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ORIGINAL ARTICLE

Immunotyping of peripheral blood lymphocytes by flow cytometry reveals Th cell as a potential prognostic biomarker for extranodal NK/T-cell lymphoma

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Abstract

This study aimed to explore the distribution, characteristics and prognostic value of baseline peripheral blood lymphocyte subsets in patients with extranodal NK/T-cell lymphoma (NKTCL). We conducted this cross-sectional study of 205 newly-diagnosed NKTCL patients receiving first-line chemotherapy and radiation at our institute between 2010 and 2020. Baseline peripheral blood lymphocytes were detected using flow cytometry, and the clinical value was analyzed. Compared with healthy controls, patients with NKTCL presented with a distinct peripheral immunity with higher levels of cytotoxic CD8+ T cells (33.230 ± 12.090% *vs.* 27.060 ± 4.010%, $p < 0.001$) and NKT cells (7.697 ± 7.219% *vs.* 3.550 ± 2.088%, $p < 0.001$) but lower proportions of suppressive regulatory T cells (Treg, 2.999 ± 1.949% *vs.* 3.420 ± 1.051%, $p = 0.003$) and CD4+ helper T cells (Th, 33.084 ± 11.361% *vs.* 37.650 ± 3.153%, $p < 0.001$). Peripheral lymphocytes were differentially distributed according to age, stage, and primary site in patients with NKTCL. The proportion of Th cells/lymphocytes was associated with tumor burden reflected by stage ($p = 0.037$), serum lactate dehydrogenase ($p = 0.0420$), primary tumor invasion ($p = 0.025$), and prognostic index for NK/T-cell lymphoma (PINK) score ($p = 0.041$). Furthermore, elevated proportions of T cells (58.9% *vs.* 76.4%, $p = 0.005$), Th cells (56.3% *vs.* 68.8%, $p = 0.047$), or Treg cells (49.5% *vs.* 68.9%, $p = 0.040$) were associated with inferior 5-year progression-free survivals (PFS) via univariable survival analysis. Multivariate cox regression revealed elevated Th cells as an independent predictor for unfavorable PFS (*HR* = 2.333, 95% *CI*, 1.030–5.288, $p = 0.042$) in NKTCL. These results suggested the proportion of Th cells positively correlated with tumor burden and was a potential non-invasive biomarker for inferior survival for patients with NKTCL.

Keywords Extranodal NK/T-cell lymphoma · Peripheral blood · Immunotyping · Lymphocyte subsets · Helper T cell

Introduction

Extranodal NK/T-cell lymphoma (NKTCL) is a highly aggressive subtype of non-Hodgkin lymphoma (NHL) derived from NK-, T-cell, or both [1]. It has a strong geographical predilection for Asians and Latin Americans

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[2–5]. In most cases, tumors are of the upper aerodigestive tract (UADT) and localized at presentation, especially nasal cavity and Waldeyer's ring (WR). And 15–20% of cases present advanced disease occurring in non-UADT sites including skin, gastrointestinal tract, testicles, and salivary glands [2, 6]. Combined chemoradiotherapy (CRT) is considered the standard treatment for early-stage NKTCL. However, approximately 20–37% cases develop treatment failure after first-line CRT and become relapsed or refractory diseases [7–10]. Prognosis of patients with advanced or relapsed/refractory NKTCL is fairly poor, with a median overall survival (OS) of 6–10 months [11, 12].

Immunotherapies have become a promising treatment option in relapsed/refractory NKTCL [13–17]. Strong expression of PD-L1 was reported to be a predictor of favorable survivals for patients receiving traditional treatment or anti-PD1/PD-L1 blockades [16–18]. Won Seog Kim and his colleagues developed an immune subtyping model (FoxP3, PD-L1, and CD68) that classifies NKTCL into four tumor immune microenvironment subgroups named immune tolerance, immune evasion-A, immune evasion-B, and immune silenced, which was proved to be a useful biomarker for immunotherapy [19]. Currently, prognostic targeting biomarkers for patients with NKTCL are primarily tumor tissue-dependent. However, sufficient tumor specimen is hard to obtain as a result of severe necrosis in lesions of NKTCL via small biopsy. Therefore, exploring new blood biomarkers especially immunotype-related factors is an urgent need for patients with NKTCL.

Lymphocyte numbers in the blood are used to evaluate the immune status in medicine [20]. Previous studies have shown that absolute lymphocyte count (ALC) is an independent prognostic indicator for survivals in multiple NHLs [21–25]. A retrospective study reported that patients with newly-diagnosed NKTCL who had high ALC ($> 1.0 \times 10^9$/L) at diagnosis achieved superior 5-year OS (60.4% *vs.* 13.1%, $P < 0.001$) and complete remission rate (CRR, 52% *vs.* 28%, $P = 0.001$) in comparison to those with low ALC [26]. In addition, EBV infects T lymphocytes, NK cells, and B lymphocytes, and patients infected with chronic active EBV showed imbalance of lymphocyte subsets and immune dysfunction [27, 28]. However, at present, the distribution of T lymphocyte subpopulations has not been fully described in NKTCL, and it is also unclear whether a specific subset of lymphocytes is associated with inferior or superior prognosis.

