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ORIGINAL ARTICLE

The correlation of clinicopathological features and prognosis in extranodal natural killer/T cell lymphoma: a report of 42 cases in the early stage

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Abstract

This study aimed to explore the clinicopathological features and prognostic correlation of extranodal natural killer (NK)/T cell lymphoma (ENKTCL) in the early stage, screen out the prognostic markers of ENKTCL, and to establish the molecular model of ENKTCL prognosis. A retrospective study was conducted in 88 patients from May 1999 to Dec 2013 in Chinese Academy of Medical Sciences Cancer Hospital, who were diagnosed with ENKTCL according to WHO lymphoid hematopoietic tumor classification (published in 2008). The clinical data and paraffin-embedded tissue blocks were collected. The expressions of CD56, MLH1, PDGFRA, VEGF, PD-L1, PD-1, CyclinD1, p53, and Ki-67 were detected by high-throughput tissue microarray and immunohistochemistry (IHC) staining. The relationship between nine protein expressions and the clinicopathological features and prognosis of patients with ENKTCL were analyzed. The survival time of the 42 patients with complete clinical and follow-up data was 0~153 months. The average survival time was 60.1 months. The survival rates of 1 year, 2 years, and 3 year were 85.7%, 78.6%, and 71.4%, respectively. Single factor survival analysis showed that the increase of serum lactate dehydrogenase (LDH ≥ 240UI/L) before treatment was associated with poor prognosis, and there was a significant difference in survival rate ($P = 0.006$). Different therapy methods were related with prognosis ($P = 0.011$); in specifically, radiotherapy alone had the best treatment effect, followed by concurrent chemoradiotherapy, and the worst was chemotherapy alone. But, multivariate statistics indicated that the LDH level and the treatment approach were not independent prognostic factors of ENKTCL. There was no statistical difference between epidemiological factors such as gender, age, and other clinicopathological factors including tumor location, B symptoms, β2-microglobulin levels before treatment, and prognosis. Survival analysis of single factor showed that the positive expression of PDGFRA and PD-L1 was, respectively, related to the poor prognosis of patients with ENKTCL ($P = 0.040$, 0.007). The patients with Ki-67 overexpression (≥ 50%) had a worse prognosis than those with lower expression (< 50%), and the difference of survival rate between the two groups has statistical significance ($P = 0.038$). The expression of CD56, MLH1, VEGF, PD-1, CyclinD1, and p53 has no effect on survival rate ($P > 0.05$). Multivariate survival analysis showed that the expression levels of PDGFRA, PD-L1, and Ki-67 were independent factors in the prognosis of patients with ENKTCL. And the positive expressions of these three proteins were risk factors for prognosis of patients with ENKTCL (PDGFRA: $P = 0.045$, HR = 8.265, 95% CI: 1.050–65.054; PD-L1: $P = 0.005$, HR = 9.369, 95% CI: 1.950–45.003; Ki-67: $P = 0.023$, HR = 3.545, 95% CI: 1.187–10.585). The elevation of serum lactate dehydrogenase (LDH ≥ 240UI/L) before treatment and the treatment approach were associated with poor prognosis, which could be used as adjunct indexes to the prognosis. However, they were not independent factors for the prognosis of patients with ENKTCL. The expressions of PDGFRA, PD-L1, and Ki-67 were independent factors in the prognosis of patients with ENKTCL and these three proteins were risk factors of prognosis. The above markers combined with clinical factors may establish the prognosis model of ENKTCL.

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Keywords Extranodal natural killer/T cell lymphoma · Pathology · Immunohistochemistry · Prognosis

Introduction

Extranodal natural killer (NK)/T cell lymphoma (ENKTCL) is a rare form of invasive non-Hodgkin's lymphoma (NHL). It occurs mainly outside of lymph nodes with the phenotype of NK cells in most cases and a few of cytotoxic T cell phenotype. ENKTCL accounts for 5–18% of all non-Hodgkin's lymphoma [1] of which 70% is from Asia, Mexico, and South America [2]. Nowadays, ENKTCL is no longer difficult to diagnose. It is done by combining morphology, immunohistochemistry, and Epstein-Barr virus-encoded RNA (EBER) in situ hybridization test. ENKTCL is characterized by low incidence, high grade of malignancy, aggressive clinical course, and poor prognosis. Due to lack of large sample retrospective researches, treatment criteria for this disease have not been set up. It is more important to take the research on the clinical practice, the molecular markers about treatment, and prognosis of ENKTCL.

In this study, we performed a retrospective analysis of ENKTCL cases in the early stage (stages I–II) obtained from the previous study [3] in the Cancer Institute and Hospital, Chinese Academy of Medical Sciences, Beijing, China, identified the clinicopathological features, and evaluated the correlation among different clinicopathological factors, immunophenotypes, and prognosis.

Materials and methods

Cases and clinical data

Included in this study were 88 pathologically diagnosed ENKTCL cases in the early stage (stages I and II) that were diagnosed in the Department of Pathology, Cancer Institute and Hospital, Chinese Academy of Medical Sciences between May 1999 and December 2013. The samples were collected from patients during routine diagnostic procedures with informed patients' consent. The study was approved by the Independent Ethics Committee of the Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China. Furthermore, the research protocol was in accordance with the Declaration of Helsinki. The clinical data were collected containing patients' age, gender, disease course, primary site, the extent of disease involvement, B symptoms, lactate dehydrogenase (LDH) levels, β2-microglobulin (β2-MG) levels, treatment approaches, therapeutic efficacy, and recurrence. All patients were followed up by phone, beginning from the date of pathological diagnosis. The reasons for discontinued follow-up were because of death, withdrawal, or termination of follow-up (October 2017). The following items were recorded during follow-up: the disease course, B symptoms, treatment, recurrence, status when follow-up was terminated (death, survival, or withdrawal), cause of death, and survival time (in months).

