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CLINICAL INVESTIGATION

Plasma Epstein-Barr Virus DNA Temporal Clearance Pattern During Induction-Concurrent (Chemo)Radiation Therapy for Risk Stratification in Nasopharyngeal Carcinoma

Zongwei Huang, MD, PhD,[a] Ying Li, MD,[a] Wenxi Wu, MM,[a] Lishui Wu, MM,[a] Zihan Chen, MM,[a] Siqi Xu, MM,[a] Yi Li, MD,[a] Jinghua Lai, MBBS,[a] Sufang Qiu, MD, PhD,[a,b,c] and Jun Lu, MBBS[a]

[a]*Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital, Fuzhou, Fujian, China;* [b]*Fujian Key Laboratory of Translational Cancer Medicine, Fuzhou, Fujian, China; and* [c]*Fujian Provincial Key Laboratory of Tumor Biotherapy, Fuzhou, Fujian, China*

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Purpose: Plasma Epstein−Barr virus (EBV) DNA is a widely used biomarker for nasopharyngeal carcinoma (NPC). Prior investigations predominantly assessed EBV DNA at a single time point, thus neglecting the differential prognostic implications of the temporal clearance pattern of EBV DNA during induction-concurrent (chemo)radiation therapy (RT).

Methods and Materials: We retrospectively reviewed EBV DNA clearance patterns during induction-concurrent chemoRT in newly diagnosed patients with nonmetastatic NPC. EBV DNA was tested at 3 time points (baseline [T0], end of induction chemotherapy [T1], and end of RT [T2]) and recorded as detectable (D) and undetectable (U). The association between EBV DNA pattern and progression-free survival was analyzed.

Results: A total of 2203 NPCs were included. Five distinct EBV DNA trajectory patterns were identified: type Ⅰ (negative-stable, U-U-U, 7.3%), type Ⅱ (induction chemotherapy-elimination, D-U-U, 42.8%), type Ⅲ (RT-elimination, D-D-U, 35.0%), type Ⅳ (persistent-positive, D-D-D, 11.7%), and type Ⅴ (resurgence, D-U-D [1.5%], U-D-U [1.2%], U-D-D [0.4%], or U-U-D [0.2%]). The median follow-up was 53.5 months (IQR, 43.1-66.9). Type Ⅱ patients displayed superior 5-year progression-free survival (82.9% [95% CI, 80.4%-85.5%]) versus type Ⅲ (75.9% [72.8%-79.1%], $P < .001$), type Ⅳ (52.5% [46.4%-59.5%], $P < .001$), and type Ⅴ (72.5% [62.2%-84.6%], $P = .028$). The 5-year progression-free survival for type V patients with "D-U-D," "U-

Corresponding authors: Jun Lu, MBBS; and Sufang Qiu, MD, PhD;; E-mail: sufangqiu@fjmu.edu.cn, fjzllujun@fjzlhospital.com

Authors responsible for statistical analysis: Zongwei Huang, MD, PhD; Email: huangzongwei@stu.fjmu.edu.cn and Ying Li, MD; Email: lying7701@163.com

Zongwei Huang and Ying Li have contributed equally to this work.

Ethics statement: The ethical clearance for this investigation was obtained from the institutional review board of Fujian Cancer Hospital, with the retrospective nature of the study rendering patient informed consent unnecessary.

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D-U," "U-D-D," and "U-U-D" patterns was 62.4% (46.2%-84.3%), 78.6% (63.1%-97.8%), 85.7% (63.3%-100.0%), and 75.0% (42.6%-100.0%), respectively. All 33 patients with the "D-U-D" pattern had stage III-IV disease at diagnosis.

Conclusions: Temporal EBV DNA clearance patterns during induction-concurrent chemoRT provide valuable prognostic insights, enabling the identification of patients with high-risk NPC and informing personalized treatment strategies. Resurgence of EBV DNA may occur occasionally (3.3%). Caution is required when considering reduced-intensity therapy in patients with locoeregionally advanced disease when EBV DNA becomes U after induction chemotherapy.

Introduction

Nasopharyngeal carcinoma (NPC) is generally a chemo/radiosensitive tumor.[1,2] Although contemporary treatments have achieved high rates of disease control and improved survival, approximately 20% of patients still experience locoregional or distant relapse.[3,4] Consequently, early detection of treatment response is essential for optimizing therapeutic strategies and improving outcomes in patients at high risk of treatment failure after primary therapy.

