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Development of a Prognostic Scoring System using MYC Expression and Soluble Interleukin Receptor -2 level for Diffuse Large B-cell Lymphoma

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ABSTRACT

Background Diffuse large B-cell lymphoma, not otherwise specified (DLBCL-NOS), is the most frequent type of lymphoid neoplasm.

Methods We investigated the relationships between clinical factors of DLBCL-NOS and MYC immunohistochemistry (IHC) staining.

Results A total of 110 patients diagnosed with DLBCL-NOS from 2012 to 2020 at Tottori University Hospital and treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) chemotherapy were included. IHC staining of MYC in formalin-fixed, paraffin-embedded tumor specimens was performed, and ROC-curve analysis revealed the cut-off value of the MYC positive rate as 55%. The 2-year overall survival (OS) rates of the MYC-negative and -positive groups were 84.7% vs 57.7% ($P = 0.0091$), and the progression-free survival rates were 77.8% vs 54.7% ($P = 0.016$), respectively. Multivariate analysis for OS showed prognostic significance of MYC positivity [hazards ratio (HR): 2.496; $P = 0.032$], and serum levels of soluble interleukin-2 receptor (sIL-2R) > 2000 U/mL (HR: 3.950; $P = 0.0019$), as well as age > 75 (HR: 2.356; $P = 0.068$). The original scoring system was developed based on these findings. By assigning one point to each item, age (> 75), MYC positivity, and sIL-2R level (> 2000), all patients were classified into three risk categories: group 1 (0 points), group 2 (1 point), and group 3 (2–3 points). The 2-year survival rates were 100%, 83.0%, and 47.1% for the groups 1, 2, and 3, respectively ($P < 0.0001$).

Conclusion We suggest that a prognostic scoring system using MYC expression and soluble interleukin receptor -2 level is useful for the prediction of prognosis, contributing to further stratification in DLBCL-NOS.

Key words diffuse large B-cell lymphoma, not otherwise specified; immunohistochemistry; MYC, Proto-Oncogene Proteins; non-Hodgkin lymphoma; R-CHOP chemotherapy

DLBCL-NOS is the most frequent type of lymphoid neoplasm and accounts for 31–34% of non-Hodgkin lymphoma.[1] It is a heterogeneous disease in terms of immunophenotype, genetic aberrations, and clinical course.[2] Although the rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) chemotherapy regimen has been the gold standard for treatment for a long time, patients still exhibit poor prognosis; thus, improvement of treatment strategies is needed.

MYC, an oncogene located at 8q24, plays a key role in cell proliferation, apoptosis, glucose metabolism, adhesion, and angiogenesis.[3] *MYC* transcription is tightly controlled to balance cell proliferation and maintenance. Upregulation of *MYC* activation has been identified in numerous types of malignancies, including lung, breast, and colon cancer and malignant lymphoma. This oncogene plays an important role in the pathogenesis of aggressive lymphomas through several mechanisms, evident by chromosomal translocation and increased copy number. In particular, it is associated with translocation of the immunoglobulin heavy chain (*IGH*) or the immunoglobulin light chain (*IGL*) gene, resulting

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Abbreviations: DEL, double-expressor lymphoma; DHL, double-hit lymphoma; DLBCL-NOS, diffuse large B-cell lymphoma, not otherwise specified; FISH, Fluorescence *in situ* hybridization; GCB, germinal center B-cell; HR, hazards ratio; IGH, immunoglobulin heavy chain; IGL, immunoglobulin light chain; IHC, immunohistochemistry; IPI, international prognostic index; LDH, lactate dehydrogenase; NCCN, National Comprehensive Cancer Network; OS, overall survival; PFS, progression-free survival; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone; R-IPI, the revised international prognostic index; ROC, receiver operating characteristic; sIL-2R, soluble interleukin-2 receptor; THL, triple-hit lymphoma

in treatment resistance, for example, in patients with double-hit lymphoma (DHL) and triple-hit lymphoma (THL).[4] Furthermore, overexpression of MYC protein even without translocation leads to aggressive disease pathogenesis and treatment resistance. Therefore, we hypothesized that MYC expression is an independent poor prognostic factor of DLBCL-NOS. This study aimed to investigate the relationship between clinical factors of DLBCL-NOS and MYC-positivity by immunohistochemical (IHC) analysis.

Furthermore, prognostic classification tools such as the international prognostic index (IPI),[5] the revised international prognostic index (R-IPI),[6] and the National Comprehensive Cancer Network IPI (NCCN-IPI)[7] have been used widely to date. They include information regarding the age, disease stage, involvement of extranodal sites, and clinical biomarkers, but not the expression of MYC. We attempted to generate a prognostic scoring system which includes MYC expression. It is essential that the novel scoring system is simple, portable, and more conducive to stratification. We attempted to develop a novel prognostic model, one that includes MYC expression, based on the analysis of MYC-positivity and clinical features.

MATERIALS AND METHODS

Patients

We included all 110 patients diagnosed with DLBCL-NOS, who were administered R-CHOP chemotherapy at our institution (Tottori University Hospital), from April 2012 to March 2020. Patients diagnosed with T-cell/histocyte-rich large B-cell lymphoma, primary DLBCL of the central nervous system, primary cutaneous DLBCL, or primary mediastinal large B-cell lymphoma, leg type, were excluded from this study. Patients with secondary DLBCL such as Richter syndrome, transformation of follicular lymphoma, and indolent B-cell lymphoma were excluded. Patients with known DHL or THL were also excluded. Clinical information, excluding personal information, was collected from the electronic medical record system.

This study was approved by the Ethical Review Committee of Tottori University School of Medicine (the ethical approval number is 20A045). The research was conducted on an opt-out basis.

Immunohistochemical staining and clinical parameters

We performed immunohistochemical staining of formalin-fixed, paraffin-embedded tumor specimens of all patients. Tumor specimens were cut into 4 μm-thick sections, subjected to heat-induced antigen retrieval in EDTA buffer at pH 9.0 (Nichrei Bioscience, Tokyo, Japan), and heated for 40 min in an automated retrieval device (Nichirei HEAT PRO II, Nichrei Bioscience). Immunological staining was performed by incubating the samples with rabbit monoclonal anti-human MYC antibody (clone: Y69, #ab32072, Abcam, Cambridge, U.K.) for 60 min at 15 °C in a 1:200 dilution. An automated immunohistochemistry staining system (Nichirei-Histostainer, Nichrei Bioscience) was used for staining and color development by reacting with diaminobenzidine solution (Nichrei Bioscience) for 10 min.