Histopathology

All specimens were fixed in 10% neutral formalin, paraffin-embedded, and sectioned into 4-μm-thick sections for hematoxylin and eosin (H&E) staining. The lymphoid hematopoietic tumors of 88 cases were classified under a light microscope according to WHO lymphoid hematopoietic tumor classification (published in 2008). The observation was made for the number of tumor cells, tumor cell distribution (diffused or scattered), size and morphology of tumor cells, vascular invasion, and the pro-epithelial or mucosal infiltration of tumor cells. Furthermore, some cases with relevant tissue necrosis, pseudoepitheliomatous hyperplasia of the mucosa, granuloma, and fungal infections were recorded.

Immunohistochemistry

Various antigenic markers in all cases were detected by high-throughput tissue microarray and immunohistochemistry (IHC) staining. The high-throughput tissue microarray was made from manual tissue arrayer MTA-1 (Beecher Instruments, Inc., USA). Immunohistochemical staining by the EnVision System (Dako Cytomation, Carpinteria, CA, USA) was applied. Positive controls were included in the immunohistochemical staining. Phosphate buffer saline (PBS) was used as negative controls. All primary antibodies used in the study are listed in Table 1.

The semi-quantitative analyses were scored as following: (1) positive intensity scoring: 0, no staining; 1, light yellow staining; 2, brownish yellow staining; and 3, brown staining. (2) Scoring based on the proportion of positive cells: 0, < 5%; 1, 5–25%; 2, 26–50%; 3, 51–75%, and 4, > 75%. The sum of the two scores was used as the final score for each case as follows: 0, negative (−); 1–4, weakly positive expression (+); 5–8, moderately positive expression (++); and 9–12, strongly positive expression (+++). Table 1 shows the corresponding location (e.g., nuclei, cell membrane, and cytoplasm) of the cells staining positive with different antibodies too. CD56, MLH1, PDGFRA, p53, VEGF, and CyclinD1 were interpreted according to that criterion. The positivity of PD-1 and PD-L1 expression by tumor cells was considered when more than 5% of all cell population was stained.

Table 1 Primary antibodies used in immunohistochemistry and antigen recognition sites

Antibody name	Clone number	Company	Positive location
CD56	UMAB83	Dako Cytomation, Carpinteria, CA, USA	Cell membrane
MLH1	ES05	Dako Cytomation, Carpinteria, CA, USA	Nuclei
PD-L1	SP142	Beijing Zhong Shan-Golden Bridge Biotechnology Co., Ltd., China	Cell membrane
PD-1	NAT	Fuzhou Maixin Biotech. Co., Ltd., China	Cytoplasm
CyclinD1	SP4	Fuzhou Maixin Biotech. Co., Ltd., China	Nuclei
p53	DO-7	Fuzhou Maixin Biotech. Co., Ltd., China	Nuclei
VEGF	Rabbit polyclonal antibody	Beijing Zhong Shan-Golden Bridge Biotechnology Co., Ltd., China	Cytoplasm
PDGFRA	Rabbit polyclonal antibody	Beijing Zhong Shan-Golden Bridge Biotechnology Co., Ltd., China	Cell membrane/cytoplasm
Ki-67	MIB-1	Fuzhou Maixin Biotech. Co., Ltd., China	Nuclei

The percentage of cells with Ki-67 expression was calculated from the number of malignant cells in the highest labeling field under high magnification (400×). The median percentage (50%) of Ki-67 expression was designated as a cutoff; thus, cases higher than the median value (≥ 50%) were defined as high Ki-67 expression, and less than the median value (< 50%) was defined as low Ki-67 expression for the survival analysis.

Statistical analysis

Data analysis and processing were done with SPSS19.0 software (SPSS Inc., Chicago, IL). The Kaplan-Meier method uses survival data summarized in table to prepare the survival curve. Multivariate analysis of potential prognostic factors was evaluated by Cox's proportional hazard regression and binary logistic regression models. Bivariate correlation analysis was used for crosstabs Chi-square test. $P < 0.05$ was considered as a statistically significant difference.

Results

Clinical features and follow-up

Among 88 cases with ENKTCL diagnosed by pathologists, the number of effective follow-up cases was 42 (47.7%) by the end of last follow-up in October 2017. Table 2 indicates the clinical data of these 42 patients. The ages of the patients were between 19 and 77 years old, with a median age of 41. ENKTCL was commonly seen in men, with the men to women ratio of 3.2:1. All tumors were localized in the upper aerodigestive tract and most commonly in the nasal cavity (31/42, 73.8%), followed by the nasopharynx (9/42, 21.4%) and oropharynx (2/42, 4.8%). Twenty-three (54.8%) out of those 42 ENKTCL patients had no B symptoms, and 19 (45.2%) of them had B symptoms (e.g., fever, night sweats, and progressive weight loss). Laboratory examinations revealed that eight patients (19.0%) had elevated serum LDH levels (240 UI/L), and 21 patients (50.0%) had elevated β2-MG levels (1.8 mg/L). All of these patients received treatment with different methods. Twenty-three of them (54.8%) treated with a combination of chemotherapy and radiotherapy (concurrent chemoradiotherapy), 18 (42.9%) underwent only radiotherapy, and one (2.4%) patient underwent only chemotherapy. Among 24 patients who received chemotherapy, 17 (70.8%) patients also received CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone)-based chemotherapy, while the others underwent personalized treatment. Complete remission (CR) was achieved in

Table 2 Clinical characteristics and univariate Kaplan-Meier survival analysis of 42 extranodal NK/T cell lymphoma patients

Characteristics	No. (%)	χ^2	*P* value
Gender		0.015	0.904
Male	32 (76.2)		
Female	10 (23.8)		
Age (range)	41 (mean)	1.947	0.163
< 41 years old	19 (45.2)		
≥ 41 years old	23 (54.8)		
Primary site		1.396	0.498
Nasal cavity	31 (73.8)		
Nasopharynx	9 (21.4)		
Oropharynx	2 (4.8)		
B symptoms		2.021	0.155
Absent	23 (54.8)		
Present	19 (45.2)		
LDH range		7.593	0.006
< 240UI/L	34 (81.0)		
≥ 240UI/L	8 (19.0)		
β2-MG		3.633	0.057
< 1.8 mg/L	21 (50.0)		
≥ 1.8 mg/L	21 (50.0)		
Treatment approach		9.098	0.011
Radiotherapy	18 (42.9)		
Chemotherapy	1 (2.4)		
Concurrent chemoradiotherapy	23 (54.8)		

LDH lactate dehydrogenase, *β2-MG* β2-microglobulin

85.7% (36/42) of patients after treatment. There were six recurrent cases (14.3%). Twenty-seven (64.3%) of the 42 patients survived and 15 (35.7%) died. The survival time of the 42 patients ranged from 0 to 153 months. The mean survival time was 60.1 months. One-, two-, and three-year survival rates were 85.7% (36/42), 78.6% (33/42), and 71.4% (30/42), respectively.

