Original Article
Clinical significance and prognostic value of STAT3 expression in patients with diffuse large B-cell lymphoma treated with rituximab-CHOP therapy

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Abstract: The clinical significance and prognostic value of Signal transducer and activator of transcription 3 (STAT3) were investigated in a cohort (n=52) of patients with diffuse large B-cell lymphoma (DLBCL) treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP), by immunohistochemical (IHC) analysis in this study. High STAT3 expression was detected in 65.38% (34/52) cases. Compared with germinal center B-cell (GCB) group, the non-GCB group had significantly higher STAT3 expression rate (82.61% versus 51.72%, P=0.02). Our data also suggest that high STAT3 expression level correlated with poor survival in DLBCL patients with respect to both 5-year OS (72.2% versus 41.2%, P=0.033) and PFS (66.7% versus 32.4%, P=0.023). The results of this study provide evidence for the ongoing researches targeting STAT3 in DLBCL.

Keywords: Diffuse large B-cell lymphoma, immunophenotype, STAT3, signaling pathway, survival

Introduction
Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma (NHL) in adults with a heterogeneous group of several variants, subgroups, and subtypes or entities [1]. Though patients with DLBCL may be curable with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) based combined chemotherapy, the disease proved to be fatal in some of them, 5-year overall survival (OS) for DLBCL patients ranges from 30% to 50% [2]. DLBCL can be divided into at least two distinct molecular subtypes by gene expression profiling (GEP), germinal center B-cell (GCB) like and activated B-cell (ABC) like DLBCL [3]. The use of anti-CD20 monoclonal antibody rituximab from late 1990s has improved the survival of DLBCL patients across all subtypes [4, 5]. But patients with ABC like DLBCL present with a worse outcome [6], which also prove there are more complex signaling pathway interaction and regulatory mechanisms among ABC like DLBCLs. Signal transducer and activator of transcription 3 (STAT3) activation has been proved to be associated with certain subtypes of DLBCLs, especially ABC like DLBCLs [7, 8].

STAT3 is an important member of the Janus kinase (JAK) and signal transducer and activator (STAT) pathway participators [9]. The inactive STAT3 is located in the cell cytoplasm, while the tyrosine residue (Tyr 705) is phosphorylated, lead to the STAT3 dimerization and translocation to the nucleus, and finally the STAT3 binds to the DNA, mediating tumor growth, invasion, cell survival, angiogenesis and so forth [10, 11]. Aberrant activation of STAT3 has been found in several tumors including some DLBCLs [7, 12, 13].

STAT3 has been identified as a target gene of B-cell lymphoma 6 (BCL6), which is a transcriptional repressor expressed in GCB cell and plays an important role during B-cell differentiation and germinal center development [14]. High levels of STAT3 expression were found in the activated B-cell and the BCL6-negative GCB DLBCL [14]. A recent study showed that the
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higher STAT3 messenger ribonucleic acid (mRNA) level and higher phosphorylated STAT3 (pSTAT3) level suggested poorer outcome in patients treated with rituximab and CHOP [15]. However, another study suggested that though STAT3 mRNA level correlated with pSTAT3 expression, it did not show significant difference with respect to OS in DLBCL or ABC like DLBCL [8]. So in this study, we evaluated the STAT3 expression through Immunohistochemical (IHC) staining, to assess its clinical significance and prognostic value in a cohort (n=52) of DLBCL patients.

Patients and methods

Patients

Between January 2007 and March 2009, 52 patients with newly diagnosed DLBCL were collected from the Department of Hematology, the First Affiliated Hospital, Zhejiang University College of Medicine. All cases were carefully reviewed by pathologists and classified according to the World Health Organization classification criteria [1]. Cases were excluded from our research with following diagnoses: DLBCL coexistent with or transformed from low-grade B-cell lymphoma, intravascular large B-cell lymphoma, DLBCL associated with chronic inflammation, primary mediastinal large B-cell lymphoma, primary cutaneous B-cell lymphoma, primary central nervous system DLBCL, T-cell or histioyte-rich large B-cell lymphoma, ALK-positive DLBCL, Epstein-Barr virus (EBV) positive DLBCL of elderly, human immunodeficiency virus related DLBCL, and DLBCL with grey zone features. All patients had comprehensive clinical data and retrievable pathological biopsy specimens. The ethics committee of our hospital approved the use of human tissue samples for this study, and informed consent was obtained from each of these patients.

Histopathologic and IHC assessment

All tissue specimens from these patients were fixed in 10% neutral buffered formalin and embedded in paraffin blocks. Then paraffin sections were used for preliminary diagnoses by hematoxylin-eosin staining. After IHC and clinical assessment, the definite diagnoses were obtained.

