

# L-asparaginase-based regimen as a first-line treatment for newly diagnosed nasal type extranodal natural killer cell/T-cell lymphoma

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Received December 12, 2014; Accepted December 15, 2015

DOI: 10.3892/etm.2016.3249

**Abstract.** The aim of the present study was to compare the efficacy of an L-asparaginase-based regimen and a CHOP regimen followed by radiotherapy as first-line treatments for newly diagnosed nasal type extranodal natural killer cell/T-cell lymphoma (ENKTL). A total of 69 patients received the CHOP regimen as the first-line treatment and 112 patients received the L-asparaginase-based regimen. All patients received radical radiotherapy following two cycles of chemotherapy. The overall response rates of the L-asparaginase-based and CHOP treatment groups were 90.18 and 72.46%, respectively ( $P=0.002$ ). The one, two, and five-year overall survival (OS) rates and progression-free survival (PFS) rates of the L-asparaginase group were 96.0, 88.3, 65.1, 94.2, 79.8 and 50.0%, respectively. The one, two, and five-year OS and PFS rates of the CHOP group were 82.6, 61.9, 25.8, 63.8, 44.0 and 21.0%, respectively ( $P<0.001$ ). Compared with CHOP treatment, L-asparaginase-based chemotherapy combined with radiotherapy was a safe and highly effective treatment for newly diagnosed ENKTL.

## Introduction

Extranodal natural killer (NK) cell/T-cell lymphoma (ENKTL) is a type of non-Hodgkin's lymphoma that presents significantly different distributions patterns according to population

and geography, and is relatively common in Asia and South America (1,2). The incidence of patients with lymphoma is as high as 11% in China (3). NK/T-cell lymphomas usually occur in middle-aged patients, and their features are characterized by localized disease in approximately two-thirds of patients, along with frequent adjacent tissue invasion, a high frequency of 'B' symptoms, despite apparently limited disease, and rare bone marrow involvement (4-6). In addition, ENKTL is highly invasive, resistant to conventional chemotherapy, highly malignant, associated with short survival time and rapid clinical progression, and has a relatively poor prognosis (7-9).

ENKTL is thought to arise from NK cells or occasionally from a subset of  $\gamma\delta$  or  $\alpha\beta$  cytotoxic T-cells, and is strongly associated with Epstein-Barr virus infection (10,11). The ENKTL phenotype is characterized by the expression of cytoplasmic CD3 $\epsilon$  in the absence of surface CD3, T-cell receptor expression, or T-cell receptor gene rearrangement, as well as an activated cytotoxic profile with expression of perforin, granzyme B, TIA-1, and frequent CD56 expression (12,13).

As ENKTL is rare, and prospective group studies are limited, no standard treatment regimen is currently available. Early NK/T-cell lymphoma is sensitive to radiotherapy if confined to the nasal cavity; however, recurrence at the primary site or distant sites occurred in 50% of patients who received radiotherapy, with a five-year survival rate of 29.8-66% (14-17). Since the multidrug resistance (MDR) gene is highly expressed in ENKTL tumor cells, the tumors are insensitive to conventional chemotherapy regimens (such as CHOP) that are applied to other pathological types of non-Hodgkin's lymphoma (18). Asparaginase is not regulated by the MDR gene, and numerous studies have demonstrated that the application of asparaginase to relapsed or refractory ENKTL induced promising responses and high survival rates (19-22). Few clinical studies on asparaginase-based regimens as first-line treatments for newly diagnosed ENKTL are available (22,23), and, to the best of our knowledge, no previous studies have compared the short-term efficacy and long-term survival impacts of asparaginase and CHOP treatment regimens in patients with ENKTL. Therefore, the present study investigated the effects of asparaginase in

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**Key words:** extranodal natural killer cell/T-cell lymphoma, CHOP, L-asparaginase, treatment

patients with previously untreated ENKTL. The clinical characteristics of 181 patients with newly-diagnosed pathologically ENKTL were retrospectively analyzed. The patients were divided into CHOP treatment regimen and asparaginase-based chemotherapy groups according to the administered treatment regimen. Short-term efficacy, long-term survival, and safety were compared in the two groups and the results are reported herein.

