Analysis of 142 Northern Chinese Patients With Peripheral T/NK-Cell Lymphomas

Subtype Distribution, Clinicopathologic Features, and Prognosis

Ya-Li Ren, MD, Lin Nong, MD, Shuang Zhang, MD, Jing Zhao, PhD, Xiao-Ming Zhang, MD, and Ting Li, MD

Key Words: Peripheral T-cell lymphoma; NK-cell lymphoma; Classification; T-bet; ETS1

DOI: 10.1309/AJCPWKJ3GPFRT7GA

Abstract

*Peripheral T- and natural killer (NK)–cell lymphomas (PTNKLs) are a heterogeneous group of lymphoid malignancies. We reclassified 142 cases and investigated their clinicopathologic features and outcome. Results showed that the most prevalent subtypes were extranodal NK/T-cell lymphoma, nasal type (eNK/T) (38.0%); angioimmunoblastic T-cell lymphoma (16.9%); and peripheral T-cell lymphoma, not otherwise specified (16.2%). Follow-up was available in 124 patients whose overall survival ranged from 3 days to 134 months, with a median of 11 months. Multivariate analysis demonstrated that thrombocytopenia (*P = *.001), elevated lactate dehydrogenase (*P = *.007), high Ki-67 index (*P = *.002), and T-bet expression in more than 20% of cells (*P = *.036) were independent factors for all cases—among which only the factor of T-bet indicated good outcome—and that thrombocytopenia (*P = *.011) and radiotherapy (*P = *.026) were significant for the eNK/T group. Thus, eNK/T was the commonest subtype in this series. The significance of T-bet in predicting outcome should be further confirmed.*

Mature (peripheral) T- and natural killer (NK)–cell lymphomas (PTNKLs) are a heterogeneous group of lymphoid malignancies with a wide variety of clinicopathologic features. They vary significantly in incidence and subtype distribution in different geographical regions or racial populations, and are found more frequently in Asian populations in general.[1] In the last several years, following an in-depth understanding of World Health Organization (WHO) classification and growing practice in this group of complicated disorders, several large studies have described PTNKLs from different Asian countries and regions,[2-6] but data from northern China are limited.

Owing to a lack of comprehensive understanding of the molecular characteristics and pathogenesis of PTNKLs, their diagnosis and classification mainly rely on clinical features in conjunction with morphologic and immunophenotypic presentations. Most PTNKLs have a highly aggressive clinical course but do not respond to aggressive chemotherapy. Their prognosis is varied and relies heavily on clinical factors, such as the international prognostic index (IPI), stage, and modes of therapy used; however, the prognostic significance of pathologic factors is controversial.[7,8] Our previous study revealed that the transcription factors *T-bet, ETS,* and *EOMES,* which engage in development of NK and T cells, were commonly expressed in nasal NK/T-cell lymphomas and some peripheral T-cell lymphomas, but not in the control group of B-cell lymphomas.[9]

In this study, we retrospectively investigated 142 cases of PTNKL diagnosed within the last 10 years and reexamined and reclassified them according to the WHO classification.[10] The resultant subtype distribution, clinicopathologic features, treatment results, and outcomes were evaluated. In addition, the expressive status of the transcription factors *T-bet* and

ETS-1 was analyzed, and their pathobiologic significance in PTNKLs was clarified.

Materials and Methods

Case Series and Clinical Data

A total of 189,834 surgical specimens were archived at the Department of Pathology, Peking University First Hospital, which serves the general population of Beijing and northern China, between January 2000 and September 2010. Six hundred eighty-eight non-Hodgkin lymphomas were diagnosed during this period, of which 164 patients were classified as T- and NK-cell lymphomas. After rejecting 12 cases of T-cell and 1 case of NK-cell lymphoblastic lymphoma for reasons related to the study, and 9 cases of mature T/NK-cell lymphoma for reasons related to the specimens, 142 cases of PTNKL were collected and retrospectively analyzed. They were reclassified according to the WHO classification.[10] In angioimmunoblastic T-cell lymphoma (AITL) cases, the diagnosis was based on the following criteria: partial or diffuse effacement of the nodal architecture, vascular proliferation with prominent arborization of high endothelial venules, extrafollicular meshwork of follicular dendritic cells, atypical population of CD3+ T cells, and large CD20+ B cells.[11]

Clinical characteristics of the patients were recorded. The information included a complete medical history and physical examination; computed tomography of the chest, abdomen, and pelvis; bone marrow involvement; and relevant laboratory data. Performance status (0 to 1 vs ≥2), and Ann Arbor stage were evaluated, too. The modes of therapy used were collected. Follow-up data on all 142 cases were collected.

Histopathology and Immunohistochemistry (IHC)

Tissue specimens were fixed in 10% formalin or neutral-buffered formalin, routinely processed, and embedded in paraffin. Sections of 4-μm thickness were stained with H&E. IHC staining was performed using DAKO EnVision detection kit (DAKO, Carpinteria, CA). The tissue sections underwent heat-induced antigen retrieval in citrate acid buffer (pH 6.0) or EDTA-Tris (pH 9.0). A standard panel of antibodies that was performed on each case included CD3 (clone Ps1, DAKO, Glostrup, Denmark), CD4 (clone 1F6, Neomarkers, Fremont, CA), CD5 (clone SP19, Neomarkers), CD8 (clone SP16, Neomarkers), CD20 (clone L26, DAKO), CD79a (clone JCB117, DAKO), CD15 (clone Car-b, DAKO), CD30 (clone BER-H2, DAKO), CD56 (clone 123C3, Zymed, South San Francisco, CA), TIA-1 (clone TIA-1, Coulter, Hialeah, FL), granzyme B (clone 11F1, Novocastra, Newcastle upon Tyne, England), TCR-β (clone G-11, Santa Cruz Biotechnology, Santa Cruz, CA), Ki-67 (clone SP6, DAKO), cutaneous lymphocyte–associated antigen (CLA; clone HECA-452, Biolegend, San Diego, USA), T-bet and ETS1 (H-210 and 1G11, Santa Cruz). The antibodies that were performed as needed included CXCL13 (clone AF801, R&D systems, Minneapolis, MN), CD10 (clone 56C6, Novocastra), bcl-6 (Clone PG-B6p, DAKO), CD21 (clone 2G9, Novocastra), anaplastic lymphoma kinase (ALK; clone ALK1, DAKO), and EMA (clone MC-5, Zhongshan Biological, Beijing, China). For the results of T-bet and ETS1 staining, the percentage of positive tumor cells was semiquantitatively estimated: negative (≤5% cells were positive), weakly positive (1+, 6%-20% cells positive), moderately positive (2+, 21%-50% cells positive), or strongly positive (3+, >50% cells positive). Pathologic review was performed by 3 pathologists (Y-L.R., L.N., T.L.) to confirm the diagnosis and each case was reclassified according to the WHO classification.[10]

In Situ Hybridization (ISH) Test for Epstein-Barr Virus (EBV)

The ISH test for detection of EBV genome was performed in 136 cases that had sufficient tissue specimens using a specific probe for EBV-encoded small RNA-1 (*EBER-1*, Zhongshan) according to manufacturer's instructions. An EBV-positive NK/T-cell lymphoma and EBV-negative lymphoid tissue were used separately as positive and negative controls. The percentage of positive tumor cells was semiquantitatively estimated as the standard in IHC.

Statistical Analysis

Clinical factors and the expression of immune markers among various subtypes were compared using the χ^2 test, Fisher exact test, or Mann-Whitney *U* test when appropriate. Treatment outcomes were measured by means of failure-free survival (FFS) and overall survival (OS). FFS was defined as the time from initial diagnosis to progression, relapse, or death from any cause. OS was calculated by the time from initial diagnosis to death from any cause or last follow-up. Estimates of FFS and OS were calculated using the Kaplan-Meier method and compared with log-rank tests. A forward stepwise Cox regression with multivariate analysis was used. Differences were considered statistically significant when the 2-sided *P* value was less than .05. All analyses were performed with SPSS 13.0 software (Chicago, IL).

Results

Subtype Distribution

According to the WHO classification, the 142 cases of PTNKLs were reclassified and the subtype distribution is listed in **Table 1**. The most prevalent subtype was extranodal NK/T-cell lymphoma, nasal type (eNK/T) (n = 54, 38%) **Image 1**, followed by AITL (n = 24, 16.9%) **Image**

DOI: 10.1309/AJCPWKJ3GPFRT7GA

Table 1
Subtype Distribution of 142 Northern Chinese Patients With Peripheral T/NK-Cell Lymphomas

Subtype of Peripheral T/NK-Cell Lymphomas	No (%)	Median Age (y)	M:F Ratio
T-cell prolymphocytic leukemia	0 (0)	NA	NA
T-cell large granular lymphocytic leukemia	0 (0)	NA	NA
Aggressive natural killer cell leukemia	0 (0)	NA	NA
EBV+ T-cell lymphoproliferative diseases of childhood	4 (2.8)	8.5	4:0
Adult T-cell leukemia/lymphoma	0 (0)	NA	NA
Extranodal NK/T-cell lymphoma, nasal type	54 (38.0)	38.5	2.2:1
Enteropathy-associated T-cell lymphoma	4 (2.8)	51.5	4:0
Hepatosplenic T-cell lymphoma	3 (2.1)	33.0	2:1
Subcutaneous panniculitis-like T-cell lymphoma	1 (0.7)	NA	0:1
Mycosis fungoides/Sézary syndrome	9 (6.3)	52	2:1
Primary cutaneous CD30+ T-cell lymphoproliferative disorders	6 (4.2)	72.5	5:1
Primary cutaneous CD8-positive aggressive epidermotropic cytotoxic T-cell lymphoma	2 (1.4)	38.0	1:1
Peripheral T-cell lymphoma, NOS	23 (16.2)	53	1.1:1
Angioimmunoblastic T-cell lymphoma	24 (16.9)	67	2:1
Anaplastic large cell lymphoma, ALK positive	7 (4.9)	10	6:1
Anaplastic large cell lymphoma, ALK negative	5 (3.5)	46	5:0
Total	142 (100)	47.5	2.2:1

ALK, anaplastic lymphoma kinase; EBV, Epstein-Barr virus; NA, not available; NK, natural killer; NOS, not otherwise specified.

