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Original Article

Prognostic Importance of the Soluble Form of IL-2 Receptorα (sIL-2Rα) and its Relationship with Surface Expression of IL-2Rα (CD25) of Lymphoma Cells in Diffuse Large B-cell Lymphoma Treated with CHOP-like Regimen with or without Rituximab : A Retrospective Analysis of 338 Cases

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We evaluated the prognostic significance of the serum level of the soluble form of interleukin-2 receptorα (sIL-2Rα) and investigated its association with CD25 expression on tumor cells in diffuse large B-cell lymphoma (DLBCL). Three hundred and thirty-eight adult patients with newly diagnosed DLBCL were eligible for this retrospective study. 32.2% of patients were treated with CHOP-like regimen and 67.8% with R-CHOP-like regimen. CD25 expression on the surface of tumor cells was evaluated in 143 cases and its relationship with sIL-2Rα level was also investigated. Both overall survival (OS) and progression-free survival (PFS) were poorer in patients with higher sIL-2Rα, in both R-CHOP and CHOP groups. sIL-2Rα > 1,000 U/mL and performance status (PS) $\geq$ 2 were independently associated with poorer OS, and sIL-2Rα > 1,000 U/mL, age > 60 years, and $\geq$ 2 extranodal sites were independently associated with poorer PFS in the R-CHOP group. The sIL-2Rα level was higher in the CD25-positive group than in the CD25-negative group in stage 3 or 4 disease (p = 0.010). Multiple linear regression analysis showed CD25 expression to be independently correlated with sIL-2Rα levels. High sIL-2Rα is an important risk factor for survival in DLBCL treated with not only CHOP-like, but also R-CHOP-like regimens, regardless of the tumor's expression of CD25. 〔*J Clin Exp Hematop 53(3) : 197-205, 2013*〕

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common subgroup of non-Hodgkin lymphoma (NHL). This type of NHL is usually very sensitive to chemotherapy and radiotherapy, and is expected to be cured in more than two-thirds of patients. Many efforts have been made to distinguish those patients with poor prognosis who need additional or alternative treatment strategies. In 1993, the International Prognostic Index (IPI) was proposed as a simple prognostic model in aggressive lymphoma, using 5 clinical factors (age > 60 years, performance status [PS] ≥ 2, stage 3 or 4, high lactate dehydrogenase [LDH], and ≥ 2 extranodal sites) to stratify patients for optimal therapy.[1] On the other hand, specific proteins and genetic changes that are useful for the prediction of prognosis have also been identified as biological markers, including β_2-microglobulin, bcl-2, bcl-6, CD10, MUM-1, and germinal center B-cell-like (GCB)/non-GCB, as well as nm23.[2,3] However, these factors are insufficient to estimate the prognosis of patients treated with monoclonal antibody (rituximab)-containing chemotherapy, including cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP), because several of these markers and models no longer predict prognosis in the rituximab era.[4,5]

The interleukin-2 receptor comprises three glycoproteins : the α, β, and γ chains. Soluble interleukin-2 receptorα (sIL-2Rα) is a soluble form of the IL-2R α-chain (CD25) that is released from activated T or B lymphocytes.[6,7] Serum sIL-2Rα is elevated in patients with hematological malignancies such as malignant lymphoma, hairy cell leukemia, and adult T-cell leukemia/lymphoma. In addition, elevated sIL-2Rα is observed in non-hematological diseases such as autoimmune disorders and various infectious diseases.[8] sIL-2Rα is now recognized as a biological marker for NHL. Several previous reports revealed the importance of sIL-2Rα as a surrogate marker of tumor burden[9] or prognosis.[10-14] Although three reports[12-14] have suggested the clinical significance of sIL-2Rα in patients with DLBCL treated with rituximab-containing chemotherapy, the rationale for this remains unclear. Furthermore, there have been no reports about the relationship between the level of sIL-2Rα and cell surface CD25 expression.

The aims of this study are 1) to evaluate the clinical usefulness of sIL-2Rα for assessment of tumor burden and prognosis in a large cohort, and 2) to investigate the association between CD25 expression and sIL-2Rα in patients with DLBCL.