Cells with positive nuclear findings for MYC immunostaining were defined as MYC-positive. The percentage of positive cells in the total tumor cells was defined as the MYC-positive rate. The MYC-positive rate was evaluated independently by two individual evaluators, and the mean value was considered the final value.

Immunostaining of proteins other than MYC had been performed as routine diagnostic workup at our hospital and the data were retrieved from all patients' medical records. These proteins investigated in the present study and their antibodies for immunostaining are as follows: CD5 (clone: SP19, Roche, Switzerland), CD10 (SP67, Roche), CD20 (L26, Roche), BCL2 (SP66, Roche), BCL6 (GI191E/A8, Roche), and MUM-1 (MRQ-43, Roche). The cut-off points for BCL2, BCL6, and MUM-1 were 50%, 30%, and 30%, respectively. Staining of CD10, BCL6, and MUM-1 was performed for the classification of germinal center B-cell (GCB) or non-GCB types based on Hans classification.[8]

Besides other clinical parameters, serum lactate dehydrogenase (LDH) and soluble interleukin-2 receptor (sIL-2R) levels were measured at our institutions. Fluorescence *in situ* hybridization (FISH) for IGH/MYC translocation and chromosome analysis (G-banding) were performed by the SRL company (Tokyo, Japan).

Statistical analysis

Overall survival (OS) was defined as the time from the date of diagnosis until the date of death by any cause or last follow-up, and progression-free survival (PFS) was defined as the time from the date of diagnosis until the date of first progression, relapse, death by any cause, or last follow-up. To evaluate the relationship between the MYC-positive rate and survival events, a significant cut-off value for sensitivity and specificity was determined using receiver operating characteristic (ROC) curve analysis. The cut-off point of the sIL-2R level was determined in the same manner. Fisher's exact test was used to evaluate the patients' clinical background. The probabilities of OS and PFS were estimated according

to the Kaplan–Meier method. Univariate and multivariate analyses for survival were performed using Cox proportional hazards regression analysis, and only variables with $P < 0.05$ in univariate analysis for OS were included in the multivariate analysis. Statistical significance was set at $P < 0.05$. Statistical analyses were performed with EZR Version1.55 (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[9]

RESULTS

Clinical characteristics of all patients

A total of 110 patients (64 males and 46 females) diagnosed with DLBCL-NOS and treated with R-CHOP chemotherapy were included in the analysis. A summary of baseline characteristics of all patients is shown in Table 1. The median age of all patients was 73.5 years (range: 32–99 years). The median follow-up period for all patients was 38.3 months (range: 1.6–100 months).

Fifty-four patients (49.1%) were in the advanced stage of the disease (Ann Arbor stage: III and IV),[10] seven patients (6.4%) developed B symptoms (mainly fever), whereas eleven patients (10.0%) had "bulky" disease (defined by lesions greater than 10 cm in size). Fifty-one patients (46.4%) had one or more extranodal lesions. LDH levels were above the upper normalized value (222 U/L) in 65 patients (59.1%) (median: 266 U/L; range: 136–15598 U/L). Furthermore, sIL-2R levels were above the upper normalized value (474 U/L) in 83 patients (75.5%) (median: 1236 U/mL; range: 310–48490 U/mL).

According to the revised international prognostic index (R-IPI) category,[6] 28 patients (25.5%) were in the very good-risk group (R-IPI score 0–1), 31 patients (28.2%) were in the good-risk group (score 2), and 51 patients (46.4%) were in the poor-risk group (score 3–5).

Survival analysis

The 2-year overall survival (OS) rate of all 110 patients was 79.3% [95% confidence interval (CI): 70.2–85.5%], and the 2-year progression-free survival (PFS) rate was 73.1% (63.7–80.5%; Figs. 1a and b). Patients aged over 75 years had a lower OS rate of 66.0% (50.1–78.0%) vs 88.7% for younger patients (77.8–94.5%), and a lower PFS rate of 62.4% (46.6–74.7%) vs 80.9% for younger patients (68.8–88.7%). The OS and PFS rates of the R-IPI categories were as follows: very good group, 2-year OS 92.9% (95%CI: 74.3–98.2%); good group, 2-year OS 79.8% (60.5–90.4%); and poor group, 2-year OS 71.1% (56.1–81.8%) ($P = 0.058$). The 2-year PFS rates of the three groups were 92.9% (74.3–98.2%), 76.8% (57.5–88.2%), and 59.7% (44.7–71.8%), with $P = 0.004$, respectively.

Immunohistochemical evaluation of MYC

Immunostaining of MYC was performed for the 110 cases. A dot plot of MYC-positive rates and representative images of MYC immunostaining are shown in Fig. 2, Figs. 3a and b, respectively. The ROC curve was used to evaluate the performance of the MYC-positive rate for survival events, and the cut-off value of the MYC-positive rate was set at 55% (Fig. 4), which was consistent with previously set cut-off values of 40–70%.[11, 12] The MYC-positive group included patients with ≥ 55% MYC-positive tumor cells, and the MYC-negative group included those with < 55% MYC-positive tumor cells. The characteristics of patients in each group are summarized in Table 1. Using Fisher's exact test, no significant difference was observed between the two groups in terms of age ($P = 0.419$), sex ($P = 0.816$), performance status > 1 ($P = 1.00$), Ann Arbor stages III & IV ($P = 0.64$), LDH levels ($P = 0.133$), sIL-2R levels ($P = 0.245$), and R-IPI groups ($P = 0.182$).

The outcomes of the MYC-positive and MYC-negative groups were compared using Kaplan–Meier analysis. The 2-year OS rates in the MYC-negative group and MYC-positive group were 84.7% (95% CI: 75.1–90.8%) and 57.7% (34.4–75.4%), respectively, and the 2-year PFS rates in these two groups were 77.8% (95% CI: 67.5–85.3%) and 54.7% (32.2–72.5%), respectively (Figs. 5 a and b). The differences in the 2-year OS rate and the 2-year PFS rate between these two groups were statistically significant (P=0.0091 and P=0.0162, respectively).

