ORIGINAL RESEARCH

A New Immunological Prognostic Model Based on Immunohistochemistry for Extranodal Natural Killer/T-Cell Lymphoma Patients After Non-Anthracycline-Based Chemotherapy

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Purpose: Programmed death ligand 1 (PD-L1) has been proposed as an important prognostic factor in many types of cancer. However, the role of predicting the prognosis of PD-L1 in extranodal natural killer/T-cell lymphoma (ENKTL) was controversial. Combining other biomarkers might enhance its predictive power. This study aims to evaluate the prognostic value of PD-L1 in conjunction with tumor-infiltrating FoxP3+Tregs for ENKTL after non-anthracycline-based chemotherapy.

Patients and Methods: A total of 81 patients with ENKTL were included in this study. Clinicopathological characteristics were collected, and prognostic significance of PD-L1 in neoplastic cells (nPD-L1) and tumor-infiltrating FoxP3+Tregs were evaluated.

Results: Patients with nPD-L1-positive had significantly inferior overall survival (OS) and progression-free survival (PFS) compared with nPD-L1-negative (3-year OS, 37.2% vs 67.3%, p = 0.014; 3-year PFS, 31.0% vs 61.8%, p =0.010, respectively). Patients who had low FoxP3+Tregs had significantly inferior OS and PFS compared with high FoxP3+Tregs (3-year OS, 36.4% vs 63.0%, p = 0.004; 3-year PFS, 31.7% vs 56.3%, p = 0.020, respectively). The results of multivariate analysis showed that nPD-L1 positivity (HR 6.629, 95% CI 1.966–22.350, p=0.002) and low FoxP3+Tregs (HR 7.317, 95% CI 2.154–24.855, p=0.001) were independent predictors of inferior OS. Using these 2 variables, we constructed a new prognostic model that singled out 3 groups with different risk profiles: group 1, no adverse factors; group 2, 1 adverse factor; and group 3, 2 adverse factors. The 3-year OS rates of group 1, group 2, and group 3 were 93.3%, 46.6% and 20.8%, respectively (p<0.001), and the 3-year PFS rates were 86.7%, 40.8% and 15.0%, respectively (p=0.001).

Conclusion: This study is the first to validate the prognostic value of nPD-L1 and tumor-infiltrating FoxP3+Tregs in ENKTL; the new immunological prognostic model might be used to stratify ENKTL patients in clinical trials for new therapeutic strategies.

Keywords: extranodal natural killer/T-cell lymphoma, PD-L1, FoxP3+Tregs, prognosis

Introduction

Extranodal natural killer/T-cell lymphoma (ENKTL) is defined as an aggressive malignancy that is strongly associated with Epstein–Barr virus (EBV) infection, it is relatively rare in western populations but has a high prevalence in East Asian populations.[1] ENKTL most commonly presents in the upper-aerodigestive tract, but it can also involve variable extranodal sites.[2] This disease is often resistant to anthracycline-based chemotherapy, such as CHOP.[3] Although some studies showed that

asparaginase-based chemotherapy improved remission and better survival than anthracycline-based chemotherapy,[4] more than 50% of patients with advanced disease experience relapse, usually within a year of completing chemotherapy.[5] Therefore, new therapeutic approaches based on molecular oncogenic mechanisms are urgently required.

The tumor immune microenvironment plays an important role in tumor development and progression through interactions between tumor cells and the immune system. Programmed death ligand 1 (PD-L1), as an immunomodulatory cell-surface glycoprotein, is expressed on antigen-presenting cells and can be induced on various tumor cells within the tumor microenvironment.[6–9] Tumor cell expression of PD-L1 results in tumor-specific T cell inactivity through engagement of PD-1-expressing T cells and serves as a way to evade immune surveillance and the immune response.[10,11] It has been shown that PD-L1 expression on tumor cells is related to a poor prognosis in many types of solid tumors.[12–17] However, the results regarding the prognostic significance of PD-L1 in ENKTL are conflicting.[18–22] Based on these observations, the role of predicting the prognosis of PD-L1 has not been fully elucidated, and its use in combination with other biomarkers might enhance its predictive power.

FOXP3, a forkhead helix transcription factor, is now recognized as a specific marker for regulatory T-cells (Tregs)[23] and appears to function as a master regulator in the development and control of Tregs.[24,25] FoxP3+Tregs are an important component of the lymphoma immune microenvironment. The prognostic effect of FoxP3+Tregs remains debatable because it is associated with a poor prognosis in various solid malignancies.[26–28] whereas it has a better prognosis for hematologic malignancies.[29–31] However, little is known about the prognostic value of FoxP3+Tregs in patients with ENKTL.