Plasma Epstein−Barr virus (EBV) DNA is currently the most prototypical and widely used biomarker for NPC.[5,6] The prognostic utility of EBV DNA is complicated by its dynamic nature.[7,8] Most prior studies have concentrated on single time point measurements of EBV DNA, such as before or after radiation therapy (RT), with limited attention to longitudinal monitoring.[9,10] Evidence suggests that dynamic changes and cumulative burden of EBV DNA over time may process independent prognostic significance in NPC.[11] For instance, Lv et al[12] demonstrated that continuous monitoring of EBV DNA levels during induction chemotherapy (IC) and chemoRT could inform the optimization of individualized chemotherapy regimens for locally advanced NPC.

A reduction in EBV DNA titer during treatment has been significantly associated with improved survival outcomes in patients with newly diagnosed or metastatic NPC.[13,14] Recent studies have emphasized the prognostic significance of plasma EBV DNA response to IC.[13,15] Clinical evidence further suggests that patients exhibiting sensitivity to IC represent ideal candidates for de-escalation therapy.[16-18] A single-arm phase 2 trial with reduced-dose RT (60 Gy) showed favorable outcomes and reduced treatment-related toxicities in patients with stage III NPC who achieved EBV DNA clearance and radiologic complete or partial response after IC.[19] These findings highlight the importance of identifying the phase of EBV DNA clearance during treatment as a practical and effective strategy for risk stratification, enabling timely adjustments to subsequent therapeutic interventions to optimize outcomes. However, whether those who achieved EBV DNA clearance after IC could remain undetectable (U) during concurrent chemoRT (CCRT) is unknown.

In this retrospective cohort study, we aimed to delineate the pattern of dynamic changes in EBV DNA during induction-CCRT and to evaluate their associations with clinical outcomes in NPC.

Methods

Patients

We conducted an analysis of a cohort of patients with NPC from Fujian Cancer Hospital, including individuals newly diagnosed with histologically confirmed NPC between January 2016 and December 2019. All patients were staged in accordance with TNM-8 NPC classification. The inclusion criteria were stages I to IVA, completed radical intensity modulated RT (IMRT), and had at least 3 plasma EBV DNA measurements: at baseline (T0), end of IC (T1), and end of RT (T2). The exclusion criteria encompassed patients who did not undergo IC, experienced treatment interruptions, exhibited disease progression during treatment, or were lost to follow-up. Ethical approval was granted by the institutional review board (K2024-311-01), and informed consent was waived because of the retrospective nature of this study.

Plasma EBV DNA quantitation

EBV DNA measurements were repeated from admission to post-RT, with the timing and intervals of these measurements varying among patients. Each participant underwent a minimum of 3 measurements: T0 (pre-IC), T1 (pre-RT), and T2 (post-RT). Pre-IC EBV DNA denoted values were obtained within 28 days prior to the initiation of IC, with a median measurement time of day 7 (IQR, day 5-8 before IC). Pre-RT EBV DNA was defined as the measurement taken closest to the initiation of RT, spanning from 24 days prior to RT to within 1 week after RT initiation (median: day 1; IQR, day 0-3 before RT). Post-RT EBV DNA referred to the measurement taken nearest to the completion of RT, within 21 days post-RT completion (median: day 3; IQR, day 1-7 after RT).

Quantitative assessment of EBV DNA was performed using a real-time quantitative polymerase chain reaction assay, which targeted the highly conserved BamHI-W region of the EBV genome.[20] The results were reported as EBV genome copies per milliliter of plasma. The quantifiable range of values in this study spanned from 20 to 4,950,000 copies/mL of the EBV genome. Additional details regarding the methodology for EBV DNA quantitation are provided in the Appendix E1.

