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HEMATOLOGY

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Prognostic impact of pre-treatment and post-treatment plasma Epstein-Barr virus DNA in peripheral T-cell lymphomas

Chu-Yi Chan[a], Tung-Liang Lin[a], Ming-Chung Kuo[a,b], Yu-Shin Hung[a], Hung Chang[a,b], Che-Wei Ou[c], Jin-Hou Wu[c], Hsuan-Jen Shih[a], Yi-Jiun Su[a], Lee-Yung Shih[a,b], Yuen-Chin Ong[a], Wen-Yu Chuang[d] and Hsiao-Wen Kao[a]

[a]Division of Hematology-Oncology, Department of Internal Medicine, Chang Gung Memorial Hospital at Linkou, Taiwan; [b]College of Medicine, Chang Gung University, Taoyuan, Taiwan; [c]Division of Hematology-Oncology, Department of Internal Medicine, New Taipei Municipal TuCheng Hospital, New Taipei City, Taiwan; [d]Department of Pathology, Chang Gung Memorial Hospital, Taoyuan, Taiwan

ABSTRACT

Background: Plasma Epstein-Barr virus (EBV) DNA levels predict the prognosis of extranodal NK/T-cell lymphoma, nasal type (NK/TCL), but its role in other peripheral T-cell lymphomas (PTCL) remains undetermined. This study aimed to determine the prognostic impact of plasma EBV DNA in PTCL patients.

Methods: We retrospectively enrolled 134 PTCL patients diagnosed between April 2008 and March 2022, with plasma EBV DNA data available at diagnosis in 124 patients and during post-treatment follow-up in 73 patients.

Results: International Prognostic Index or prognostic index for T-cell lymphoma scores > 1 was associated with higher median plasma EBV DNA levels in all analyzed patients. Plasma EBV DNA positivity at the time of diagnosis was not associated with treatment response, overall survival (OS), or progression-free survival (PFS) in non-NK/TCL patients. In NK/TCL patients, an EBV DNA level < 3255 copies/mL at diagnosis was significantly associated with higher five-year PFS (64.2% vs. 16.7%, $p<0.001$) and OS rates (64.4% vs. 20.8%, $p<0.001$). Plasma EBV DNA positivity at the time of complete remission and during post-treatment follow-up was significantly linked to lower PFS and OS rates in NK/TCL patients. Multivariate analysis revealed that advanced-stage disease, elevated β2-microglobulin, and EBV DNA level ≥ 3255 copies/mL at diagnosis were independent predictors for OS and PFS in NK/TCL patients.

Conclusions: Plasma EBV DNA at diagnosis and during follow-up predict survival for NK/TCL patients but not for patients with other PTCL subtypes. Detection and monitoring of plasma EBV DNA levels at diagnosis and post-treatment follow-up for NK/TCL patients is recommended.

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1. Introduction

Peripheral T-cell lymphomas (PTCLs) encompass a heterogeneous group of rare and aggressive malignancies originating from mature T cells or natural killer (NK) cells. These neoplasms account for approximately 6-10% of all non-Hodgkin lymphomas in Western populations, with a higher prevalence of 12-20% observed in Asian countries [1,2]. Specifically, systemic PTCLs are associated with a poor prognosis and present significant challenges in terms of diagnosis and therapeutic management [3]. Recent studies have proposed certain clinical factors as predictors of outcomes in these patients, but few prognostic biomarkers were identified [4–6]. Among the various factors contributing to the pathogenesis and poor prognosis of PTCLs, Epstein–Barr virus (EBV) has emerged as an important focus of the investigation.

EBV is a member of the herpesvirus family and is a ubiquitous human virus infecting approximately 90% of the population, with a remarkable ability to establish lifelong latency within the host's B lymphocytes. EBV is associated with specific B-cell malignancies, such as Burkitt lymphoma and Hodgkin lymphoma [7–10], and plays a pivotal role in the tumorigenesis of

CONTACT Hsiao-Wen Kao hsiaowen@cgmh.org.tw Division of Hematology-Oncology, Department of Internal Medicine, Chang Gung Memorial Hospital at Linkou, No.5, Fuxing Street, Guishan Dist., Taoyuan City 333423, Taiwan (R.O.C.)
Wen-Yu Chuang is now affiliated to Department of Pathology, Cathay General Hospital, Taipei, Taiwan.

NK/T-cell lymphomas by directly infecting T and NK cells [11].

EBV-encoded small RNAs (EBERs) are abundantly expressed in EBV-infected cells and can be detected through *in situ* hybridization (ISH) in tissue specimens [12]. While EBER ISH can directly identify infected cells and remains the gold standard for confirmation of the EBV presence in tumor cells, its clinical application is limited due to the requirement of tissue samples and specialized techniques. Another tool for viral load evaluation is the detection of circulating EBV DNA using real-time quantitative polymerase chain reaction (PCR). Previous research has revealed that EBV DNA level in the peripheral blood serves as a valuable biomarker for evaluating treatment response and predicting prognosis in patients with NK/T-cell lymphoma [13–16], but its role in other PTCLs remains incompletely understood.

Our study aims to analyze the clinical features, treatment modalities, and outcomes of different subtypes of systemic PTCLs as well as their association with the presence and quantity of plasma EBV DNA at the time of diagnosis and throughout the subsequent clinical course. Our goal is to elucidate the role of EBV DNA levels in the prognosis of systemic PTCLs.

2. Materials and methods

2.1. Patients

Between April 2008 and March 2022, a total of 169 patients diagnosed with PTCLs at Chang Gung Memorial Hospital, Linkou branch were retrospectively analysed. Inclusion criteria were patients with the pathological diagnosis of systemic PTCLs by 2016 WHO classification [17], including extranodal NK/T-cell lymphoma, nasal type (NK/TCL), angioimmunoblastic T-cell lymphoma (AITL), peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), and anaplastic large cell lymphoma (ALCL). Cutaneous lymphomas, adult T-cell leukemia/lymphoma, and other less common subtypes including monomorphic epitheliotropic intestinal T-cell lymphoma and enteropathy-associated T-cell lymphoma were excluded. Patients without available plasma EBV DNA data at the time of initial diagnosis and throughout the follow-up period were excluded. The flowchart of this research was shown in Figure 1. EBER ISH was performed at the time of diagnosis if requested by the pathologist. EBER positivity was defined as nuclear positivity in 100% of lymphoma cells in NK/TCL and above 50% in other PTCLs. This study was approved by the Investigational Review Board (IRB) of Chang Gung Memorial Hospital (201502568B0, 201601360B0), and informed consent was waived by the IRB due to retrospective observational design of the study with minimal risk to the patients. The study was conducted in accordance with the Declaration of Helsinki and was adherent to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for observational retrospective studies [18].