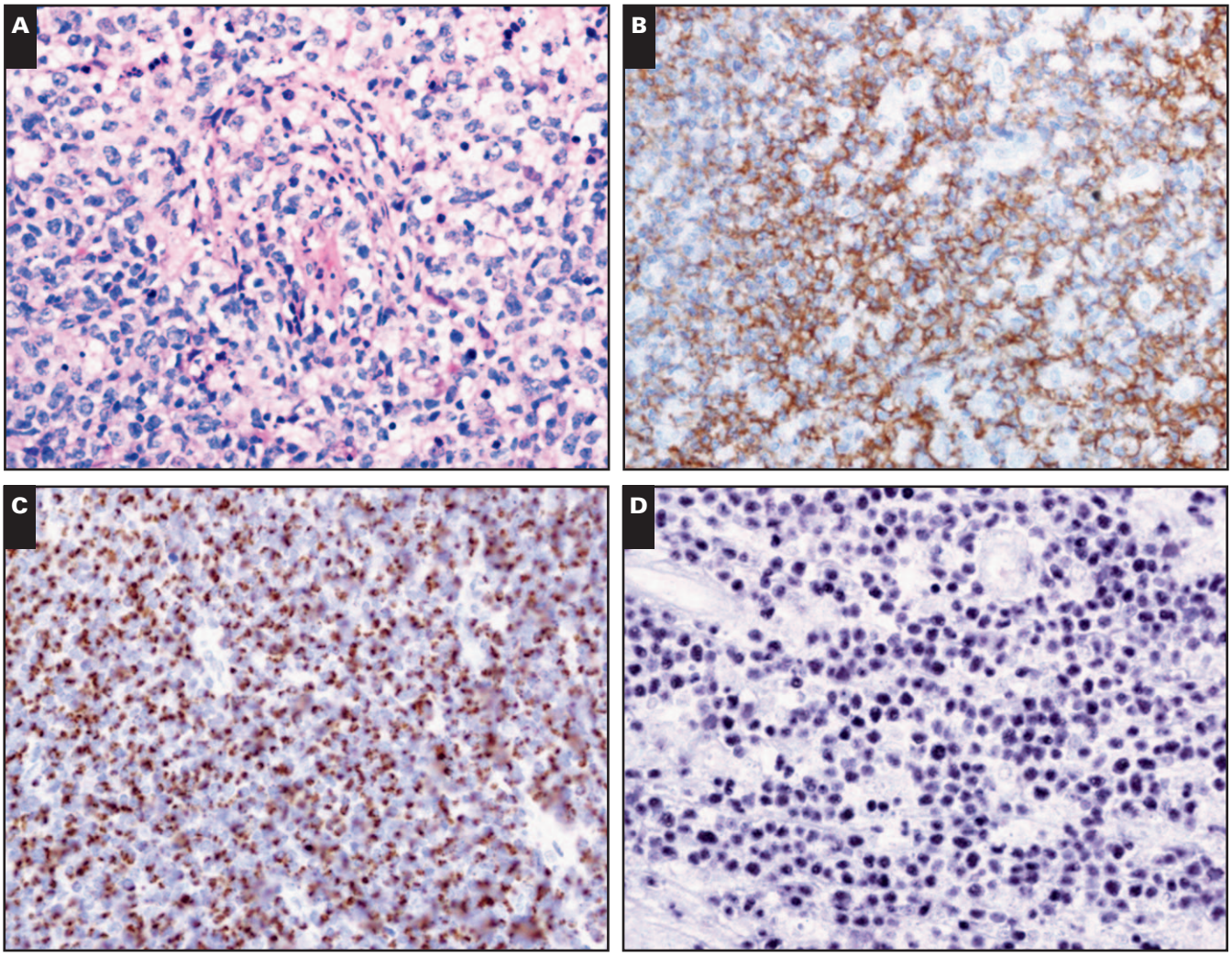

Image 1 Histopathologic features and immunophenotyping of extranodal natural killer (NK)/T-cell lymphoma, nasal type. **A**, Tumor cells were composed predominantly of polymorphic medium-sized to large cells with irregular nuclei and inconspicuous nucleoli. An angiocentric and angiodestructive growth pattern was present (H&E, ×400). On immunohistochemical staining, tumor cells were strongly positive for CD56 (**B**, ×400) and TIA-1 (**C**, ×400). **D**, In situ hybridization with Epstein-Barr virus–encoded small RNA (EBER-1) probe revealed positive signals in the nuclei of numerous tumor cells (×400).

DOI: 10.1309/AJCPWKJ3GPFRT7GA

2, peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) (n = 23, 16.2%), mycosis fungoides (MF)/Sézary syndrome (n = 8 and 1, respectively, 6.3%), anaplastic large-cell lymphoma, ALK-positive (ALCL, ALK+) (n = 7, 4.9%), primary cutaneous ALCL (C-ALCL) (n = 6, 4.2%), ALCL, ALK– (n = 5, 3.5%), EBV+ T-cell lymphoproliferative diseases of childhood (EBV + TLPD) and enteropathy-associated T-cell lymphoma (n = 4 each, 2.8%), and hepatosplenic T-cell lymphoma (n = 3, 2.1%). Several subtypes of PTNKLs were not found in our series, such as T-cell prolymphocytic leukemia and adult T-cell lymphoma/leukemia.

Clinical Characteristics

Median age for the 142 patients was 47.5 years, with a range of 2 to 95 years. A male predominance was noted, with a male-to-female ratio of 2.3:1 (98:44). For subtypes, the median age was younger in eNK/T (38.5 y), ALCL, ALK+ (10 y), and EBV+ TLPD (8.5 y), but older in AITL (67 y) and C-ALCL (72.5 y) (Table 1).

The most common sites for this series were upper aerodigestive tract (n = 56, 39.4%), lymph node (n = 47, 33.1%), and skin (n = 26, 18.3%). Thirteen cases (9.2%) arose at other sites including gastrointestinal tract, spleen, liver, soft tissue, and bone marrow. Forty-six (85.2%) of 54 eNK/T cases arose