Treatment

All patients received IC combined with IMRT, with or without concurrent or adjuvant chemotherapy (AC). Detailed descriptions of the IMRT protocol are provided in the Appendix E1. The prescribed doses ranged from 69.30 to 72.00 Gy to the planning target volumes of gross tumor volumes (GTVs) for the primary tumor and from 66.00 to 72.00 Gy GTV for lymph nodes. Doses of 54.90 to 66.00 Gy were delivered to the planning target volume of clinical target volume 1, and doses of 48.00 to 56.00 Gy were delivered to the planning target volumes of clinical target volume 2 and clinical target volume lymph nodes. These doses were administered in 31 to 35 fractions, once daily, 5 days per week. Residual disease identified near the completion of RT, based on clinical assessment (including endoscopic evaluation) and imaging (computed tomography or magnetic resonance imaging) was treated with boost irradiation, delivering an additional dose of 2.0 to 6.3 Gy over 1 to 3 fractions.

All patients received platinum-based IC. The regimens comprised gemcitabine (1000 mg/m^2) plus platinum (GP), paclitaxel (100-150 mg/m^2) or docetaxel (60-75 mg/m^2) plus platinum (TP), platinum plus 5-fluorouracil (800-2500 mg/m^2) (PF), and a combination of paclitaxel or docetaxel, platinum, and 5-fluorouracil (TPF). Specifically, 55.2% (1217/2203) of patients received the GP regimen, 40.1% (883/2203) received TP, and 0.2% (4 cases) received TPF, with all administered every 3 weeks. The PF regimen was used in 2.5% (56/2203) of patients, including the conventional triweekly regimen (cisplatin, 100 mg/m^2 on day 1, followed by continuous infusion of 5-fluorouracil, 800-1000 mg/m^2/d on days 1-4, repeated every 3 weeks for 3 cycles) and a weekly regimen (cisplatin, 60 mg/m^2 on days 1, 15, 29, 43, and 57, and 5-fluorouracil, 2500 mg/m^2, plus leucovorin, 250 mg/m^2, on days 8, 22, 36, 50, and 64, for a total of 10 doses). Forty-three patients switched their IC regimen because of insufficient therapeutic response or intolerable adverse effects.

Concurrent chemotherapy was administered to 69.8% (1537/2203) of patients, primarily consisting of cisplatin (80-100 mg/m^2) or alternative platinum-based agents, given every 3 weeks in combination with IMRT. Patients who experienced severe hematologic toxicities during IC or those with low-risk factors—such as low tumor burden, minimal EBV DNA load, or complete tumor regression after IC—were treated with IC combined with IMRT alone. AC was reserved for high-risk patients, including those with residual tumor, detectable (D) EBV DNA post-RT, or a high tumor burden (eg, T4N2 or N3 disease) and was typically given for 1 to 3 cycles, with decisions guided by clinical factors and patient preferences. The most frequently administered adjuvant regimens were GP and TP.

Surveillance protocol and outcome

After the completion of treatment, all patients underwent follow-up evaluations at 3-month intervals during the first 2 years, transitioning to 6-month intervals over the subsequent 3 years, and annually thereafter. Nasopharyngoscopy was conducted every 3 to 6 months during the follow-up visits, whereas magnetic resonance imaging of the head and neck was performed biannually. Whole-body bone emission computed tomography was administered annually. Additionally, ^{18}F-fluorodeoxyglucose positron emission tomography and computed tomography could be also employed in cases where disease progression was suspected. The follow-up period for this investigation concluded on February 20, 2024. The median follow-up was defined as the median time to the last recorded survival status—whether alive or deceased—or to the final clinical or radiographic assessment.

The primary endpoint of this study was progression-free survival (PFS), with progression defined to encompass death, locoregional recurrence, and distant metastasis. Secondary endpoints comprised overall survival (OS), locoregional failure-free survival (LRFS), and distant metastasis failure-free survival (DMFS). LRFS and DMFS were defined as the interval from the completion of RT to the occurrence of locoregional relapse or distant metastasis or death from any cause. For patients whose first event was distant metastasis, they were conserved at the time of distant metastasis for LRFS; similarly, for patients whose first event was a locoregional relapse, they were conserved at the time of locoregional relapse for DMFS.