A comprehensive evaluation encompassed a full blood count with differential, assessments of renal and liver function, serum albumin, total protein, lactate dehydrogenase (LDH), β2-microglobulin, uric acid levels, bone marrow biopsy, as well as computed tomography (CT) scans of the brain, chest, abdomen, and pelvis or Positron Emission Tomography (PET). Patients were staged according to the Ann Arbor staging system [19]. The performance status was assessed using the Eastern Cooperative Oncology Group (ECOG) scale (0-4) [20]. The International Prognostic Index (IPI) [21] and the prognostic index for T-cell lymphoma (PIT) score [22] were used to determine the risk stratification.

2.2. Treatment and response evaluation

Frontline treatments included anthracycline-based regimens such as CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone) or CHOP-like regimens and L-asparaginase-based regimens including LVP (L-asparaginase, vincristine, and prednisolone) and SMILE (dexamethasone, methotrexate, ifosfamide, L-asparaginase, etoposide). Combined radiotherapy was adopted based on different treatment protocols. High-intensity chemotherapy followed by autologous or allogeneic hematopoietic stem cell transplantation was introduced as a salvage therapy in the event of relapse or refractory disease.

Response to treatment was assessed through interim PET scans after three to four cycles of chemotherapy and upon completion of therapy. After the end of treatment, physical examinations and laboratory tests were conducted during follow-up visits, which were scheduled quarterly in the first one to two years, biannually in the next two to three years and annually thereafter. Whole body CT scans of the brain, chest, abdomen, and pelvis or PET/CT were arranged every three to six months during the first two years after therapy. The patient's response to treatment was categorized as complete remission (CR), partial response, stable disease, or progressive disease, in accordance with the revised response criteria for malignant lymphoma [23]. The overall response rate (ORR) was defined as the proportion of patients who achieved a complete or partial response. Progression-free survival (PFS) was defined as the time from diagnosis to progression, relapse, or death from any cause.

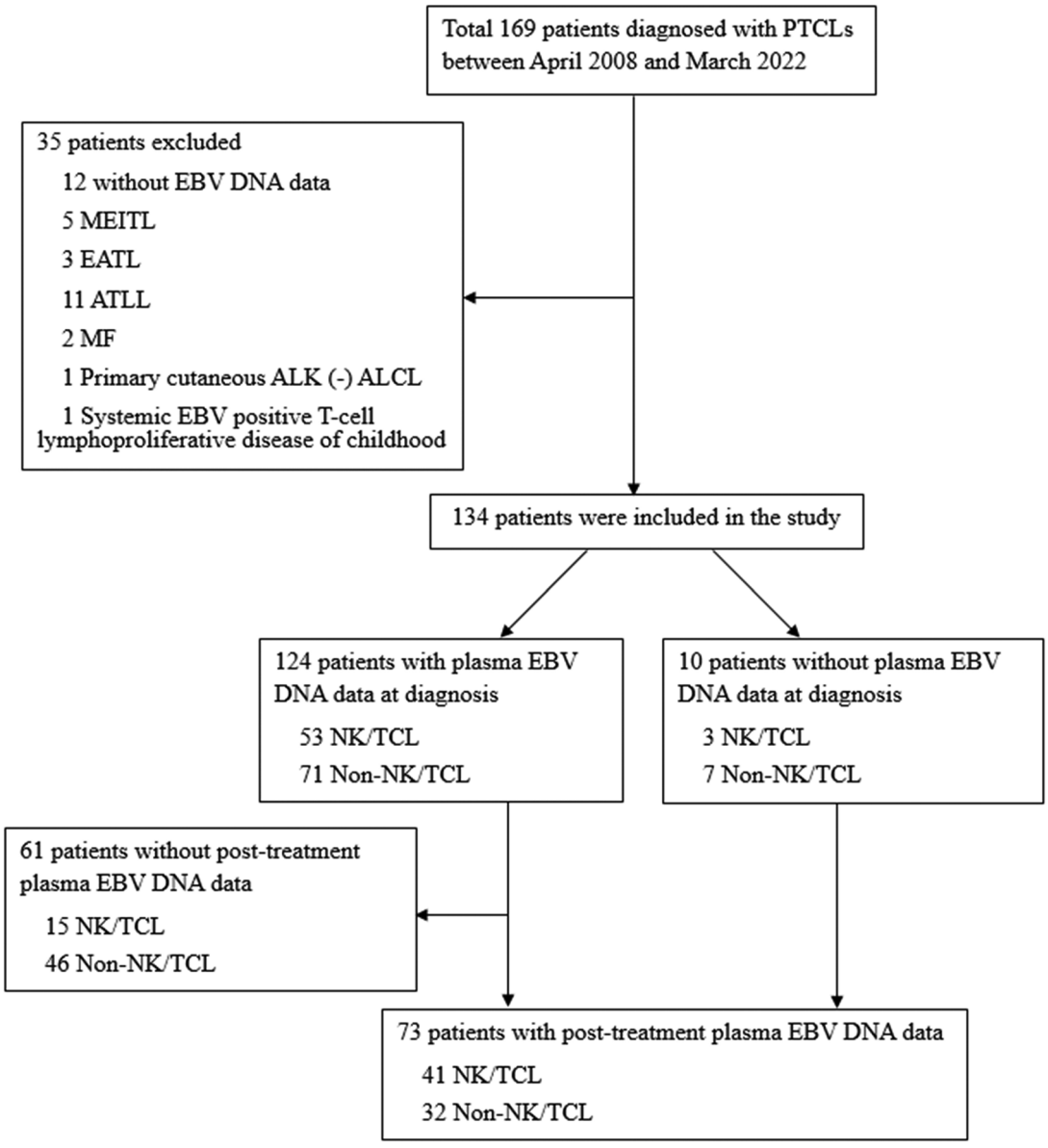


Figure 1. Study flowchart.
Abbreviations: MEITL: monomorphic epitheliotropic intestinal T-cell lymphoma; EATL: enteropathy-associated T-cell lymphoma; ATLL: adult T-cell leukemia/lymphoma; MF: mycosis fungoides; ALCL: anaplastic large cell lymphoma; NK/TCL: extranodal NK/T-cell lymphoma, nasal type.

Overall survival (OS) was defined as the time from diagnosis to death from any cause. Confirmation of disease relapse was established through comprehensive imaging studies such as PET, CT, or magnetic resonance imaging, or via tissue biopsy [24].

