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Specific Soft-Tissue Invasion and LMP1 Expression Are Potential Indicators of Extranodal NK/T Cell Lymphoma, Nasal Type

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Background: Extranodal NK/T cell lymphoma, nasal type (ENKTL-NT) is difficult to distinguish from nasal polyps and inverted papilloma, leading to its high misdiagnosis ratio. The aim of this study was to investigate its potential prognostic indicators.

Material/Methods: Kaplan-Meier method was used to calculate overall survival (OS) rate. Cox proportional hazards regression was used to analyze risk ratios (ORs) with 95% confidence intervals (CIs).

Results: Nasal ala infiltration and nasal floor thickness >2.0 mm or nasal septum thickness >2.5 mm were potential prognostic factors for OS (p=0.0323 and 0.0072, respectively). Cox proportional-hazards regression indicated that high LMP1 expression and the nasal floor thickness >2.0 mm or nasal septum thickness >2.5 mm were the independent risk factors for poor OS of ENKTL-NT (HR=3.0655, p=0.028; HR=2.3650, p=0.0452, respectively). In the subgroup analysis, the OS rate was lower when the nasal floor thickness >2.0 mm or nasal septum thickness >2.5 mm in the patients who had high expression of LMP1 (p=0.0651), whereas high LMP1 expression increased the risk of worse prognostic outcome in patients with deep infiltration thickness. Thus, high LMP1 expression may contribute to the tissue invasion of ENKTL-NT.

Conclusions: Any patient with nasal ala soft-tissue invasion, nasal floor thickness >2.0 mm/nasal septum thickness >2.5 mm on CT imaging or high LMP1 expression should prompt immediate histopathologic diagnosis to rule out ENKTL-NT in clinical practice.

MeSH Keywords: Leukemia-Lymphoma, Adult T-Cell • Lymphoma, Extranodal NK-T-Cell • TNF Receptor-Associated Factor 2

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Background

Extranodal NK/T cell lymphoma is a rare and aggressive non-Hodgkin's lymphoma, which is accompanied by poor survival [1]. It is more prevalent in Southeast Asia and in South and Central America, and is less common in Africa and Europe [2,3]. ENKTL accounts for 6–7% of all non-Hodgkin lymphoma in Southeast Asia and is associated with Epstein-Barr Virus (EBV) infection [4,5]. In addition, compared with females, males are more susceptible to ENKTL [3]. ENKTL can affect extra-nasal areas such as skin, soft tissue, and testes, as well as the gastrointestinal and upper respiratory tracts [3]. However, it resembles other destructive nasal diseases, thereby leading to the failure of diagnosis and treatment [6,7]. Thus, it is very difficult to distinguish between nasal type (ENKTL-NT) and other benign nasal diseases in clinical symptoms [6,8]. If not treated promptly, it can lead to total destruction of the nasopharyngeal region, including the midface, nasopalatine, and orbital wall. Accordingly, the diagnosis of ENKTL-NT is a challenge because of its nonspecific clinical history. Therefore, early diagnosis and treatment will help increase the overall quality of life and survival of patients with this disease [6].

Plain radiography of the paranasal sinuses is widely used in the diagnosis of paranasal sinus diseases. However, it is very limited because of the minimal information about delicate bony structures and mucosal changes of the ostiomeatal complex [9]. Computed tomography (CT) is the first imaging modality in use to differentiate sinonasal disease of the head and neck, which contributes to evaluating the size, morphology, and extent of ENKTL locally, distant metastases, and pre-treatment staging after the histopathologic diagnoses [8]. Due to its ability to display bone and soft tissues, CT is the current diagnostic modality of choice for evaluating the ostiomeatal complex, but MRI [2,10] is the first choice for detecting the extent of disease progression [11,12]. Moreover, CT is used both as a diagnostic tool to identify anatomical anomalies and mucosal pathology and to preoperatively map and guide through the challenging, convoluted, and variable anatomy of the area [13].

Epstein-Barr virus (EBV) has been recognized as a "class I cancer-causing virus" by the WHO [14]. Various lymphomas (e.g., Burkett's lymphoma, Hodgkin's lymphoma, Lymphoepithelioma-like carcinoma, and NK/T cell lymphoma) have been demonstrated to be associated with EBV [15]. In particular, there is a very strong relationship between EBV infection and ENKTL [16]. Multiple molecules are involved in EBV latent infection, including latent membrane protein (LMP) 1. LAMP1 regulates proliferation and invasion of lymphoma cells, playing oncogenic roles in the progression of lymphomas [17–19].

In the present study we investigated the specific prognostic indicators of ENKTL-NT by analysis of CT imaging and LMP1 expression of the patients, thereby providing valuable references for accurate clinical diagnosis of ENKTL-NT.