Statistical analysis

The study incorporated several covariates, including age, sex, tumor category, node category, histological subtype, IC regimens, GTV for the primary tumor and GTV for lymph nodes, and the use of concurrent and AC. To maintain the representativeness of the data set, the "MICE" R package was used for the imputation of missing data in 85 cases (3.9%) with unavailable histological subtype information.[21] Comparisons of characteristics among groups were conducted using Kruskal−Wallis tests for continuous variables and χ^2 tests for categorical variables. Survival analyses were performed using Kaplan−Meier curves, with comparisons via log-rank tests. EBV DNA results were classified as U or D for trajectory analysis. A multivariable analysis with Cox proportional hazards models was used to evaluate associations between trajectory grouping and survival outcomes, adjusting for age, sex, tumor characteristics, and treatment regimens. Furthermore, exploratory subgroup analyses were undertaken to examine the association between survival outcomes and treatment intensity across various subpopulations. Statistical analyses were conducted using SPSS Statistics v25.0 or R (version 4.3.0, R Foundation for Statistical Computing), supplemented by Zstats v0.90 (www.medsta.cn/software). All tests were 2-sided with statistical significance defined as $P < .05$.

Results

Patient characteristics

A total of 2203 out of 2613 patients with M0 NPC were included. The clinical characteristics of these patients are shown in Table E1. The cohort comprised 1612 (73.2%) male patients, with a median age of 48 years (IQR, 40-56). All patients received IC, and 1537 (69.8%) and 666 (30.2%) patients received CCRT and IMRT alone, respectively. Additionally, 203 (9.2%) patients also received AC.

The median follow-up was 53.5 months (IQR, 43.1-66.9). Five-year PFS, OS, LRFS, and DMFS rates were 76.3% (95% CI, 74.4%-78.2%), 85.5% (83.9%-87.2%), 83.8% (82.1%-85.5%), and 84.5% (82.9%-86.2%), respectively.

EBV DNA clearance trajectory analysis

Patients were categorized into 8 groups and 5 types according to the binary classification of EBV DNA levels at distinct time points as either "U" or "D" (Table 1): type Ⅰ (negative-stable type, U-U-U): 160 (7.3%); type Ⅱ (IC-elimination type, D-U-U): 942 (42.8%); type Ⅲ (RT-elimination type, D-D-U): 771 (35.0%), type Ⅳ (persistent-positive type, D-D-D): 258 (11.7%), and type Ⅴ (resurgence type, including D-U-D [33, 1.5%], U-D-U [26, 1.2%], U-D-D [8, 0.4%], and U-U-D [5, 0.2%]): 72 (3.3%).

Type Ⅰ was characterized by consistent U EBV DNA levels throughout treatment. Type Ⅱ exhibited rapid clearance of EBV DNA after IC with subsequent stable U levels. Type Ⅲ demonstrated incomplete EBV DNA clearance after IC (median: 290 copies/mL [IQR, 102-1130]), yet complete clearance was achieved post-RT. Conversely, type IV was distinguished by persistent detectability of EBV DNA throughout treatment. The remaining 4 groups shared the common feature of the reappearance of D EBV DNA after a period of undetectability, a phenomenon unlikely attributable to testing variability. These were classified as type Ⅴ or the "resurgence type." The clinicopathologic characteristics of the trajectory groups are presented in Table E2.

EBV DNA trajectory types inform on prognosis in patients with NPC

Type Ⅳ exhibited the lowest 5-year PFS (52.5% [95% CI, 46.4%-59.5%]) versus type I (79.4% [72.9%-86.5%], $P < .001$), type Ⅱ (82.9% [80.4%-85.5%], $P < .001$), type Ⅲ (75.9% [72.8%-79.1%], $P < .001$), and type Ⅴ (72.5% [62.2%-84.6%], $P = .005$) (Fig. 1A). Similar trends were observed for OS, LRFS, and DMFS (Fig. 1B-D). The poor PFS with type Ⅳ was confirmed in multivariable analysis after adjusted for age, sex, tumor category, node category, histologic subtype, and AC (Table 2). Additionally, compared with type Ⅲ, type Ⅱ shows superior 5-year PFS (82.9% [80.4%-85.5%] vs 75.9% [72.8%-79.1%]) and LRFS (88.6% [86.4%-90.9%] vs 81.8% [78.8%-84.8%]) (all $P < .001$).