2.3. EBV DNA detection

Plasma EBV DNA detection was performed using the in-house TaqMan real-time quantitative PCR technique, specifically targeting the BamHI-W sequence, with the Namalwa cell line used as an internal standard. The lower limit of detection was 200 copies/mL (range 200 – 2 x 10^6 copies/mL). A DNA level measured below 200 copies/mL was determined through an extrapolation technique. EBV negativity was defined as the absence of detectable plasma EBV DNA. Plasma EBV DNA levels were documented at the time of diagnosis, after achieving CR, during follow-up, and at the time of relapse. Post-treatment EBV DNA positivity was defined as the presence of detectable EBV DNA levels at least once after first-line treatment, either at the time of CR, during follow-up, or at the time of relapse.

2.4. Statistical analysis

The plasma EBV DNA levels, characterized by a non-normal distribution, were presented as median values with interquartile ranges, and non-parametric statistical methods were applied for analysis. Categorical

variables between groups were compared using Chi-square tests or Fisher's exact tests, while differences in numerical variables were assessed using the Mann–Whitney U-test or the Kruskal–Wallis test.

The primary objective of this study was to assess the association between plasma EBV DNA positivity and survival outcomes. Previous studies reported a plasma EBV DNA positivity rate (defined as >500 copies/mL) of approximately 80% in NK/TCL patients and 40% in non-NK/TCL patients [25,26]. Using Schoenfeld's method for sample size calculation, with a 95% confidence interval, and 90% power to detect a statistically significant median survival difference of 20% between groups, the required sample size was estimated to be 27 patients for the NK/TCL group and 35 patients for the non-NK/TCL group.

The OS and PFS were estimated using the Kaplan–Meier method. The comparison of OS or PFS between groups was analysed using the log-rank test. Univariate and multivariate analyses were performed to evaluate the prognostic impact of different variables on OS and PFS using the Cox proportional hazard model. A two-tailed *p*-value less than 0.05 was considered statistically significant. The optimal cutoff value for EBV DNA levels in discriminating OS and PFS was analyzed using the maximally selected rank statistics. All statistical analyses were conducted using R statistical software for Windows, version 4.2.1, R Foundation for Statistical Computing, Vienna, Austria.

3. Results

3.1. Patient characteristics

A total of 134 patients were included in the study, with a median age of 59.5 (range 24-90) years at diagnosis. More than half of the patients had elevated levels of LDH (60.4%) and β2-microglobulin (57.5%) exceeding the upper limit of normal. Histological subtypes of PTCL included NK/TCL ($n=56$, 41.8%), AITL ($n=34$, 25.4%), PTCL-NOS ($n=28$, 20.9%), and ALCL ($n=16$, 11.9%). EBER ISH was conducted in 63 patients, and EBER positivity was observed in 42 patients (97.7%) from the NK/TCL group and in two patients (25%) from the AITL group. None of the patients with other histological subtypes were tested positive for EBER. At the time of diagnosis, plasma EBV DNA positivity was identified in 41 patients (77.1%) in the NK/TCL group and in 44 patients (62.0%) in the non-NK/TCL group. Patients with NK/TCL and AITL tended to present with a higher frequency of plasma EBV DNA positivity, along with higher EBV DNA levels compared to other PTCL subtypes. The detailed characteristics of the entire cohort and different PTCLs were summarized in Table 1.

Table 1. Characteristics of all peripheral T-cell lymphoma (PTCL) patients.

Parameter	All N (%)	NK/TCL n (%)	AITL n (%)	ALCL n (%)	PTCL-NOS n (%)
Case number (N)	134	56	34	16	28
Age, median (range)	59.5 (24-90)	59.5 (28-85)	66.0 (46-90)	48.5 (28-73)	56.0 (24-83)
Age > 60 years	65 (48.5)	26 (46.4)	26 (76.5)	5 (31.3)	8 (28.6)
Male sex	76 (56.7)	30 (53.6)	19 (55.9)	9 (56.3)	18 (64.3)
Ann Arbor Stage					
I-II	50 (37.3)	39 (69.6)	2 (5.9)	4 (25.0)	5 (17.9)
III-IV	84 (62.7)	17 (30.4)	32 (94.1)	12 (75.0)	23 (82.1)
ECOG score ≥ 2	33 (24.6)	14 (25.0)	10 (29.4)	3 (18.8)	6 (21.4)
IPI score					
0	24 (17.9)	20 (35.7)	0 (0)	2 (12.5)	2 (7.1)
1	21 (15.7)	11 (19.6)	2 (5.9)	3 (18.8)	5 (17.9)
≥2	89 (66.4)	25 (44.6)	32 (94.1)	11 (68.8)	21 (75.0)
PIT score					
0	33 (24.6)	18 (32.1)	3 (8.8)	3 (18.8)	9 (32.1)
1	39 (29.1)	17 (30.4)	7 (20.6)	7 (43.8)	8 (28.6)
≥2	62 (46.3)	21 (37.5)	24 (70.6)	6 (37.5)	11 (39.3)
B symptoms	55 (41.0)	20 (35.7)	15 (44.1)	5 (31.3)	15 (53.6)
LDH ≥ ULN	81 (60.4)	25 (44.6)	28 (82.4)	10 (62.5)	18 (64.3)
β2-microglobulin ≥ ULN	77 (57.5)	28 (50.0)	28 (82.4)	7 (43.8)	14 (50.0)
EBER ISH positive n/N (%)	44/63 (69.8)	42/43 (97.7)	2/8 (25.0)	0/2 (0)	0/10 (0)
Treatment					
Chemotherapy	121 (90.3)	50 (89.3)	32 (94.1)	15 (93.8)	24 (85.7)
CHOP/CHOP-like	94 (70.1)	26 (46.4)	30 (88.2)	15 (93.8)	23 (82.1)
LVP/LVP-like	19 (14.2)	19 (33.9)	0 (0)	0 (0)	0 (0)
SMILE	4 (3.0)	4 (7.1)	0 (0)	0 (0)	0 (0)
Palliative	4 (3.0)	1 (1.8)	2 (5.9)	0 (0)	1 (3.6)
Radiotherapy	35 (26.1)	31 (55.4)	1 (2.9)	2 (12.5)	1 (3.6)

Abbreviations: ECOG: Eastern Cooperative Oncology Group performance status; IPI: International Prognostic Index; PIT: prognostic index for T-cell lymphoma; LDH: lactate dehydrogenase; ULN: upper limit of normal; EBER ISH: EBER *in situ* hybridization; CHOP: cyclophosphamide, doxorubicin, vincristine, and prednisolone; LVP: L-asparaginase, vincristine, and prednisolone; SMILE: methotrexate, dexamethasone, ifosfamide, L-asparaginase, etoposide; NK/TCL: extranodal NK/T-cell lymphoma, nasal type; AITL: angioimmunoblastic T-cell lymphoma; PTCL-NOS: peripheral T-cell lymphoma, not otherwise specified; ALCL: anaplastic large cell lymphoma.