PATIENTS AND METHODS

Patients

A total of 338 adult patients with DLBCL newly diagnosed between January 2001 and July 2008 were eligible for this retrospective study. Pathological diagnosis was made according to WHO 2008 classification.[15] Patients with intravascular lymphoma, human immunodeficiency virus-associated lymphoma, and DLBCL with transformation from indolent lymphoma were excluded. All patients were treated with combination chemotherapy with CHOP or CHOP-like regimen, with or without rituximab. Response to the therapy was evaluated using the criteria of Cheson *et al*.[16] Overall survival (OS) and progression-free survival (PFS) were measured in both R-CHOP- and CHOP-administered patients. OS was defined as the period from the date of diagnosis until last follow-up or death from any cause. PFS was defined as the period from the date of diagnosis to last follow-up or to one of the following events : documented disease progression, relapse, or death from any cause. Clinical stage was evaluated according to the Ann-Arbor system[17] (computed tomography scan, physical examination, and bone-marrow examination), and then IPI was evaluated.[1] The serum level of sIL-2Rα was evaluated by enzyme-linked immunosorbent assay at diagnosis (Kyowa Medex Co., Ltd., Tokyo, Japan). The study was approved by the local Institutional Review Board of each hospital, and the committees waived the informed consent requirement because of the observational nature of the protocol. The primary endpoints of this study are 3-year %OS (3OS) and 3-year %PFS (3PFS).

Immunophenotyping of lymphoma cells

In 143 of the 338 patients, CD25 expression was evaluated on the tumor cells at diagnosis using three-color flow cytometry on a lymphoma sample from lymph node, bone marrow, peripheral blood, or another extranodal organ. To estimate CD25, CD10, and CD5 expression on tumor cells using three-color flow cytometry, CD45 bright cells (lymphocyte gate) were gated and considered positive if the positivity was ≥ 20% of the population, excluding CD4-positive cells.[18]

Statistical analysis

Patients' characteristics were compared using Fisher's exact tests. sIL-2Rα levels were compared between two groups using the Mann-Whitney U test. Correlations between log-transformed sIL-2Rα level and log-transformed LDH, stage, and CD25 expression were determined by Pearson's or Spearman's correlation coefficient. Multiple linear regression analysis was performed between sIL-2Rα and correlated factors. Survival curves were generated by the Kaplan-Meier

method and compared between two groups by log-rank test. To estimate the impact of several factors on survival, including sIL-2Rα level, age > 60 years, PS ≥ 2, stage 3 or 4, high LDH, and ≥ 2 extranodal sites, we performed multivariate analysis using Cox proportional hazards. A probability value of $p < 0.05$ was considered to indicate statistical significance. Univariate and multivariate analyses were performed using SPSS software version 17.0 for Windows (SPSS, Chicago, IL).

RESULTS

Clinical characteristics of patients

The characteristics of the 338 patients (183 men and 155 women) are presented in Table 1. The median age was 67 years (range 17-90). One hundred and seventy-four patients (51.5%) had advanced-stage disease (stage 3 or 4) ; 109 patients (32.2%) were treated with a CHOP-like regimen, while 229 patients (67.8%) were treated with an R-CHOP-like regimen. There was no significant difference in any factor between the CHOP-like group and the R-CHOP-like group. Median follow-up time was 847.0 days : 809.0 days in the R-CHOP group and 1,131.0 days in the CHOP group. A total of 33 patients received high-dose chemotherapy with autologous peripheral blood stem cell transplantation : 29 patients on first remission (initial treatment : 25 R-CHOP, 4 CHOP) and 4 patients on second remission (3 R-CHOP, 1 CHOP).

Prognosis and sIL-2Rα

First, we analyzed 3OS and 3PFS of all DLBCL patients categorized by IPI treated with a CHOP-like regimen with or without rituximab. The IPI score was predictive of outcome in the CHOP group (low vs. low-intermediate vs. high-intermediate vs. high : 3OS 85.7%, 73.7%, 44.4%, and 18.3% ; 3 PFS 68.2%, 57.5%, 38.9%, and 6.0%, respectively). However, it was less predictive in the R-CHOP group

