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*Original Article*

# Serum Soluble Tumor Necrosis Factor Receptor 1 Level is Associated with the Outcome of Diffuse Large B-Cell Lymphoma Patients Treated with the CHOP or R-CHOP Regimen

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Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease, with patients exhibiting a wide range of outcomes. Many investigators have searched for prognostic factors for DLBCL and, recently, the concentrations of several cytokines were identified to predict the clinical outcome of patients with aggressive non-Hodgkin's lymphomas, including DLBCL. Tumor necrosis factor receptor 1 (TNFR1), which is a member of the TNFR superfamily, has a soluble form (sTNFR1). In this study, we focused on sTNFR1 as a candidate prognostic factor that can be measured readily. We evaluated the prognostic significance of serum sTNFR1 in 213 patients with DLBCL (72 treated with CHOP and 141 with R-CHOP). In the CHOP-treated group, serum sTNFR1 concentration was one of the prognostic factors found. In the R-CHOP group, 5-year overall survival (OS) rates for those having sTNFR1 $\geq$ 4.25 ng/mL and < 4.25 ng/mL were 28.6% and 77.0% ($p < 0.0001$), and 5-year progression-free survival (PFS) rates were 26.7% and 69.2% ($p < 0.0001$), respectively. In multivariate analyses, serum sTNFR1 was an independent prognostic factor for OS and PFS in the CHOP group. In the R-CHOP group, serum sTNFR1 was also an independent prognostic factor for both OS and PFS, as was poor PS for PFS. The prognosis of patients with high-intermediate risk or high risk, according to the International Prognostic Index, who also had high serum sTNFR1, was especially poor. Serum sTNFR1 level is a reliable prognostic factor for patients with DLBCL. 〔*J Clin Exp Hematop 54(2) : 117-127, 2014*〕

**Keywords:** diffuse large B-cell lymphoma, lymphoma, prognostic factor, soluble tumor necrosis factor receptor 1

## INTRODUCTION

The incidence of malignant lymphoma is reported as 1/10,000 people in Japan and exhibits an increasing trend. Diffuse large B-cell lymphoma (DLBCL) accounts for 30-40% of non-Hodgkin's lymphoma (NHL), being the most common lymphoma in the Japanese population. On the basis of factors such as prognosis, pathology, and treatment response, DLBCL is a heterogeneous disease. Thus, the high-risk groups among patients with DLBCL need to be accurately identified to select an appropriate therapeutic strategy. Before the rituximab (R) era, the International Prognostic Index (IPI) was considered the standard index for patients with NHL treated with the CHOP regimen, consisting of cyclophosphamide (CPA), doxorubicin (DOX), vincristine (VCR), and prednisolone (PSL), or similar CHOP-like regimens.[1] Since the introduction of R, the R-CHOP regimen has become the standard treatment for patients with DLBCL.[2-4] However, another concern subsequently arose regarding the utility of previously identified prognostic factors, such as the IPI, for prediction of the outcome of R-CHOP treatment.

Tumor necrosis factor (TNF) is one of the earliest cytokines to be produced in the inflammatory process, and plays a key role in initiating cytokine cascades.[5] Furthermore, TNF

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and its receptors (TNFRs) are also important in the regulation of growth, differentiation, and/or apoptosis of malignant cells in chronic lymphocytic leukemia,[6] hairy cell leukemia,[7] Hodgkin's lymphoma, and NHL.[5] Two types of TNFR have been identified: 55-kDa (p55 TNFR; TNFR1) and 75-kDa varieties (p75 TNFR; TNFR2). While both receptors are simultaneously expressed to varying degrees on many cell types, activation of TNFR1 induces, through its intracellular death domain, the most common TNF responses, such as activation of nuclear factor-κB, cell cytotoxicity, and proliferation, in contrast to TNFR2, which lacks the intracellular death domain.[8] In addition, the extracellular domains of these two receptors can be cleaved into soluble TNFRs (sTNFR1, sTNFR2),[9] and then released into the serum. In this study, we focus on the relationship of sTNFR1, which has been well studied with regard to its activation and mechanisms in the common TNF activity cascade, with the prognosis of DLBCL patients.

**Table 1.** Patient's characteristics

| | CHOP | | R-CHOP | |
|---|---|---|---|---|
| | N = 72 | | N = 141 | |
| Age, Median (range) | 67 (22–80) | | 68 (24–80) | |
| | N | % | N | % |
| Male | 43 | 59.7 | 79 | 56.0 |
| Age > 60 | 47 | 65.3 | 104 | 73.8 |
| PS > 1 | 22 | 30.5 | 29 | 20.6 |
| CS III/IV | 48 | 66.7 | 90 | 63.8 |
| Number of extra nodal sites > 1 | 25 | 34.7 | 54 | 38.3 |
| Elevated LDH | 57 | 79.2 | 96 | 68.1 |
| IPI | | | | |
| Low risk | 10 | 13.9 | 33 | 23.4 |
| Low intermediate risk | 19 | 26.4 | 35 | 24.8 |
| High intermediate risk | 25 | 34.7 | 29 | 20.6 |
| High risk | 18 | 25.0 | 44 | 31.2 |

CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; PS, performance status; CS, clinical stage; IPI, International prognostic index; LDH, lactate dehydrogenase