Plasma EBV DNA data at diagnosis was obtainable in 124 patients, with 85 (68.5%) of them tested positive, indicating a concordance rate of 65.0% with EBER ISH. Patients presented with detectable plasma EBV DNA at diagnosis were significantly associated with older age and elevated LDH levels above the upper limit of normal when compared to EBV-negative patients. There were no statistically significant differences between these two groups regarding disease staging or risk stratification as assessed by the IPI score and PIT score. Three patients had EBV DNA levels marked as positive without specific quantification and were therefore excluded from the analysis regarding EBV DNA quantification. Patients with older age, B symptoms, poor performance status, IPI or PIT scores of two or more, elevated LDH levels, or increased β2-macroglobulin levels above the upper limit of normal exhibited significantly higher plasma EBV DNA levels compared to patients without these factors. The association of EBV DNA positivity and DNA level with clinical and laboratory characteristics at diagnosis was summarized in Table 2.

3.2. Treatment response

The CR rate was 56.0% (65/116) and the ORR was 75.0% (87/116) among the 116 patients with obtainable treatment responses. Twenty-one (18.1%) patients experienced disease progression after first-line treatment. Plasma EBV DNA positivity at the time of diagnosis was not associated with CR rate (50.7% vs. 60.0%, $p=0.49$) or ORR (71.8% vs. 80.0%, $p=0.50$). In subgroup analysis, an EBV DNA level of 3255 copies/mL was identified as the best cutoff value for discriminating survival in patients with NK/TCL, and the CR rate (88.0% vs. 38.9%, $p=0.002$) and ORR (92.0% vs. 55.6%, $p=0.02$) of these patients with EBV DNA < 3255 copies/mL at diagnosis were significantly higher than those with EBV DNA ≥ 3255 copies/mL.

3.3. The outcome of PTCLs stratified by different EBV DNA cut-off values

With a median follow-up time of 60.2 (95% confidence interval [CI] 55.8-78.0) months for all patients, the

Table 2. Association of plasma EBV DNA positivity and EBV DNA levels with PTCL patient characteristics at diagnosis.

Variables	EBV DNA Positive n/N (%)	EBV DNA Negative n/N (%)	*p*-values	Median EBV DNA (IQR) (copies/mL)	*p*-values
All	85/124 (68.5)	39/124 (31.5)		531 (0-14680)	
Sex			0.396		0.157
Male	46/85 (54.1)	25/39 (64.1)		271 (0-9688)	
Female	39/85 (45.9)	14/39 (35.9)		632 (0-44497)	
Age (years)			0.038*		0.008*
>60	47/85 (55.3)	13/39 (33.3)		1980 (36-32347)	
≤60	38/85 (44.7)	26/39 (66.7)		226 (0-3312)	
B symptoms			0.131		<0.001*
Positive	40/85 (47.1)	12/39 (30.8)		4247 (207-36671)	
Negative	45/85 (52.9)	27/39 (69.2)		53 (0-2888)	
ECOG			0.315		<0.001*
≥2	24/85 (28.2)	7/39 (17.9)		18492 (337-69488)	
0–1	61/85 (71.8)	32/39 (82.1)		270 (0-3936)	
Stage			1		0.019*
Stage III-IV	55/85 (64.7)	25/39 (64.1)		1790 (0-31736)	
Stage I-II	30/85 (35.3)	14/39 (35.9)		157 (0-825)	
IPI score			0.138		<0.001*
≥2	61/85 (71.8)	22/39 (56.4)		3072 (0-32622)	
0–1	24/85 (28.2)	17/39 (43.6)		40 (0-708)	
PIT score			0.066		<0.001*
≥2	45/85 (52.9)	13/39 (33.3)		4020 (264-34062)	
0–1	40/85 (47.1)	26/39 (66.7)		52 (0-932)	
LDH			0.044*		<0.001*
Elevated	57/85 (67.1)	18/39 (46.2)		3384 (29-33476)	
Normal	28/85 (32.9)	21/39 (53.8)		40 (0-571)	
β2-microglobulin			0.108		0.002*
Elevated	54/78 (69.2)	18/35 (51.4)		1580 (25-33451)	
Normal	24/78 (30.8)	17/35 (48.6)		30 (0-1352)	
Extranodal sites			1		0.429
≥2	24/85 (28.2)	11/39 (28.2)		1542 (0-32030)	
0–1	61/85 (71.8)	28/39 (71.8)		485 (0-10128)	
Histological subtypes			<0.001*		<0.001*
NK/TCL	41/53 (77.4)	12/53 (22.6)		840 (11-31516)	
AITL	27/31 (87.1)	4/31 (12.9)		5040 (732-32032)	
ALCL	3/12 (25.0)	9/12 (75.0)		0 (0-8)	
PTCL-NOS	14/28 (50.0)	14/28 (50.0)		0 (0-359)	

Abbreviations: IQR: interquartile range; ECOG: Eastern Cooperative Oncology Group performance status; IPI: International Prognostic Index; PIT: prognostic index for T-cell lymphoma; LDH: lactate dehydrogenase; NK/TCL: extranodal NK/T-cell lymphoma, nasal type; AITL: angioimmunoblastic T-cell lymphoma; ALCL: anaplastic large cell lymphoma; PTCL-NOS: peripheral T-cell lymphoma, not otherwise specified.

