Expression of c-Myc and p53 Correlates With Clinical Outcome in Diffuse Large B-Cell Lymphomas

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Abstract

We performed a retrospective immunohistochemical study of the relationships between clinical manifestations and outcomes of diffuse large B-cell lymphoma (DLBCL) and expression of oncogenic proteins in 21 cases of DLBCL at various clinical stages. Cases of nodal origin expressed p53 more often and presented with high clinical stage more frequently than those of extranodal origin. Expression of c-Myc or p53, but not Bcl-6, Bcl-2, or Bcl-1, showed a statistically significant positive correlation with high clinical stage at presentation and with high or high-intermediate risk. Coexpression of c-Myc and p53 occurred in 7 of 12 patients with high clinical stage but was absent in patients with low clinical stage; coexpression was more frequent in patients with high or high-intermediate risk than in patients with low or low-intermediate risk. Four patients with this coexpression pattern demonstrated an unusually aggressive clinical course (median survival, 7 months). Coexpression of c-Myc and p53 seems to be a better indicator than the MIB1 proliferative index for identification of a cohort of aggressive disease in patients with DLBCL.

In the proposed revised European-American classification of lymphoid neoplasms, diffuse large B-cell lymphomas (DLBCLs) are much more heterogeneous in terms of clinical behavior and histomorphologic features than other groups of lymphomas. This type of lymphoma constitutes 30% to 40% of non-Hodgkin lymphoma. DLBCLs occur over a broad range of age and manifest at nodal or extranodal sites.1 These tumors usually respond to chemotherapy, with reported 5-year survival rates of about 50%, although some patients have a more aggressive course than others.2 Histomorphologically, DLBCLs consist of several variants including anaplastic features or a T-cell or histiocyte-rich background. Nuclear cytologic features also vary with centroblastic, large cleaved, spindle, polylobated, or immunoblastic appearances.1 However, no distinct prognostic significance has been correlated with histomorphologic pattern.

As a reflection of the morphologic and clinical heterogeneity of DLBCLs, the molecular events in DLBCL also are very complicated. Rearrangements of bcl-6 are found in up to 35% of DLBCLs. The reported incidence of t(14;18)(q32;q21) translocations (resulting in overexpression of Bcl-2) in DLBCLs is lower than that of follicular lymphoma, with a range of 12% to 30%. Rearrangement of the c-myc gene, frequently involving t(8;14)(q24;q32), leading to deregulation and overexpression of the transcription factor c-Myc, can be observed in up to 15% of large cell lymphomas.3

The clinical significance of these molecular lesions remains uncertain despite extensive studies. Offit et al4 reported that bcl-6 rearrangements were found relatively frequently in extranodal DLBCL and correlated with a favorable clinical outcome, but this could not be confirmed by others.5 Rearrangements of bcl-2 often are confined to nodal...
DLBCL, and here also, conflicting results are reported about the clinical effects.6-11 Rearrangement of c-myc seems to be more frequent in extranodal DLBCLs than in nodal DLBCLs. Previous studies suggested that c-myc rearrangement had no prognostic significance in DLBCL.12,13 Kramer et al15 studied 156 patients with de novo DLBCL for rearrangements of the bcl-2, bcl-6, and c-myc oncogenes by Southern blot analysis and concluded that, thus far, these genetic rearrangements fail to provide significant information as prognostic markers.3 Similar results have been suggested by others.14

However, to our knowledge, information on clinical significance of the overexpression of oncogenic proteins by immunohistochemistry in DLBCL is sparse in the literature. Recent studies have shown that the expression of the oncogenes, such as Bcl-2 and Bcl-6, can occur independently of the presence of molecular lesions of oncogenes.15 Furthermore, immunohistochemical analysis is used routinely in many laboratories and is relatively easy to perform on paraffin section compared with molecular and cytogenetic methods. Thus, our goal was to perform a comprehensive study of the relationship between the clinical features, specifically nodal vs extranodal origin, low or low-intermediate risk vs high or high-intermediate risk by the international prognostic index (IPI),16 and localized (low clinical stage) vs high or high-intermediate risk by the international study of the relationship between the clinical features, specifically nodal vs extranodal origin, low or low-intermediate risk vs high or high-intermediate risk by the international prognostic index (IPI),16 and localized (low clinical stage) vs high or high-intermediate risk by the international

