Original Article

The phenotypes of germinal center B-cell-like (GCB) and non-GCB failed to predict the survival of patients with diffuse large B-cell lymphoma in the rituximab era

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Received December 15, 2016; Accepted July 6, 2017; Epub September 15, 2017; Published September 30, 2017

Abstract: The prognostic significance of the phenotypes of germinal center B-cell-like (GCB) and non-GCB in diffuse large B-cell lymphoma (DLBCL) remains uncertain. In this study, we investigated the prognostic role of the phenotypes of GCB and non-GCB and explored whether it could be improved when combined with international prognostic index (IPI) and BCL2 in patients with diffuse large B-cell lymphoma (DLBCL) treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP). One hundred and six cases with de novo DLBCL were examined, and 50 patients had the GCB and 56 had the non-GCB phenotypes. The 2-year progression free survival (PFS) and overall survival (OS) were 67% and 85% in the GCB group, 55% and 82% in the non-GCB group. No significant differences of progression free survival (PFS) and overall survival (OS) were observed between these two phenotypes (P>0.05). When based on the same international prognostic index (IPI) risk group, the PFS and OS between the phenotypes of GCB and non-GCB still had no significant differences (P>0.05). Besides, patients with GCB and non-GCB showed no significant differences in PFS and OS in the BCL2 positive group and so did in the BCL2 negative group. The phenotypes of GCB and non-GCB failed to predict the survival of patients with DLBCL in the rituximab era.

Keywords: International prognostic index, diffuse large B-cell lymphoma, germinal-center phenotype, BCL2, prognosis

Introduction

Diffuse large B-cell lymphoma (DLBCL), accounting for approximately 30% to 40% of all non-Hodgkin lymphoma (NHL) cases [1], is a clinically and biologically heterogeneous [2] neoplasm. Two molecular phenotypes of DLBCL, germinal center B-cell-like (GCB) and non-GCB, which were based on the expression of CD10, bcl-6 and MUM-1 [3], had been proved to be a powerful method to predict the survival of patients with DLBCL. Patients with GCB subtype had a favorable outcome than the non-GCB [2-4]. However, this result was under controversy in various reports. Some studies [5, 6] found that the prognostic value of GCB and non-GCB was eliminated by rituximab-containing chemotherapy. Besides, other studies [7, 8] showed that the phenotypes of GCB and non-GCB had little clinical value and could not improve the prognosis of patients with DLBCL. But we found that these studies did not include the clinical characteristics of international prognostic index (IPI) in evaluating the prognostic role of GCB and non-GCB. Patients with a high IPI score had a worse outcome than the low [9]. The phenotype of GCB with a high IPI score may not have a favorable outcome than the non-GCB with a low IPI score in patients with DLBCL. Therefore, it would be better if we determined the prognostic value of GCB and non-GCB that based on the same IPI risk group.

BCL2, a mitochondrial inner-membrane protein, plays a significant role in response assessment and outcome evaluation for patients with DLBCL. Various reports demonstrated that patients with BCL2 positive had a worse outcome than the negative [10, 11]. Iqbal et al. [12] demonstrated that BCL2 was a prognostic marker.
Lost prognostic role of GCB and non-GCB in DLBCL patients

Table 1. Patients’ characteristics and the difference of survival between the phenotypes of GCB and non-GCB in related clinical features

