Original Article

Development and validation of prognostic scoring in primary intestinal diffuse large B-cell lymphoma: a single-institution study of 184 patients

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Background: The incidence of primary intestinal diffuse large B-cell lymphoma (PI-DLBCL) is much lower than primary gastric DLBCL, and large-scale analyses on the clinical characteristics, molecular features, therapeutic strategies, and risk stratification have been seldomly performed in PI-DLBCL.

Methods: To assess prognostic model development, 107 PI-DLBCL patients diagnosed before 2014 were studied for prognosis factors including different primary involved sites and treatment strategies. For internal validation, a non-random split sample set with 77 PI-DLBCL patients after 2014 was included for validation of the prognosis factors.

Results: Patients with an ileocecal lesion presented with better survival time than those with non-ileocecal sites, with surgical resection significantly influencing the prognosis. Non-ileocecal patients who underwent surgery with lymphadenectomy had superior overall survival (OS) and progression-free survival (PFS) compared to those receiving surgery without lymphadenectomy or those not receiving (without) surgery. For ileocecal patients, surgery with or without lymphadenectomy resulted in better OS and PFS than those without surgery. For biomarker analysis, only BCL-2 >50% or Ki67 >80% on tumor cells indicated poor clinical outcome. In multivariate analysis, age, Eastern Cooperative Oncology Group (ECOG) score, and site of origin were independent prognostic factors for inferior OS in PI-DLBCL. A prognosis model was set up based on age, ECOG score, and site of origin, and validated well.

Conclusions: The prognosis in patients with PI-DLBCL with ileocecal involvement showed was better than those with non-ileocecal involvement. Surgical strategy can impact the clinical outcome of PI-DLBCL patients.

Keywords: Primary intestinal diffuse large B-cell lymphoma (PI-DLBCL); ileocecal site; surgery strategy; prognostic factors

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma (NHL), with the gastrointestinal (GI) tract the most frequent site of extranodal involvement (1-3). Approximately 5–20% of extranodal lymphomas occur in the GI, and GI lymphoma accounts for 1–4% of total GI malignancies (4). The incidence of primary intestinal DLBCL (PI-DLBCL) is much lower than that of primary gastric DLBCL. Accumulating evidence has shown that PI-DLBCL is a distinct entity with biological characteristics different from primary gastric DLBCL (5-8) and warrants further investigation. The prevalent involved sites are the small intestine, followed by the large intestine and ileum (1-3). Recently, a study of 50 PI-DLBCL indicated that immunochemotherapy plus surgery was associated with a superior prognosis compared with immunochemotherapy (9). However, analysis based on a large cohort of PI-DLBCL is still lacking.

Previous reports have shown several molecules such as BCL-2, BCL-6, and MYC are essential indicators of DLBCL (10,11). However, none of these molecules have been systematically studied in PI-DLBCL. Various treatment approaches have been attempted, but few compared the data in terms of different strategies (12-16), and the therapeutic strategies and optimal prognostic scoring specific for PI-DLBCL have not been established. In the present study, we analyzed the relevance of clinical characteristics, molecule features, and therapeutic strategies with clinical outcomes in a discovery cohort of 107 patients and evaluated these in a validation cohort of 77 patients with PI-DLBCL. We present the following article in accordance with the TRIPOD reporting checklist (available at https://dx.doi.org/10.21037/atm-21-4761).

Methods

Patients

All patients were from the Shanghai Jiaotong University, School of Medicine, Rui Jin Hospital. A total of 107 patients who were newly diagnosed PI-DLBCL from January 2003 to October 2014 were included in the discovery cohort. A further 77 PI-DLBCL patients enrolled in NCT01852435, which is a clinical trial for primary DLBCL patients, who were diagnosed between November 2014 to December 2017, were included in the validation cohort (Figure S1). All available data on the database were used to maximize the power and generalizability of the results. Patients with PI-DLBCL were defined as those having either apparent alimentary tract lesions or those with initial GI symptoms proven to be caused by lymphoma. The study was performed in accordance with the Declaration of Helsinki (as revised in 2013) and the protocol was approved by the Ethics Committee of Shanghai Rui Jin Hospital (2012-26). Informed consent was obtained from all patients.

Overall survival (OS) and progression-free survival (PFS) were followed over time using a variety of methods, including annual telephone interviews, triennial field center examinations, review of death certificates, physician questionnaires, and informant interviews. OS and PFS were ascertained by physicians blinded to the predictor variables.

The following data were extracted for each patient: gender, age, Ann Arbor stage, Lugano stage, performance status, B symptoms, extranodal involvement, lactate dehydrogenase (LDH), and international prognostic index (IPI) score, site of origin, and surgery method, as well as Ki67, MYC, BCL-2, and BCL-6 expressions on tumor cells. Two physicians and two surgeons classified the site of origin and surgery method with a structured standardized format and were blinded to other predictor variables and each patients' OS and PFS. To ensure reliability of data, we excluded patients who had missing key information, including age, gender, IPI score, site of origin, OS, and PFS.

Diagnosis and treatment

Pathological diagnosis was established according to the World Health Organization (WHO) classification (17). Patients who underwent surgery were reclassified into two groups; surgery with or without lymphadenectomy. Patients received surgery alone, or surgery followed by six cycles of RCHOP regimens (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone) which is the first line standard treatment for all the DLBCL patients, including PI-DLBCL. The treatment response was evaluated according to the WHO response criteria.

Immunohistochemistry assay

Immunohistochemistry was performed on 5 μm-paraffin sections with an indirect method (EnVison) using the primary antibody against MYC (Abcam, Cambridge, MA, USA, 1:100), BCL-2 (Abcam, 1:100), BCL-6 (Abcam, 1:100), and anti-rabbit/rat-IgG antibody (Dako,

Carpinteria, CA, USA) as the second antibody. BCL-2, BCL-6, and MYC positives were determined as previously reported (18-21). Interpretation of immunostains was performed by two certified hematopathologists.

Statistical analysis

OS was defined as the time from the date of diagnosis to either death or the last date of follow-up. PFS was defined as the time from the date of diagnosis to the time of disease progression or last follow-up. Continuous predictors, including age, were analyzed both with continuous numbers and with converted categorical forms (like cut off point of 60 years for age). The clinical data of patients with different treatment was calculated by Chi-square, while the survival analysis was calculated by the Kaplan-Meier method and compared by the log-rank test. The restricted mean survival time (RMST) was performed if there was a late crossing in the Kaplan-Meier survival curves. The multivariate survival analysis was performed by a Cox regression model, and only significant variables in the univariate analysis were selected for the multivariate analysis. A forest plot was performed with the risk factors in the univariate and multivariate analysis, and calibration of the risk score predictions was assessed. $P<0.05$ was considered statistically significant. All statistical analyses were evaluated using Statistical Package for the Social Sciences (SPSS) 23.0 software (SPSS Inc., Chicago, IL, USA) and R 4.0.3 (Foundation for Statistical Computing, Vienna, Austria).

Results

Clinical characteristics

Patient characteristics are summarized in Table S1. In the discovery cohort, the median follow-up duration was 42.0 months (0.6–162.4 months), the median age was 62.0 years (16–82 years), and the male-to-female ratio was 2.06:1. For these patients, 72.9% had good performance status [Eastern Cooperative Oncology Group (ECOG) 0–1] and 59.9% had localized disease (Lugano stage I/II-1/II-2). In the validation cohort, the median follow-up duration was 29.4 months (0.4–66.3 months), the median age was 61.0 years (29–83 years), and the male-to-female ratio was 2.5:1. For these patients, 48.1% had good performance status (ECOG 0–1) and 33.8% had localized disease. Among the patients of discovery cohort, 74 had surgery combined with chemotherapy, while 28 patients received chemotherapy alone, and five received surgery alone. Among the patients of the validation cohort, 40 patients had surgery combined with chemotherapy, while 37 patients received chemotherapy alone.

