CD5 expression is potentially predictive of poor outcome among biomarkers in patients with diffuse large B-cell lymphoma receiving rituximab plus CHOP therapy

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Background: Several biomarkers indicating poor prognosis have been reassessed in patients receiving rituximab combination chemotherapy for diffuse large B-cell lymphoma (DLBCL). However, few studies have investigated outcome in relation to a combination of these biomarkers. In addition, no large-scale studies have reassessed the outcome of patients with CD5-positive DLBCL treated with rituximab.

Patients and methods: We conducted a retrospective study and investigated the predictive value of three biomarkers—BCL2, germinal center (GC) phenotype and CD5—in 121 DLBCL patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone.

Results: CD5-positive patients showed significantly poorer event-free survival (EFS) and overall survival (OS) than CD5-negative patients (2-year EFS, 18% versus 73%, \( P < 0.001 \); 2-year OS, 45% versus 91%, \( P = 0.001 \)). However, no significant difference in outcome according to BCL2 or GC phenotype was observed. Multivariate analysis revealed that CD5 expression was a significant prognostic factor for EFS (hazard ratio 14.2, 95% confidence interval (CI) 4.7–43.2) and OS (hazard ratio 20.3, 95% CI 3.6–114.4).

Conclusions: CD5 expression was the only significant prognostic factor among the biomarkers examined in this study. Further studies with larger numbers are warranted to confirm the prognostic significance of CD5 expression for patients with DLBCL receiving rituximab-containing chemotherapy.

Key words: biomarker, CD5, diffuse large B-cell lymphoma, rituximab

introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin’s lymphoma (NHL) [1]. It shows an aggressive clinical course and comprises a heterogeneous group of lymphomas in terms of morphology, immunophenotype, molecular abnormality and clinical behavior. Although the cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) regimen has been the mainstay of treatment for aggressive lymphomas for several decades [2], a significantly improved outcome has been obtained in both young and elderly patients by combining the CHOP regimen with rituximab (an anti-CD20 chimeric antibody) [3–5].

In the era when CHOP was used alone, the International Prognostic Index (IPI) was the primary clinical tool employed for prediction of outcome in patients with aggressive NHL [6]. Although the IPI is considered to be the most important prognostic factor for DLBCL, the five risk factors used for assessing it do not provide any information about biologic features. To date, several biomarkers have been shown to predict the outcome and responsiveness of DLBCL to therapy. Overexpression of BCL2 family proteins has also been shown to indicate resistance to chemotherapy both \textit{in vitro} and \textit{in vivo} [7, 8]. BCL6 family proteins are reportedly associated with a better prognosis, and patients with BCL6-positive DLBCL have a relatively favorable outcome when treated with the CHOP regimen [9]. On the other hand, it has been reported that CD5-positive DLBCL has a very poor prognosis and high stage with more extranodal sites in comparison with CD5-negative DLBCL [10, 11]. Moreover, on the basis of the data obtained using complementary DNA (cDNA) microarray, DLBCL has been divided into two distinct subtypes that reflect the different stages of B-cell differentiation, i.e. germinal center.
B-cell-like (GCB) and activated B-cell like (ABC) [12]. The ABC subtype is associated with a poorer prognosis than the GCB subtype. It has been reported that the immunostaining patterns of CD10, BCL6 and MUM1 are an alternative means of identifying germinal center (GC) or non-GC DLBCL including the ABC subtypes and that non-GC DLBCL shows poor responsiveness to anthracycline-based regimens [13].

Recently, it has been recognized that addition of rituximab to anthracycline-based regimens may alter the previously identified prognostic factors, in view of the markedly improved outcome of patients with DLBCL. The study from British Colombia demonstrated that the IPI remained predictive, but reclassified patients into three prognostic groups after reassessing the five prognostic factors [14]. Moreover, several studies have investigated whether these biomarkers predict responsiveness to rituximab combination chemotherapy and outcome. The prognosis of BCL2- or BCL6-overexpressing DLBCL, and GC phenotype has been reassessed in patients receiving rituximab combination chemotherapy [15–17]. On the other hand, no large-scale studies of CD5 expression in the rituximab era have been reported.

