MYC and BCL-2 adjusted-International Prognostic Index (A-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP

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Summary. The International Prognostic Index (IPI) has been the basis for determining prognosis in patients with diffuse large B-cell lymphoma (DLBCL) for the past 20 years. The utility of the IPI must be reassessed in the era of immunochemotherapy. Seven risk factors at diagnosis were identified, and a maximum of 7 points were assigned to each patient. Four risk groups were created: low (0-1), low-intermediate (2-3), high-intermediate (4), and high (5-7). Using MYC and BCL-2 clinical data from the Drum Tower Hospital collected during the rituximab era, we performed a retrospective analysis of patients with DLBCL treated with R-CHOP and built an biological markers adjusted IPI with the goal of improving risk stratification.Clinical features from 60 adults with de novo DLBCL diagnosed from 2008-2013 were assessed for their prognostic significance. The IPI remains predictive, but it cannot identify the high-risk subgroup. Compared with the IPI, the MYC and BCL-2 adjusted-IPI (A-IPI) better discriminated patients in the high-risk subgroup (4-year overall survival [OS]: 33.3%) than did the IPI (4 year OS: 48.0%). In the era of R-CHOP treatment, MYC and BCL-2 adjusted-IPI is more powerful than the IPI for helping guide treatment planning and interpretation of clinical trials.

Key words: Diffuse large B-cell lymphoma, MYC, BCL-2, IPI

Introduction

Diffuse large B cell lymphomas (DLBCL), which represent approximately 35-40 % of aggressive B cell non-Hodgkin's lymphoma in adulthood, are a heterogeneous group of neoplasms with various immunophenotypes, different clinical and genetic abnormalities, and highly variable prognoses (Smith et al., 2010). The introduction of the monoclonal CD20 antibody rituximab in chemotherapies has significantly improved the prognosis of DLBCL patients; therefore, the prognostic index based exclusively on traditional chemotherapies must be re-evaluated.

The International Prognostic Index (IPI) has been the primary clinical tool used to predict the prognoses of patients with aggressive NHL (Project TIN-HsLPF, 1993). Its predictive capacity has been validated in the past two decades, but it has become less powerful in the era of immunochemotherapy because of its limited ability to identify patients in the high-risk subgroup (Sehn et al., 2007; Sehn, 2012; Sengar et al., 2013) . More recently, as with solid organ malignancies (Paik et al., 2004), there has been a shift towards incorporating tumor molecular profiling into prognostication and treatment stratification for DLBCL. An increasing number of studies have indicated that MYC protein detected by immunohistochemistry could be used to identify distinctive subgroups of DLBCL (Ruzinova et al., 2010; Tapia et al., 2011; Kluk et al., 2012).We have also created a novel clinicopathologic prognostic model (combination of MYC and IPI) to predict the prognosis of DLBCL patients (Zhou et al., 2014). Based on the

number of negative prognostic factors present at the time of diagnosis (IPI>2, MYC>50%), 3 discrete outcome groups were identified with a 3-year overall survival ranging from 39.1% in the high risk group to 100% in the low risk group (Zhou et al., 2014). BCL-2 protein expression was also associated with an inferior outcome (Visco et al., 2013). Most studies agree that DLBCL with both MYC and BCL-2 protein expression has an aggressive clinical course and a poor outcome (Green et al., 2012; Johnson et al., 2012; Hu et al., 2013; Perry et al., 2014). We performed a retrospective analysis of patients with DLBCL treated with R-CHOP in China to investigate whether the addition of biological markers MYC and BCL-2 to the IPI would enhance its prognostic capacity.