## METHODS

### *Patients*

A total of 213 consecutive biopsy-confirmed DLBCL patients (122 males, 91 females) who were treated between September 1995 and June 2008 were enrolled in this study. None of the patients had previously been treated for DLBCL and none was infected with the human immunodeficiency virus or human T-cell lymphotropic virus type I. All cases were reclassified according to the World Health Organization (WHO) classification[10] by three pathologists (TT, TY, and NG). R was introduced into our institution in October 2002. Between September 1995 and September 2002, 75 patients received chemotherapy that did not include rituximab (CHOP). Between December 2002 and June 2008, 144 patients received chemotherapy that did include it (R-CHOP). The clinical stage (CS) of the disease was evaluated according to the Ann Arbor classification,[11] based on clinical findings and tumor measurements obtained before excisional biopsy. Mediastinal bulky disease was defined as a mediastinal mass with a maximal diameter exceeding one-third of the maximal chest diameter or any other mass 10 cm or more in maximal diameter. Staging and disease evaluation were based on results of the following procedures: physical examination; chest radiology; computed tomography or magnetic resonance imaging, if necessary, of the brain, neck, chest, abdomen, and pelvis; bone marrow aspiration and biopsy; gallium scintigraphy or fluorodeoxyglucose-positron emission tomography (FDG-PET) with computed tomography; and laboratory measurements of serum aspartate aminotransferase, total bilirubin, alkaline phosphatase, creatinine, lactate dehydrogenase (LDH), and peripheral blood counts. Patients who did not receive chemotherapy for any reason were excluded. Our Institutional Review Board approved the study protocol. Written informed consent was obtained from each patient at study entry. The study was conducted in accordance with the human and ethical principles of research set forth in the Helsinki guidelines. Characteristics of the enrolled patients are presented in Table 1.

### *Serum sTNFR1 determination*

To evaluate serum levels of sTNFR1, venous blood samples were drawn from patients immediately before the initiation of treatment. Serum sTNFR1 was determined using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (BioSource sTNF-R1 ELISA kit, BioSource Europe SA, Nivelles, Belgium). The detection limit of the ELISA test was 0.05 ng/mL. In healthy control subjects (N = 21), the median sTNF-R1 level was 1.88 ng/mL (range 0.6 to 2.56). No significant differences in serum sTNFR1 levels were observed with respect to gender or age (data not shown).

### *Treatment*

Patients in CS I received three cycles of CHOP (N = 9) or R-CHOP therapy (N = 16) followed by involved-field radiotherapy from 30 to 40 Gy. Patients in CS II received six cycles of CHOP (N = 15) or R-CHOP therapy (N = 34). Patients in advanced CS (III or IV) received six or eight cycles of CHOP (N = 48) or R-CHOP therapy (N = 90). In advanced CS cases, except cases refractory to initial treatment, there was no difference in survival between patients who received six or eight cycles of CHOP or R-CHOP. The attending physician made the selection of six or eight cycles of treatment. All cases, except chemotherapy-refractory ones,

completed the previously planned cycles of treatment. The standard CHOP regimen consisted of cyclophosphamide at 750 mg/m$^2$ intravenously (IV), doxorubicin at 50 mg/m$^2$ IV, and vincristine at 1.4 mg/m$^2$ (maximum dose of 2.0 mg) IV on day 1, and then prednisolone at 100 mg orally on days 1 to 5. For patients enrolled after October 2002, R at 375 mg/m$^2$ IV was administered 2 days prior to each cycle of CHOP. After chemotherapy, patients with bulky disease underwent radiotherapy ranging from 30 to 40 Gy. Patients who relapsed or whose disease progressed after CHOP or R-CHOP, and those who were resistant to CHOP or R-CHOP, received the P-IMVP-16/CBDCA (methylprednisolone, ifosfamide, methotrexate, etoposide, and carboplatin) regimen[12] with or without R as a second-line therapy.

### *Response criteria*

Tumor response was evaluated after cycles 2, 4, and 6, and again after the final cycle of chemotherapy. Tumor progression at any of these time points indicated treatment failure. Responses to treatment were categorized after repeated physical examinations, radiological studies, gallium scintigraphy, and bone marrow aspiration, as defined by Cheson *et al*.[13]

### *Statistical analyses*

Data are expressed as the median and range and differences in median values were tested using the nonparametric Mann-Whitney U test and the Bartlett test. The cut-off value for serum sTNFR1 was determined by a receiver operating characteristic analysis. Overall survival (OS) was measured from the start of chemotherapy until death from any cause. Progression-free survival (PFS) was measured from the start of chemotherapy until relapse or death from DLBCL. Univariate analyses of the effects of several pretreatment characteristics upon achieving complete remission (CR) were performed using the chi-square test. Univariate analyses of the effects of several pretreatment characteristics, including sTNFR1, upon survival were performed using the Kaplan-Meier method and log-rank test. A multivariate analysis was performed using the Cox proportional-hazards regression technique. $P$ values $< 0.05$ indicated significance. All statistical analyses were conducted using JMP 7.0.2 (SAS Institute Inc., Cary, NC, USA).

## RESULTS

### *Characteristics of enrolled patients and serum sTNFR1 levels in DLBCL*

In the CHOP group, the median serum sTNFR1 level was 3.32 ng/mL (range 1.06 to 38.31 ng/mL), and in the R-CHOP group, 3.12 ng/mL (range 1.29 to 18.18 ng/mL) (Table 2) (Fig. 1a). Various poor prognostic indicators were strongly associated with high serum sTNFR1 level, as follows: in the CHOP group, poor performance status (PS), advanced CS, and the existence of B symptoms (night sweats, fever, and body-weight loss); and in the R-CHOP group, advanced age, poor PS, elevated LDH, multiple extranodal involvement sites, advanced CS, the existence of B symptoms, and no achievement of CR (Table 2). Serum sTNFR1 levels significantly correlated with an increasing IPI score ($p = 0.0021$ in the CHOP group and $p < 0.0001$ in the R-CHOP group) (Table 2).

### *Cut-off value for serum sTNFR1*

The cut-off value for sTNFR1 was determined by a receiver operating characteristic analysis to be 4.25 ng/mL (Fig. 1b).

