Clinical Research Paper

Short-term efficacy of rituximab-CHOP and CHOP regimens on two subtypes of diffuse large B-cell lymphoma

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Background and Objective: Diffuse large B-cell lymphoma (DLBCL) can be divided into two subgroups as germinal center B-cell like (GCB) and non-GCB DLBCL, according to the origin of tumor cells. The prognosis of GCB DLBCL is better than that of non-GCB DLBCL after receiving the CHOP regimen on initial therapy. This study was to compare the short-term efficacy of rituximab(R)-CHOP and CHOP regimens on GCB and non-GCB DLBCL, thus to explore the optimal first-line regimen for the initial treatment of DLBCL patients. Methods: In total, eighty-three patients with de novo DLBCL initially treated at Sun Yat-sen University Cancer Center from November 2006 to February 2008 were enrolled. Patients were divided into GCB and non-GCB groups. The short-term efficacy of the CHOP or R-CHOP regimen as the first-line therapy on the two groups was evaluated according to the revised response criteria for lymphoma. Bcl-2 expression and its correlation to the short-term efficacy of the two groups were assessed. Results: There were 35 cases (42.2%) in the GCB group and 48 cases (57.8%) in the non-GCB group. The total remission rate was 74.3% in the GCB group and 60.4% in the non-GCB group (p = 0.006). There was no significant difference in Bcl-2 expression in the two groups. The remission rate was higher in Bcl-2 positive patients receiving R-CHOP regimen than those receiving CHOP regimen (75.6% vs. 47.8%, p = 0.023). There was no significant difference in the remission rate of Bcl-2 negative patients, regardless of chemotherapy regimen. Conclusion: The prognosis is better in the GCB group than in the non-GCB group. Addition of rituximab to CHOP could improve the short-term efficacy of Bcl-2 positive patients.

In recent years, using gene chips and immunohistochemistry (IHC), diffuse large B-cell lymphoma (DLBCL) is classified into two subtypes according to the origin of tumor cells as germinal center B-cell like (GCB) and non-GCB DLBCL. Although prognosis of GCB group is significantly better than that of non-GCB group,1-4 these studies are all based on CHOP chemotherapy. Currently, several large scale clinical trials have shown that combination of CD20 monoclonal antibody rituximab (R) with CHOP chemotherapy (R-CHOP) could improve the overall efficacy by 15–20% in treating DLBCL.5,6 However, it is still not clear whether the R-CHOP regimen is effective for both GCB and non-GCB patients. In this study, we classified DLBCL patients into the two aforementioned subgroups by IHC, and compared the short-term remission rate of R-CHOP or CHOP as the first-line chemotherapy on GCB and non-GCB DLBCL, thus to explore an optimal regimen to improve the therapeutic efficacy for DLBCL.

Data and Methods

Clinical data. From November 2006 to February 2008, in total 83 patients with primary DLBCL were initially diagnosed and pathologically conformed in the Department of Medical Oncology of Sun Yat-sen University Cancer Center. Diagnostic biopsies were obtained from all patients before the treatment. Specimens were fixed in 10% neutral formalin, routinely dehydrated, embedded in paraffin, sectioned and stained by hematoxylin-eosin. Diagnosis was confirmed based on the updated classification criteria proposed by World Health Organization (WHO) by two pathologists specialized in lymphoma. Measurable lesions on images were detected in all 83 patients prior to treatment. Clinical characteristics of patients are shown in Table 1. Routine examinations were conducted in all patients before and during the treatment, including blood count, biochemical tests, bone marrow examination, imaging emanation (enhanced CT of neck, chest, abdomen and pelvis or whole body PET/CT) and so on. All patients received the R-CHOP or the CHOP regimen, repeated every three weeks. The CHOP regimen was administered as follows: intravenous injection of cyclophosphamide 750 mg/m² on day 1, intravenous injection of Adriamycin 50 mg/m² on day 1, intravenous injection of vincristine 1.4 mg/m² (up to 2 mg) on day 1, oral administration of prednisone 60 mg/m² on days 1–5. The R-CHOP regimen was administered as follows: intra-
venous infusion of rituximab 375 mg/m² on day 1, and the dose and usage of the CHOP regimen were given as above mentioned on days 2–6. Only four patients received less than four courses of chemotherapy. Two were due to disease progression and the other two cases were due to severe toxicity of chemotherapy.