Survival analysis

In the discovery cohort, the 5-year OS and PFS of patients were 62.5% and 58.3%, respectively. Analysis of the primary sites showed 54 had small intestine lesions, 27 had large intestine lesions, and 26 had ileocecal lesions. Of the five patients with multiple involvement, three had combined lesions of the ileocecal and large intestine, and two had ileocecal and small intestine involvements. Since the survival status of these five patients was compared with the large intestine group and small intestine group (Figure S2), they were grouped to the large and small intestine groups, respectively. In the validation cohort, the 3-year OS and PFS were 62.5% and 55.6%, respectively, and 32 had small intestine lesions, 25 had large intestine lesions, and 20 patients had ileocecal lesions as the primary site.

In the discovery cohort, patients of the large intestine group had similar outcomes as those of the small intestine group (5-year OS 56.0% *vs.* 57.0% and 5-year PFS 54.8% *vs.* 51.0%), while 5-year OS and PFS of the ileocecal group was 86.7% and 81.2%, respectively. Thus, we considered both large and small intestine groups as a non-ileocecal group (*Figure 1A,1B*). The 5-year OS and PFS of the ileocecal group were 86.7% and 81.2%, respectively, which was significantly higher than those of the non-ileocecal group (56.6% and 52.4%, P=0.0174 and P=0.0044), (*Figure 1C,1D*).

Similar to the discovery cohort, the 3-year OS and PFS of the ileocecal group were 91.7% and 85.7%, respectively, which were significantly higher than those of the non-ileocecal group (52.2% and 47.1%, P=0.0019 and P=0.0053), (*Figure 1E,1F*).

The clinical features and survival time of patients with ileocecal or non-ileocecal involvement are summarized in Table S2. In both the discovery and validation cohorts, no significant difference in clinical and pathological parameters was observed between the ileocecal and non-ileocecal groups, including gender, age, Ann Arbor stage, Lugano stage, performance status, B symptoms, extranodal involvement, and IPI score, as well as the Ki67 expression

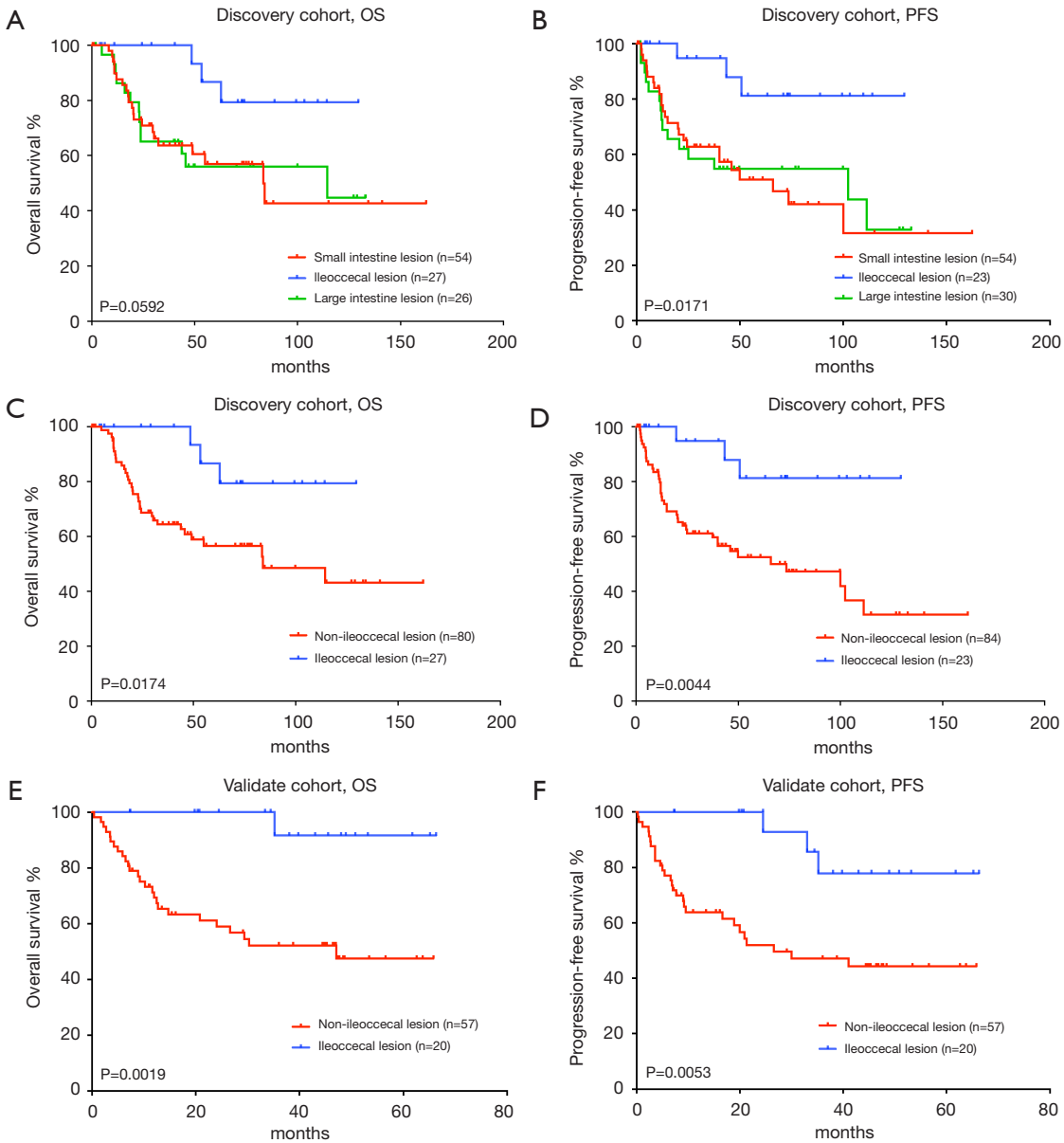

Figure 1 OS and PFS according to primary involved sites in PI-DLBCL. (A,B) OS (A) and PFS (B) in the discovery cohort by three subgroups based on primary involved sites: small intestine, ileoccecal, and large intestine lesions. (C,D) OS (C) and PFS (D) in the discovery cohort by two subgroups based on primary involved sites: ileocecal and non-ileocecal lesions. (E,F) OS (E) and PFS (F) in the validation cohort by two subgroups based on primary involved sites: small intestine, ileoccecal, and large intestine lesions. PI-DLBCL, primary intestinal diffuse large B-cell lymphoma; OS, overall survival; PFS, progression-free survival.

on tumor cells.

Surgical strategies

Of note, surgery significantly affected the clinical outcomes of patients. In the non-ileocecal group of the discovery cohort, subgroup 1 received surgery with lymphadenectomy, and subgroup 2 received surgery without lymphadenectomy, or did not undergo surgery, and the results showed subgroup 1 had significantly longer survival time than subgroup 2 (5-year OS as 83.1%, 52.4%, and 26.1%, respectively, P=0.0033, *Figure 2A*; and 5-year

PFS as 79.7%, 45.4%, and 25.2%, respectively, P=0.0049, *Figure 2B*). We then divided the non-ileocecal group into two subgroups: (I) surgery with lymphadenectomy, (II) surgery without lymphadenectomy or no surgery (5-year OS 83.1% *vs.* 39.9%, P=0.0018, *Figure 2C*; 5-year PFS 79.7% *vs.* 36.3%, P=0.0040, *Figure 2D*). Since there is late-stage crossover between subgroups in the PFS analysis of the validate cohort, we further performed RMST survival analysis to verify the difference between subgroups (Figure S3). Similar results were observed in the validation cohort: patients with non-ileocecal involvements who received surgery with lymphadenectomy had a longer survival time than those who underwent either surgery without lymphadenectomy or those without surgery (3-year OS 84.6%, 49.1%, and 36.3%, respectively, P=0.0095; and 3-year PFS as 82.5%, 42.4%, and 32.5%, respectively, P=0.0090). Further, by reclassifying patients who underwent surgery without lymphadenectomy and those without surgery into a group without lymphadenectomy, the group without lymphadenectomy showed a noticeably worse prognosis than those with surgery and lymphadenectomy (3-year OS 40.1% *vs.* 84.6%, P=0.0033, *Figure 2E*; 3-year PFS 38.3% *vs.* 82.5%, P=0.0023, *Figure 2F*).