## Materials and methods

**Clinical data.** A total of 181 patients with newly diagnosed ENKTL received either CHOP treatment or L-asparaginase-based chemotherapy along with radiotherapy between October 2003 and June 2013 at the Henan Tumor Hospital, First Affiliated Hospital of Henan University of Science and Technology (Luoyang, China), the Xinzheng City People's Hospital (Xinzheng, China), and the Jiaozuo City Second People's Hospital (Jiaozuo, China). Of these patients, 69 were included in the CHOP treatment group and 112 were included in the L-asparaginase treatment group. All patients were diagnosed via imaging studies and pathological analyses. Differences in the main clinical characteristics between the two groups of patients were not statistically significant ( $P > 0.05$ ), as shown in Table I.

**Prognostic factors.** The International Prognostic Index (IPI) clinical tool was used to predict the prognosis of patients (24). IPI factors include: Age,  $>60$  years; serum lactate dehydrogenase (LDH) level above normal; performance status, 2–4; stage of disease, III or IV; and extranodal involvement,  $>1$  site (24). In addition, the gender, B-symptoms (including unexplained fever,  $>10\%$  weight loss or sweating), Eastern Cooperative Oncology Group performance status and primary anatomic sites of lymphomatous involvement for all patients were evaluated for their potential prognostic importance. The serum LDH and  $\beta_2$ -microglobulin levels were analyzed using an automated biochemical analyzer (Modular P800; Hitachi, Ltd., Tokyo, Japan), and the expression of Ki67 in ENKTL tumor samples were assessed by immunohistochemistry. Briefly, the tumor samples were cut into sections using a microtome (Leica RM2235; Leica Microsystems GmbH, Wetzlar, Germany), followed by incubation with mouse anti-Ki67 monoclonal antibody (1:200; cat. no. M7240; Dako, Glostrup, Denmark), and then ready-to-use EnVision reagent (cat. no. K5007; Dako). Subsequently, positive cells (yellow/tan color) were counted in 10 fields of view under a microscope (Olympus IX81; Olympus Corporation, Tokyo, Japan). Phosphate-buffered saline was used as a negative control.

**Treatment.** The CHOP treatment regimen included  $750 \text{ mg/m}^2$  ( $d_1$ ) cyclophosphamide (Baxter Oncology GmbH, Halle, Germany),  $50 \text{ mg/m}^2$  ( $d_1$ ) adriamycin (Pfizer, New York, USA),  $1.4 \text{ mg/m}^2$  ( $d_1$ ) vincristine (Zhejiang Hisun Pharmaceutical, Co., Ltd., Taizhou, China) and  $40 \text{ mg/m}^2$  ( $d_{1-5}$ ) prednisone (Zhejiang Xianju Pharmaceutical Co., Ltd., Taizhou, China), which was administered to 69 patients. L-asparaginase (Guangzhou Baiyun Shan Ming Xing Pharmaceutical, Co., Ltd., Guangzhou, China) treatment was given to 112 patients. The patients who received L-asparaginase