The univariate survival analysis included Kaplan-Meier plots and Log-rank tests to analyze the correlations between patient gender, age, primary site, the presence of B symptoms, pretreatment LDH and β2-MG levels, treatment approaches, and prognosis. The results showed that the high lactate dehydrogenase level (LDH ≥ 240 UI/L) before treatment (P = 0.006) and different treatment approaches (P = 0.011) were statistically significant associated with patient prognosis (Table 2). Specifically, radiotherapy alone had the best treatment effect, followed by concurrent chemoradiotherapy, and the worst was chemotherapy alone (Fig. 1a, b).

Histopathological features

Microscopically, the morphology of ENKTCL at different localizations was similar. ENKTCL is characterized by the background of coagulation necrosis and the mixed infiltration of a variety of inflammatory cells (e.g., small lymphocytes, tissue cells, eosinophils, and plasma cells) and atypical lymphoid cells (ALCs) were scattered or diffusely distributed (Fig. 2a). Small vessel fibrinoid necrosis and vasculitis were found adjacent to regions of ulceration and tissue necrosis [4].

Immunohistochemical stain

As shown in Table 3, cluster of differentiation (CD) 56, MLH1, platelet-derived growth factor receptor-alpha (PDGFRA), programmed cell death ligand 1 (PD-L1), and p53 were highly expressed, with staining rates of 73.8% (31/42), 66.7%(28/42), 73.8% (31/42), 59.5% (25/42), and 69.0% (29/42), respectively (Fig. 2b–f). On the other hand, programmed cell death 1 (PD-1) expression rate in the ENKTCL specimens was very low (2.4%) (Fig. 2g). Vascular endothelial growth factor (VEGF) (26.2%) and CyclinD1 were also expressed in some samples (47.6%) (Fig. 2h, i). In this study, we used 50% as the threshold to divide Ki-67 positive specimens into two groups: underexpression group (< 50%) and overexpression group (50%). Sixteen samples (38.1%) overexpressed Ki-67. Univariate Kaplan-Meier analysis and Log-rank tests were used to analyze the correlation between all positively expressed biomarkers and prognosis in the 42 patients with ENKTCL. PDGFRA and PD-L1 expression was significantly associated with a poor prognosis (P = 0.040 and 0.007, respectively; Fig. 1c, d). Ki-67 overexpression (≥ 50%) was also significantly correlated with a poor prognosis (P = 0.017; Fig. 1e).

Multivariate analyses

The Cox proportional hazard regression and binary logistic regression models were achieved for multivariate prognostic analyses. The results showed that the LDH level and the treatment methods were not independent prognostic factors for ENKTCL. But multivariate survival analysis showed that the expression levels of PDGFRA, PD-L1, and Ki-67 were independent factors in the prognosis of patients with ENKTCL. Furthermore, the positive expression of these three markers was the risk factors for prognosis of patients with ENKTCL (PDGFRA: P = 0.045, HR = 8.265, 95% CI: 1.050–65.054; PD-L1: P = 0.005, HR = 9.369, 95% CI: 1.950–45.003; Ki-67: P = 0.023, HR = 3.545, 95% CI: 1.187–10.585) (Table 4).

Discussion

In many retrospective studies, LDH has been identified as a valuable predictive and/or prognostic biomarker for different types of carcinoma. Lactate dehydrogenase (LDH), as a key enzyme in the glycolysis pathway, catalyzes the redox reaction between pyruvate and lactic acid in the body and maintains the sustained production of glycolytic ATP [5]. It is known that specific changes in the tumor microenvironment and hypoxia can enhance glycolysis, which becomes a major source for the production of ATP in cancer cells. It is confirmed that LDH plays a crucial role in tumor maintenance and that serum LDH is commonly increased in patients with hematopoietic malignancies, such as Hodgkin's lymphoma (HL), non-Hodgkin's lymphoma (NHL), or multiple myeloma [5, 6]. LDH is one of the risk factors included in the International Prognostic Index (IPI), and it is considered a strong predictor of survival of patients with ENKTCL [7, 8]. In this study, the univariate Kaplan-Meier analysis showed that the elevation of serum lactate dehydrogenase (LDH ≥ 240 UI/L) before treatment was associated with poor prognosis, which could be used as adjunct indexes to the prognosis. However, multivariate prognostic analyses showed that it was not independent factors for the prognosis of patients with ENKTCL. This could be correlated with the different cut-off levels applied in the different studies.