### *Serum sTNFR1 on achievement of CR*

The CR rates of patients with sTNFR1 level $< 4.25$ ng/mL and $\geq 4.25$ ng/mL were 79.6% and 57.7% in the CHOP group ($p = 0.0475$) and 83.7% and 55% in the R-CHOP group ($p = 0.0005$), respectively. In addition, the CR rate was significantly worse in patients having multiple extranodal involvement sites ($> 1$), advanced CS (III or IV), presence of B symptoms, unfavorable IPI score (high-intermediate [HI]- or high [H]-risk groups) in the CHOP group, and advanced age, poor PS, elevated LDH, multiple extranodal involvement sites, unfavorable IPI, and poor revised IPI score in the R-CHOP group (Table 3).

### *Univariate analyses for effects of various factors on OS and PFS*

Patients with sTNFR1 level $< 4.25$ ng/mL and $\geq 4.25$ ng/mL had 5-year OS rates of 61.4% and 6.1% in the CHOP group ($p < 0.0001$) and 77.0% and 28.6% in the R-CHOP group ($p < 0.0001$), and PFS rates of 34.5% and 7.7% in the CHOP group ($p < 0.0001$) and 69.2% and 26.7% in the R-CHOP group ($p < 0.0001$), respectively (Table 3; Fig. 1a, 1b; Fig. 2a, 2b). We then analyzed OS and PFS by separating limited- (CSI, II) from advanced-stage patients (CSIII, IV). The limited-stage patients with high sTNFR1 also had poorer OS and PFS than early-stage patients with low sTNFR1 (Fig. 1c, 1d; Fig. 2c, 2d), indicating that those with high sTNFR1 have a poor prognosis even in the limited-stage setting. In the CHOP group, the OS and PFS rates were significantly worse in elderly patients and patients with poor PS, multiple extranodal involvement sites, advanced CS, B symptoms, unfavorable IPI score, and no achievement of CR. Elevated LDH was a prognostic factor only for PFS (Table 3).

**Table 2.** Serum soluble tumor necrosis factor receptor 1 (TNFR 1) level (ng/mL) according to some conventional prognostic factors

| Factor | | CHOP No. | CHOP Median | CHOP Range | CHOP *p*-value | R-CHOP No. | R-CHOP Median | R-CHOP Range | R-CHOP *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| All patients | | 72 | 3.32 | 1.06−38.31 | | 141 | 3.12 | 1.29−18.18 | |
| Gender | | | | | | | | | |
| | Male | 43 | 3.36 | 1.32−38.31 | N.S. | 79 | 3.13 | 1.47−18.18 | N.S. |
| | Female | 29 | 3.32 | 1.06−17.42 | | 62 | 2.97 | 1.29−10.01 | |
| Age | | | | | | | | | |
| | < 60 | 25 | 3.22 | 1.35−17.42 | N.S. | 37 | 2.33 | 1.29−13.51 | < 0. 0001 |
| | ≥ 60 | 47 | 3.675 | 1.06−38.31 | | 104 | 3.7 | 1.29−18.18 | |
| PS | | | | | | | | | |
| | 0, 1 | 50 | 3.15 | 1.06−17.42 | 0.0099 | 112 | 2.795 | 1.29−11.39 | < 0. 0001 |
| | ≥ 2 | 22 | 5.75 | 1.68−38.31 | | 29 | 5.24 | 2.13−18.18 | |
| LDH | | | | | | | | | |
| | Normal | 15 | 3.22 | 1.9 −38.31 | N.S. | 45 | 2.37 | 1.29− 5.81 | 0.0002 |
| | Elevated | 57 | 3.555 | 1.06−17.42 | | 96 | 3.77 | 1.55−18.18 | |
| Extranodal sites | | | | | | | | | |
| | 0, 1 | 47 | 3.285 | 1.06−17.42 | N.S. | 87 | 2.68 | 1.29−12.2 | < 0. 0001 |
| | ≥ 2 | 25 | 3.9 | 1.68−38.41 | | 54 | 4.365 | 1.35−18.18 | |
| CS | | | | | | | | | |
| | I, II | 24 | 2.83 | 1.32− 7.42 | 0.0056 | 51 | 2.46 | 1.29− 5.91 | < 0.0001 |
| | III, IV | 48 | 3.74 | 1.06−38.41 | | 90 | 3.91 | 1.29−18.18 | |
| IPI | | | | | | | | | |
| | Low | 10 | 2.56 | 1.35− 4.25 | 0.0021 | 33 | 2.17 | 1.29− 3.09 | < 0.0001 |
| | Low-Intermedate | 19 | 3.32 | 1.32−17.42 | | 35 | 2.85 | 1.35− 5.91 | |
| | High-Intermedate | 25 | 3.205 | 1.06−10.19 | | 29 | 5.085 | 1.5 −18.18 | |
| | High | 18 | 5.93 | 1.68−38.31 | | 44 | 8.7175 | 2.01−13.51 | |
| B symptom | | | | | | | | | |
| | (−) | 48 | 3.06 | 1.06−11.06 | < 0. 0001 | 94 | 2.735 | 1.35−18.18 | < 0. 0001 |
| | (+) | 24 | 6.61 | 2.26−38.31 | | 47 | 5 | 1.29−13.51 | |
| Therapy response | | | | | | | | | |
| | CR | 52 | 3.29 | 1.06−38.31 | N.S. | 108 | 2.83 | 1.29−13.51 | < 0.0001 |
| | not CR | 20 | 5.53 | 1.68−17.42 | | 33 | 4.5 | 1.98−18.18 | |

CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; PS, performance status; LDH, lactate dehydrogenase; CS, clinical stage; IPI, International prognostic index; CR, complete remission; N.S., no significant difference

In the R-CHOP group, the OS and PFS rates were significantly worse in elderly patients and patients with poor PS, elevated LDH, multiple extranodal involvement sites, B symptoms, unfavorable IPI score, and no achievement of CR. Advanced CS was a prognostic factor for only PFS (Table 3).