Although several studies analyzing the prognostic significance of individual biomarkers have been carried out since the introduction of rituximab, none have investigated outcome by considering these biomarkers together. The aims of the present study were to reassess the predictive values of these biomarkers at a single institution and to investigate which factor among BCL2 expression, GC phenotype and CD5 expression has the greatest influence on the outcome of DLBCL patients.

patients and methods

patient characteristics

In the present study, we reviewed the medical records of patients with CD20-positive DLBCL who received CHOP with or without rituximab as a first-line therapy at the Cancer Institute Hospital from April 2004 to May 2007 and were followed until January 2008. The study protocol and sampling were approved by the Institutional Review Board of the Cancer Institute Hospital. Informed consent for retrospective analysis and sampling were approved by the Institutional Review Board of the Cancer Institute Hospital. Informed consent for retrospective analysis and additional immunophenotypic analysis and gene rearrangement studies was obtained.

Patients were analyzed if they were older than 18 years and had a performance status (PS) of zero to three according to the criteria of the European Cooperative Oncology Group. Patients were excluded if they had clinically relevant cardiac diseases or positivity for antibodies against human immunodeficiency virus-1 or -2. Patients with primary mediastinal large B-cell lymphoma, primary central nervous system lymphoma and primary testicular lymphoma were also not included in this study.

The disease stage was evaluated according to the Ann Arbor staging system. All patients had undergone staging investigations, including physical examinations, blood and serum analysis, bone marrow aspiration and biopsy and computed tomography of the neck, chest, abdomen and pelvis. Magnetic resonance imaging was used for evaluation of involved organs in the head and neck. The following clinical and laboratory data were available at the time of diagnosis: age, sex, serum lactate dehydrogenase level, PS, presence of B symptoms, clinical stage and number of extranodal sites. This information allowed IPI scores to be determined in the included patients. Patients were categorized into either a low-risk group (IPI score, 0–2) or a high-risk group (IPI score, 3–5).

Treatment

All patients received rituximab plus CHOP (RCHOP) chemotherapy. For patients with stage IB–IV, rituximab was administered at the standard dose of 375 mg/m² once weekly for 8 weeks and CHOP chemotherapy was given concurrently triweekly, as described previously [18]. CHOP chemotherapy was given for a total of six cycles. For patients with stage IA, CHOP chemotherapy was repeated for three cycles and rituximab was continued in the same way as for patients with stages IB–IV, with subsequent radiotherapy.

pathological studies

Biopsy samples collected at the time of diagnosis were fixed in formalin, embedded in paraffin, sliced and stained with hematoxylin and eosin for morphological analysis. Immunohistochemical analysis was carried out using the dextran-polymer method (Envision; Dako, Glostup, Denmark) using mAbs against CD10 (56C6, Novocastra, Newcastle-upon-Tyne, UK), BCL6 (PG-Bep, Dako), MUM1 (MUM1p, Dako), BCL2 (124, Dako), CD5 (4C7, Novocastra) and cyclin D1 (P2D11F11, Novocastra) at our institution. For all the antibodies, heat-induced antigen retrieval pretreatment using Target Retrieval Solution, pH 9 (Dako) was carried out. BCL6, MUM1 and BCL2 were designated as positive when the proportion of stained lymphoma cells was 30% or higher. CD5 and CD10 were considered to be immunohistochemically positive when at least a small population of the neoplastic cells was positive. To classify the samples into immunohistochemically defined GC or non-GC phenotypes, we used an algorithm previously described by Hans et al. [13].