Other immunostainings and clinical parameters

Immunostaining of BCL2, BCL6, MUM-1, CD5, CD10, and CD20 was also performed. A total of 74 patients were BCL2-positive (76.2% of all evaluable cases), 70 were BCL6-positive (73.7%), 72 were MUM-1 positive (75.8%), and 29 were CD10-positive (26.9%). There was no clear tendency for MYC-positivity between the BCL2-, BCL6-, MUM-1-, and CD10-positive and -negative groups. Association of CD5 and CD20 positivity with MYC-positivity was also evaluated, but the sample size was too small to conduct such an analysis; there was only one CD20-negative case and eight CD5-positive cases.

According to the Hans classification,[8] 34 cases of GCB-type and 67 non-GCB cases (9 cases were not determinable due to lack of staining) were observed, and the survival rates were similar in both groups and no statistically significant difference was found. Regarding double-expressor lymphoma (DEL), defined as double-positive for BCL2 and MYC, there was no statistically significant difference in the OS and PFS rates between

Table 1. Patient characteristics

Variables	Total (n = 110)	MYC-negative (n = 87)	MYC-positive (n = 23)	P value
Age, median (range)	73.5 (32–99)	73 (32–87)	76 (57–99)	0.419
Sex (%)				
Female	46 (41.8)	37 (42.5)	9 (39.1)	0.816
Male	64 (58.2)	50 (57.5)	14 (60.9)	
PS (%)				
0–1	77 (70.0)	61 (70.1)	16 (69.6)	1.000
2–4	33 (30.0)	26 (29.9)	7 (30.4)	
Ann Arbor stage (%)				
I-II	55 (50.5)	45 (51.7)	10 (45.5)	0.64
III-IV	54 (49.5)	42 (48.3)	12 (54.5)	
Bulky mass (%)				
Yes	7 (6.4)	7 (8.0)	0 (0.0)	0.341
No	103 (93.6)	80 (92.0)	23 (100.0)	
B symptom (%)				
Yes	11 (10.0)	10 (11.5)	1 (4.3)	0.453
No	99 (90.0)	77 (88.5)	22 (95.7)	
DLBCL type (%)				
GCB	34 (33.7)	28 (35.4)	6 (27.3)	0.612
non-GCB	67 (66.3)	51 (64.6)	16 (72.7)	
LDH, median (range)	266 (136–15598)	262 (136–5721)	315 (157–15598)	0.133
sIL-2R, median (range)	1236 (310–48490)	1139 (143–23721)	1236 (309–48490)	0.245
R-IPI (%)				
Very good	28 (25.5)	24 (27.6)	4 (17.4)	0.182
Good	31 (28.2)	21 (24.1)	10 (43.5)	
Poor	51 (46.4)	42 (48.3)	9 (39.1)	
BCL2 (%)				
positive	72 (75.8)	58 (77.3)	14 (70.0)	0.560
negative	23 (24.2)	17 (22.7)	6 (30.0)	
BCL6 (%)				
positive	69 (73.4)	53 (72.6)	16 (76.2)	1.000
negative	25 (26.6)	20 (27.4)	5 (23.8)	
MUM-1				
positive	70 (76.0)	54 (76.1)	16 (76.2)	1.000
negative	22 (23.9)	17 (23.9)	5 (23.8)	
FISH IGH-MYC (%)				
positive	4 (16.0)	1 (5.0)	3 (60.0)	0.0162
negative	21 (84.0)	19 (95.0)	2 (40.0)	

FISH, fluorescence *in situ* hybridization; GCB, germinal center B cell; IGH, immunoglobulin heavy chain gene; LDH, lactate dehydrogenase; PS, performance status; R-IPI, revised international prognostic index; sIL-2R, soluble interleukin-2 receptor.

14 DEL patients and others.

For the evaluation of IGH-MYC-translocation, FISH analysis was performed for the samples of 25 cases, 4 of which were positive. Three cases had a MYC-positivity rate of 55% or higher, while only one MYC-negative case showed the fusion signal.

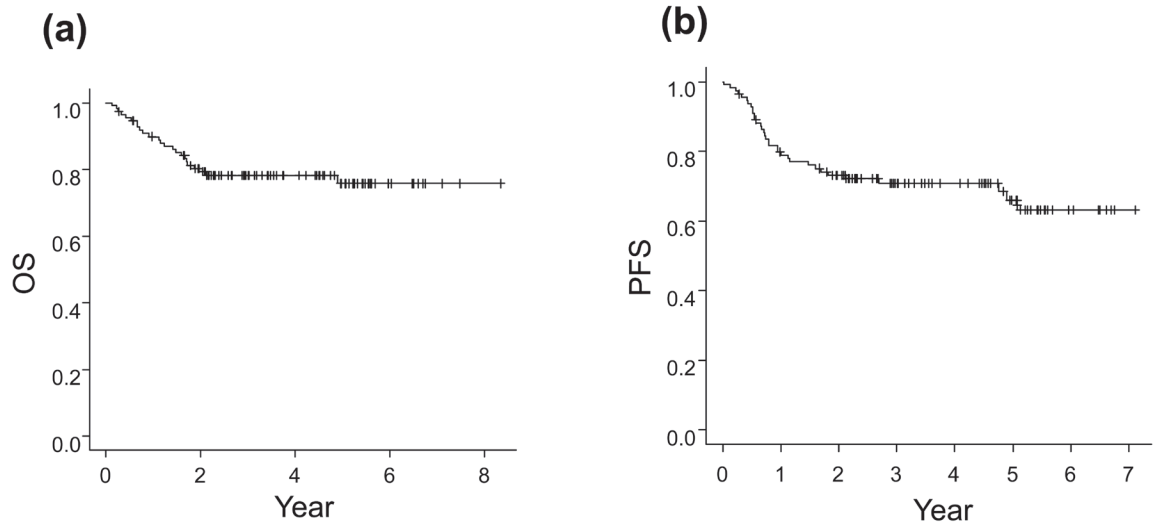

Fig. 1. Survival rate of all the patients. (**a**) Overall survival (OS). 2-year OS was 79.3%. (**b**) Progression-free survival rate (PFS). The 2-year PFS rate was 73.1%.