### *Multivariate analyses on OS and PFS*

Multivariate analyses employing IPI-related factors and serum sTNFR1 level demonstrated that serum sTNFR1 was an independent prognostic factor for OS and PFS in the CHOP group. In the R-CHOP group, serum sTNFR1 was also an independent prognostic factor for both OS and PFS, as was poor PS for PFS (Table 4).

### *Combination of IPI score and sTNFR1 level on OS and PFS*

As demonstrated above, by univariate analysis, sTNFR1 was closely associated with the prognosis of DLBCL and, by multivariate analysis, had a higher propensity to associate with OS and PFS. Risk categories per IPI score were then divided into low- and high-sTNFR1 groups, resulting in four subgroups as follows: low and low-intermediate risk with high sTNFR1; low and low-intermediate risk with low sTNFR1; high-intermediate and high risk with high sTNFR1; and high-intermediate and high risk with low sTNFR1. As shown in Fig. 3, a highly significant difference was seen among these subgroups in both the CHOP and the R-CHOP groups. In particular, the high-intermediate- and high-risk with high-sTNFR1 subgroup had the poorest prognosis.

## DISCUSSION

Before the R era, age, PS, CS, elevated LDH, and number of extranodal lesions were recognized as conventional prognostic factors incorporated into the IPI for patients with aggressive NHL.[1] The IPI was based on patient characteristics that are directly associated with their condition, such as age and PS, and variables indirectly reflecting tumor biology, such as CS, LDH, and the number of extranodal involvement

**Table 3.** Univariate analyses on remission rate and survival in diffuse large B-cell lymphoma

| Factor | | CHOP | | | | | | | R-CHOP | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | CR rate | | 5-year OS | | 5-year PFS | | No. | CR rate | | 5-year OS | | 5-year PFS | |
| | | | % | *p*-value | % | *p*-value | % | *p*-value | | % | *p*-value | % | *p*-value | % | *p*-value |
| sTNFR 1 | < 4. 25 ng/mL | 47 | 79.6 | 0.0475 | 61.4 | < 0.0001 | 34.5 | < 0.0001 | 103 | 83.7 | 0.0005 | 77.0 | < 0.0001 | 69.2 | < 0.0001 |
| | ≥ 4.25 ng/mL | 25 | 57.7 | | 6.1 | | 7.7 | | 38 | 55.0 | | 28.6 | | 26.7 | |
| Gender | Male | 43 | 68.9 | N.S. | 44.9 | N.S. | 45.2 | N.S. | 79 | 77.0 | N.S. | 63.5 | N.S. | 60.5 | N.S. |
| | Female | 29 | 75.6 | | 37.9 | | 41.5 | | 62 | 74.6 | | 65.7 | | 56.7 | |
| Age | < 60 | 25 | 68.0 | N.S. | 54.6 | 0.026 | 54.7 | 0.0196 | 37 | 94.6 | 0.0005 | 82.3 | 0.0087 | 70.3 | 0.045 |
| | ≥ 60 | 47 | 74.0 | | 36.7 | | 31.8 | | 104 | 69.2 | | 58.6 | | 54.7 | |
| PS | 0, 1 | 50 | 76.5 | N.S. | 51.7 | 0.0005 | 48.6 | 0.0003 | 112 | 84.5 | < 0.0001 | 71.1 | 0.0024 | 66.1 | < 0.0001 |
| | ≥ 2 | 22 | 62.5 | | 25 | | 20.8 | | 29 | 46.8 | | 42.0 | | 28.0 | |
| LDH | Normal | 15 | 86.7 | N.S. | 64.7 | N.S. | 57.3 | 0.0415 | 45 | 95.6 | < 0.0001 | 81.1 | 0.0188 | 68.8 | 0.03 |
| | Elevated | 57 | 68.3 | | 37.4 | | 35.1 | | 96 | 67.0 | | 56.9 | | 53.6 | |
| Extranodal Sites | 0, 1 | 47 | 81.3 | 0.0188 | 56 | 0.0085 | 51.7 | 0.0014 | 87 | 82.4 | 0.02 | 72.4 | 0.0242 | 65.6 | 0.0192 |
| | ≥ 2 | 25 | 55.6 | | 18.7 | | 18.5 | | 54 | 66.1 | | 52.5 | | 45.7 | |
| CS | I, II | 24 | 95.8 | 0.0004 | 68.8 | 0.0306 | 64.7 | 0.0395 | 51 | 82.7 | N.S. | 74.8 | N.S. | 70.1 | 0.0263 |
| | III, IV | 48 | 60.8 | | 30.7 | | 27.9 | | 90 | 71.4 | | 57.9 | | 51.3 | |
| IPI | Low | 10 | 100.0 | 0.0106 | 100 | < 0.0001 | 100 | < 0.0001 | 33 | 96.9 | < 0.0001 | 86.8 | 0.0032 | 76.9 | 0.0009 |
| | Low-Intermedate | 19 | 73.7 | | 45.3 | | 39.9 | | 35 | 80.6 | | 68.6 | | 63.0 | |
| | High-Intermedate | 25 | 76.9 | | 44.1 | | 40.1 | | 29 | 82.8 | | 62.8 | | 64.2 | |
| | High | 18 | 50.0 | | 10.9 | | 10 | | 44 | 52.2 | | 44.9 | | 36.6 | |
| B symptom | (−) | 48 | 79.2 | 0.0303 | 59.1 | < 0.0001 | 55 | < 0.0001 | 94 | 80.3 | N.S. | 66.2 | N.S. | 58.4 | N.S. |
| | (+) | 24 | 54.2 | | 9.4 | | 8.8 | | 47 | 68.1 | | 58.2 | | 53.4 | |
| Therapy response | CR | 52 | | | 60.1 | < 0.0001 | 55.2 | < 0.0001 | 108 | | | 78.9 | < 0.0001 | 70.8 | < 0.0001 |
| | not CR | 20 | | | 0 | | 0 | | 33 | | | 19.0 | | 17.4 | |