However, in the ileocecal group, patients receiving surgery, regardless of having lymphadenectomy or not, had similar OS and PFS, which was better than those without surgery (5-year OS as 91.7%, 100.0%, and 0%, P=0.0136, *Figure 3A*; 5-year PFS as 92.9%, 100.0%, and 0%, P=0.0174, *Figure 3B*). Since the surgical strategies did not interrupt the outcome of patients with ileocecal involvement, these patients were divided into two subgroups: surgery and non-surgery (5-year OS, 92.3% *vs.* 0%, P=0.0034, *Figure 3C*; 5-year PFS, 93.8% *vs.* 0%, P=0.0045, *Figure 3D*). The analysis of the validation cohort showed a similar trend, that among patients with ileocecal involvement, the surgery subgroup tended to display a favorable prognosis (as for surgery with lymphadenectomy, surgery without lymphadenectomy, and no surgery groups, 3-year OS as 100.0%, 100.0%, and 66.7%, P=0.2231; 3-year PFS as 100.0%, 100.0%, and 50.0%, P=0.2844). When patients were divided into surgery and non-surgery subgroups, 3-year OS were 100.0% and 66.7% (P=0.0833), and 3-year PFS were 100.0% *vs.* 50.0%, respectively (P=0.1267), (*Figure 3E,3F*).

Tumor biomarkers

As previously reported (22), the prognosis of DLBCL patients with non-germinal center B cell-like (non-GCB) subtype is usually worse than those with germinal center B cell-like (GCB) subtype. However, in our cohort, according to the algorithm of Hans classification, no statistical difference in survival was observed between GCB and non-GCB, nor in the discovery cohort (3-year OS, 82.2% *vs.* 70.6%, P=0.468; 3-year PFS, 77.6% *vs.* 56.8%, P=0.5249), or in the validation cohort (3-year OS, 76.1% *vs.* 57.8%, P=0.3610; 3-year PFS, 77.0% *vs.* 50.2%, P=0.2116).

We further evaluated the prognostic effect of pathological molecular markers. Among these, 131 patients had available results for BCL-2 and MYC, 106 patients had results of BCL-6, and 147 patients had results of Ki67. Overexpression of BCL-2 (BCL-2 >50%) and Ki67 (Ki67 >80%) on tumor cells correlated with the poor prognosis of patients (Table S3). Patients with BCL-2 >50% had worse outcomes than those with BCL-2 ≤50% (3-year OS, 56.1% *vs.* 84.2%, P=0.0004, *Figure 4A*; 3-year PFS, 52.9% *vs.* 79.1%, P=0.0034, *Figure 4B*). In addition, following Ki67 staining, patients with Ki67 >80% on tumor cells had worse outcomes than those with Ki67 ≤80% (3-year OS, 52.5% *vs.* 80.4%, P=0.0002, *Figure 4C*; 3-year PFS, 48.3% *vs.* 74.5%, P=0.0007, *Figure 4D*).

Prognostic factors

Clinical and pathological characteristics, and molecular biomarkers were included in the univariate analysis, and the results showed that age, Ann Arbor stage, Lugano stage, performance status, number of extranodal involvements, Ki67, BCL-2, and primary involved sites had significant predictive abilities for OS and PFS (Table S4). These parameters were further included in the multivariate analysis, and the results showed that age, performance status, and primary involved sites were independent prognostic factors for OS and PFS in PI-DLBCL (*Table 1*). However, overexpression of BCL-2 (BCL-2 >50%) on tumor cells was an independent unfavorable factor for OS.