Material and Methods

Patients and samples

From 2010 to 2015, we enrolled 52 patients with histologically proven ENKTL-NT, 134 patients with diagnosis of nasal polyps, and 24 patients with inverted papilloma. The background data of the patients is presented in Table 1. Patients with previously untreated, histologically diagnosed disease of the nasopharynx without coexisting disease were selected. The included patients had no previous history of head and neck cancer. Informed consent was obtained from all participating patients in clinical trials. The study was conducted with approval of the Zhenjiang First People's Hospital, Affiliated People's Hospital of Jiangsu University's Review Board. Biopsy tissue examination was used to diagnose of ENKTL, nasal polyps, and inverted papilloma. The staging of ENKTL was performed according to the Ann Arbor classification system [20]. Clinical history, pathological report, and CT image with report were collected and retrospectively reviewed.

Treatment of ENKTL-NT patients and the evaluation criteria of efficacy

All the ENKTL-NT patients in this study received combined CHOP regimen therapy and radiation therapy: cyclophosphamide (750 mg/m^2), intravenous drip, day 1; epirubicin (80 mg/m2), intravenous drip, day 1; vincristine sulphate (1.4 mg/m^2), intravenous drip, day 1; prednisone acetate (40 mg/m^2), intravenous drip, days 1–5. After 2 cycles of chemotherapy, radiation therapy was administered. The radiation dose was

Table 1. Demographic details.

	Total number of patients	Age (mean ± sd)	Male: Female
ENKTL	52	52.83±13.69	7: 6 (53.8% Male)
Nasal polyps	134	47±15.65	85: 49 (63% Male)
Inverted papilloma	24	54.08±10.72	14: 10 (58.3% Male)

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40–60 Gy; 2 Gy/day. The lesion site and the edge of the infiltration zone were radiotherapy target areas. According to the WHO criteria [21], the treatment efficiency was rated as follows: complete response (CR), partial response (PR), and no response (NR).

CT imaging

All patients underwent standard CT scans before treatment. The CT images were evaluated by head and neck specialist radiologists. Patients were examined with standard CT scan of the nasal, accessory nasal sinuses, and nasopharynx region in the axial and coronal planes on either of the 2 scanner machines. Parameters were as below:

I. Siemens Somatom sensation 64 (Siemens, Germany): 120 kVp, 150–200 mAs, collimation of 64*0.625 mm, pitch of 0.8 mm and primary reconstruction of 0.4 mm, 1.5 ml/kg of intravenous contrast medium, and flow rate of 3 ml/s. A 3-mm-thick axial reconstruction and a 3-mm coronal multiplanar reconstruction (MRP) were obtained with bone and soft tissue.
II. Brilliance iCT 256 slice (Philips Medical Systems, USA): 120 kVp, 300 mAs, pitch of 0.391 with 100 ml of intravenous contrast medium, flow rate of 1.0 ml/s +50 mL saline flush, collimation of 256 * 0.5 mm, 3-mm axial thickness reconstruction, and 3-mm coronal thickness reconstruction.

Patients were placed in a supine position and scans were obtained parallel to the occlusal line from the hard palate to the end of the frontal sinus in paranasal sinus (PNS) setting in axial plane and coronal plane, followed by reconstruction.

Interpretation of CT images

CT images were retrospectively reviewed by 2 head and neck specialist radiologist in a double-blind manner. The interpretation of results included the description of tumor location (unilateral *vs.* bilateral nasal cavity), morphological pattern of the tumor (polypoidal *vs.* infiltrative lesion), tumor signal intensity (homogeneous *vs.* heterogeneous), bone destruction/erosion, bone sclerosis, involvement of the sinuses (maxillary, ethmoid, frontal and sphenoid), involvement of the soft-tissue and nasal vestibule, involvement of the nasopharynx and surrounding structures, and nasal turbinate and nasal septum destruction.

LMP1 expression detection

The blood samples from ENKTL-NT patients were collected and stored for further examinations. DNA were isolated with a QIAamp Blood kit (Qiagen, Germany) following the manufacturer's instructions. Real-time quantitative DNA PCR for LAMP1 DNA levels was carried out according to previous studies [22]. The TaqMan probe sequence for LMP1 DNA was as follows: 5'-FAM-TGATCTCCTTTGGCTCCTCCTGTTT-TAMRA-3'. The primer used was sense primer 5'-AAAACTGGTGGACTCTATTG-3'; antisense primer 5'-TCGTTGGAGTTAGAGTCAGA-3'. The ABI 7700 Sequence Detection System was used to perform the PCR reactions. The plasmid-containing LMP1 fragment was used to run a calibration curve. The concentration (copies/ml) was calculated according to the following equation [23]: $C=Q\times[V_{DNA}/V_{PCR}]\times[1/Vext]$, C=target concentration in plasma (copies/ml); Q = target quantity (copies) determined by sequence detector in a PCR; V_{DNA}=total volume of DNA obtained following extraction; V_{PCR}=volume of DNA solution used for PCR; V_{ext}=volume of plasma extracted. Four copies/ml of LMP1DNA level were set as the lower limits of detection for LMP1 DNA. Values below the detection limit were regarded as zero.

