Histological vascular invasion is a novel prognostic indicator in extranodal natural killer/T-cell lymphoma, nasal type

HUA WANG[1-3*], PENGFEI LI[1-3*], XINKE ZHANG[1,3,4], ZHONGJUN XIA[1-3], YUE LU[1-3] and HUIQIANG HUANG[1,3,5]

[1]State Key Laboratory of Oncology in South China; [2]Department of Hematological Oncology;
[3]Collaborative Innovation Center of Cancer Medicine; Departments of [4]Pathology and [5]Medical Oncology,
Sun Yat-Sen University Cancer Center, Guangzhou, Guangdong 510060, P.R. China

Abstract. Extranodal natural killer (NK)/T-cell lymphoma, (ENKTL), nasal type, is an aggressive lymphoma with no validated prognostic parameters, to date. In the present study, vascular invasion by this tumor was retrospectively analyzed in 214 patients with untreated ENKTL to evaluate its association with clinical features, treatment response and prognosis. Histological vascular invasion by the tumor was confirmed in 32.7% of patients with ENKTL. The presence of vascular invasion significantly correlated with poor performance status, B symptoms, extranodal involved sites, advanced stage, elevated serum lactate dehydrogenase, D-dimer and cluster of differentiation 68[+] tumor-associated macrophages. Upon treatment termination, the complete remission (CR) rate and overall response rate were significantly lower for the vascular invasion group compared with the non-vascular invasion group. Furthermore, vascular invasion resulted in significantly reduced 5-year progression-free survival (PFS; 21.8 vs. 60.1%) and overall survival (OS; 36.8 vs. 77.0%) rates. Using the multivariate Cox regression model, vascular invasion, stage III/IV and CR after chemotherapy were independent prognostic factors for OS and PFS. Thus, histological vascular invasion by the tumor affected the response to treatment, and was also an independent prognostic factor for OS and PFS in ENKTL, nasal type, suggesting a role for vascular invasion in disease progression.

Correspondence to: Dr Hua Wang, Department of Hematological Oncology, Sun Yat-Sen University Cancer Center, 651 Dong Feng Road East, Guangzhou, Guangdong 510060, P.R. China
E-mail: wanghua@sysucc.org.cn

*Contributed equally

Introduction

Extranodal natural killer (NK)/T-cell lymphoma (ENKTL), nasal type, is relatively more common in Asia and Latin America compared with Western countries (1,2), and accounts for 5-10% of all malignant lymphomas in China (3). ENKTL is characterized by angiodestruction, obvious necrosis and association with the Epstein-Barr virus (EBV). Considering its poor prognosis, extensive clinical and pathological research has been conducted to investigate possible prognostic markers in ENKTL. Clinically, two major clinical prognostic models are applied in NK/T-cell lymphoma: The International Prognostic Index (IPI) and the Korean Prognostic Index (KPI). The IPI has been widely used for predicting prognosis and selecting therapeutic strategies in patients with aggressive non-Hodgkin's lymphoma. However, the IPI has not been approved for use in ENKTL, as almost 60% of patients with ENKTL belong to the low IPI risk group (score, 0-1), in which significant heterogeneity exists. The KPI was developed in the era of anthracycline-based chemotherapy and appears to be more useful than IPI for predicting ENKTL prognosis (4,5). However, the prognostic value of KPI could not be repeated in certain studies, particularly in the era of asparaginase-based chemotherapy (6), suggesting that both the IPI and KPI scoring systems should be further improved. These prognostic models are also primarily based on pretreatment clinical characteristics.

Numerous studies have demonstrated that histological vascular invasion is associated with poor prognosis for various types of solid tumor, such as thyroid carcinoma (7), nodular melanoma (8), colorectal cancer (9), gastric carcinoma (10), hepatocellular carcinoma (11), renal cell carcinoma (12) and breast cancer (13). However, the prognostic significance of vascular invasion in ENKTL is unclear. ENKTL is a distinct and heterogeneous histopathological subtype of non-Hodgkin's lymphoma that shares the following characteristics with solid tumors: i) Originates from local tissue (nasal cavity, skin or gastrointestinal tract) outside the lymph nodes that contains numerous blood vessels; and ii) as the disease progresses, lesions spread to surrounding lymph nodes, and migrate to distant tissues and organs. Based on the aforementioned characteristics, we hypothesize that histological vascular invasion by the tumor is a risk factor for disease progression and distant

metastasis in ENKTL. In the present study, the vascular invasion status of the tumor in patients with untreated ENKTL was retrospectively examined to investigate its association with clinical features, treatment response and prognosis.

Patients and methods

Ethics statement. Written informed consent was obtained from all patients for the use of patient tissue samples and other medical information to be stored in our hospital database. The current study was performed in accordance with the Declaration of Helsinki and the institutional guidelines of the ethics committee of Sun Yat-Sen University Cancer Center (Guangzhou, China). The study was approved by the Institutional Review Board of the National Cancer Institute and the ethics committees of Sun Yat-Sen University Cancer Center.

Patient selection. A total of 214 patients with histologically diagnosed ENKTL, nasal type, were selected for inclusion in the present study between June 2002 and July 2013 at the Sun Yat-Sen University Cancer Center. Patient selection was based on the following criteria: i) Histologically confirmed diagnosis of ENKTL; ii) NK/T-cell type proven by immunophenotypes and EBV status; iii) no previous malignant tumor or second primary tumor; iv) previously untreated; and v) adequate clinical information and follow-up data. The primary site of the tumor was classified into two subtypes: i) Upper aerodigestive tract ENKTL (UNKTL), including nasal cavity, nasopharynx, paranasal sinuses, tonsils, hypopharynx and larynx; and ii) extra UNKTL (EUNKTL), including all sites excluding the upper aerodigestive tract (14). Patients with primary lesions within the nasal cavity and with secondary spread to other organs were also categorized as UNKTL.