Therefore, we conducted this study aiming to explore the distribution and characteristics of baseline peripheral blood (PB) lymphocytes and to explore its applicability in predicting survivals among patients with NKTCL.

Materials and methods

Patient eligibility

Consecutive patients with newly-diagnosed NKTCL from 2010 to 2020 at Peking University Cancer Hospital were initially considered. Inclusion criteria were: pathologically diagnosed NKTCL with typical morphology and immunophenotype including CD20/CD79α, CD3ε, CD56, TIA-1, Gram-B, perforin, and EBV-encoded RNA in situ hybridization, according to WHO classification; patients who received at least one cycle of systemic therapy with or without local RT at our institute; patients who had baseline flow cytometry of PB lymphocyte analysis; and at least one measurable lesion. Patients with bone marrow involvement at diagnosis, active infection, autoimmune diseases, who had prior treatment, or with incomplete clinicopathologic or follow-up information, or those with no baseline peripheral lymphocyte cytometry were excluded. PB samples were also collected from healthy volunteers (33 males and 17 females) as control with a median age of 45 years.

Medical records of eligible patients were reviewed including physical examination, imaging studies, nasopharyngeal endoscopies, bone marrow aspirations, and laboratory tests (complete blood count, liver and renal function analysis, β2-M, serum lactate dehydrogenase [LDH], and EBV DNA tilter). Patients were stratified according to the International Prognostic Index (IPI) [29], the Nomogram-revised Risk Index (NRI) [30], Korea Prognostic Index (KPI) [31], and the Prognostic Index of NK lymphoma (PINK) [32].

Treatment and follow-up

Patients with localized NKTCL received combined chemotherapy (CT) and radiation therapy (RT); and those with advanced diseases received CT alone. RT was started after 2–3 cycles of induction CT and followed by additional 2–3 cycles of consolidation CT. The clinical target volume (CTV) included the whole nasal cavity, the entire ipsilateral maxillary sinus, the bilateral anterior ethmoid sinus, and the hard palate and involved paranasal organ/tissues. The CTV was extended to cover the bilateral cervical region (levels II–V) when the regional lymph nodes were involved. A 3 to 5-mm isotropic expansion of the CTV was used to create the planning target volume (PTV). The median RT dose was 50 Gy (range, 45–56 Gy; dose per fraction, 1.8–2.0 Gy).

All enrolled patients underwent asparaginase (ASP)-based regimens including COEPL (cyclophosphamide, etoposide, vincristine, prednisone, and ASP; 87.3%),

CHOPL/CHOPEL (cyclophosphamide, doxorubicin, vincristine, prednisone, and ASP ± etoposide; 9.3%), and GELOX/GDPL (gemcitabine, oxaliplatin and ASP/gemcitabine, and dexamethasone and cisplatin; 3.4%). Twenty-one patients with disseminate or refractory disease received autologous stem-cell transplantation (ASCT). Response was assessed every 2–3 cycles of CT, at the completion of RT, at the end of the entire treatment. Patients were reevaluated every 3 months for the first 2 years, every 6 months for the following 3 years, and yearly or when clinically required thereafter.

Flow cytometry

Two tubes of 5-mL-venous blood were collected from patients prior to treatment. One tube was used for measurement of white blood cell count, lymphocyte count, and monocyte cell count using automatic biochemical analyzer. The other tube of PB sample was measured for levels of lymphocyte subsets. Staining of antigens included CD123 (anti-human CD123 antibody), CD19 (anti-human CD19 antibody), CD3 (anti-human CD3 antibody), CD4 (anti-human CD4 antibody), CD8 (anti-human CD8 antibody), CD28 (anti-human CD28 antibody, APC), CD25 (anti-human CD25 antibody), CD16 (anti-human CD16 antibody), and CD56 (anti-human CD56 antibody). Fluorochrome-conjugated monoclonal antibodies were added to 100 μL suspension and then mixed and incubated at room temperature for 20 min. Samples were subjected to flow cytometry for measurement of B cells (CD19+), T cells (CD3+), T helper cells (Th and CD3+CD4+), CD8+ T cells (CD3+CD8+), T cytotoxic cells (Tc, CD3+CD8+CD28+), T suppressor cells (Ts, CD3+CD8+CD28−), T regulatory cells (Treg, CD4+CD25+), NK cells (CD3–CD16+CD56+), and NKT cells (CD3+CD16+CD56+). Data were acquired on flow cytometer (Beckman Coulter, Brea, CA, USA) and analyzed using CXP Analysis software (Beckman Coulter, Brea, CA, USA).

Statistical analysis

Mean ± standard deviation ($\bar{x} \pm sd$) was used for description of lymphocyte subsets due to the conformance with normal distribution of our data. Comparison among multiple groups was conducted using one-way analysis of variance followed by *t* test for pairwise comparison. Correlations between lymphocyte subsets were analyzed using linear regression. Progression free survival (PFS) was defined as time interval from the date of treatment to the date of progression, relapse, last follow-up or death from any cause. Estimates of survivals were calculated using the Kaplan-Meier method, and survival differences were assessed using the log-rank test. Cox regression was used for multivariate analysis of indicators with p values < 0.05 in the univariate analysis for PFS. A two-tail $P < 0.05$ was determined to be with statistical difference. IBM SPSS (version 26.0) was used for statistical analysis, and GraphPad Prism (version 9.0) was used to graph the statistics.