PD-L1 was found to have a pivotal role in enhancing and sustaining FoxP3 expression, regulating FoxP3+Treg development and sustaining FoxP3+Treg function.[32,33] However, knowledge of the prognostic role of neoplastic PD-L1 (nPD-L1) expression in conjunction with tumor-infiltrating FoxP3 +Tregs in ENKTL is limited. Therefore, in this study, we investigated the prognostic value of nPD-L1 combined with tumor-infiltrating FoxP3+Tregs in ENKTL patients after receiving non-anthracycline-based chemotherapy.

Materials and Methods
Patients
A total of 365 consecutive patients newly diagnosed with ENKTL according to the 2008 WHO criteria of lymphoma between 2008 and 2017 were collected from Sun Yat-sen University Cancer Center. Cases with a relapsed course, previous anti-cancer treatment and inadequate/insufficient samples were excluded. A total of 81 patients with formalin-fixed, paraffin-embedded (FFPE) samples available, complete clinicopathologic and follow-up data were analyzed in this study. Patient clinical information such as demographic features, performance status, B symptoms, serum lactate dehydrogenase (LDH), stage, International prognostic index (IPI),[34] Korean prognostic index (KPI),[35] the prognostic index of natural killer lymphoma (PINK) score,[36] nasal and nonnasal types, treatment modalities and treatment response were recorded. All tissue samples were acquired at initial presentation prior to treatment. This work was approved by the ethics committee of Sun Yat-sen University Cancer Center. Written informed consent were provided from all patients prior to collecting patient tissue samples and clinical information.

Immunohistochemistry
All the diagnoses were confirmed by two experienced pathologists after reviewing hematoxylin and eosin-stained tissue sections, immunohistochemistry (IHC) results and clinical data. IHC examinations including CD3, CD56, T-cell intracytoplasmic antigen-1 (TIA-1), CD4, PD1, CD30 and in situ hybridization for EBV-encoded small RNAs (EBERs) were performed on sections of 4-μm thickness. Sections cut from FFPE blocks 4-μm thick underwent deparaffinization and rehydration through changes in xylene, graded ethanol and water. Antigen retrieval was performed with microwave pressure-cooking at 1000 W for 2.5 mins in tris-ethylenediaminetetraacetic acid (EDTA) buffer (pH 9) before staining with each primary antibody. After cooling and washing with buffer, 3% hydrogen peroxide solution was used for blocking endogenous peroxidase in tissues for 2 mins. Then, slides were incubated with an anti-PD-L1 mouse monoclonal antibody (UMAB228 clone, ZM-0170, 1:100 dilution) and with a mouse anti-FoxP3 monoclonal antibody (236A/E7 clone, Abcam, 1:100 dilution) in Dako antibody diluent at 4°C overnight. Subsequently, the slides were serially rinsed and incubated with secondary antibodies at 37°C in an incubator for 20 mins. Dako Real Envision 3,3′-diaminobenzidine (DAB) was applied for 1–2 mins for the visualization of antigen-antibody binding, and the sections were counterstained with Mayer hematoxylin, dehydrated with ethanol, and coverslipped for evaluation. The slides were reviewed by two experienced pathologists independently who were blinded to the clinical data. The expression

of PD-L1 was scored semiquantitatively based on the proportion of staining regardless of staining intensity. A representative tumor area was defined as having tumor cells in >80% of the area. A tumor area was avoided if it showed marked necrosis in >20% of the area or had an ulcer and inflamed granulation tissue. Four high-power fields (HPFs, 400× magnification level) of representative areas with similar total cell numbers were evaluated for the expression of PD-L1 of tumor cells and the number of tumor-infiltrating FoxP3+Tregs. In cases of disagreement, a consensus was reached by two blinded observers who re-examined the slides with a two-headed microscope.

Treatment and Response Evaluation

Patients received one of the following initial treatment modalities: (1) chemotherapy (CT) alone or (2) CT combined with RT. All patients received non-anthracycline-based chemotherapy. The treatment response was evaluated according to standard response criteria.[37] The chemotherapeutic regimens used were as following: (1) GELOX (gemcitabine, L-asparaginase and oxaliplatin); (2) SMILE (corticosteroid, methotrexate, ifosfamide, L-asparaginase, and etoposide).