Clinical data of patients were obtained from the hospital discharge database, mortality registry and electronic medical records, which contained the following information: Patient demographics, physical examination, Eastern Cooperative Oncology Group (ECOG) performance status (PS) (15), B symptoms (including unexplained fever with temperature >38˚C, night sweating or weight loss of >10% within 6 months), primary tumor site, involved sites, involvement of regional lymph nodes, serum lactate dehydrogenase (LDH), serum $\beta2$ microglobulin ($\beta2$ M), serum D-dimer (D-D), Ann Arbor stage (16), IPI (age, stage, LDH, extranodal sites and PS) and KPI (adverse factors: Stage >2, >normal LDH, presence of B symptoms and involvement of regional lymph nodes).

Pathological evaluation
Vascular invasion. Patient pathological records and original histopathological slides were independently reviewed by two pathologists (Professors Xin-Ke Zhang and Wan-Ming Hu) with experience in lymphoma pathology. The pathologists were blinded to the pathological diagnoses and outcome data. Discrepancies were resolved by mutual consensus following simultaneous re-examination of the slides by both pathologists using a BX41 double-headed microscope (Olympus corporation, Tokyo, Japan). To assess vascular invasion, 4-μm thick sections of paraffin-embedded tissues were cut, placed on slides, deparaffinized in xylene, hydrated in a graded alcohol series and hematoxylin and eosin-stained was performed

(C0105, Beyotime Insititute of Biotechnology, Inc., Shanghai, China). A mean of 4 (range, 2-8) conventional histopathological sections per tumor sample were available for evaluation. Vascular invasion was defined as infiltration of vessel walls or the existence of tumor emboli (Fig. 1A). Any equivocal focuses, in which tumor cells merely encroached on a vascular lumen, were considered negative (Fig. 1B).

Tumor-associated macrophages (TAMs). Immunohistochemical staining with a polyclonal antibody against cluster of differentiation (CD)68 was performed on 4-μm-thick paraffin-embedded sections to identify and quantify TAMs in ENKTL. CD68 was detected using a rabbit polyclonal antibody (cat no. 11192-RP02; dilution, 1:1,000; final concentration, 1.08 μg/ml; Sino Biological, Inc., Beijing, China). The secondary antibody used was goat anti-rabbit IgG (polyclonal antibody (cat no. SSA018, Sino Biological, Inc.; 1:1,000 dilution). The staining protocol and TAM assessment were conducted according to previously described methods (17). TAMs were detected in 71 cases and, using a cut-off value of 56 TAMs per sample, 28 cases showed high numbers of TAMs (Fig. 1C and D). Sections were assessed on a BX41 microscope (Olympus corporation).

Treatment and response evaluation. Patients received the following treatment strategies: i) Patients with early-stage ENKTL received chemotherapy followed by involved-field radiotherapy (IFRT); and ii) patients with advanced stage ENKTL received chemotherapy alone. The chemotherapy regimens were as follows: i) CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) or CHOP-like therapy (18); ii) alternating triple therapy (ATT), consisting of CHOP-B (cyclophosphamide 750 mg/m^2, adriamycin 50 mg/m^2, vincristine 1.4 mg/m^2 and bleomycin 5 mg/m^2;intravenously infused on day 1, prednisolone 100 mg was administered orally on days 1-5), IMVP-16 (ifosfamide, methotrexate and etoposide) (19) and DHAP (dexamethasone, cytarabine and cisplatin) (20); iii) EPOCH (etoposide, doxorubicin, vincristine, cyclophosphamide and prednisone) (21); and iv) GELOX (gemcitabine, oxaliplatin and L-asparaginase) (21). Following a minimum of 2 cycles of chemotherapy, patients received IFRT. IFRT of 36-60 Gy was delivered in daily fractions of 1.8-2.0 Gy (5 fractions per week). Computed tomography (CT) or 18F-fluorodeoxyglucose positron emission tomography/CT were performed to investigate the curative effect every 2 courses of chemotherapy. Routine follow-up imaging analyses, as well as hematology and biochemical blood serum tests, were performed every 3 months for the first 2 years, then every 6 months for the next 3 years, and annually thereafter or when clinically indicated.

Statistical analysis. Treatment response was assessed according to the International Working Group recommendations for response criteria for non-Hodgkin's lymphoma (22). Overall survival (OS) was measured from the date of diagnosis to the date of mortality or last follow-up visit. Progression-free survival (PFS) was calculated from the date of diagnosis to the date of disease progression, relapse, mortality or last follow-up visit. Local relapse-free survival (LRFS) was calculated from the start of treatment to the date of locoregional relapse in patients that responded completely. Distant
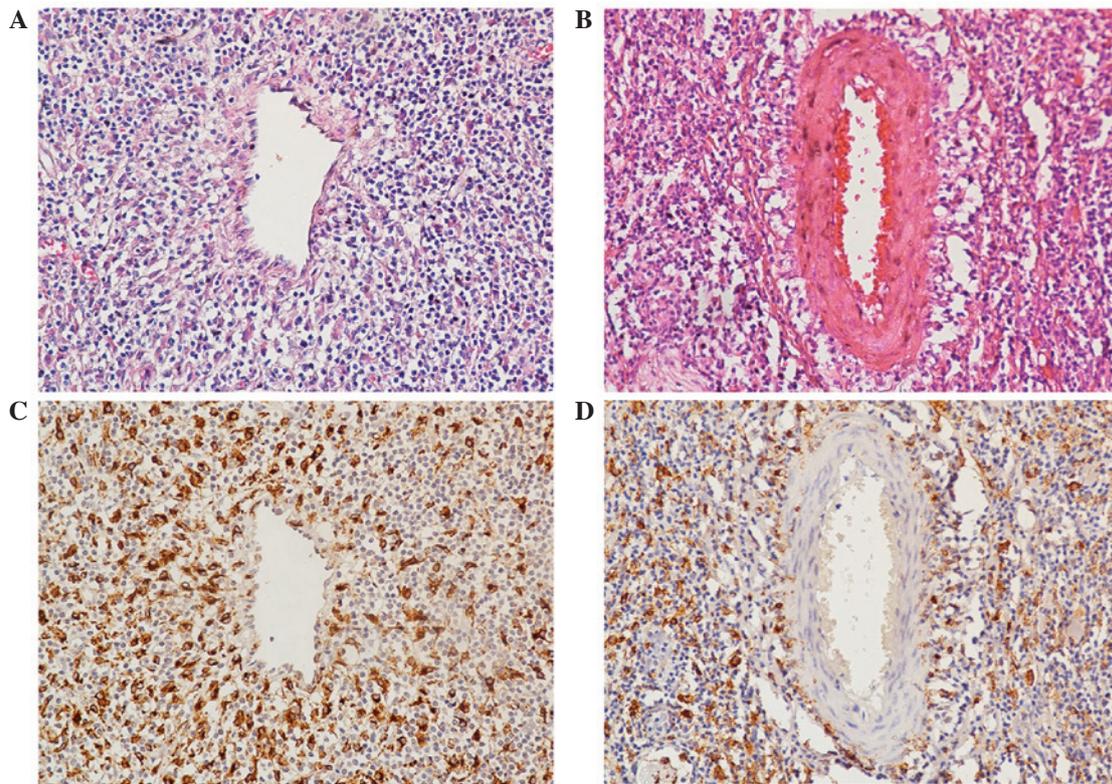
Figure 1. Results of (A and B) hematoxylin and eosin staining for vascular invasion and (C and D) immunohistochemical staining of cluster of differentiation 68 for tumor-associated macrophages (TAMs) in extranodal natural killer/T-cell lymphoma (ENKTL). (A) Present and (B) absent vascular invasion in ENKTL. (C) Tissue section with vascular invasion showed a high number of TAMs via positivity of CD68. (D) Tissue section without vascular invasion showed a high number of TAMs. Magnification, x200.

