The Clinical Significance of CD10 Antigen Expression in Diffuse Large B-Cell Lymphoma

Patricia Uherova, MD, Charles W. Ross, MD, Bertram Schnitzer, MD, Timothy P. Singleton, MD, and William G. Finn, MD

Key Words: Diffuse large B-cell lymphoma; CD10 antigen; International prognostic index

Abstract

The clinical significance and prognostic value of CD10 in de novo diffuse large B-cell lymphoma (DLBCL) is largely unknown. We retrospectively studied 19 men and 9 women based on the following criteria: (1) DLBCL with no evidence of concomitant or antecedent follicular lymphoma; (2) available flow cytometric immunophenotyping data, including CD10 status; (3) older than 15 years; (4) specific exclusion of high-grade, Burkitt-like lymphoma; and (5) exclusion of primary cutaneous DLBCL. When available, clinical data at diagnosis, including components of the international prognostic index, were reviewed. Eleven cases were CD10+, and 17 were CD10–. There was no significant difference between the CD10+ and CD10– groups in age, sex, stage, performance status, extranodal involvement, or serum lactate dehydrogenase levels at diagnosis. However, in the 26 cases for which follow-up data were available, the CD10+ group displayed a shorter overall survival than the CD10– group (8 vs 30 months). Although the clinical findings at diagnosis are similar in CD10+ and CD10– DLBCL, CD10 expression is associated with shortened overall survival. Therefore, our data suggest CD10 expression may have prognostic importance in adults with de novo DLBCL.

Diffuse large B-cell lymphoma (DLBCL) accounts for approximately 30% to 40% of newly diagnosed non-Hodgkin lymphomas in the United States. Morphologically, this entity is characterized by a diffuse proliferation of large lymphoid cells with vesicular nuclei and prominent nucleoli. It represents a clinically and histologically diverse group of lymphomas, which includes diffuse centroblastic and immunoblastic B-cell lymphoma in the Kiel classification and malignant lymphoma, diffuse large cell, and immunoblastic types in the Working Formulation. In the Revised European-American Lymphoma (REAL) and upcoming World Health Organization classifications, diffuse large B-cell lymphomas are not subclassified further and include cases previously classified as immunoblastic lymphoma. This category also comprises de novo DLBCLs and those transformed from low-grade B-cell lymphomas.

In general, DLBCL is an aggressive disease with a median survival of 1 to 2 years if untreated. Standard systemic chemotherapy generally includes doxorubicin-containing regimens such as CHOP (cyclophosphamide, doxorubicin, vincristine [Oncovin], and prednisone) and produces complete remission in 50% to 60% of cases. Several attempts to improve therapy of the intermediate- and high-grade lymphomas during the past 20 years have been based largely on empiric changes in the treatment regimens. However, randomized trials have not confirmed any significant improvement compared with CHOP, and, overall, only one third of the patients with high-stage disease are cured.

Several clinical factors have prognostic significance in DLBCL, including age, stage of the disease, systemic symptoms, performance status, tumor burden, and serum lactate dehydrogenase levels.
dehydrogenase (LDH) levels, all of them essentially incorporated into the international prognostic index (IPI). The IPI was developed for patients with intermediate- and high-grade non-Hodgkin lymphomas as defined in the Working Formulation and successfully predicts outcome based on the patients’ clinical characteristics before treatment.

In contrast, biologic markers (histologic type, immunophenotype, and genetic findings) have not been reliable for predicting the outcome in patients with DLBCL. Histologic subclassification of DLBCL has been problematic owing to lack of reproducible criteria. The attempts to link certain immunophenotypic and genetic findings with different clinical outcome have provided controversial results. Anecdotal studies have found that the absence of CD20, CD22, or HLA-DR correlated with poorer survival, and CD24 expression or lack of CD38 was associated with better relapse-free survival in patients with DLBCL. More aggressive clinical course and more frequent bone marrow involvement was observed in CD5+ compared with CD5− DLBCL. An isofrom of the CD44 adhesion molecule involved in cell-cell interactions and metastasis, emerged as a significant parameter for poorer overall survival in the patients with primary nodal DLBCL as reported by Inagaki et al. In another study, evaluation of the proliferative index did not explain the differences in outcome between patient groups defined by the IPI. Conflicting results are reported on the clinical significance of bcl-2, bcl-6, and c-myc oncogenes and tumor suppressor gene p53 in DLBCL. Recently, distinct types of DLBCL based on gene expression profiling were identified.

