Early Relapse Is Associated with High Serum Soluble Interleukin-2 Receptor after the Sixth Cycle of R-CHOP Chemotherapy in Patients with Advanced Diffuse Large B-Cell Lymphoma

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Abstract. The clinical significance of serum soluble interleukin-2 receptor (sIL2R) levels was retrospectively assessed in patients with advanced diffuse large B-cell lymphoma (DLBCL). Twenty-one patients, who were newly-diagnosed with advanced DLBCL (stage III and IV) between 2006 and 2009, were evaluated. The median follow-up period was 37 months. All patients received 6-8 cycles of chemotherapy with rituximab in combination with doxorubicin, cyclophosphamide, vincristine, and prednisolone (CHOP)-like regimens and attained complete remission. Although all patients reached complete remission, six patients experienced disease relapse within 1 year after treatment completion. The overall survival was significantly poorer in patients with relapse than in patients with durable remission. The sIL2R levels after the sixth cycle of treatment were significantly higher in the relapse group than in the non-relapse group. Thus, the present study suggests sIL2R levels to be a valuable predictor for the prognosis of patients with advanced DLBCL.

Diffuse large B-cell lymphoma (DLBCL) is the most common histological subtype of non-Hodgkin’s lymphoma (NHL), accounting for approximately 30% of NHL cases (1). The standard chemotherapy, R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) provides a nearly 60% rate of 3-year overall survival even in patients in the worst initial prognostic group (2-4). Nevertheless, patients who are initially refractory to treatment or who experience relapse have a poor prognosis (5). Prediction of prognosis is, thus, needed to further optimize treatment for each patient.

The International Prognostic Index (IPI) is useful as an initial prognostic determinant of aggressive lymphoma including DLBCL (6). The IPI is calculated from the patients’ clinical characteristics including age, performance status, clinical stage, serum lactate dehydrogenase (LDH) levels, and number of extranodal lesions. The original IPI is still useful for stratifying patients with DLBCL using rituximab therapy (4, 7). However, IPI-alone may not always be sufficient to categorize patients with DLBCL in order to determine the therapeutic strategy. Apart from the clinical status-based IPI, several biological prognostic parameters have been sought (8-15). Among them, interleukin-2 receptor (IL2R) is expressed not only on the surface of activated lymphocytes but also on some types of malignant lymphoid cells (16). Soluble IL2R (sIL2R) is released from the cell membrane by the cleavage of IL2R, and it is elevated in the serum of such patients. Therefore, it has been suggested that sIL2R levels may also be a prognostic factor for lymphoma.

In the present study, serum sIL2R levels were measured in 21 patients with advanced DLBCL who achieved complete remission after 6-8 cycles of standard chemotherapy using rituximab with CHOP-like regimens. The primary objective was to evaluate sIL2R levels serially and to seek for any association between sIL2R levels and early relapse within one year after the completion of chemotherapy with complete remission.
Patients and Methods

Patients. Patients who presented at the University of Fukui Hospital between 2006 and 2009 were included in the study. They were newly-diagnosed with advanced DLBCL (stage III and IV) based on the pathological findings in biopsy specimens and radiographic determinations, using computed-tomography and positron-emission tomography. All patients received 6-8 cycles of R-CHOP every 21 days (rituximab on day 1, 50 mg/m² doxorubicin on day 1, 750 mg/m² cyclophosphamide on day 1, 1.4 mg/m² vincristine on day 1, and 100 mg prednisolone on days 1-5) or R-THP-COP (rituximab on day 1, 50 mg/m² tetrahydropyranyl-doxorubicin on day 1, 750 mg/m² cyclophosphamide on day 1, 1.4 mg/m² vincristine on day 1, and 100 mg prednisolone on days 1-5) with a substitution of tetrahydropyranyl-doxorubicin for doxorubicin. Those who attained complete response at the end of the treatment were evaluated for early relapse within one year after the completion of chemotherapy. Response-to-treatment and relapse were defined according to the International Workshop for NHL (17). This retrospective study was approved by the Ethics Committee of the University of Fukui Hospital.