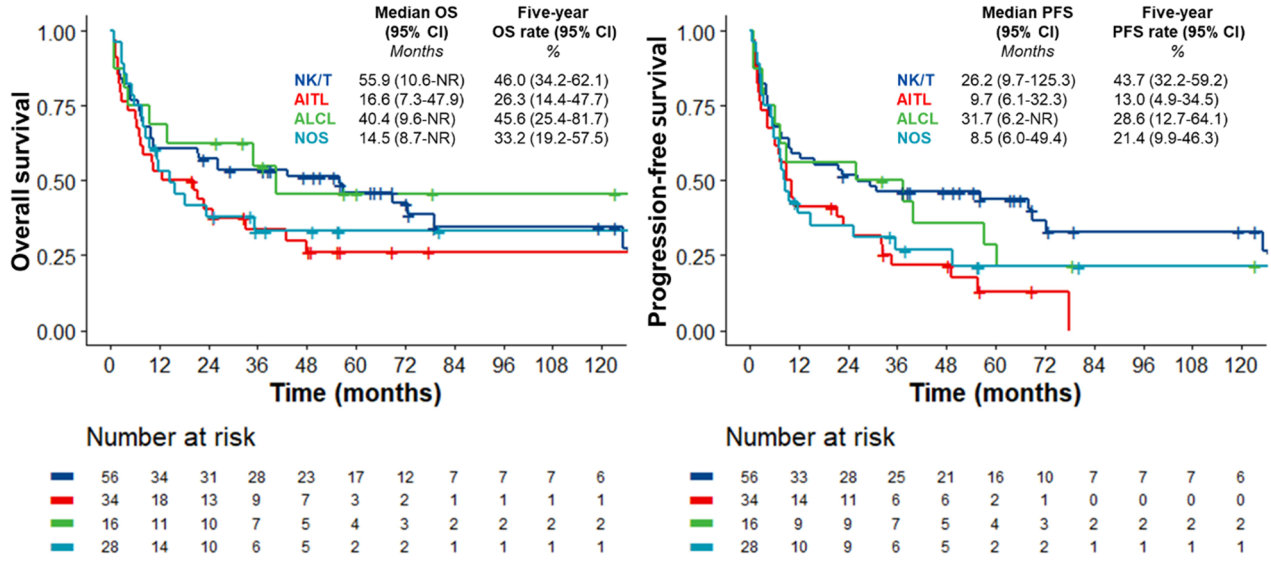


Figure 2. Survival of patients with different peripheral T-cell lymphoma subtypes.

five-year PFS rates were 43.7% (95% CI 32.2%–59.2%), 13.0% (95% CI 4.9%–34.5%), 28.6% (95% CI 12.7%–64.1%), and 21.4% (95% CI 9.9%–46.3%) for NK/TCL, AITL, ALCL, and PTCL-NOS patients, respectively. The five-year OS rates were 46.0% (95% CI 34.2%–62.1%), 26.3% (95% CI 14.4%–47.7%), 45.6% (95% CI 25.4%–81.7%), and 33.2% (95% CI 19.2%–57.5%) for NK/TCL, AITL, ALCL, and PTCL-NOS patients, respectively (Figure 2).

Using maximally selected rank statistics, we identified the optimal EBV DNA cutoff values for discriminating survival outcomes of all patients as 840 copies/mL ($p = 0.003$) for OS and 829 copies/mL ($p = 0.006$) for PFS. In NK/TCL patients with positive plasma EBV DNA at diagnosis, the OS (five-year OS rate 34.8% [95% CI 22.5%–53.8%] vs. 83.3% [95% CI 64.7%–100.0%], $p = 0.04$) and PFS (five-year PFS rate 31.0% [95% CI 19.5%–49.4%] vs. 83.3% [95% CI 64.7%–100.0%], $p = 0.02$) were significantly lower compared to those with negative plasma EBV DNA (Figure 3(A)). The best cut-off level was 3255 copies/mL for predicting both PFS and OS in NK/TCL patients. In patients with NK/TCL presented with an EBV DNA level < 3255 copies/mL, the OS (five-year OS rate 64.4% [95% CI 46.7%–89.1%] vs. 20.8% [95% CI 9.6%–45.4%], $p < 0.001$) and PFS (five-year PFS rate 64.2% [95% CI 47.5%–86.8%] vs. 16.7% [95% CI 6.8%–40.8%], $p < 0.001$) were significantly higher than those with EBV DNA level ≥ 3255 copies/mL at diagnosis (Figure 3(B)). In non-NK/TCL patients including AITL, ALCL, and PTCL-NOS, the pre-treatment plasma EBV DNA positivity or EBV DNA ≥ 840 copies/mL both did not significantly predict survival (Figure 3(C)).

In univariate analysis, age >60 years, the presence of B symptoms, poor ECOG performance status, advanced-stage disease, an IPI score > 1, a PIT score > 1, elevated LDH levels, elevated β2-microglobulin levels, EBV DNA positivity, and EBV DNA ≥ 3255 copies/mL at diagnosis were risk factors associated with lower PFS and OS in NK/TCL patients (Figure 4). In multivariate analysis, advanced-stage disease, an elevated β2-microglobulin level, and plasma EBV DNA ≥ 3255 copies/mL at diagnosis remained independent prognostic factors negatively impacting both OS and PFS in NK/TCL patients (Figure 4).

3.4. Post-treatment plasma EBV DNA positivity and the risk of disease progression

Post-treatment EBV DNA levels either at the time of CR, during follow-up, or at the time of relapse were available in 73 patients. Plasma EBV DNA positivity at CR was associated with significantly lower OS (two-year OS rate 60.0% [95% CI 36.2%–99.5%] vs. 94.1% [95% CI 83.6%–100.0%], $p = 0.01$) and PFS (two-year PFS rate 40.0% [95% CI 18.7%–85.5%] vs. 88.2% [95% CI 74.2%–100.0%], $p < 0.001$) in the NK/TCL group (Figure 5(A)). Significantly lower OS (two-year OS rate 54.8% [95% CI 39.8%–75.5%] vs. 100.0% [95% CI 100.0%–100.0%], $p = 0.003$) and PFS (two-year PFS rate 45.2% [95% CI 30.6%–66.6%] vs. 100.0% [95% CI 100.0%–100.0%], $p = 0.001$) were also found among NK/TCL patients when post-treatment plasma EBV DNA remained detectable (Figure 5(B)). In non-NK/TCL PTCL patients, plasma EBV DNA positivity at CR or plasma EBV DNA positivity at any time after first-line treatment were both not associated with survival (Figure 5(A–B)).