Statistical Analysis

Categoric data were compared via Pearson's chi-square or Fisher's test, whereas continuous variables were compared via Mann–Whitney and Kruskal–Wallis tests. Optimal cutoff values of nPD-L1 level and FoxP3+Treg level for predicting survival were determined using receiver operating characteristics (ROC) curve analysis. Progression-free survival (PFS) was defined as the period of time from the date of initial diagnosis to the date of disease progression or death. Overall survival (OS) was defined as the period of time from the date of initial diagnosis to the date of death or last follow-up. OS and PFS were compared using the Kaplan-Meier method with a Log rank test. The multivariate analysis of the variables predicting prognosis was performed by logistic regression. The Statistical Package for Social Sciences software, version 20.0 (IBM, USA) was used for statistical analyses, and two-sided p values less than 0.05 were considered significant based on a two-sided statistical analysis.

Results
Clinical Characteristics

Table 1 summarizes the main clinical and histologic features of the 81 patients at diagnosis. Briefly, more male

Table 1 Main Clinical and Histopathologic Features of 81 Patients

Characteristics	Patients, n=81, No. (%)
Male/Female Median age(range),years	59(72.8)/22(27.2) 45(16–79)
ECOG PS 　0–1 　≥2	 73(90.1) 8(9.9)
B symptoms 　yes 　no	 44(54.3) 37(45.7)
LDH level 　Normal 　Increased	 64(79.0) 17(21.0)
Ann Arbor stage 　I/II 　III/IV	 39(48.1) 42(51.9)
IPI 　0–1 　≥2	 35(43.2) 46(56.8)
KPI 　0–1 　≥2	 33(40.7) 48(59.3)
PINK 　low-risk 　intermediate-risk 　high-risk	 23(28.4) 24(29.6) 34(42.0)
UAT/non-UAT CR after primary treatment	66(81.5)/15(18.5) 46(56.8)
Therapy pattern 　CT 　CT + RT	 44 (54.3) 37 (45.7)
Chemotherapy regimen 　GELOX 　SMILE	 51 (63.0) 30 (37.0)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; IPI, International Prognostic Index; KPI, Korean prognostic index; PINK, prognostic index of natural killer lymphoma; UAT, upper aerodigestive tract; CR, complete remission; RT, radiotherapy; CT, chemotherapy; GELOX, gemcitabine, L-asparaginase, and oxaliplatin; SMILE, corticosteroid, methotrexate, ifosfamide, L-asparaginase, and etoposide.

patients than female patients were included (72.8%). More than half of the patients had advanced-stage disease (51.9%). The most frequently involved sites were the upper aerodigestive tract (UAT) (81.5%). More than half of the patients had high-risk IPI scores (56.8% had an IPI score of ≥2), and high-risk KPI scores (59.3% had a KPI score of ≥2) and were in intermediate- and high-risk groups based on PINK scores (71.6%). Among the various

non-anthracycline-based chemotherapy regimens, GELOX (63.0%) was the most commonly used.

Pathological and Immunophenotypic Characteristics

Only 3 of the 78 patients (3.8%) expressed PD-1 in tumor-infiltrating immune cells, however, none of the patients expressed PD-1 in tumor cells. CD3 was positive in 76 of 80 cases (95.0%), CD4 was positive in 8 of 42 cases (19.0%).The percentage of PD-L1-positive malignant cells varied widely from 0–70%, with a median value of 10% (Figure 1A and B). Based on the ROC curve for clinical outcome, the optimal cutoff value of neoplastic PD-L1 (nPD-L1) positive was 10%, which makes nPD-L1 positive defined as 10% or more of lymphoma cells stained and allowed groups nPD-L1-positive group and nPD-L1 negative group to be distinguished. The number of FoxP3+Tregs varied widely from 0–468.25/HPF (400× magnification), with a median value of 52.5/HPF (Figure 1C and D). Based on the ROC curve for clinical outcome, the optimal cutoff value of FoxP3+Tregs was approximately 46/HPF, which allowed groups with low and high levels of FoxP3 +Tregs to be distinguished.

Clinicopathological Comparison Between nPD-L1 Positivity and Negativity

In our study, the nPD-L1-positive group showed a trend toward an older median age (48 y vs 42 y, p=0.066) and higher PINK score (45.4% vs 37.8%, p=0.071) and a trend toward a lower CR rate after initial treatment (47.7% vs 67.6%, p=0.073) (Table 2). The high FoxP3+Tregs group had more female patients (36.4% vs 16.2%, p=0.046) and fewer patients with a high PINK score (29.5% vs 56.7%, p=0.021) than the low FoxP3+Treg group, both of which were significant (Table 2).