metastasis-free survival (DMFS) was calculated from the start of treatment to the date of distant metastasis relapse in patients that responded completely. The correlation between vascular invasion and clinicopathological features was evaluated using the χ^2 test. The Kaplan-Meier method was used to calculate the probability of survival and survival curves were compared by the log-rank test. Univariate and multivariate analyses were performed using the Cox proportional-hazards model. Two-sided P<0.05 was considered to indicate a statistically significant difference. Statistical analysis was performed using SPSS software (version 19.0; IBM SPSS, Chicago, IL, USA).

Results

Patient characteristics. The clinical characteristics of the 214 patients are summarized in Table I. The median age was 41 years, with a range of 17-89 years. The majority of patients were aged <60 years and the male:female ratio was 2:1. In addition, 201 patients (93.9%) had a good PS (ECOG 0-1). The majority of the patients initially presented with UNKTL tumors (n=169, 79.0%) and localized diseases (stage I/II; n=164, 76.6%). In EUNKTL patients (n=45, 21.0%), the primary lesion sites included the small bowel, colon, lungs, skin, testes and soft tissues. Half of the patients presented with B symptoms (n=106, 49.5%). A total of 87 patients had elevated serum LDH levels. Serum β2-M was detected in 123 cases; 74 of which were found be higher than normal level. In 106 cases examined, 30 patients had increasing serum D-dimer level. Furthermore, over half of the patients were classified in the

low-risk group according to the IPI (n=134, 62.6%) or KPI (n=109, 50.9%) scores.

Association between vascular invasion and clinicopathological characteristics. Vascular invasion was observed in 70 patients (32.7%). The majority of these patients were aged <60 years old, excluding only 4 patients, and greater than two-thirds were male (n=49, 70.0%). In addition, 45 (64.3%) and 32 (45.7%) patients in the vascular invasion group were classified in the high-intermediate and high-risk categories, respectively, based on the KPI score and IPI score. Table I indicates the association between vascular invasion and clinicopathological characteristics. In brief, vascular invasion was significantly associated with ECOG PS≥2 (P=0.011), EUNKTL (P=0.003), B symptoms (P=0.015), ≥2 extranodal sites (P=0.017), elevated serum LDH (P=0.011), elevated serum D-D (P=0.003), increased numbers of CD68+ TAMs (P=0.009), stage III/IV (P=0.001) and KPI score ≥2 (P=0.002). However, age, gender, regional lymphadenopathy and elevated serum β2 M exhibited no significant correlation with vascular invasion (Table I).

Treatment outcome and response rate. Chemotherapy followed by IFRT was administered to 147 patients, while 67 patients received chemotherapy alone. No statistical difference in vascular invasion at diagnosis was identified between the different treatment modalities (P=0.199) or chemotherapy regimens (P=0.153) (Table I). The treatment response was evaluated in each patient. Table II shows that 96 patients

Table I. Clinical characteristics according to the presentation of vascular invasion at diagnosis.

	Patients, n (%)		
Characteristic	Vascular invasion (n=70)	No vascular invasion (n=144)	P-value
Age at diagnosis, years			0.075
≤60	66 (94.3)	124 (86.1)	
>60	4 (5.7)	20 (13.9)	
Gender			0.431
Male	49 (70.0)	93 (64.6)	
Female	21 (30.0)	51 (35.4)	
ECOG PS			0.011
0,1	61 (87.1)	140 (97.2)	
≥2	9 (12.9)	4 (2.8)	
Subtype			0.003
UNKTL	47 (67.1)	122 (84.7)	
EUNKTL	23 (32.9)	21 (15.3)	
B-symptoms	43 (61.4)	63 (43.8)	0.015
Extranodal sites ≥2	48 (68.6)	74 (51.4)	0.017
Regional lymphadenopathy	44 (62.9)	78 (54.2)	0.228
Elevated serum LDH	37 (52.9)	50 (34.7)	0.011
Elevated serum β2 M[a]			0.331
Yes	29 (65.9)	45 (57.0)	
No	15 (34.1)	34 (43.0)	
Elevated serum D-dimer[b]			0.003
Yes	14 (50.0)	16 (20.5)	
No	14 (50.0)	62 (79.5)	
CD68^{+} TAMs[c]			
High	15 (40.0)	13 (28.3)	0.009
Low	10 (60.0)	33 (71.7)	
Ann Arbor stage			0.001
I/II	44 (62.9)	120 (83.3)	
III/IV	26 (37.1)	24 (16.7)	
IPI score			0.079
0/1	38 (54.3)	96 (66.7)	
≥2	32 (45.7)	48 (33.3)	
KPI score			0.002
0/1	25 (35.7)	84 (58.3)	
≥2	45 (64.3)	60 (41.7)	
Treatment			0.199
Chemotherapy alone	26 (37.1)	41 (28.5)	
Chemotherapy + radiotherapy	44 (62.9)	103 (71.5)	
Chemotherapy regimens			0.153
CHOP	18 (25.7)	25.0 (17.4)	
ATT	16 (22.9)	40.0 (27.8)	
EPOCH	21 (30.0)	43.0 (29.8)	
GELOX	15 (21.4)	36.0 (25.0)	