Immunohistochemistry (IHC)

Paraffin sections were dewaxed and rehydrated. The sections in citrate buffer were microwaved for antigen retrieval. The endogenous peroxidase was inactivated by 0.3% hydrogen peroxide solution. After nonspecific binding with 5% goat serum for 30 min, the sections were incubated with monoclonal anti-LMP1 (Abcam) antibodies. Then, the sections were incubated with horseradish peroxidase complex (DAKO). The results were estimated by immunohistochemistry score based on staining density and intensity, as previously reported [24].

The IHS evaluation was independently performed by 2 investigators.

Statistical analysis

Chi-square test or Fisher's exact test was used to analyze the data, as appropriate, with SPSS 16.0 software (SPSS, USA). $p<0.05$ was considered statistically significant. Kaplan-Meier method was used to calculate the overall survival rate followed by the log-rank test. Multivariate analyses were performed using the Cox proportional hazards model. The survival analysis was carried out with MedCalc software.

Results

CT imaging analysis of ENKTL-NT, nasal polyps, and inverted papilloma

As shown in Tables 2, 3, unilateral tumors were usually found in the patients with ENKTL-NT (82.7%, Figure 1A) or inverted papilloma (95.8%). Nevertheless, for the 134 patients with nasal polyps, 70.8% patients had bilateral tumor lesion. There was a significant difference between the ENKTL-NT and nasal polyps (p=0.000). Heterogeneous or homogeneous enhancement imaging (Figure 1B) were both observed in ENKTL-NT, nasal

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Table 2. Difference in CT imaging finding between ENKTL and nasal polyps.

	ENKTL (n=52)	Nasal polyps (n=134)	p Value
Tumor location			
Unilateral	43	39	p=0.000
Bilateral	9	95	
Tumor density			
Heterogeneous	21	48	p=0.339
Homogeneous	31	86	
Bone remodeling			
Bone sclerosis	8	13	p=0.305
Bone erosion	10	9	p=0.016
Tumor morphology			
Polypoid	35	46	p=0.000
Infiltrative	17	88	
Sinus involvement			
Maxillary	50	130	p=0.673
Ethmoid	48	113	p=0.205
Frontal	28	96	p=0.025
Sphenoid	16	59	p=0.133
Soft tissue infiltration			
Nasal vestibule	47	58	p=0.000
Choana & nasopharynx	1	50	p=0.000
Nasal ala	28	2	p=0.000
Nasopharyngeal wall	9	1	P=0.000
Nasal floor thickness >2.0 mmor nasal septum thickness >2.5 mm	28	8	P=0.000

polyps, and inverted papilloma. There was no significant difference between ENKTL-NT *vs.* nasal polyp (p=0.339) and ENKTL *vs.* inverted papilloma (P=1.000). Bone erosion was more common in ENKTL-NT (Figure 1C) than in nasal polyps (p=0.016). Polypoidal tumor lesion was often noticed both in ENKTL-NT (Figure 1D) and inverted papilloma. Sinus involvement was found in most cases of ENKTL-NT, inverted papilloma, and nasal polyps (Figure 1E). Moreover, sinus involvement was more common in nasal polyps (p=0.025) compared to ENKTL-NT. Soft-tissue infiltration (except for infiltration of choana and nasopharynx) was the special feature for ENKTL-NT. Infiltration of nasal vestibule was most common in patients with ENKTL-NT or nasal polyps. Over half of the patients with ENKTL-NT had infiltration of nasal ala (Figure 1F), whereas minor cases were found in nasal polyps (p=0.000) and inverted papilloma (p=0.000). Some cases of nasopharyngeal wall infiltration were noticed in ENKTL-NT and nasal polyps, whereas no patients with inverted papilloma had this infiltration (p=0.05). Furthermore, 53.8% of patients with ENKTL-NT had either nasal floor thickness >2.0 mm or nasal septum thickness >2.5 mm. In contrast, 8 of 134 patients with nasal polyps and no patients with inverted papilloma had nasal mucosal thickening.