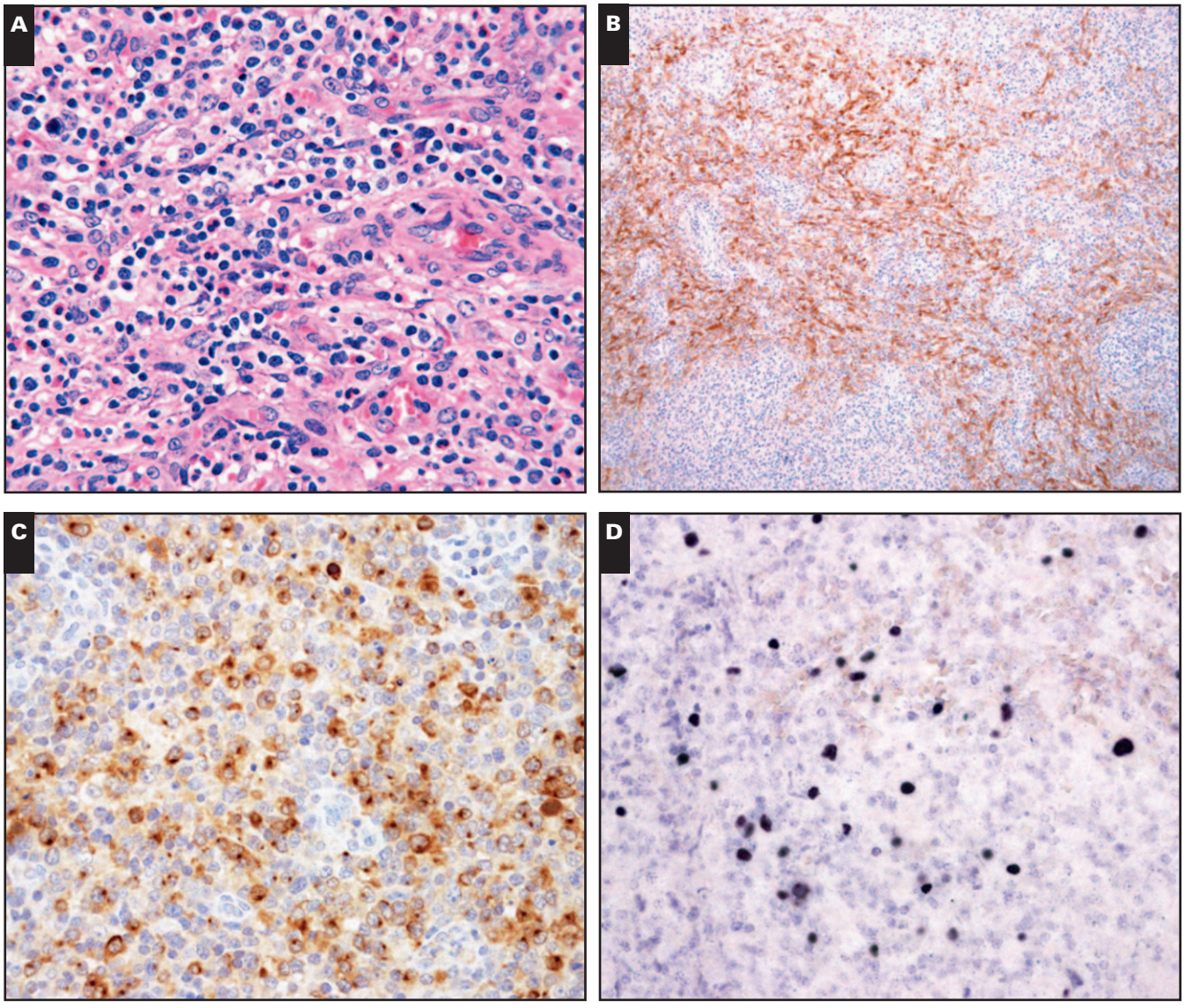

Image 2 Histopathologic features and immunophenotyping of angioimmunoblastic T-cell lymphoma. **A**, Diffuse infiltration of medium-sized atypical lymphoid cells with abundant clear cytoplasm, usually around marked proliferation of arborizing high endothelial venules (H&E, ×400). On immunohistochemical staining, CD21 exhibited the expanded follicular dendritic cell meshworks (**B**, ×100); characteristically, tumor cells were strongly positive for CXCL13 (**C**, ×400). **D**, In situ hybridization with Epstein-Barr virus–encoded small RNA (EBER-1) probe revealed positive signals in the nuclei of scattered large B cells (×400).

DOI: 10.1309/AJCPWKJ3GPFRT7GA

in the upper respiratory tract including nasal cavity (n = 31, 67.4%), oral cavity (n = 7, 15.2%), nasopharynx (n = 3, 6.5%), vocal cords (n = 2, 4.3%), and other sites (n = 4, 8.7%) such as paranasal sinuses, and pyriform. Another 8 (14.8%) of 54 eNK/T cases occurred in the skin (n = 6, 11.1%), intestine (n = 1, 1.8%), and lymph node (n = 1, 1.8%). Twenty-four (100.0%) of 24 and 12 (52.2%) of 23 cases arose in the lymph nodes in patients with AITL and PTCL-NOS, respectively. Another 11 cases of PTCL-NOS occurred in the upper aerodigestive tract, gastrointestinal tract, and soft tissue separately.

The clinical characteristics of patients are summarized in **Table 2**. More than two thirds of patients in this series had advanced stage (III/IV) disease or high level of β_2-microglobulin, while poor performance status (>1), bone marrow involvement, or effusion/edema/ascites was seen in fewer than one quarter of the patients. Bone marrow puncture or biopsy of 98 cases showed involvement in 12 cases. The following features, including age more than 60 years ($P < .001$), performance status ($P = .012$), effusion/edema/ascites ($P < .001$), splenomegaly ($P = .002$), hepatomegaly ($P = .001$), enlarged mediastinal lymph node ($P = .003$), enlarged abdominal lymph node ($P < .001$), and elevated lactate dehydrogenase (LDH) ($P = .020$) demonstrated difference among the 3 major subtypes with LDH seen most frequently in PTCL-NOS and the others most frequently in AITL.

Immunophenotyping

Immunohistochemically, all 54 cases of eNK/T were positive for CD3ε and cytotoxic proteins (90.7% for TIA-1, 90.7% for granzyme B, and 83.3% for both), and 43/53 (81.1%) positive for CD56. For 24 cases of AITL, moderate or strong immunoreaction of tumor cells for CXCL13, bcl-6, and CD10 were observed in 20/24 (83.3%), 16/24 (66.7%) and 10/24 (41.7%) cases respectively. All 24 cases were positive for TIA-1 with weak or moderate degree and only 1 was positive for granzyme B. CD21 staining exhibited the expanded follicular dendritic cell meshworks in 17 (70.8%) of 24 cases. In PTCL-NOS group, CD56 expression was observed in 8 (34.8%) and cytotoxic proteins in 16 (69.6%) (16 for TIA-1, 4 for granzyme B, and 4 for both) of 23 cases. Strong expression of CD30 was seen in all 3 cases of ALCL subtypes (7 cases of ALCL, ALK+, 6 cases of C-ALCL, and 5 cases of ALCL, ALK–), while ALK was demonstrated in only 7 cases of ALCL, ALK+ with staining patterns being cytoplasmic-nuclear in 2 cases, membranous cytoplasmic in 2, nuclear in 2, and cytoplasmic in 1 case. In addition, most of the ALCL, ALK+ cases lost the expression of T-cell markers, with only 3 cases positive for CD3 reaction, but all positive for cytotoxic proteins. In our series, all 3 cases of hepatosplenic T-cell lymphoma displayed γδ origin (TCRβ–) and CD56 negativity. CLA staining manifested in 22.7% cases of the large group and covered various subsets including eNK/T (36.4%), PTCL-NOS (16.7%), AITL (5.3%), ALCL, ALK+ (40.0%), MF (40.0%), and C-ALCL (66.7%), and was associated with skin disease ($P = .004$).

The expression of T-bet **Image 3** and ETS-1 **Image 4** was detected in all subsets in various proportions **Table 3**, with total positive rates of 84.3% and 58.6%, respectively. The proportion of more than 20% immunostaining for T-bet and ETS-1 in some groups was as follows: 88.0% and 38.8% in eNK/T group, 33.3% and 0.0% in PTCL-NOS, 54.2% and

Table 2
Clinical Characteristics of Major Subtypes of 142 Northern Chinese Patients With Peripheral T/NK-Cell Lymphomas*

				ALCL					
	eNK/T	PTCL-NOS	AITL	ALK+	ALK–	C-ALCL	MF	Other Subtypes	All
B symptoms	29/49 (59.2)	8/23 (34.8)	16/23 (69.6)	6/7 (85.7)	2/5 (40.0)	0/5 (0.0)	2/7 (28.6)	14/15 (93.3)	77/134 (57.5)
PS >1	2/49 (4.1)	3/22 (13.6)	6/22 (27.3)	0/7 (0.0)	0/5 (0.0)	0/5 (0.0)	0/7 (0.0)	4/15 (26.7)	15/132 (11.4)
Stage III/IV	31/50 (62.0)	17/23 (73.9)	17/19 (89.5)	7/7 (100.0)	5/5 (100.0)	1/5 (20.0)	3/7 (42.9)	15/15 (100.0)	96/131 (73.3)
Enlarged lymph nodes >1 site	17/40 (42.5)	11/19 (57.9)	20/20 (100.0)	7/7 (100.0)	3/4 (75.0)	2/4 (50.0)	3/6 (50.0)	7/15 (46.7)	70/115 (60.9)
Effusion/edema/ascites	4/42 (9.5)	2/20 (10.0)	11/18 (61.1)	1/7 (14.3)	1/4 (25.0)	0/4 (0.0)	0/7 (0.0)	7/14 (50.0)	26/116 (22.4)
Leukopenia	4/37 (10.8)	2/17 (11.8)	2/18 (11.1)	2/7 (28.6)	0/3 (0.0)	0/2 (0.0)	1/5 (20.0)	4/15 (26.7)	15/104 (14.4)
Thrombocytopenia	5/37 (13.5)	3/17 (17.6)	2/18 (11.1)	1/7 (14.3)	0/3 (0.0)	0/2 (0.0)	0/5 (0.0)	5/15 (33.3)	16/104 (15.4)
Lymphopenia	18/37 (48.6)	5/17 (29.4)	7/18 (38.9)	2/7 (28.6)	1/3 (33.3)	1/2 (50.0)	1/5 (20.0)	5/15 (33.3)	40/104 (38.5)
LDH >normal	5/21 (23.8)	6/9 (66.7)	11/17 (64.7)	3/5 (60.0)	1/2 (50.0)	0/1 (0.0)	2/4 (50.0)	7/12 (58.3)	35/71 (49.3)
Albumin <normal	20/37 (54.1)	8/17 (47.1)	14/18 (77.8)	3/7 (42.9)	1/3 (33.3)	0/2 (0.0)	3/5 (60.0)	10/15 (66.7)	59/104 (56.7)
γ-globulin >normal	2/5 (40.0)	3/5 (60.0)	6/9 (66.7)	0/0 (0.0)	0/1 (0.0)	0/0 (0.0)	0/1 (0.0)	3/5 (60.0)	14/26 (53.8)
β_2-microglobin >normal	12/16 (75.0)	4/6 (66.7)	9/10 (90.0)	2/2 (100.0)	1/1 (100.0)	1/2 (50.0)	2/3 (66.7)	3/3 (100.0)	34/43 (79.1)
BM involvement	1/34 (2.9)	3/16 (18.8)	1/16 (6.3)	2/6 (33.3)	1/5 (20.0)	0/3 (0.0)	0/6 (0.0)	4/12 (33.3)	12/98 (12.2)

AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; ALK, anaplastic lymphoma kinase; BM, bone marrow; C-ALCL, primary cutaneous anaplastic large cell lymphoma; eNK/T, extranodal NK/T-cell lymphoma, nasal type; LDH: serum lactate dehydrogenase; MF, mycosis fungoides; NK, natural killer; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified; PS, performance status.
* Data show number of positive cases/total number of cases examined (percentage).