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n, %)</th>
<th>GCB (n, %)</th>
<th>Non-GCB (n, %)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>106 (100%)</td>
<td>50 (47%)</td>
<td>56 (53%)</td>
<td>0.317</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.158</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>65 (61%)</td>
<td>35 (70%)</td>
<td>30 (54%)</td>
<td>0.357</td>
</tr>
<tr>
<td>Female</td>
<td>41 (39%)</td>
<td>15 (30%)</td>
<td>26 (46%)</td>
<td>0.124</td>
</tr>
<tr>
<td>Ann arbor stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>29 (27%)</td>
<td>16 (32%)</td>
<td>13 (23%)</td>
<td>0.253</td>
</tr>
<tr>
<td>III-IV</td>
<td>77 (73%)</td>
<td>34 (68%)</td>
<td>43 (77%)</td>
<td>0.496</td>
</tr>
<tr>
<td>Age (Year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>80 (75%)</td>
<td>44 (88%)</td>
<td>36 (64%)</td>
<td>0.159</td>
</tr>
<tr>
<td>≥60</td>
<td>26 (25%)</td>
<td>6 (12%)</td>
<td>20 (36%)</td>
<td>0.697</td>
</tr>
<tr>
<td>ECOG score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>74 (70%)</td>
<td>39 (78%)</td>
<td>35 (63%)</td>
<td>0.114</td>
</tr>
<tr>
<td>≥2</td>
<td>32 (30%)</td>
<td>11 (22%)</td>
<td>21 (37%)</td>
<td>0.574</td>
</tr>
<tr>
<td>LDH level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;ULN</td>
<td>62 (58%)</td>
<td>30 (60%)</td>
<td>32 (57%)</td>
<td>0.060</td>
</tr>
<tr>
<td>≥ULN</td>
<td>44 (42%)</td>
<td>20 (40%)</td>
<td>24 (43%)</td>
<td>0.956</td>
</tr>
<tr>
<td>Extranodal sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>54 (51%)</td>
<td>30 (60%)</td>
<td>24 (43%)</td>
<td>0.409</td>
</tr>
<tr>
<td>≥2</td>
<td>52 (49%)</td>
<td>20 (40%)</td>
<td>32 (57%)</td>
<td>0.660</td>
</tr>
<tr>
<td>IPI score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (0-2)</td>
<td>61 (58%)</td>
<td>32 (64%)</td>
<td>29 (52%)</td>
<td>0.192</td>
</tr>
<tr>
<td>High (3-5)</td>
<td>45 (42%)</td>
<td>18 (36%)</td>
<td>27 (48%)</td>
<td>0.829</td>
</tr>
<tr>
<td>BCL2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>35 (33%)</td>
<td>17 (34%)</td>
<td>18 (32%)</td>
<td>0.711</td>
</tr>
<tr>
<td>Positive</td>
<td>71 (67%)</td>
<td>33 (66%)</td>
<td>38 (68%)</td>
<td>0.265</td>
</tr>
</tbody>
</table>

Materials and methods

Patients

We retrospectively analyzed 106 patients with newly diagnosed DLBCL. All patients were treated with rituximab combined with cyclophosphamide, vincristine, doxorubicin, and prednisone (R-CHOP) chemotherapy between Jan 2006 to Dec 2013. Patients were excluded if the clinical information and histological features were not available. The patients’ characteristics of sex, age, Eastern cooperative oncology group (ECOG) performance status, Ann arbor stage, number of extranodal sites involvements, LDH level and IPI score were summarized in Table 1. This study was approved by the Ethics Committee of the institution of the southern medical university, Guangzhou, China. Informed consent was waived because the nature of this retrospective study.

Immunohistochemistry

All biopsy specimens were analyzed independently by 2 pathologists (Yang XJ and Liu ZX). Fresh 4 micrometer sections were obtained from the formalin-fixed, paraffin embedded tissue. The presences of DLBCL were confirmed by the sections that stained with hematoxylin and eosin. Stainings for BCL2 (clone 124, Dako, Denmark), BCL-6 (clone P1F6, Novocastra, UK), CD10 (clone 56C6, Novocastra, UK), and MUM-1 (clone MUM1p, Dako, Denmark) were performed using antibody dilutions 1:10, 1:20, 1:20, and 1:100, respectively. According to previous study, if 30% or more of the tumor cells were stained with an antibody (BCL2, CD10, BCL-6 and MUM1), the samples were scored positive. Immunohistochemical features for CD10, BCL-6, and MUM1 were used to identify the phenotypes of GCB and non-GCB according to Hans’ criteria [3].

for patients with activated B-cell-like (ABC) DLBCL treated with CHOP-like regimens. But later, they [13] determined that the prognostic role of BCL2 had changed in DLBCL patients owing to the addition of rituximab to CHOP. The GCB DLBCL patients with BCL2 positive received less benefit from R-CHOP-like regimens. These results indicated that BCL2 was a prognostic parameter in patients with GCB or non-GCB in related clinical features. However, they didn’t show whether the phenotypes of GCB and non-GCB were valid prognostic markers or not when combined with BCL2 in DLBCL patients.

In this setting, we determined the prognostic value of the phenotypes of GCB and non-GCB and explored whether it could be improved when combined with IPI and BCL2 in patients with DLBCL treated with R-CHOP.
Lost prognostic role of GCB and non-GCB in DLBCL patients

Figure 1. Progression-free survival (A) and Overall survival (B) curves according to the phenotypes of germinal center B-cell-like (GCB) and non-GCB in DLBCL patients.