Results

Patient characteristics

A total of 398 patients with stage I(E)–IV(E) NKTCL were treated at Peking University Cancer Hospital from 2010 to 2020. Finally, 205 eligible cases met the inclusion and exclusion criteria and were included into further analysis. Patient basic characteristics are summarized in Table 1. The median age was 43 (range, 15–85) years. The male to female ratio was 2.01:1. Majority of patients presented UADT NKTCL (189/205, 92.2%) and localized disease (165/205, 80.5%). Sixteen cases were extra-UADT NKTCL with primary lesions located in gastrointestinal tract ($n = 4$), skin ($n = 3$), testis ($n = 3$), lung ($n = 1$), adrenal gland ($n = 1$), pelvic ($n = 1$), and lymph nodes ($n = 3$). At diagnosis, 72.2%, 30.8%, and 80.0% of enrolled patients were classified as high-intermediate/high-risk according to KPI, PINK, and NRI scoring systems, respectively.

NKTCL patients presented a distinct peripheral immunity from healthy donors

Patients with NKTCL had comparable baseline peripheral lymphocytes (1.478 ± 0.972 × 10^9/L *vs.* 1.350 ± 0.278 × 10^9/L, $p = 0.060$) and neutrophils (3.837 ± 1.754 *vs.* 4.050 ± 1.148 × 10^9/L, $p = 0.080$) with healthy controls (Fig. 1A). The baseline distribution of lymphocyte subsets in NKTCL is summarized in Fig. 1B. Though patients with NKTCL had similar levels of T cells/lymphocytes as healthy controls (71.560 ± 13.429% *vs.* 70.510 ± 3.974%, $p = 0.263$), lymphocyte subsets were significantly differently distributed between the two groups. Higher levels of effect T cells were detected in NKTCL than healthy controls: CD8+ T cells, Tc cells, and NKT cells accounted for 33.230 ± 12.090%, 14.305 ± 6.299%, and 7.697 ± 7.219% of peripheral lymphocytes in NKTCL, and 27.060 ± 4.010% ($p < 0.001$), 12.035 ± 2.191% ($p < 0.001$), and 3.550 ± 2.088% ($p < 0.001$) in health controls, respectively. Whereas, lower levels of Th cells (33.084 ± 11.361% *vs.* 37.650 ± 3.153%, $p < 0.001$) and Treg cells (2.999 ± 1.949% *vs.* 3.420 ± 1.051%, $p = 0.003$) were documented in patients with NKTCL.

Table 1 Baseline characteristics of enrolled patients.

Characteristics	*N*	%
Gender		
Male	137	66.8
Female	68	33.2
Age		
≤ 60	179	87.3
> 60	26	12.7
Primary site		
UADT	189	92.2
Extra-UADT	16	7.8
Ann Arbor stage		
I/II	165	80.5
III/IV	40	19.5
ECOG		
0–1	200	97.6
>1	5	2.4
B symptoms		
No	122	59.5
Yes	83	40.5
Serum LDH		
Normal	151	73.7
Elevated	54	26.3
EBV copies		
Normal	118	57.6
Elevated	48	23.4
Undetected	39	19.0
Primary tumor invasion		
No	83	40.5
Yes	122	59.5
Treatment modality		
CT	41	20.0
CRT	164	80.0
KPI		
Low-risk	57	27.8
Intermediate-risk	65	31.7
High-risk	83	40.5
PINK		
Low-risk	142	69.2
Intermediate-risk	45	22.0
High-risk	18	8.8
NRI		
Low-risk	41	20.0
Intermediate-risk	96	46.8
High-risk	68	33.2

UADT upper aerodigestive tract, *ECOG* Eastern Cooperative Oncology Group, *LDH* lactate dehydrogenase, *CT* chemotherapy, *CRT* chemoradiation, *PINK* prognostic index for NK cell lymphoma, *KPI* Korea prognostic index, *NRI* nomogram-revised index

Correlation of peripheral lymphocyte subsets and clinical variations in NKTCL

We further explored the relationship between lymphocyte subsets and age, stage, primary site, and risk score of patients with NKTCL, respectively. Compared with elderly patients aged ≥ 60 years, those young cases (< 60 years) presented enhanced peripheral immunity with higher levels of T cells (72.294 ± 12.681% vs. 66.476 ± 17.220%, $p = 0.039$) and Tc cells (10.314 ± 4.227% *vs.* 15.063 ± 6.268%, $p = 0.002$, Fig. 1C). Besides, patients with advanced NKTCL had higher levels of T cells (76.037 ± 13.333% *vs.* 70.481 ± 13.267%, $p = 0.018$) and Th cells (36.490 ± 12.083% *vs.* 32.263 ± 11.183%, $p = 0.036$, Fig. 1D) than those with stage I/II diseases. In terms of primary site, higher levels of Treg cells (4.094 ± 2.680% *vs.* 32.902 ± 1.849%, $p = 0.019$) and NKT cells (11.154 ± 10.957% *vs.* 7.391 ± 6.752%, $p = 0.045$, Fig. 1E) were detected in patients with extra-UADT NKTCL than UADT. The lymphocyte subsets were identical regardless of EBV infections (Fig. 1F). Additionally, baseline proportion of Th cells was associated with tumor burden reflected by disease stage ($p = 0.037$), PTI ($p = 0.025$), and serum LDH ($p = 0.042$) in NKTCL (Fig. 2). In this study, only a very small proportion had comorbidities, and further analysis revealed they had similar peripheral lymphocytes with those without comorbidity.