Characteristics and outcomes of EBV DNA resurgence patients

Patients in type Ⅴ exhibited a higher risk of cancer progression compared with those in type Ⅱ (5-year PFS, 72.5% [62.2%-84.6%] vs 82.9% [80.4%-85.5%], $P = .028$). Further analysis of prognostic outcomes within the type Ⅴ group revealed comparable 5-year PFS rates between patients with the "U-D-U" and "U-U-U" patterns (78.6% [63.1%-97.8%] vs 79.4% [72.9%-86.5%], $P = .963$) nor between "U-U-D" and "U-U-U" (75.0% [42.6%-100.0%] vs 79.4% [72.9%-86.5%]; $P = .760$). Similarly, the 5-year PFS rate among those with the "U-D-D" pattern did not differ significantly from that of the "U-U-U" (85.7% [63.3%-100.0%] vs 79.4% [72.9%-86.5%], $P = .643$), but marginally higher than that of "D-D-D" pattern (52.5% [95% CI, 46.4%-59.5%], $P = .098$). Notably, patients with the "D-U-D" pattern exhibited markedly inferior 5-year PFS (57.6% [41.8%-79.4%] vs 82.9% [80.4%-85.5%], $P = .002$) and DMFS (77.9% [64.6%-93.9%] vs 88.4% [86.2%-90.7%], $P < .001$) compared with those with the "D-U-U" pattern (ie, type II). Furthermore, the "D-U-D" pattern was associated with significantly worse 5-year PFS (57.6% [41.8%-79.4%] vs 78.6% [63.1%-97.8%], $P = .036$) and DMFS (77.9% [64.6%-93.9%] vs incalculable, $P = .018$) relative to the "U-D-U" pattern.

Among the 33 patients with the "D-U-D" pattern, all of whom had locoregionally advanced disease, 3 (9.1%) exhibited recurrent disease and relocated in the initial diagnostic imaging and IMRT plan (Table 3). One patient with T3N2 disease experienced a local failure in the posterior nasopharyngeal parietal wall, classified as in-field failure (prescribed dose ≥ 66 Gy). Another patient with T3N1 disease experienced the sphenoid sinus, cavernous sinus, and skull base recurrence, categorized as out-field failure with a prescribed dose of 60 to 66 Gy aimed at optic nerve protection. A third patient experienced both local and regional recurrences, with local failure classified as out-field failure (60-66 Gy) and regional failure classified as in-field failure (≥66 Gy). Furthermore, 8 patients (24.2%) with the "D-U-D" pattern developed distant metastases. Among the 26 patients with the "U-D-U" pattern, 4 (15.4%) developed recurrent disease, comprising 3 cases of regional lymph node recurrence and 1 case of local recurrence in the nasopharyngeal mucosa, all of which were classified as in-field failure (≥66 Gy). No distant metastases were observed in this subgroup. Additional clinical characteristics of these patients are detailed in Tables 3 and 4.

Exploratory analysis: Impact of treatment intensity on outcomes of various EBV DNA trajectory patterns

To investigate whether patients who achieved EBV DNA clearance after IC could be treated with RT alone, we compared outcomes in the "D-U-U" and "D-U-D" subtypes and found comparable 5-year PFS between CCRT (81.2%

Table 1 The characteristics of the EBV DNA clearance trajectory during induction-concurrent chemoRT in NPC