Figure 2. Outcomes according to the phenotypes of GCB and non-GCB DLBCL which based on the same IPI risk groups. (A-D) are the PFS and OS curves of patients with GCB and non-GCB DLBCL in the low (score 0-2) and high (score 3-5) risk groups of S-IPI, respectively.

Statistical analysis

Descriptive statistics of clinical characteristics were generated as proportions. The differences in the frequency of individual prognostic factors were assessed by chi-square test. End points were progression free survival (PFS; defined as time from diagnosis to progression, relapse, or death from any cause) and overall survival (OS; defined as time from diagnosis to death from any cause). The survival curves of PFS and OS were determined by Kaplan-Meier analysis. The log-rank test was used for the comparison of statistical differences between the survival curves in clinical and pathological parameters. All tests were considered significant at the two-sided 0.05 significance level. GraphPad Prism version 5.0 (GraphPad software, Inc., La Jolla, CA, USA) was used to perform statistical analysis.

Results

The patients’ outcome and related clinical information

The clinical features and outcomes of 106 patients with DLBCL were summarized in Table 1. Patients (n=106) with DLBCL had a median age of 61 years (range, 20-82 years). The 2-year PFS and OS in the GCB group were 67% and 82%, respectively. In the non-GCB group, the 2-year PFS and OS were 55% and 82%, respectively. No significant differences of PFS and OS were observed between these two phenotypes ($\chi^2=1.997$, $P=0.158$ and $\chi^2=1.000$, $P=0.317$; respectively) (Figure 1A and 1B). In univariate analysis, no significant differences of PFS and OS were observed between the phenotypes of GCB and non-GCB which were based on the same clinical features, such as sex (male or female), age (<60 or ≥60 years), clinic stage (stage IV), LDH level (normal or high), ECOG score (<2 or ≥2) and number of extranodal sites (<2 or ≥2) (Table 1).

S-IPI and the phenotypes of GCB and non-GCB for predicting the survival of patients with DLBCL

According to the IPI score, out of the total 106 patients, 21 patients (20%) were sub-classified as score 0, 39 (37%) as score 1-2, 22 (21%) as score 3 and 24 (22%) as score 4-5. We combined the patients with score 0 and score 1-2 as a low risk group, score 3 and score 4-5 as a high risk group. Obviously, significant differences were observed between the low (score 0-2) and high (score 3-5) risk groups in PFS ($\chi^2=16.17$, $P<0.001$) and OS ($\chi^2=10.83$, $P=0.001$). In the low risk group (score 0-2), 31
Lost prognostic role of GCB and non-GCB in DLBCL patients

patients were sub-classified as GCB group and 29 patients as non-GCB group, and no significant differences of PFS and OS were observed between GCB and non-GCB groups (both $P>0.05$, Figure 2A and 2B). Similarly, 19 patients were sub-classified as GCB group and 27 patients as non-GCB group in the high risk group (score 3-5), and no significant differences of PFS and OS were observed between these two phenotypes (both $P>0.05$, Figure 2C and 2D).

**BCL2 and the phenotypes of GCB and non-GCB for predicting the survival of patients with DLBCL**

Various reports showed that patients with BCL2 positive revealed a poor outcome. In our results, 71 patients were BCL2 positive and 35 patients were BCL2 negative. The 2-year PFS and OS were 52% and 79% in the BCL2 positive group. And in the BCL2 negative group, the 2-year PFS and OS were 79% and 84%, respectively. Statistical analysis showed that patients with BCL2 positive indicated a poor PFS ($P<0.01$), but not in OS ($P>0.05$). In the BCL2 positive group, 29 patients were sub-classified as GCB group and 42 patients as non-GCB group, and no significant differences of PFS and OS were observed between GCB and non-GCB groups (both $P>0.05$, Figure 3A and 3B). In the BCL2 negative group, 17 patients were sub-classified as GCB group and 18 patients as non-GCB group, and no significant differences of PFS and OS were observed between these two phenotypes (both $P>0.05$, Figure 3C and 3D).