treatment were subdivided into the following treatment groups: CHOP-L (65 patients), VLP (22 patients), COP-L (10 patients), or L-asparaginase alone (15 patients). The patients in the CHOP-L group received the entire CHOP treatment regimen, whereas the patients in the VLP group received the vincristine and prednisone only, and the patients in the COP-L group received the cyclophosphamide, vincristine and prednisone only. All patients received  $6,000 \text{ U/m}^2$  ( $d_{1-7}$ ) L-asparaginase and the other drugs at the reference doses described for the CHOP treatment. The patients who responded positively to an L-asparaginase skin test (25 patients), which was indicated by the presence of erythemas or wheals, were treated with  $2,500 \text{ U/m}^2$  pegaspargase (Jiang Su Heng Rui Medicine, Co., Ltd., Lianyungang, China). During chemotherapy, the heart, liver and kidneys were protected by intravenous injection with  $5 \text{ mg}$  tropisetron hydrochloride (Jiang Su Heng Rui Medicine, Co., Ltd.) 30 min prior to chemotherapy, hydration ( $250\text{--}300 \text{ ml/m}^2 \text{ H}_2\text{O}$ ) and  $1.25\%$  sodium bicarbonate (Tianjin Pharmaceuticals Group Co., Ltd., Tianjin, China) intravenous injection. Following chemotherapy, patients with leukocytopenia (white blood cells count,  $<4.0 \times 10^9/\text{l}$ ) were treated with granulocyte colony-stimulating factor (Harbin Pharmaceutical Group Bioengineering, Co., Ltd., Harbin, China) to elevate their white blood cell counts, patients with anaemia were injected with red blood cells, and patients with low platelets were transfused with platelets (both Henan Red Cross Blood Centre, Zhengzhou, China). All patients underwent involved-field radiation therapy (TrueBeam™ Radiotherapy System; Varian Medical Systems, Inc., Palo Alto, CA, USA) following two cycles of chemotherapy. Three-dimensional radiotherapy counting was performed with a 6–8 MV photon ray at a median dose of  $52 \text{ Gy}$  (range,  $40\text{--}60 \text{ Gy}$ ) and was administered in fractionated doses of  $2 \text{ Gy}$  five times/week. The radiotherapy target covered the edge of the lesion and the infiltration. Subsequent chemotherapy continued until completion following radiotherapy. All patients provided signed written-informed consent prior to treatment.

**Evaluation of short-term efficacy and adverse reactions.** All patients underwent at least six cycles of chemotherapy combined with radiotherapy. Following two cycles of chemotherapy and the entire treatment regimen, the efficacies of the treatments in all patients were evaluated by a physical examination, B-ultrasound and computed tomography (CT; GE LightSpeed Pro 32; GE Healthcare Life Sciences, Chalfont, UK) or positron emission/CT (GE Discovery STE 16; GE Healthcare Life Sciences). Clinical efficacy was assessed according to World Health Organization (WHO) criteria (25), which classify responses as complete remission (CR), partial remission (PR), stable disease and progressive disease. The overall response rate (ORR) was calculated as  $\text{CR} + \text{PR}$ . Based on the WHO evaluation criteria for adverse reactions, the adverse reactions were divided into 0–IV degrees of severity. Toxicity assessments were conducted at the end of each chemotherapy cycle.

**Evaluation of survival.** Overall survival (OS) was defined as the time between the date of diagnosis and the date on which the patient succumbed to the disease or the end of the follow-up period. Progression-free survival (PFS) was defined as the time between the date of diagnosis and the date of tumor

Table I. Clinical characteristics of patients with newly diagnosed ENKTL.

Characteristic	CHOP	L-asparaginase	P-value
Gender			0.685
Male	47	73	
Female	22	39	
Age, years			0.571
≤60	59	99	
>60	10	13	
Ann Arbor stage			0.89
I/II	58	95	
III/IV	11	17	
IPI score			0.679
0-1	52	92	
≥2	17	20	
ECOG performance status			0.88
0-1	67	109	
≥2	2	3	
B symptoms			0.229
Yes	30	59	
No	39	53	
ENKTL subtype			0.639
UNKTL	66	107	
EUNKTL	3	5	
Bony perforation	18	28	0.87
Elevated serum LDH (>245 IU/l)	42	53	0.076
Elevated β <sub>2</sub> -microglobulin (>2.2 U/l)	52	76	0.281
Leukocytopenia (<4.0x10 <sup>9</sup> /l)	15	26	0.818
Haemoglobin (<110 g/l)	14	21	0.799
CD56 <sup>+</sup>	57	97	0.463
EBV <sup>+</sup>	43	63	0.421
Ki67 expression (median)	60	60	0.846

ENKTL, extranodal natural killer cell/T-cell lymphoma; IPI, international prognostic index; ECOG, Eastern Cooperative Oncology Group; UNKTL, upper aerodigestive tract natural killer cell/T-cell lymphoma; EUNKTL, non-upper aerodigestive tract natural killer cell/T-cell lymphoma; LDH, lactate dehydrogenase; EBV, Epstein-Barr virus.