EBV DNA clearance types* (T0-T1-T2)	EBV DNA trajectory types	No. of cases (%)	Copy no. of EBV DNA (median [IQR], copies/mL)	5-year outcome (95% CI) (%)
U-U-U	Type Ⅰ (negative-stable type)	160 (7.3%)	T0: 0 (0-0) T1: 0 (0-0) T2: 0 (0-0)	PFS: 79.4 (72.9-86.5) OS: 86.3 (80.7-92.3) LRFS: 85.3 (79.4-91.6) DMFS: 87.0 (81.3-93.1)
D-U-U	Type Ⅱ (IC-elimination type)	942 (42.8%)	T0: 2325 (485.75-10,800) T1: 0 (0-0) T2: 0 (0-0)	PFS: 82.9 (80.4-85.5) OS: 89.0 (86.8-91.3) LRFS: 88.6 (86.4-90.9) DMFS: 88.4 (86.2-90.7)
D-D-U	Type Ⅲ (RT-elimination type)	771 (35.0%)	T0: 5220 (853.5-26,400) T1: 290 (102-1130) T2: 0 (0-0)	PFS: 75.9 (72.8-79.1) OS: 87.1 (84.5-89.7) LRFS: 81.8 (78.8-84.8) DMFS: 86.6 (84.0-89.3)
D-D-D	Type Ⅳ (persistent-positivity type)	258 (11.7%)	T0: 11,650 (1947.5-48,625) T1: 1340 (282.5-7657.5) T2: 144.5 (62.45-491)	PFS: 52.5 (46.4-59.5) OS: 68.3 (62.4-74.6) LRFS: 69.6 (63.2-76.6) DMFS: 63.4 (57.3-70.1)
D-U-D	Type Ⅴ (resurgence type)	33 (1.5%)	T0: 3110 (392-12,900) T1: 0 (0-0) T2: 120 (48.9-319)	PFS: 57.6 (41.8-79.4) OS: 83.5 (68.9-100.0) LRFS: 88.1 (76.3-100.0) DMFS: 77.9 (64.6-93.9)
U-D-U		26 (1.2%)	T0: 0 (0-0) T1: 247 (128-914.5) T2: 0 (0-0)	PFS: 78.6 (63.1-97.8) OS: 82.4 (67.8-100.0) LRFS: 78.6 (63.1-97.8) DMFS: Incalculably
U-D-D		8 (0.4%)	T0: 0 (0-0) T1: 1160 (114.88-3640) T2: 78.9 (57.5-155.5)	PFS: 85.7 (63.3-100.0) OS: Incalculably LRFS: 85.7 (63.3-100.0) DMFS: Incalculably
U-U-D		5 (0.2%)	T0: 0 (0-0) T1: 0 (0-0) T2: 213 (170-259)	PFS: 75.0 (42.6-100.0) OS: Incalculably LRFS: 75.0 (42.6-100.0) DMFS: Incalculably

Abbreviations: D = detectable; DMFS = distant metastasis failure-free survival; EBV = Epstein−Barr virus; IC = induction chemotherapy; LRFS = locoregional failure-free survival; NPC = nasopharyngeal carcinoma; OS = overall survival; PFS = progression-free survival; RT = radiation therapy; U = undetectable.

* EBV DNA clearance types: EBV DNA dichotomization method, which classifies patients as "U" or "D" at each time point (including baseline [T0], end of IC [T1], and end of RT [T2]).

[78.0%-84.6%]) and RT alone (83.5% [79.4%-87.9%]) (P = .128, Fig. 2A). Removing the "D-U-D" subset, the 5-year PFS remained comparable (82.2% [79.0%-85.5] for CCRT vs 83.6% [79.4%-88.0%] for RT, P = .352, Fig. 2B). However, in patients whose EBV DNA remained D prior to RT (including the "D-D-U" and "D-D-D" types), CCRT (71.6% [68.4%-75.0%]) demonstrated an improved 5-year PFS compared with RT alone (65.1% [59.1%-71.8%]) (P = .044, Fig. 2C). We further explore the value of AC using the same IC regimen (31 of 38) in patients with type IV pattern (ie, "D-D-D") and found no significant improvement in 5-year PFS between AC versus observation (51.2% [35.2%-74.4%] vs 52.3% [45.6%-59.9%], P = .938, Fig. 2D).