Table 1. Patients characters

Clinical findings		All	Group 1	Group 2	Group 3	*p* value
Number of patients		338	142	116	80	
Age	< 60	112	54	39	19	0.094
	> 60	226	88	77	61	
Gender	Male	183	76	65	42	0.871
	Female	155	66	51	38	
Stage	1-2	164	102	53	9	< 0.001
	3-4	174	40	63	71	
IPI	Low	114	81	31	2	< 0.001
	LI	81	37	33	11	
	HI	59	15	25	19	
	High	83	9	27	47	
	Unknown	1	0	0	1	
PS	0-1	265	125	90	50	< 0.001
	2-4	72	17	26	29	
	Unknown	1	0	0	1	
LDH	Normal	143	100	35	8	< 0.001
	High	195	42	81	72	
Extranodal site	0-1	254	123	89	42	< 0.001
	≥ 2	84	19	27	38	
Bulky mass	no	276	131	93	52	< 0.001
	yes	59	9	23	27	
	Unknown	3	2	1	1	
B sympton	A	266	133	95	38	< 0.001
	B	69	8	20	41	
	Unknown	3	1	1	1	
Treatment	R-CHOP	229	101	82	46	0.080
	CHOP	109	41	34	34	

IPI, international prognostic index; PS, performance status; LDH, lactate dehydrogenase; LI, low-intermediate; HI, high-intermediate; R-CHOP, monoclonal antibody (rituximab)-containing chemotherapy including cyclophosphamide, doxorubicin, vincristine, and predonisolone;

Table 2. Survival in patients treated with R-CHOP or CHOP

sIL-2Rα	R-CHOP			CHOP		
	Number of cases	OS (%)	PFS (%)	No	OS (%)	PFS (%)
All	229	75.4	67.6	109	59.5	45.8
< 1,000	101	88.6	83.4	41	81.6	65.3
1,000-3,500	82	71.5	60.6	34	63.6	51.7
> 3,500	46	57.1	47.1	34	28.7	16.5

R-CHOP, monoclonal antibody (rituximab)-containing chemotherapy including cyclophosphamide, doxorubicin, vincristine, and predonisolone; OS, overall survival; PFS, progression-free survival; sIL-2Rα, soluble interleukin-2 receptor α

(low vs. low-intermediate vs. high-intermediate vs. high : 3OS 89.4%, 76.6%, 71.1%, and 58.7% ; 3PFS 85.4%, 68.7%, 57.7%, and 47.5%, respectively). 3OS and 3PFS were significantly better in the R-CHOP group than in the CHOP group (R-CHOP vs. CHOP : 3OS 75.4% vs. 59.5% [$p < 0.001$], 3PFS 67.6% vs. 45.8% [$p < 0.001$], respectively) (Table 2).

The median sIL-2Rα value of all patients was 1,427 U/mL (range 253-51,600). To analyze the prognostic significance of sIL-2Rα, we divided the patients into 3 groups according to the serum concentration of sIL-2Rα (group 1, sIL-2Rα < 1,000 U/mL ; group 2, sIL-2Rα 1,000-3,500 U/mL ; group 3, sIL-2Rα > 3,500 U/mL). The characteristics of the patients in these 3 groups are presented in Table 1. In patients receiving high-dose chemotherapy with autologous peripheral blood stem cell transplantation in first remission, the numbers of patients in groups 1, 2, and 3 were 5, 12, and 8 in patients receiving R-CHOP and 1, 1, and 2 in those receiving CHOP, respectively. In patients receiving R-CHOP, 3OS was 88.6% in group 1 (n = 101), 71.5% in group 2 (n = 82), and 57.1% in group 3 (n = 46). 3PFS was 83.4%, 60.6%, and 47.1%, respectively. Group 1 had significantly better 3OS than groups 2 and 3 (group 1 vs. group 2, $p = 0.026$; group 1 vs. group 3, $p < 0.001$; group 2 vs. group 3, $p = 0.133$). Likewise, group 1 had better PFS than group 2 and group 3 (group 1 vs. group 2, $p = 0.002$; group 1 vs. group 3, $p < 0.001$; group 2 vs. group 3, $p = 0.104$) (Fig. 1a & 1c). In patients receiving CHOP, 3OS was 81.6% in group 1 (n = 41), 63.6% in group 2 (n = 34), and 28.7% in group 3 (n = 34). 3PFS was 65.3%, 51.7%, and 16.5%, respectively. Group 3 had significantly worse 3OS than groups 1 and 2 (group 1 vs. group 2, $p = 0.051$; group 1 vs. group 3, $p < 0.001$; group 2 vs. group 3, $p = 0.001$). Group 3 had poorer 3PFS than groups 1 and 2 (group 1 vs. group 2, $p = 0.244$; group 1 vs. group 3, $p < 0.001$; group 2 vs. group 3, $p = 0.001$) (Fig. 1b & 1d).