Survival Analysis According to the Expression of nPD-L1 and FoxP3+Tregs

The median follow-up period of the entire cohort was 28.9 months (range 1–129 months). The 3-year PFS and 3-year OS were 45.1% and 51.0%, respectively. Patients with
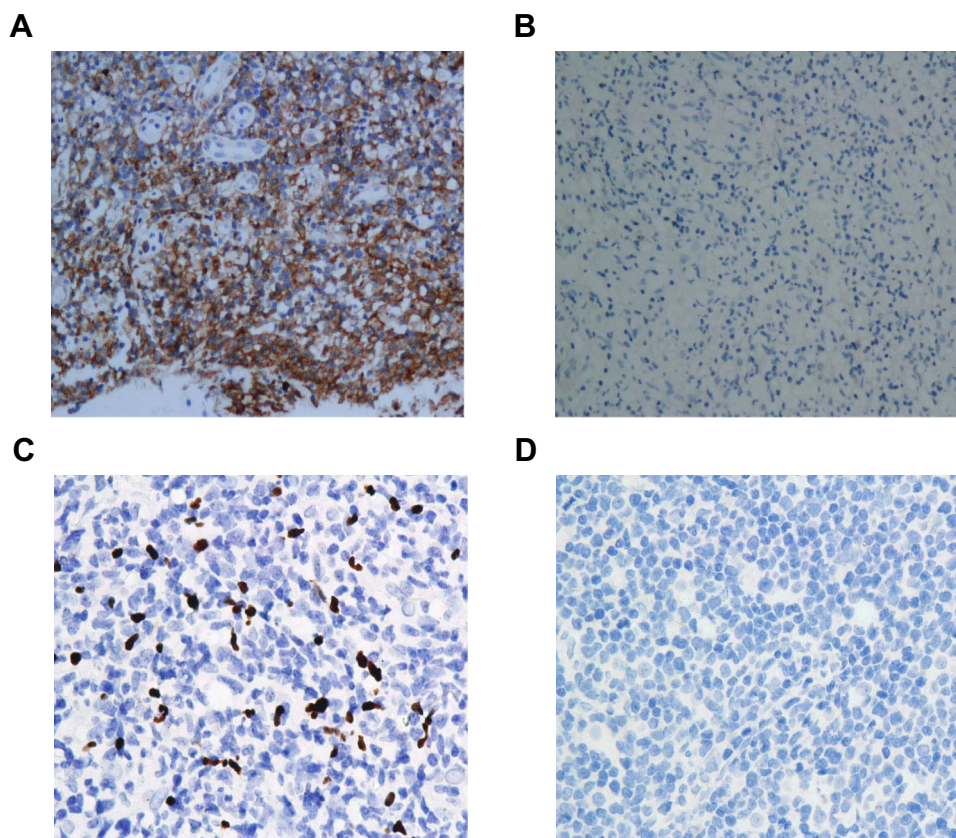

Figure 1 Representative immunohistochemical analysis of PD-L1 expression in neoplastic cells. (**A**) PD-L1 positive in neoplastic cells (×200); (**B**) PD-L1 negative in neoplastic cells (×200). Representative immunohistochemical analysis of tumor-infiltrating FoxP3+Tregs in ENKTL. (**C**) High tumor-infiltrating FoxP3+Tregs (×200); (**D**) Low tumor-infiltrating FoxP3+Tregs (×200).

Table 2 Correlation of Neoplastic PD-L1 Expression and FoxP3+ Tregs with Clinicopathological Features