Association between LMP1 expression and clinicopathological characteristics

All ENKTL patients received combined chemotherapy and radiation therapy. Twenty-seven patients (51.9%) experienced CR,

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Table 3. Difference in CT imaging findings between ENKTL and inverted papilloma.

	ENKT (n=52)	Inverted papilloma (n=24)	p Value
Tumor location	43	23	p=0.156
Unilateral	9	1	
Bilateral			
Tumor density	21	10	p=1.000
Heterogeneous	31	14	
Homogeneous			
Bone remodeling	8	8	p=0.128
Bone sclerosis	10	2	p=0.319
Bone erosion			
Tumor morphology	17	23	p=0.000
Polypoid	35	1	
Infiltrative			
Sinus involvement	50	20	p=0.075
Maxillary	48	18	p=0.064
Ethmoid	28	10	p=0.46
Frontal	16	5	p=0.42
Sphenoid			
Soft tissue infiltration	47	6	p=0.000
Nasal vestibule	1	14	p=0.000
Choana & nasopharynx	28	0	p=0.000
Nasal ala	9	0	p=0.051
Nasopharyngeal wall	28	0	p=0.000
Nasal floor thickness >2.0 mmor nasal septum thickness >2.5 mm			

21 patients (40.4%) experienced partial remission (PR), and 4 patients (7.7%) had no response (NR) (Table 4). LMP1 is implicated in EBV-mediated invasion in lymphoma cells [25]. Thus, the expression of LMP1 was evaluated next. The expression of LMP1 was divided into 2 groups: a high LMP1 expression group and a low expression group, based on a previous study [22]: 40 copies/ml was the cut-off value to define the low (<40 copies/ml) and high (>40 copies/ml) expression. There were 29 patients with ENKTL-NT who were determined to have high LMP1 expression. IHC was conducted to further estimate the expression of LMP1 in ENKTL-NT tissues. Notably, the distribution of LMP1 was mainly located in cell membrane and cytoplasm. Furthermore, high expression of LMP1 was observed in 27 of 52 ENKTL-NT tissues (51.9%) and 48.1% presented low LMP1 expression (Figure 2A–2C). The association of LMP1 expression with the clinicopathological characteristics with ENKTL-NT patients is displayed in Table 5. No significant relationship was found between LMP1 expression and sex, age, or IPI. Poor prognosis was strongly associated with high expression of LMP1.

Patient survival and multivariate analyses

Kaplan-Meier analysis was used to analyze the survival rate of ENKTL-NT patients. The overall (OS) survival probability was significantly lower in patients with nasal ala infiltration, the nasal floor thickness >2.0 mm, or nasal septum thickness >2.5 mm, or high LMP1 expression (p<0.01, Figure 3A–3C).

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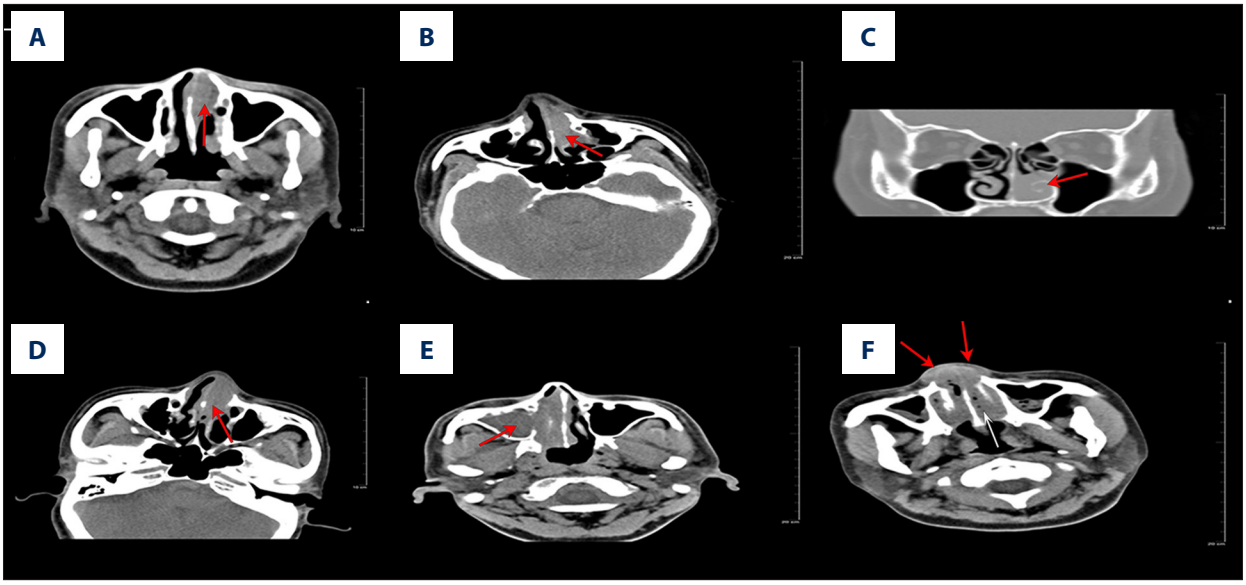