Nomogram validation

Forest plot was performed with the risk factors in

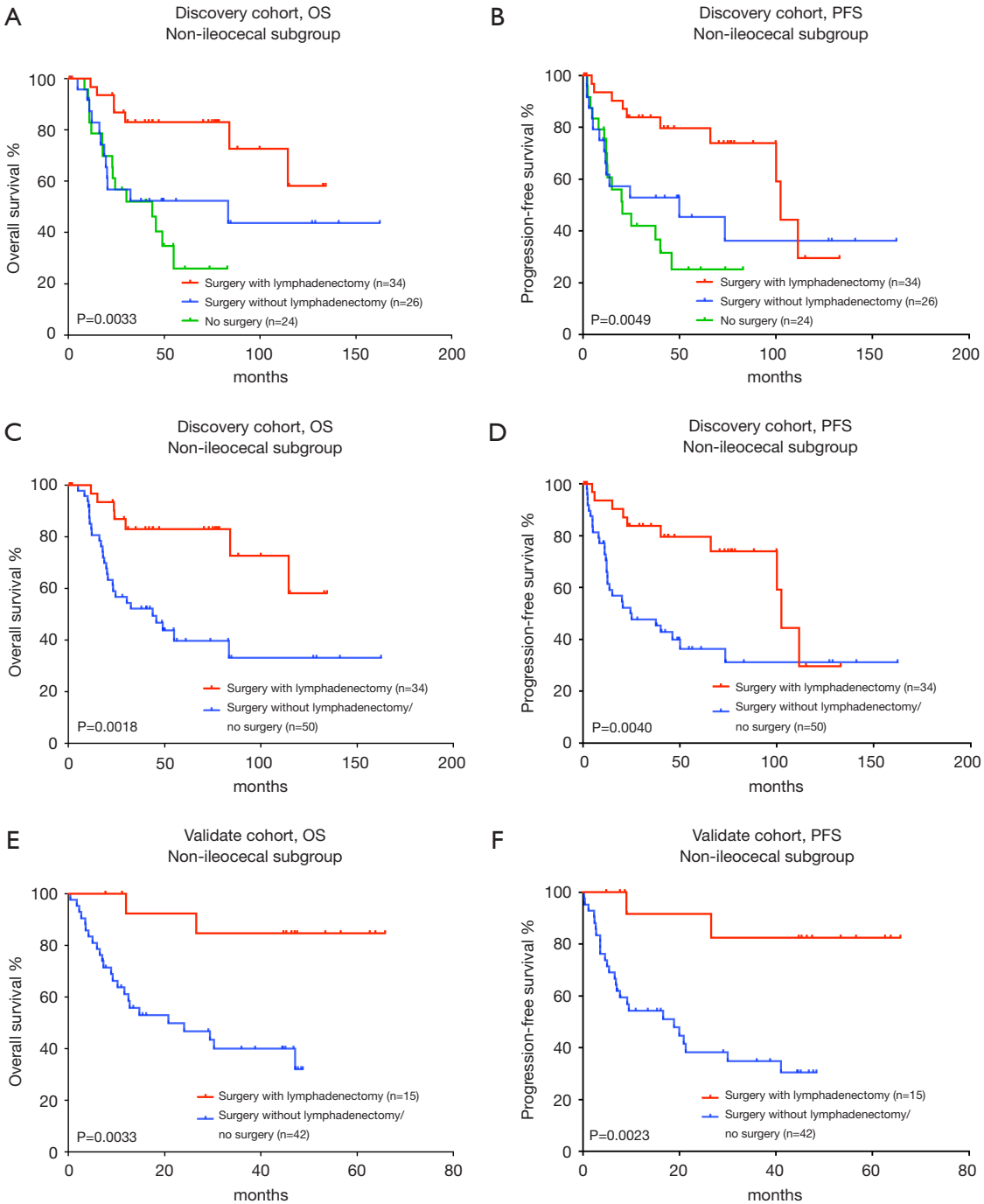

Figure 2 OS and PFS according to therapeutic strategies in PI-DLBCL with non-ileocecal involvements. (A,B) OS (A) and PFS (B) in non-ileocecal patients of the discovery cohort by three subgroups based on surgery approaches: surgery with lymphadenectomy, surgery without lymphadenectomy, and no surgery. (C,D) OS (C) and PFS (D) in non-ileocecal patients of the discovery cohort by two subgroups based on surgery approaches: surgery with lymphadenectomy and surgery without lymphadenectomy/no surgery. (E,F) OS (E) and PFS (F) in non-ileocecal patients of the validation cohort by two subgroups based on surgery approaches: surgery with lymphadenectomy, surgery without lymphadenectomy, and no surgery. OS, overall survival; PFS, progression-free survival; PI-DLBCL, primary intestinal diffuse large B-cell lymphoma.

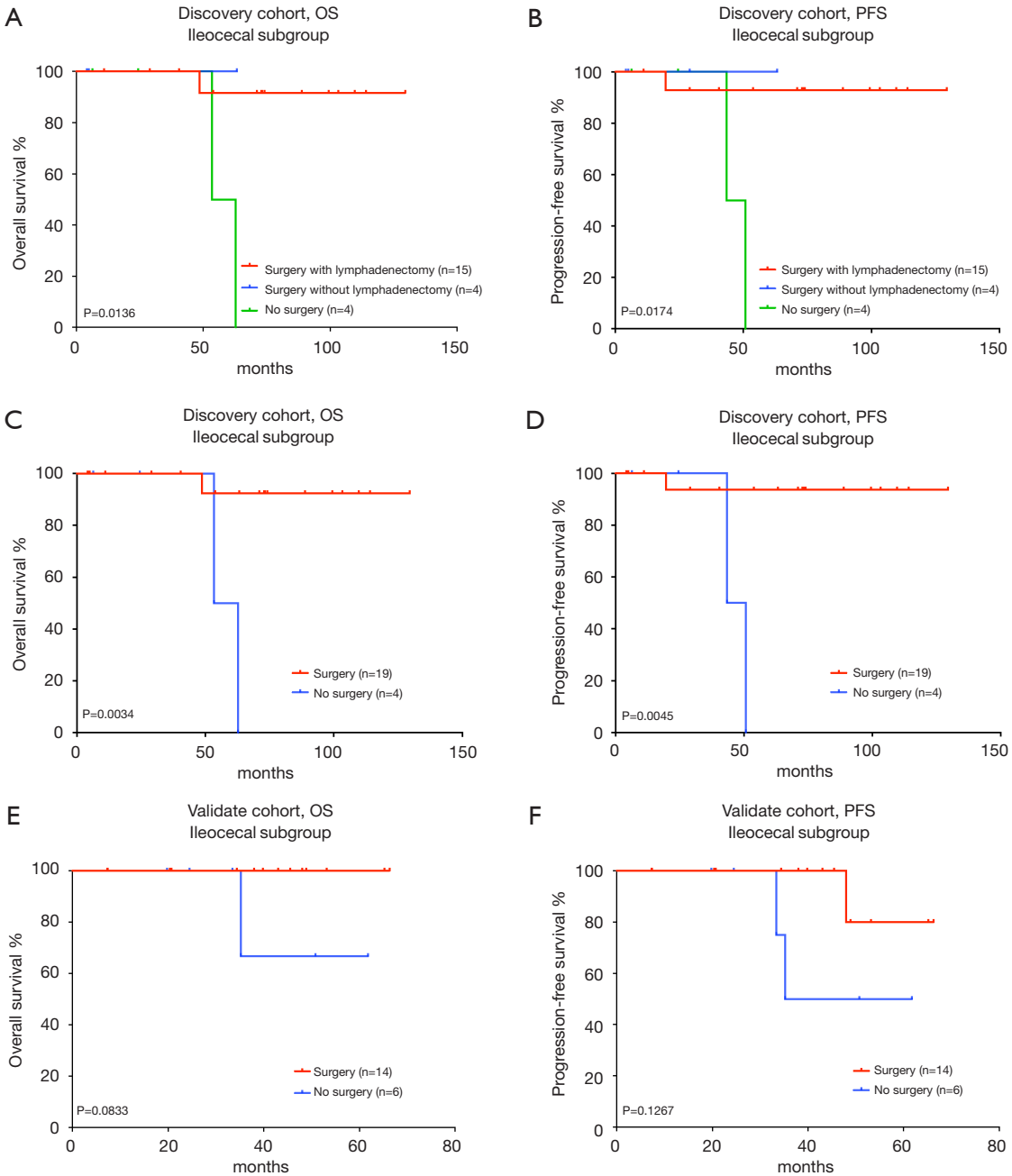

Figure 3 OS and PFS based on treatment strategy in ileocecal involvements of PI-DLBCL. (A,B) OS (A) and PFS (B) in ileocecal patients of the discovery cohort by three subgroups based on surgery approaches: surgery with lymphadenectomy, surgery without lymphadenectomy, and no surgery. (C,D) OS (C) and PFS (D) in ileocecal patients of the discovery cohort by two subgroups based on surgery approaches: surgery and no surgery. (E,F) OS (E) and PFS (F) in ileocecal patients of the validation cohort by two subgroups based on surgery approaches: surgery with lymphadenectomy, surgery without lymphadenectomy, and no surgery. OS, overall survival; PFS, progression-free survival; PI-DLBCL, primary intestinal diffuse large B-cell lymphoma.