Patients were stratified as low-, intermediate-, and high-risk group according to three risk scoring systems which were widely used in NKTCL. As shown in Fig. 3 A, patients with high-risk NKTCL have higher levels of T cells ($p = 0.035$), Th cells ($p = 0.041$) and NK cells ($p = 0.003$) in peripheral according to PINK. Similarly, higher levels of T cells ($p = 0.055$) and Ts cells ($p = 0.027$) were detected in high-risk group compared with low-risk group according to KPI (Fig. 3B). NRI-high-risk patients in this study exhibited higher level of Th cells ($p = 0.041$) in peripheral than low-risk cases at diagnosis (Fig. 3C). Conclusively, lymphocyte subsets correlated with risk stratifications in NKTCL, suggesting its potential role in prognosis predictions.

Correlation of peripheral lymphocyte subsets in NKTCL

We also investigated the relations between different cell groups in baseline PB in NKTCL. Lymphocyte was positively correlated with monocyte and neutrophil (Supplementary Figure 1A–B). However, a negative linear correlation was observed between T lymphocytes and NK cells ($R^2 = 0.584$, $p < 0.001$, Supplementary Figure 1C). These results

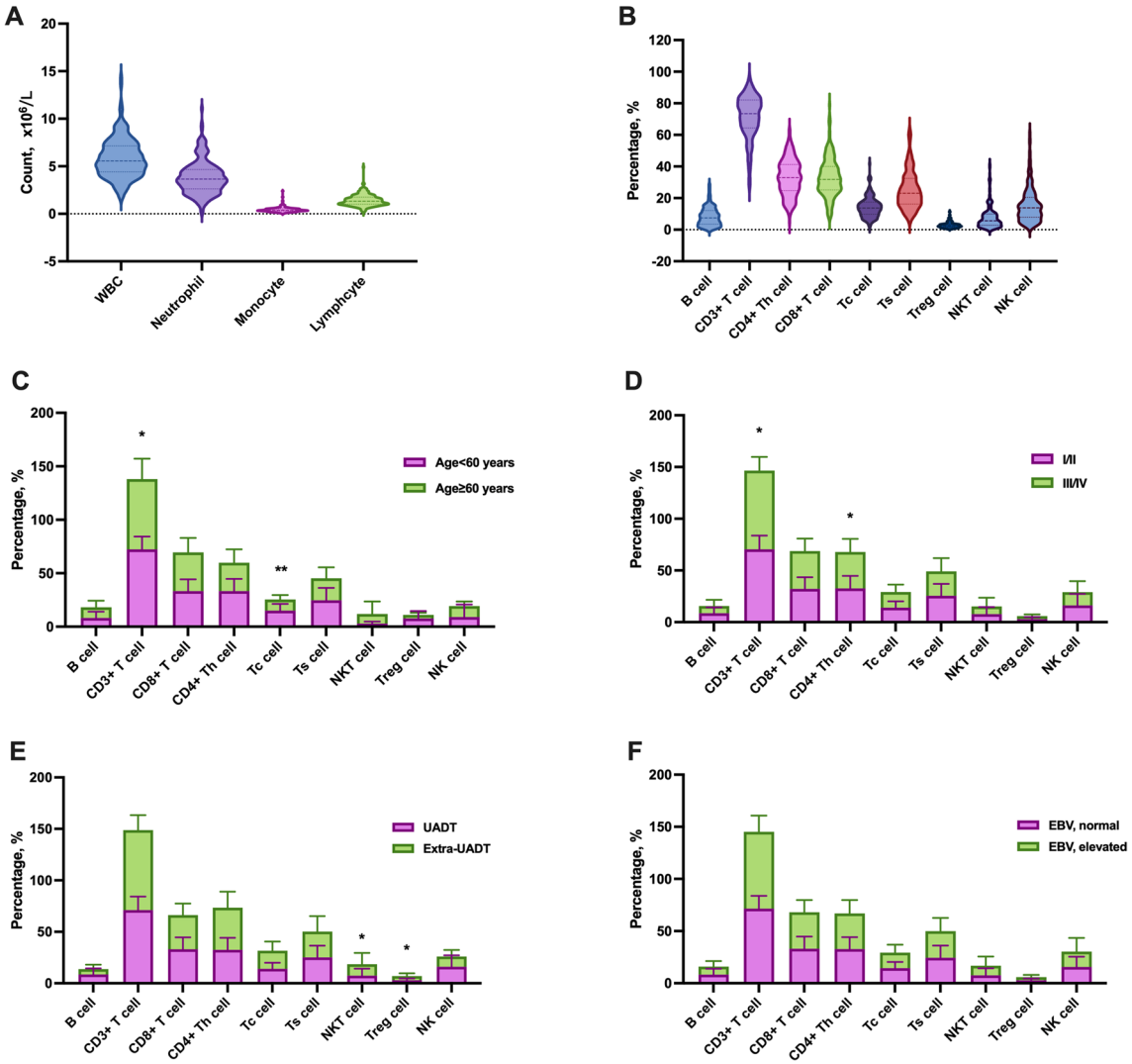

Fig. 1 Distributions of peripheral lymphocyte subsets in NKTCL patients. **A** Violin plot of white blood cell (WBC), neutrophil, monocyte, and lymphocyte in peripheral blood at diagnosis in NKTCL; **B** distribution of lymphocyte subsets in peripheral blood in NKTCL patients; **C**, **D**, **E**, **and F**, distributions of lymphocyte subsets with regard to age (**C**), stage (**D**), primary site (**E**), and EBV infection (**F**). UADT, upper aerodigestive tract. * $p < 0.05$; ** $p < 0.01$

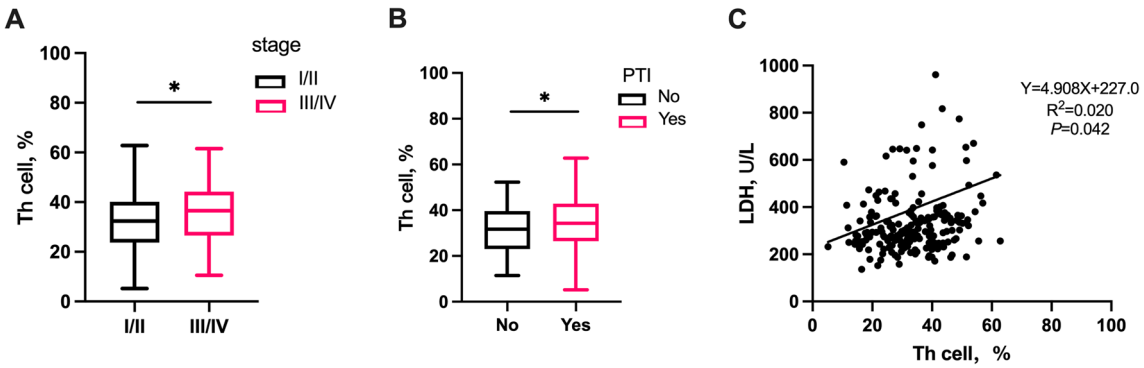

Fig. 2 Linear correlation of the proportion of Th cells in peripheral blood and disease stage, primary tumor invasion (PTI), and serum LDH in patients with NKTCL.