[a]Serum β_2 M was detected in 123 cases; [b]Serum D-Dimer was detected in 106 cases; [c]CD68 + TAMs was detected in 71 cases. ECOG PS, Eastern Cooperative Oncology Group performance status; UNKTL, upper aerodigestive tract NK/T-cell lymphoma; EUNKTL, extra upper aerodigestive tract NK/T-cell lymphoma; LDH, lactate dehydrogenase; CD, cluster of differentiation; TAMs, Tumor-associated macrophages; IPI, International Prognostic Index; KPI, Korean Prognostic Index; ATT, alternating triple therapy (CHOP-B + IMVP-16 + DHAP); CHOP-B, cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin; IMVP-16, ifosfamide, methotrexate and etoposide; DHAP, dexamethasone, cytarabine and cisplatin; EPOCH, etoposide, doxorubicin, vincristine, cyclophosphamide and prednisone; GELOX, gemcitabine, oxaliplatin and L-asparaginase.

Table II. Treatment outcomes according to the presentation of vascular invasion.

Vascular invasion	PR after CT (%)	P	CR after CT, n (%)	P	ORR after CT, n (%)	P	CR at end, n (%)	P	ORR at end, n (%)	P	5-year PFS rate, %	P	5-year OS rate, %	P
Present[a]	24 (34.3)	0.879	20 (28.6)	0.001	44 (62.9)	<0.001	28 (40.0)	<0.001	44 (62.9)	<0.001	21.8	<0.001	36.8	<0.001
Absent[b]	52 (36.1)		76 (52.8)		128 (88.9)		100 (69.4)		129 (89.6)		60.1		77.0	
Total	76 (35.5)		96 (44.9)		172 (80.4)		128 (59.8)		173 (80.8)		47.5		63.5	

[a]n=70; [b]n=144. CR, complete response; CT, chemotherapy; ORR, overall response rate; PFS, progression-free survival; OS, overall survival.
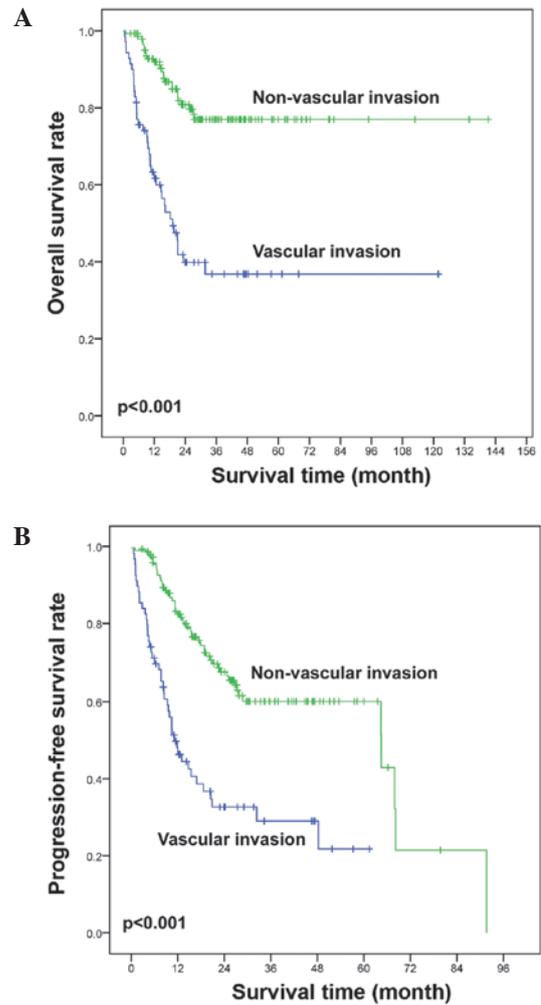

Figure 2. Kaplan-Meier plots of (A) overall and (B) progression-free survival for 214 patients with extranodal natural killer/T-cell lymphoma, according to vascular invasion versus non-vascular invasion at diagnosis.

(44.9%) achieved complete remission (CR) and 76 patients (35.5%) achieved partial response following chemotherapy; thus, the overall response rate (ORR) was 80.4% following chemotherapy. For the non-vascular invasion group, CR and ORR rates following chemotherapy were significantly higher than those in the vascular invasion group (52.8 vs. 28.6%, P=0.001 for CR; 88.9 vs. 62.9%, P<0.001 for ORR). At the end of treatment, the CR and ORR rates for the non-vascular invasion group were also significantly higher than those in the vascular invasion group (69.4 vs. 40.0%, P<0.001 for CR; 89.6 vs. 62.9%, P<0.001 for ORR). The median follow-up time was 26 months (range, 2-141 months), and the 5-year OS and PFS rates were 63.5% [95% confidence interval (CI), 56.1-70.9%] and 47.5% (95% CI, 38.7-56.3%), respectively. Vascular invasion was significantly associated with OS and PFS. The 5-year OS (36.8 vs. 77.0%, P<0.001; Fig. 2A) and PFS (21.8 vs. 60.1%, P<0.001; Fig. 2B) rates were lower in the vascular invasion group compared with the non-vascular invasion group (Table II).

In stage I/II patients (n=164), CR rates were significantly higher for the non-vascular invasion group than those for the vascular invasion group following chemotherapy alone (58.3 vs. 40.9%, P=0.047). The non-vascular invasion group also

Table III. Treatment outcome and response rate for stage I/II patients.