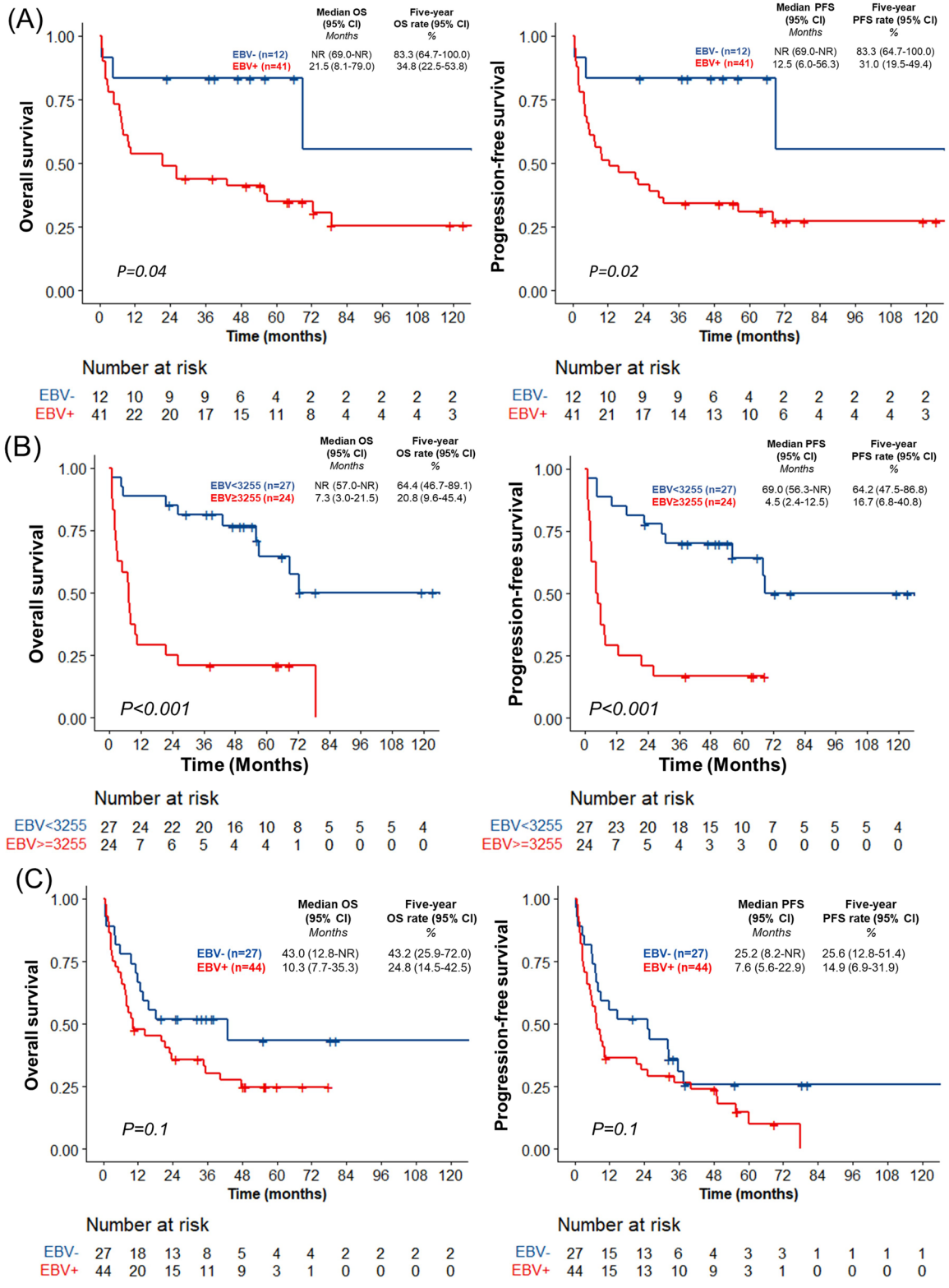
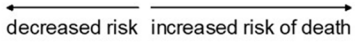


Figure 3. Survival of extranodal NK/T-cell lymphoma, nasal type (NK/TCL) patients and non-NK/TCL patients according to plasma EBV DNA status at diagnosis **(A)** OS and PFS of NK/TCL patients by plasma EBV DNA positivity. **(B)** OS and PFS of NK/TCL patients by plasma EBV DNA level of 3255 copies/mL as cutoff value. **(C)** OS and PFS of non-NK/TCL patients by plasma EBV DNA positivity.

4. Discussion

To the best of our knowledge, our study presents one of the relatively large PTCL cohorts with long follow-up period and includes plasma EBV DNA data at different time points. In this study, we conducted a comprehensive analysis to evaluate the association between

Factors	Overall Survival HR	95% CI	P	Progression-free Survival HR	95% CI	P
Univariate analysis						
Age > 60 years	2.26	1.14 - 4.51	0.020	2.17	1.11 - 4.25	0.020
B symptoms	4.93	2.36 - 10.30	<0.001	4.32	2.15 - 8.68	<0.001
ECOG >1	4.16	1.98 - 8.73	<0.001	3.69	1.80 - 7.56	<0.001
Stage III/IV	5.97	2.82 - 12.66	<0.001	5.60	2.72 - 11.53	<0.001
IPI score >1	3.04	1.51 - 6.10	0.001	2.63	1.34 - 5.14	0.004
PIT score >1	2.73	1.38 - 5.44	0.003	2.37	1.21 - 4.63	0.009
Elevated LDH	3.03	1.51 - 6.10	0.001	2.68	1.37 - 5.24	0.003
Elevated beta2-microglobulin	6.26	2.53 - 15.50	<0.001	4.92	2.19 - 11.08	<0.001
Diagnostic EBV-DNA positive	3.30	1.00 - 10.86	0.040	3.83	1.16 - 12.61	0.020
Diagnostic EBV-DNA ≥ 3255 cp/ml	4.78	2.17 - 10.51	<0.001	4.82	2.20 - 10.54	<0.001
Multivariate analysis						
Stage III/IV	5.44	1.02 - 28.92	0.047	15.31	2.43 - 96.44	0.004
Elevated beta2-microglobulin	15.06	3.39 - 66.91	<0.001	17.85	3.90 - 81.73	<0.001
Diagnostic EBV-DNA ≥3255 cp/ml	12.16	2.36 - 62.77	0.003	19.27	3.35 - 110.74	<0.001



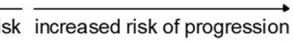


Figure 4. Univariate and multivariate regression analysis for survival of extranodal NK/T-cell lymphoma, nasal type patients.
Abbreviations: HR: hazard ratio; CI: confidence interval; ECOG: Eastern Cooperative Oncology Group performance status; IPI: International Prognostic Index; PIT: prognostic index for T-cell lymphoma; LDH: lactate dehydrogenase; cp/ml: copies/mL.