Characteristic	nPD-L1 Positive (n=44)	nPD-L1 Negative (n=37)	P value	High FoxP3+ Tregs (n=44)	Low FoxP3+ Tregs (n=37)	P value
Male/Female	29(65.9)/15(34.1)	30(81.1)/7(18.9)	0.142	28(63.6)/16(36.4)	31(83.8)/6(16.2)	0.042*
Median age(range),years	48 (16–79)	42 (16–70)	0.066	45.5 (16–70)	45.0 (16–79)	0.435
ECOG/PS						
0–1	39 (88.6)	34 (91.9)	0.721	41 (93.2)	32 (86.5)	0.314
≥2	5 (11.4)	3 (8.1)		3 (6.8)	5 (13.5)	
B symptoms						
yes	21 (47.7)	16 (43.2)	0.823	24 (54.5)	20 (54.1)	0.965
no	23 (52.3)	21 (56.8)		20 (45.5)	17 (45.9)	
LDH level						
Normal	35 (79.5)	31 (83.8)	0.625	36 (81.8)	30 (81.1)	0.932
Increased	9 (20.5)	6 (16.2)		8 (18.2)	7 (18.9)	
Ann Arbor stage						
I/II	21 (47.7)	18 (48.6)	0.934	22 (50.0)	17 (45.9)	0.716
III/ IV	23 (52.3)	19 (51.4)		22 (50.0)	20 (54.1)	
IPI						
0–1	16 (36.4)	19 (51.4)	0.175	19 (43.2)	16 (43.2)	0.996
≥2	28 (63.6)	18 (48.6)		25 (56.8)	21 (56.8)	
KPI						
0–1	19 (43.2)	14 (37.8)	0.626	17 (38.6)	16 (43.3)	0.674
≥2	25 (56.8)	23 (62.2)		27 (61.4)	21 (56.7)	
PINK						
low-risk	8 (18.2)	15 (40.6)	0.071	13 (29.5)	10 (27.1)	0.021*
Intermediate-risk	16 (36.4)	8 (21.6)		18 (41.0)	6 (16.2)	
High-risk	20 (45.4)	14 (37.8)		13 (29.5)	21 (56.7)	
UAT/non-UAT	33 (75.0)/11 (25.0)	33 (89.2)/4 (10.8)	0.151	37 (84.1)/7 (15.9)	29 (78.3)/8 (21.7)	0.510
CR after primary treatment	21 (47.7)/23 (52.3)	25 (67.6)/12 (32.4)	0.073	27 (47.7)/17 (52.3)	19 (67.6)/18 (32.4)	0.365
Therapy pattern						
CT	25 (56.8)	19 (51.3)	0.623	26 (59.1)	18 (48.6)	0.347
CT + RT	19 (43.2)	18 (48.7)		18 (42.9)	19 (51.4)	
Chemotherapy regimen						
GELOX	31 (70.5)	20 (54.1)	0.387	27 (61.4)	24 (64.9)	0.384
SMILE	13 (29.5)	17 (45.9)		17 (38.6)	13 (35.1)	

Notes: *P<0.05, the difference was statistically significant.
Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; IPI, International Prognostic Index; KPI, Korean prognostic index; PINK, prognostic index of natural killer lymphoma; UAT, upper aerodigestive tract; CR, complete remission; RT, radiotherapy; CT, chemotherapy; GELOX, gemcitabine, L-asparaginase, and oxaliplatin; SMILE, corticosteroid, methotrexate, ifosfamide, L-asparaginase, and etoposide.

nPD-L1-positive had significantly inferior OS and PFS compared with patients with nPD-L1-negative (3-year OS, 37.2% vs 67.3%, p=0.014; 3-year PFS, 31.0% vs 61.8%, p=0.010, respectively) (Figure 2A and B). Patients who had low FoxP3+Tregs had significantly inferior OS and PFS compared with high FoxP3+Tregs (3-year OS, 36.4% vs 63.0%, p=0.004; 3-year PFS, 31.7% vs 56.3%, respectively) (Figure 2C and D).