For further confirmation of the prognostic significance of sIL-2Rα in the rituximab era, we performed Cox proportional hazard analysis. In patients who had received R-CHOP, univariate analysis revealed that sIL-2Rα as well as IPI-related factors (age > 60 years, high LDH, stage 3 or 4, PS ≥ 2, ≥ 2 extranodal sites) was a significant prognostic factor for both OS and PFS. Multivariate analysis revealed that sIL-2Rα > 1,000 U/mL and PS ≥ 2 were independently associated with poor OS, and sIL-2Rα > 1,000 U/mL, age > 60 years, and ≥ 2 extranodal sites were associated with poor PFS in this group (Table 3).

CD25 expression and sIL-2Rα in DLBCL patients

CD25 expression on tumor cells was evaluated in 143 (66 [46.2%] CD25-positive and 77 [53.8%] CD25-negative) patients. There was no significant difference in any factor except number of high-LDH patients between the CD25-positive and -negative groups (Table 4).

We compared the sIL-2Rα level in the CD25-positive group (median 1,853 U/mL, range 253-37,200) with that in the negative group (median 1,370 U/mL, range 264-19,200) and found the former to be higher in stage 3 or 4 disease ($p = 0.010$), but the difference was insignificant in stages 1 and 2 (Fig. 2a-2c).

Correlation between sIL-2Rα and multiple factors including CD25

To examine the influence of CD25 expression, we calculated correlation coefficients between sIL-2Rα and CD25 expression on tumor cells. In the 143 CD25-measured patients, multiple linear regression analysis confirmed the correlation between sIL-2Rα levels and multiple factors including stage, LDH, and CD25 expression (Table 5).

Prognosis and sIL-2Rα in CD25-positive and negative groups

We compared the survival curves of DLBCL patients treated with R-CHOP according to the CD25 expression status of their tumor cells (Fig. 3). When patients were divided into 3 groups according to the serum value of sIL-2Rα (group 1, sIL-2Rα < 1,000 U/mL ; group 2, 1,000-3,500 U/mL ;