Figure 1. (**A**) Axial CT scan of a 47-year-old man with ENKTL-NT and a left-sided nasal mass (red arrow); (**B**) Axial CT scan of a 38-year-old with ENKTL-NT having homogeneous enhancement of left nasal cavity mass with invasion of nasal ala (red arrow); (**C**) Coronal CT scan of a 47-year-old man with ENKTL-NT having nasal septum and inferior turbinate erosion (red arrow); (**D**) Axial CT scan of a 48-year-old man, with a polypoidal mass seen in the left nasal cavity in ENKTL-NT (red arrow); (**E**) Axial scan of a 60-year-old man with ENKTL-NT, right-sided nasal mass in the nasal cavity, and involvement of the maxillary sinus (red arrow); (**F**) Axial CT scan of a 63-year-old woman with ENKTL-NT, bilateral nasal cavity mass and infiltration of the nasal ala (red arrow), small bubbles in lesion area (white arrow).

Table 4. The response to treatment.

Response to treatment	Total number of patients	Percentage (%)
CR	27	51.9
PR	21	40.4
NR	4	7.7

CR – complete remission; PR – partial remission; NR – no response.

Clinical features that were statistically significant risk factors of ENKTL-NT and poor prognosis ($p<0.05$) were included in the multivariate analysis. Multivariate analysis showed that high LMP1 expression and the nasal floor thickness >2.0 mm or nasal septum thickness >2.5 mm were the independent risk factors for the prognostic outcome of ENKTL-NT patients (Table 6, HR=3.0655, p=0.028; HR=2.3650, p=0.0452, respectively).

Subgroup analysis

In consideration of the tight relationship between LMP1 expression and tissue invasion, a subgroup analysis was performed to determine the association of LMP1 expression with nasal ala infiltration and infiltration thickness. The analysis showed that the OS was slightly lower in patients with high LMP1 expression when the nasal floor thickness was >2.0 mm or nasal septum thickness was >2.5 mm (p=0.0581, Figure 4A). There were no poor outcomes observed in patients with high LMP1 expression when the nasal ala infiltration occurred (p=0.303). On the other hand, worse prognostic outcome was noticed in patients with high LMP1 expression when the nasal floor thickness was >2.0 mm or nasal septum thickness was >2.5 mm, although the difference was not significant (p=0.0651, Figure 4B).

Discussion

ENKTL is the most aggressive malignant disease of the nasal cavity. It progresses rapidly and has a poor prognosis, which can only be improved by early diagnosis and early management. However, the clinical phenotype of ENKTL is very similar to nasal polyps and inverted papilloma, which are the most common benign diseases occurring in the nasopharynx [11]. Thus, the misdiagnosis rate of ENKTL-NT is extremely high [26]. Accurate clinical diagnosis is desirable for choosing timely and optimal treatment modalities. Compared to conventional plain radiography, CT scanning has become a valuable tool for diagnosing, comparing, and staging of ENKTL [14]. Many studies have pointed out that LMP1 may serve as a potential indicator for ENKTL [27–29]. Therefore, we aimed to investigate whether ENKTL can differentiate between nasal polyps