### Materials and Methods

#### Patients

We retrospectively studied 21 de novo DLBCL cases (8 women, 13 men; mean ± SD age, 55 ± 16 years) accessioned from July 1993 through August 1998 at the Department of Pathology, MetroHealth Medical Center, Cleveland, OH, and at the Department of Pathology, University of Utah Health Science Center, Salt Lake City. Cases with history of AIDS, receiving immunosuppressive therapy, or a preceding diagnosis of low-grade lymphoma, such as follicular lymphoma or chronic lymphocytic leukemia/small lymphocytic lymphoma, were excluded from the study. The cases included in the study were reviewed to confirm that morphologic characteristics fulfilled the criteria of the revised European-American classification of lymphoid neoplasms for DLBCL. In addition, all cases had immunophenotyping by immunohistochemical stain, flow cytometry, or both to confirm the B-cell lineage.

The pertinent clinical information for the cases was obtained by reviewing the tumor registry records, patients’ medical charts, or both. The Ann-Arbor staging system was used. Nine of 21 cases presented with low clinical stage (stage I, 4 cases; stage II, 5 cases) and 12 with high clinical stage (stage III, 1 case; stage IV, 11 cases). Five patients were in the high or high-intermediate risk groups, and 15 were in the low or low-intermediate risk groups by IPI.16 Nodal origin was assigned to cases with a clinical presentation mainly in lymph nodes, spleen, or bone marrow according to the criteria of Kramer et al.17 Extranodal origin was defined as primary presentation in other sites with or without local lymph node involvement.17 Ten cases presented with nodal disease (low clinical stage, 2 cases; high clinical stage, 8 cases) and 11 cases with extranodal disease (low clinical stage, 7 cases; high clinical stage, 4 cases). The median length of follow-up was 11.5 months (range, 1-47 months). The end point of follow-up was the date of the last visit or the date of death through August 1998. Clinical presentation and outcome data are given in Table 1.

#### Immunohistochemical Stains

We performed immunohistochemical stains on formalin- or B-5-fixed paraffin-embedded tissue with antibodies against p53 and a panel of oncoproteins: Bcl-2, Bcl-6, c-Myc, and Bcl-1. In addition, MIB1 (Ki-67) staining was performed to evaluate the proliferative activity of large neoplastic lymphocytes as previously described.18 Paraffin section immunohistochemistry was performed using the avidin-biotin-peroxidase technique with antigen epitope enhancement by pressure cooker heating, enzyme pretreatment, or both. Table 2 shows the characteristics of antibodies used and the methods of antigen epitope enhancement for each antibody. After antigen epitope enhancement, the staining was performed using an automated immunostainer (Ventana ES, Ventana, Tucson, AZ).

#### Interpretation of Staining Results

Expression of p53 and each oncoprotein was reviewed systematically by 2 of us (C.-C.C. and S.L.P.) independently without the knowledge of clinical information. The intensity of staining was graded as follows: strongly positive when the positive staining could be recognized readily at low power (<40), moderately positive when positive staining could be recognized at intermediate power (40-100) but not at low power, weakly positive when recognized at high power (>400) only, and negative when not recognized even at high power. Each case also was evaluated to determine percentages of the large neoplastic lymphocytes that stained positively for p53 and each oncoprotein. As some background small reactive lymphocytes may sometimes show occasional cells that stain weakly positive for p53 and

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oncoproteins, we defined positive expression of oncoproteins and p53 as any degree of strong or moderate staining intensity. In samples showing only weak staining intensity, we required more than 20% of large neoplastic lymphocytes to stain positively to be considered positive expression. This definition provided the best intraobserver consistency and interobserver agreement. For the MIB1 stain, the percentages of large neoplastic cells stained were evaluated using the method described previously.18

Results

The results of the immunohistochemical staining of oncoproteins and p53 are shown in Table 1. The overall frequencies of expression for c-Myc, Bcl-2, Bcl-6, Bcl-1, and p53 in the cases studied were as follows: 57%, 48%, 33%, 5%, and 38%, respectively. Chi-square analysis revealed that the tumors of primary nodal origin presented more frequently as high clinical stage (8/10 cases) than those of extranodal origin (4/11 cases) ($P < .05$). No significant difference was found in high or high-intermediate and low or low-intermediate risk between the tumors of primary nodal origin and extranodal origin. The cases of nodal origin more frequently expressed p53 (6/10 cases) than those of extranodal origin (2/10 cases) ($P < .05$). There were no significant differences in the expression of Bcl-6, Bcl-2, or c-Myc between the tumors of nodal origin and those of extranodal origin.