Li et al. [9] conducted the study of a meta-analysis of 11 randomized controlled trials including 871 ENKTCL Chinese patients with early-stage (IE-IIE) disease. They showed that the therapeutic effects of radiotherapy alone in the early clinical stages of ENKTCL (I/IIE) were good. Despite an apparent good initial overall response rate (ORR) and complete response (CR) rate, the use of radiotherapy alone is associated with an incidence of systemic relapse that is alarmingly high for early-stage patients [10]. Therefore, radiotherapy alone is needed to be combined with chemotherapy in an appropriate

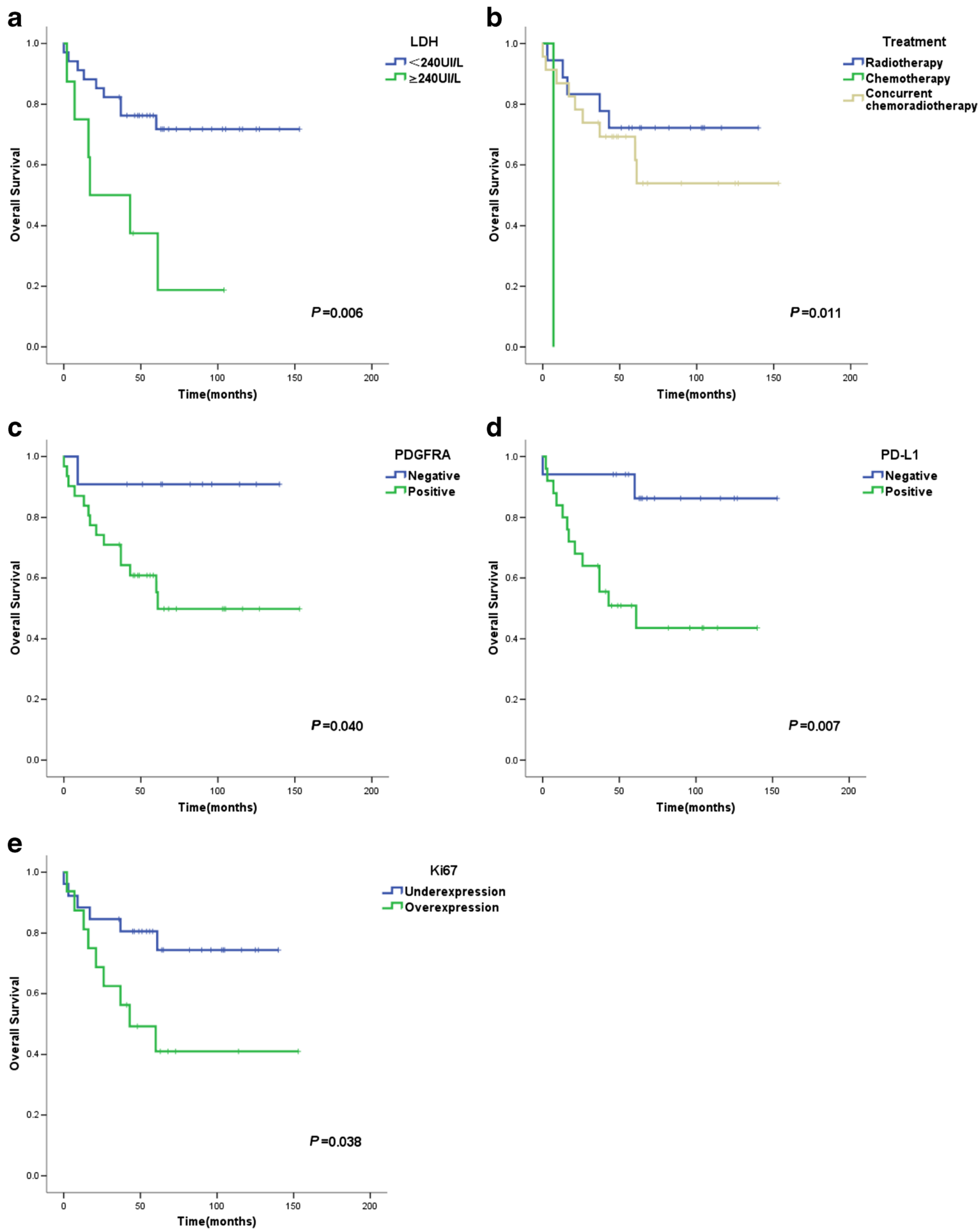

Fig. 1 Kaplan-Meier survival curves. Survival following **a** LDH and **b** different treatment approaches are shown. The effects of expression of **c** PDGFRA, **d** PD-L1, and **e** Ki-67 on survival are also compared

sequence [11]. Among the combined treatment modalities, concurrent chemoradiotherapy (CCRT) using multidrug resistance (MDR) unrestricted agents [12] and radiation sensitizers [13] can improve the CR rate to 82% and 77%, respectively. In the current work, single-factor survival analysis and log-rank tests indicated that different treatment methods were associated with patient prognosis ($P = 0.011$). Specifically, radiotherapy alone had the best treatment effect, followed by concurrent chemoradiotherapy, and the worst was chemotherapy alone. The reason why the result of this study is contrary to