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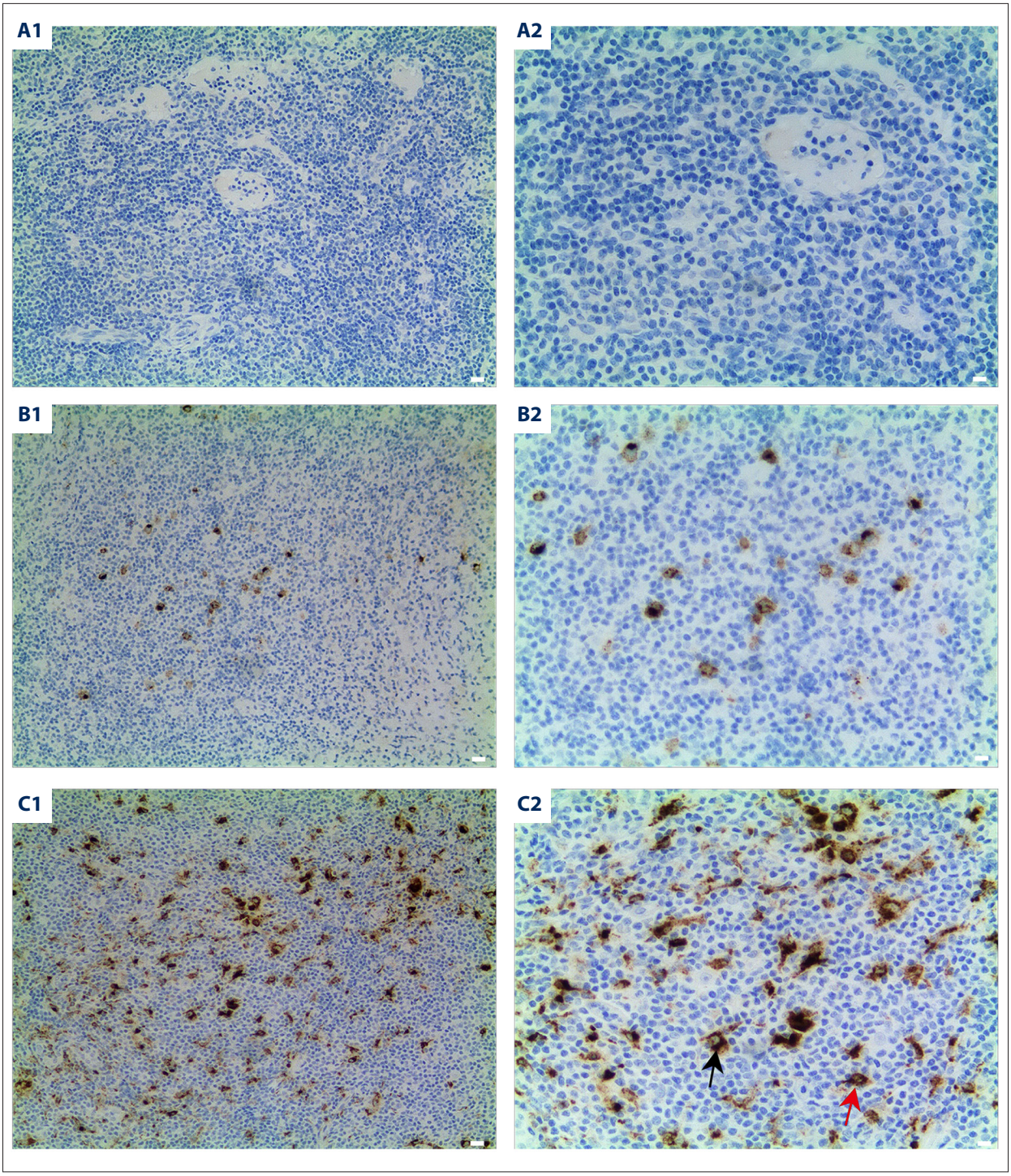

Figure 2. (**A**) Negative IHC staining of adjacent normal tissue. (**B**) Weak IHC staining for LMP1 in ENKTL-NT samples. (**C**) Strong IHC staining for LMP1 in ENKTL-NT samples.

and inverted papilloma arising in the nasal cavity by comparison of the CT imaging and the expression of LMP1.

In the present study, most of the lesions in ENKTL-NT were present in only 1 nasal cavity, which was similar to unilateral nasal cavity lesions found by previous studies [2,30]. Unilateral nasal cavity was also found in most cases of inverted papilloma, but the situation was the opposite in nasal polyps patients. Sclerosis and bone erosion have been observed in ENKTL [31,32]. More cases of ENKTL-NT had bone erosion, with a significant

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Table 5. Association of LMP1 expression with clinical features.

	High	Low	χ^2
Gender			
Male	15 (14)	13 (14)	1.000 (0.788)
Female	14 (13)	10 (11)	
Age			
≤50	9 (6)	7 (6)	1.000 (1.000)
>50	20 (21)	16 (19)	
IPI			
Low/Low-intermediate	21 (22)	16 (19)	0.548 (0.74)
High-intermediate/High	8 (5)	7 (6)	
Prognosis			
Live	6 (5)	16 (20)	0.001 (<0.001)
Dead	23 (22)	7 (5)	

Data in "()" were from IHC for LMP1 expression.