| | | No. | CR rate | *p*-value | 5-year OS | *p*-value | 5-year PFS | *p*-value |
|---|---|---|---|---|---|---|---|---|
| Revised IPI | Very Good | 7 | 100.0 | 0.0009 | 100.0 | 0.011 | 68.6 | 0.0295 |
| | Good | 61 | 87.0 | | 74.7 | | 70.3 | |
| | Poor | 73 | 64.0 | | 52.1 | | 47.7 | |

sTNFR 1, soluble tumor necrosis factor receptor 1; PS, performance status; LDH, lactate dehydrogenase; CS, clinical stage; IPI, International prognostic index; CR, complete remission; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; OS, overall survival; PFS, progression-free survival; N.S., no significant difference;

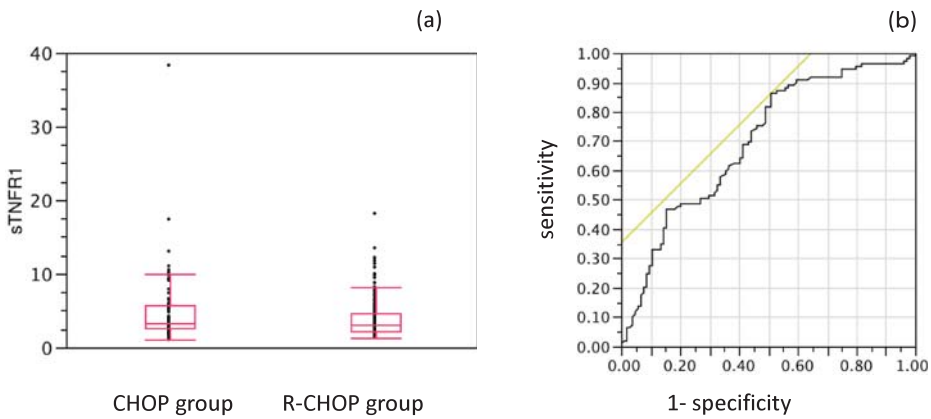


**Fig. 1.** Receiver operating characteristic curve and distributions of the value of measured soluble tumor necrosis factor receptor 1 (TNFR 1). (***1a***) Receiver operating characteristic curve shows that 4.25 ng/mL is a suitable cut-off (sensitivity 0.86, 1-specificity 0.3528). (***1b***) Distributions of the value of measured sTNFR 1 are shown (CHOP group, R-CHOP group).

**Table 4.** Multivariate analyses on overall survival and progression free survival in diffuse large B-cell lymphoma patients

| **CHOP** | Odd's ratio | 95% confidence interval | | | *p*-value |
|---|---|---|---|---|---|
| **< OS >** | | | | | |
| High sTNFR 1 | 3.99 | 1.97 | - | 8.1 | 0.0001 |
| Age > 60 | 1.45 | 0.7 | - | 3.16 | 0.318 |
| Extranodal sites > 1 | 1.12 | 0.54 | - | 2.4 | 0.757 |
| PS > 1 | 1.51 | 0.73 | - | 3.09 | 0.26 |
| Advanced CS | 1.83 | 0.81 | - | 4.21 | 0.14 |
| **< PFS >** | | | | | |
| Extranodal sites > 1 | 1.12 | 0.53 | - | 2.4 | 0.8895 |
| High sTNFR 1 | 3.63 | 1.81 | - | 7.35 | 0.0003 |
| Age > 60 | 1.51 | 0.72 | - | 3.31 | 0.664 |
| Elavated LDH | 2.22 | 0.97 | - | 6.02 | 0.451 |
| PS > 1 | 1.64 | 0.79 | - | 3.4 | 0.608 |
| Advanced CS | 2.17 | 0.93 | - | 5.21 | 0.461 |

| **R-CHOP** | Odd's ratio | 95% confidence interval | | | *p*-value |
|---|---|---|---|---|---|
| **< OS >** | | | | | |
| High sTNFR 1 | 2.89 | 1.44 | - | 5.89 | 0.0029 |
| Extranodal sites > 1 | 1.03 | 0.53 | - | 2.03 | 0.6779 |
| Age > 60 | 2.11 | 0.94 | - | 5.66 | 0.0725 |
| Elavated LDH | 1.19 | 0.53 | - | 2.87 | 0.6779 |
| PS > 1 | 1.52 | 0.76 | - | 2.94 | 0.6228 |
| **< PFS >** | | | | | |
| High sTNFR 1 | 2.39 | 1.26 | - | 4.56 | 0.008 |
| Elavated LDH | 0.88 | 0.43 | - | 1.83 | 0.7498 |
| PS > 1 | 2.15 | 1.16 | - | 3.93 | 0.0164 |
| Extranodal sites > 1 | 1.11 | 0.6 | - | 2.05 | 0.7498 |
| Age > 60 | 1.56 | 0.8 | - | 3.34 | 0.2001 |

CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; OS, overall survival; sTNFR 1, soluble tumor necrosis factor receptor 1; PS, performance status; CS, clinical stage; PFS, progression-free survival; LDH, lactate dehydrogenase; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone

lesions. Beginning in 2000, using a cDNA microarray profiling method, patients with DLBCL were divided into two groups: those with a germ center B-cell type (GCB type) and those with an activated B-cell-like type (ABC type).[14] The ABC-type patients had poorer prognosis than the GCB-type patients treated without R. Currently, the DNA microarray profiling method for patients with DLBCL is not available in most diagnostic pathology departments. Instead, immunohistochemistry is often used clinically as a prognostic tool. For example, Hans *et al*.[15] showed that immunohistochemistry analysis for CD10, Bcl-6, and MUM-1 could be used to classify DLBCL into GCB and non-GCB subgroups, including ABC types, and was prognostically correlated with the groups defined by the cDNA microarray method. This immunohistochemical diagnostic method has been followed in some other studies.[16-19]