Table II. Response assessments in the two treatment groups at various time points.

Group	N	Following the second cycle		Following all cycles and radiation	
		CR	ORR	CR	ORR
CHOP	69	10 (14.5%)	32 (46.4%)	29 (42.03%)	50 (72.46%)
L-asparaginase	112	38 (31.3%)	76 (67.9%)	72 (64.28%)	101 (90.18%)
P-value		0.011	0.004	0.003	0.002

CR, complete remission; ORR, overall response rate.

progression/relapse, the date on which the patient succumbed to the disease, or the end of the follow-up period. For patients who were lost to follow-up, survival ended at the time of the last follow-up.

*Statistical analysis.* Statistical analysis was conducted with SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). Categorical variables were compared between the groups using a  $\chi^2$  test, and continuous variables were compared between groups using a

Table III. Clinical characteristics and response rates following CHOP and L-asparaginase-based treatment regimens followed by radiation.

Characteristic	CHOP			L-asparaginase		
	N	ORR	P-value	N	ORR	P-value
Gender			0.973			1.000
Male	47	34		73	66	
Female	22	16		39	35	
Age			0.000			0.225
≤60	59	48		99	91	
>60	10	2		13	10	
Ann Arbor stage			0.000			0.012
I/II	58	48		95	89	
III/IV	11	2		17	12	
IPI score			0.000			0.036
0-1	52	46		92	86	
≥2	17	4		20	15	
ECOG performance status			0.073			0.025
0-1	67	50		109	100	
≥2	2	0		3	1	
B symptoms			0.344			0.896
Yes	30	20		53	48	
No	39	30		59	53	
ENKTL subtype			0.182			0.006
UNKTL	66	49		107	99	
EUNKTL	3	1		5	2	
Bony perforation	18	12	0.739	28	26	0.855
Elevated LDH level	42	28	0.179	53	45	0.076
Elevated β <sub>2</sub> -microglobulin	52	38	1.00	76	68	0.981
Leukocytopenia	15	10	0.809	26	20	0.027
Haemoglobin	14	7	0.076	21	18	0.722
CD56 <sup>+</sup>	57	41	1.000	97	88	0.980
EBV <sup>+</sup>	43	29	0.23	63	55	0.401
Ki67 <sup>*</sup>			0.919			0.075
Low	37	27		73	69	
High	32	23		39	32	
Pegaspargase replacement	-	-	-	25	22	0.973

IPI, international prognostic index; ECOG, Eastern Cooperative Oncology Group; ENKTL, extranodal natural killer cell/T-cell lymphoma; UNKTL, upper aerodigestive tract natural killer cell/T-cell lymphoma; EUNKTL, non-upper aerodigestive tract natural killer cell/T-cell lymphoma; LDH, lactate dehydrogenase; EBV, Epstein-Barr virus; ORR, overall response rate.

t-test for independent samples. Kaplan-Meier survival analysis was applied to calculate the patient survival rates. The survival rates were compared between groups using a log-rank test. Variables with  $P < 0.05$  were included in the Cox proportional hazards regression model for multivariate analysis.  $P < 0.05$  was considered to indicate a statistically significant result.

## Results

*Short-term efficacy and long-term survival.* Treatment efficacies were evaluated in the 181 patients, and the overall

efficacies in the two treatment groups are shown in Table II. The 69 patients in the CHOP treatment group included 10 cases of CR and 22 cases of PR for an ORR of 46.4% following two cycles of chemotherapy; 29 cases of CR and 21 cases of PR for an ORR of 72.46% was shown at the end of the treatment. The  $\chi^2$  test results (Table III) demonstrated that among the various clinical factors, patient age, clinical stage, and IPI score significantly affected the ORR ( $P < 0.001$ ) at the end of the treatment period. The 112 patients in the asparaginase group included 38 cases of CR and 38 cases of PR for an ORR of 67.9% following two cycles of chemotherapy, and 72 cases

Table IV. Survival data of the two groups.