CD10, the common acute lymphoblastic leukemia antigen (CALLA), is a single-chain, 100-kd glycoprotein with a sequence identical to that of neutral endopeptidase. CD10 might have a role in inactivating regulatory peptides with a sequence identical to that of neutral endopeptidase. Approximately 20% to 30% of de novo DLBCLs express the CD10 antigen. Previous studies have examined the prognostic value of other possible markers of follicle center differentiation in DLBCL, but the clinical significance of CD10 in DLBCL is largely unknown. Only a few reports on its prognostic relevance have emerged in the literature, without a clear consensus. Our aim was to study the prognostic value of CD10 in de novo DLBCL by examining the relationship of CD10 expression to the components of the IPI and to overall survival.

Materials and Methods

Patients

We reviewed cases of DLBCL diagnosed in the Department of Pathology, University of Michigan Medical Center, Ann Arbor, between March 1991 and July 1999, for which flow cytometric immunophenotyping data were available. The diagnosis of DLBCL was established by a consensus conference in the presence of all authors. We selected 28 cases for study based on the following criteria: (1) DLBCL with no evidence of concomitant or antecedent follicular lymphoma; (2) available flow cytometric immunophenotyping data, including CD10 status; (3) older than 15 years of age; (4) specific exclusion of high-grade, Burkitt-like lymphoma as defined by REAL classification guidelines; and (5) exclusion of primary cutaneous DLBCL.

Clinical Characteristics

When available, clinical data at diagnosis, including components of the IPI (age, Ann Arbor stage, extranodal involvement, performance status, and serum LDH levels) were obtained from medical records. Patients were grouped as follows: males vs females; age 60 years or older vs younger than 60 years; stage I or II vs stage III or IV disease; 0 or 1 vs more than 1 extranodal site; serum LDH level 200 U/L or less vs more than 200 U/L; and ambulatory vs nonambulatory performance status. Performance status classified as ambulatory corresponds to the 0 or 1 and nonambulatory to the 2, 3, or 4 performance status classification in the IPI. Patients were treated with standard chemotherapy regimens, which included CHOP or its modifications. Six patients received additional local radiation. Five patients received high-dose chemotherapy with hematopoietic stem-cell support.

Light Microscopy and Flow Cytometry

For histologic evaluation, tissue was fixed with 10% formalin or B-5 fixative and processed routinely for paraffin embedding. Sections of paraffin-embedded tissue (3-5 µm thick) were stained with H&E.

Flow cytometric immunophenotyping was performed by using 2- and 3-color immunofluorescence staining. Peripheral blood, cerebrospinal fluid, pleural effusion, or cell suspension obtained from lymph nodes was incubated with antibody cocktails according to the manufacturer’s recommendation. Samples were analyzed using an EPICS XL-MCL flow cytometer (Beckman-Coulter, Hialeah, FL). Surface immunophenotyping of lymphoma cells was performed using a standard lymphoma panel of monoclonal antibodies directed against the following antigens: CD2,
CD3, CD4, CD5, CD7, CD8, CD10, CD11c, CD14, CD19, CD20, CD22, CD23, CD45, FMC7, and kappa and lambda immunoglobulin light chains. B-cell monoclonality was confirmed in every case by coexpression of CD19 (Immunotech-Coulter, Miami, FL) and kappa or lambda immunoglobulin light chain (Becton-Dickinson, San Jose, CA). CD10 surface marker (clone W8E7, Becton-Dickinson) expression was assessed in combination with CD19 on the basis of detection of a distinct cell population with a fluorescence surface intensity greater than internal control.