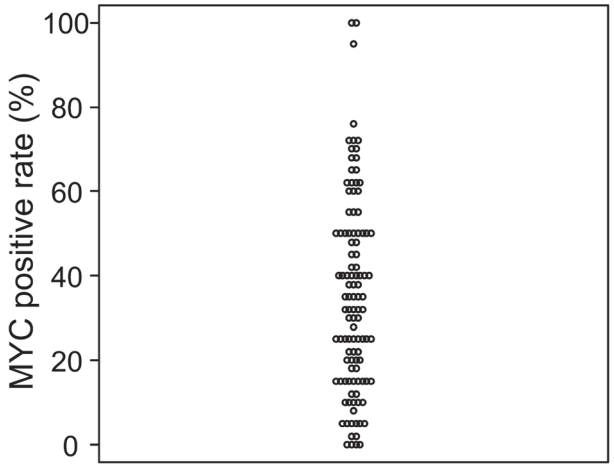

Fig. 2. Dot plot of positivity rates for MYC.

To determine the significant value for sIL-2R, we performed the ROC curve analysis and found a cut-off value of 1957 U/mL. The Kaplan–Meier analysis curve of the 2-year OS rate showed that the groups that had sIL-2R levels higher than 2000 U/mL had a 2-year OS of 56.0% (37.0–69.5%), and those that had values lower than 2000 U/mL had a 2-year OS of 91.4% (95% CI: 81.9–96.0%, $P = 0.00002$). The 2-year PFS rate of the group that had sIL-2R values higher than 2000 U/mL was 48.3% (31.5–63.2%), whereas that of the group that had values lower than 2000 U/mL was 85.9% (75.4–92.2%, $P = 0.00022$). In patients with highly elevated sIL-2R levels, the survival prognosis was considerably poor.

Univariate and multivariate analysis

Cox proportional hazards regression analysis was performed for univariate analysis of OS and PFS in terms of all clinical variables (evaluable enough). Regarding OS, four variables resulted in statistical significance ($P < 0.05$): MYC-positivity, age > 75, PS > 1, and sIL-2R > 2000. Univariate analysis of PFS revealed statistical significance as follows: MYC-positivity, age > 75, PS > 1, and sIL-2R > 2000. Results for univariate analysis for OS and PFS are listed in Tables 2 (a) and (b).

Multivariate analysis for OS was performed for four variables: MYC-positivity, age > 75, PS > 1, and sIL-2R > 2000. Multivariate analysis revealed that MYC-positivity and high sIL-2R levels were significant factors for survival events.

A prognostic model of combined MYC, sIL-2R, and age

We attempted to create an original scoring system for prognosis of DLBCL-NOS. We assigned one point each to age > 75, MYC-positivity (≥ 55%), and sIL-2R level > 2000 U/mL. All patients were classified into three risk categories: group 1 (0 point), group 2 (1 point), and group 3 (2–3 points). In the Kaplan–Meier analysis, the 2-year survival rate of each group was as follows: group 1: 100%, group 2: 83.0% (95% CI: 65.8–92.0%), and group 3: 47.1% (28.7–63.4%) ($P < 0.0001$). The 2-year PFS rate was as follows: group 1: 92.5% (95% CI: 78.5–97.5%), group 2: 75.5% (58.1–86.4%), and group 3: 45.3% (27.5–61.5%) ($P < 0.0001$) (Figs. 5a and b).

The prognosis of group 1 (patients without any factor) was optimal (nearly 100% survival rate); in contrast,

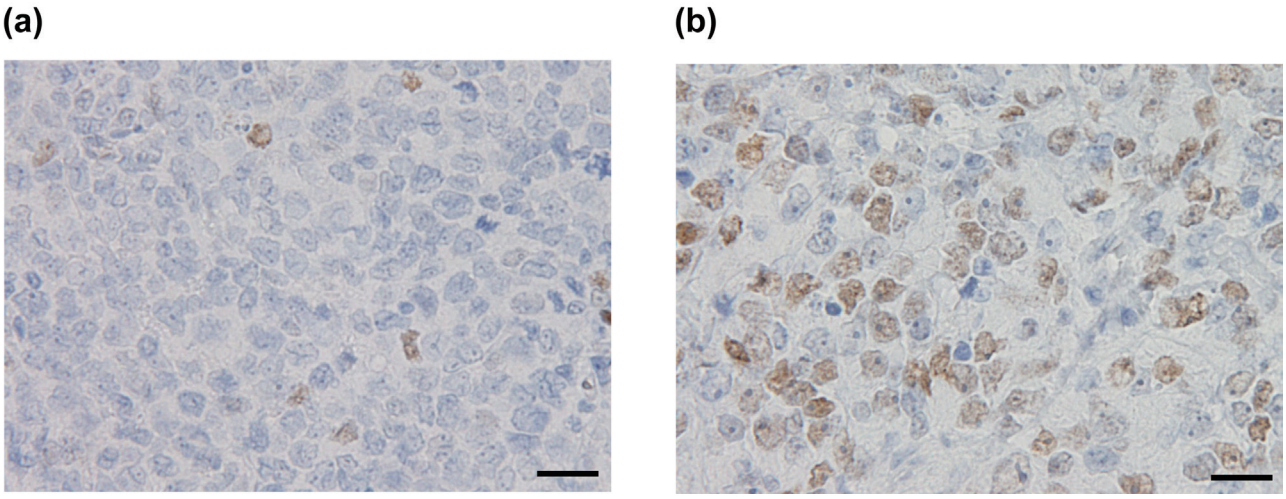

Fig. 3. Representative immunohistochemistry cases of high and low MYC protein expression. Positive tumor cells are those with brown-stained nuclei. **(a)** the low positive rate (MYC positive rate: 5%). **(b)** the high positive rate (70%). Bar = 20 μm.