Group	OS rate (%)			PFS rate (%)		
	One-year	Two-year	Five-year	One-year	Two-year	Five-year
CHOP	82.6	61.9	28.4	63.8	44.0	21.0
L-asp	96.0	88.3	65.1	94.2	79.8	50.0
P-value	0.00	0.00	0.00	0.00	0.00	0.00

OS, overall survival; PFS, progression-free survival; L-asp, L-asparaginase.

Table V. Univariate analysis of prognostic factors.

Factor	CHOP		L-asparaginase	
	OS	PFS	OS	PFS
Gender	0.556	0.611	0.332	0.212
Age	0.000	0.000	0.027	0.038
Ann Arbor stage	0.000	0.000	0.000	0.000
IPI score	0.000	0.000	0.005	0.018
ECOG performance status	0.000	0.000	0.000	0.000
B symptoms	0.575	0.439	0.593	0.575
ENKTL subtype	0.000	0.000	0.000	0.000
Bony perforation	0.314	0.248	0.973	0.898
Elevated LDH	0.025	0.008	0.014	0.001
Elevated $\beta_2$ -MG	0.300	0.356	0.550	0.242
Leukopenia	0.034	0.006	0.006	0.020
Haemoglobin	0.295	0.134	0.959	0.077
CD56	0.982	0.896	0.717	0.422
EBV	0.217	0.268	0.493	0.418
Ki67	0.024	0.035	0.001	0.001
Early efficacy	0.000	0.000	0.000	0.000
Pegaspargase replacement	-	-	0.191	0.638

Data are P-values. IPI, international prognostic index; ECOG, Eastern Cooperative Oncology Group; ENKTL, extranodal natural killer cell/T-cell lymphoma; LDH, lactate dehydrogenase;  $\beta_2$ -MG,  $\beta_2$ -microglobulin; EBV, Epstein-Barr virus; OS, overall survival; PFS, progression-free survival.

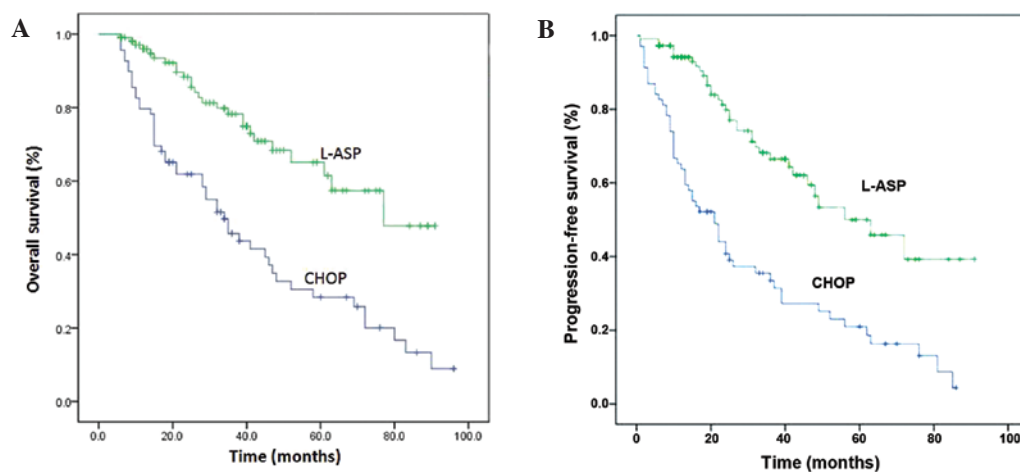


Figure 1. Kaplan-Meier survival curves for all patients with extranodal natural killer cell/T-cell lymphoma treated with CHOP or L-asparaginase-based regimens combined with radiation. (A) Overall survival. (B) Progression-free survival.

Table VI. Cox proportional hazard regression analysis for the CHOP and L-asparaginase treatment groups.