Materials and methods

Patient selection

This study is a retrospective analysis of an unselected population of patients with DLBCL. We retrospectively examined 60 cases of de novo DLBCL patients from 2008 to 2013 in the Nanjing Drum Tower Hospital. All cases were diagnosed according to World Health Organization (WHO) classification criteria. All patients were treated with 4 to 8 cycles of R-CHOP regimen at 21-day intervals. Patients underwent (18)F-FDG PET/CT before, after cycle four, and after completion of R-CHOP treatment. This study was approved by the institutional review board, and all patients gave written informed consent.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue (FFPE) sections of 3 μm were placed on adhesive-coated slides. Heated antigen retrieval was completed by immersing slides in EDTA buffer (pH 8.0) and heating for 2 min in a steamer. An antibody against BCL-2 (124, ×100; Dako, Glostrup, Denmark) was used as well as an autostainer with the standard polymer method (Dako Autostainer Plus). MYC staining was completed as our previously reported (Zhou et al., 2014). Immuno-histochemistry was evaluated by 2 experienced hematopathologists on a multihead microscope. Cutoff values of 30% for BCL-2 staining and 50% for MYC staining were considered high respectively (Perry et al., 2014).

MYC and BCL-2 adjusted IPI

Seven risk factors (age>60 years, stage III/IV disease, elevated lactate dehydrogenase level, Eastern Cooperative Oncology Group [ECOG] performance status≥2, more than one extranodal site of disease, high MYC expression, and high BCL-2 expression) at diagnosis were identified, and a maximum of 7 points assigned were assigned to each patient. Four risk groups

were created: low (0-1), low-intermediate (2-3), high-intermediate (4), and high (5-7).

Statistical analysis

This analysis is based on follow-up data obtained through August 05, 2014. This was an intention-to-treat analysis including all patients. Progression-free survival (PFS) was calculated from the date of diagnosis to documented disease progression; observations were censored on the date the patient was last known to be alive or for patients dying as a result of causes unrelated to lymphoma or treatment. Overall survival (OS) was calculated from the date of diagnosis until death as a result of any cause or date last known alive. PFS and OS were assessed using the Kaplan-Meier method and compared among risk groups using the log-rank test. Data were analyzed using SPSS version 11.0. Differences were considered significant when the P-value was <0.05.

Results

Patients

Clinical characteristics of patients at diagnosis, including the distribution of the individual IPI factors, are listed in Table 1. The median age at diagnosis was 58 years (range: 18 to 81).

MYC and BCL-2 protein expression and prognosis

The expression and prognostic role of MYC has been shown and discussed in our previously study (Zhou et al., 2014). High BCL-2 protein expression was shown in 31 patients (52%), whereas 29 patients (48%) were considered to have low BCL-2 protein expression (Fig. 1). For all patients, the 4-year estimated OS was 76% and PFS was 51.7%. The low BCL-2 group showed a

Table 1. Patient characteristics.

Patient Characteristic	Value
Number	60
Median age, y (range)	58 (18- 81)
Male sex, %	58
IPI factors	
Age > 60 y, %	45
ECOG ≥ 2, %	20
Elevated LDH, %	50
More than 1 extranodal site, %	21
Stage III/IV, %	65
Tumor characteristics	
High MYC expression, %	35
High BCL-2 expression, %	52

IPI, International Prognostic Index; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase.

significantly better survival compared to the high BCL-2 group in both 4-year estimated OS (87.6% vs. 53.3%, P=0.006) and PFS (64.8% vs. 34.7%, P= 0.003) (Fig. 2). We found 16 cases with MYC and BCL-2 protein coexpression. BCL-2 was not a significant predictor of PFS (P =0.104) and OS (P =0.084) in the MYC group.