Vascular invasion	CR after CT, n (%)	P-value	CR at the end of treatment, n (%)	P-value	5-year PFS rate, %	P-value	5-year OS rate, %	P-value
Present (n=44)	18 (40.9)	0.047	26 (59.1)	0.152	42.0	<0.001	48.7	<0.001
Absent (n=120)	70 (58.3)		92 (76.7)		68.2		87.9	
Total (n=164)	88 (53.7)		118 (72.0)		61.3		75.0	

CR, complete response; CT, chemotherapy; ORR, overall response rate; PFS, progression-free survival; OS, overall survival.
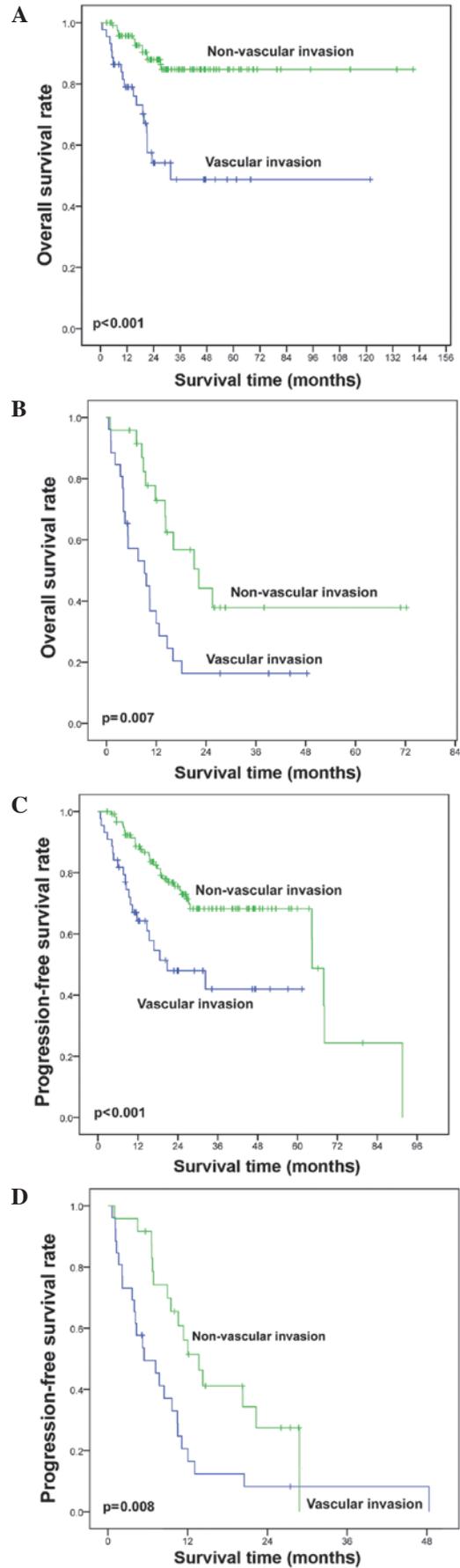

Figure 3. Kaplan-Meier plots of overall survival for stage (A) I/II and (B) III/IV patients and of progression-free survival for stage (C) I/II and (D) III/IV with extranodal natural killer/T-cell lymphoma, according to vascular invasion vs. non-vascular invasion at diagnosis.

Table IV. Relapse rate for stage I/II patients that achieved CR at the end of treatment.

Vascular invasion	LR rate, n (%)	P-value	DMR rate, n (%)	P-value	LR and DMR rate, n (%)	P-value
Present (n=26)	4 (15.4)	0.087	11 (42.3)	0.035	3 (11.5)	0.344
Absent (n=92)	10 (10.9)		14 (15.2)		3 (3.3)	
Total (n=118)	14 (11.9)		25 (21.2)		6 (5.1)	

LR and DLR occur simultaneously. CR, complete response, LR, local relapse; DMR, distant metastasis relapse; LR, locoregional relapse; DMR, distant metastatic relapse.
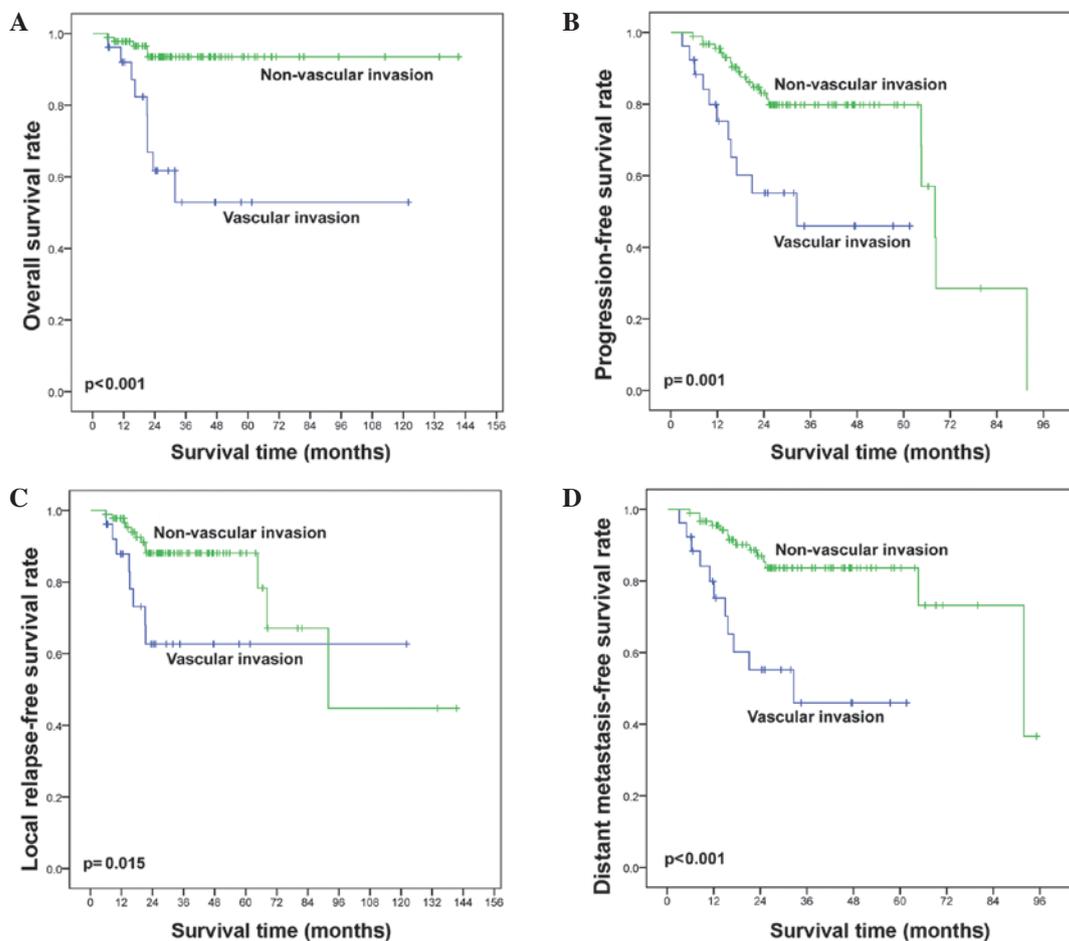