In the R era, prognostic factors were re-examined, and the utility of the IPI was validated in the R era by Ziepert *et al*.[20] They reported that IPI retained its prognostic value in three prospective clinical trials with varying sample sizes. Sehn *et al*.[21] reported that the revised IPI can discriminate patients with DLBCL into three prognostic groups : very good, good, and poor. However, both investigator teams reported that OS of the poor group was approximately 50%. In order to select more strictly appropriate indications for various conventional, high-dose, and dose-dense regimens, including stem cell transplantation, the poorer prognostic groups (i.e., HI- and H-risk groups according to the IPI before the R era) need to be discriminated with additional prognostic factors. As for DLBCL subtyping, the difference in the prognostic significance between the GCB type and the ABC type remains controversial in the R era.[22-25]

Examination of the cancer microenvironment has recently become a focus of clinical research. After performing clinical analyses on prognostic factors, some investigators suggested that serum levels of cytokines and their soluble receptors might reflect tumor growth and host responses.[26,27] Recently, many studies on DLBCL by gene expression profiling have been carried out, and characteristic gene expression of microenvironment cells was also identified in a similar manner to that of tumor cells. In whole-genome arrays and multiple clustering analyses reported by Monti *et al*.[28] and microarray

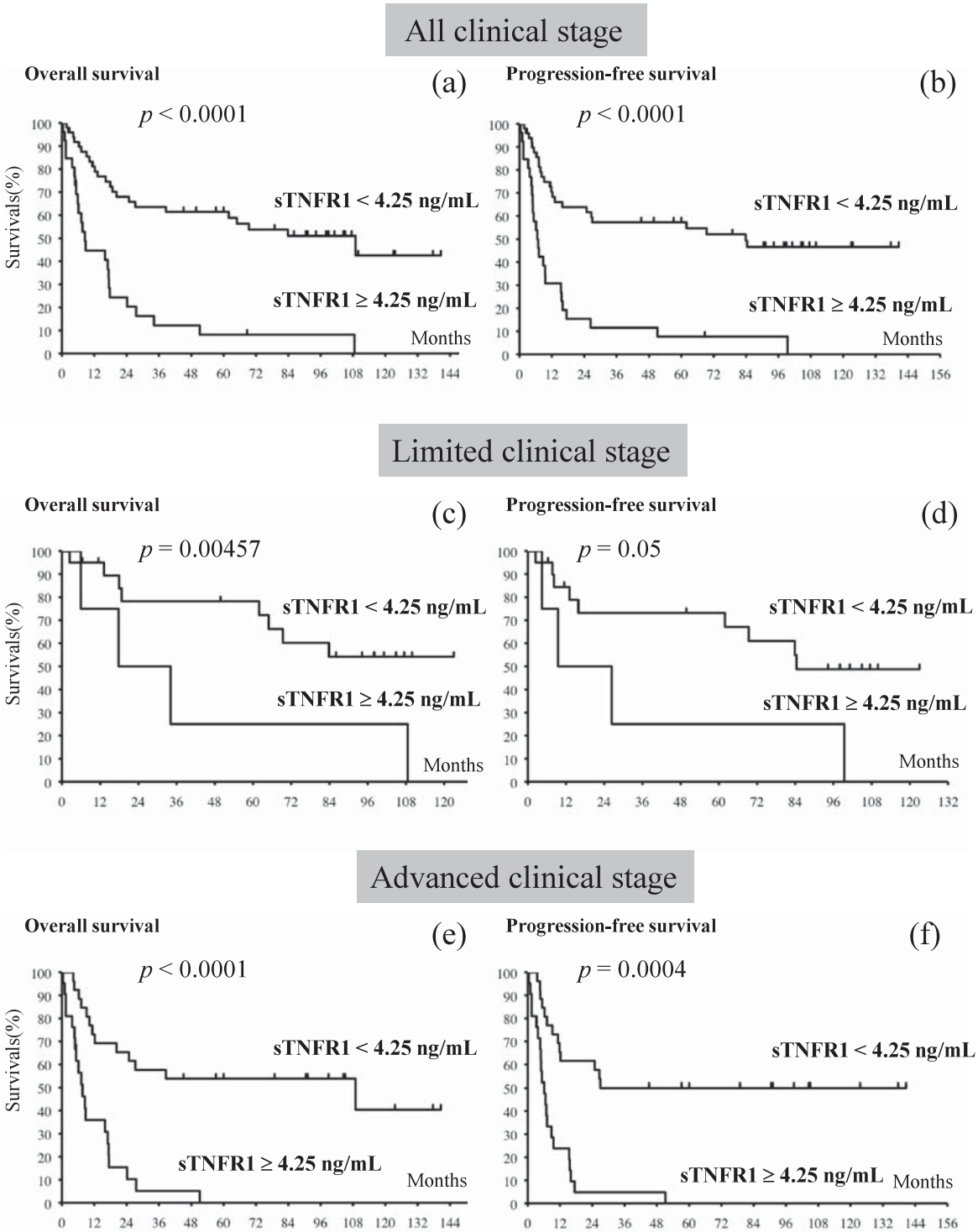


**Fig. 2.** Overall survival (OS) and progression-free survival (PFS) for diffuse large B-cell lymphoma (DLBCL) patients treated with CHOP. OS (*2a*) and PFS (*2b*) curves for DLBCL patients treated with CHOP using serum soluble tumor necrosis factor receptor 1 (TNFR1) levels of < 4.25 ng/mL and ≥ 4.25 ng/mL ($p < 0.0001$ for OS; $p < 0.0001$ for PFS). OS (*2c*) and PFS (*2d*) curves for patients with limited clinical stage ($p = 0.00457$ for OS; $p = 0.05$ for PFS). OS (*2e*) and PFS (*2f*) curves for patients with advanced clinical stage ($p < 0.0001$ for OS; $p = 0.0004$ for PFS).