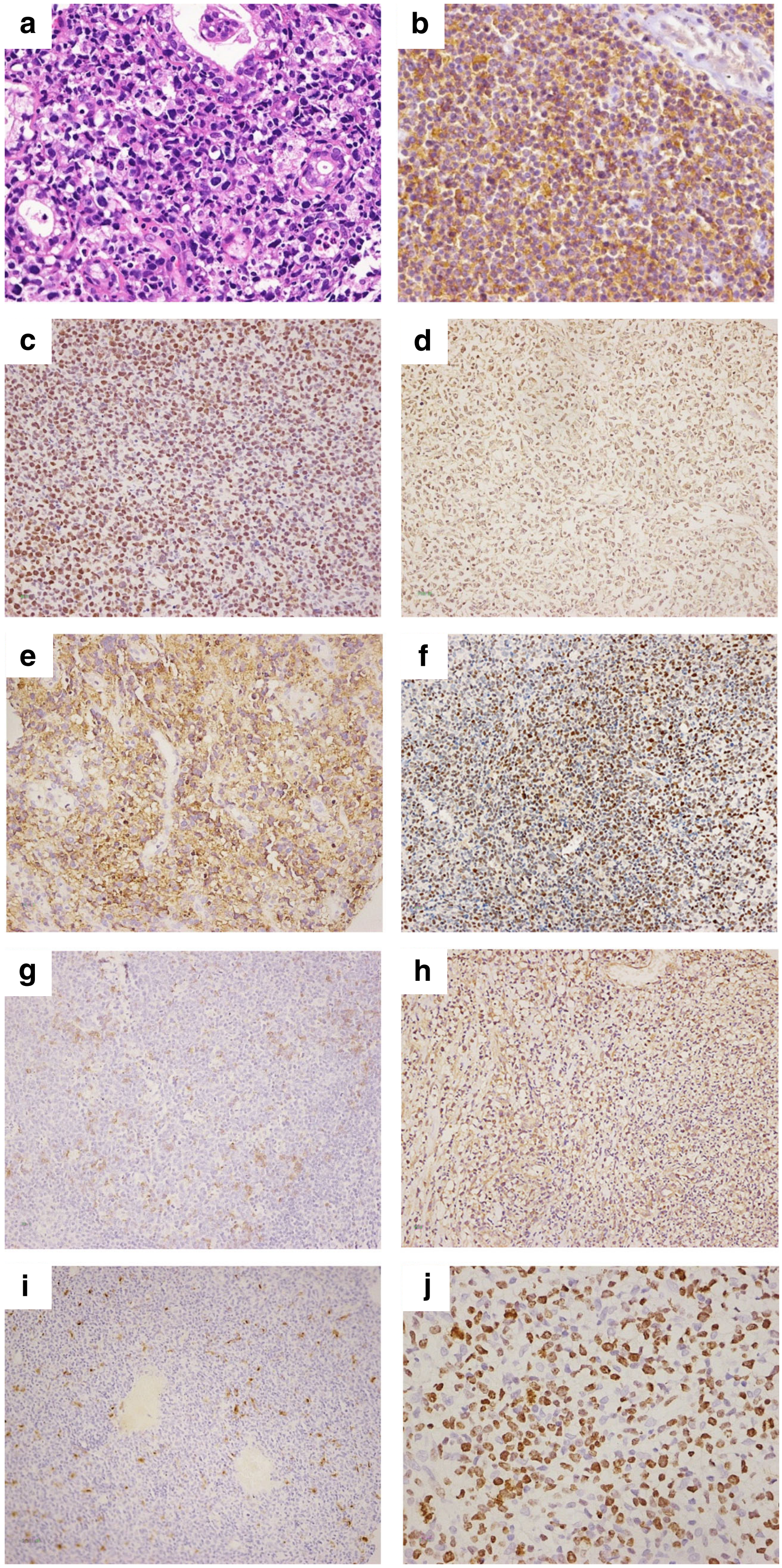

Fig. 2 Histopathology of extranodal natural killer /T cell lymphoma. **a** H&E staining of extranodal natural killer /T cell lymphoma. Immunoreactivity for CD56 (**b**), MLH1 (**c**), PDGFRA (**d**), PD-L1 (**e**), p53 (**f**), PD1 (**g**), VEGF (**h**), CyclinD1 (**i**), and Ki-67 (**j**). Magnification: × 200 for **b**–**i**); × 400 for **a**, **j**

the above studies may be that most ENKTCL patients in this study received CHOP regimen. However, the optimal treatment of early stages nasal ENKTCL remains to be determined. The multivariate analysis showed that the treatment approach was not independent prognostic factors for ENKTCL. Considering that the incidence of this disease is small and the sample size is too small to influence the prognosis of survival analysis, it is necessary to confirm the results in large-scale studies.

So far, ENKTCL has no standard treatment plan and its efficacy and prognosis are poor. One of the reasons may be that lymphoma cells escape the organism's immune surveillance and kill through some mechanism [14]. The programmed cell death 1 (PD-1)/PD-1 ligand 1 (PD-L1) pathway plays an important role as immune checkpoint contributing to the maintenance of peripheral tolerance and control of excessive immune responses to prevent harmful tissue damage [15]. PD-1 is expressed in immune cells, particularly in T cells following T cell activation [16], whereas PD-L1 is expressed in variable immune and non-immune cells including antigen-presenting cells (APCs), epithelial cells, and mesenchymal cells [17]. PD-1 delivers inhibitory signals to T cells upon engagement by PD-Ls and suppresses T cell activation and effector function, thereby inducing T cell exhaustion [18]. Cancer utilizes the PD1/PD-L pathway to escape antitumor immunity by suppressing T cells, particularly CD8+ T cell response against the tumor [19]. Many previous studies [20, 21] have found that high expression of PD-L1 was found in liver cancer, gastric cancer, melanoma, renal cancer, and other solid tumors, as well as multiple myeloma, leukemia, myelodysplastic syndrome, and other hematological tumors. The expression of PD-L1 in solid cancers was associated with poorer outcomes [22, 23]. Many reports suggest that the PD-1/PD-L1 axis is a reasonable target for therapeutic intervention [24]. It has been reported that blockade of PD-1 or PDL1 by monoclonal antibodies may lead to significant antitumor effects in malignancies [25, 26]. However, the prognosis with respect to PD-L1 expression in lymphoproliferative disease has not been determined [27–29]. Thus, to determine the biological significance of the PD-1/PD-L1 pathway and its potential as a therapeutic target in ENKTL, we investigated immunohistochemically the expression of PD-1 and PD-L1 in ENKTL tissues and analyzed the results according to clinicopathological features and patient outcome.

In our study, PD-1 expression rates in ENKTL tumor cells were very low (2.4%), and PD-L1 expression rates of 59.5% were observed. The reason for this may be that PD-1 as a cell surface receptor belonging to the immunoglobulin superfamily is expressed on T cells and pro-B cells, while it is not expressed on NK cell [30]. Univariate and multivariate survival analysis showed that the expression status of PD-L1 was an independent risk factor in the prognosis of patients with ENKTCL ($P = 0.005$, HR = 9.369, 95% CI: 1.950–45.003). It suggested that PD-L1 is highly expressed in ENKTCL and has a certain clinical significance. It is further speculated that PD-L/PD-1 signaling pathway may be involved in the immune escape of ENKTCL, which provides an important clue to reveal the mechanism of immune deficiency in ENKTCL

Table 3 Immunohistochemical expression and univariate analysis of prognostic factors for 42 extranodal NK/T cell lymphoma patients