The immunophenotype was analyzed by using rabbit monoclonal antibodies to detect cellular antigens in paraffin sections according to the scoring criteria from the Lunenberg Lymphoma Biomarker Consortium [16]. The antibodies used were as follows: STAT3 (Santa Cruz Biotechnology, CA, USA, 1:100), CD20, CD10, BCL-6 and MUM-1 (Abcam, Cambridge, Britain, 1:100). The STAT3 staining intensity was scored from 0 to 3, representing lack of staining (0, if 0-5%), mild staining (1, if 6-33%), intermediate staining (2, if 34-66%) and strong staining (3, if 66-100%). IHC staining in DLBCLs was defined as low expression (score 0-1) and high expression (score 2-3). CD20 was used to determine the lymphoma cells, CD10, Bcl-6 and MUM-1 were considered to classify DLBCLs into GCB type and non-GCB type [17].

Clinical assessment and follow-up

All patients underwent a physical examination, complete blood count, serum chemistry assays of liver and kidney function, ultrasound of lymph nodes, computed tomography of chest and abdomen before each course of chemotherapy. Special attention was also given to hepatitis B (HBV) surface antigen (HBsAg). Bone marrow aspirates and biopsy were performed to evaluate bone marrow involvement status. Disease staging was evaluated according to the Ann Arbor staging system, which was originally used in Hodgkin lymphomas [18]. Performance status (PS) was based on the Eastern Cooperative Oncology Group (ECOG) scale. Then International Prognostic Index (IPI) scores were determined by age, stage of disease, lactate dehydrogenase (LDH) level, PS and the number of extranodal sites of disease (Disease in bone marrow, CNS, liver, gastrointestinal tract, or lung) [19]. A recently reported IPI scoring system named National Comprehensive Cancer Network (NCCN)-IPI was also used in this research [20].

Complete remission (CR) or unconfirmed (CRu), and partial remission (PR) were evaluated according to the International Working Group (IWG) response criteria for NHL [21]. OS was defined from the date of diagnosis to the date of last follow-up or death. Moreover, progression-free survival (PFS) was defined from the date of diagnosis to the date of progression, second tumor or death.

Treatment regimens

The chemotherapy regimens were employed including R-CHOP (rituximab 375 mg/m², day 0,
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Table 1. Clinical characteristics of 52 patients of DLBCL with regard to STAT3 expression

<table>
<thead>
<tr>
<th>Patients characteristics</th>
<th>STAT3 High expression N (%)</th>
<th>STAT3 Low expression N (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>34 (65.38)</td>
<td>18 (33.62)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>15 (44.12)</td>
<td>4 (22.22)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>19 (55.88)</td>
<td>14 (77.78)</td>
<td>0.119</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 or younger</td>
<td>21 (61.76)</td>
<td>10 (55.56)</td>
<td></td>
</tr>
<tr>
<td>Older than 60</td>
<td>13 (38.24)</td>
<td>8 (44.44)</td>
<td>0.664</td>
</tr>
<tr>
<td>B symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>23 (67.65)</td>
<td>15 (83.33)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>11 (32.35)</td>
<td>3 (16.67)</td>
<td>0.376</td>
</tr>
<tr>
<td>ECOG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>15 (44.12)</td>
<td>13 (72.22)</td>
<td></td>
</tr>
<tr>
<td>2–4</td>
<td>19 (55.88)</td>
<td>5 (27.78)</td>
<td>0.053</td>
</tr>
<tr>
<td>Stage</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>17 (50.00)</td>
<td>11 (61.11)</td>
<td></td>
</tr>
<tr>
<td>III/IV</td>
<td>17 (50.00)</td>
<td>7 (38.89)</td>
<td>0.444</td>
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<td>Extranodal site</td>
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<td>19 (55.88)</td>
<td>10 (55.56)</td>
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<tr>
<td>Present</td>
<td>15 (44.12)</td>
<td>8 (44.44)</td>
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<td>LDH</td>
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<tr>
<td>Normal</td>
<td>26 (76.47)</td>
<td>14 (77.78)</td>
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</tr>
<tr>
<td>Elevated</td>
<td>8 (23.53)</td>
<td>4 (22.22)</td>
<td>1</td>
</tr>
<tr>
<td>HBsAg</td>
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<td></td>
</tr>
<tr>
<td>Negative</td>
<td>27 (72.97)</td>
<td>15 (83.33)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>7 (27.03)</td>
<td>3 (16.67)</td>
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</tr>
<tr>
<td>BCL-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>10 (29.41)</td>
<td>8 (44.44)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>24 (70.59)</td>
<td>10 (55.56)</td>
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<tr>
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<tr>
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<td>14 (77.78)</td>
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<td>Non-GCB</td>
<td>19 (55.88)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0–2</td>
<td>22 (64.71)</td>
<td>15 (83.33)</td>
<td></td>
</tr>
<tr>
<td>3–5</td>
<td>12 (35.29)</td>
<td>3 (16.67)</td>
<td>0.158</td>
</tr>
<tr>
<td>NCCN IPI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3</td>
<td>20 (58.82)</td>
<td>15 (83.33)</td>
<td></td>
</tr>
<tr>
<td>4–8</td>
<td>14 (41.18)</td>
<td>3 (16.67)</td>
<td>0.073</td>
</tr>
<tr>
<td>Response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR/CRu/PR</td>
<td>27 (79.41)</td>
<td>17 (94.44)</td>
<td></td>
</tr>
<tr>
<td>No Response</td>
<td>7 (20.59)</td>
<td>1 (5.56)</td>
<td>0.305</td>
</tr>
</tbody>
</table>