Statistical Analysis

Comparison between patient groups based on components of the IPI (age, sex, stage of disease, number of extranodal sites, serum LDH, and performance status) was performed by using the Fisher exact test. Survival was calculated from the date of the diagnosis until the last follow-up or patient’s death. Overall survival in CD10+ and CD10– groups was determined by using Kaplan-Meier analysis. Survival duration in studied groups was compared by using the log-rank test.

**Results**

**Patient Characteristics**

The clinical features of each analyzed case are listed in [Table 1](#) and summarized in [Table 2](#). CD10 expression was documented in 11 of 28 cases [Image 1](#) and [Image 2](#). The present study included 19 men and 9 women (mean age, 59 years; range, 18-91 years). Of patients for whom data were available, 19 (70%) of 27 patients had advanced stage of disease (Ann Arbor stage III or IV) at diagnosis. The recorded sites of extranodal involvement included the bone marrow, spleen, liver, gastrointestinal tract, lung, adrenal gland, central nervous system, and eye. Primary cutaneous DLBCL was excluded from the study. Overall, 9 (33%) of 27 patients with data available had more than 1 extranodal site of disease at the time of diagnosis. Of 20 patients for whom data were available, 16 (80%) had increased LDH levels (>200 U/L) at diagnosis. Performance status was classified as ambulatory or nonambulatory; 16 of 26 patients were nonambulatory at the time of diagnosis. None of the patients was diagnosed with a second neoplasm during the follow-up interval. Eleven of 26 patients for whom follow-up data were available.