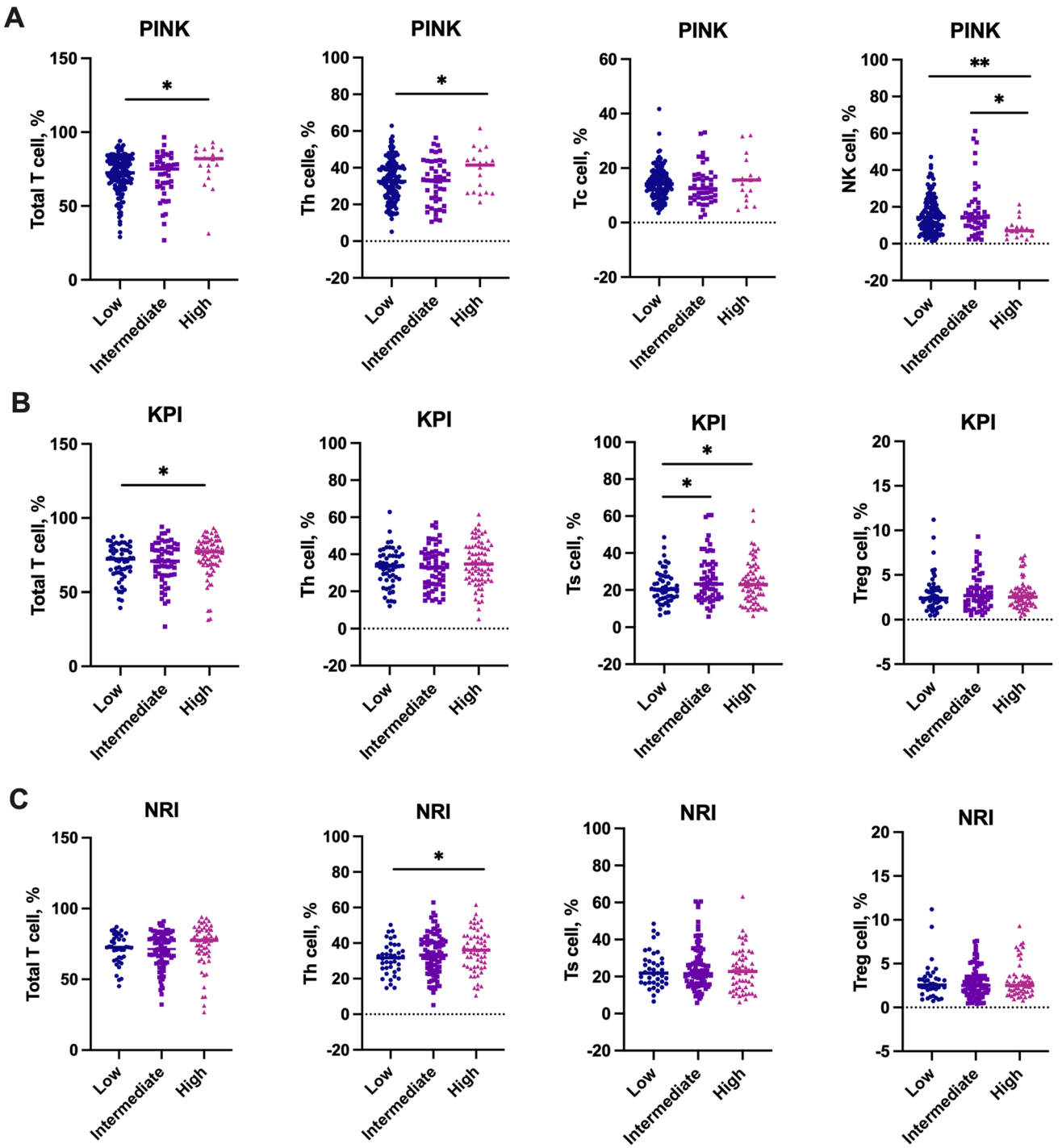

Fig. 3 Correlation between lymphocyte and prognostic stratification according to different risk indexes. PINK, prognostic index for NK cell lymphoma; KPI, Korea prognostic index; NRI, nomogram-revised index

might indicate a balance between peripheral adaptive and natural immunity in NKTCL. The correlations of various lymphocyte subsets were further analyzed (Supplementary Figure 1D–I). Our results revealed negative linear regressions between Tc cells and Treg cells, indicating the suppressive effect of Treg cells on proliferation of cytotoxic T cells.

Table 2 Univariate and multivariate analyses of survival for patients with NKTCL.

Variable	Univariate analysis			Multivariate analysis		
	HR	95% *CI*	*P*-value	*HR*	95% *CI*	*P*-value
Age (> 60 *vs.*≤ 60 years)	1.871	0.994–3.518	0.052	0.602	0.208–1.742	0.349
Stage (III/IV *vs.* I/II)	3.141	1.847–5.343	< 0.001	2.111	1.061–4.202	0.033*
LDH (elevated *vs.* normal)	2.979	1.792–4.951	< 0.001	2.410	1.148–5.058	0.020*
ECOG (> 1 *vs.* 0–1)	1.874	4.702–29.99	< 0.001	48.44	9.061–259.0	< 0.001*
B symptom (yes *vs.* no)	2.102	1.270–3.481	0.004	1.159	0.825–2.796	0.179
PTI (yes *vs.* no)	2.361	1.317–4.230	0.004	1.692	0.737–3.888	0.215
Primary site (extra-UADT *vs.* UADT)	2.079	0.988–4.374	0.054	1.004	0.364–2.768	0.993
Plasma EBV-DNA copies (elevated *vs.* normal)	2.841	1.583–5.102	< 0.001	1.421	0.607–3.329	0.418
Total T cells (elevated *vs.* normal)	2.094	1.261–3.479	0.004	1.158	0.530–2.527	0.713
Th cells (elevated *vs.* normal)	1.839	1.021–3.313	0.042	2.333	1.030–5.288	0.042*
CD8+ T cells (elevated *vs.* normal)	2.196	1.222–3.947	0.009	1.929	0.832–4.469	0.125
Tc cells (elevated *vs.* normal)	1.817	1.047–3.154	0.034	1.230	0.560–2.695	0.605
Ts cells (elevated *vs.* normal)	1.373	0.923–2.041	0.118			