Survival	CHOP (95% CI)				L-asparaginase (95% CI)			
	RR	Lower	Upper	P-value	RR	Lower	Upper	P-value
<b>OS</b>								
Early efficacy	6.596	2.365	18.397	0.000	8.760	2.879	26.657	<0.001
Ann Arbor stage	2.003	0.372	10.771	0.418	10.588	2.553	43.910	0.001
Ki67	1.578	0.843	2.954	0.154	6.517	2.165	19.615	0.001
LDH	1.330	0.632	2.800	0.452	3.525	1.092	11.373	0.035
<b>PFS</b>								
Early efficacy	7.144	2.853	17.890	0.000	4.093	1.635	10.245	0.003
ENKTL subtype	8.597	1.677	44.085	0.010	4.962	0.621	39.655	0.131
Ki67	1.438	0.758	2.727	0.266	3.554	1.582	7.984	0.002
Ann Arbor stage	1.580	0.300	8.330	0.590	7.405	2.045	26.811	0.002
Age	5.260	1.351	20.480	0.017	5.480	0.595	50.433	0.133
LDH	1.348	0.646	2.811	0.426	3.554	1.582	7.984	0.001

RR, risk ratio; 95% CI, 95% confidence interval; OS, overall survival; LDH, lactate dehydrogenase; PFS, progression-free survival; ENKTL, extranodal natural killer cell/T-cell lymphoma.

Table VII. Toxicity profiles of the CHOP and L-asparaginase treatment groups.

Toxicity	CHOP		L-asparaginase		$\chi^2$	P-value
	I-II	III-IV	I-II	III-IV		
Leukopenia	36 (52.2)	13 (18.8)	59 (52.7)	25 (22.3)	0.348	0.555
Anaemia	13 (18.8)	2 (2.9)	24 (21.4)	5 (4.5)	0.400	0.527
Thrombocytopenia	8 (11.6)	1 (1.4)	13 (11.6)	2 (1.8)	0.005	0.946
Increase in ALT/AST	11 (15.9)	3 (4.3)	31 (27.7)	5 (4.5)	3.000	0.083
Increase in bilirubin	8 (11.6)	2 (2.9)	25 (22.3)	4 (3.6)	3.283	0.070
Increase in creatinine	10 (14.5)	0	16 (14.3)	0	0.001	0.969
Increase in BUN	6 (8.7)	0	11 (9.8)	0	0.064	0.801
Anaphylaxis	3 (4.3)	0	29 (25.9)	0	13.618	<0.001
Nausea, vomiting	41 (59.4)	3	76 (67.9)	10 (8.9)	1.647	0.199
Infection	15 (21.7)		23 (20.5)		0.037	0.847
Reduction in FIB	5 (7.2)		68 (60.7)		50.721	<0.001

ALT/AST, alanine aminotransferase/aspartate aminotransferase; BUN, blood urea nitrogen; FIB, fibrinogen.

of CR and 29 cases of PR for an ORR of 90.18% at the end of the treatment period. Among the various clinical factors, clinical staging, IPI score, ECOG score, primary lesion site, and leukocytopenia affected the ORR ( $P<0.05$ ).

The patients were followed-up until 31 October 2013, with a median follow-up time of 29 months (range, 6-96 months). A total of 10 cases were lost to follow-up. In the CHOP treatment group, the median OS duration was 34 months, the median PFS duration was 21 months, the one, two, and five-year OS rates were 82.6, 61.9 and 28.4%, respectively, and the one, two, and five-year PFS rates were 63.8, 44.0 and 21.0%, respectively (Table IV, Fig. 1A and B). Univariate analysis demonstrated that patient age, clinical staging, IPI score, ECOG score, primary lesion site, LDH levels, leukocytopenia, Ki67 levels and early