Efficacy evaluation criteria. According to Revised Response Criteria for Malignant Lymphoma, treatment efficacy is categorized into complete remission (CR), complete remission uncertain (CRu), partial remission (PR), stable disease (SD) and progressive disease (PD). Short-term remission includes CR and CRu.

IHC and classification criteria. IHC was performed using the EnVision two-step method. The EnVision kit was purchased from Dako Corporation (Japan). Antibodies used included CD10 (Novocastra Corp., UK), Bcl-6 (Zhongshan Golden Bridge Corp., P.R. China), Mum1 (Dako Corp., Japan) and Bcl-2 (Zhongshan Golden Bridge Corp., P.R. China). Positive staining of CD10 was localized on the cell membrane, that of Bcl-6 and Mum1 was in the nucleus, and that of Bcl-2 was in cytoplasm. All positive staining were light yellow to brown granules. Five fields of each slide were selected under high magnification, and the average percentage of positive tumor cells was calculated. The ratio of $\geq 30\%$ positive cells was regarded positive (+), and that of $< 30\%$ was regarded negative (-).

The subtype of DLBCL was classified according to the staining results of CD10, Bcl-6 and Mum1, as well as the classification criteria of Hans. CD10 and Bcl-6 were used as markers for GCB DLBCL and Mum1 was used as the marker for non-GCB DLBCL. Criteria for GCB subtype was as follows: CD10+ or CD10- Bcl-6+ Mum1-. Criteria for non-GCB subtype was as follows: CD10- Bcl-6+/-, Mum1+ or CD10- Bcl-6- Mum1-.

Statistical analysis. Normal distributions of the baseline values in two groups were compared using the chi-square test. Efficacy was analyzed using the chi-square test, the Fisher exact probability method and the Row Mean Scores Differ Cochran-Mantel-Haenszel (CMH) statistic. All tests were bilateral and $p < 0.05$ was considered statistically different. SPSS13.0 and SAS8.01 were used for data analyses.

Results

Classification of DLBCL. Expression of CD 10 was detected in 21.7% patients (18/83), Bcl-6 in 42.2% (35/83) and Mum1 in 56.6% (47/83) patients. According to the IHC results, 35 cases (42.2%) were classified into GCB group while 48 cases (57.8%) into non-GCB group. Clinical features of patients in different subgroups are listed in Table 1.

Short-term efficacy of different subtypes. Twenty-six patients (74.3%) achieved CR/CRu, eight (22.9%) achieved PR and one (2.8%) had PD in GCB group while 29 patients (60.4%) achieved CR/CRu, 14 (29.2%) achieved PR and five (10.4%) had PD in non-GCB group. Among 28 patients who received the CHOP regimen as the initial treatment, the CR/CRu rate was 66.7% in GCB group and 30.8% in non-GCB group. Among the 55 patients received R-CHOP regimen as the initial treatment, the CR/CRu rate was 80.0% in GCB group and 71.4% in non-GCB group (Table 2).

Expression of Bcl-2 in two subtypes of DLBCL. Among 68 patients with positive expression of Bcl-2, 26 cases were classified into GCB group and 42 cases into non-GCB group. The Bcl-2 positive rate in non-GCB group and GCB group were 87.5% and 75.6% respectively, without significant difference ($p = 0.153$).

Correlation of Bcl-2 expression to treatment efficacy. The CR/CRu rates and overall response rates (ORR) were 75.6% vs. 47.8%, 95.6% vs. 83.3% in 45 positive Bcl-2 patients receiving the R-CHOP regimen and 23 positive Bcl-2 patients receiving the CHOP regimen. The short-term remission rate was significantly higher in patients with positive expression of Bcl-2 treated by the R-CHOP regimen than those treated by the CHOP regimen ($p = 0.031$). In contrast, the ORR in Bcl-2 negative patients was 100% and no cases of PD occurred, regardless of chemotherapy regimen (Table 3).