Markers	No. (%)	χ^2	*P* value
CD56		0.007	0.935
Positive	31 (73.8)		
Negative	11 (26.2)		
MLH1		0.006	0.940
Positive	28 (66.7)		
Negative	14 (33.3)		
PDGFRA		4.207	0.040
Positive	31 (73.8)		
Negative	11 (26.3)		
VEGF		2.139	0.144
Positive	11 (26.2)		
Negative	31 (73.8)		
PD-L1		7.317	0.007
Positive	25 (59.5)		
Negative	17 (40.5)		
PD-1		0.598	0.439
Positive	1 (2.4)		
Negative	41 (97.6)		
Cyclin D1		0.526	0.468
Positive	20 (47.6)		
Negative	22 (52.4)		
p53		2.405	0.121
Positive	29 (69.0)		
Negative	13 (31.0)		
Ki-67		4.312	0.038
Overexpression (≥ 50%)	16 (38.1)		
Under expression (< 50%)	26 (61.9)		

Table 4 Multivariate survival analyses of 42 extranodal NK/T cell lymphoma patients

Item	Overall survival		
	HR	95%CI	*P* value
LDH	1.360	0.417–4.436	0.610
Treatment approaches	2.241	0.734–6.844	0.157
PDGFRA	8.265	1.050–65.054	0.045
PD-L1	9.369	1.950–45.003	0.005
Ki-67	3.545	1.187–10.585	0.023

cells. It is expected to become a new target for the immunotherapy of ENKTCL.

Platelet-derived growth factor receptor (PDGFR) belongs to the class III receptor tyrosine kinases (RTKs). PDGFRA known to interact with PI3K/AKT and STAT proteins mediates important cell functions, such as migration, proliferation, and cell survival. PDGFRA plays an important role in growth, development, repair, and other physiological processes [31]. PDGFRA as an angiogenic factor is particularly relevant in tumorigenesis and progression in PDGF/PDGFRA pathway. A number of studies on the prognostic significance of PDGFRA expression in various human malignant solid tumors including gastrointestinal stromal tumors (GISTs) [32], glioblastoma [33], and gastric cancer [34] have been published. Studies have demonstrated that PDGFRA expression is also highly expressed in T cell lymphoma, including angioimmunoblastic lymphoma, lymphocytic leukemia, and T/NK cell-derived lymphoma [35, 36]. Huang et al. [37] have shown overexpression of PDGFRA in ENKTCL and imatinib-induced inhibition of PDGFRA expression in MEC04 cells, suggesting that PDGFRA could be a therapeutic target. Li et al. [38] demonstrated that high expression of PDGFRA was evaluated to be correlated with worse progression-free survival (PFS) and was a statistically independent prognostic factor for patients' PFS.

In the current study, our results showed that OS was poor for patients with high PDGFRA expression. In addition, multivariate analysis showed that high PDGFRA expression had an independent prognostic influence in patients with ENKTCL. Our results were consistent with the above-mentioned previous reports. PDGFRA plays a vital role in the pathogenesis of NKTCL. It may be a target of anti-angiogenic therapy for NKTCL and provide a new way for treatment of ENKTCL patients. However, there were some limitations in this study. A limited number of patients were enrolled with incomplete information, which could have resulted in selection bias. Follow-up periods were also short, and the study was a retrospective single center analysis. Therefore, a large prospective study is required to confirm the prognostic significance of PDGFRA in ENKTCL.

Ki-67 is a nuclear and nucleolar protein antigen present in proliferating cells during the G1 to M phases of the cell cycle, but is undetected in resting cells. The Ki-67 expression evaluated through immunohistochemistry is useful as one of the most reliable markers of the proliferative status of cancer cells [39]. In the recent years, a number of studies have found that the expression of ki-67 was closely related to the prognosis of lymphoma, but the prognostic value was different in various subtypes of lymphoma. He et al. [40] conducted a meta-analysis that pooled data from 27 relevant studies to explore the correlation between Ki-67 expression and the survival outcome in lymphoma. The results revealed that high Ki-67 expression in patients with lymphoma was associated with worse prognosis. Subgroup analysis on the different subtypes of lymphoma suggested that the association between high Ki-67 expression and OS in Hodgkin lymphoma was absent, while high Ki-67 expression was highly associated with worse OS for non-Hodgkin lymphoma and its various subtypes, including NK/T lymphoma, DLBCL, and MCL.

Kim et al. first studied the prognostic value of Ki-67 in ENKTL in 2007. The OS rate and disease-free survival rate in patients with ki-67 overexpression ($\geq 65\%$) were significantly lower than those in patients with ki-67 underexpression ($< 65\%$). Ki-67 expression is predictive of prognosis and became a useful tool for predicting prognosis in patients with stage I/II ENKTCL [41]. Subsequent studies also confirmed that Ki-67 high expression ($\geq 50\%$) is associated with lower OS and PFS ($P =$ 0.002, 0.027), and Ki-67 high expression may be an independently prognostic factor for ENKTCL [42, 43].

In our present study, we use 50% as the threshold to divide the Ki-67 positive specimens into the under-expression group ($< 50\%$) and the overexpression group (50%). Sixteen of the valuable samples (38.1%) highly expressed Ki-67. Univariate and multivariate prognostic analyses were performed and found that Ki-67 was an independent risk factor for ENKTCL ($P = 0.023$, HR = 3.545, 95% CI: 1.187–10.585). Consistent with the above literature, it is clear that it is necessary to combine Ki-67 as a biological index with clinical factors to establish a prognostic model for ENKTCL.

In conclusion, our study suggests that the elevation of serum LDH and the treatment approach were associated with poor prognosis, which could be used as adjunct indexes to the prognosis. However, they were not independent factors for the prognosis of patients with ENKTCL. In addition, the expression levels of PDGFRA, PD-L1, and Ki-67 were independent factors in the prognosis of patients with ENKTCL and these 3 proteins were risk factors of prognosis. The above markers combined with clinical factors may establish the prognosis model of ENKTCL. Since the sample size of this study is smaller, these results should be confirmed in a large prospective study.