In univariate analysis, factors predicting inferior OS included advanced stage (Ann Arbor stage III/IV p=0.029), nPD-L1 positivity (p=0.014) and low FoxP3+Tregs (p=0.005). In multivariate analysis, nPD-L1 positivity (HR 6.629, 95% CI 1.966–22.350, p=0.002) and low FoxP3+Tregs (HR 7.317, 95% CI 2.154–24.855, p=0.001) were independent predictors of inferior OS (Table 3).
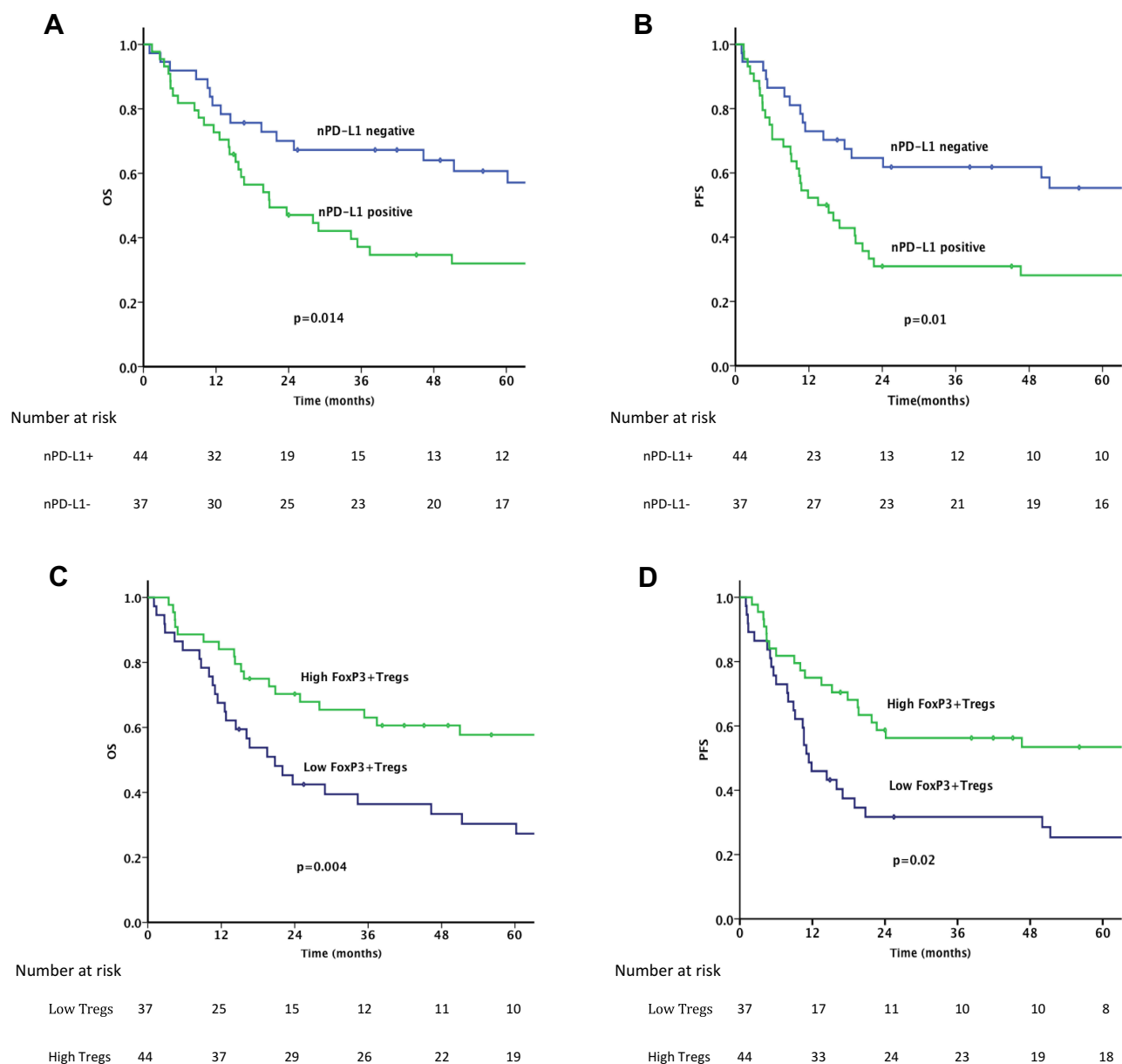
Figure 2 Survival of patients with nPD-L1 positive and nPD-L1 negative. (**A**) OS; (**B**) PFS. Survival of patients with high FoxP3+ Tregs and low FoxP3+Treg. (**C**) OS; (**D**) PFS.

Univariate survival analysis revealed that the male sex (p=0.039), presence of systemic B symptoms (p=0.016), advanced stage (Ann Arbor stage III/IV p=0.026), bone marrow involvement (p=0.017), regional lymphadenopathy involvement (p=0.006), nPD-L1 positivity (p=0.016) and low FoxP3+Tregs (p=0.022) were significantly associated with a poor PFS. Then, a multivariate analysis was performed including all variables found to be significant in the univariate analysis, which identified nPD-L1 positivity (HR 5.266, 95% CI 1.668–16.626, p=0.005) and low FoxP3 +Tregs (HR 3.598, 95% CI 1.127–11.493, p=0.031) as independent prognostic factors for poor PFS (Table 3).

Using these two variables (nPD-L1 and FoxP3+Tregs), we constructed a new prognostic model that stratified the patients into 3 groups with different risk profiles: group 1, no adverse factors; group 2, 1 adverse factor; and group 3, 2 adverse factors. The 3-year OS rates of group 1, group 2, and group 3 were 93.3%, 46.6% and 20.8%, respectively (p<0.001) (Figure 3A), and 3-year PFS rates were 86.7%, 40.8% and 15.0%, respectively (p=0.001) (Figure 3B).

Additionally, we used a ROC analysis to compare the sensitivity and specificity of survival prediction. Our new prognostic model showed a better performance in predicting survival than the PINK model with regard to OS (our

Table 3 Univariate and Multivariate Analysis of Factors Associated with Overall Survival and Progression-Free Survival