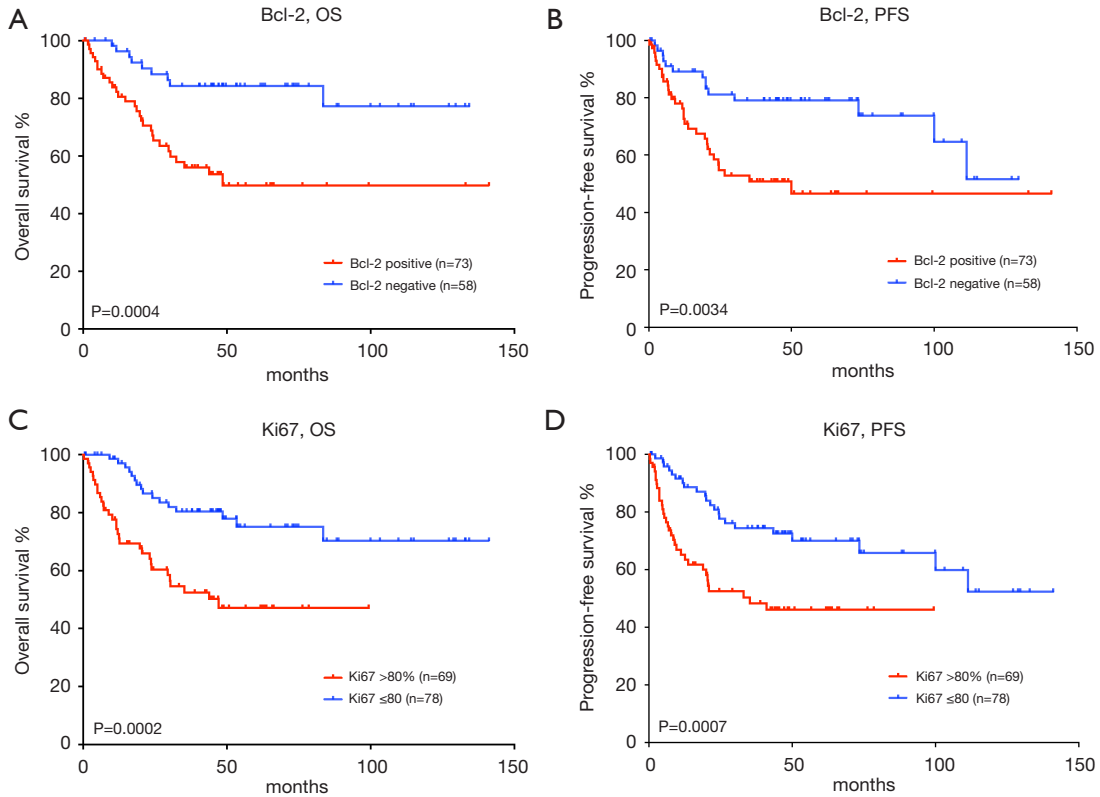

Figure 4 OS and PFS based on molecular biomarkers in PI-DLBCL. (A,B) OS (A) and PFS (B) in PI-DLBCL patients based on BCL-2 expression: BCL-2 >50% and BCL-2 ≤50%. (C,D) OS (C) and PFS (D) in PI-DLBCL patients based on Ki67 expression: Ki67 >80% and Ki67 ≤80%. OS, overall survival; PFS, progression-free survival; PI-DLBCL, primary intestinal diffuse large B-cell lymphoma.

Table 1 Multivariate analysis of prognostic factors for OS and PFS in patients with PI-DLBCL

Factors	OS			PFS		
	P	HR	95% CI	P	HR	95% CI
Age, y (≤60 *vs.* >60 y)	0.005	3.041	1.402–6.596	0.016	2.330	1.172–4.634
Ann Arbor (I/II/III/IV)	0.811	1.070	0.614–1.865	0.127	1.478	0.895–2.440
Lugano (I/II1/II2/IIE/IV)	0.310	1.278	0.796–2.051	0.803	1.055	0.691–1.612
ECOG (0/1/2/3/4)	0.001	2.126	1.362–3.318	0.004	1.808	1.214–2.693
No. of extranodal involvements (<2 *vs.* ≥2)	0.774	0.884	0.380–2.054	0.561	0.795	0.366–1.726
Ki67 (≤80% *vs.* >80%)	0.200	1.631	0.772–3.447	0.239	1.496	0.765–2.924
BCL-2 (≤50% *vs.* >50%)	0.025	2.646	1.132–6.185	0.108	1.800	0.879–3.690
Primary sites (ileocecal *vs.* non-ileocecal)	0.001	3.349	1.604–6.992	0.001	2.836	1.550–5.191

OS, overall survival; PFS, progression-free survival; PI-DLBCL, primary intestinal diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group.

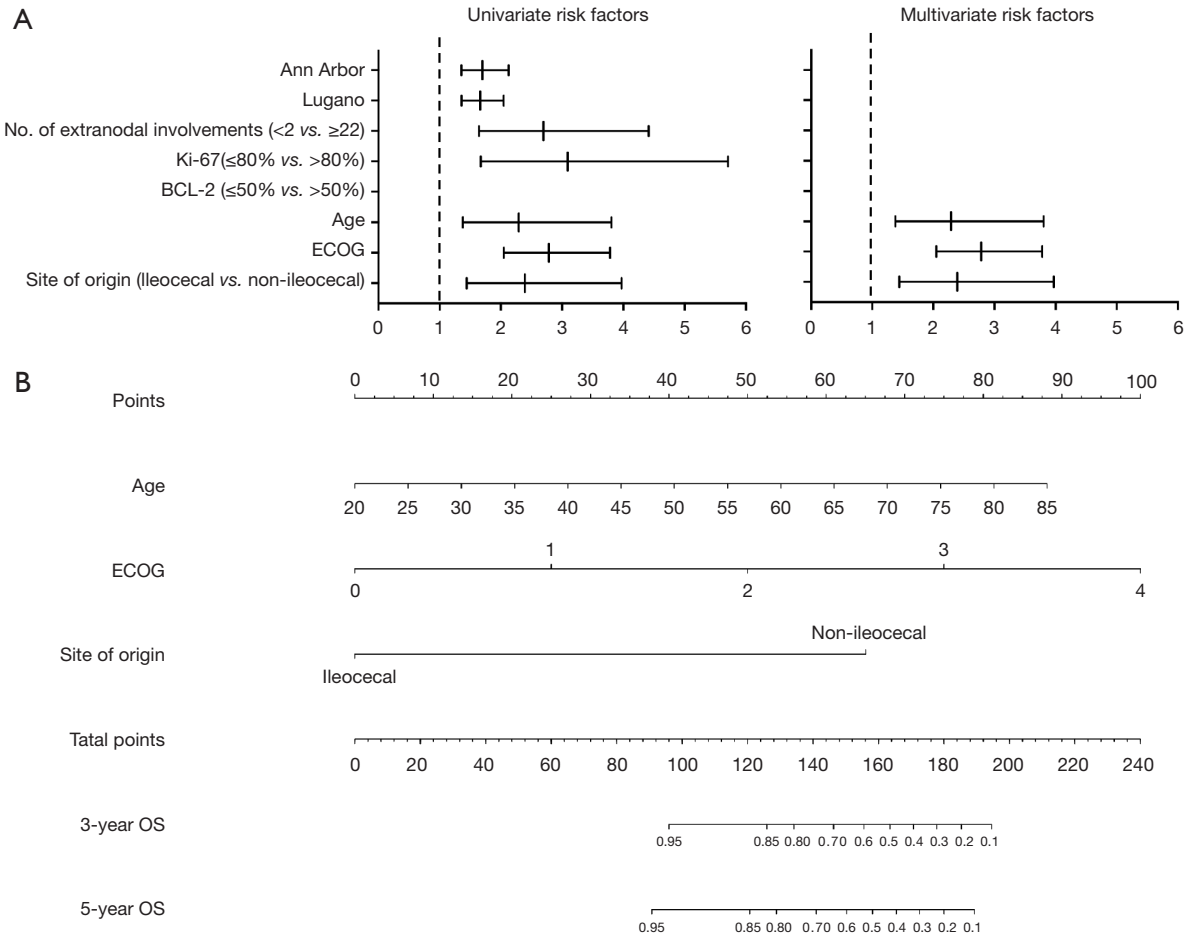

Figure 5 Nomogram predicting and calibration curves. (A) Forest plot with the risk factors in the univariate and multivariate analysis. (B) Nomogram predicting 3- and 5-year survival in the discovery cohort.

the univariate and multivariate analysis (*Figure 5A*). Independent prognostic factors associated with the OS rate identified in the multivariate analysis were incorporated into the nomogram internal validation in the discovery cohort. The nomogram was performed by drawing a vertical line up to the points row to obtain the points for each variable, the total points obtained by adding up the points for all the variables, and a vertical line was drawn down from the total points row to obtain the 3- and 5-year OS rates (*Figure 5B*). The C index was 0.8 (95% CI, 0.774–0.832). The validation cohort showed a C index 0.8 (95% CI, 0.741–0.817) with the nomogram model based on the discovery cohort. In addition, as presented in Figure S4A,S4B, the predicted 5-year OS rate probabilities for the discovery and validation cohorts were highly closed to the actual observations.