We further grouped the patients into low clinical stage and high clinical stage and into high or high-intermediate and low or low-intermediate risk groups. By simple regression analysis, expression of c-Myc or p53 showed statistically significant positive correlation with high clinical stage at presentation ($R^2 = .37$ and $P < .005$; $R^2 = .23$ and $P < .03$, respectively). Similarly, expression of c-Myc or p53...
showed statistically significant positive correlations with high or high-intermediate risk groups by the IPI ($R^2 = .22$ and $P < .04$ for both). In contrast, expression of Bcl-6, Bcl-2, and Bcl-1 showed no significant correlation with stage or risk groups at presentation.

In addition, we studied whether the different combinations of expression of oncoproteins and p53 correlated with clinical stage or risk groups at presentation. We found that coexpression of c-Myc and p53 occurred in 7 (58%) of 12 patients with high clinical stage, but coexpression of c-Myc and p53 were absent in patients with low clinical stage ($P < .02$, chi-square). Similarly, coexpression of c-Myc and p53 tended to occur more frequently in patients with high or high-intermediate risk than in patients with low or low-intermediate risk by IPI (80% vs 20%; $P = 0.06$, chi-square).

**Table 3**

<table>
<thead>
<tr>
<th>No. (%) of Cases</th>
<th>LCS (n = 9)</th>
<th>HCS (n = 12)</th>
<th>$R^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Myc</td>
<td>2 (22)</td>
<td>10 (83)</td>
<td>0.37</td>
<td>.003</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>4 (44)</td>
<td>6 (50)</td>
<td>0.003</td>
<td>.81</td>
</tr>
<tr>
<td>Bcl-6</td>
<td>2 (22)</td>
<td>5 (42)</td>
<td>0.04</td>
<td>.37</td>
</tr>
<tr>
<td>Bcl-1</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>0.04</td>
<td>.40</td>
</tr>
<tr>
<td>p53</td>
<td>1/9 (11)</td>
<td>7 (58)</td>
<td>0.23</td>
<td>&lt;.03</td>
</tr>
</tbody>
</table>

HCS, high clinical stage (III or IV); LCS, low clinical stage (I or II).

Furthermore, 4 of 7 patients (cases 14, 15, 19, and 20) with this coexpression demonstrated an unusually aggressive clinical course. All died of disease within 1 year of diagnosis despite chemotherapy. The median survival was 7 months. Importantly, only 1 case showed high (80%) proliferation activity with MIB1 staining, and 2 patients were in the high or high-intermediate risk groups by IPI. The remaining 3 patients (cases 11, 17, and 18) with this coexpression were diagnosed near the time of the present study, and the clinical outcome is undetermined. In contrast, the median survival of the patients with stage IV disease but without c-Myc and p53 coexpression was 26 months (Table 1).

**Discussion**

The clinical significance of molecular lesions involving tumor suppressor gene p53 and several oncoproteins, including bcl-1, bcl-2, bcl-6, and c-myc, as well as the expression of these genes in patients with DLBCL has been the subject of numerous studies. To date, no clear-cut conclusions for clinical significance or prognostic importance have emerged. Our findings using immunohistochemical staining to evaluate the expression of p53 and oncoproteins in patients with DLBCL indicate that the expression of p53, c-Myc, or both correlates with clinical manifestation, specifically nodal vs extranodal disease, high or high-intermediate risk group vs low or low-intermediate risk group, and low clinical stage vs high clinical stage disease, and outcome.

It has been suggested that DLBCLs of nodal and extranodal origin may be different disease entities. In the present study, cases of nodal origin more often expressed p53 and more frequently presented as high clinical stage than those of extranodal origin. However, our results suggested that there is no significant differences in the expression of Bcl-2, Bcl-6, or c-Myc proteins between cases of nodal and extranodal origin, in contrast with the results of previous studies using molecular methods to determine the rearrangement of the corresponding genes. This difference most likely reflects that detectable protein expression of the oncoproteins is independent of detectable molecular rearrangement.