Clinical Characteristic	OS			PFS		
	Univariate Analysis	Multivariate Analysis		Univariate Analysis	Multivariate Analysis	
	P value	HR (95% CI)	P value	P value	HR (95% CI)	P value
Gender,male	0.169			0.039*		
Age>60	0.063			0.149		
ECOG/PS ≥2	0.258			0.552		
B symptoms	0.112			0.016*	3.047 (0.989–9.387)	0.052
LDH ≥ 245U/L	0.346			0.208		
Ann Arbor stage(III/IV)	0.029*	4.148 (0.980–17.562)	0.053	0.026*	6.161 (0.697–54.501)	0.102
Bone marrow involvement	0.420			0.017*		
Regional LN involvement	0.303			0.006*		
UAT	0.133			0.199		
nPD-L1+	0.014*	6.629 (1.966–22.350)	0.002*	0.016*	5.266 (1.668–16.626)	0.005*
FoxP3+Tregs(low)	0.005*	7.317 (2.154–24.855)	0.001*	0.022*	3.598 (1.127–11.493)	0.031*

Notes: *P<0.05, the difference was statistically significant. The multivariate analysis was performed by logistic regression.
Abbreviations: OS, overall survival; PFS, progression-free survival; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; LN, lymph node; UAT, upper aerodigestive tract.

model ROC area, 0.741; 95% CI, 0.630–0.851; PINK ROC area, 0.697; 95% CI, 0.583–0.811).

Discussion

In this study, we identified two risk factors for PD-L1 expression in tumor cells and tumor-infiltrating FoxP3 +Tregs among various clinical parameters that were identified as being independent predictors of OS and PFS. Our new immunological prognostic model that included these two factors could stratify the prognosis of ENKTL patients after non-anthracycline-based chemotherapy with statistical significance. Furthermore, our method uses immunohistochemistry, which is inexpensive and simple to perform in clinical studies and in general
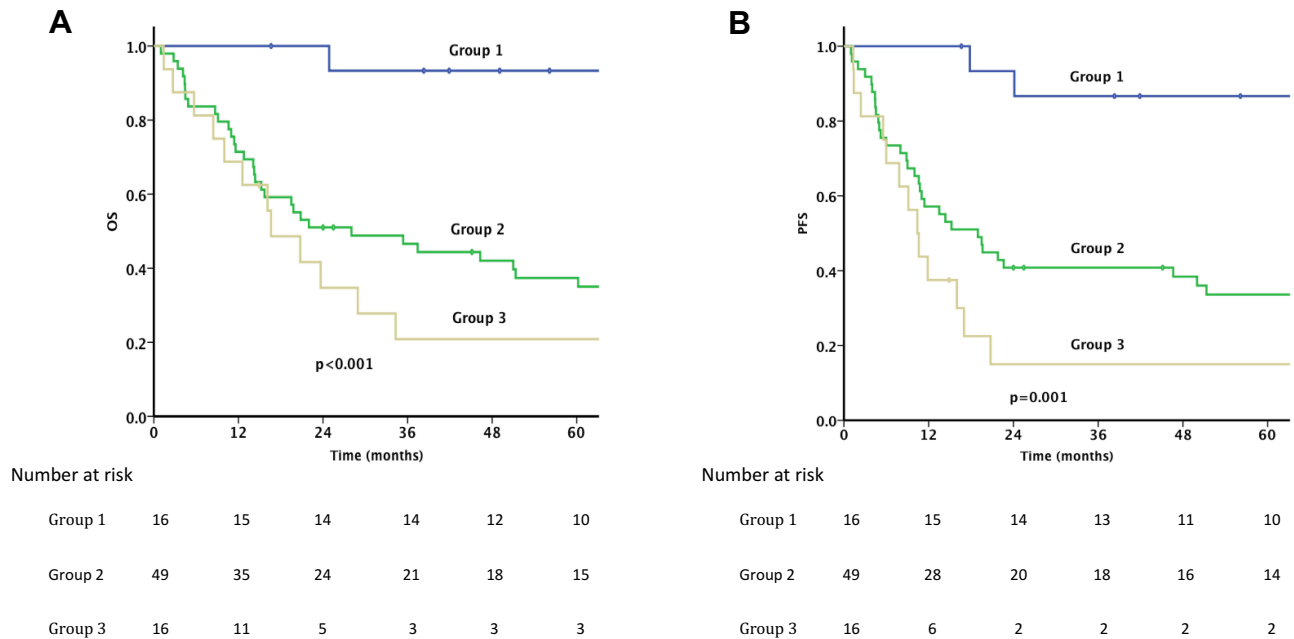

Figure 3 Survival of patients with combined nPD-L1 and FoxP3+Tregs. (**A**) OS; (**B**) PFS.

practice. Our research provides support for the use of these biomarkers as a supplement for predicting prognosis.