Treg cells (elevated *vs.* normal)	1.914	1.037–3.534	0.038	0.839	0.359–1.957	0.684
LMR (decreased *vs.* normal)	0.610	0.364–1.024	0.062	0.885	0.418–1.751	0.669
Hb (decreased *vs.* normal)	1.737	0.992–3.042	0.053	1.139	0.957–1.393	0.831

UADT upper aerodigestive tract, *ECOG* Eastern Cooperative Oncology Group, *LDH* lactate dehydrogenase, *LMR* lymphocyte/monocyte ratio

Peripheral lymphocyte subsets and survival in NKTCL

Till the last visit in February 2023, the median follow-up interval was 52.8 months. The 5-year PFS and OS were 66.4% and 80.9%, respectively. Prognostic analysis revealed that age > 60 years, advanced stage, elevated serum LDH, Eastern Cooperative Oncology Group (ECOG) performance status > 1, B symptoms, primary tumor invasion (PTI), plasma EBV-DNA copies, and extra-UADT lesions were associated with unfavorable PFS in NKTCL (Table 2). Lymphocytes of NKTCL beyond the upper limit of normal from healthy controls were defined as elevated subsets. Patients with elevated level of T cells (55.3% *vs.* 72.7%, $p = 0.003$), Th cells (56.3% *vs.* 68.6%, $p = 0.047$), CD8+ cells (58.9% *vs.* 76.4%, $p = 0.005$), Ts cells (60.8% *vs.* 76.4%, $p = 0.023$), or Treg cells (49.5% *vs.* 68.9%, $p = 0.040$) at diagnosis had inferior 5-year PFS in comparison to normal levels of corresponding subsets (Fig. 4A–D and F; Table 2). Patients with lymphocyte-monocyte ratio (LMR) < 2.34 (58.7% *vs.* 69.6%, $p = 0.048$, Fig. 4G) or Hb < 120 g/L (54.8% *vs.* 68.6%, $p = 0.027$, Fig. 4G–H) had inferior 5-year PFS. Apart from advanced stage, elevated serum LDH and ECOG > 1, multivariate analysis also revealed elevated levels of Th cells in peripheral as an independent indicator for inferior survival (*HR* = 2.333, 95% CI, 1.030–5.288, $p = 0.042$) in NKTCL (Table 2).