For examination of CD5 expression, we reviewed the results of flow cytometry analysis. Cases were defined as CD5 positive if CD5 expression was detected by flow cytometry, irrespective of the result of CD5 immunohistochemistry. Excluded were those positive for cyclin D1 or those with a history of chronic lymphocytic leukemia/small lymphocytic lymphoma. Patients with a small-cell component implying transformation from low-grade/indolent B-cell lymphoma were also excluded. All the histopathology samples were reviewed by an expert hematopathologist (KT), and flow cytometric analyses were reviewed by two of the authors independently (DE and KT).

statistical analysis

The main outcomes of this study were event-free survival (EFS) and overall survival (OS). EFS was calculated from the date of diagnosis to the date of documented disease progression, relapse or death from any cause or to the date on which the study was stopped. OS was calculated from the date of diagnosis until death from any cause or the last follow-up. If the stopping date was not reached, the data were censored at the date of the last follow-up evaluation. Survival curves were estimated by the Kaplan–Meier method, and overall differences were compared by the log-rank test. Cox multivariate analysis was carried out to estimate the prognostic impacts of the biomarkers and IPI risk factors on EFS and OS. Comparisons of basic characteristics between the CD5-positive and -negative groups were tested by Fisher’s exact test and Student’s t-test. Data were analyzed using SPSS software version 11.0 for Windows (SPSS, Chicago, IL).

results

patient characteristics

During the study period, 180 patients were included, and data for all three biomarkers and flow cytometric analysis were available for 121 patients. The characteristics of these patients are listed in Table 1. CD5 was expressed in 11 of 121 patients with DLBCL (9%). None of the CD5-positive patients
had a history of other lymphoproliferative disorders, and all were found to have de novo CD5-positive DLBCL. Of these 11 patients, seven were positive by both flow cytometry and immunohistochemistry and four were positive only by flow cytometry. In all the seven cases defined as CD5 positive by both methods, the lymphoma cells expressed less CD5 than normal T cells in the background. Expression of BCL2 was detected in 79 of 121 cases (65%). CD10 was expressed in 45 cases (37%), BCL6 in 89 (66%) and MUM1 in 55 (45%). Overall, 48 of 121 cases (40%) were categorized into the non-GC group. No significant difference in basic characteristics was found between the 121 and 59 patients for whom all biomarkers were and were not available, respectively. No significant difference in survival was detected in relation to BCL2 and immunohistochemically defined GC phenotype.

### discussion

This analysis of biomarkers in 121 DLBCL patients receiving RCHOP highlighted the potentially poor outcome of patients with CD5-positive DLBCL. Multivariate analysis including the IPI revealed that CD5 expression and IPI were independent factors associated with poor prognosis. On the other hand, significant differences in survival were not detected in relation to BCL2 and immunohistochemically defined GC phenotype.

**De novo** CD5-positive DLBCL, a distinct subgroup that accounts for 5%–10% of all DLBCL, has been reported to be associated with elderly onset, female predominance, frequent involvement of extranodal sites and inferior survival [10, 11]. The largest study of CD5-positive DLBCL demonstrated a 5-year survival rate of 34% for CD5-positive DLBCL treated with an anthracycline-based regimen [11]. The Nordic Lymphoma Study Group also demonstrated that CD5 expression was associated with significantly inferior OS and failure-free survival [19]. In contrast, other authors showed that...
CD5-positive DLBCL did not show distinctive clinical features or inferior survival [20]. In the present study, patients who received immunochemotherapy showed significantly poor OS and EFS, whereas the factors comprising the IPI were similar between the patients who were positive for CD5 and those who were negative. We consider that this poor prognosis of CD5-positive DLBCL in the rituximab era is noteworthy and that a large-scale study is warranted.