Previous studies have shown that PD-L1 expression on tumor cells is associated with poor prognosis in various solid malignant tumors and some types of hematologic malignancies.[12–17] However, the results regarding the role of PD-L1 expression in ENKTL remain controversial. Kim and his colleagues found that PD-L1 expression was the only significant independent predictor for longer OS in patients with advanced stage ENKTL.[18] Another recent study also found that ENKTL patients with PD-L1 positivity in tumor cells had a trend toward improved OS compared with the negative control.[21] Contrary to these results, Bi et al and Muhamad et al found that PD-L1 expression in lymphoma cell tissue exhibited remarkably worse OS in ENKTL patients,[19] which was similar to the results of our study. Therefore, a single biomarker of PD-L1 for predicting the prognosis of ENKTL patients might have some limitations. More studies are needed to elucidate the role of PD-L1 or the incorporation of other biomarkers to PD-L1 to gain good prognostic capacity in ENKTL patients.

Currently, the mechanism of upregulation of PD-L1 expression in ENKTL tumor cells remains unclear. Previous data have shown that the expression of PD-L1 in tumor cells could be upregulated by 9p24.1 amplification in classical Hodgkin's lymphomas (HL) and diffuse large B cell lymphoma (DLBCL).[38,39] Moreover, Green et al demonstrated that PD-L1 is expressed in HL- and EBV-positive posttransplant lymphoproliferative disorders (PTLD) as a result of latent membrane protein 1 (LMP1)-mediated JAK/STAT-dependent promoter and AP-1-associated enhancer activity.[38] ENKTL, which is closely related to EBV infection, is thought to be related to the expression of PD-L1 in tumor cells of ENKTL and LMP1. In a recent study of NK cell lines, PD-L1 is upregulated by EBV-driven LMP1 through the NF-κB pathway.[19] The mechanism of PD-L1 expression on tumor cells affecting the prognosis of ENKTL remains unclear. It is thought that the PD-1/PD-L1 pathway might contribute to a poor prognosis in ENKTL patients with PD-L1 expression on tumor cells. The expression of PD-L1 in tumor cells may engage PD-1 receptors on tumor-infiltrating T cells, induce PD-1 signaling and associated T-cell "exhaustion", and mediate tumors to escape the host immune response.[40] Therefore, it is speculated that PD-1/PD-L1 blockade may benefit ENKTL patients. In a retrospective study, Kwong YL et al reported that PD1 blockade with pembrolizumab is effective in relapsed or refractory NK/T-cell lymphoma failing L-asparaginase regimens, which provide proof-of-concept data that the PD-L1/PD1 axis is highly relevant in this malignancy.[41]

PD-L1 was found to have a pivotal role in enhancing and sustaining FoxP3 expression, regulating FoxP3+Treg development and sustaining FoxP3+Treg function.[32,33] In this study, we demonstrated that low frequency FoxP3+Treg infiltration in the tumor microenvironment was an independent prognostic factor for poor outcome in patients with ENKTL, which is similar to the results reported in other hematologic malignancies, such as follicular lymphomas, DLBCL and HL.[29–31] However, many studies showed that a low density of FoxP3+Tregs infiltrating the tumor microenvironment correlated with improved clinical outcomes in various human solid malignancies.[23,26-28] This evidence suggests that the role of FoxP3+Tregs in the pathogenesis of solid tumors and hematological malignancies is different. FoxP3+Tregs may represent important lymphoma/host microenvironment modulators that participate in the regulation of the host immune response and biologic behavior of ENKTL. As important independent immune biomarkers, our study combined nPD-L1 and tumor-infiltrating FoxP3 +Tregs to form a new immunological prognostic model that yielded prognostic stratification with significance in ENKTL patients. This result suggested that these two biomarkers might serve as a supplement for each other when combined to predict prognosis in ENKTL patients. Moreover, the PINK model has been found to be useful at predicting outcomes in patients with ENKTL,[36] but our new prognostic model showed similar predictive accuracy as the PINK model, which indicated that our model successfully reflects the clinical outcome in ENKTL patients. However, how PD-LI expression on tumor cells and tumor-infiltrating FoxP3+Tregs counteract and impact the development of ENKTL remains unclear and should be explored in future studies.

There are some limitations to our study. First, being a retrospective study utilizing registry data is an inherent limitation. Second, the sample size is relatively small, which may limit the power to detect differences between groups.Third, circulating EBV-DNA was not included as variables in our study due to patients series that are collected over long periods and some of which are without EBV-DNA result. Therefore, further prospective studies including larger samples are needed to validate our results.

Conclusion

In conclusion, our new immunological prognostic model based on immunohistochemistry could stratify the prognosis of ENKTL patients after non-anthracycline-based chemotherapy and might be used to stratify patients in clinical trials for new therapeutic strategies or risk-adapted therapies.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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