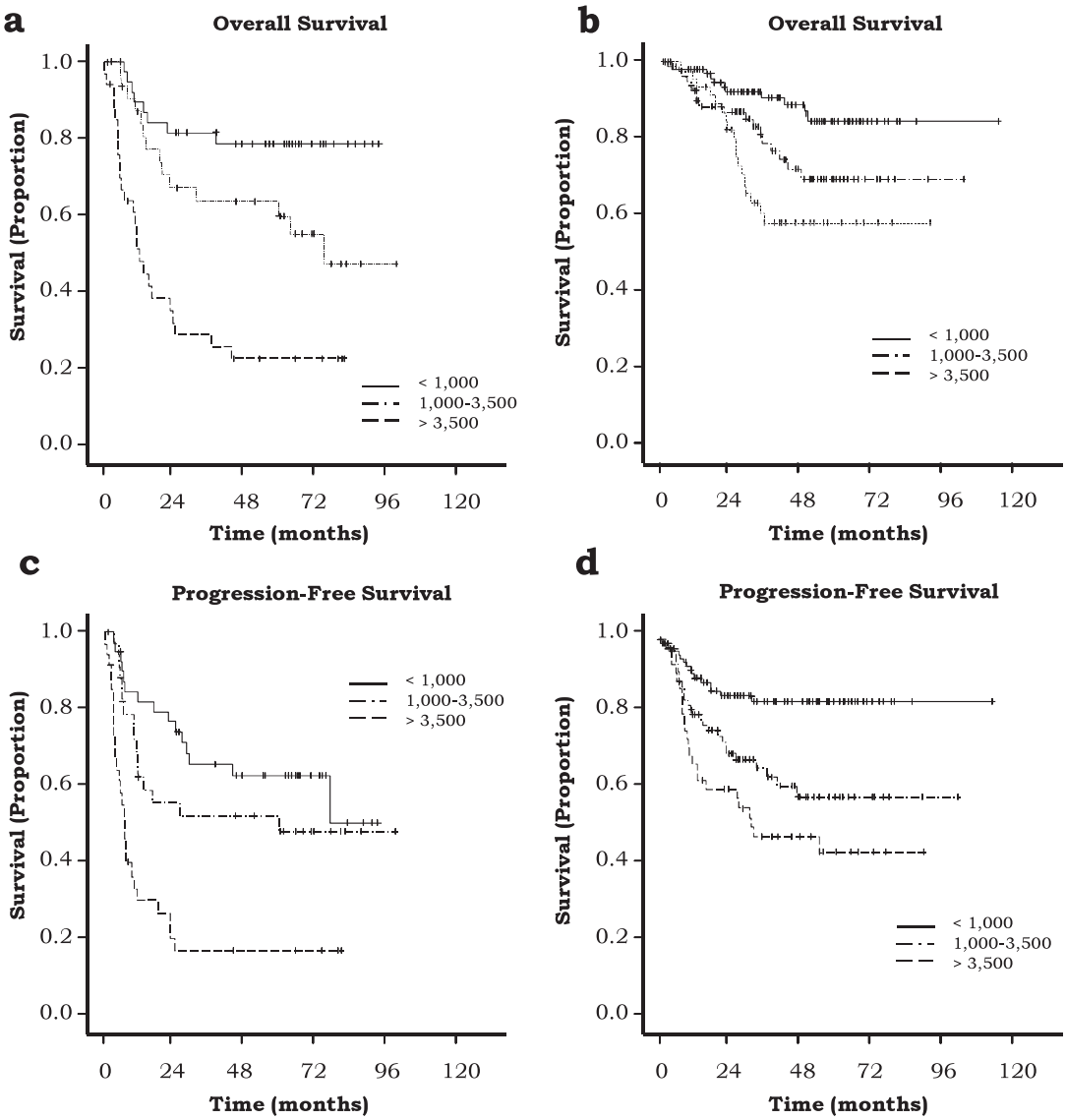

Fig. 1. Overall (*1a* & *1b*) and progression-free survival (*1c* & *1d*) according to soluble interleukin-2 receptorα (sIL-2Rα) value in diffuse large B-cell lymphoma patients treated with R-CHOP (*1b* & *1d*) or CHOP (*1a* & *1c*). Overall and progression-free survival was poor in patients with sIL-2Rα > 1,000 U/mL in both groups.

Table 3. Univariate and multivariate analysis about prognostic factors

Clinical findings		Univariate analysis			Multivariate analysis		
		Hazard ratio	95%CI	*p* value	Hazard ratio	95%CI	*p* value
OS	sIL-2R > 1,000	2.863	1.457-5.627	0.002	2.438	1.220-4.871	0.012
	PS ≥ 2	2.642	1.466-4.760	0.001	2.142	1.172-3.915	0.013
PFS	sIL-2R > 1,000	3.169	1.784-5.632	< 0.001	2.882	1.613-5.151	< 0.001
	age > 60 y	2.026	1.124-3.654	0.019	1.876	1.038-3.390	0.037
	extranodal ≥ 2	2.177	1.330-3.563	0.002	1.778	1.079-2.928	0.024

OS, overall survival; PFS, progression-free survival; sIL-2Rα, soluble interleukin-2 receptor α

Table 4. Characters of CD25-positive and negative patients

Clinical findings		CD25-positive	CD25-negative	*p* value
Patients		66	77	
Age	< 60	18	29	0.187
	> 60	48	48	
Gender	Male	33	41	0.699
	Female	33	36	
Stage	1-2	31	33	0.622
	3-4	35	44	
IPI	Low	26	23	0.376
	LI	11	19	
	HI	12	19	
	High	17	16	
	Unknown	0	0	
PS	0-1	48	56	1.000
	2-4	18	21	
	Unknown	0	0	
LDH	Normal	33	26	0.049
	High	33	51	
Extranodal site	0-1	51	62	0.635
	≥ 2	15	15	
Bulky mass	no	57	58	0.05
	yes	7	18	
	Unknown	2	1	
B sympton	A	53	62	0.847
	B	13	14	
	Unknown	0	1	
Treatment	R-CHOP	45	56	0.552
	CHOP	21	21	