Expression of c-Myc or p53 showed statistically significant positive correlation with high clinical stage and high or high-intermediate risk groups at presentation. Rao et al, using comparative genomic hybridization analysis, reported that c-myc amplification was associated with advanced stage disease in patients with DLBCL. To our knowledge, the present study is the first to further confirm their findings using the alternative method of immunohistochemical staining to determine c-Myc expression. Our results also agree with the results of a study by Kramer et al, which suggested that p53 protein expression is related to high tumor burden. Importantly, using our panel of stains, we further found that coexpression of c-Myc and p53 occurred frequently (58%) in patients with high clinical stage, but coexpression of c-Myc and p53 was absent in patients with low clinical stage. Similarly, coexpression of c-Myc and p53
tended to occur more frequently in patients with high or high-intermediate risk than in patients with low or low-intermediate risk by IPI (80% vs 20%; \( P = 0.06 \), chi-square).

The most interesting finding of the present study was that cases with coexpression of c-Myc and p53 demonstrated an unusually aggressive clinical course, with a median survival of 7 months among patients with stage IV disease. Furthermore, this coexpression seems a better indicator of disease aggressiveness than proliferative activity determined by MIB1 staining. Only 25% (1 of 4) patients with this coexpression showed high proliferative activity (defined as MIB1 staining 80% of neoplastic lymphocytes), although previous studies have indicated that DLBCL cases with an aggressive clinical course usually demonstrated high proliferation indices by MIB1 staining.\(^18\) Also, only 2 cases were in the high or high-intermediate risk groups by IPI scoring. Our results suggested that determination of the expression of p53 and c-Myc by immunohistochemistry may provide additional prognostic information for patients with stage IV DLBCL.

The mechanism of this coexpression leading to an aggressive clinical course remains uncertain. Expression of p53 alone has not proved to be an independent risk factor for survival in cases of DLBCL.\(^17\) Previous studies also suggested that c-myc rearrangement had no prognostic significance in DLBCL.\(^12,13\) A key role in apoptosis has been shown for p53. The physiologic function of c-myc is only partially known. Its product has DNA binding properties and has a role in the control of proliferation and differentiation. In general, increased c-Myc expression leads to proliferation and abolishment of differentiation.\(^21\) There also may be a role for c-myc in regulating the apoptosis of lymphocytes.\(^22\) In addition, p53 mutations, c-myc activation by translocation, or both have been reported to involve in tumor progression.

\[\text{A} \quad \text{B} \quad \text{C}\]

\[\text{Image 1} \quad \text{Diffuse large B-cell lymphoma showing coexpression of c-Myc and p53 (A, H&E, ×400). B, Neoplastic lymphocytes show strongly positive staining with p53 (×400). Endothelial cells are negative. C, Neoplastic lymphocytes show moderately positive staining with c-Myc (×400).}\]
from overt follicular lymphoma to DLBCL. Thus, it is likely that an unknown interaction between c-Myc and p53 is at least partially responsible for the aggressiveness of these lymphomas, perhaps by action on the apoptotic or tumor progression pathway.

In the present study, the expression of Bcl-6 and Bcl-2 did not show a significant correlation with stage at presentation. Others have shown Bcl-2 oncoprotein expression to be a possible indicator for a poor prognosis in DLBCL, independent of clinical stage. This suggests that the prognostic significance of Bcl-2 may be related to the inhibition of chemotherapy-induced apoptosis rather than tumor burden or extent of disease. The lack of the correlation of Bcl-6 expression and clinical stage at presentation in the present study and the lack of prognostic significance found in other studies suggests that Bcl-6 should be considered as a marker of histogenesis of DLBCL rather than a marker of disease extent or prognosis.

Expression of Bcl-1, an oncoprotein associated with t(11;14) that is commonly present in mantle cell lymphoma, was very uncommon in DLBCL. In the present study, only 1 case showed expression of Bcl-1 (case 21, Table 1). This single case presented as stage IV disease, but the patient was alive without evidence of disease 26 months after diagnosis. Expression of c-Myc and Bcl-2 but not p53 were noted in this case as well.

We found that cases of nodal origin more often expressed p53 and more frequently presented as high clinical stage than those of extranodal origin. Expression of c-Myc or p53 showed a statistically significant positive correlation with high clinical stage and with high or high-intermediate risk groups at presentation. Coexpression of c-Myc and p53 occurred frequently (53%) in patients with high clinical stage but was absent in patients with low clinical stage. In addition, patients with stage IV disease and this coexpression pattern demonstrated an unusually aggressive clinical course. Coexpression of c-Myc and p53 seemed to be a better indicator than MIB1 proliferative index or IPI scoring for identification of a cohort of patients with aggressive disease. Further studies with a larger sample and multivariate analysis are indicated to confirm our results and to further study whether coexpression of c-Myc and p53 is an independent prognostic indicator in DLBCL.

References