Serum sIL2R determination. To evaluate serum levels of sIL2R, venous blood samples were drawn from patients serially at least at the onset and after the sixth cycle of chemotherapy. Serum sIL2R levels were determined by a chemiluminescent enzyme immunoassay using a Siemens Immulyze IL-2RII kit (Siemens Medical Solutions Diagnostics, Tokyo, Japan), according to the manufacturer's instructions. The normal range is between 144 U/ml and 518 U/ml. The limit of detection of the kit was 50 U/ml.

Figure 1. Serum soluble interleukin 2 receptor (sIL2R) concentrations according to patient characteristics. The serum sIL2R levels were compared between patients with and those without B symptoms (A), patients in stage III or IV (B), and patients in different International Prognostic index (IPI) risk groups (C). The bars represent the mean±SD. A statistical correlation was found between the serum sIL2R concentration and the serum lactate dehydrogenase (LDH) concentration at onset (D).
Follow-up. After the completion of chemotherapy and the confirmation of complete response, the patients returned periodically for physical examinations, blood tests, and computed-tomography to check the disease status.

Statistical analyses. All statistical analyses were performed using the Microsoft Excel 2007 software (Microsoft, Redmond, WA, USA). All of the graphs, linear regression lines, and curves were generated using the GraphPad Prism software (version 5.0) (GraphPad Software, Inc. San Diego, CA, USA). Each comparison was evaluated statistically by using Mann-Whitney two-tailed test. Values of \( p \leq 0.05 \) were considered statistically significant.

Results

Patients. The study population consisted of 21 patients with advanced DLBCL (stage III and IV) who received 6-8 cycles of R-CHOP or R-THP-COP and achieved complete remission. The group included 14 males and seven females with a median age of 73 years, ranging between 56 and 87 years. The patient characteristics are summarized in Table I, demonstrating the clinical stage and the IPI for each patient. The follow-up period following completion of treatment ranged between 12 months and 73 months, with a median of 37 months.

Serum sIL2R concentrations according to patient characteristics. The sIL2R levels at onset ranged from 416 to 21,300 U/ml, suggesting a wide variability among patients. The serum sIL2R levels were compared between several patients categories. The patient group with B symptoms had higher sIL2R levels (mean=9495 U/ml) than the group without B symptoms \( (p=0.006) \) (Figure 1A). The stage IV patient group had higher sIL2R levels (mean=6452 U/ml) than the stage III group \( (p=0.07) \) (Figure 1B). The sIL2R levels \( (p=0.024) \) (mean=5478 U/ml) of the high-risk group (high or high-intermediate risk of IPI) were higher than those \( (p=0.15) \) (mean=4414 U/ml) of the low-risk group (low or low-intermediate risk) (Figure 1C). A statistical correlation was found between the serum sIL2R and serum LDH concentrations at onset \( (R=0.49, p=0.024) \) (Figure 1D). These results suggest that the more aggressive disease tended to exhibit higher sIL2R levels at onset.

The time course of serum sIL2R concentrations. Figure 2 shows the time course of serum sIL2R concentrations in 21 patients with diffuse large B-cell lymphoma during treatment. The sIL2R values were determined at least at onset and after the sixth cycle of the chemotherapy.

Correlation between early relapse and serum sIL2R level. Out of the 21 enrolled patients, six patients experienced relapse within one year from the completion of the first-line chemotherapy, although they all achieved complete remission. We determined whether the early relapse was correlated with sIL2R levels (Figure 3). The sIL2R at onset

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Patient & Age, years & Gender & Stage & IPI \\
\hline
1 & 68 & M & IIIA & High-int \\
2 & 79 & M & IVA & High \\
3 & 77 & M & IVA & Low-int \\
4 & 72 & M & IVA & High \\
5 & 73 & M & IIIA & High-int \\
6 & 69 & M & IIIA & High-int \\
7 & 75 & F & IVB & High \\
8 & 74 & M & IVA & High \\
9 & 78 & M & IVA & Low-int \\
10 & 73 & M & IVB & High \\
11 & 87 & F & IVB & High \\
12 & 74 & F & IVB & High \\
13 & 71 & F & IIIA & Low-int \\
14 & 59 & F & IIIA & Low-int \\
15 & 61 & M & IVB & High \\
16 & 74 & M & IIIB & High \\
17 & 60 & M & IIIA & Low-int \\
18 & 75 & F & IVA & High \\
19 & 74 & F & IVA & High \\
20 & 68 & M & IIIA & Low-int \\
21 & 56 & M & IIIA & Low-int \\
\hline
\end{tabular}
\caption{Patients' characteristics.}
\end{table}