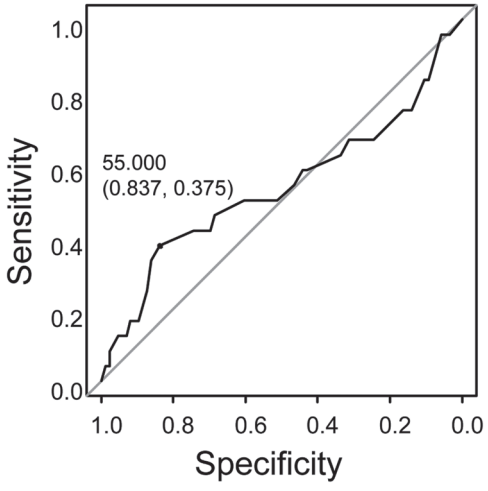

Fig. 4. ROC-curve analysis of OS. The cut-off value of the MYC-positive rate was 55%.

the prognosis of group 3 was considerably poor.

DISCUSSION

In this study, we found that MYC-positivity observed by IHC is an important prognostic factor for DLBCL-NOS.

The *MYC* oncogene has multiple functions in the proliferation and metabolism of lymphocytes.[13] In pre-B cell maturation, MYC expression is induced in response to B-cell receptor stimulation,[14, 15] For appropriate maturation and proliferation, *MYC* transcription must be tightly controlled. Although the mechanism is not fully understood, *MYC* deregulation and the overexpression of MYC protein significantly affects lymphomagenesis of mature B cell.[16] In DLBCL-NOS, MYC overexpression is caused by translocation, increase of copy number by gene amplification or transcription, and mutation.[15, 17] Translocation is associated with double and triple-hit lymphomas and the frequency of *MYC*-translocation with the immunoglobulin gene (Ig) is ~10% in patients with DLBCL,[11] which is associated with poor prognosis.[4] However, in some cases without Ig translocation, lymphoma cell nuclei exhibit high MYC protein expression. Approximately 30% of DLBCL patients show high MYC expression.[11] This was explained to be caused by translocation events, gene amplification, and abnormal transcription of *MYC*. Increased copy number of *MYC* is observed in about 2–20% of DLBCL, and those cases had poor prognosis.[18, 19] MYC protein is degraded with glycogen synthase kinase (GSK-3β)-mediated phosphorylation, though in DLBCL-NOS, the activation of phosphoinositide 3-kinases (PI3K) impairs downregulation of MYC.[20]

Previous reports have indicated that MYC protein expression is associated with poor prognosis in DLBCL-NOS. The meta-analysis by Zhou K. reported that MYC aberration was an independent prognostic factor for DLBCL.[21] Zhou M. et al. developed a combination scoring system with *MYC* expression and IPI score, which showed that MYC-positive patients (> 50%) and patients in the high IPI group showed worse prognosis in terms of the 3-year OS.[22] We performed the same stratification using our current patients and checked for reproducibility (data not shown). The multivariate analysis confirmed that the hazard ratio of sIL-2R > 2000 was high, and that the classification including age in this analysis was more reflective of prognosis.

Additionally, in the analyses of the association

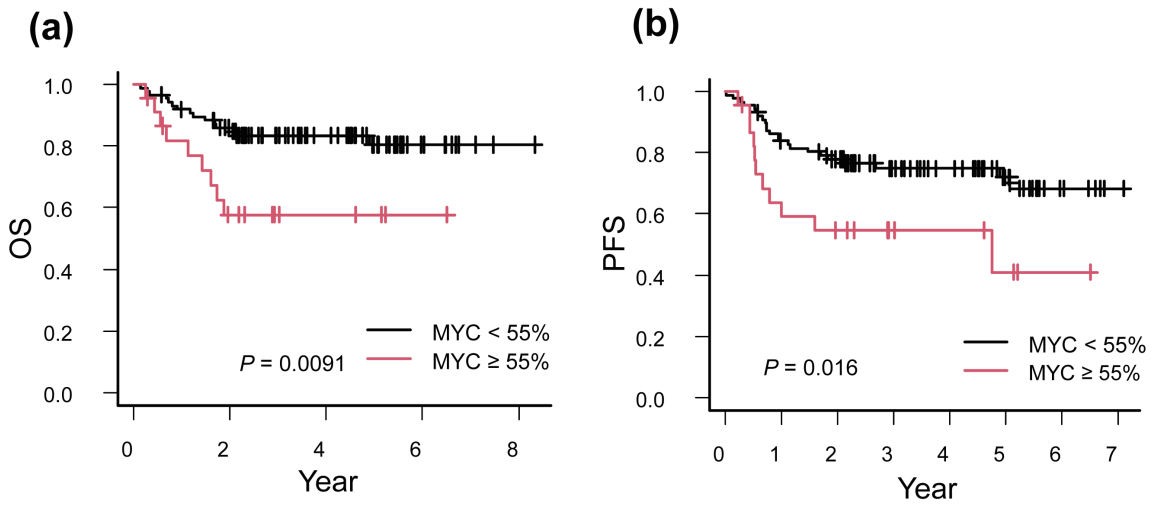

Fig. 5. Survival rate of MYC-positive and negative groups. **(a)** OS of MYC-positive (≥ 55%) group and MYC-negative (< 55%) group. The 2-year OS rates were 47.1% and 83.0%, respectively. **(b)** PFS of MYC-positive and MYC-negative groups. The 2-year OS rates were 54.7% and 77.8%, respectively.

between MYC, BCL2, and BCL6 protein expression, the researchers found that the MYC-positive, BCL2-positive, and BCL6-low population had an inferior survival rate.[11, 12] In CD5-positive cases, MYC-positivity also resulted in poor outcomes.[23]

MYC-positivity is associated with poor prognosis. In this study, patients with an MYC-positive rate ≥ 55% had a significantly inferior prognosis to those with an MYC-positive rate < 55%. Standardization of evaluation is limited, and there are minor differences in the methods of retrieval and staining. Furthermore, the cut-off points of MYC-positivity vary in the range of 30–70% in previous reports; therefore, they are generally set at 50–60%.[12, 24, 25] The cut-off value used in our study was the median of this range.