Discussion

PI-DLBCL is a unique entity of DLBCL with distinct clinical and biological features. Due to its low incidence, PI-DLBCL is frequently analyzed as a rare extranodal subtype both in DLBCL and in NHL (5,7,12,14-16,23). The prognosis of PI-DLBCL is poor as compared to primary gastric DLBCL (3) and its optimal management remains elusive.

Surgery combined with chemotherapy has shown to be more preferable than chemotherapy alone in PI-DLBCL (24). However, in another study of primary colon lymphoma, surgery resection failed to improve survival compared to chemotherapy alone (25). To better review PI-DLBCL, we searched PubMed using the terms “intestinal

diffuse large B-cell lymphoma" for studies published in English from 2000 and found 10 articles, which are summarized in Table S5. Among these, four studies involved research on outcomes and treatment modalities, such as surgery, chemotherapy, and radiotherapy, with two concluding surgery improved patient outcomes (5,26) and two finding it did not (15,16). Two studies analyzed the impact of primary involved sites on the prognosis of PI-DLBCL, one of which showed non-ileocecal involvement had a poor prognosis (24), while the other reached a contrary conclusion (14). To our knowledge, no data was presented to compare the surgical strategies according to the different primary sites in PI-DLBCL. Our results revealed that primary sites of PI-DLBCL significantly impacted on the prognosis of the patients both in the discovery and validation cohorts. Patients with ileocecal involvement had better outcome, and surgery, either with or without lymphadenectomy, could improve the prognosis of PI-DLBCL. For non-ileocecal DLBCL, only surgery with lymphadenectomy combined with chemotherapy prolonged OS and PFS. We also notice that if the patients with clinical features of GI bleeding, obstruction or perforation, they mostly underwent urgent surgery without lymphadenectomy which can also affect the prognosis. These results suggest that surgery should be stratified according to primary involved sites in PI-DLBCL.

Molecule biomarkers are not only essential for optimizing treatment strategies, but also for developing new bio-therapeutic agents. BCL-2, BCL-6, MYC, and Ki67 are important prognostic factors of DLBCL (19-21,27-31). As previously reported, GCB and non-GCB subtypes shared similar prognosis in primary GI DLBCL (32). In addition, we found that overexpression of BCL-2 (BCL-2 >50%) and Ki67 (Ki67 >80%) on tumor cells predicted poor patient outcomes, suggesting the importance of BCL-2 and Ki67 in the biological features of PI-DLBCL. While previous publications have indicated therapeutic approaches and primary sites related to PI-DLBCL prognosis (5,24,26), to the best of our knowledge, ours is the first study to analyze multivariate predictors for survival of the disease. In multivariate analysis, overexpression of BCL-2 was an independent prognostic factor for OS, providing a clinical rationale of using BCL-2 inhibitor to treat PI-DLBCL.

Different staging systems have been developed to improve risk stratification of DLBCL in recent times. In a Korean study of 106 PI-DLBCL patients, IPI and R-IPI staging could predict prognosis (7), while other studies of small cohorts of patients in Asia and Switzerland reported that IPI staging might not stratify PI-DLBCL patients and the NCCN-IPI scoring system might be more accurate (33,34). But the NCCN-IPI scoring system needs many more clinical parameters which limits its application in practice. In our study, using multivariate analysis we showed that primary involved sites were independent factors for both OS and PFS, in addition to age and performance status. The nomogram model based on the three independent factors was further validated in both cohorts. These results provide a new and convenient clinical parameter: primary involved sites, to value the prognosis of PI-DLBCL patients independent of IPI and might contribute to a new scoring system for PI-DLBCL patients after further validation.

As this study was conducted at a single center, validation through multi center analyses is required to verify the results and support further application in clinical practice.

In conclusion, our findings suggest that PI-DLBCL is a distinct entity with unique clinical and biological features. Primary involved sites are important for survival prediction in the clinical management of PI-DLBCL.

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Footnote

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Data Sharing Statement: Available at https://dx.doi.org/10.21037/atm-21-4761

Conflicts of Interest: All authors have completed the ICMJE

uniform disclosure form (available at https://dx.doi.org/10.21037/atm-21-4761). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was performed in accordance with the Declaration of Helsinki (as revised in 2013) and the protocol was approved by the Ethics Committee of Shanghai Rui Jin Hospital (2012-26). Informed consent was obtained from all patients.

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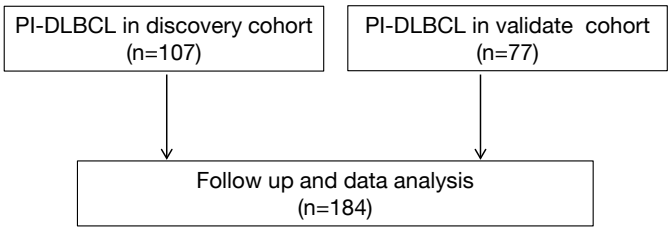

Figure S1 Flow chart of the clinical studies. PI-DLBCL, primary intestinal diffuse large B-cell lymphoma.

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Table S1 Clinical characteristics of patients with PI-DLBCL

Characteristics	Discovery cohort		Validation cohort	
	Cases	%	Cases	%
Sex				
Male	72	67.3	55	71.4
Female	35	32.7	22	28.6
Age				
>60 y	47	43.9	41	53.2
≤60 y	60	56.1	26	46.7
Ann Arbor stage				
I	33	30.8	10	13.0
II	44	41.1	31	40.3
III	7	6.5	13	16.9
IV	23	21.5	23	30.0
Lugano stage				
I	28	26.2	6	7.8
II1	31	29.0	15	19.5
II2	5	4.7	5	6.5
IIE	20	18.7	40	52.0
IV	23	21.5	11	14.3
ECOG score				
0	10	9.3	3	3.9
1	68	63.6	34	44.2
2	24	22.4	29	37.7
3	4	3.7	9	11.7
4	1	0.9	2	2.6
B symptom				
Present	46	43.0	24	31.2
Absent	61	57.0	53	68.8
LDH (U/L)				
>200	35	32.7	34	44.2
≤200	72	67.3	43	55.8
Ki67 expression				
>80%	17	15.9	52	67.5
≤80%	53	49.5	25	32.5
NA	37	34.6	0	0
IPI score				
0	29	27.1	8	10.4
1	30	28.0	15	19.5
2	21	19.6	29	37.7
3	16	15.0	14	18.2
4	8	7.5	11	14.3
5	3	2.8	0	0
Extranodal involvement				
≥2	27	25.2	37	48.1
<2	80	74.8	40	51.9

PI-DLBCL, primary intestinal diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; IPI, international prognostic index.

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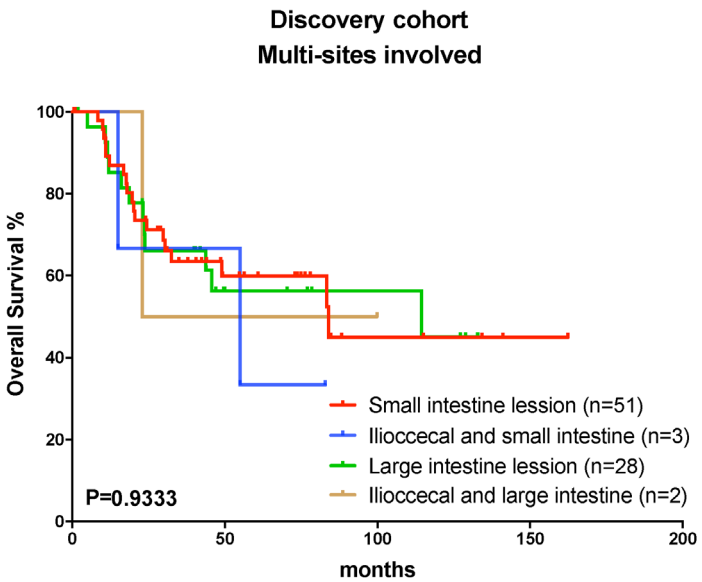

Figure S2 OS of multi-site involved patients. Kaplan-Meier estimates of OS of the PI-DLBCL patients in the discovery cohort with different sites involved. Log rank test was used to test the significance, and censored patients were indicated by crosses. OS, overall survival; PI-DLBCL, primary intestinal diffuse large B-cell lymphoma.

