenotype. The mean Ki-67 expressive fraction for B cell DLCL was 40.9% and the mean for the T cell DLCL was 34.4%; a difference which is not statistically significant (P = .28).

**DISCUSSION**

In this study high proliferative activity as defined by a Ki-67 > 60% was an important pretreatment predictor of poor survival among clinically similar patients with DLCL. The relationship of Ki-67 to survival persisted even after adjusting for extranodal disease and phenotype. Other well known clinical prognostic factors including age, stage, B symptoms, tumor bulk, and LDH had comparable distribution between the two groups. Multivariate analysis indicates Ki-67 > 60% is an independent prognostic factor (multivariate P = .006) with a median survival of 8 months compared with 39 months for those ≤60%. Prediction of poor outcome by Ki-67 > 60 (P = .002) was retained among our largest homogeneous subgroup of B cell DLCL treated with CHOP. This indicates that Ki-67 may be a very useful, easily performed, and readily quantifiable laboratory marker which improves prediction of clinical outcome of DLCL.

Ki-67 is a monoclonal antibody which detects a nuclear antigen associated with proliferation. This antigen is found throughout the cell cycle and is absent in resting (G0) cells, hence its utility in establishing the growth fraction of neoplasms. Previous study of Ki-67 has established its comparability to the tritiated thymidine labeling index. Recent preliminary study of Ki-67 in malignant non-Hodgkin's lymphomas has found a variable relationship of Ki-67 to outcome. Since the latter study of Ki-67 did not take into account clinical prognostic factors or treatment, a comparison with the present study cannot be made. On this basis, this appears to be the first study of the relevance of Ki-67 to survival within a single histologic category of non-Hodgkin's lymphoma with statistical adjustment for clinical prognostic factors.
Other investigators using either flow cytometry assessment of DNA content and/or radioactive thymidine labeling have previously found a correlation between high proliferation and poor prognosis in both high grade and large cell lymphomas. Our data are in concert with these findings. In contrast with the indirect nature of assessment by flow cytometry and the labor intensity of the radioactive method, the Ki-67 determination on frozen sections affords an inexpensive, quickly performed alternative of more practical utility in hospital practice. In conclusion, the monoclonal antibody Ki-67 is a useful and practical independent determinant of prognosis in DLCL which should aid in the laboratory prediction of survival. This antibody places growth fraction assessment within the realm of daily hospital practice. The very poor prognosis of Ki-67 > 60% patients is comparable with DLCL patients with no treatment suggesting that Ki-67 may identify DLCL patients requiring alternative therapy.

REFERENCES


Table 1. Demographic and Clinical Characteristics Stratified by Ki-67

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>≤60 (n = 86)</th>
<th>&gt;60 (n = 19)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>57.8 (12.3-86.6)</td>
<td>56.2 (14.4-75.4)</td>
<td>0.60</td>
</tr>
<tr>
<td>Phenotypes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>T cell</td>
<td>18 (21.4%)</td>
<td>2 (10.5%)</td>
<td>0.28</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>54 (62.8%)</td>
<td>10 (62.6%)</td>
<td>0.41</td>
</tr>
<tr>
<td>Stage I, II, III, or IV</td>
<td>26 (30.2%)</td>
<td>5 (26.3%)</td>
<td>0.74</td>
</tr>
<tr>
<td>B symptoms</td>
<td>37 (43.0%)</td>
<td>9 (47.4%)</td>
<td>0.73</td>
</tr>
<tr>
<td>Tumor bulk ≥ 10 cm</td>
<td>17 (20.2%)</td>
<td>5 (26.3%)</td>
<td>0.56</td>
</tr>
<tr>
<td>LDH ≥ 225</td>
<td>35 (61.4%)</td>
<td>7 (53.9%)</td>
<td>0.35</td>
</tr>
<tr>
<td>LDH ≥ 226-500</td>
<td>12 (21.1%)</td>
<td>5 (38.5%)</td>
<td></td>
</tr>
<tr>
<td>Not performed</td>
<td>10 (17.5%)</td>
<td>1 (7.7%)</td>
<td></td>
</tr>
<tr>
<td>Extranodal disease</td>
<td>71 (82.6%)</td>
<td>18 (94.7%)</td>
<td>0.18</td>
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<tr>
<td>Curative treatment</td>
<td>73 (84.9%)</td>
<td>15 (79.0%)</td>
<td>0.53</td>
</tr>
<tr>
<td>Complete response</td>
<td>53 (62.4%)</td>
<td>9 (52.9%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Median (range)</td>
<td>65 (1090)</td>
<td>60 (98.9)</td>
<td></td>
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<tr>
<td>Median (range)</td>
<td>58:45, 1986</td>
<td>65:1090, 1985</td>
<td></td>
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