DOI: 10.1309/AJCPWKJ3GPFRT7GA

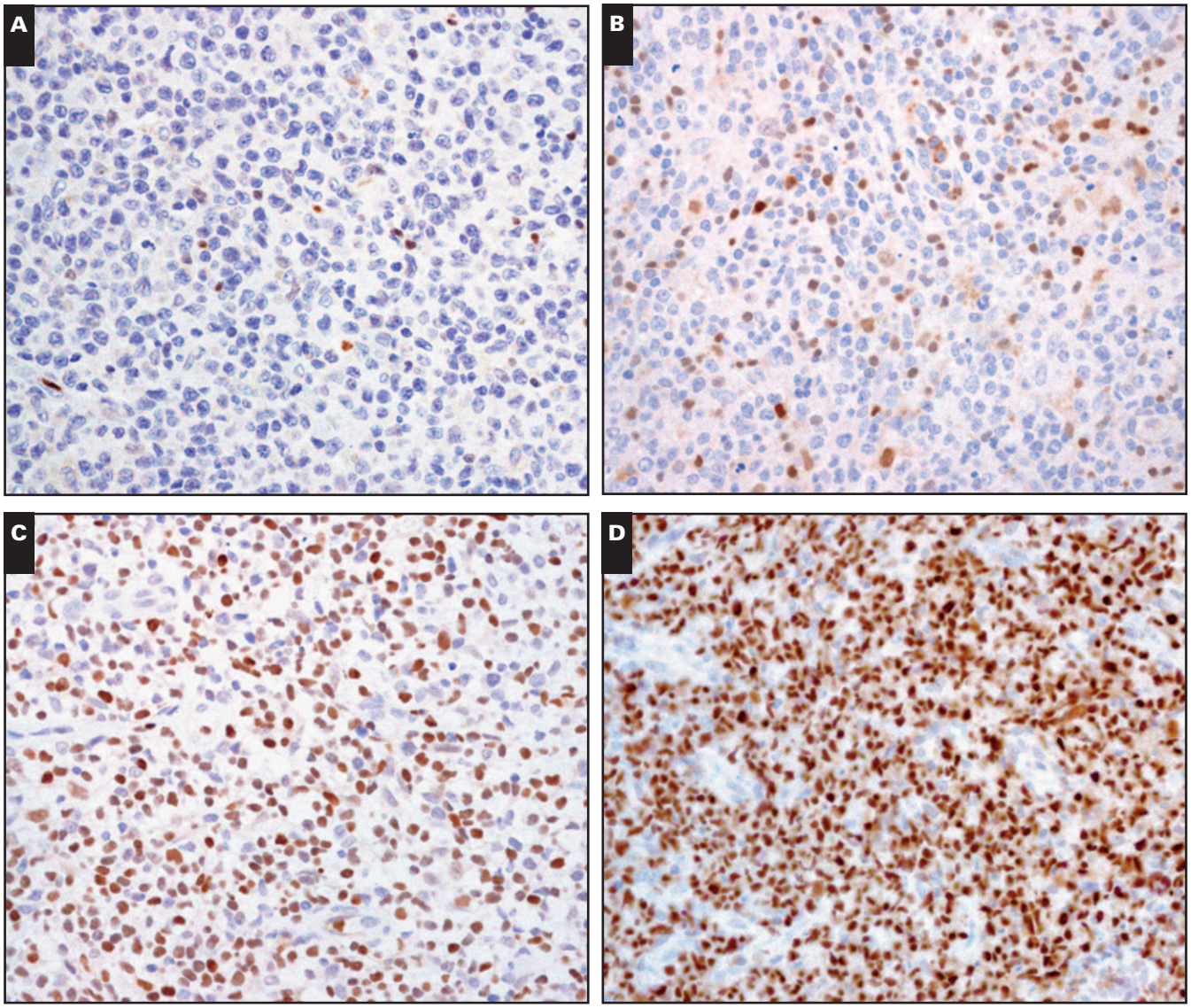

Image 3 Different degrees of immunostaining with T-bet. **A**, Negative reactivity (–, ≤5% cells were positive), a case of peripheral T cell lymphoma, not otherwise specified. **B**, Weakly positive (1+, 6%-20% cells positive), a case of angioimmunoblastic T-cell lymphoma. **C**, Moderately positive (2+, 21%-50% cells positive), a case of mycosis fungoides. **D**, Strongly positive (3+, >50% cells positive), a case of extranodal natural killer (NK)/T-cell lymphoma, nasal type.

12.5% in AITL, 50.0% and 16.7% in ALCL, ALK+, 60.0% and 20.0% in ALCL, ALK–, 14.3% in MF in both, 33.3% in C-ALCL in both, and 53.3% and 20.0% in the others. For reasons related to sample size, we further analyzed their expressive status only in the 3 major subtypes. Statistical analysis showed differences between any 2 subsets, ie, eNK/T and PTCL-NOS ($P < .001$ for both markers), eNK/T and AITL ($P = .002$ for T-bet and $P = .008$ for ETS-1), and PTCL-NOS and AITL ($P = .023$ for T-bet and $P = .015$ for ETS-1). For the eNK/T group, receiver operating characteristic analysis among the 3 major subsets showed an area value of 0.762 for T-bet and 0.734 for ETS-1 with $P < .001$ for both. Further grading of staining was analyzed in the 3 major subtypes. Strong staining of T-bet was observed most frequently in eNK/T (62.0%), second in AITL (29.2%), and least in PTCL-NOS (9.5%). Similar tendency was found in ETS-1 for its strong immunoreaction with a frequency of 22.4% in eNK/T, 4.2% in AITL, and none in PTCL-NOS. Moreover, all PTCL-NOS cases were negative or weakly positive. Pairwise comparison of staining status of the 2 markers was performed among the 3 major subtypes. A significant difference was seen between eNK/T and PTCL-NOS in any staining grade for both markers (all $P < .01$). The difference between eNK/T and AITL was significant in that more than 20% staining was seen for both markers ($P = .002$ for T-bet, and $P = .029$ for ETS-1) and significantly strong staining was seen for T-bet ($P = .013$).

DOI: 10.1309/AJCPWKJ3GPFRT7GA

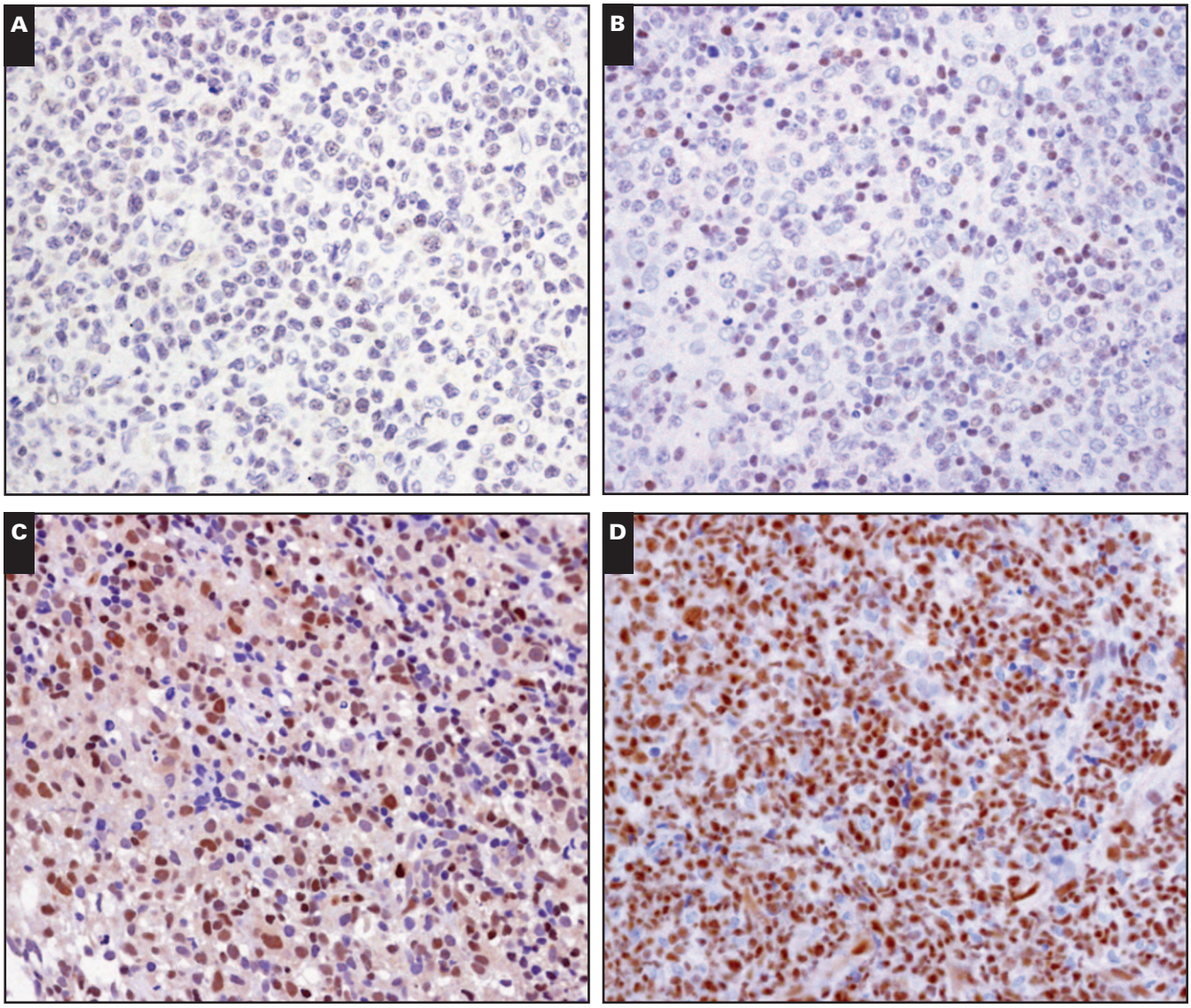

Image 4 Different degrees of immunostaining with ETS-1. **A**, Negative (–, ≤5% cells were positive), a case of peripheral T-cell lymphoma, not otherwise specified. **B**, Weakly positive (1+, 6%-20% cells positive), a case of angioimmunoblastic T-cell lymphoma. **C**, Moderately positive (2+, 21%-50% cells positive), a case of extranodal natural killer (NK)/T-cell lymphoma, nasal type. **D**, Strongly positive (3+, >50% cells positive), another case of extranodal NK/T-cell lymphoma, nasal type.