Figure 4. Kaplan-Meier plots of (A) overall survival, (B) progression-free survival, (C) local relapse-free survival and (D) distant metastasis-free survival in stage I/II patients with extranodal natural killer/T-cell lymphoma that achieved complete remission at the end of treatment (n=118), according to vascular invasion versus non-vascular invasion at diagnosis.

had a higher CR rate than the vascular invasion group at the end of treatment (76.7 vs. 59.1%), however, no statistical difference existed (P=0.152). In stage I/II patients, vascular invasion was associated with shorter OS (48.7 vs. 87.9%, P<0.001; Fig. 3A) rates compared with non-vascular invasion. In stage III/IV patients (n=50), vascular invasion at diagnosis was also associated with inferior OS (Fig. 3B). Similarly, vascular invasion was associated with shorter PFS rates in stage I/II (42.0 vs. 68.2%, P<0.001; Fig. 3C) and stage III/IV (Fig. 3D) patients compared with non-vascular invasion (Table III).

In stage I/II patients that achieved CR at the end of treatment (n=118), vascular invasion resulted in a high distant

metastatic relapse (DMR) rate (42.3 vs. 15.2%, P=0.035) but did not significantly effect the locoregional relapse (LR) rate (15.4 vs. 10.9%, P=0.087) or the simultaneous DMR and LR rate (11.5 vs. 3.3%, P=0.344; Table IV). The OS and PFS were reduced in patients with vascular invasion compared with those without vascular invasion (P<0.001 for OS, Fig. 4A; P=0.001 for PFS, Fig. 4B). The LRFS and DMFS were significantly shorter for patients with vascular invasion compared with those without vascular invasion (P=0.015, Fig. 4C and P<0.001, Fig. 4D, respectively). CR was achieved in 88 stage I/II patients following chemotherapy alone. The OS (52.6 vs. 96.2%, P<0.001; Fig. 5A), PFS (37.9 vs. 79.6%,

Table V. Results of univariate and multivariate analyses of prognostic factors for PFS and OS in patients with ENKTL.

	PFS				OS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
Parameter	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, >60 years	0.865	0.652			1.623	0.159		
Gender, male	1.222	0.347			1.101	0.711		
ECOG PS ≥2	5.816	<0.001			5.300	<0.001		
Subtype, EUNKTL	2.614	<0.001			2.727	<0.001		
B symptoms	2.415	<0.001			2.427	0.001		
≥2 extranodal sites	2.532	<0.001			2.577	0.001		
Regional lymphadenopathy	2.618	<0.001			2.398	0.002		
Elevated serum LDH	2.803	<0.001			3.225	<0.001		
Vascular invasion	3.125	<0.001	2.141 (1.354-3.347)	<0.001	4.274	<0.001	3.042 (1.743-5.162)	<0.001
Stage III/IV	4.895	<0.001	2.032 (1.089-3.731)	0.019	5.434	<0.001	2.411 (1.141-5.493)	0.016
CR after CT	0.293	<0.001	2.043 (1.192-3.328)	0.005	0.206	<0.001	2.768 (1.265-5.412)	0.002

PFS, progression-free survival; OS, overall survival; RR, relative risk; CI, confidence interval; ECOG PS, eastern cooperative oncology group performance status; EUNKTL, extraupper aerodigestive tract NK/T-cell lymphoma; LDH, lactate dehydrogenase; CR, scomplete response, CT, chemotherapy.
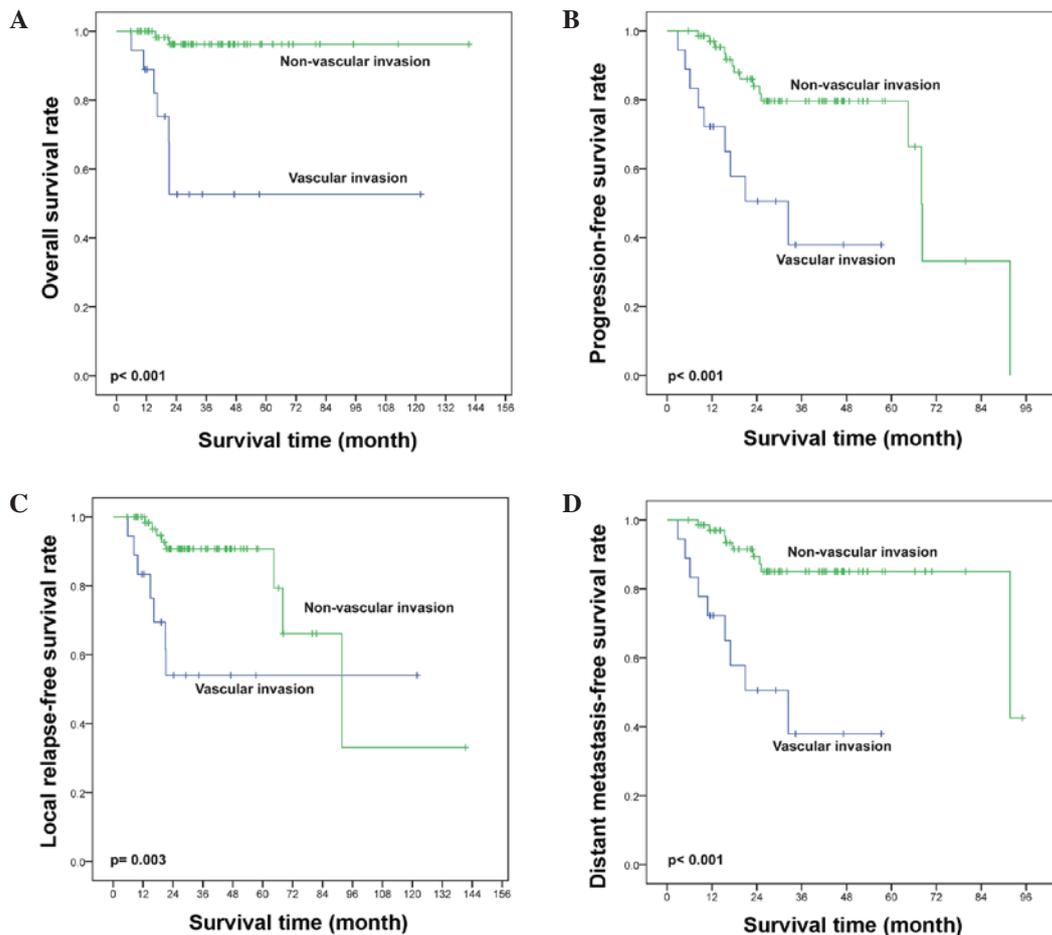
Figure 5. Kaplan-Meier plots of (A) overall survival, (B) progression-free survival, (C) local relapse-free survival and (D) distant metastasis-free survival in stage I/II patients with extranodal natural killer/T-cell lymphoma that achieved complete remission following only chemotherapy (n=88), according to vascular invasion versus non-vascular invasion at diagnosis.