Discussion

DLBCL is the most common type of non-Hodgkin's lymphoma, and is a group of diseases with high heterogeneity. Currently, the International Prognostic Index (IPI) is commonly used as an index that predicts the prognosis of DLBCL. However, a considerable number of patients with the same IPI score respond differently to the same chemotherapy regimen and achieve different prognosis,
suggesting that IPI can not adequately identify high-risk patients. Further studies find biological heterogeneity is the main reason for the unreliability of IPI in predicting short-term efficacy and prognosis. Gene expression profiling established in 2000 can reveal the expression status of disease-relevant genes at the genetic level. When combining gene expression profiling with clinical predictors, the overall biological characteristics of DLBCL and similar diseases could be comprehensively reflected.1-4

Gene chip technology is used to classify highly heterogeneous DLBCL into three subtypes, including GCB, activated peripheral B cell-like (ABC) and Type3. The prognosis of ABC and Type3 are significantly worse than that of GCB. Therefore, these two subtypes with poor prognosis are designated as non-GCB group. Gene chip involves intricate technology, requires fresh tissues for the test and is expensive, which limits its clinical application. In contrast, IHC is a well-established, simple to perform and widely used clinical examination method. DLBCL can be categorized into GCB and non-GCB groups using IHC based on the expression of characteristic proteins at different differentiation stages of DLBCL cells. CD10 and Bcl-6 are markers for normal lymph node germinal center B cells. Positive expressions of these two markers suggest a good prognosis of DLBCL. Mum1 is a marker for post-germinal center B cells, whose positive expression implies poor prognosis. Most studies have confirmed the significance of immunohistologic phenotyping in predicting prognosis independent of IPI. Chang et al.10 classify DLBCL into GCB subtype, late GC or early GC post-B cell subtype and post-GC cell subtype. The prognosis of GCB subtype is much better than that of the latter two subtypes. Colomo et al.11 also confirm the clinical significance of immunohistochromaticonotyping According to Hans et al.,8 results obtained from immunohistochonomicontyping are highly consistent with those acquired from gene chips. We identified 35 cases of GCB and 48 cases of non-GCB in 83 cases of newly diagnosed DLBCL patients. The proportion of the two subtypes was consistent with other studies.8,11,12 This study is a perspective one, and the median follow-up time of involved patients was shorter than two years. Therefore we only analyzed the short-term efficacy, but not the survival of these patients. Whether DLBCL patients can achieve CR after the standard first-line chemotherapy is closely related to their progression-free survival (PFS) and prognosis.

CHOP regimen has been proven to be the standard treatment for DLBCL over the years, although it can only achieve a short-term remission rate of 50% and a long-term survival rate of less than 40%. Many large-scale clinical studies have confirmed that using rituximab plus the CHOP regimen can increase the short-term remission rate, improve prognosis and prolong survival time for DLBCL patients.5-6 Our study showed that patients in GCB group achieved a higher CR/CRu rate than those in non-GCB group, regardless of the regimen (p = 0.006), even though this effect was more apparent when the CHOP regimen was used. The short-term remission rate of non-GCB patients was significantly higher in those treated by the R-CHOP regimen (71.4%) than those treated by the CHOP regimen (30.8%).

<table>
<thead>
<tr>
<th>Group</th>
<th>Regimen</th>
<th>CR/CRu [cases (%)]</th>
<th>PR [cases (%)]</th>
<th>PD [cases (%)]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCB group</td>
<td>CHOP</td>
<td>10 (66.7)</td>
<td>4 (26.7)</td>
<td>1 (6.6)</td>
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<tr>
<td></td>
<td>R-CHOP</td>
<td>16 (80.0)</td>
<td>4 (20.0)</td>
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<td></td>
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<tr>
<td></td>
<td>CHOP plus R-CHOP</td>
<td>26 (74.3)</td>
<td>8 (22.9)</td>
<td>1 (2.8)</td>
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<tr>
<td>Non-GCB group</td>
<td>CHOP</td>
<td>4 (30.8)</td>
<td>6 (46.2)</td>
<td>3 (23.0)</td>
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<tr>
<td></td>
<td>R-CHOP</td>
<td>25 (71.4)</td>
<td>8 (22.9)</td>
<td>2 (5.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHOP plus R-CHOP</td>
<td>29 (60.4)</td>
<td>14 (21.2)</td>
<td>5 (10.4)</td>
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<tr>
<td>Total</td>
<td></td>
<td>55 (66.3)</td>
<td>22 (26.5)</td>
<td>6 (7.8)</td>
<td>0.006*</td>
</tr>
</tbody>
</table>