CD5 is a 67-kDa transmembrane glycoprotein that is expressed by most normal T cells and less brightly by a subset of B cells known as B1 cells [21]. Reflecting this difference in expression level, neoplastic CD5-positive B cells also usually express less CD5. Therefore, even if successfully stained by immunohistochemistry, these cells are usually stained less strongly than normal background T cells with anti-CD5 antibody. In the authors’ experience, until the introduction of antigen retrieval techniques and effective antibodies like mAb 4C7, it was very difficult to detect CD5-positive B cells immunohistochemically on formalin-fixed paraffin-embedded sections [22]. However, even since the introduction of these techniques, CD5 immunohistochemistry using paraffin sections still remains less sensitive than flow cytometry and frozen section immunohistochemistry [23]. In fact, in the present study, only seven cases of DLBCL were positive for CD5 by immunohistochemistry out of 11 cases that were CD5 positive by flow cytometry. In an attempt to overcome this lower sensitivity of CD5 immunohistochemistry for CD5-positive DLBCL, de Jong et al. examined the usefulness of recently developed immunohistochemical enhancement techniques (Powervision; Immunovision Technologies, Duiven, The Netherlands and ChemMate; Dako). However, although they acquired higher sensitivity, there was also a loss of

Figure 1. Event-free survival (EFS) and overall survival (OS) curves for diffuse large B-cell lymphoma patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone according to CD5 expression and clinical factors. EFS (A) and OS (B) curves according to positive (n = 11) versus negative (n = 110) CD5 expression. EFS (C) and OS (D) curves according to the IPI (0–2, n = 89 versus 3–5, n = 32).

Table 2. Patient characteristics in relation to CD5 expression

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CD5 positive (n = 11), n (%)</th>
<th>CD5 negative (n = 110), n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: male</td>
<td>6 (55)</td>
<td>59 (53)</td>
<td>1.0</td>
</tr>
<tr>
<td>Age: median, range</td>
<td>68, 33–76</td>
<td>66, 23–88</td>
<td>0.27</td>
</tr>
<tr>
<td>IPI score 3–5</td>
<td>4 (36)</td>
<td>28 (25)</td>
<td>0.47</td>
</tr>
<tr>
<td>Stages III–IV</td>
<td>4 (36)</td>
<td>32 (30)</td>
<td>0.74</td>
</tr>
<tr>
<td>Elevated LDH level</td>
<td>7 (64)</td>
<td>49 (44)</td>
<td>0.54</td>
</tr>
<tr>
<td>More than one extranodal site</td>
<td>5 (45)</td>
<td>22 (20)</td>
<td>0.38</td>
</tr>
<tr>
<td>PS &gt;1</td>
<td>5 (45)</td>
<td>12 (11)</td>
<td>0.013</td>
</tr>
<tr>
<td>BCL2 positive</td>
<td>10 (91)</td>
<td>69 (62)</td>
<td>0.095</td>
</tr>
<tr>
<td>Non-GC type</td>
<td>6 (55)</td>
<td>42 (38)</td>
<td>0.54</td>
</tr>
</tbody>
</table>

IPI, International Prognostic Index; LDH, lactate dehydrogenase; PS, performance status; GC, germinal center.
with DLBCL was demonstrated [10, 11]. The present study between CD5 expression and BCL2 overexpression in patients previous studies of CD5-positive DLBCL, no association CD5 expression and BCL2 overexpression. Moreover, in these studies did not reveal any data on the association between significance of BCL2 overexpression in DLBCL [15]. However, rituximab to chemotherapy eliminated the prognostic conducted in the rituximab era demonstrated that addition of the prerituximab era [7, 8]. In contrast, several studies handling of these risk factors in the rituximab era. IPI risk factors. A consensus will be required for accurate values were retained, although we did not evaluate each of the division into two risk groups [14]. In the present study, IPI differences were also demonstrated in the same cohort upon treated with RCHOP and divided them into three distinct groups—low or low intermediate and high or high intermediate—and this remained a predictive tool in DLBCL [27]. In some studies, IPI category was divided into two risk groups—low or low intermediate and high or high intermediate. Other authors have reassessed the IPI risk factors of DLBCL patients receiving immunohistochemistry is less sensitive for these B cells. For these reasons, we consider that flow cytometric analysis or frozen section immunohistochemistry needs to be carried out for detection of CD5 in DLBCL. Differences in the method of CD5 detection might lead to differences among studies in the apparent impact of CD5 on prognosis.