Table 3
Expression of T-bet and ETS-1 in 142 Northern Chinese Patients With Peripheral T/NK-Cell Lymphomas by Immunohistochemistry*

	T-bet					ETS-1				
	No.	–	1+	2+	3+	No.	–	1+	2+	3+
eNK/T	50	1 (2.0)	5 (10.0)	12 (26.0)	31 (62.0)	49	8 (16.3)	22 (44.9)	8 (16.3)	11 (22.4)
PTCL-NOS	21	7 (33.3)	7 (33.3)	5 (23.8)	2 (9.5)	21	15 (71.4)	6 (28.6)	0 (0.0)	0 (0.0)
AITL	23	1 (4.2)	10 (41.7)	6 (25.0)	7 (29.2)	23	9 (37.5)	12 (50.0)	2 (8.3)	1 (4.2)
ALCL-ALK+	6	2 (33.3)	1 (16.7)	3 (50.0)	0	6	4 (66.7)	1 (16.7)	1 (16.7)	0
ALCL-ALK–	5	0 (0.0)	2 (40.0)	0 (0.0)	3 (60.0)	5	2 (40.0)	2 (40.0)	1 (20.0)	0
C-ALCL	6	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)	6	4 (66.7)	0 (0.0)	1 (16.7)	1 (16.7)
MF	7	5 (71.4)	1 (14.3)	0 (0.0)	1 (14.3)	7	5 (71.4)	1 (14.3)	0 (0.0)	1 (14.3)
Others	15	3 (20.0)	4 (26.7)	2 (13.3)	6 (40.0)	15	8 (53.3)	4 (26.7)	2 (13.3)	1 (6.7)
Total	133	21 (15.7)	32 (23.9)	29 (22.4)	51 (38.1)	132	55 (41.4)	48 (36.1)	15 (11.3)	15 (11.3)

AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; ALK, anaplastic lymphoma kinase; C-ALCL, primary cutaneous anaplastic large cell lymphoma; eNK/T, extranodal NK/T-cell lymphoma, nasal type; MF, mycosis fungoides; NK, natural killer; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified; –, ≤5% of cells were positive; 1+, 6%-20% of cells were positive; 2+, 21%-50% of cells were positive; 3+, >50% of cells were positive.
* Data show number of cases (percentage).

DOI: 10.1309/AJCPWKJ3GPFRT7GA

However, difference between PTCL-NOS and AITL was only demonstrated in general positive staining ($P = .017$ for T-bet, and $P = .036$ for ETS-1).

EBV

All 54 cases (100%) of eNK/T were positive for EBER with strong or moderate reaction in 45. All 4 cases of EBV-TLPD showed a moderate or better reaction. EBV-positive large B cells were identified in 9 of 24 cases (37.5%) of AITL. Positive EBV reaction was also observed in 3 (14.3%) of 21 PTCL-NOS cases.

Treatment

We obtained the treatment status of 103 cases, of which 7 received no treatment and 96 (36 eNK/T, 17 PTCL-NOS, 17 AITL, and 26 others) received various modes of therapy. The treatment modalities included cyclophosphamide, hydroxydaunorubicin, vincristine (Oncovin), prednisone (CHOP)/CHOP-type chemotherapy, radiotherapy, hematopoietic stem cell transplantation (HSCT), Chinese medicine, or a combination of them. Most of the cases received CHOP/CHOP-type chemotherapy (61 cases), radiotherapy combined with or without chemotherapy (21 vs 3 cases), or chemotherapy and/or radiotherapy followed by HSCT (5 cases) with radiotherapy mainly for eNK/T group (16 cases). Among the 3 major subtypes, 35 cases with eNK/T received chemotherapy alone (19 cases) or chemoradiotherapy (16 cases) and 1 had chemotherapy followed by HSCT. For PTCL-NOS, 16 cases had chemotherapy, of which 5 had combination therapy (3 with radiotherapy and 2 with HSCT) and 1 received only Chinese medicine. All 17 AITL cases received chemotherapy alone.

Outcome and Survival Analysis

Follow-up data were available for 124 patients whose OS ranged from 3 days to 134 months, with a median of 11 months; of these, 43 patients (34.7%) had eNK/T, 22 had PTCL-NOS (17.7%) and 21 had AITL (16.9%). A total of 58 (46.7%) patients died of disease 3 days to 67 months after diagnosis, with a median of 8 months, including 20 (46.5%) of 43 cases of eNK/T, 12 (54.5%) of 22 cases of PTCL-NOS, 10 (47.6%) of 21 cases of AITL, and 16 (42.1%) of 38 other cases. The cause of death included tumor progression (n = 37, 63.8%) or related complications such as treatment toxicity or infection (n = 12, 20.7%), bleeding (n = 3, 5.2%), respiratory failure (n = 2, 3.4%), or other unknown reasons (n = 4, 6.9%). Sixty-six patients were alive, with a median survival of 18.5 months; of these, 48 patients were living with illness, with overall survival periods of 1 to 134 months (mean, 20.1 mo); 18 patients were alive without disease for periods ranging from 8 to 134 months; and 3 survived longer than 10 years (1 case each of eNK/T, AITL, and MF).

As shown in **Figure 1**, the 2- and 5-year OS rates were 53.4% and 42.5% in all cases, 56.1% and 56.1% in eNK/T cases, 50.5% and 21.6% in PTCL-NOS cases, 54.3% and 37.3% in AITL cases, respectively, reaching an apparent plateau level around 5 years. The 2- and 5-year FFS rates were 44.3% and 25.1% in all cases, 43.7% and 22.4% in eNK/T, 43.3% and 14.4% in PTCL-NOS, 44.5% and 25.4% in AITL, respectively. For the small sample size of other subtypes, we did not perform further analyses on their outcomes.

Univariate and Multivariate Analyses of OS and FFS

The pathologic and clinical factors were evaluated for prediction of survival by means of univariate analysis. Advanced stage (III/IV) disease and thrombocytopenia **Figure 2A** were found to be significant predictors ($P < .001$) for poor OS. Other predictors for poor OS included more than 1 extranodal sites involved ($P = .032$), enlarged abdominal lymph nodes ($P = .037$), leucopenia ($P = .001$), lymphopenia ($P = .024$), low serum albumin ($P = .008$), elevated LDH ($P = .008$) **Figure 2B**, and Ki-67 index above 70% ($P = .019$) **Figure 2C**. CLA expression ($P = .018$) was a good predictor. The analysis of T-bet showed that only positive staining above 20% displayed effect on good outcome ($P = .031$) for the patients in the large group of PTNKLs **Figure 2D** but not for any individual subset, including eNK/T **Figure 3**. ETS-1 showed significance for neither all PTNKLs nor any subset **Figure 4**. The tendency of FFS curves was almost identical with OS. Multivariate analysis of the aforementioned indexes showed that thrombocytopenia ($P = .001$), elevated LDH ($P = .007$), and high Ki-67 index ($P = .002$) were significant factors for predicting poor outcome, while T-bet expression in more than 20% of tumor cells ($P = .036$) was a protective factor.

In the group of eNK/T cases, advanced stage ($P = .006$), leucopenia ($P = .017$), thrombocytopenia ($P < .001$), low serum albumin ($P = .020$), elevated LDH ($P = .001$), and CD56 expression ($P = .008$) were poor predictors, and radiotherapy ($P = .008$) was the only good predictor. Multivariate analysis showed thrombocytopenia ($P = .011$) and radiotherapy ($P = .026$) were significant. AITL cases had 2 predictors of poor survival, ie, thrombocytopenia ($P = .037$) and high Ki-67 value ($P = .019$). We did not find any predictor for PTCL-NOS cases.