cyclophosphamide 750 mg/m², day 1, doxorubicin 50 mg/m², day 1, vincristine 1.4 mg/m², day 1, and prednisolone 100 mg, days 1-5) and R-mini-CHOP (rituximab 375 mg/m², day 0, cyclophosphamide 400 mg/m², day 1, doxorubicin 25 mg/m², day 1, vincristine 1.4 mg/m², day 1, and prednisolone 100 mg, days 1-5). Both chemotherapy regimens were conducted every 21 days.

Patients with involvement of the paranasal sinus, testes and bone marrow, or those having more than one extranodal site were given intrathecal cytarabine for prophyaxis [22, 23].

Statistical analysis

OS and PFS were analyzed by Kaplan-Meier method, with difference compared by the log-rank test. Chi-square test was used to examine the relationships between STAT3 and clinical characteristics. The Spearman analytical methods were used to analysis the correlation between the variables. A P value less than 0.05 was considered statistically significant. SPSS Statistics V22 (IBM) was used for statistical analyses.

Results

Patient characteristics

The detailed clinical and pathological data of 52 patients with newly diagnosed DLBCL were shown in Table 1.

DLBCL subtypes

29 cases (55.77%) were evaluated as GCB type and 23 cases (44.23%) were evaluated as non-GCB type according to Hans algorithm [17]. Among 29 cases of GCB types, 21 cases expressed CD10 and 8 cases expressed BCL-6 alone. Among 23 cases of non-GCB types, 15 cases expressed both MUM-1 and BCL-6, while the other 8 were negative for CD10 and BCL-6 with 4 of them positive for MUM-1.

The expression of STAT3 in DLBCL patients and its clinical significance

The intensity of STAT3 expression in large atypical lymphoid cells was observed and shown in Figure 1 and Table 1. High STAT3 expression was detected in 34 cases (65.38%), while low expression was detected in 18 cases (33.62%). High expression of STAT3 was found...
Expression of STAT3 in DLBCL

in 15 cases with GCB type (51.72%), and 19 cases with non-GCB type (82.61%). High STAT3 expression was observed easily in non-GCB type with statistical significance ($P=0.02$).

There were no significant relations between expression of STAT3 and other clinical data such as age, gender, B-symptoms, PS, clinical stage, extranodal infiltrations, LDH lever, hepatitis B surface antigen (HBsAg), the expression of BCL-6, IPI and NCCN IPI, which listed in Table 1.

Refer to the relations between expression of STAT3 and treatment response, 44 cases reached CR, CRu or PR with 27 cases of high STAT3 expression. Though there was no statistical significance ($P=0.305$).

The prognostic value of STAT3

No patients were lost to follow-up, 27 cases died before the time of closeout. Among the 8 cases who did not respond to chemotherapy, they all died 1-31 months later. Then, among the 44 cases who responded to the chemotherapy, 16 cases were confirmed relapse or disease progression 19-64 months later, 1 case had chronic myeloid leukemia 61 months after lymphoma onset, by taking Imatinib, the patient complete molecular response and still survived. 1 case had hepatocellular carcinoma 57 months after lymphoma onset and died 4 months later. 1 case diagnosed with pancreatic cancer and died 34 months after lymphoma onset. 1 case had signet ring cell carcinoma of the stomach 32 months after lymphoma onset and died in 8 months.

By the time of closeout, with a median follow-up of 66.5 months, for all the 52 patients, the 5-year OS was 51.9% and the 5-year PFS was 46.2%. For cases of low STAT3 expression, the 5-year OS was 72.2%, which was significantly higher than cases of high STAT3 expression (41.2%, $P=0.033$). The 5-year PFS for low and high STAT3 expression group was 66.7% and 32.4%, respectively, with statistical significance ($P=0.023$). These data were shown in Figure 2.