Outcome according to standard IPI, NCCN-IPI and MYC and BCL-2 adjusted IPI

The median follow-up time for living patients was 36 months (range: 5 to 70 months). All patients were classified into one of 4 risk groups (low, low-intermediate, high-intermediate, and high). Outcomes according to the standard IPI are listed in Table 2. Although the IPI remains predictive, it no longer distinguishes among the 4 risk groups (Table 2, Fig. 3). Outcome according to the NCCN-IPI (Zhou et al., 2014)

Table 2. Comparison of MYC and BCL-2 Adjusted-IPI to IPI and NCCN-IPI for risk stratification and outcomes of 4-y PFS and OS.

| Risk Group	No. of prognostic factors	Patients (%)	4y PFS (%)	4y OS (%)
Standard IPI				
Low	0,1	13.3	87.5	100
Low-intermediate	2	33.3	53.7	88.9
High-intermediate	3	36.7	36.4	43.5
High	4,5	16.7	24.0	48.0
NCCN-IPI				
Low	0,1	11.7	100	100
Low-intermediate	2,3	51.7	47.2	59.5
High-intermediate	4,5	28.3	44.1	58.8
High	6,7,8	8.3	0	30
MYC and BCL-2 adjusted IPI				
Low	0,1	8.3	100	100
Low-intermediate	2,3	43.4	59.8	90.9
High-intermediate	4	15	53.3	59.3
High	5,6,7	33.3	16.9	33.3
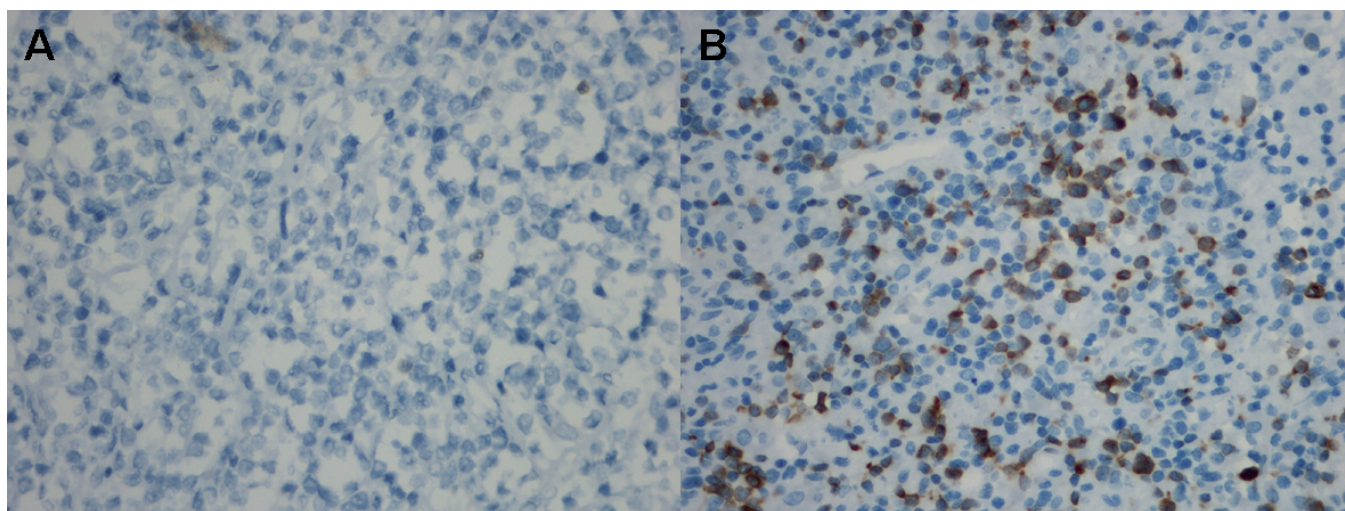
Fig 1. BCL-2 protein expression was exclusively brown cytoplasm. **A.** Low BCL-2 protein expression (≤30% BCL-2-positive tumor cells). **B.** High BCL-2 protein expression (>30% BCL-2-positive tumor cells). x 400