Discussion

To our knowledge, this is a real-world study which at the first time focused on the frequency and prognostic significance of peripheral T lymphocyte subsets in NKTCL. Our results revealed that NKTCL presented a distinct peripheral immunity with significantly higher levels of CD8+ T especially Tc cell and NKT cells but lower levels of suppressive Treg and Th cells than healthy controls. Furthermore, the proportion of Th cells in peripheral, positively correlated with tumor burden reflected by stage, PTI and serum LDH, was demonstrated as reliable predictive biomarker for inferior survival in NKTCL.

Patients with diffuse large B cell lymphoma (DLBCL), the most common pathology type of NHL, had distinct peripheral lymphocytes distribution to healthy donors [33]. However, the peripheral immunity of NKTCL has not been extensively explored so far. Our study revealed comparable ALC ($p = 0.060$) and T cells/lymphocytes ($p = 0.263$) in NKTCL, but an enhanced adaptive immunity with high levels of cytotoxic CD8+ T cells (33.351±11.349% *vs.* 26.385±12.559%, $p = 0.010$), especially activated CD8+CD28+ Tc cells (15.063 ± 6.268% *vs.* 10.314 ± 4.227%, $p = 0.002$), but lower proportions of suppressive Treg cells (2.999±1.949% *vs.* 3.420±1.051%, p=0.003) and Th cells (33.084 ±1 1.361% *vs.* 37.650 ± 3.153%, $p < 0.001$) at diagnosis than healthy controls. Since patients with bone