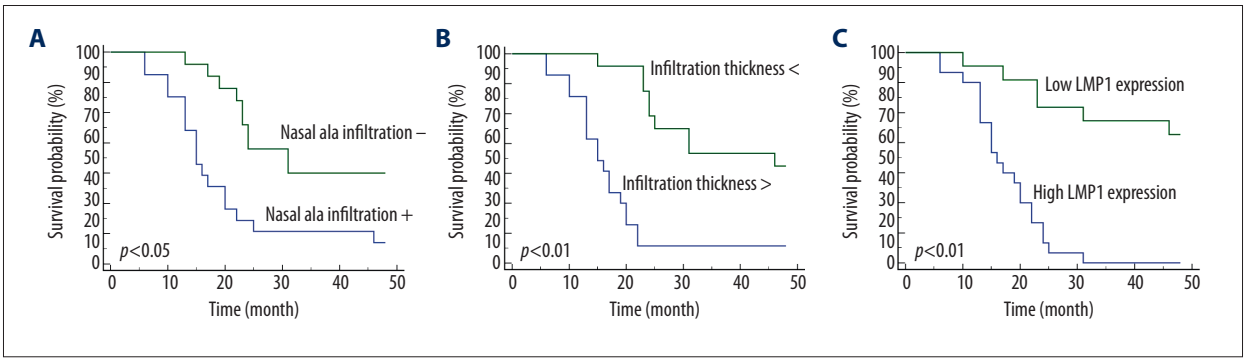

Figure 3. Kaplan-Meier analysis of overall survival for patients with ENKTL-NT according to nasal ala infiltration at CT imaging (**A**), infiltration thickness > or < at CT imaging (nasal floor thickness >2.0 mm or nasal septum thickness >2.5 mm) (**B**) and high/low LMP1 expression (**C**).

Table 6. Results of multivariate analyses of prognostic factors for OS in patients with ENKTL-NT.

	Multivariate analysis	
	HR (95% CI)	p Value
High LMP1 expression	3.0655 (1.1344 to 0.82841)	0.028
Nasal ala infiltration	1.1875 (0.5095 to 2.7678)	0.692
Nasal floor thickness >2 mm or nasal septum thickness >2.5 mm	2.3650 (1.0229 to 5.4683)	0.0452
Nasopharyngeal wall	0.4520 (0.115 to 1.1708)	0.2565
Nasal vestibule	1.1708 (0.2268 to 6.0438)	0.8514

difference with nasal polyps but no significant difference with inverted papilloma. In terms of tumor morphology, about 70% of patients had a polypoidal tumor pattern in ENKTL-NT. There was a significant difference with the nasal polyps, but polypoidal pattern were also observed in many inverted papilloma patients. As for sinus involvement, ethmoid sinus was reported as the most commonly involved in ENKTL [32]. However, in the present study, maxillary sinus involvement was the most commonly involved in ENKTL-NT patients, which was identical to a previous finding [33]. There was no significant difference in

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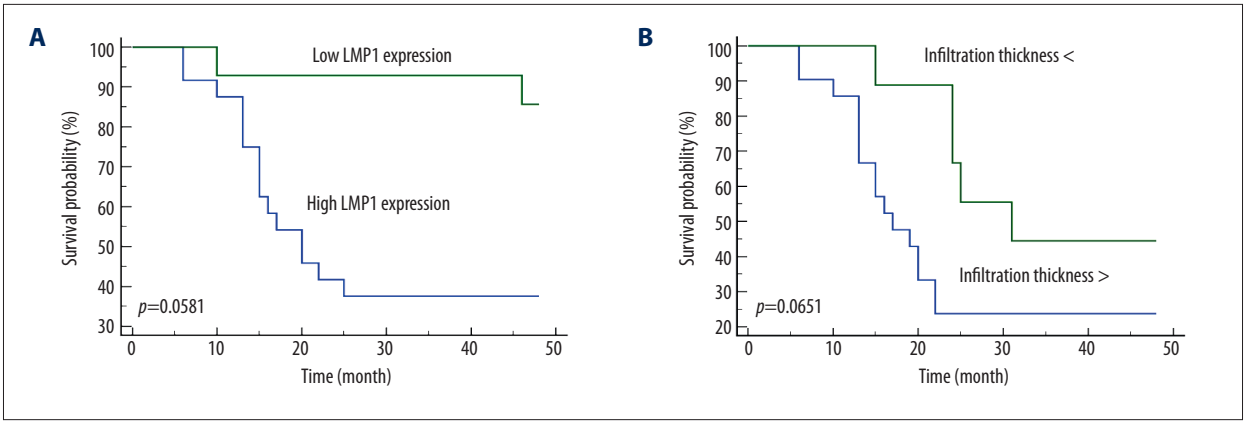

Figure 4. (**A**) Kaplan-Meier analysis of overall survival for the ENKTL-NT patients with nasal floor thickness > 2.0 mm or nasal septum thickness >2.5 mm according to LMP1 expression; (**B**) Kaplan-Meier analysis of overall survival for the ENKTL-NT patients with high LMP1 expression according to infiltration thickness.