IPI, international prognostic index; PS, performance status; LDH, lactate dehydrogenase; LI, low-intermediate; HI, high-intermediate; R-CHOP, monoclonal antibody (rituximab)-containing chemotherapy including cyclophosphamide, doxorubicin, vincristine, and predonisolone

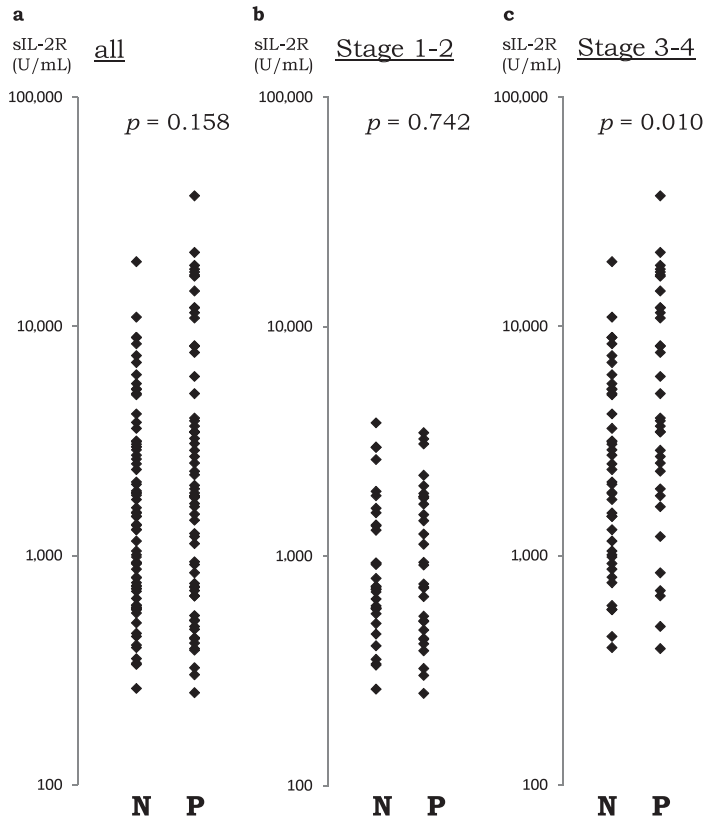

Fig. 2. Soluble interleukin-2 receptorα (sIL-2Rα) level in CD25-positive and -negative DLBCL (*2a*). In an analysis divided by stage, sIL-2Rα level showed no significant difference between the CD25-positive and -negative groups in stage 1-2 patients (*2b*). On the other hand, sIL-2Rα level was significantly higher in the CD25-positive group than in the negative group in stage 3-4 patients (*2c* : p = 0.010).

Table 5. Multiple linear regression analysis for evaluating the influence on sIL-2Rα

Clinical findings	Partial regression coefficient	95%CI	Standardized partial regression coefficient	*p* value
LDH	0.937	0.711-1.164	0.525	< 0.001
Stage	0.305	0.183-0.427	0.318	< 0.001
CD25 expression	0.443	0.189-0.696	0.195	0.001

sIL-2Rα, soluble interleukin-2 receptor α; LDH, lactate dehydrogenase

group 3 > 3,500 U/mL), CD25-positive patients had better 3OS and 3PFS than CD25-negative patients in each group, except for group 1 ; however, there was no significant difference between the CD25-positive and -negative groups according to the log-rank test (Fig. 3a-3f).