Discussion

PTNKL belongs to a disease group of high heterogeneity, and shows significant geographic variations in subtype distribution. A recent international collaborative study of 1,153 cases of PTNKLs from 22 centers worldwide[1] further validated the geographic variation seen in the various

DOI: 10.1309/AJCPWKJ3GPFRT7GA

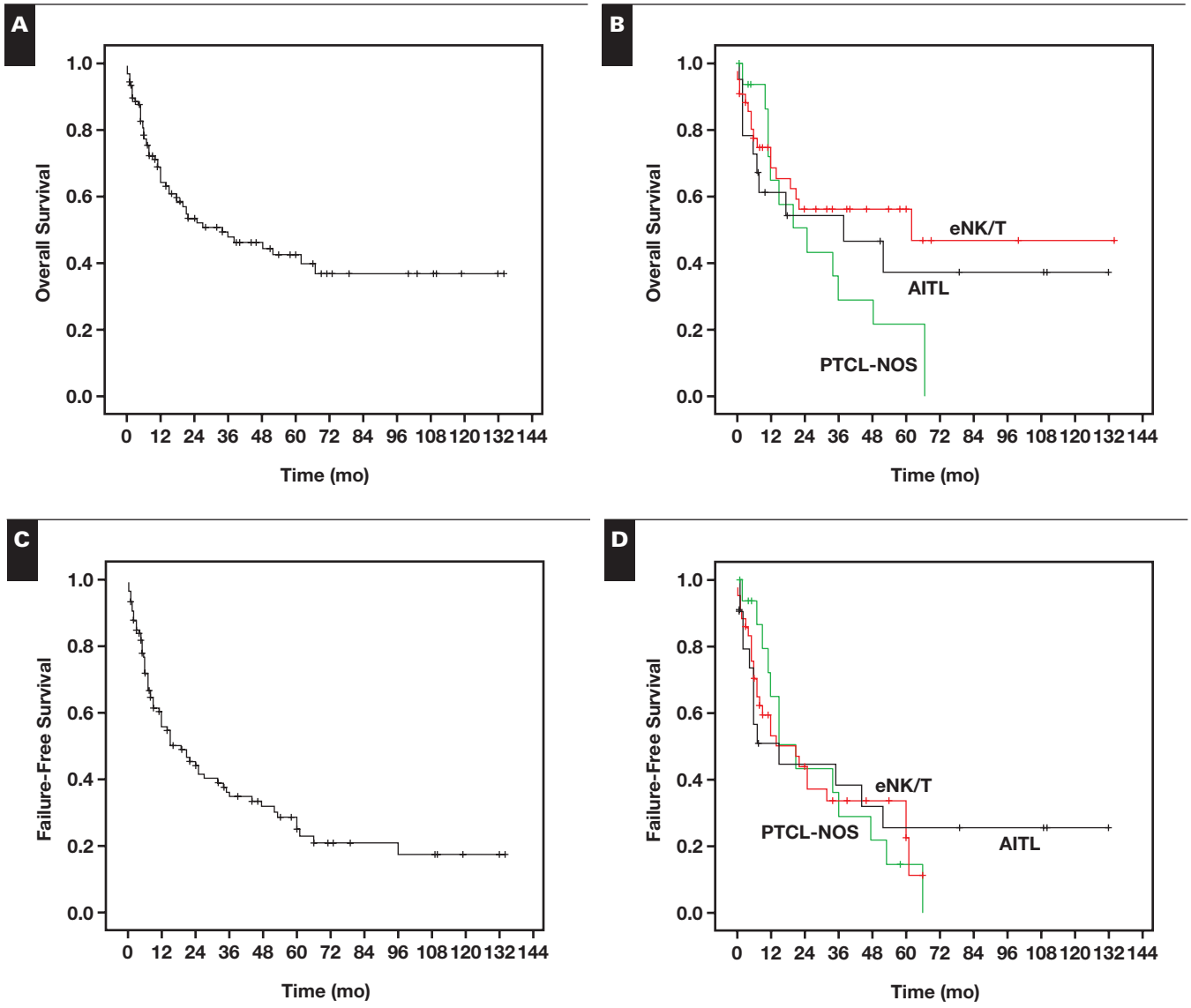

Figure 1 Survival analysis of 142 Northern Chinese patients with peripheral T/natural killer (NK)–cell lymphomas (PTNKLs). **A** and **C**, Overall and failure-free survival of all 142 Northern Chinese patients with PTNKLs. **B** and **D**, Overall ($P = .383$) and failure-free survival ($P = .962$) according to 3 major subtypes of PTNKLs. (AITL, angioimmunoblastic T-cell lymphoma; eNK/T, extranodal NK/T cell lymphoma, nasal type; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified.)

subtypes of PTNKLs. Their data showed that PTCL-NOS was the most common subtype in North America and Europe (34.4% and 34.3% each), whereas eNK/T was common (22.4%) in Asia. PTNKLs accounted for 21.9% of non-Hodgkin lymphomas in the present series, with eNK/T (38.0%), AITL (16.9%), and PTCL-NOS (16.2%) being the 3 most prevalent subtypes. Our data displayed a much similar set to that from Hong Kong,[4] in which eNK/T reached the highest frequency, exceeding one third of cases. Thus, the high exposure or genetic susceptibility to environmental agents should be considered as a potential contributor to the selective development of PTNKLs.

As a ubiquitous human herpesvirus worldwide, EBV is found to be associated with a broad spectrum of T- and NK-cell lymphomas.[12-14] EBV-associated PTNKLs, especially those that are most directly implicated, were seen significantly more frequently in our series and in other studies from Asian countries compared with western countries. This epidemiologic feature of strong geographic variations and racial predisposition has been ascribed to a genetic-based impaired immune response mechanism against EBV.

Transcription factors are important proteins that have crucial influences on differentiation and effective function of cells of the immune system. Changes in levels of their

DOI: 10.1309/AJCPWKJ3GPFRT7GA

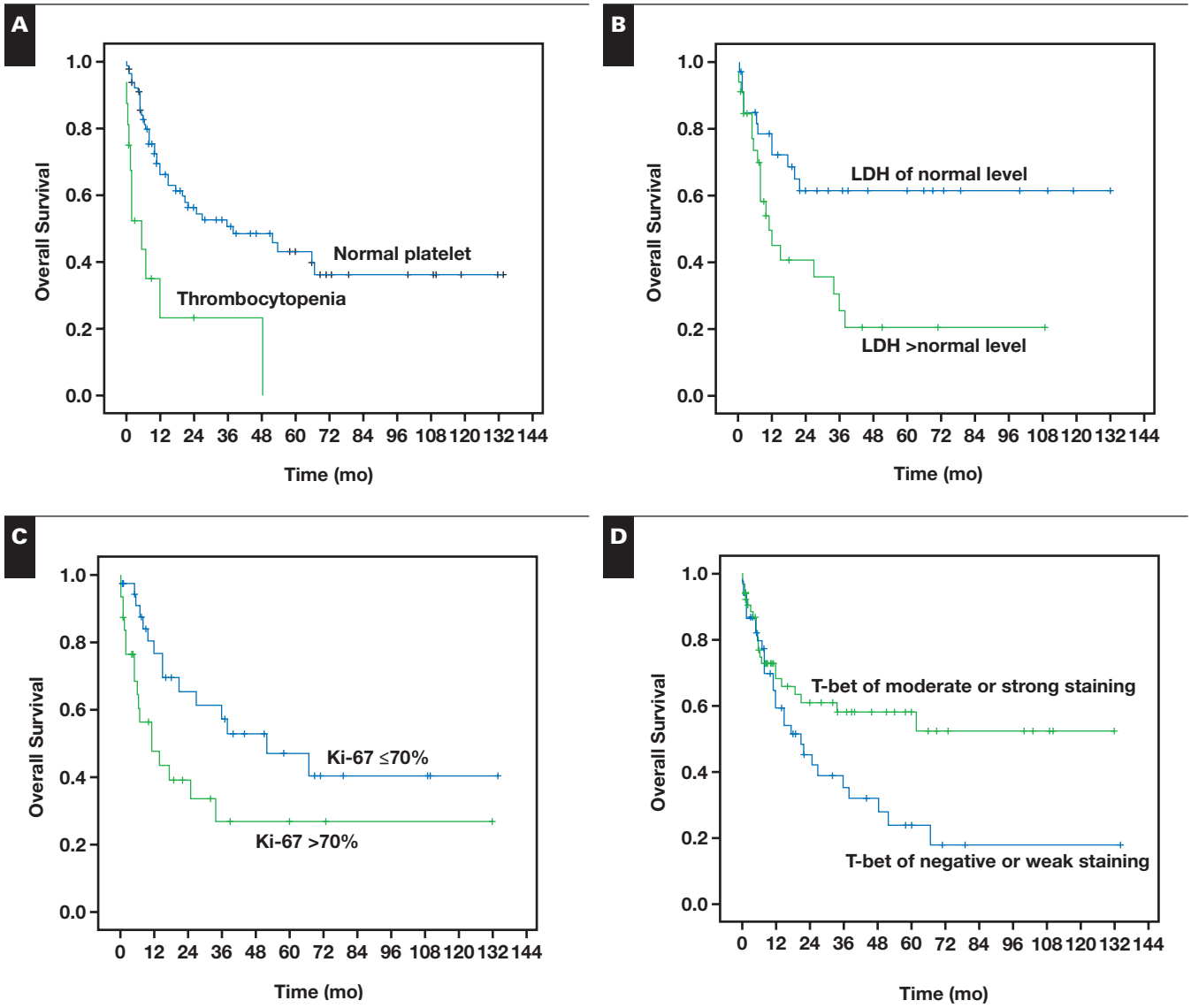

Figure 2 Overall survival of 142 Northern Chinese patients with peripheral T/natural killer (NK)–cell lymphomas correlated to **A,** thrombocytopenia ($P < .001$); **B,** serum lactate dehydrogenase (LDH) level ($P < .008$); **C,** Ki-67 index ($P = .019$); and **D,** the expressive level of T-bet ($P = .031$).

expression or activity result in diverse and manifold effects on the whole transcriptome of cells, therefore they are of special interests in physiologic and pathologic processes, particularly in tumor development and progression. As transcription factors, *T-bet* cooperates functionally with its family member *EOMES* or *ETS* family members (*ETS-1* and *MEF*), and positively regulates the development and cytotoxic function of NK cells and T lymphocytes.[15-22] In our previous studies, by using IHC and ISH we demonstrated that these factors were expressed predominantly in eNK/T and PTCL.[9,23] In the current study, we further analyzed the expression of *T-bet* and *ETS-1* with IHC in the heterogeneous large group of PTNKLs to investigate their expression status, role, and clinical relevance. The results showed that the 2 markers were expressed more frequently in eNK/T than in other subtypes of PTNKLs.