**Discussion**

Our results demonstrated that the phenotypes of germinal center B-cell-like (GCB) and non-GCB which were based on the expression of CD10, Bcl-6 and MUM1 failed to predict the survival of patients with diffuse large B-cell lymphoma in the rituximab era. The IPI showed a powerful method to predict the survival of patients with DLBCL. Patients with BCL2 positive revealed a poor PFS, but not in OS. However, even though patients based on the same IPI risk group or the same BCL2 risk group, the phenotypes of GCB and non-GCB still had no ability to differentiate the outcome of patients with DLBCL.

During the past decades, people have realized that DLBCL is not only a clinically heterogeneous, but also a molecular heterogeneity [2]. At first, DLBCL was divided into 3 subgroups termed germinal center B-cell-like (GCB), activate B-cell-like (ABC) and a type 3 by gene expression profiling [14]. Owing to the similar outcome of type 3 and ABC subtype, both of them were aggregated together as non-GCB group. The most widely used criteria to designate patients as GCB and non-GCB was Hans' algorithm [3] which were based on the expression of CD10, bcl-6, or MUM1 by immunohistochemistry. Several studies [3, 4, 15] confirmed that the phenotype of GCB in DLBCL had a significantly better survival than the non-GCB, but not in all [7, 8, 16]. Nyman et al. [6] revealed that the prognostic role of GCB and non-GCB in DLBCL patients was eliminated in the rituximab era. Ilic et al. [7] also found that no significant differences of the survival were observed between the phenotypes of GCB and non-GCB, irrespectively whether they had treated with or without rituximab. In our study, no significant difference of survival was observed between the phenotypes of GCB and non-GCB.
From the constituent elements, we found that the phenotypes of GCB and non-GCB were not associated with the clinical features, such as age, clinic stage, performance status, LDH level, and extranodal involvements. The international prognostic index (IPI) [17], owing to include these five risk factors, is the most commonly used prognostic factor in survival for patients with DLBCL in the last two decades, even though in the rituximab era [9]. Our results indicated that the IPI had a significant prognostic role to differentiate DLBCL patients into the low and high risk groups. As no significant difference of survival was observed between the phenotypes of GCB and non-GCB in DLBCL patients, we supposed it might be equitable if there were based on the same IPI risk group. However, in our study, we found that even in the same low or high risk group, there still were no significant differences between GCB and non-GCB phenotypes in PFS and OS. Besides, we also found that there were no significant differences with regard to the clinical features of sex, age, performance status, stage, extranodal sites and LDH level.

BCL2 plays an important role in outcome evaluation of patients with DLBCL. Patients with BCL2 positive had a worse outcome than the negative [10, 11]. Iqbal et al. [12] demonstrated that BCL2 was a prognostic marker for patients with ABC DLBCL treated with CHOP-like regimens. But later, they [13] determined that the prognostic role of BCL2 had changed in DLBCL patients owing to the addition of rituximab to CHOP. However, they didn’t show whether the phenotypes of GCB and non-GCB were valid prognostic phenotypes or not when combined with BCL2 in DLBCL patients. In our results, patients with BCL2 positive had a worse PFS in DLBCL patients, but not in OS. The phenotypes of GCB and non-GCB still had no significant role in predicting the survival of patients with DLBCL when based on the same BCL2 risk group.

Recently, studies have indicated that the expression of CD40 is a positive prognostic factor of patients with DLBCL treated with R-CHOP [18]. Besides, other studies have showed that the expression of CD23 and CD40 may identify a favorable subgroup of DLBCL [19]. Moreover, numerous studies have demonstrated that the MYC rearrangement is associated with a poorer survival in DLBCL patients treated with R-CHOP [20, 21]. Now, more studies [22-24] have indicated that the role of MYC is forcefully influenced by BCL2. Patients with MYC and BCL2 translocation may have poor outcomes in DLBCL patients. Therefore, using only these three markers (CD10, Bcl-6 and MUM1) to designate patients as GCB and non-GCB is insufficient. The heterogeneity of DLBCL is remarkable complexity and we need to integrate all these molecular targets to seek a more effective method to improve the prognostication and to allow for the possibility of personalized therapy.

In conclusion, the phenotypes of GCB and non-GCB based on the expression of CD10, Bcl-6 and MUM1 have failed to predict the outcome of patients with DLBCL in the rituximab era. The classification of GCB and non-GCB should not be used to guide the clinical decisions for patients with DLBCL.

Disclosure of conflict of interest

None.

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References

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