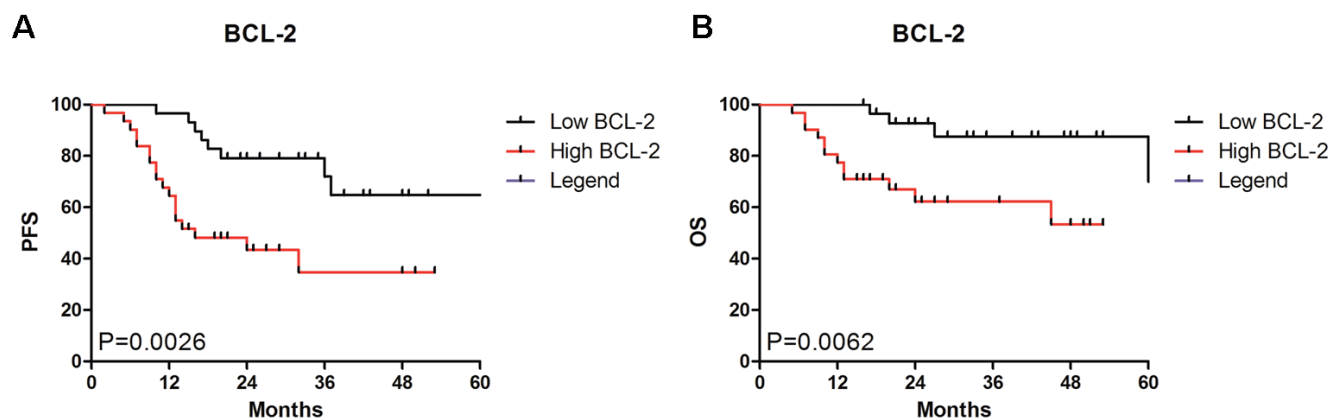**Fig 2.** Survival according to BCL-2 protein expression. **A.** Progression-free survival. **B.** Overall survival.
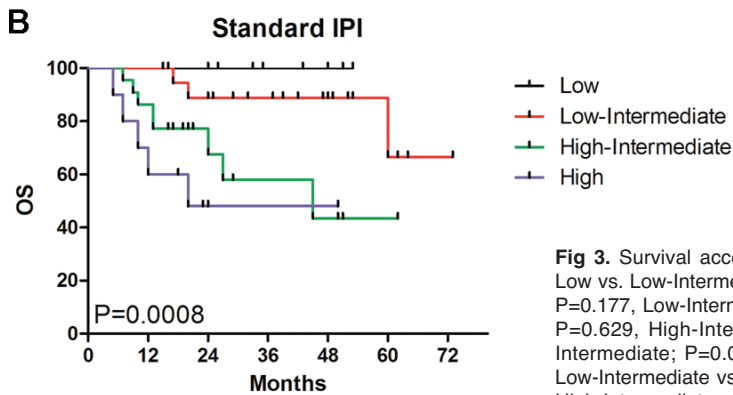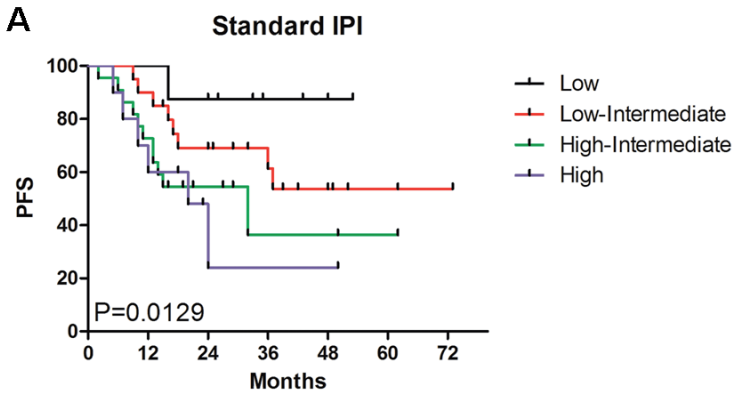
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Fig 3. Survival according to the standard IPI. **A.** Progression-free survival (P=0.206, Low vs. Low-Intermediate; P=0.048, Low vs. High-Intermediate; P=0.025, Low vs. High; P=0.177, Low-Intermediate vs. High-Intermediate; P=0.122, Low-Intermediate vs. High; P=0.629, High-Intermediate vs. High). **B.** Overall survival (P=0.339, Low vs. Low-Intermediate; P=0.03, Low vs. High-Intermediate; P=0.021, Low vs. High; P=0.034, Low-Intermediate vs. High-Intermediate; P=0.008, Low-Intermediate vs. High; P=0.264, High-Intermediate vs. High).
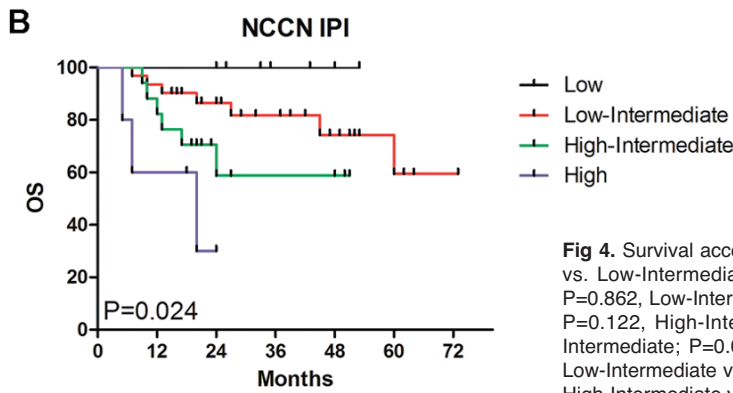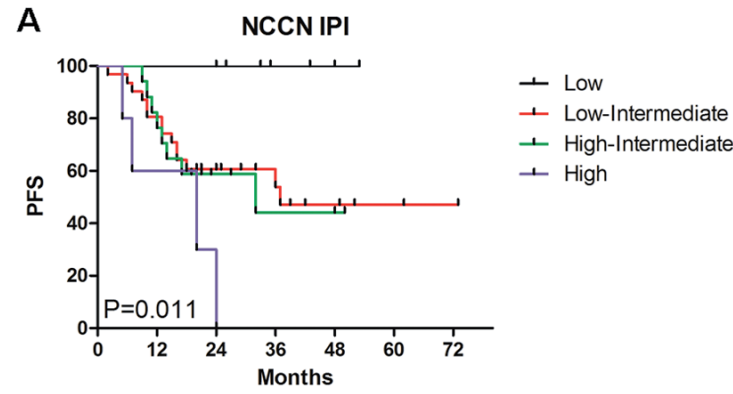
A