sinus involvement between ENKTL-NT and nasal polyps or inverted papilloma, but a significant difference was observed in the frontal sinus compared to nasal polyps. A major difference between ENKTL-NT and nasal polyps or inverted papilloma was noted in soft-tissue infiltration. Choana and nasopharynx infiltration was rare in ENKTL-NT patients. Higher incidence of infiltration into nasal ala and nasopharyngeal wall and the deeper infiltration thickness (nasal floor thickness >2.0 mm or nasal septum thickness >2.5 mm) were common in ENKTL-NT compared to nasal polyps or inverted papilloma. These results are supported by previous studies [33,34]. Taken together, these results confirm the aggressive features of ENKTL-NT and indicate that the soft-tissue infiltration (except from choana and nasopharynx infiltration) observed from CT imaging may predict the risk of ENKTL-NT. Moreover, as a most important oncogenic factor in EBV-induced transformation and invasion [35], the expression of LMP1 was estimated as well. The results showed that high LMP1 expression was highly associated with poor prognosis of patients with ENKTL-NT. It was previously reported that patients with nasal polyps and inverted papilloma tend to be EBV-negative [36]. It was suggested that the soft-tissue invasion may be related to the high expression of LMP1. This mechanism needs further investigation for a deeper understanding of the pathogenesis of ENKTL-NT.

There is a lack of consensus among experts in the treatment protocol of ENKTL. No therapy is considered standard and various treatment methods are used for this disease [37]. Radiotherapy is initiated for patients with localized disease [3]. Common anthracycline (called as CHOP regimen) is used for chemotherapy [38]. However, there is a significant relapse of the disease in patients receiving chemotherapy treatment, which may be due to the expression of multi-drug resistant 1 (MDR1) gene, leading to overproduction of p-glycoprotein [39]. Chemotherapy followed by radiotherapy is the best choice to decrease relapse rate [38,39], but the result is far from satisfactory. Recently, combination regimens of dexamethasone (a steroid), methotrexate, ifosfamide, L-asparaginase, and etoposide (SMILE) have been tried on different relapsed or refractory cases [3]. The outcomes of this new therapy were quite remarkable. In addition, multiple therapy regimens such as Gelox [40], Gemox [41], DDGP [31], and AspaMet Dex [42] were reported. However, definitive long-term results are not yet available [39].

Kaplan-Meier survival analysis revealed that nasal ala infiltration and the nasal floor thickness >2.0 mm or nasal septum thickness >2.5 mm, or higher LMP1 expression can independently predict low overall survival rate. However, a previous study found no significant difference between LMP1 expression and prognosis in ENKTL-NT [43]. These conflicting results may be due to differences in sample origin, detection methods for LMP1, and sample size. In addition, to predict the independent risk factors of ENKTL-NT, clinical factors that were significant risk factors of ENKTL-NT and poor prognosis ($p<0.05$) were enrolled in the multivariate analysis. The results showed that high LMP1 expression and nasal floor thickness >2.0 mm or nasal septum thickness >2.5 mm were independent prognostic factors for OS (HR=3.0655, p=0.028; HR=2.3650, p=0.0452, respectively). ENKTL-NT patients who had high expression of LMP1, nasal ala infiltration, the nasal floor thickness >2.0 mm/nasal septum thickness >2.5 mm had higher possibility of poor OS. Although no significant difference was found and further exploration is still needed, the subgroup analysis revealed that high LMP1 expression may be related with nasal ala infiltration and deeper infiltration thickness. The distinction in CT imaging and the detection of LMP1 expression may be beneficial to the timely diagnosis for ENKTL-NT.

However, further investigations with larger samples of ENKTL-NT patients are needed to confirm our results, and it is also required to gain a better understanding of the mechanisms

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underlying the association of soft-tissue infiltration and clinical outcomes. In addition, MRI has a good resolution in differentiating various soft-tissue structures and also has a multiplanar scanning function [11,12,44]. It was reported that PET/CT was useful in staging and detecting ENKTL in patients that were missed by conventional staging methods [45–47]. Therefore, CT/MRI or PET/CT may be included in diagnosis and routine staging modality [11,48,49].

Conclusions

In conclusion, infiltration of nasal ala and the infiltration thickness (nasal floor thickness >2.0 mm/nasal septum thickness >2.5 mm) were the independent risk factors of ENKTL compared to nasal polyps and inverted papilloma. If any patient has these symptoms in adjacent nasal soft-tissue invasion on CT imaging, prompt histopathologic diagnosis is needed. Our study may provide an early diagnostic indicator for patients with ENKTL.

Conflict of interest

None.

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