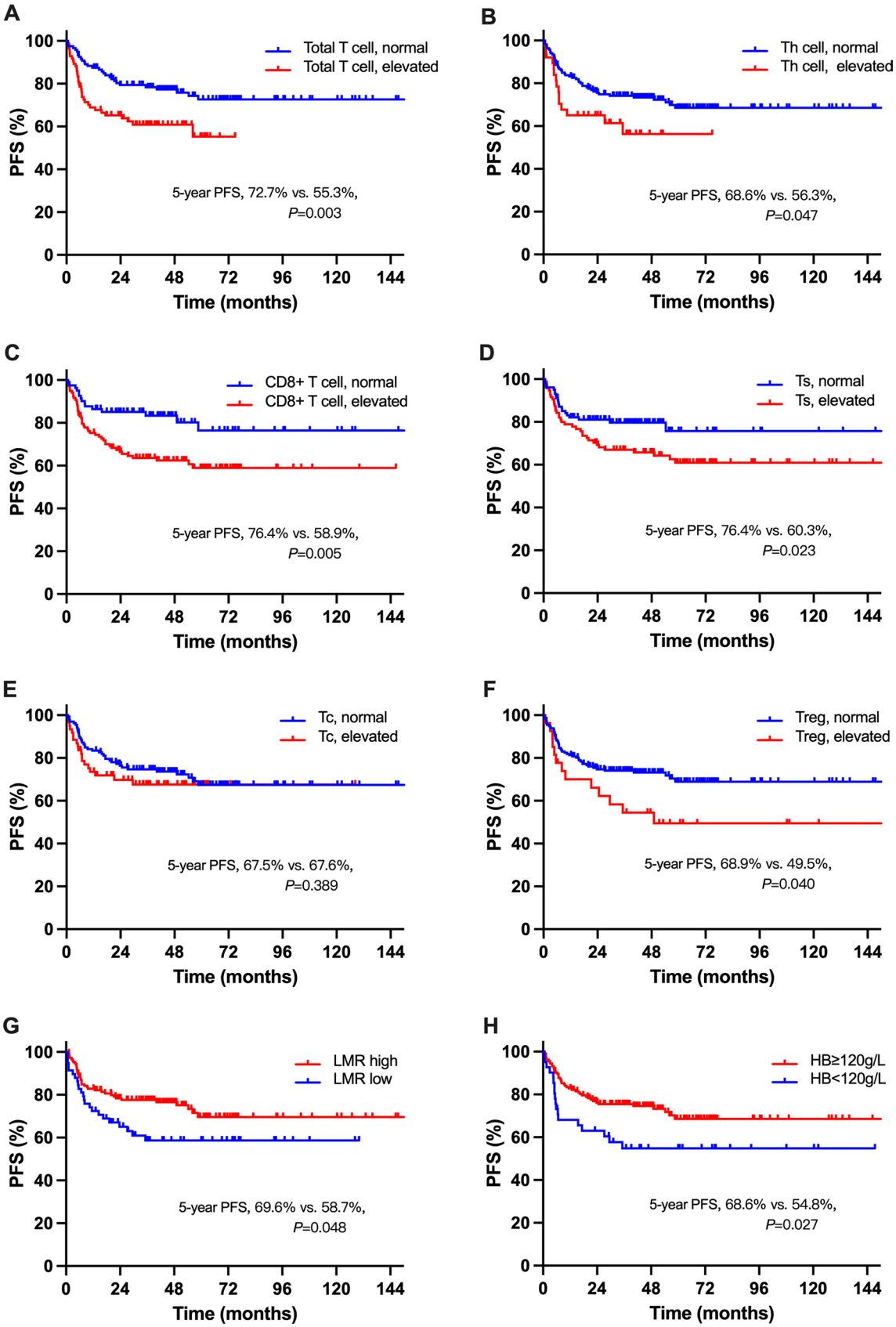

Fig. 4 Survival curves of NKTCL patients with different lymphocyte subset distribution

marrow involvement at diagnosis were excluded from this study, these elevated lymphocytes suggested an activated immunity status in face of tumor in patients with NKTCL rather than the infiltration of malignant T or NK cells in PB. Furthermore, since the distribution of peripheral T lymphocytes was identical regardless of EBV infection status, we supposed the activated immunity might not be established against antigens of EBV but the tumor cells itself. Similar to EBV-associated gastric cancer, HL, Burkitt lymphoma, the virus establishes a latent infection in NKTCL [34]. We have previously demonstrated that lytic antigens of EBV were silenced and only few latent antigens were expressed in NKTCL cell lines (NKYS, YT, and YTS) via real-time quantitative PCR (RT-qPCR). Noteworthy, since the functions of T lymphocytes were various and complex, further more functional tests need to be done in lymphocytes.

At present, the correlation between peripheral immunity and tumor immunity remains controversial. Francesco Gaudio and his colleagues demonstrated the positive relationship between circulating blood CD3+ and CD4+ lymphocytes and T cells in the TME in patients with DLBCL [35]. Additionally, the circulating lymphocytes, CD4+ cells, CD8+ cells, and the ratio of CD4+/CD8+ cells were previously reported as predictive biomarkers in various malignancies including follicular lymphoma, mantle lymphoma, myeloma, and DLBCL [21, 22, 26, 33, 36]. In contrast to CD8+ T cells which are the main cytotoxic participants in anti-tumor response, CD4+ T cells play more of an immunemodulatory and mainly inhibit the augment of body's immune response. CD4+ T cell count in PB predicted poor prognosis in patients with DLBCL who received first-line R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) [37]. The present study revealed that CD4+ Th cells in PB at diagnosis were positively correlated with tumor stage ($p = 0.037$), PTI ($p = 0.025$), and serum LDH ($p = 0.042$) and was demonstrated as an independent indicator for unfavorable survival in NKTCL (*HR* = 2.333, 95% CI, 1.030–5.288, $p = 0.042$). Conclusively, CD4+ Th cells might be a potential invasive biomarker for disease evaluation and prognostic prediction in NKTCL.

Besides, survival analysis revealed elevated level of CD8+ T cells in PB was associated with inferior survival in this group of patients (58.9% *vs.* 76.4%, $p = 0.005$, Fig. 4C). Since CD8+ T cells are considered the main effector cells in anti-tumor response, this result seemed to be unreasonable in our study. We subsequently explored the prognostic effect of the two subgroups of CD8+ T cells including suppressive Ts and cytotoxic Tc cells, and found Ts rather than Tc cells were negatively correlated with PFS in NKTCL (60.3% *vs.* 76.4%, $p = 0.023$). These results revealed that the level of CD8+ T cells was not a feasible indicator for survivals, and the suppressive CD8+ T cells (Ts cells) which played a negative role in anti-tumor immunity might be more suitable in predicting survivals in NKTCLs.

This study had some limitations that need to be addressed. Our cohort is monocentric, and a validation cohort is missing. Besides, CD4+ Th cells are classified into several different subgroups including Th1, Th2, Th17, and others, and the exact roles of different Th cells in NKTCL were not assessed in this study. Furthermore, the correlation between peripheral immunity and tumor immunity were not analyzed in this study due to the difficulty in obtaining fresh sufficient tumor specimens. Nevertheless, this study is the first to highlight the distribution and potential prognostic value of lymphocyte subsets at diagnosis for NKTCL patients, and our results opened new perspectives in patient risk stratification in NKTCL. We are building a new prognostic model incorporating Th cell in PB with established predicting index used in NKTCL. The new index might be of better value in predicting survivals. Relevant prospective studies are warranted in the future.

Conclusion

Patients with NKTCL presented an activated peripheral immunity status. The proportion of Th cells/lymphocytes positively correlated with tumor burden and was an independent risk factor for survival in NKTCL receiving first-line chemotherapy and radiation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00277-023-05605-8.

Author contribution Jun Zhu, Yuqin Song: conceptualization and methodology. Fei Qi: data curation. Fei Qi: writing original draft preparation. Yuce Wei: revising the manuscript and editing. Meng Wu, Yan Sun, and Yan Xie: formal analysis. Weiping Liu and Weihu Wang: supervision.

Declarations

Human ethics and consent to participate Not applicable.

Competing interests The authors declare no competing interests.

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