B

Fig 4. Survival according to the NCCN-IPI. **A.** Progression-free survival (P=0.042, Low vs. Low-Intermediate; P=0.030, Low vs. High-Intermediate; P=0.001, Low vs. High; P=0.862, Low-Intermediate vs. High-Intermediate; P=0.085, Low-Intermediate vs. High; P=0.122, High-Intermediate vs. High). **B.** Overall survival (P=0.228, Low vs. Low-Intermediate; P=0.064, Low vs. High-Intermediate; P=0.013, Low vs. High; P=0.163, Low-Intermediate vs. High-Intermediate; P=0.004, Low-Intermediate vs. High; P=0.201, High-Intermediate vs. High).

is listed in Table 2 and shown in Fig. 4. When outcome is plotted according to the number of seven prognostic factors present at diagnosis (age>60 years, stage III/IV disease, elevated lactate dehydrogenase level, Eastern Cooperative Oncology Group [ECOG] performance status≥2, more than one extranodal site of disease, high MYC expression, and high BCL-2 expression), 4 discrete outcome groups were identified, respectively (Table 2, Fig. 5). Patients with 0 or 1 risk factors had the best outcomes, patients with 2 or 3 risk factors had good outcomes, patients with 4 risk factors had moderate outcomes, and patients with more than 4 risk factors had the poorest outcomes. Outcomes according to the MYC and BCL-2 adjusted IPI are listed in Table 2.