**Table 1**

<table>
<thead>
<tr>
<th>Case No./Sex/ Age (y)</th>
<th>Stage *</th>
<th>Extranodal Sites</th>
<th>LDH Level (U/L)</th>
<th>Performance Status</th>
<th>Follow-up Status</th>
<th>Survival Time (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD10+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/M/91</td>
<td>III</td>
<td>1</td>
<td>282</td>
<td>Nonambulatory</td>
<td>Dead</td>
<td>1</td>
</tr>
<tr>
<td>2/M/20</td>
<td>I</td>
<td>0</td>
<td>160</td>
<td>Ambulatory</td>
<td>Alive</td>
<td>13</td>
</tr>
<tr>
<td>3/F/79</td>
<td>IV</td>
<td>1</td>
<td>354</td>
<td>Nonambulatory</td>
<td>Dead</td>
<td>1</td>
</tr>
<tr>
<td>4/M/70</td>
<td>III</td>
<td>4</td>
<td>843</td>
<td>Nonambulatory</td>
<td>Dead</td>
<td>1</td>
</tr>
<tr>
<td>5/F/41</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>6/M/29</td>
<td>II</td>
<td>2</td>
<td>395</td>
<td>Ambulatory</td>
<td>Dead</td>
<td>8</td>
</tr>
<tr>
<td>7/F/57</td>
<td>II</td>
<td>1</td>
<td>141</td>
<td>Ambulatory</td>
<td>Alive</td>
<td>20</td>
</tr>
<tr>
<td>8/F/75</td>
<td>III</td>
<td>2</td>
<td>293</td>
<td>Ambulatory</td>
<td>Alive</td>
<td>9</td>
</tr>
<tr>
<td>9/M/58</td>
<td>III</td>
<td>1</td>
<td>306</td>
<td>Nonambulatory</td>
<td>Dead</td>
<td>7</td>
</tr>
<tr>
<td>10/F/65</td>
<td>III</td>
<td>1</td>
<td>352</td>
<td>Nonambulatory</td>
<td>Dead</td>
<td>19</td>
</tr>
<tr>
<td>11/M/54</td>
<td>IV</td>
<td>4</td>
<td>1,063</td>
<td>Nonambulatory</td>
<td>Alive</td>
<td>8</td>
</tr>
<tr>
<td>CD10–</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/M/30</td>
<td>I</td>
<td>0</td>
<td>202</td>
<td>Ambulatory</td>
<td>Alive</td>
<td>60</td>
</tr>
<tr>
<td>2/M/81</td>
<td>IV</td>
<td>4</td>
<td>NA</td>
<td>Nonambulatory</td>
<td>Alive</td>
<td>12</td>
</tr>
<tr>
<td>3/M/72</td>
<td>IV</td>
<td>1</td>
<td>313</td>
<td>Nonambulatory</td>
<td>Dead</td>
<td>30</td>
</tr>
<tr>
<td>4/M/76</td>
<td>IV</td>
<td>4</td>
<td>477</td>
<td>Nonambulatory</td>
<td>Alive</td>
<td>25</td>
</tr>
<tr>
<td>5/M/66</td>
<td>IV</td>
<td>1</td>
<td>180</td>
<td>Nonambulatory</td>
<td>Alive</td>
<td>9</td>
</tr>
<tr>
<td>6/F/73</td>
<td>IV</td>
<td>1</td>
<td>NA</td>
<td>Nonambulatory</td>
<td>Dead</td>
<td>1</td>
</tr>
<tr>
<td>7/M/52</td>
<td>I</td>
<td>0</td>
<td>356</td>
<td>Ambulatory</td>
<td>Alive</td>
<td>13</td>
</tr>
<tr>
<td>8/F/72</td>
<td>II</td>
<td>2</td>
<td>NA</td>
<td>Nonambulatory</td>
<td>Alive</td>
<td>1</td>
</tr>
<tr>
<td>9/M/18</td>
<td>I</td>
<td>0</td>
<td>156</td>
<td>Ambulatory</td>
<td>Alive</td>
<td>28</td>
</tr>
<tr>
<td>10/M/76</td>
<td>IV</td>
<td>1</td>
<td>NA</td>
<td>Nonambulatory</td>
<td>Dead</td>
<td>21</td>
</tr>
<tr>
<td>11/M/67</td>
<td>I</td>
<td>0</td>
<td>251</td>
<td>Ambulatory</td>
<td>Alive</td>
<td>25</td>
</tr>
<tr>
<td>12/F/41</td>
<td>II</td>
<td>1</td>
<td>511</td>
<td>Nonambulatory</td>
<td>Dead</td>
<td>14</td>
</tr>
<tr>
<td>13/M/70</td>
<td>IV</td>
<td>1</td>
<td>380</td>
<td>Nonambulatory</td>
<td>Alive</td>
<td>6</td>
</tr>
<tr>
<td>14/M/54</td>
<td>IV</td>
<td>2</td>
<td>NA</td>
<td>Nonambulatory</td>
<td>Alive</td>
<td>1</td>
</tr>
<tr>
<td>15/M/71</td>
<td>IV</td>
<td>2</td>
<td>326</td>
<td>Ambulatory</td>
<td>Dead</td>
<td>22</td>
</tr>
<tr>
<td>16/F/39</td>
<td>III</td>
<td>1</td>
<td>NA</td>
<td>Nonambulatory</td>
<td>Alive</td>
<td>9</td>
</tr>
<tr>
<td>17/M/53</td>
<td>IV</td>
<td>1</td>
<td>NA</td>
<td>Nonambulatory</td>
<td>Alive</td>
<td>9</td>
</tr>
</tbody>
</table>

LDH, lactate dehydrogenase; NA, information not available.

* Ann Arbor stage classification.
available died of lymphoma. Survival duration ranged from 1 to more than 60 months [Figure 1].

Comparison of Clinical Characteristics Between CD10+ and CD10– Groups

Comparison of components of the IPI for CD10+ and CD10– groups is summarized in Table 3. Five of 11 patients in the CD10+ group compared with 10 of 17 in the CD10– group were older than 60 years; however, the difference was not significant. Slightly more men were included in the CD10– group (13/17 [76%] in the CD10– group vs 6/11 [54%] in the CD10+ group; P = .411). In the CD10+ group, 6 (40%) of 10 patients for whom data were available and 5 (29%) of 17 in the CD10– group had more than one extranodal site at diagnosis (P = .683). Studied groups were virtually homogeneous considering stage, serum LDH level, and performance status (P = 1.00). In summary, clinical findings at diagnosis were similar in CD10+ and CD10– DLBCL.