Table S2 Clinical characteristics of patients with PI-DLBC

Characteristics	Discovery cohort			Validation cohort		
	Ileocecal group	Nonileocecal group	P value	Ileocecal group	Nonileocecal group	P value
Sex						0.324
Male	17	55	0.445	16	39	
Female	6	29		4	18	
Age						0.482
>60 y	13	34	0.170	12	29	
≤60 y	10	50		8	28	
Ann Arbor stage						0.106
I–II	20	57	0.071	15	31	
III–IV	3	27		5	26	
Lugano stage						0.074
I/II1/II2	17	47	0.120	10	16	
IIE/IV	6	37		10	41	
ECOG score						0.214
0–1	18	60	0.514	12	25	
2–3	5	24		8	32	
B symptom						0.489
Present	9	37	0.673	5	19	
Absent	14	47		15	38	
Extranodal involvement						0.175
≥2	5	22	0.663	7	30	
<2	18	62		13	27	
Ki67 expression						0.779
>80%	2	15	0.122	13	39	
≤80%	18	35		7	18	
NA	3	34		0	0	
IPI score						0.802
0	8	21	0.825	5	3	
1	6	24		4	11	
2	4	17		4	25	
3	5	11		4	10	
4	0	8		3	8	
5	0	3		0	0	
OS						
5 years	86.7%	56.6%	0.017			
3 years				91.7%	52.2%	0.002
PFS						
5 years	81.2%	52.4%	0.004			
3 years				85.7%	47.1%	0.005

PI-DLBCL, primary intestinal diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; IPI, international prognostic index; OS, overall survival; PFS, progression-free survival.

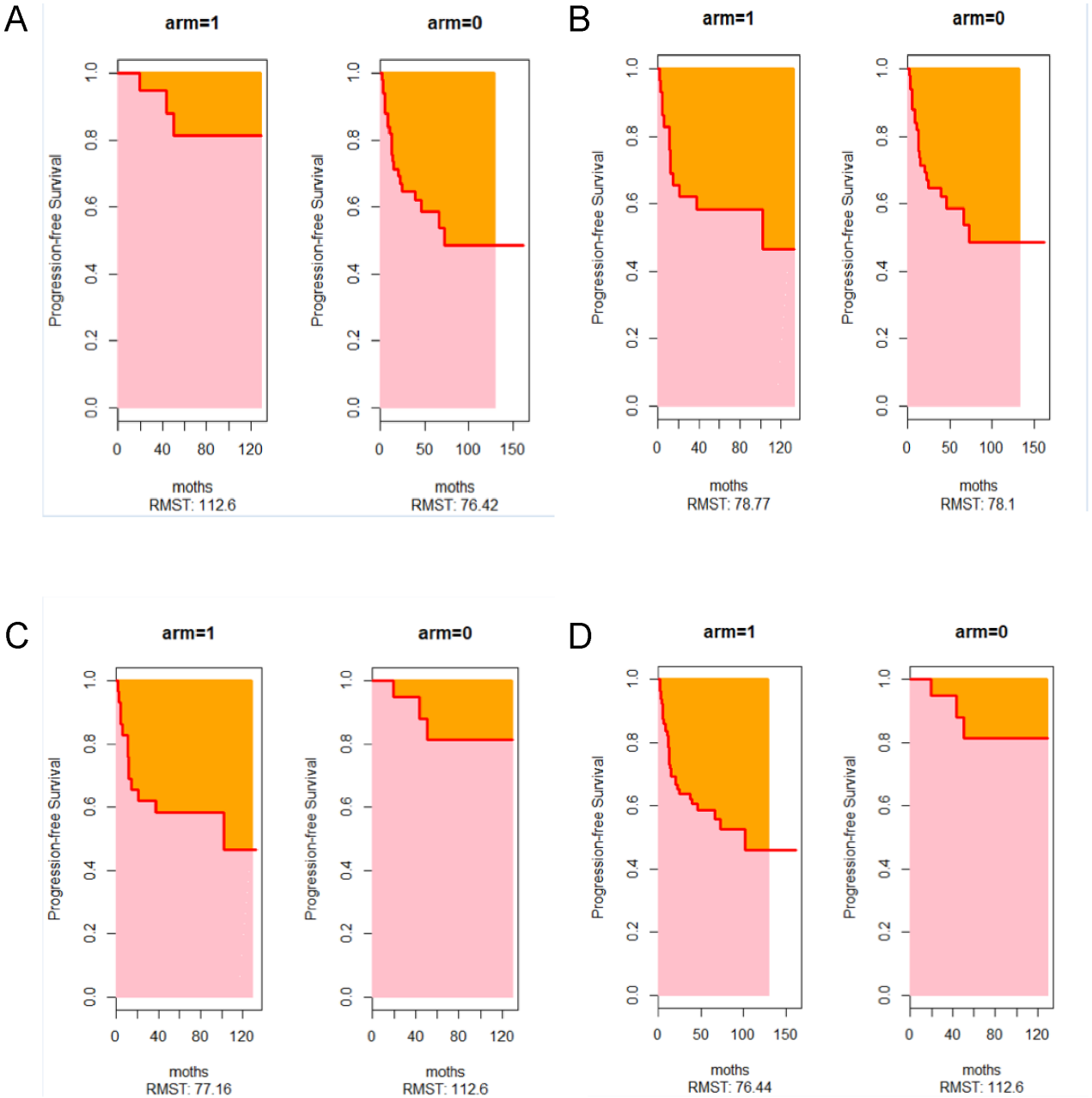

Figure S3 RMST analysis of the non-ileocecal group in the discovery cohort. (A) Comparison between surgery with the lymphadenectomy subgroup and surgery without lymphadenectomy subgroup, P=0.003. (B) Comparison between no surgery subgroup and surgery without lymphadenectomy subgroup, P=0.962. (C) Comparison between surgery with lymphadenectomy subgroup and no surgery subgroup, P=0.011. (D) Comparison between surgery with lymphadenectomy subgroup and surgery without lymphadenectomy or no surgery subgroup, P=0.001. RMST, restricted mean survival time.

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Table S3 Biomarkers of patients with PI-DLBCL

Biomarkers	3-yr-OS	3-yr-PFS	P value
MYC			
>40%	73.6	69.4	0.274
≤40%	58.9	54.4	0.413
BCL-2			
>50%	56.1	52.9	<0.001
≤50%	84.2	79.1	0.003
BCL-6			
>30%	63.8	55.7	0.961
≤30%	67.6	60.4	0.578
Ki67			
>80%	52.5	48.3	<0.001
≤80%	80.4	74.5	0.001

PI-DLBCL, primary intestinal diffuse large B-cell lymphoma; OS, overall survival; PFS, progression-free survival.