Discussion

The IPI has been used as the primary prognostic model in the management of patients with DLBCL for nearly two decades. Because of its accessibility and predictive capacity, the IPI has gained universal acceptance. However, its value in the era of rituximab should been re-evaluated because of its limited ability to identify patients in the high-risk subgroup (Sehn et al., 2007; Sehn, 2012; Sengar et al., 2013). Many efforts have been made to improve the model's discrimination.

Sehn et al. regrouped the original IPI score (R-IPI) (Sehn et al., 2007), and Advani et al. focused on elderly patients (E-IPI) (Advani et al., 2010). Zhou et al. divided 5 factors among 8 scoring points (NCCN-IPI) (Zhou et al., 2014), and Cox et al. added a new clinical prognostic factor to the initial index (Cox et al., 2008). When combining IPI score and MYC protein expression, we found that the composite model could effectively stratify patients into prognostically relevant subgroups (Zhou et al., 2014). However, the combined model was still unable to distinguish the group of low MYC/high IPI from the group of high MYC/low IPI.

MYC and BCL-2 protein expression levels correlate with an aggressive clinical course and poor outcome (Green et al., 2012; Johnson et al., 2012; Hu et al., 2013; Perry et al., 2014). In this study, we investigated whether the addition of the biological markers MYC and BCL-2 to the IPI would enhance its capacity to prognosticate. The addition of MYC and BCL-2 to the IPI might provides a more clinically relevant outcome prediction. The MYC and BCL-2 adjusted-IPI identifies 4 distinct prognostic groups with significantly different outcomes. Patients with 0 or 1 risk factors fall into a "Low" risk group with a 100% chance of long-term OS. R-CHOP will remain the standard regimen for this group. Patients with 2 or 3 risk factors fall into a "Low-intermediate"
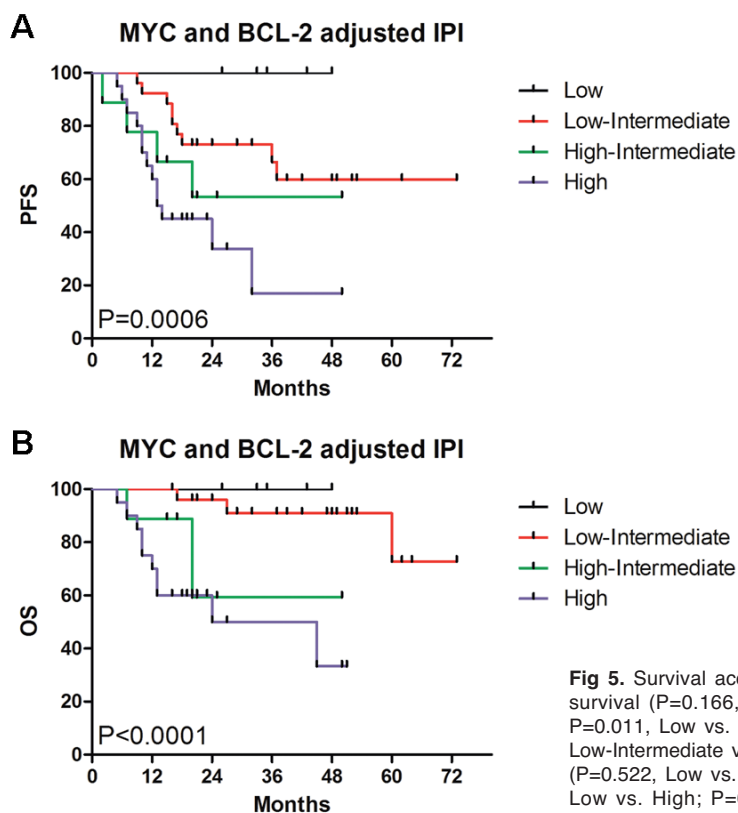