A summary of therapeutic modalities for the patients in the study is given in Table 4. Twenty-three patients were treated with chemotherapy. In 17 cases, this included CHOP, and in 6 cases, other regimens were used. Six patients received local radiation. Chemotherapeutic salvage regimens for relapsed disease were administered in 8 cases. Five patients received high-dose chemotherapy with hematopoietic stem-cell support. There were no significant differences in treatment modalities between the CD10+ and CD10– groups.

Overall Survival Comparison Between CD10+ and CD10– Groups

Five (31%) of 16 patients in the CD10– group and 6 (60%) of 10 patients in the CD10+ group died of lymphoma. None of the patients included in the study died of unrelated causes. The median overall survival in the CD10+ group was 8 months compared with 30 months in the CD10– group. The Kaplan-Meier survival curves for the groups are shown in Figure 1. The CD10+ group displayed a significantly shorter overall survival duration than the CD10– group (P = .009).

To ensure that differences in survival between the CD10+ and CD10– groups in our study were not attributed to the histologic type, we compared the proportion of cases with the diagnosis of immunoblastic lymphoma in both groups and found no significant difference (5 of 17 in the CD10– group and 2 of 11 in the CD10+ group; P = .668).

Table 2
Summarized Patient Characteristics*

| Number (%) |
|------------------|------------------|
| Number (%)       |
| Sex (n = 28)     |                  |
| Male             | 19 (68)          |
| Female           | 9 (32)           |
| Age (y; n = 28; mean, 59; range 18-91) |                  |
| Younger than 60  | 13 (46)          |
| 60 or older      | 15 (54)          |
| Ann Arbor stage (n = 27) |              |
| I or II          | 8 (30)           |
| III or IV        | 19 (70)          |
| Extranodal sites (n = 27) |              |
| 0 or 1           | 18 (67)          |
| >1               | 9 (33)           |
| Serum lactate dehydrogenase level (n = 20) |                 |
| ≤ 200 U/L        | 4 (20)           |
| >200 U/L         | 16 (80)          |
| Performance status (n = 26) |              |
| Ambulatory       | 10 (38)          |
| Nonambulatory    | 16 (62)          |

* For numbers less than 10, the information was not available for the remaining patients.

Image 1A, A representative case of CD10+ diffuse large B-cell lymphoma, including (B) flow cytometric documentation of CD10 expression.
Discussion

DLBCL is a heterogeneous entity with a variable clinical course. The only reliable prognostic indicator so far has been the IPI, which successfully predicts the outcome based on patients’ clinical characteristics. In the present retrospective study, we detected no difference between CD10+ and CD10– DLBCL in the components of the IPI at diagnosis (age, stage, extranodal involvement, serum LDH level, and performance status). The number of cases studied was likely too low to detect small differences among subgroups; however, there was a significantly shorter survival duration in the CD10+ group than in the CD10– group.

Our studied cases were defined based on the REAL classification and included both DLBCL and immunoblastic lymphoma, which in the Working Formulation are lymphomas of different grade (intermediate vs high). Previous studies yielded contradictory conclusions about the prognostic importance of immunoblastic histology in large cell lymphoma, and the REAL and upcoming World Health Organization classifications do not include a formal category of immunoblastic lymphoma. However, a recent study demonstrated a significantly higher relative risk of death in immunoblastic lymphoma compared with other diffuse large cell lymphomas.