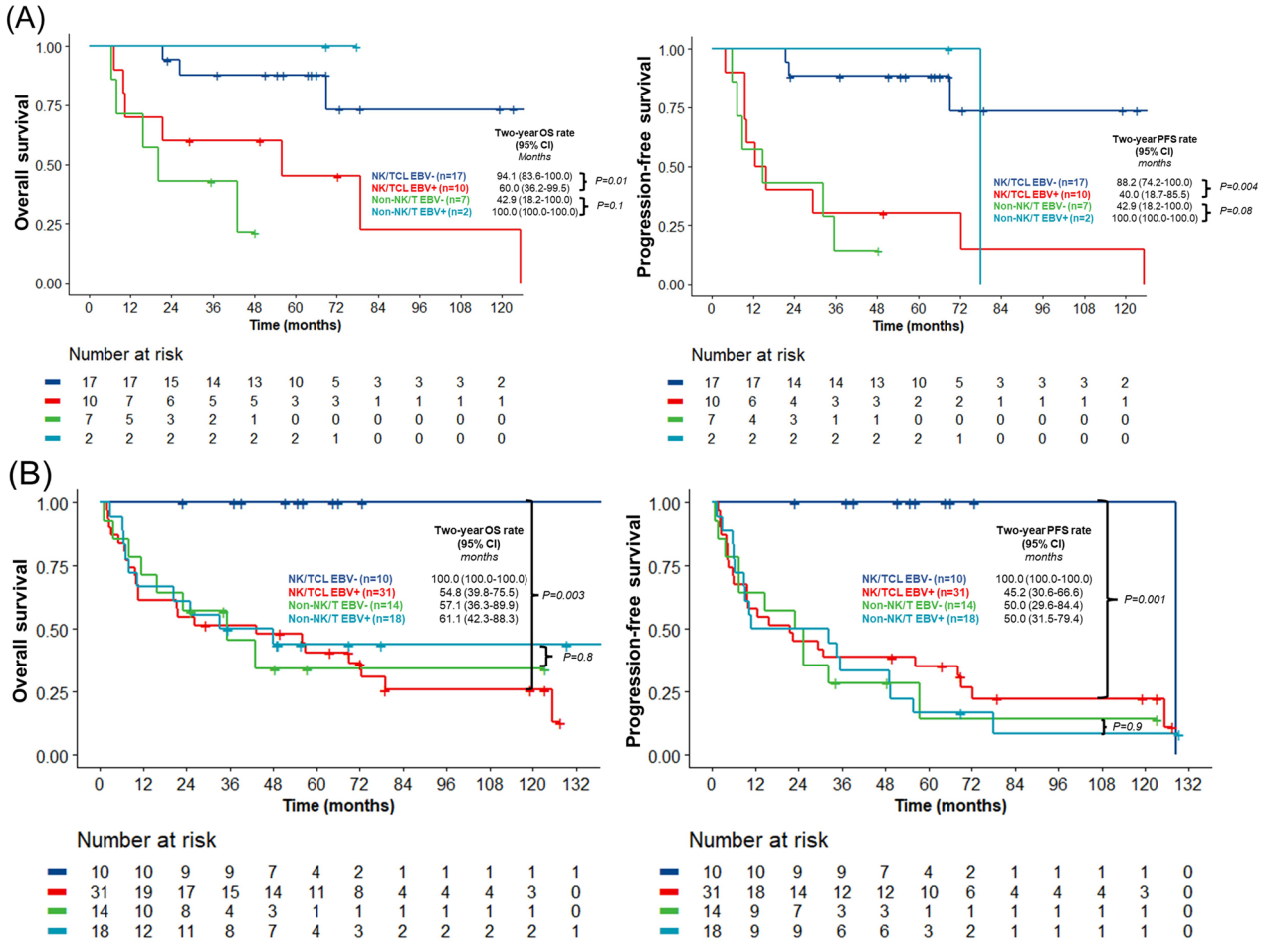


Figure 5. Survival of extranodal NK/T-cell lymphoma, nasal type (NK/TCL) vs. non-NK/TCL patients by post-treatment plasma EBV DNA positivity. **(A)** OS and PFS by plasma EBV DNA positivity at complete remission. **(B)** OS and PFS by post-treatment plasma EBV DNA positivity at any time after first-line treatment.

pre-treatment plasma EBV DNA levels and clinical characters, as well as the relationship between EBV DNA positivity, EBV DNA levels, treatment response, and prognosis in PTCL patients. Among different histological subtypes, patients with AITL had the highest rates of EBV DNA positivity and the highest plasma EBV DNA levels at the time of diagnosis, followed by those with NK/TCL. In patients with NK/TCL, an EBV DNA ≥ 3255 copies/mL at diagnosis was identified as the best cutoff value that negatively predicted OS and PFS, which also significantly correlated with lower CR rate and ORR. A detectable plasma EBV DNA at the time of CR and during follow-up after first-line treatment was associated with significantly lower PFS and OS in NK/TCL patients. For patients with PTCL other than NK/TCL, plasma EBV DNA at diagnosis or after treatment did not have an impact on treatment response, PFS, and OS.

In previous studies, inconsistent findings have been noticed regarding the prevalence of EBV DNA positivity and its correlation with various clinical and laboratory characteristics among different PTCL subtypes [25–30]. The pre-treatment EBV DNA positivity rates varied from 25% to 82% between different studies, with AITL and NK/TCL consistently showing the highest rates of EBV DNA positivity, followed by PTCL-NOS. In contrast, patients with ALCL were rarely tested positive with EBV DNA at diagnosis, and among the few positive cases, all were ALK-negative ALCL. In our study, we observed that higher median EBV DNA levels were associated with older age, the presence of B symptoms, poorer ECOG status, advanced-stage disease, higher IPI scores, higher PIT scores, as well as elevated LDH and β2-microglobulin levels in PTCL patients. In a cohort study by Kim et al., plasma EBV DNA positivity was found to be associated with advanced-stage disease, elevated LDH, and lower albumin levels [28]. Zhao et al. also demonstrated in their study that a higher plasma EBV DNA level has been linked to advanced age (> 60 years), elevated LDH, IPI > 3, and PIT ≥ 2 [26]. However, in two other studies, there were no distinct clinical characteristics among PTCL patients with positive plasma EBV DNA or higher EBV DNA levels at diagnosis [29,30].

In previous studies involving patients with NK/TCL, pre-treatment or post-treatment plasma EBV DNA positivity has been identified as a significant prognostic factor [13,15, 31,32]. However, some studies focused primarily on limited stage or extranodal NK/TCL, with widely diverse treatment regimen adopted across different studies. In our cohort, we incorporated advanced-staged and nodal NK/TCL in our analysis, as all of these patients were classified as extranodal NK/TCL, nasal type according to 2016 WHO classification [17]. The disparities in clinicopathological features and molecular signatures between nodal and extranodal NK/TCLs [33,34], as well as variations in the choice of first-line treatment among these patients, may lead to distinct survival outcomes. In another cohort of 32 advanced-stage extranodal NK/TCL patients, DNA positivity in the whole blood was found to be a significant prognostic factor for both PFS and OS, with a cutoff level of 5000 copies/mL for EBV DNA positivity [35]. We also found in our study that a higher EBV DNA cutoff level of 3255 copies/mL is an independent prognostic factor for PFS and OS in patients with NK/TCL. These findings suggest that the EBV DNA level may correlate with disease severity, and that a higher pre-treatment EBV DNA level, rather than just EBV DNA positivity, is required to discriminate survival outcomes in general NK/TCL patients.