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Compliance with ethical standards

All patients or their guardians provided written informed consent to allow collection of personal data in accordance with the Declaration of Helsinki.

Conflicts of interests The authors declare that they have no conflicts of interest

References

1. Pongpruttipan T, Sukpanichnant S, Assanasen T, Wannakrairot P, Boonsakan P, Kanoksil W, Kayasut K, Mitarnun W, Khuhapinant A, Bunworasate U, Puavilai T, Bedavanija A, Garcia-Herrera A, Campo E, Cook JR, Choi J, Swerdlow SH (2012) Extranodal NK/T-cell lymphoma, nasal type, includes cases of natural killer cell and alphabeta, gammadelta, and alphabeta/gammadelta T-cell origin: a comprehensive clinicopathologic and phenotypic study. Am J Surg Pathol 36(4):481–499
2. Au WY, Weisenburger DD, Intragumtornchai T, Nakamura S, Kim WS, Sng I, Vose J, Armitage JO, Liang R, for the International Peripheral T-Cell Lymphoma Project (2009) Clinical differences between nasal and extranasal natural killer/T-cell lymphoma: a study of 136 cases from the international peripheral T-cell lymphoma project. Blood 113(17):3931–3937
3. Zeng LS, Huang WT, Qiu T, Shan L, Guo L, Ying JM, Lyu N, Feng XL (2017) Correlation between the clinicopathological features and prognosis in patients with extranodal natural killer/T cell lymphoma. Chronic Dis Transl Med 3(4):252–259
4. Asano N, Kato S, Nakamura S (2013) Epstein-Barr virus-associated natural killer/T-cell lymphomas[J]. Best Pract Res Clin Haematol 26(1):15–21
5. Augoff K, Hryniewicz-Jankowska A, Tabola R (2015) Lactate dehydrogenase 5: an old friend and a new hope in the war on cancer. Cancer Lett 358(1):1–7
6. Lu R, Jiang M, Chen Z, Xu X, Hu H, Zhao X, Gao X, Guo L (2013) Lactate dehydrogenase 5 expression in non-Hodgkin lymphoma is associated with the induced hypoxia regulated protein and poor prognosis. PLoS One 8(9):e74853
7. Hanakawa H, Orita Y, Sato Y, Takao S, Marunaka H, Morishita T, Yamashita Y, Hori Y, Domae S, Inokuchi I, Akagi S, Kondo E, Iwaki N, Motomiya K, Okumura H, Yoshino T, Nishizaki K (2014) Novel and simple prognostic index for nasal natural killer/T-cell lymphoma. Head Neck 36(4):551–556
8. Niu SQ, Yang Y, Li YY, Wen G, Wang L, Li ZM, Wang HY, Zhang LL, Xia YF, Zhang YJ (2016) Primary site and regional lymph node involvement are independent prognostic factors for early-stage extranodal nasal-type natural killer/T cell lymphoma. Chin J Cancer 35:34
9. Li H, Wang CS, Wang XD (2014) Meta analysis of treatment for stage IE~IIE extranodal natural killer /T cell lymphomas in China. Asian Pac J Cancer Prev 15(5):2297–2302
10. Tse E, Kwong YL (2015) Nasal NK/T-cell lymphoma: RT, CT, or both. Blood 126(12):1400–1401
11. Tse E, Kwong YL (2017) The diagnosis and management of NK/T-cell lymphomas. J Hematol Oncol 10(1):85
12. Yamaguchi M, Suzuki R, Oguchi M, Asano N, Amaki J, Akiba T, Maeda T, Itasaka S, Kubota N, Saito Y, Kobayashi Y, Itami J, Ueda K, Miyazaki K, Ii N, Tomita N, Sekiguchi N, Takizawa J, Saito B, Murayama T, Ando T, Wada H, Hyo R, Ejima Y, Hasegawa M, Katayama N (2017) Treatments and outcomes of patients with Extranodal natural killer/T-cell lymphoma diagnosed between 2000 and 2013: a cooperative study in Japan. J Clin Oncol 35(1):32–39
13. Yamaguchi M, Tobinai K, Oguchi M, Ishizuka N, Kobayashi Y, Isobe Y, Ishizawa K, Maseki N, Itoh K, Usui N, Wasada I, Kinoshita T, Hotta T, Tsukasaki K, Oshimi K (2012) Concurrent chemoradiotherapy for localized nasal natural killer/T-cell lymphoma: an updated analysis of the Japan clinical oncology group study JCOG0211. J Clin Oncol 30(32):4044–4046
14. Shi L, Chen S, Yang L, Li Y (2013) The role of PD-1 and PD-L1 in T-cell immune suppression in patients with hematological malignancies. J Hematol Oncol 6(1):74
15. Pardoll DM (2012) The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12(4):252–264
16. Lyford-Pike S, Peng S, Young GD, Taube JM, Westra WH, Akpeng B, Bruno TC, Richmon JD, Wang H, Bishop JA, Chen L, Drake CG, Topalian SL, Pardoll DM, Pai SI (2013) Evidence for a role of the PD-1:PD-L1 pathway in immune resistance of HPV-associated head and neck squamous cell carcinoma. Cancer Res 73(6):1733–1741
17. Carter L, Fouser LA, Jussif J et al (2002) PD-1:PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2. Eur J Immunol 32(3):634–643
18. Droeser RA, Hirt C, Viehl CT, Frey DM, Nebiker C, Huber X, Zlobec I, Eppenberger-Castori S, Tzankov A, Rosso R, Zuber M, Muraro MG, Amicarella F, Cremonesi E, Heberer M, Iezzi G, Lugli A, Terracciano L, Sconocchia G, Oertli D, Spagnoli GC, Tornillo L (2013) Clinical impact of programmed cell death ligand 1 expression in colorectal cancer. Eur J Cancer 49(9):2233–2242
19. Mamalis A, Garcha M, Jagdeo J (2014) Targeting the PD-1 pathway: a promising future for the treatment of melanoma. Arch Dermatol Res 306(6):511–519
20. Ghebeh H, Mohammed S, Al-Omair A et al (2006) The B7-H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. Neoplasia 8(3):190–198
21. Sznol M, Chen L (2013) Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. Clin Cancer Res 19(5):1021–1034
22. Huang B, Chen L, Bao C et al (2015) The expression status and prognostic significance of programmed cell death 1 ligand 1 in gastrointestinal tract cancer: a systematic review and meta-analysis. Onco Targets Ther 8:2617–2625
23. Wu P, Wu D, Li L et al (2015) PD-L1 and survival in solid tumors: a Meta-analysis. PLoS One 10(6):e131403
24. Hamid O, Carvajal RD (2013) Anti-programmed death-1 and anti-programmed death-ligand 1 antibodies in cancer therapy. Expert Opin Biol Ther 13(6):847–861
25. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366(26):2443–2454
26. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, Schuster SJ, Millenson MM, Cattry D, Freeman GJ, Rodig SJ, Chapuy B, Ligon AH, Zhu L, Grosso JF, Kim SY, Timmerman JM, Shipp MA, Armand P (2015) PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med 372(4):311–319
27. Hawkes EA, Grigg A, Chong G (2015) Programmed cell death-1 inhibition in lymphoma. Lancet Oncol 16(5):e234–e245
28. Kim WY, Jung HY, Nam SJ, Kim TM, Heo DS, Kim CW, Jeon YK (2016) Expression of programmed cell death ligand 1 (PD-L1) in advanced stage EBV-associated extranodal NK/T cell lymphoma is associated with better prognosis. Virchows Arch 469(5):581–590
29. Nagato T, Ohkuri T, Ohara K, Hirata Y, Kishibe K, Komabayashi Y, Ueda S, Takahara M, Kumai T, Ishibashi K, Kosaka A, Aoki N, Oikawa K, Uno Y, Akiyama N, Sado M, Takei H, Celis E, Harabuchi Y, Kobayashi H (2017) Programmed death-ligand 1 and its soluble form are highly expressed in nasal natural killer/T-cell lymphoma: a potential rationale for immunotherapy. Cancer Immunol Immunother 66(7):877–890
30. Jo JC, Kim M, Choi Y, Kim HJ, Kim JE, Chae SW, Kim H, Cha HJ (2017) Expression of programmed cell death 1 and programmed