Generally, DLBCL-NOS is a heterogeneous group, especially in terms of the clinical course. Major prognostic indexes have successfully stratified the patient outcome in terms of IPI,[5] R-IPI,[6] NCCN-IPI,[7] and elderly IPI.[26] Moreover, several dozens of prognostic items have been reported. Patients with the GCB-type disease have been reported to have a better prognosis than those with non-GCB-type disease; however, this was not found in this study.

Several biomarkers such as LDH and sIL-2R reflect the course of the disease. LDH has been established as an item of IPI and other indexes; furthermore, an elevation of sIL-2R levels is associated with a poor prognosis. Soluble IL-2R is involved in the activation of T-cell lymphocytes[27] and is a marker for lymphoma.[28] Soluble IL-2R levels reflect the tumor burden and aggressive course of the disease.[29, 30] Several studies on the DLBCL prognoses have reported cut-off values of sIL-2R to be 800–3000 U/mL, particularly 2000 U/mL.[31, 32] We found that an increase in the sIL-2R levels (> 2000 U/mL) is a significant prognostic factor in multivariate analysis.

As shown in Fig. 6, our original scoring system using MYC expression, sIL-2R level, and age was an even more useful assessment method. Group 1 (patients without any risk factor) showed a very good survival rate (nearly 100% survival rate) with standard R-CHOP treatment. In contrast, the patients in group 3 had a poor prognosis. Therefore, this novel prognostic model may contribute to stratifying the precision of the prognosis of patients with DLBCL-NOS.

So far, almost all DLBCL patients in this institute have received R-CHOP chemotherapy independent of risk classifications. Although the international prognostic indexes and other factors have been successful in stratifying prognosis, therapeutic interventions for patients with poor prognosis remain insufficient. Recently, a novel treatment comprising rituximab, cyclophosphamide, prednisolone, and polatuzumab vedotin improved the prognosis of intermediate and poor IPI patients.[33] Improvements in the accuracy of DLBCL risk scoring, which can be done anywhere, are still needed to further improve treatment strategies.

This study had certain limitations; the patients in our institutions were relatively older than the patients in other studies.[34] Thus, several patients who were not treated with R-CHOP chemotherapy due to age or comorbidities were excluded beforehand. Unsatisfactorily, the number of patients who underwent FISH analysis

Table 2. Univariate and multivariate analysis of OS (a) and PFS (b)
(a) OS

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
MYC positive	2.869 (1.25–6.57)	0.013	2.496 (1.080–5.766)	0.032
Age > 75	3.900 (1.616–9.412)	0.0025	2.356 (0.938–5.916)	0.068
PS > 1	2.932 (1.316–6.533)	0.0085	1.939 (0.844–5.453)	0.119
sIL-2R > 2000	5.280 (2.252–12.38)	0.00013	3.950 (1.657–9.415)	0.0019
Sex (male)	1.746 (0.274–4.212)	0.215		
Stage III & IV	1.853 (0.801–4.284)	0.149		
LDH > normal	2.430 (0.964–6.126)	0.060		
R-IPI poor	2.260 (0.988–5.168)	0.053		
Bulky	0.540 (0.0073–4.005)	0.547		
B symptom	2.106 (0.719–6.167)	0.174		
Extranodal site > 1	1.328 (0.495–3.563)	0.573		
CD10 positive	1.751 (0.757–4.05)	0.191		
BCL2 positive	0.953 (0.342–2.556)	0.897		
BCL6 positive	0.822 (0.316–2.139)	0.191		
MUM-1	1.106 (0.408–2.998)	0.843		
Non-GCB type	0.869 (0.362–2.063)	0.741		
DEL	1.811 (0.613–5.357)	0.283		

(b) PFS

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
MYC positive	2.357 (1.147–4.846)	0.020	2.156 (1.036–4.488)	0.040
Age > 75	2.496 (1.249–4.987)	0.0096	1.453 (0.689–3.064)	0.327
PS > 1	3.186 (1.623–6.254)	0.00076	2.404 (1.177–4.911)	0.016
sIL-2R > 2000	3.346 (1.696–6.602)	0.00049	2.567 (1.270–5.188)	0.0086
Sex(male)	1.779 (0.738–4.292)	0.199		
Stage III & IV	2.996 (1.424–6.304)	0.0038		
LDH > normal	3.289 (1.431–7.563)	0.0051		
R-IPI poor	2.938 (1.431–6.031)	0.0033		
Bulky	0.835 (0.199–3.5)	0.805		
B symptom	2.998 (1.234–7.281)	0.015		
Extranodal site > 1	2.152 (0.997–4.648)	0.051		
CD10 positive	1.097 (0.505–2.384)	0.816		
BCL2 positive	1.148 (0.468–2.812)	0.764		
BCL6 positive	1.193 (0.512–2.781)	0.682		
MUM-1	1.838 (0.7051–4.789)	0.213		
Non-GCB type	1.299 (0.601–2.810)	0.506		
DEL	1.884 (0.775–4.583)	0.163		

DEL, double expressor lymphoma (both BCL2 and MYC positive); HR, hazard ratio; OS, overall survival; PFS, progression free survival; 95% CI, 95% confidence interval.