CLA is a receptor for T and NK cells homing to the skin.[24,25] Expression of CLA was reported in various types of epidermotropic T-cell lymphomas, including MF, ATLL, C-ALCL, nodal ALCL with secondary cutaneous involvement, some nodal non-nasal NK-cell lymphomas, and eNK/T.[26-28] The value of CLA as a predictor for prognosis is controversial. Yoshino et al[28] reported that CLA positivity was associated with a poor outcome of eNK/T regardless of the primary site or clinical staging. Chang et al,[26] however, did not find any statistical significance between CLA expression and tumor spreading. Our result showed that besides eNK/T, MF, and C-ALCL, CLA was observed in some other cases of

DOI: 10.1309/AJCPWKJ3GPFRT7GA

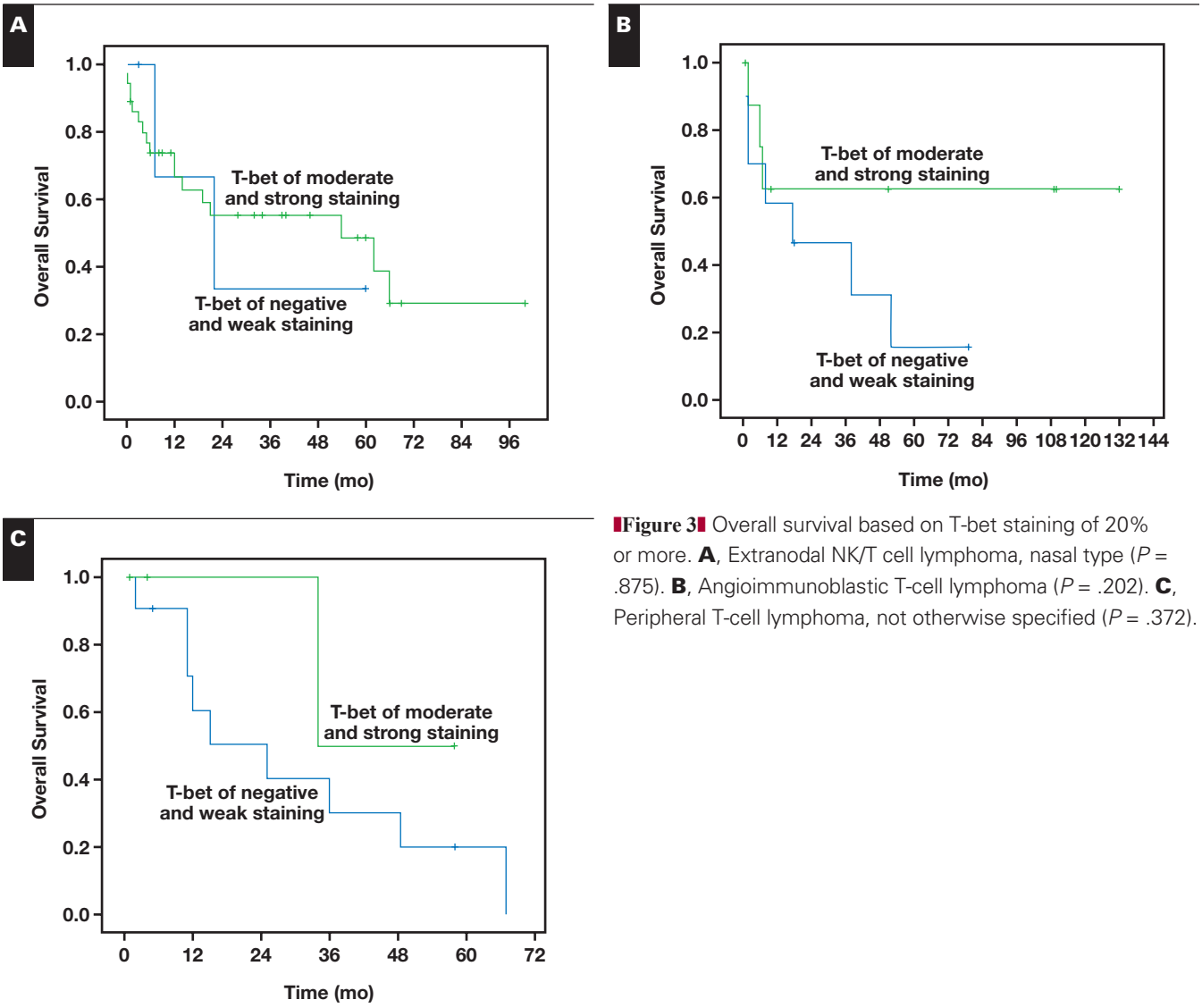

Figure 3 Overall survival based on T-bet staining of 20% or more. **A**, Extranodal NK/T cell lymphoma, nasal type (P = .875). **B**, Angioimmunoblastic T-cell lymphoma (P = .202). **C**, Peripheral T-cell lymphoma, not otherwise specified (P = .372).

PTCL-NOS, AITL, and ALCL and was associated with skin disease. In addition, the statistical analysis showed that CLA was a predictor for good outcome in univariate (P = .018) but not in multivariate analysis.

PTNKLs are a heterogeneous group of neoplasms presenting as advanced disease and characterized by widespread dissemination, aggressive behavior, and a poor outcome, with the 5-year OS rate ranging from 10.2% to 62.0%.[5,6] In this study, the 5-year OS was 42.5% for the whole group of PTNKL, 56.1% for eNK/T, 37.3% for AITL, and 21.6% for PTCL-NOS. Compared with a multinational study[1] that found follow-up 5-year OS rates of 42% for eNK/T, 32% for AITL, and 32% for PTCL-NOS, we found a variation in the prognosis of these subtypes—a much better outcome for eNK/T and a worse result for PTCL-NOS in our series than in the multinational study. Further investigation is needed for the association of the survival status of various subtypes with the factors of geography and race.

To date, the prognosis for PTNKL relies heavily on the clinical factors, ie, IPI, stage, and modes of therapy used, whereas the prognostic significance of the pathologic factors is controversial. The standard IPI is helpful in prognosticating some subtypes or cases of PTNKL, but not helpful in others.[2,7,29-32] There is a growing desire and demand to identify more objective and reliable prognostic factors, especially molecular markers. In this study, thrombocytopenia, elevated LDH, and high Ki-67 index were reconfirmed to be independent prognostic factors, indicating their positive role in evaluating the clinical outcome of PTNKL. In addition, our research also suggested a role for T-bet staining in clinical prediction, because a significant correlation was observed with good outcome in multivariate analysis (P = .031) when more than 20% of tumor cells expressed this marker. However, a similar effect was not observed in any individual subtype even in eNK/T. The reason for the positive effect of T-bet on predicting all PTNKLs and its role in

DOI: 10.1309/AJCPWKJ3GPFRT7GA

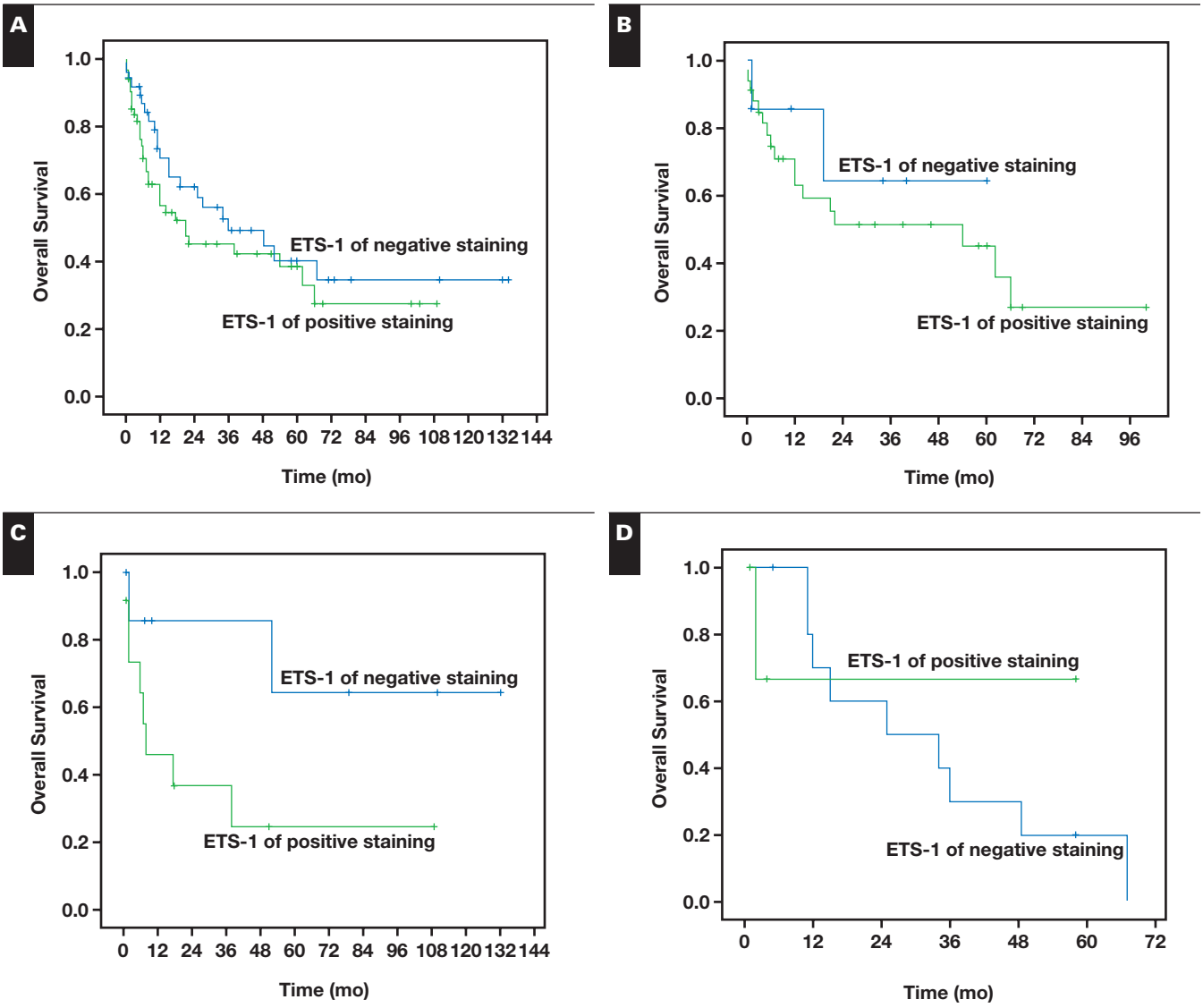

Figure 4 Overall survival by positive staining for ETS-1. **A**, All 142 Peripheral T/natural killer (NK) cell lymphomas ($P = .283$). **B**, Extranodal NK/T cell lymphoma, nasal type ($P = .540$). **C**, Angioimmunoblastic T-cell lymphoma ($P = .061$). **D**, Peripheral T cell lymphoma, not otherwise specified ($P = .713$).