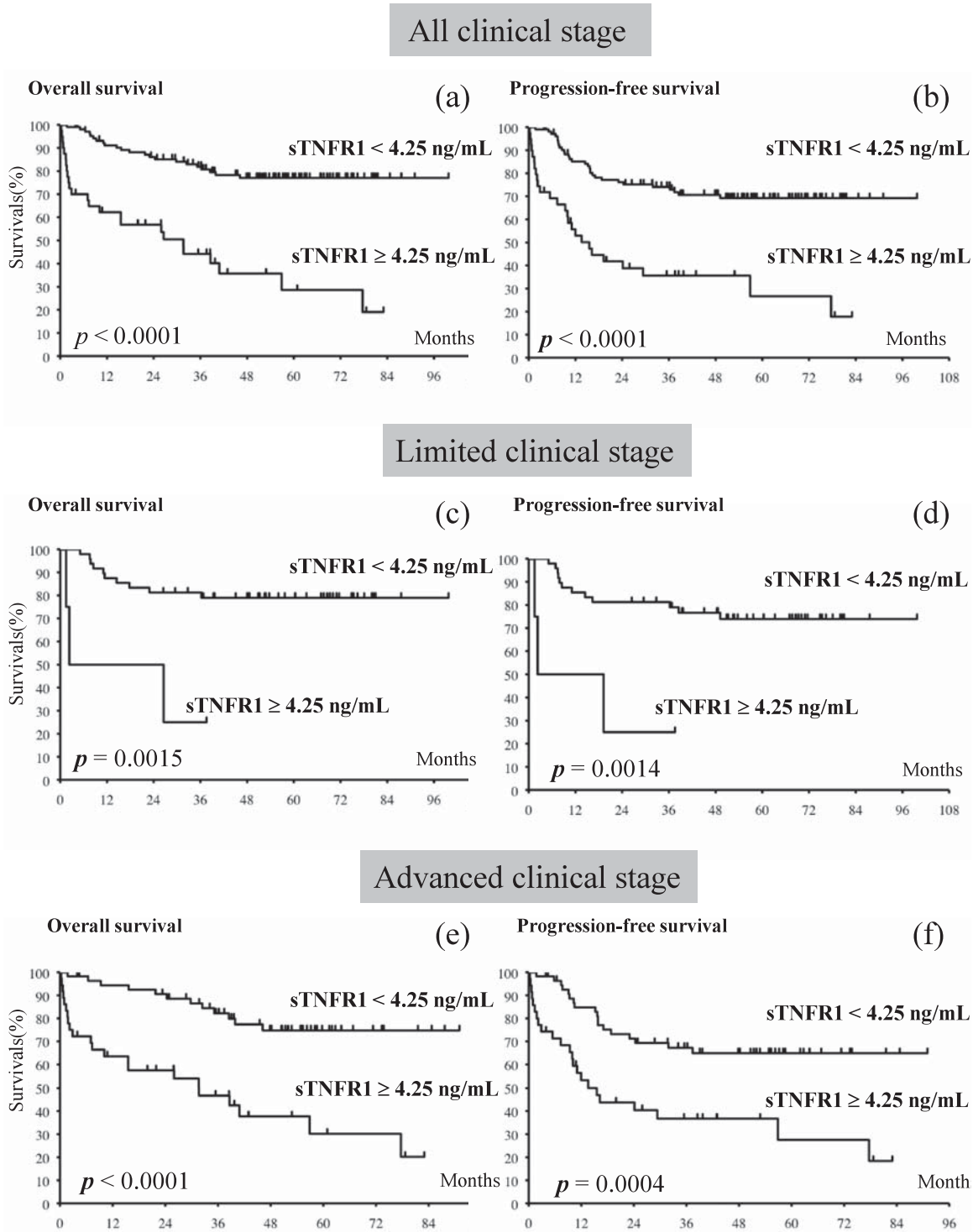


**Fig. 3.** Overall survival (OS) and progression-free survival (PFS) for diffuse large B-cell lymphoma (DLBCL) patients treated with R-CHOP. OS (*3a*) and PFS (*3b*) curves for DLBCL patients treated with R-CHOP using serum soluble tumor necrosis factor receptor 1 (TNFR1) levels of < 4.25 ng/mL and ≥ 4.25 ng/mL ($p < 0.0001$ for OS; $p < 0.0001$ for PFS). OS (*3c*) and PFS (*3d*) curves for patients with limited clinical stage ($p = 0.0015$ for OS; $p = 0.0014$ for PFS). OS (*3e*) and PFS (*3f*) curves for patients with advanced clinical stage ($p < 0.0001$ for OS; $p = 0.0004$ for PFS).