In addition to these differences in clinical aspects, there are several lines of evidence for genetic differences between CD5-positive and -negative DLBCL. Microarray studies have suggested that integrin beta-1 in tumor cells and CD36 in vascular endothelium are expressed more frequently in CD5-positive than in CD5-negative DLBCL [25]. Comparative genomic hybridization studies have revealed that CD5-positive DLBCL has a different pattern of chromosomal gain and loss compared with CD5-negative DLBCL [20, 26]. Loss of 9q21 (p16 INK4a), which is strongly associated with lymphoma progression, has been observed more frequently in CD5-positive DLBCL [27].

Previous studies also showed that IPI values remained in patients with DLBCL receiving immunochemotherapy [14–17]. In some studies, IPI category was divided into two risk groups—low or low intermediate and high or high intermediate—and this remained a predictive tool in DLBCL patients receiving immunohistochemistry [15–17]. Other authors have reassessed the IPI risk factors of DLBCL patients treated with RCHOP and divided them into three distinct prognostic groups referred to as R-IPI groups. Significant differences were also demonstrated in the same cohort upon division into two risk groups [14]. In the present study, IPI values were used to delineate two risk groups, and prognostic values were retained, although we did not evaluate each of the IPI risk factors. A consensus will be required for accurate handling of these risk factors in the rituximab era.

BCL2 overexpression was associated with poorer survival in the prerituximab era [7, 8]. In contrast, several studies conducted in the rituximab era demonstrated that addition of rituximab to chemotherapy eliminated the prognostic significance of BCL2 overexpression in DLBCL [15]. However, these studies did not reveal any data on the association between CD5 expression and BCL2 overexpression. Moreover, in previous studies of CD5-positive DLBCL, no association between CD5 expression and BCL2 overexpression in patients with DLBCL was demonstrated [10, 11]. The present study demonstrated that 10 of 11 CD5-positive patients had BCL2 overexpression. The OS and EFS of BCL2-positive, CD5-negative patients were significantly superior to those of patients positive for both BCL2 and CD5 (data not shown), suggesting that the poorer survival trend of patients with BCL2 overexpression in the present series may have been influenced by CD5 expression. A large-scale analysis of BCL2 expression in CD5-positive DLBCL will be needed to clarify the association between expressions of BCL2 and CD5.

There have been several studies of the relationship between CD5 expression and GC/ABC phenotype [27–29]. An analysis of genomic imbalance showed that most cases of CD5-positive DLBCL were included in the ABC type [27], and another study of somatic mutations of the immunoglobulin heavy chain variable region suggested that the cells from which CD5-positive DLBCL arise are predominantly of post-GC origin [28, 29]. These conclusions were based on molecular-based analyses and not by immunohistochemistry. Our study found no association between CD5 expression and GC phenotype. This may be because GC phenotype in the present study was defined by an immunophenotypic algorithm, which reproduced ~80% of the GC phenotype defined by cDNA microarray [13]. A new algorithm using five types of immunostaining—GCET1, MUM1, CD10, BCL6 and FOXP1—has been introduced recently and provided an improved GC/ABC subclassification [30]. Application of this approach to our series might lead to a consistent result.

In conclusion, we have investigated the outcome of DLBCL patients receiving rituximab combination chemotherapy by considering several biomarkers together and demonstrated that CD5 expression is a potentially useful indicator of poor prognosis. To accurately confirm whether CD5 expression influences the outcome of patients receiving RCHOP, further large-scale and prospective studies of CD5-positive patients will be required.

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**References**