treatment response significantly affected patient OS and PFS ( $P<0.05$ ). Other factors such as gender, B symptoms, and  $\beta_2$ -microglobulin had no impact on the OS and PFS ( $P>0.05$ ; Table V). Multivariate analysis demonstrated that early treatment response was an independent predictor of OS ( $P<0.001$ ), whereas primary lesion site, early treatment response, and age were independent predictors of PFS ( $P=0.010$ ,  $P<0.001$ , and  $P=0.017$ , respectively; Table VI). In the asparaginase treatment group, the median OS duration was 77 months, the median PFS duration was 56 months, the one, two, and five-year OS rates were 96.0, 88.3 and 65.1%, respectively, and the one, two, and five-year PFS rates were 94.2, 79.8 and 50%, respectively. Univariate analysis demonstrated that patient age, clinical stage, IPI score, ECOG score, primary lesion site, LDH level,



leukocytopenia, Ki67 level, and early treatment response affected patient OS and PFS ( $P < 0.05$ ); other factors such as gender, B symptoms, and  $\beta_2$ -microglobulin had no impact on OS and PFS ( $P > 0.05$ ). Multivariate analysis demonstrated that early treatment, clinical staging, LDH levels, and Ki67 levels were independent predictors of OS and PFS ( $P < 0.001$ ,  $P = 0.001$ , 0.035 and 0.001, and  $P = 0.011$ , 0.002, 0.002 and 0.02, respectively).

**Adverse reactions.** The adverse reactions observed in the patients in the two groups are shown in Table VII. The primary adverse reactions to chemotherapy included bone marrow suppression, gastrointestinal reactions, infections, and liver dysfunction, the majority of which were rapidly relieved following symptomatic treatment. In the asparaginase treatment group, 68 cases (60.7%) showed a statistically significant reduction in fibrinogen levels, as compared with the CHOP treatment group ( $P < 0.001$ ). Associated bleeding and thrombotic events occurred in two cases; these were of low severity and improved following active treatment. During L-asparaginase treatment, a rash occurred in 29 patients and improved following symptomatic treatment; 25 of these patients were treated with pegaspargase subsequent to receiving positive results (detection of erythemas or wheals) from an L-asparaginase skin test. No treatment interruptions or treatment-associated mortality occurred in either group.

## Discussion

ENKTL is a type of non-Hodgkin's lymphoma that accounts for 11% of lymphoma cases in China (3). Given the rapid course of clinical progression and relatively poor prognosis of ENKTL, the optimal treatment strategy for this disease remains unclear. Previously, either CHOP chemotherapy alone or radiotherapy alone was not able to achieve satisfactory results. Efficacy of the CHOP chemotherapy regimen for ENKTL is poor (26-29). Several studies have reported that radiotherapy is an important treatment method that is beneficial to local lesion control (15,28,30,31). However, the rates of recurrence and distant metastasis were high, and the five-year survival rate was low in the early-stage patients who received radiotherapy alone (28,32). As chemoradiotherapy strategies have progressed, chemotherapeutic regimens based on CHOP-class anthracycline compounds have been reported (29). However, the efficacy remained low even following an increase in cyclophosphamide dose or the combination with other drugs such as semustine. In addition, these treatment regimens did not significantly improve patient survival in the early stages of ENKTL (30).

The insensitivity of ENKTL to conventional CHOP-class chemotherapy may be associated with tumor cell overexpression of P-glycoprotein, which is encoded by the MDR1 (33). Doxorubicin and vincristine, components of the CHOP-class treatment, may be actively pumped out by P-glycoprotein, thereby reducing the therapeutic effect. L-asparaginase hydrolyses asparaginase in the serum thereby reducing the levels of amino acids required in certain tumor cells that do not produce asparagine synthetase, resulting in the inhibition of DNA, RNA, and protein synthesis, which acts as an