an individual subset, especially in eNK/T, need to be investigated. In addition, because of the predominant expression of T-bet in eNK/T, future research needs to focus on the downstream signaling triggered by T-bet.

In conclusion, our data showed that the commonest subtype of PTNKLs in northern China was eNK/T, which constituted more than one third of all PTNKLs, followed by AILT and PTCL-NOS. Among the 4 predictors, ie, thrombocytopenia, elevated LDH, high Ki-67 index, and T-bet staining of more than 20%, only T-bet staining had good prognostic value for PTNKLs. The significance of this last finding is worth confirming in a future study.

From the Department of Pathology, Peking University First Hospital, Beijing, China.

Supported by research grants 81071944 and 30770910 from National Natural Sciences Foundation of China, Beijing.

Address reprint requests to Dr Li: Department of Pathology, Peking University First Hospital, No. 7 Xishiku Street, Xicheng District, Beijing 100034, China; lixiaoting12@hotmail.com.

Acknowledgments: We thank Ying Zhang and Ying Wang for their technical assistance and Xue-Ying Li for her guidance in statistical analysis.

References

1. Vose J, Armitage J, Weisenburger D. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. *J Clin Oncol*. 2008;26:4124-4130.
2. Xu PP, Wang Y, Shen Y, et al. Prognostic factors of Chinese patients with T/NK-cell lymphoma: a single institution study of 170 patients. *Med Oncol*. June 27, 2011.

DOI: 10.1309/AJCPWKJ3GPFRT7GA

3. Niitsu N, Okamoto M, Nakamine H, et al. Clinicopathologic features and outcome of Japanese patients with peripheral T-cell lymphomas. *Hematol Oncol.* 2008;26:152-158.
4. Au WY, Ma SY, Chim CS, et al. Clinicopathologic features and treatment outcome of mature T-cell and natural killer-cell lymphomas diagnosed according to the World Health Organization classification scheme: a single center experience of 10 years. *Ann Oncol.* 2005;16:206-214.
5. Ko OB, Lee DH, Kim SW, et al. Clinicopathologic characteristics of T-cell non-Hodgkin's lymphoma: a single institution experience. *Korean J Intern Med.* 2009;24:128-134.
6. Lu D, Lin CN, Chuang SS, et al. T-cell and NK/T-cell lymphomas in southern Taiwan: a study of 72 cases in a single institute. *Leuk Lymphoma.* 2004;45:923-928.
7. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. *N Engl J Med.* 1993;329:987-994.
8. Yang DH, Kim WS, Kim SJ, et al. Prognostic factors and clinical outcomes of high-dose chemotherapy followed by autologous stem cell transplantation in patients with peripheral T cell lymphoma, unspecified: complete remission at transplantation and the prognostic index of peripheral T cell lymphoma are the major factors predictive of outcome. *Biol Blood Marrow Transplant.* 2009;15:118-125.
9. Zhang S, Li T, Zhang B, et al. Transcription factors engaged in development of NK cells are commonly expressed in nasal NK/T-cell lymphomas. *Hum Pathol.* 2011;42:1319-1328.
10. Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* 4th ed: Lyon, France: IARC Press; 2008.
11. Mourad N, Mounier N, Briere J, et al. Clinical, biologic, and pathologic features in 157 patients with angioimmunoblastic T-cell lymphoma treated within the Groupe d'Etude des Lymphomes de l'Adulte (GELA) trials. *Blood.* 2008;111:4463-4470.
12. Cohen JI, Kimura H, Nakamura S, et al. Epstein-Barr virus-associated lymphoproliferative disease in non-immunocompromised hosts: a status report and summary of an international meeting, 8-9 September 2008. *Ann Oncol.* 2009;20:1472-1482.
13. Kim YC, Yang WI, Lee MG, et al. Epstein-Barr virus in CD30 anaplastic large cell lymphoma involving the skin and lymphomatoid papulosis in South Korea. *Int J Dermatol.* 2006;45:1312-1316.
14. Novelli M, Merlino C, Ponti R, et al. Epstein-Barr virus in cutaneous T-cell lymphomas: evaluation of the viral presence and significance in skin and peripheral blood. *J Invest Dermatol.* 2009;129:1556-1561.
15. Intlekofer AM, Takemoto N, Wherry EJ, et al. Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. *Nat Immunol.* 2005;6:1236-1244.
16. Grenningloh R, Kang BY, Ho IC. Ets-1, a functional cofactor of T-bet, is essential for Th1 inflammatory responses. *J Exp Med.* 2005;201:615-626.
17. Barton K, Muthusamy N, Fischer C, et al. The Ets-1 transcription factor is required for the development of natural killer cells in mice. *Immunity.* 1998;9:555-563.
18. Anderson MK, Hernandez-Hoyos G, Diamond RA, et al. Precise developmental regulation of Ets family transcription factors during specification and commitment to the T cell lineage. *Development.* 1999;126:3131-3148.
19. Lacorazza HD, Nimer SD. The emerging role of the myeloid Elf-1 like transcription factor in hematopoiesis. *Blood Cells Mol Dis.* 2003;31:342-350.
20. Showell C, Binder O, Conlon FL. T-box genes in early embryogenesis. *Dev Dyn.* 2004;229:201-218.
21. Suto A, Wurster AL, Reiner SL, et al. IL-21 inhibits IFN-gamma production in developing Th1 cells through the repression of Eomesodermin expression. *J Immunol.* 2006;177:3721-3727.
22. Takemoto N, Intlekofer AM, Northrup JT, et al. Cutting edge: IL-12 inversely regulates T-bet and eomesodermin expression during pathogen-induced CD8+ T cell differentiation. *J Immunol.* 2006;177:7515-7519.
23. Ye Y, Li T, Zhang B, et al. Amplification and specific expression of T-bet gene in nasal NK/T-cell lymphoma. *Leuk Lymphoma.* 2007;48:168-173.
24. Tsuchiyama J, Yoshino T, Toba K, et al. Induction and characterization of cutaneous lymphocyte antigen on natural killer cells. *Br J Haematol.* 2002;118:654-662.
25. Alon R, Rossiter H, Wang X, et al. Distinct cell surface ligands mediate T lymphocyte attachment and rolling on P and E selectin under physiological flow. *J Cell Biol.* 1994;127:1485-1495.
26. Chang ST, Liao YL, Lin SH, et al. NK-cell lymphoma with nodal presentation and expression of cutaneous lymphocyte-associated antigen. *Pathol Res Pract.* 2010;206:463-466.
27. Magro CM, Dyrsen ME. Cutaneous lymphocyte antigen expression in benign and neoplastic cutaneous B- and T-cell lymphoid infiltrates. *J Cutan Pathol.* 2008;35:1040-1049.
28. Yoshino T, Nakamura S, Suzumiya J, et al. Expression of cutaneous lymphocyte antigen is associated with a poor outcome of nasal-type natural killer-cell lymphoma. *Br J Haematol.* 2002;118:482-487.
29. Gisselbrecht C, Gaulard P, Lepage E, et al. Prognostic significance of T-cell phenotype in aggressive non-Hodgkin's lymphomas. Groupe d'Etudes des Lymphomes de l'Adulte (GELA). *Blood.* 1998;92:76-82.
30. Kim YR, Kim JS, Kim SJ, et al. Lymphopenia is an important prognostic factor in peripheral T-cell lymphoma (NOS) treated with anthracycline-containing chemotherapy. *J Hematol Oncol.* 2011;4:34.
31. Went P, Agostinelli C, Gallamini A, et al. Marker expression in peripheral T-cell lymphoma: a proposed clinical-pathologic prognostic score. *J Clin Oncol.* 2006;24:2472-2479.
32. Gallamini A, Stelitano C, Calvi R, et al. Peripheral T-cell lymphoma unspecified (PTCL-U): a new prognostic model from a retrospective multicentric clinical study. *Blood.* 2004;103:2474-2479.

DOI: 10.1309/AJCPWKJ3GPFRT7GA

HOLOGIC®

First and Only FDA Cleared
Digital Cytology System

Genius™ Cervical AI

Genius™ Review Station

Genius™ Digital Imager

Empower Your Genius With Ours

Make a Greater Impact on Cervical Cancer
with the Advanced Technology of the
Genius™ Digital Diagnostics System

Click or Scan
to discover more

genius™
DIGITAL DIAGNOSTICS