Table S4 Univariate analysis of prognostic factors for OS and PFS in patients with PI-DLBCL

Factors	OS			PFS		
	P	HR	95% CI	P	HR	95% CI
Age, y (≤60 *vs.* >60 y)	0.001	2.289	1.378–3.804	0.012	1.913	1.153–3.716
Sex (male *vs.* female)	0.185	0.710	0.428–1.178	0.192	0.709	0.423–1.188
Ann Arbor (I/II/III/IV)	<0.001	1.696	1.355–2.124	<0.001	1.621	1.292–2.034
Lugano (I/II1/II2/IIE/IV)	<0.001	1.665	1.357–2.044	<0.001	1.651	1.343–2.030
B symptom (absent *vs.* present)	0.850	1.050	0.635–1.736	0.956	1.014	0.608–1.692
ECOG (0/1/2/3/4)	<0.001	2.782	2.048–3.779	<0.001	2.504	1.822–3.442
No. of extranodal involvements (<2 *vs.* ≥2)	<0.001	2.693	1.643–4.413	<0.001	2.712	1.639–4.488
LDH (≤200 *vs.* >200)	0.703	1.000	1.000–1.000	0.414	1.000	1.000–1.000
Serum ALB (<32 *vs.* ≥32 g/L)	0.904	1.000	1.000–1.000	0.914	1.000	1.000–1.000
β2 microglobulin (normal *vs.* abnormal)	0.749	1.000	1.000–1.000	0.782	1.000	1.000–1.000
CRP (normal *vs.* abnormal)	0.520	1.000	1.000–1.000	0.577	1.000	1.000–1.000
Ki67 (≤80% *vs.* >80%)	<0.001	3.090	1.673–5.707	0.003	2.521	1.372–4.630
BCL-2 (≤50% *vs.* >50%)	0.025	1.000	1.000–1.000	0.003	3.037	1.464–6.300
Phenotype (GCB *vs.* non-GCB)	0.241	1.000	1.000–1.000	0.229	1.000	1.000–1.000
Serum CA125 (normal *vs.* abnormal)	0.858	1.000	1.000–1.000	0.937	1.000	1.000–1.000
Serum CA199 (normal *vs.* abnormal)	0.927	1.000	1.000–1.000	0.700	1.000	1.000–1.000
Primary involved sites (ileocecal *vs.* non-ileocecal)	0.001	2.392	1.441–3.970	0.001	2.109	1.335–3.332

OS, overall survival; PFS, progression-free survival; PI-DLBCL, primary intestinal diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; ALB, albumin; CRP, C-reactive protein; GCB, germinal center B cell-like.

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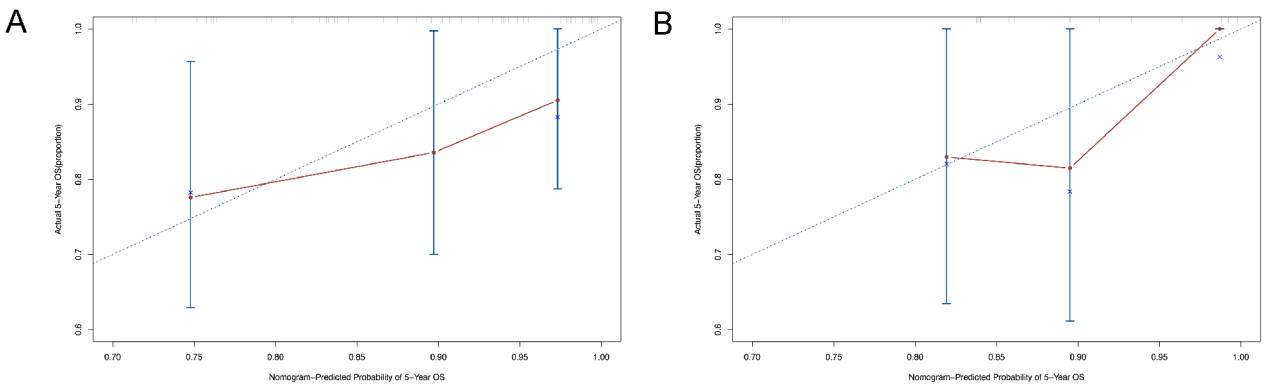

Figure S4 Calibration curves for the nomogram in discovery cohort and validate cohorts. Calibration curves for the nomogram in the discovery cohort (A) and validation cohort (B). The X-axis is the predicted survival calculated by the nomogram, and the Y-axis is the actual survival estimated by the Kaplan-Meier method. The 95% CIs of the Kaplan-Meier estimates are indicated by blue vertical lines at each point. The blue dashed line represents the reference line and is a 45° diagonal.

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Table S5 Review of literature on PI-DLBCL

No. (reference)	Number of primary intestinal NHL cases	Country or region	Observation factors	Survival (OS)	P value	Time of publication	Publication
1 (7)	345 (PI-DLBCL)	Korea	The surgery/chemotherapy group in localized DLBCL (n=163)	3y-OS: 91%	<0.001	2011	Blood
			The chemotherapy group in localized DLBCL (n=87)	3y-OS: 62%			
			The surgery/chemotherapy group in disseminated DLBCL (n=25)	3y-OS: 47%	0.3034		
			The chemotherapy group in disseminated DLBCL (n=52)				
2 (29)	581 (PI-NHL)	Korea	The ileocecal region of NHL (n=231)	–	0.006	2011	BMC Cancer
			The small intestinal region of NHL (n=162)				
			The large intestinal region of NHL (n=125)				
			Multiple intestinal of NHL (n=3)				
3 (9)	106 (PI-DLBCL)	Korea	IPI risk group	–	0.021	2014	J Korean Med Sci
			ECOG performance score	–	0.045		
			Modified Ann Arbor stage	–	0.358		
			Lugano stage	–	0.401		
4 (28)	40 (PI-DLBCL)	Korea	Modified Ann Arbor stage	–	<0.001	2007	Leukemia Research
5 (31)	76 (DLBCL)	Korea	The surgery + RCHOP group in DLBCL (n=47)	3y-OS: 94.2%	0.049	2014	American Journal of Clinical Oncology
			The RCHOP group in DLBCL (n=29)	3y-OS: 80.7%			
6 (20)	40 (PI-NHL)	China	Ann Arbor stage	–	0.64	2008	Int J Hematol
			IPI score	–	0.73		
			Surgery (n=5)	–	0.62		
			Surgery + chemotherapy (n=24)	–			
			Surgery + radiotherapy (n=4)	–			
			Surgery + chemotherapy + radiotherapy (n=6)	–			
7 (18)	66 (PI-DLBCL)	Switzerland	Small intestinal region (n=37)	5y-OS: 56%	0.78	2001	Annals of Oncology
			Colon-rectal region (n=8)	5y-OS: 50%			
			Small and large intestinal region (n=21)	5y-OS: 57%			
			IPI score	–	0.27		
			ECOG score	–	0.23		
			Serum ALB level normal (n=27)	5y-OS: 70%	0.004		
			Serum ALB level low (n=31)	5y-OS: 39%			
8 (19)	23 (PI-NHL)	Japan	LDH normal or abnormal (primary small intestinal NHL)	–	0.050	2009	Leuk Lymphoma
			Ann Arbor stage (primary small intestinal NHL)	–	0.413		
			IPI score (primary small intestinal NHL)	–	0.090		
			Surgery or not (primary small intestinal NHL)	–	0.103		
			Chemotherapy or not (primary small intestinal NHL)	–	0.782		
9 (16)	21 (PI-NHL)	Germany	Intestinal B-cell lymphoma	2y-OS: 94%	–	2003	Journal of Clinical Oncology
10 (3)	96 (PI-NHL)	Japan	The primary intestinal lymphoma (n=94)	3y-OS: ~63%	–	2003	Cancer

PI-DLBCL, primary intestinal diffuse large B-cell lymphoma; NHL, non-Hodgkin lymphoma; OS, overall survival; IPI, international prognostic index; ECOG, Eastern Cooperative Oncology Group; ALB, albumin; LDH, lactate dehydrogenase.