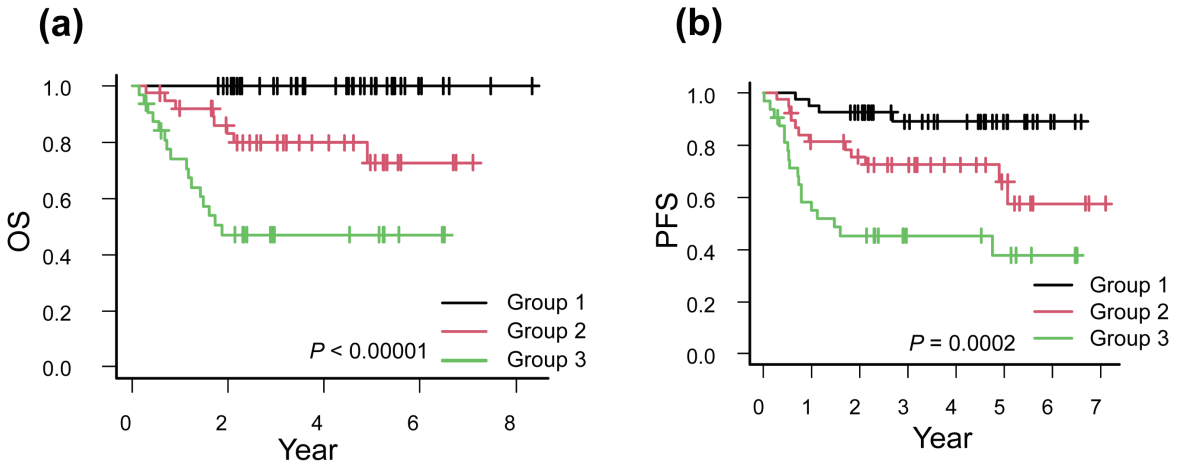

Fig. 6. Novel clinicopathologic prognostic model, with combined effects of age (> 75), MYC-positivity (≥ 55%), and sIL-2R levels (> 2000 U/mL). Allotment: one point each to age > 75, MYC positivity (≥ 55%), and sIL-2R level > 2000 U/mL. All patients were classified into three risk categories: group 1 (0 points), group 2 (1 point), and group 3 (2–3 points) **(a)** Overall survival (OS). **(b)** Progression-free survival rate (PFS).

was small, and there were a few cases of MYC-translocation; thus, we could not analyze the relationship between MYC-translocation and other clinical variables.

In conclusion, MYC-positivity by IHC is an independent poor prognostic factor and has a potential for better accuracy for risk stratification in patients with DLBCL-NOS. The evaluation of MYC by IHC staining is a simple method and is available at all institutions. The addition of sIL-2R level to MYC expression was found to be an even more useful assessment method. Our original scoring system has the potential to detect populations with inferior prognosis. We recommend the use of this simple, highly useful evaluation method, which would contribute to improved therapeutics.

AUTHOR CONTRIBUTION

Contribution: S.S., T.F., and S.K. designed the study; S.K. and M.M. supported technical methods, and S.S. and M.M. performed IHC staining; K.K. planned the statistical analysis, S.S. and K.K. performed the statistical analysis; S.S. wrote the manuscript; T.M., Y.H., M.M., R.H. and K.H. provided the patient data; S.S collected the patient data; all authors interpreted the data and reviewed and approved the final manuscript.

CONSENT STATEMENT

This study was conducted on an opt-out basis, and we used the tissues of formalin-fixed specimens stored at Department of Pathology, Tottori University Hospital.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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The authors declare no conflict of interest.

REFERENCES

1 Al-Hamadani M, Habermann TM, Cerhan JR, Macon WR, Maurer MJ, Go RS. Non-Hodgkin lymphoma subtype distribution, geodemographic patterns, and survival in the US: A longitudinal analysis of the National Cancer Data Base from 1998 to 2011. Am J Hematol. 2015;90:790-5. DOI: 10.1002/ajh.24086, PMID: 26096944

2 Liu Y, Barta SK. Diffuse large B-cell lymphoma: 2019 update on diagnosis, risk stratification, and treatment. Am J Hematol. 2019;94:604-16. DOI: 10.1002/ajh.25460, PMID: 30859597

3 Dang CV. MYC on the path to cancer. Cell. 2012;149:22-35. DOI: 10.1016/j.cell.2012.03.003, PMID: 22464321

4 Riedell PA, Smith SM. Double hit and double expressors in lymphoma: definition and treatment. Cancer. 2018;124:4622-32. DOI: 10.1002/cncr.31646, PMID: 30252929

5 International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med. 1993;329:987-94. DOI: 10.1056/NEJM199309303291402, PMID: 8141877

6 Sehn LH, Berry B, Chhanabhai M, Fitzgerald C, Gill K, Hoskins P, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood. 2007;109:1857-61. DOI: 10.1182/blood-2006-08-038257, PMID: 17105812

7 Zhou Z, Sehn LH, Rademaker AW, Gordon LI, LaCasce AS, Crosby-Thompson A, et al. An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era. Blood. 2014;123:837-42. DOI: 10.1182/blood-2013-09-524108, PMID: 24264230

8 Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004;103:275-82. DOI: 10.1182/blood-2003-05-1545, PMID: 14504078

9 Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48:452-8. DOI: 10.1038/bmt.2012.244, PMID: 23208313

10 Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana M. Report of the Committee on Hodgkin's Disease Staging Classification. Cancer Res. 1971;31:1860-1. PMID: 5121694

11 Horn H, Ziepert M, Becher C, Barth TFE, Bernd HW, Feller AC, et al.; German High-Grade Non-Hodgkin Lymphoma Study Group. MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. Blood. 2013;121:2253-63. DOI: 10.1182/blood-2012-06-435842, PMID: 23335369

12 Li L, Zhang X, Zhang T, Song Z, Hu G, Li W, et al. Prognostic Significance of BCL-2 and BCL-6 Expression in MYC-positive DLBCL. Clin Lymphoma Myeloma Leuk. 2018;18:e381-9. DOI: 10.1016/j.clml.2018.06.010, PMID: 29983382

13 Chou C, Egawa T. Myc or no Myc, that is the question. EMBO J. 2015;34:1990-1. DOI: 10.15252/embj.201592267, PMID: 26139535

14 Larsson LG, Schena M, Carlsson M, Sällström J, Nilsson K. Expression of the c-myc protein is down-regulated at the terminal stages during in vitro differentiation of B-type chronic lymphocytic leukemia cells. Blood. 1991;77:1025-32. DOI: 10.1182/blood.V77.5.1025.1025, PMID: 1899807

15 de Barrios O, Meler A, Parra M. MYC's Fine Line Between B Cell Development and Malignancy. Cells. 2020;9:523. DOI: 10.3390/cells9020523, PMID: 32102485

16 Nguyen L, Papenhausen P, Shao H. The Role of c-MYC in B-Cell Lymphomas: Diagnostic and Molecular Aspects. Genes (Basel). 2017;8:116. DOI: 10.3390/genes8040116, PMID: 28379189

17 Ahmadi SE, Rahimi S, Zarandi B, Chegeni R, Safa M. MYC: a multipurpose oncogene with prognostic and therapeutic implications in blood malignancies. J Hematol Oncol. 2021;14:121. DOI: 10.1186/s13045-021-01111-4, PMID: 34372899