On the other hand, the studies regarding the impact of EBV DNA on non-NK/TCL PTCL patients were rare and mostly conducted in a retrospective design. Some studies have shown that different cutoffs of EBV DNA levels, such as ≥ 500 copies/mL or ≥ 2000 copies/mL at diagnosis, were associated with lower OS or PFS in non-NK/TCL PTCLs [26,28–30], while a few other studies reported no impact on survival [25,27]. One study, in which NK/TCL patients were excluded, also showed a significantly decreased CR rate in PTCL patients with positive EBV DNA at diagnosis [29]. In our cohort, we found that plasma EBV DNA positivity or any cutoff levels either at diagnosis or after treatment did not have an impact on treatment response or survivals in 78 non-NK/TCL PTCL patients. The discrepancy between different studies might result from the variable EBV DNA positivity rate, heterogeneity in PTCL histological subtypes, and a relatively small number of non-NK/TCL PTCL patients in this study. Larger cohort studies are warranted to determine the role of EBV DNA in non-NK/TCL PTCL patients.

The different results between our study and earlier research can be analyzed from several perspectives. Previous research has demonstrated that plasma EBV DNA serves as a better marker than cellular EBV DNA for disease monitoring in EBV-positive diseases due to its higher sensitivity and specificity, including PTCLs [36]. Another crucial aspect is the estimation of EBV viral load using PCR techniques. The selection of the specific viral genes or sequences targeted by PCR can impact the sensitivity and accuracy of the assay. Additionally, the lower limits and detection range for EBV DNA also vary among laboratories. This variability could lead to different interpretations regarding the definition of EBV DNA positivity and possible

discrepancies in quantitative results, especially when the data falls beyond the reliable range of detection. In our study, the best cutoff value of EBV DNA for OS and PFS estimated by the maximally selected rank statistics are both higher than the lower limit of detection. This may explain why a high EBV viral load at diagnosis, rather than mere EBV positivity, was identified as an independent prognostic factor for the poor outcomes in these patients.

We found that the prognostic effect of EBV DNA regarding treatment response, survival, and the risk of progression was only observed in patients with NK/TCL rather than in patients with other PTCL subtypes. The distinct role of EBV in each PTCL subtype may partially explain the results of our study. In NK/TCL, EBV directly infects the T or NK-cells, transforming these cells into tumour cells. The proliferating tumour releases EBV DNA after apoptosis and necrosis, making it detectable by PCR [37,38]. As a result, the plasma EBV DNA viral load serves as an ideal marker for estimating the disease burden and monitoring treatment response in NK/TCL. In AITL, EBV-infected cells were the background B-cells with immunoblastic/plasmablastic immunophenotype, which were distinct from the neoplastic T-cells. In a study by Zhou et al. a higher EBV viral load in the EBV-positive AITL tissue was associated with more severe histological patterns and an increased incidence of B-cell clonality [39]. Another recent study regarding EBV transcriptome in 14 AITLs compared to 21 other lymphoma samples showed that Bam-HI A rightward transcripts, which have been reported to be highly expressed by carcinoma cells, were frequently found in AITLs and other lymphomas. Additionally, specific EBV genes associated with immune surveillance evasion and the promotion of tumor survival were found to be strongly expressed in patients with AITL [40]. These findings suggest that EBV may play a role in the pathogenesis of AITL by altering the tumour microenvironment and compromising the immune system. In patients with PTCL-NOS, EBER positivity in the tissue has been reported to be associated with the presence of B-cell proliferation but not increased B-cell clonality [41]. The role of EBV in the tumorigenesis of PTCL-NOS is probably through its effect on the immune system, similar to its role in AITL. These indirect mechanisms of tumorigenesis may help explain the dissociation between plasma EBV DNA levels and the prognosis of AITL or PTCL-NOS in our study.

The major limitations of this study are the retrospective and single-center design, heterogeneity of PTCL histology subtypes, relatively small case number of non-NK/TCL patients, as well as the incomplete EBV viral load data during CR and follow-up after treatment, which may interfere with the analysis.

5. Conclusion

Our study analysed plasma EBV DNA levels both at the time of diagnosis and during follow-up in a relatively large cohort of PTCL patients. In patients with NK/TCL, plasma EBV DNA ≥ 3255 copies/mL at diagnosis is an independent prognostic factor for both OS and PFS. Plasma EBV DNA positivity during CR and at any time of post-treatment follow-up was associated with significantly lower OS and PFS, but this association was not observed in non-NK/TCL patients. Detection and monitoring of plasma EBV DNA levels at diagnosis and during post-treatment follow-up is recommended for NK/TCL patients. In NK/TCL patients, a positive conversion of plasma EBV DNA after achieving CR or persistently detectable plasma EBV DNA during follow-up may raise concerns about disease progression, and further imaging studies may be indicated for early detection of relapse. Further large-scale studies are warranted to identify the prognostic role of plasma EBV DNA in PTCLs other than NK/TCL.

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Author contributions

HWK conceptualized, designed and supervised the study. CYC performed data collection, statistical analysis, and wrote the manuscript. HWK collected data, reviewed and edited the manuscript. HWK, TLL, MCK, YSH, HC, CWO, JHW, HJS, YJS, LYS, and YCO provided clinical data. WYC contributed to the pathology review. All authors reviewed and approved the manuscript.

Ethics statement

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the IRB of Chang Gung Memorial Hospital (201502568B0, 201601360B0). Informed consent was waived by the same IRB due to retrospective observational design of the study with minimal risk to the patients.

Consent for publication

All authors agreed to the publication.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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ORCID

Chu-Yi Chan http://orcid.org/0009-0009-6694-6265
Tung-Liang Lin http://orcid.org/0000-0002-9532-749X
Hung Chang http://orcid.org/0000-0001-8451-0304
Yi-Jiun Su http://orcid.org/0000-0002-8372-1166
Lee-Yung Shih http://orcid.org/0000-0003-1866-7922
Wen-Yu Chuang http://orcid.org/0000-0002-3729-073X
Hsiao-Wen Kao http://orcid.org/0000-0002-3586-5317

Data availability statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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