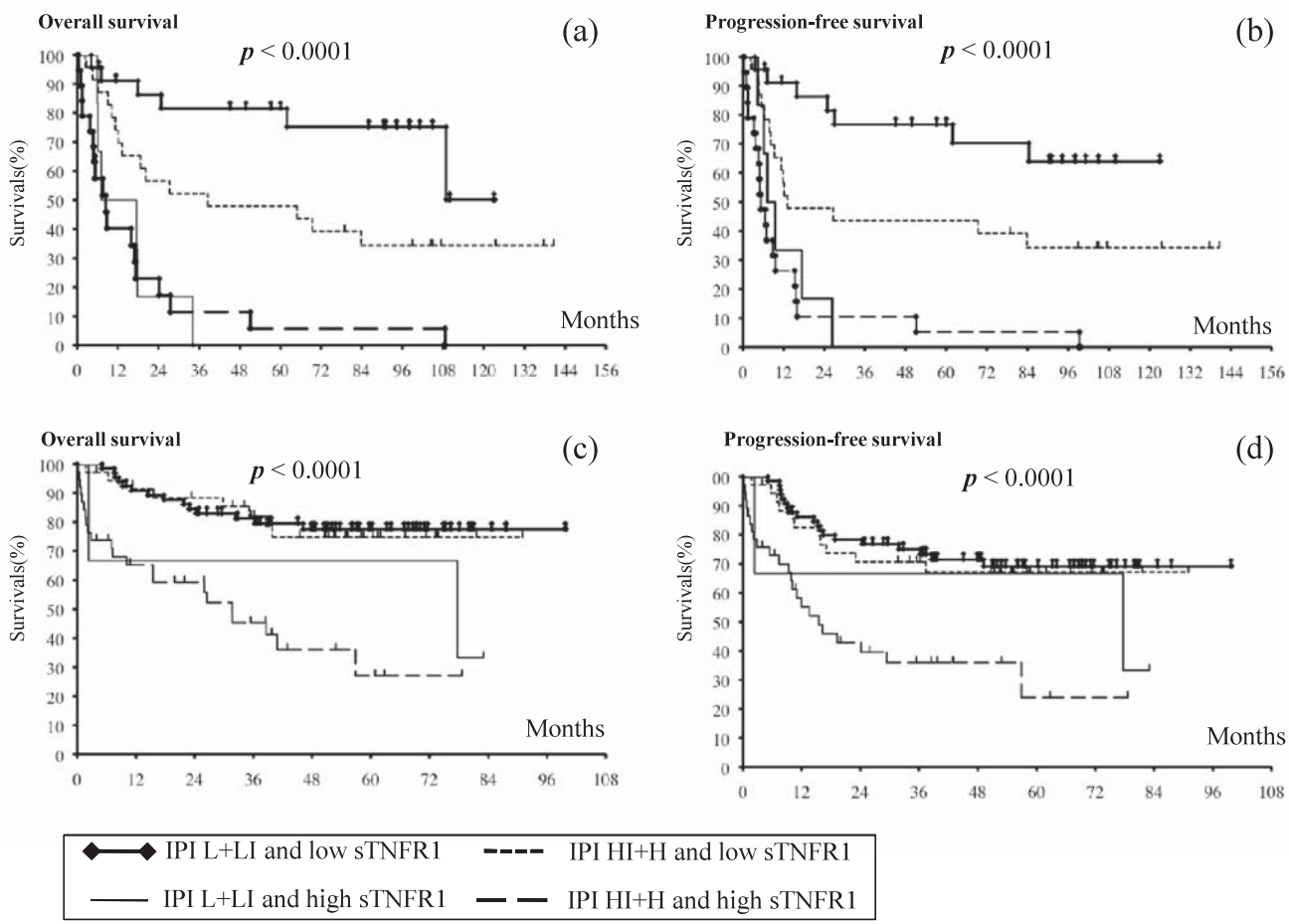


**Fig. 4.** OS (*4a*) and PFS (*4b*) curves in patients with diffuse large B-cell lymphoma (DLBCL) treated with CHOP, classified according to the combination of International Prognostic Index (IPI) results and soluble tumor necrosis factor receptor 1 (sTNF-R1) level. OS (*4c*) and PFS (*4d*) curves in DLBCL patients treated with R-CHOP, classified according to the combination of IPI results and sTNF-R1 level. L, low-risk group; LI, low-intermediate-risk group; HI, high-intermediate-risk group; H, high-risk group

expression profiling of apoptosis-related genes reported by Muris *et al*.,[29] TNFRSF1A, the gene for TNFR1, was identified in the DLBCL cohort. In both reported studies, TNFRSF1A was found to be upregulated as a molecule related to an immune response rather than to the tumor growth per se. Therefore, we hypothesized that serum sTNFR1 was related to an immune reaction against lymphoma. TNFRSF1B, the gene of TNFR2, and the TNF receptor-associated death domain (TRADD) gene are also similarly expressed in DLBCL. TRADD is an intracellular death domain that directly binds to only TNFR1, but not to TNFR2. This finding suggests that common activation of the TNF cascade through TNFR1 is closely related to the immune response against the tumor. Soluble TNFR1 is shed from the cell surface due to cell activation,[8] thus reflecting the immune response. The IPI contains factors that reflect tumor growth (CS, LDH, extranodal sites) and factors that reflect a patient's ability to tolerate intensive therapy (age and PS), but no factors that reflect the immune response to lymphoma. In our analysis, sTNFR1 was a strong and independent prognostic factor from each index of IPI both before and after the R era. In addition, we found a more prognostically poor subgroup (high-intermediate and high risk by IPI score and high-serum sTNFR1) by using a combination of sTNFR1 and IPI results, even in the R-CHOP group. Refractory or relapsed cases after R-CHOP do not have a good response to salvage therapy.[30-32] If we could predict refractory or relapsed cases before initial R-CHOP therapy, these cases could possibly be given an alternative therapy for improved prognosis.

It is difficult to use sTNFR1, which is a continuous variable, to determine a precise prognostic cut-off value. However, its measurement, as a prognostic factor, is simple and inexpensive when compared with gene expression profiling and FDG-PET. Therefore, we think those problems of

cut-off determination could be resolved by examining an increased number of cases.

In conclusion, serum sTNFR1 might be a significant prognostic factor for patients with DLBCL treated with either CHOP or R-CHOP regimens. The current findings should be confirmed in other patient cohorts in the future in order to reach more definitive conclusions regarding differences among various patient subgroups. The most reliable prognostic factor and the best combination of some prognostic factors for DLBCL should be further clarified in order to assist in selecting the appropriate rituximab-based treatment regimen.

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