18 Stasik CJ, Nitta H, Zhang W, Mosher CH, Cook JR, Tubbs RR, et al. Increased MYC gene copy number correlates with increased mRNA levels in diffuse large B-cell lymphoma. Haematologica. 2010;95:597-603. DOI: 10.3324/haematol.2009.012864, PMID: 20378577

19 Lu TX, Fan L, Wang L, Wu JZ, Miao KR, Liang JH, et al. *MYC* or *BCL2* copy number aberration is a strong predictor of outcome in patients with diffuse large B-cell lymphoma. Oncotarget. 2015;6:18374-88. DOI: 10.18632/oncotarget.4073, PMID: 26158410

20 Wang WG, Liu ZB, Jiang XN, Lee J, Zhou XY, Li XQ. MYC protein dysregulation is driven by BCR-PI3K signalling in diffuse large B-cell lymphoma. Histopathology. 2017;71:778-85. DOI: 10.1111/his.13287, PMID: 28639315

21 Zhou K, Xu D, Cao Y, Wang J, Yang Y, Huang M. C-MYC aberrations as prognostic factors in diffuse large B-cell lymphoma: a meta-analysis of epidemiological studies. PLoS One. 2014;9:e95020. DOI: 10.1371/journal.pone.0095020, PMID: 24740248

22 Zhou M, Wang J, Ouyang J, Xu J, Chen B, Zhang Q, et al. MYC protein expression is associated with poor prognosis in diffuse large B cell lymphoma patients treated with RCHOP chemotherapy. Tumour Biol. 2014;35:6757-62. DOI: 10.1007/s13277-014-1907-z, PMID: 24719189

23 Lu TX, Wu S, Zhou XY, Zhang Y, Hong TT, Cai DY, et al. $CD5^{+}MYC^{+}$ predicts worse prognosis in diffuse large B-cell lymphoma. Exp Mol Pathol. 2020;112:104326. DOI: 10.1016/j.yexmp.2019.104326, PMID: 31706988

24 Agarwal R, Lade S, Liew D, Rogers TM, Byrne D, Feleppa F, et al. Role of immunohistochemistry in the era of genetic testing in *MYC* -positive aggressive B-cell lymphomas: a study of 209 cases. J Clin Pathol. 2016;69:266-70. DOI: 10.1136/jclinpath-2015-203002, PMID: 26307073

25 Nwanze J, Siddiqui MT, Stevens KA, Saxe D, Cohen C. MYC Immunohistochemistry Predicts *MYC* Rearrangements by FISH. Front Oncol. 2017;7:209. DOI: 10.3389/fonc.2017.00209, PMID: 28983465

26 Advani RH, Chen H, Habermann TM, Morrison VA, Weller EA, Fisher RI, et al.; Eastern Cooperative Oncology Group; Cancer and Leukemia Group B; Southwest Oncology Group. Comparison of conventional prognostic indices in patients older than 60 years with diffuse large B-cell lymphoma treated with R-CHOP in the US Intergroup Study (ECOG 4494, CALGB 9793): consideration of age greater than 70 years in an elderly prognostic in. Br J Haematol. 2010;151:143-51. DOI: 10.1111/j.1365-2141.2010.08331.x, PMID: 20735398

27 Damoiseaux J. The IL-2 – IL-2 receptor pathway in health and disease: the role of the soluble IL-2 receptor. Clin Immunol. 2020;218:108515. DOI: 10.1016/j.clim.2020.108515, PMID: 32619646

28 Motokura T, Kobayashi Y, Fujita A, Nakamura Y, Taniguchi T, Uchimaru K, et al. Clinical significance of serial measurement of the serum levels of soluble interleukin-2 receptor and soluble CD8 in malignant lymphoma. Leuk Lymphoma. 1995;16:355-62. DOI: 10.3109/10428199509049776, PMID: 7719243

29 Yang ZZ, Grote DM, Ziesmer SC, Manske MK, Witzig TE, Novak AJ, et al. Soluble IL-2Rα facilitates IL-2–mediated immune responses and predicts reduced survival in follicular B-cell non-Hodgkin lymphoma. Blood. 2011;118:2809-20. DOI: 10.1182/blood-2011-03-340885, PMID: 21719603

30 Tomita N, Sakai R, Fujisawa S, Fujimaki K, Taguchi J, Hashimoto C, et al. SIL index, comprising stage, soluble interleukin-2 receptor, and lactate dehydrogenase, is a useful prognostic predictor in diffuse large B-cell lymphoma. Cancer Sci. 2012;103:1518-23. DOI: 10.1111/j.1349-7006.2012.02331.x, PMID: 22587388

31 Goto H, Tsurumi H, Takemura M, Ino-Shimomura Y, Kasahara S, Sawada M, et al. Serum-soluble interleukin-2 receptor (sIL-2R) level determines clinical outcome in patients with aggressive non-Hodgkin?s lymphoma: in combination with the International Prognostic Index. J Cancer Res Clin Oncol. 2005;131:73-9. DOI: 10.1007/s00432-004-0600-9, PMID: 15503137

32 Goto N, Tsurumi H, Goto H, Shimomura YI, Kasahara S, Hara T, et al. Serum soluble interleukin-2 receptor (sIL-2R) level is associated with the outcome of patients with diffuse large B cell lymphoma treated with R-CHOP regimens. Ann Hematol. 2012;91:705-14. DOI: 10.1007/s00277-011-1363-4, PMID: 22183251

33 Tilly H, Morschhauser F, Sehn LH, Friedberg JW, Trněný M, Sharman JP, et al. Polatuzumab Vedotin in Previously Untreated Diffuse Large B-Cell Lymphoma. N Engl J Med. 2022;386:351-63. DOI: 10.1056/NEJMoa2115304, PMID: 34904799

34 Martelli M, Ferreri AJM, Agostinelli C, Di Rocco A, Pfreundschuh M, Pileri SA. Diffuse large B-cell lymphoma. Crit Rev Oncol Hematol. 2013;87:146-71. DOI: 10.1016/j.critrevonc.2012.12.009, PMID: 23375551