DISCUSSION

The prognosis of patients with DLBCL has improved since the introduction of rituximab-combined chemotherapy.[19,20] However, about one-third of patients still have poor prognoses and die from their disease, so it is very important to develop reliable prognostic markers in the rituxi-

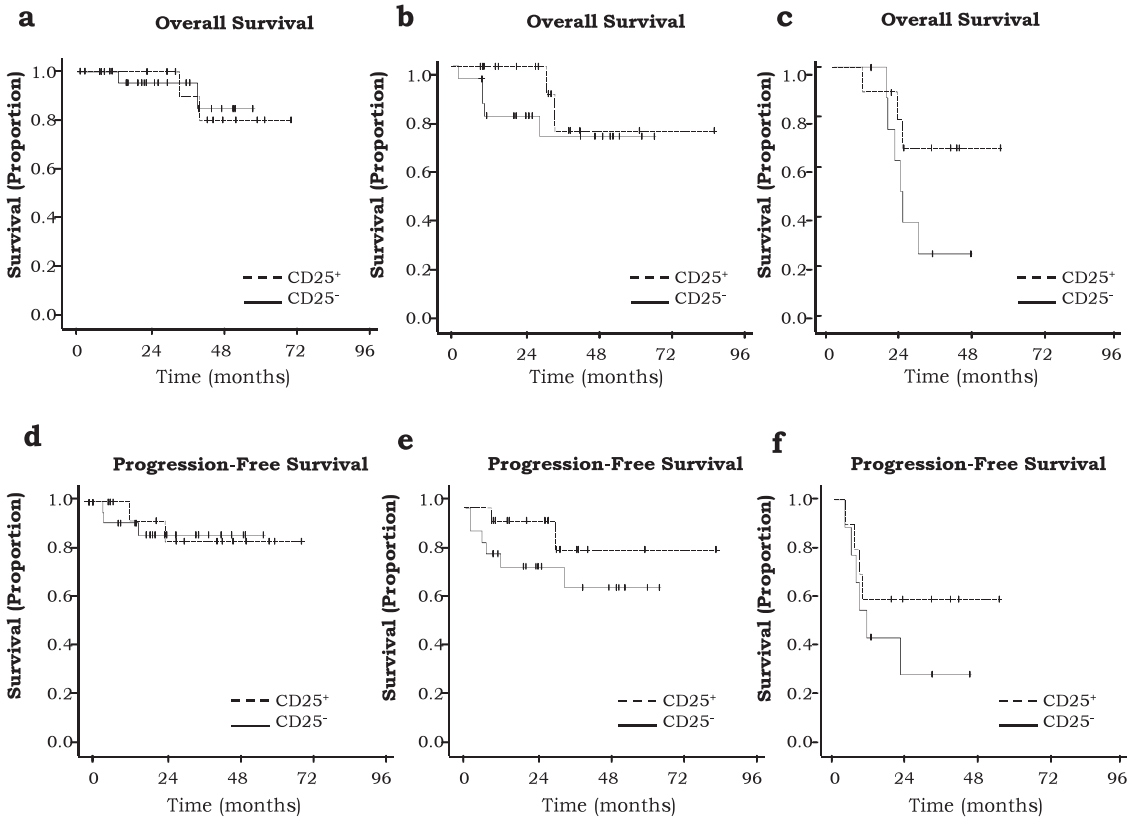

Fig. 3. Overall and progression-free survival according to the soluble interleukin-2 receptorα (sIL-2Rα) value in CD25-positive and negative diffuse large B-cell lymphoma patients [group 1 (*3a* & *3d*), group 2 (*3b* & *3e*), and group 3 (*3c* & *3f*)]. CD25-positive patients had better 3-year % overall survival and 3-year % progression-free survival than CD25-negative patients in groups 2 and 3 ; however, there was no significant difference between the CD25-positive and negative groups according to the log-rank test.

mab era to identify those patients who are unlikely to be cured and who need the optimal treatment strategy. IPI is one of the most popular and reliable prognostic models in aggressive NHL.[1] After the establishment of rituximab-containing chemotherapy for aggressive B-cell lymphoma, the revised version IPI (R-IPI) has been reported to be a better predictive model than IPI.[21] Here, we also found IPI to be less predictive in the R-CHOP group. Likewise, although several biological prognostic factors using immunohistochemical staining or gene-expression profiling have been proposed,[4,5] many lose their ability to predict prognosis after the induction of rituximab. For example, R-CHOP treatment overcame the poor prognosis of patients with bcl-2 expression, which was a representative biological prognostic factor of poor prognosis in DLBCL when treated with CHOP.[22] However, there are few reliable biological prognostic factors for patients treated with rituximab plus chemotherapy.