During lymphocyte differentiation, CD10 first appears on pro-B cells and is lost during maturation to naïve B cells. CD10 reappears on the cell surface during antigen-dependent germinal center maturation. Therefore, CD10 expression in DLBCL has been postulated to be a marker of germinal center B-cell origin. Various CD10+ lymphomas correspond to different stages of B-cell differentiation, which might be reflected in their clinical behavior. CD10 is expressed in indolent follicular lymphomas and in aggressive Burkitt lymphomas. Approximately 20% to 30% of DLBCLs are CD10+. Previously, it was suggested that a subset of DLBCL with follicle center cell features (eg, CD10 expression) might behave clinically in a manner resembling low-grade follicular lymphomas with indolent course and a high rate of relapse. However, data regarding the relationship between CD10 expression and clinical behavior in DLBCL are inconsistent. We specifically excluded cases with evidence of antecedent or concomitant follicular lymphoma.
in order to assess the significance of CD10 expression in de novo DLBCL. We also specifically excluded Burkitt and high-grade Burkitt-like lymphomas from the present study. Nevertheless, some lymphomas are classifiable cytologically as DLBCL but display certain features in common with high-grade Burkitt-like lymphoma, including high mitotic rate, frequent single cell necrosis, and a prominent starry-sky pattern of benign phagocytic histiocytes. Chai et al24 recently studied a group of such cases and found this subset of DLBCL to be an aggressive disease, clinically similar to Burkitt or high-grade Burkitt-like lymphoma, with a median survival of 8 months from diagnosis. This histologic variant of DLBCL was not excluded from the present study.

Our data showed that despite similar clinical findings at diagnosis, patients with CD10+ DLBCL had significantly shortened overall survival duration. Harada et al10 studied 3 groups of de novo DLBCL based on immunophenotype: CD5+, CD5–CD10+, and CD5–CD10–. The latter 2 groups are immunophenotypically identical to ours, with comparable clinical characteristics at diagnosis except for a slightly larger proportion of high-stage lymphomas in our studied group. However, in the study by Harada et al,10 CD10+ and CD10– groups showed identical survival. Findings comparable to ours were reported by Xu et al.19 In their study, there was no difference in age, sex, extranodal presentation, “B symptoms,” clinical stage, or morphologic features between CD10+ and CD10– groups. Patients with CD10+ tumors showed a trend toward poorer overall survival. In addition, a significantly higher proportion of patients in the CD10– group achieved complete remission with therapy (83% compared with 44% in the CD10+ group).

By using gene expression profiling with high-density DNA microarray technology, Alizadeh et al16 classified DLBCL into 2 groups: germinal center B-like and activated B-like groups. In the low clinical risk category, patients from the activated B-like DLBCL group had a distinctly worse survival than those in the germinal center B-like group. Overall, 76% of patients with germinal center B-like DLBCL were still alive after 5 years compared with 16% of patients with activated B-like DLBCL. CD10 was positive by immunochemical study in a subset of lymphoma cells with gene expression signatures similar to those of normal germinal center B cells (germinal center B-like group).16

The data of Alizadeh et al16 do not seem to support our findings of shortened survival in CD10+ DLBCL. However,
Despite the fact that subclassifying lymphomas based on gene expression profiling is quite complex, the germinal center and activated B-like groups in the study of Alizadeh et al.\textsuperscript{16} were not completely homogeneous. Half of the surviving patients in the activated B-like group with a less favorable prognosis were still alive after 5 years. The germinal center B-like group with an overall favorable prognosis included some cases with very poor survival. Most of the patients in this group who died did so within 2 years of their diagnosis. Given the variability of the reported data, it is possible, therefore, that CD10 expression not only reflects follicle center differentiation, but also a subset of more aggressive large cell lymphomas. In essence, these tumors may be more clinically similar to Burkitt-like lymphoma but with cytologic features of large cell lymphoma as described by Chai et al.\textsuperscript{24}

This retrospective study demonstrates a significantly shortened survival in CD10+ de novo DLBCL but no difference in clinical features at diagnosis. However, the inclusion of CD10 status in large prospective clinical trials will be necessary to study the possibility of small but significant differences in clinical manifestations and to more definitively assess the general clinical significance of CD10 expression in this common type of non-Hodgkin lymphoma.

From the Department of Pathology, University of Michigan Medical School.

Address reprint requests to Dr Finn: University of Michigan, Room M5242 Medical Science I, 1301 Catherine Rd, Ann Arbor, MI 48109-0602.

References