Discussion

Increasing research data have suggested that these were strong links between DLBCL and

Figure 1. The intensity of STAT3 expression in DLBCL. (A) Lack of staining, score 0, (B) mild staining score 1, (C) intermediate staining, score 2, and (D) strong staining, score 3.
Expression of STAT3 in DLBCL

JAK/STAT signaling pathway, especially STAT3 [7, 8]. Higher phosphorylated STAT3 (pSTAT3) expression rate was found in non-GCB group than in GCB group [7]. And it is also showed

Figure 2. Survival data in DLBCL. A and B. The OS and PFS for all the patients respectively. C and D. Survival impact of GCB type and non-GCB type in DLBCL, the subtype did not show different outcome in OS and PFS. E and F. Survival impact of STAT3 expression in DLBCL, higher STAT3 expression was correlated with poorer outcome in OS and PFS.
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that the higher phosphorylated STAT3 expression level suggested poorer outcome in patient treated with rituximab and CHOP [15]. Ding and his colleague found that high levels of total STAT3 expression were preferentially observed in the ABC-like and BCL6 negative GCB-like lymphoma cell lines [14]. However, the clinical relationship between the total STAT3 expression and DLBCL have never been described in a cohort of DLBCL patients in previous researches. So this study was designed to investigate the clinical significance and prognostic value of STAT3 expression in DLBCL.

Our result showed that high STAT3 expression was detected in 65.38% (34/52) of all the patients with DLBCLs. The prevalence of total STAT3 expression in DLBCL is higher than the prevalence of pSTAT3 in earlier studies which ranged from 30% to 40% [15, 24-26]. Furthermore we observed that high expression of STAT3 was found in 51.72% cases with GCB type (15/29), and 82.61% cases with non-GCB type (19/23), these data suggested that high STAT3 expression was observed easily in non-GCB type with statistical significance (P=0.02), which was consistent with previous researches. These researches showed that pSTAT3 expressed more commonly in ABC like DLBCL [15, 24, 25, 27]. This phenomenon can be explained by the fact that STAT3 gene transcription was negatively regulated by BCL6 [14]. However, in our study there were no significant relations between expression of STAT3 and BCL6, which might be due to the small cohort, and furthermore, recent researches documented that JAK-STAT signaling pathway was also regulated by other genes such as myeloid differentiation factor 88 (MYD88) oncogenic mutations [28].

China is an HBV prevalent area, the epidemiologic association between HBV infection and DLBCL was confirmed by several studies [29-32]. STAT3 plays an important role in HBV related hepatocellular carcinoma [33]. STAT3 interacts with interleukin 6, which moderately suppresses HBV transcription and protein levels [34]. HBV expression endows hepatocellular carcinoma cells with resistance to STAT3 inactivation on proliferation. Whether there was connection between STAT3 and HBV infection in DLBCL patients, was never explored in previous research. Our data suggested that HBSAg positive DLBCLs were more frequently observed in high STAT3 expression cases without significance (27.03% versus 16.67%, P=1). The IPI has served as risk stratification in patients with DLBCL for more than two decades. Recently, an enhanced NCCN-IPI was used to stratify patients with newly diagnosed DLBCL into different risk groups, and discriminated patients in the different risk subgroups better than the former IPI [20, 35]. Our data showed that high STAT3 expression was observed easily in both higher risk groups by IPI (35.29% versus 16.67%, P=0.158) and NCCN-IPI (41.18% versus 16.67%, P=0.073), though there were no statistical significance. More cases will be needed in future study concerning these issues.

The 5-year OS and PFS was 51.9% and 46.2%, respectively, in our study, which were similar to those of earlier studies [8, 15, 27, 30, 31]. Although early study suggested that STAT3 mRNA level correlated with pSTAT3 expression, it did not show significant difference with respect to OS in DLBCL or ABC like DLBCL [8], and moreover, another study found that pSTAT3 IHC assessment was not an useful prognostic factor for DLBCL patients treated with epratuzumab or rituximab plus CHOP [27]. But lots of evidences supported that higher pSTAT3 level suggested poorer outcome in DLBCL patients [8, 15]. Our data suggested that higher STAT3 expression was also correlated with poorer outcome in DLBCL patients with respect to both 5-year OS (72.2% versus 41.2%, P=0.033) and PFS (66.7% versus 32.4%, P=0.023).

In summary, we have demonstrated in this study that STAT3 expression using IHC method is correlated with poor prognosis in patients with DLBCL, which may be explained that high STAT3 expression was observed easily in non-GCB type. Our findings support the therapies targeting the STAT3 signaling pathway, and moreover, our finding offers a useful prognostic indicator for DLBCL treatment.

Acknowledgements

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Disclosure of conflict of interest

None.

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