P<0.001; Fig. 5B), LRFS (54.0 vs. 90.7%, P=0.003; Fig. 5C), and DMFS (37.9 vs. 85.0%, P<0.001; Fig. 5D) of patients with vascular invasion were all significantly shorter than those without vascular invasion.

The effects of vascular invasion on OS and PFS in patients with IPI 0-1 (n=134) were also verified in subgroup analysis. IPI 0-1 patients were further categorized into different prognostic groups by vascular invasion. Vascular invasion was significantly associated with shorter OS (P<0.001; Fig. 6A) and PFS (P<0.001; Fig. 6B). Similarly, in the KPI 0-1 subgroup patients (n=109), vascular invasion at diagnosis was significantly associated with inferior OS (P<0.001; Fig. 6C) and PFS (P=0.004; Fig. 6D).

Univariate and multivariate analyses. Univariate analysis revealed that ECOG PS >2, EUNKTL, B symptoms, ≥2 extranodal sites, regional lymphadenopathy, elevated serum LDH, vascular invasion, advanced stage (III/IV) and CR after CT could significantly predict shorter OS and PFS. Clinical factors that were statistically significant predictors of OS and PFS (P<0.05) were included in the multivariate analysis. IPI and KPI values were not included in the univariate and multivariate analyses due to their overlap with several other clinical variables. Multivariate analysis revealed that vascular invasion, stage III/IV and CR after chemotherapy were

independent prognostic factors for OS (P<0.001, P=0.016 and P=0.002, respectively) and PFS (P<0.001, P=0.019 and P=0.005, respectively) (Table V).

Discussion

ENKTL is an aggressive type of non-Hodgkin's lymphoma that is characterized by poor survival. Pathological and molecular markers for predicting the outcome of ENKTL have yet to be established, although Ki-67 proliferation rate was correlated recently with shorter disease-free survival and OS (P<0.05) (23). Loss of granzyme B protease inhibitor, cyclooxygenase-2 expression and decreased quantity of tumor-infiltrating forkhead box P3-positive regulatory T-cells have also been associated with poor prognosis of ENKTL, nasal type or UNKTL (24-26). Furthermore, tumor cell nuclear diameter and CD30 expression may be potential prognostic parameters in patients with ENKTL, nasal type (27). Vascular invasion is an associated prognostic factor for various types of malignant tumor. For example, Cuadra-Garcia *et al* reported that easily recognizable vascular invasion and occlusion by tumor cells exists in 20% (12/58) of patients with ENKTL (28). However, reports regarding the association between histological vascular invasion and ENKTL prognosis are limited (29).
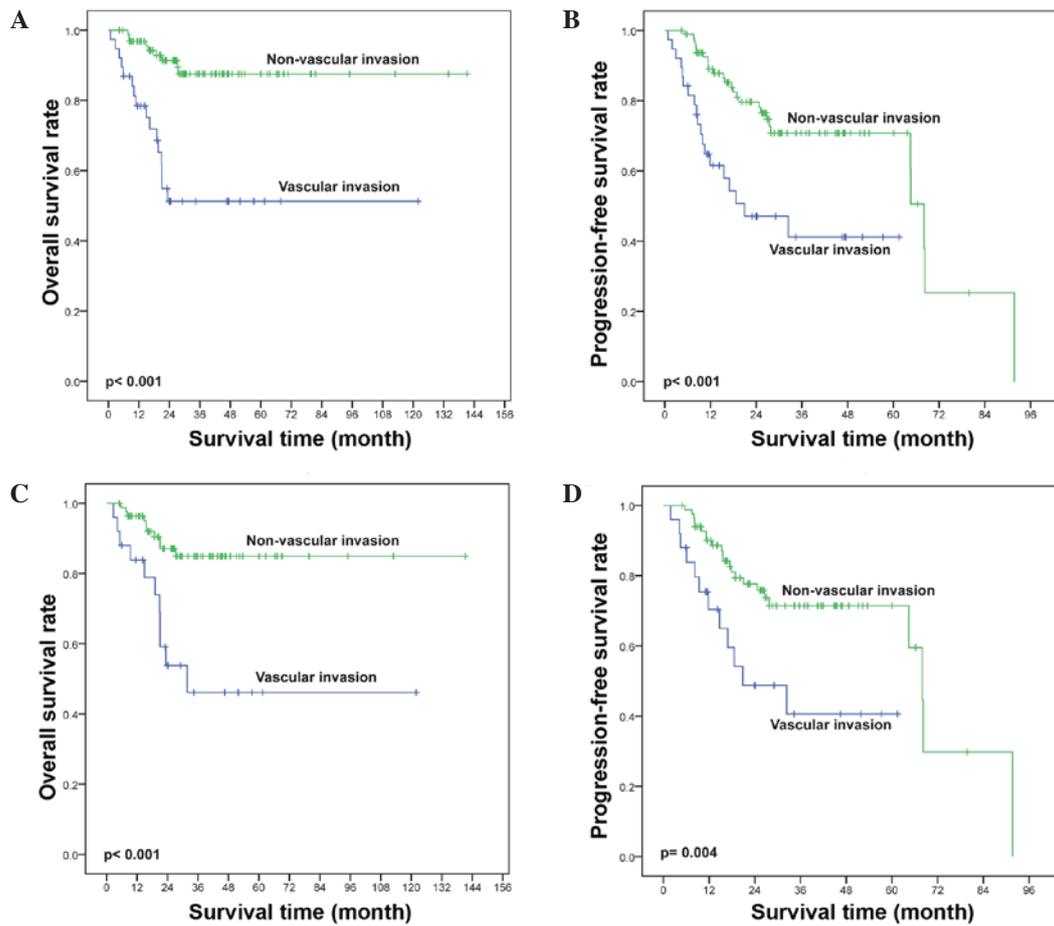
Figure 6. Kaplan-Meier plots of (A) overall survival (OS) and (B) progression-free survival (PFS) for extranodal natural killer/T-cell lymphoma (ENKTL) patients with a International Prognostic Index of 0/1, and (C) OS and (D) PFS for ENKTL patients with a Korean Prognostic Index of 0/1, according to vascular invasion versus non-vascular invasion at diagnosis.