IPI, International Prognostic Index.
was insignificantly greater in the group of patients with early relapse (mean=6813 U/ml) than for the group without relapse (mean=4448 U/ml) ($p=0.259$) (Figure 3A). The ratio between the sIL2R values at onset and after the second cycle, which might represent the initial response, did not differ between the groups with (mean=0.30) and without (mean=0.36) early relapse ($p=0.27$) (Figure 3B). Nevertheless, the sIL2R levels after the sixth cycle were significantly higher for the patient group with early relapse (mean=722 U/ml) than for the group without relapse (mean=496 U/ml) ($p=0.007$) (Figure 3C). However, the LDH levels after the sixth cycle did not differ between the two groups ($p=0.483$) (Figure 3D), even though the LDH levels were proportional to the sIL2R levels at onset, suggesting its insensitivity. Thus, the results suggest that the sIL2R level after the sixth cycle would predict early relapse for patients who had achieved complete response after first-line treatment with R-CHOP or similar regimens.

**Survival analysis.** According to the Kaplan–Meier method, the 3-year overall survival rate and the 3-year relapse-free survival rate were 80.3%, and 69.0%, respectively (Figures 4A, B). When the cases were divided into the complete-response group and the early-relapse group, the former group had a higher survival rate and a higher relapse-free survival rate than the early-relapse group (Figures 4C, D). These results suggest that the prediction of early relapse would be critical from the viewpoint of prognosis.
Discussion

Despite significant advances in treatment efficacy, the majority of patients with DLBCL are not cured with conventional chemotherapy. Although most patients with relapse are sensitive to second-line chemotherapy, it is difficult for these patients to maintain long-term remission (5). Therefore, a predictor for early relapse may be able to stratify patients according to risk, allowing improved treatment schedules and thus improved clinical outcomes.

Serum sIL2R levels have been clinically investigated in the context of DLBCL (11, 13, 15). Overall, the serum sIL2R level was reported to be an independent predictor for progression-free survival and overall survival even after the rituximab era. The sIL2R levels were also found to be a prognostic marker for other diseases, including acute lymphoblastic leukemia and peripheral T-cell lymphoma (18, 19). However, these studies mostly measured the sIL2R concentrations at onset. Jo et al. measured sIL2R concentrations serially in patients with NHL (14). The authors found that sIL2R levels decreased during complete remission and were elevated during disease progression or relapse (14). Nevertheless, they did not evaluate serial sIL2R levels in terms of the prediction of relapse.

In the present study, high sIL2R levels were significantly associated with the presence of B symptoms and with a high
LDH level (Figure 1). sIL2R levels also appeared to be higher in the high-risk group and the stage IV group (Figure 1). These findings were consistent with those of previous reports (12, 14, 15). Most importantly, the present study clearly demonstrated that sIL2R levels after the sixth cycle of chemotherapy (Figure 2), a parameter that has not been evaluated previously, were significantly correlated with early relapse in DLBCL (Figure 3). Moreover, the early relapse was directly associated with the shortened relapse-free survival and overall survival (Figure 4). These results thus suggest that serum sIL2R levels, not only as measured at onset but also serially determined, would be a biological marker for predicting the future of patients with DLBCL after completion of chemotherapy using rituximab-CHOP-like regimens.

There are several limitations to the present study. Firstly, the number of the patients evaluated was relatively small. Some of the insignificant differences in Figures 1 and 3 might become significant with a larger number of patients. Secondly, the achievement of complete remission was confirmed by computed-tomography after the completion of the chemotherapy. Positron-emission tomography was not undertaken because this was not covered by health insurance in Japan. The last limitation is related to risk stratification. It is still unclear whether the use of additional modalities, such as high-dose chemotherapy with autologous hematopoietic stem cell transplantation, to first-line treatment would improve the outcome of the patients who are likely to experience early relapse (20).

In conclusion, serum sIL2R after the sixth cycle of rituximab plus CHOP-like regimens might predict relapse within a year from the completion of treatment despite achievement of complete remission. sIL2R, in addition to the IPI, may be a variable predictor that will enable the individualization of treatment for patients with DLBCL.

References