*p was calculated using Row Mean Scores Differ CMH statistic by comparing GCB group with non-GCB group treated by CHOP or R-CHOP regimen. CR, complete remission; CRu, complete remission uncertain; PR, partial remission; PD, progressive disease; CHOP, cyclophosphamide+vincristine+doxorubicin+prednisone; R-CHOP, rituximab+CHOP; GCB, germinal center B-cell like.

<table>
<thead>
<tr>
<th>Group</th>
<th>Regimen</th>
<th>CR/CRu [cases (%)]</th>
<th>PR [cases (%)]</th>
<th>PD [cases (%)]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bel-2(+)</td>
<td>CHOP</td>
<td>11 (47.8)</td>
<td>8 (34.8)</td>
<td>4 (17.4)</td>
<td>0.031*</td>
</tr>
<tr>
<td></td>
<td>R-CHOP</td>
<td>34 (75.6)</td>
<td>9 (20.0)</td>
<td>2 (4.4)</td>
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<tr>
<td>Bel-2(-)</td>
<td>CHOP</td>
<td>3 (60.0)</td>
<td>2 (40.0)</td>
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<td></td>
<td>R-CHOP</td>
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<td>3 (30.0)</td>
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<tr>
<td>Total</td>
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<td>22 (26.5)</td>
<td>6 (7.2)</td>
<td>0.016*</td>
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</table>

*p was calculated by comparing Bel-2(+) patients receiving CHOP or R-CHOP regimen using the Fisher’s exact test; P was calculated using Row Mean Scores Differ CMH statistic by comparing Bel-2(+) group with Bel-2(-) group treated by CHOP or R-CHOP regimen. CHOP, cyclophosphamide+vincristine+doxorubicin+prednisone; R-CHOP, rituximab+CHOP; GCB, germinal center B-cell like.
result suggests that rituximab can improve the short-term remission rate of non-GCB subtype. However, whether R-CHOP can improve the progression-free survival (PFS) and prognosis of non-GCB patients requires further long-term follow-up studies.

Positive expression of Bcl-2 has been found to indicate a poor prognosis in DLBCL patients,13-15 which may attribute to resistance of cancer cells towards CHOP chemotherapy.16 Addition of rituximab in CHOP regimen can partially reverse chemotherapy resistance of Bcl-2 positive patients.16 In this study, the Bcl-2 positive rate was not significantly different in the GCB and non-GCB groups. The molecular mechanisms of Bcl-2 protein expression are found different between these two groups, but no significant difference in the positive rate has been reported.17-18 We found 81.9% patients had Bcl-2 expression. The CR/CRu rate was significantly higher in Bcl-2 positive patients receiving R-CHOP regimen (75.6%) than those treated with CHOP regimen (47.8%), with a nearly 30% increase. This suggests that rituximab can increase the chemosensitivity of Bcl-2 positive patients and increase the CR/CRu rate, which is in line with other reports.16 However, the Bcl-2 positive rate in this study was higher than that reported in other studies, which may be due to the subject selection and a small sample size.

In summary, using IHC to classify DLBCL can effectively predict the short-term efficacy. The short-term remission rate is higher in GCB subtype than in non-GCB subtype. In addition, rituximab can improve the short-term remission rate of Bcl-2 positive patients, and may improve that of non-GCB patients, suggesting that R-CHOP may be the optimal first-line chemotherapy regimen for DLBCL patients.

References:

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