cell death ligand 1 in extranodal NK/T-cell lymphoma, nasal type. Ann Hematol 96(1):25–31
31. Board R, Jayson GC (2005) Platelet-derived growth factor receptor (PDGFR): a target for anticancer therapeutics. Drug Resist Updat 8(1–2):75–83
32. Yan W, Zhang A, Powell MJ (2016) Genetic alteration and mutation profiling of circulating cell-free tumor DNA (cfDNA) for diagnosis and targeted therapy of gastrointestinal stromal tumors. Chin J Cancer 35(1):68
33. Karsy M, Neil JA, Guan J et al (2015) A practical review of prognostic correlations of molecular biomarkers in glioblastoma. Neurosurg Focus 38(3):E4
34. Kurokawa Y, Matsuura N, Kawabata R et al (2014) Prognostic impact of major receptor tyrosine kinase expression in gastric cancer. Ann Surg Oncol 21(Suppl 4):S584–S590
35. Piccaluga PP, Agostinelli C, Califano A, Carbone A, Fantoni L, Ferrari S, Gazzola A, Gloghini A, Righi S, Rossi M, Tagliafico E, Zinzani PL, Zupo S, Baccarani M, Pileri SA (2007) Gene expression analysis of angioimmunoblastic lymphoma indicates derivation from T follicular helper cells and vascular endothelial growth factor deregulation. Cancer Res 67(22):10703–10710
36. Huang Q, Snyder DS, Chu P, Gaal KK, Chang KL, Weiss LM (2011) PDGFRA rearrangement leading to hyper-eosinophilia, T-lymphoblastic lymphoma, myeloproliferative neoplasm and precursor B-cell acute lymphoblastic leukemia. Leukemia 25(2):371–375
37. Huang Y, de Reynies A, de Leval L, Ghazi B, Martin-Garcia N, Travert M, Bosq J, Briere J, Petit B, Thomas E, Coppo P, Marafioti T, Emile JF, Delfau-Larue MH, Schmitt C, Gaulard P (2010) Gene expression profiling identifies emerging oncogenic pathways operating in extranodal NK/T-cell lymphoma, nasal type. Blood 115(6):1226–1237
38. Li J, Chen P, Liu W, Xia Z, Shi F, Zhong M (2016) Expression and significance of Ku80 and PDGFR-alpha in nasal NK/T-cell lymphoma. Pathol Res Pract 212(3):204–209
39. Czyzewska J, Guzinska-Ustymowicz K, Lebelt A et al (2004) Evaluation of proliferating markers Ki-67, PCNA in gastric cancers. Rocz Akad Med Bialymst 49(Suppl 1):64–66
40. He X, Chen Z, Fu T, Jin X, Yu T, Liang Y, Zhao X, Huang L (2014) Ki-67 is a valuable prognostic predictor of lymphoma but its utility varies in lymphoma subtypes: evidence from a systematic meta-analysis. BMC Cancer 14:153
41. Kim SJ, Kim BS, Choi CW, Choi J, Kim I, Lee YH, Kim JS (2007) Ki-67 expression is predictive of prognosis in patients with stage I/II extranodal NK/T-cell lymphoma, nasal type. Ann Oncol 18(8):1382–1387
42. Huang X, Sun Q, Fu H, Zhou X, Guan X, Wang J (2014) Both c-Myc and Ki-67 expression are predictive markers in patients with extranodal NK/T-cell lymphoma, nasal type: a retrospective study in China. Pathol Res Pract 210(6):351–356
43. Jiang L, Li P, Wang H, Liu J, Zhang X, Qiu H, Zhang B (2014) Prognostic significance of Ki-67 antigen expression in extranodal natural killer/T-cell lymphoma, nasal type. Med Oncol 31(10):218