sIL-2Rα has been proposed as a prognostic indicator of NHL. sIL-2Rα is widely used because it is simple to measure, but its significance is not completely established. Kono *et al*.[10] reported that serum sIL-2Rα > 1,000 U/mL at diagnosis was associated with a high incidence of treatment failure and poor OS in patients with NHL. Niitsu *et al*.[11] proposed that OS was significantly poorer when the sIL-2Rα level exceeded 2,000 U/mL in patients with aggressive lymphoma. However, these reports were published before the introduction of rituximab. Oki *et al*.[12] reported sIL-2Rα to have prognostic value in 94 DLBCL patients treated with R-CHOP, where sIL-2Rα > 1,000 U/mL was associated with shorter PFS and OS. Ennishi *et al*.[13] also reported that sIL-2Rα > 1,000 U/mL showed significantly poorer PFS and OS in 141 DLBCL patients treated with R-CHOP. Morito *et al*.[14] analyzed the prognosis of DLBCL patients with sIL-2Rα < 1,500 or > 1,500. In this report, we divided the patients into 3 groups according to sIL-2Rα value (< 1,000, 1,000-3,500, > 3,500 U/mL) to estimate the appropriate cut-off value using this large retrospective cohort. As described above, several reports showed sIL-2Rα > 1,000 U/mL to be the cut-off point for prognosis. Our analysis confirmed this level even in patients treated with R-CHOP. Moreover, these three levels of sIL-2Rα were clearly separated in terms of survival for both OS and PFS, with statistical significance. We revealed

that the higher the sIL-2Rα level, the poorer the OS and PFS in patients treated with both R-CHOP and CHOP. Furthermore, patients with sIL-2Rα > 3,500 U/mL (3PFS 47.1%, 3OS 57.1%) might have poor prognoses even with R-CHOP, although we were not able to reveal a significant survival difference between the sIL-2Rα > 3,500 group and the sIL-2Rα 1,000-3,500 group, probably because the number of patients was small. These could be candidates for an alternative treatment approach, including new drugs and stem cell transplantation.

Elevation of sIL-2Rα probably results from its release from lymphoma cells and/or their surrounding reactive inflammatory cells. Various complications, including infections and autoimmune mechanisms, influence its serum level.[8] Concerning lymphoma, the release from neoplastic cells has been reported in B-cell chronic lymphocytic leukemia[23] and anaplastic large cell lymphoma.[24] Furthermore, the correlation between strong positive expression of CD25 on the tumor-cell surface and high levels of serum sIL-2Rα in hairy cell leukemia and adult T-cell leukemia/lymphoma has also been reported. These results probably reflect the release of sIL-2Rα from tumor cells.[25-28] Regarding the mechanism of release, CD25 has been shown on activated T-cells, B-cells, and recently DLBCL cells peeled from the cell surface by a membrane proteolytic mechanism related to matrix metallopeptidase 9.[29-31] On the basis of these findings, we assumed that sIL-2Rα might be higher in CD25-positive DLBCL by release from the lymphoma cell surface ; as such, because CD25-positive patients have higher sIL-2Rα than CD25-negative patients with the same tumor burden, survival might be better at the same level of sIL-2Rα for the CD25-positive group. In our study, CD25 was expressed in about half of patients, and there was no significant difference in terms of patient characteristics between CD25-positive and negative DLBCL. Indeed, sIL-2Rα was significantly higher in the CD25-positive group, as expected, suggesting that some proportion of the serum sIL-2Rα originated from the lymphoma cells. In our analyses, CD25-positive patients had better %OS and %PFS than CD25-negative patients in groups 2 and 3, and we believe that these results somewhat reflect our hypothesis in this analysis. However, we could not show a significant difference in sIL-2Rα level between CD25-positive and -negative patients with low tumor burden (stages 1-2). This probably means that the proportion of sIL-2Rα that originates from lymphoma cells is small. For that reason, we were not able to show a significant difference in survival rate between CD25-positive and negative patients with similar sIL-2Rα levels.

In conclusion, high sIL-2Rα remains an important risk factor with respect to survival and relapse of DLBCL, not only for patients being treated with CHOP-like regimens, but also for those treated with R-CHOP. We revealed the close association between sIL-2Rα level and the expression of CD25 in DLBCL. sIL-2Rα was shown to be a useful biological marker for the prognosis of DLBCL patients, regardless of CD25 expression.

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