In the current study, the histological vascular invasion status was investigated in tumor samples from 214 patients with untreated ENKTL. The present study is the first to report on the prognostic role of vascular invasion in hematopoietic malignancies. The results showed a significant difference in clinical behavior between the vascular invasion and non-vascular invasion groups. Patients with vascular invasion had more adverse clinical features, such as poor PS, B symptoms, bulky disease and advanced stage. Notable among these features was elevated serum D-D. Blood vessels were compressed and partially filled by tumor cells during tumor cell invasion. Under these circumstances, thrombosis is more likely to occur. Consequently, serum D-D levels were markedly higher in ENKTL tumor samples with histological vascular invasion than in those without vascular invasion. Wróbel *et al* identified that elevated serum D-D levels are associated with poor prognosis in non-Hodgkin's lymphoma (30). Similarly, vascular invasion was associated with poor responses to chemotherapy in the present study. According to the Cox regression model, which included ECOG PS, B symptoms, local tumor invasion, elevated serum LDH, advanced stage (III/IV), histological vascular invasion and CR after CT, vascular invasion was an independent prognostic factor for both OS and PFS.

In the present study, the CR and ORR rates of the vascular invasion group were significantly lower following chemotherapy

and at the end of treatment. Patients with vascular invasion have a distinctive tumor microenvironment with an elevated number of TAMs (31). Numerous studies have indicated that TAMs produce a vast diversity of growth factors, including proteolytic enzymes, pro-angiogenic cytokines and inflammatory mediators, which not only directly stimulate tumor cell growth and/or facilitate tumor metastatic invasion but also induce immune suppression of host defenses against tumors (32,33). We propose that the aforementioned factors may be attributed to the responses to radiation and chemotherapy.

In the present study, survival analysis indicated that the 5-year OS and PFS rates in the vascular invasion group were significantly lower than those in the non-vascular invasion group (36.8 vs. 77.0% for OS, P<0.001; 21.8 vs. 60.1% for PFS, P<0.001). Further analysis identified that, following treatment, stage I/II UNKTL patients with histological vascular invasion were significantly more likely to metastasize distally than patients without vascular invasion (DMR rate: 42.3 vs. 15.2%, P=0.035). Notably, the DMFS of patients with vascular invasion was inferior to that of non-vascular invasion patients (37.9 vs. 85.0%, P<0.001). Several large retrospective studies have shown that radiotherapy is an important treatment modality for ENKTL (34-37). Radiotherapy is beneficial in controlling local lesions but may lead to distant dissemination. Although chemotherapy combined with radiotherapy can reduce the risk

of recurrence, distant metastasis still commonly occurs, which is a fatal sign in patients with ENKTL following the completion of treatment (38,39). Intravascular invasion of a tumor is a prerequisite for the occurrence of metastasis. The current results identified more stage III/IV patients in the vascular invasion group than the non-vascular invasion group (P<0.001). A higher proportion of EUNKTL patients also existed in the vascular invasion group, indicating strong invasiveness and easy spread of disease through the body. Thus, the histological vascular invasion status of a tumor may result in distant metastasis, leading to shorter patient survival. The multiple factor analysis performed in the present study revealed CR following chemotherapy as a significant favorable prognostic factor in patients with ENKTL. However, even if the stage I/II ENKTL patients with vascular invasion achieved CR following chemotherapy alone, their prognosis remained worse than that for patients without vascular invasion at the same stage. This finding indicates that vascular invasion is a poor independent prognostic factor of patients' response to chemotherapy.

Two major clinical prognostic models, termed IPI and KPI, were applied in NK/T-cell lymphoma. The distribution of patients within risk groups based on IPI and KPI scores is presented in Table I. Based on IPI scores, >60% of the cases belonged to the low-risk category (with 0 or 1 adverse factor). The KPI model balanced the distribution of patients into different risk groups better than IPI did. However, both prognostic models failed to differentiate patients with different outcomes in the low-risk group. The vascular invasion group can be divided based on IPI or KPI scores of 0-1 into two subgroups with significant differences in OS and PFS (IPI: P<0.001 and P<0.001, respectively; KPI: P<0.001 and P<0.004, respectively) (Fig. 6). Thus, vascular invasion may be a good independent prognostic factor for determining OS and PFS in the whole group of ENKTL patients, as well as in those with low-risk IPI or KPI scores.

In conclusion, histological vascular invasion was an independent prognostic factor for OS and PFS in patients with ENKTL, nasal type. Further investigation is required to gain a better understanding of the mechanisms underlying the association between vascular invasion and clinical outcomes. UNKTL derived from the nasal cavity and its surroundings is also characterized by metastasis to the cervical lymph nodes or distant organs, and is commonly accompanied by histological vascular invasion. This distinction may be useful for encouraging clinicians to refer to nasopharyngeal carcinoma research methods to explore diagnostic and therapeutic methods for ENKTL.

Acknowledgements

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