anti-tumor agent (18,34). *In vitro* studies have demonstrated that asparaginase is able to reduce the activity of normal NK cells and induce apoptosis in NK tumor cells, whereas the conventional chemotherapy drugs used to treat lymphoma do not have this property (35,36). Yong *et al* (37) reported the use of an L-asparaginase-based treatment as the primary therapy for 11 patients with refractory midline peripheral T-cell lymphoma, and reported an efficacy of 63.6% and a two-year survival rate of 45.5%. Subsequently, this L-asparaginase treatment was applied in 45 patients with relapsed or refractory ENKTCL and led to a CR rate of 56%, an ORR of 82%, and a five-year survival rate of 67% (19). A recent study on CHOP-L treatment combined with radiotherapy for newly diagnosed ENKTL demonstrated that, among 38 newly diagnosed patients with ENKTL who received CHOP-L chemotherapy and sequential radiotherapy, the CR rate was 81.6%, the ORR rate was 84.2%, and the two-year OS rate was 80.1% (23). The results of the present study demonstrated that, compared with the CHOP treatment group, the ENKTL patient group that received an L-asparaginase-based treatment combined with radiotherapy had significantly improved remission rate and improved long-term survival. Among the 112 patients in the L-asparaginase treatment group, the overall efficacy following two cycles of chemotherapy was 67.9%, the overall efficacy at the end of the treatment was 90.18%, the median OS duration was 77 months, the median PFS duration was 56 months, the one, two, and five-year OS rates were 96.0, 88.3 and 65.1%, respectively, and the one, two, and five-year PFS rates were 94.2, 79.8 and 50.0%, respectively.

The incidence of allergic reactions in patients who were treated with L-asparaginase has been reported to be 30% (38). Studies have reported that pegaspargase therapy had a similar efficacy to L-asparaginase as a treatment in children with acute lymphoblastic leukaemia (39,40). In the present study, allergic reactions occurred in 29 patients in the asparaginase treatment group (25.9%) during the application of L-asparaginase, and 25 of these patients (22.3%) switched to treatment with pegaspargase following a positive L-asparaginase skin test results. The statistical results indicated that switching to pegaspargase did not affect the CR rate, OS, or PFS. A study on treatment with pegaspargase in newly diagnosed ENKTL patients is currently on-going in China. Pegaspargase may be another treatment option for ENKTL patients.

Prognostic factors that have been reported to be closely associated with ENKTL include patient age, B symptoms, ECOG score, regional lymph node invasion, clinical staging, CR rate, leukocytopenia, IPI score and Ki67 expression levels (41-43). In the present study, a univariate analysis of the 112 patients in the asparaginase regimen group demonstrated that patient age, clinical staging, IPI score, ECOG score, primary lesion site, LDH, leukocytopenia, Ki67 levels, and early treatment response affected patient OS and PFS ( $P < 0.05$ ). Other factors such as gender, B symptoms, and  $\beta_2$ -microglobulin had no impact on OS and PFS ( $P > 0.05$ ). Multivariate analysis demonstrated that early treatment response ( $P < 0.001$ ), clinical staging ( $P < 0.001$ ), and LDH level ( $P = 0.006$ ) were independent predictors of OS, whereas Ki67 expression levels ( $P = 0.011$ ), early treatment response ( $P = 0.001$ ), clinical staging ( $P = 0.002$ ), and LDH level ( $P = 0.001$ ) were independent predictors of PFS.

In the present study the primary adverse reactions to treatment experienced by the patients in the two groups included bone marrow suppression, gastrointestinal reactions, infections, and liver dysfunction, most of which were rapidly relieved following symptomatic treatment. The incidence of hypofibrinogenemia was significantly higher in the asparaginase treatment group compared with the CHOP treatment group, although associated bleeding and thrombotic events occurred in only two cases and at a low severity, and no treatment-associated mortality was recorded.

In conclusion, L-asparaginase-based chemotherapy combined with radiotherapy is effective as a first-line treatment for ENKTL. The patients who received this type of treatment showed significantly increased improvements compared with those treated with the CHOP regimen combined with radiotherapy, with regard to short-term efficacy and long-term survival, as well as safety performance.

## Acknowledgements

The present study was supported by grants from the National Natural Science Foundation of China (grant no. 81170520), the Henan Department of Health/Provincial Departmental collaboration project (grant no. 2011010014), and the National Natural Science Foundation of Henan Province (grant nos. 81170520 and 81000921).

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