Fig 5. Survival according to the MYC and BCL-2 adjusted IPI. **A.** Progression-free survival (P=0.166, Low vs. Low-Intermediate; P=0.09, Low vs. High-Intermediate; P=0.011, Low vs. High; P=0.279, Low-Intermediate vs. High-Intermediate; P=0.003, Low-Intermediate vs. High; P=0.375, High-Intermediate vs. High). **B.** Overall survival (P=0.522, Low vs. Low-Intermediate; P=0.124, Low vs. High-Intermediate; P=0.058, Low vs. High; P=0.014, Low-Intermediate vs. High-Intermediate; P=0.001, Low-Intermediate vs. High; P=0.442, High-Intermediate vs. High).

risk group with a 90.9% chance of long-term OS. Patients with 4 risk factors fall into a "High-intermediate" risk group with an approximately 60% chance of long-term survival. Finally, patients with more than 4 risk factors fall into a "High" risk group with a 33% chance of long-term survival; this group of high risk patients remains a therapeutic challenge. Clinical studies including novel approaches are needed, such as the combination of rituximab with dose-dense chemotherapy (Brusamolino et al., 2006; Glass et al., 2006; Gisselbrecht, 2008; Vitolo et al., 2009) or a combination of new biological drugs with conventional chemo-immunotherapy, such as bortezomib (Elstrom et al., 2012), a new humanized anti-CD20 mAb (GA101) (Alduaij et al., 2011), lenalidomide (Wiernik et al., 2008), mTOR inhibitors (Witzig et al., 2011), and enzastaurin (Forsyth et al., 2013). The small number of patients in our study may contribute to the lack of significance. However, in contrast to the standard IPI, NCCN-IPI and the MYC and BCL-2 adjusted IPI have shown an improved capacity to discriminate among the 4 risk groups. The NCCN-IPI was developed in a cohort of 1650 patients and validated in a second cohort with 1138 patients (Zhou et al., 2014). Our study showed that NCCN-IPI and the MYC and BCL-2 adjusted IPI substantially is better for determination of high risk patients.

Our study added new IHC prognostic markers to the IPI to create a new prognostic model based on a previously validated model that is widely used in current treatment. Although IHC has been controversial because of its technical limitations, it is a widely available, inexpensive, rapid and reproducible method. Immunohistochemical stains, in contrast to fluorescence in situ hybridization (FISH) cytogenetics, detect cases that have increased protein expression through mechanisms other than translocations. FISH is not routinely performed at most institutions. IHC assessment for MYC and BCL-2 expression provides a practical approach to effectively stratify DLBCL into prognostically relevant subgroups. A wide range of cut-off points have been reported for MYC and BCL-2 expression (Brusamolino et al., 2006; Tzankov et al., 2010; Salles et al., 2011; Green et al., 2012; Johnson et al., 2012; Horn et al., 2013; Hu et al., 2013; Valera et al., 2013; Perry et al., 2014). In this study, we used the cut-off points of >30% and >50% for BCL-2 and MYC, respectively. They are based on Perry et al. study (Perry et al., 2014),where the survival tree method using P - values from adjusted logrank statistics was used to find the optimal cut-off points for BCL-2 and MYC expression to predict survival. However, these rates need to be validated in a large prospective cohort of DLBCL patients. This is a retrospective study with a small number of patients, and therefore, the patient cohort might not be representative of the general population of DLBCL patients treated with R-CHOP. However, our findings could be validated prospectively in a larger patient population.

In conclusion, we have demonstrated that the MYC and BCL-2 adjusted IPI model with seven prognostic factors present at diagnosis (age>60 years, stage III/IV disease, elevated lactate dehydrogenase level, Eastern Cooperative Oncology Group [ECOG] performance status≥2, more than one extranodal site of disease, high MYC expression, high BCL-2 expression) might be a practical approach to predict survival of DLBCL patients treated with R-CHOP.

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