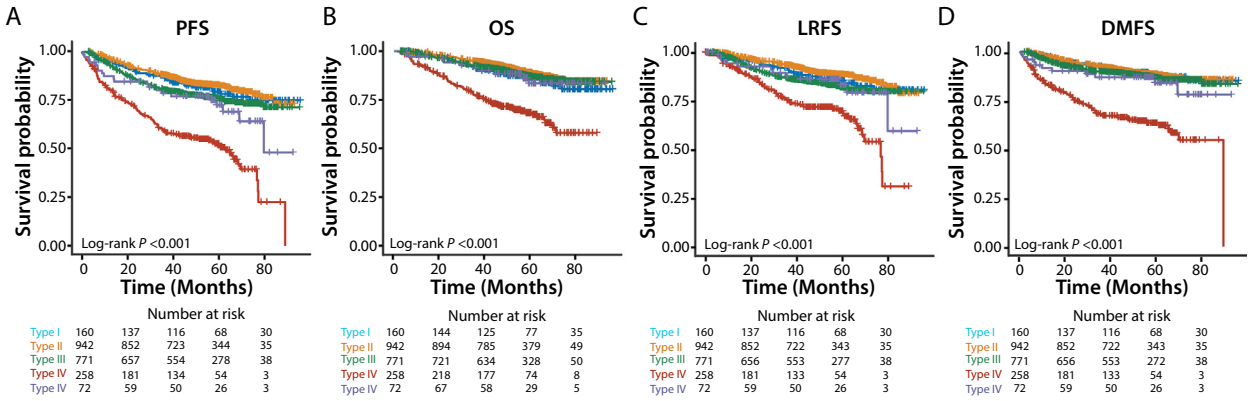

Fig. 1. Longitudinal EBV DNA trajectory types and NPC outcomes. Kaplan−Meier curves of (A) PFS, (B) OS, (C) LRFS, and (D) DMFS for longitudinal EBV DNA trajectory types. *Abbreviations:* DMFS = distant metastasis failure-free survival; EBV = Epstein−Barr virus; LRFS = locoregional failure-free survival; NPC = nasopharyngeal carcinoma; OS = overall survival; PFS = progression-free survival.

Discussion

This study described the longitudinal EBV DNA clearance trajectories in patients with NPC during induction-CCRT, identifying 5 distinct patterns: negative-stable type (type Ⅰ), IC-elimination type (type Ⅱ), RT-elimination type (type Ⅲ), persistent-positivity type (type Ⅳ), and resurgence type (type Ⅴ), defined by tumor marker clearance and treatment sensitivity. These trajectories exhibited varying risks of disease progression, with type Ⅳ patients demonstrating significantly worse PFS, OS, LRFS, and DMFS compared with other groups. Notably, type Ⅱ patients displayed superior 5-year PFS and LRFS rates relative to type Ⅲ, underscoring the prognostic advantage of rapid EBV DNA clearance after IC. Patients with the "D-U-D" pattern in type V all had stage Ⅲ to Ⅳ disease, and they had a higher risk of disease progression, primarily due to distant metastases. Therefore, caution is required when considering reduced-intensity therapy in patients with sensitivity to IC. These findings highlight the critical role of dynamic EBV DNA monitoring in guiding individualized therapeutic strategies to optimize outcomes in NPC.

Our study aligns with existing literature, revealing that dynamic changes in EBV DNA levels are helpful for treatment evaluation and prognosis surveillance in NPC.[11,12,22-24] Recent research has shown that EBV DNA levels measured 8 to 12 weeks post-RT are more predictive of 2-year relapse incidence than those assessed within 0 to 2 weeks post-RT.[11] Although earlier longitudinal studies, such as those by Neo et al,[11] concentrated on post-RT EBV DNA trends, Lv et al[12] identified distinct EBV DNA remission patterns during IC that correlate with treatment sensitivity and recurrence risk in locally advanced NPC. These observations have been further substantiated by the prospective EP-SEASON study (NCT03855020), underscoring the prognostic value of dynamic EBV DNA changes in predicting treatment response and disease recurrence.[23] More recently, Liu et al[24] also delineated 4 response clusters based on multipoint assessments of EBV DNA and tumor response,

Table 2 Association of EBV DNA trajectory types* and survival outcomes in patients with NPC by Cox proportional hazard regression analysis†

EBV DNA trajectory type	PFS		OS		LRFS		DMFS	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Type Ⅳ	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Type Ⅰ	0.35 (0.23-0.52)	<.001	0.44 (0.27-0.70)	<.001	0.40 (0.25-0.65)	<.001	0.31 (0.19-0.52)	<.001
Type Ⅱ	0.29 (0.23-0.37)	<.001	0.31 (0.23-0.41)	<.001	0.32 (0.23-0.43)	<.001	0.28 (0.21-0.37)	<.001
Type Ⅲ	0.40 (0.32-0.51)	<.001	0.34 (0.25-0.46)	<.001	0.48 (0.36-0.65)	<.001	0.30 (0.23-0.41)	<.001
Type Ⅴ	0.51 (0.32-0.82)	.002	0.38 (0.20-0.74)	.004	0.47 (0.26-0.88)	.018	0.41 (0.22-0.76)	.005

Abbreviations: DMFS = distant metastasis failure-free survival; EBV = Epstein−Barr virus DNA; IC = induction chemotherapy; LRFS = locoregional failure-free survival; NPC = nasopharyngeal carcinoma; OS = overall survival; PFS = progression-free survival; RT = radiation therapy.

* EBV DNA trajectory types: type Ⅰ (negative-stable type), type Ⅱ (IC-elimination type), type Ⅲ (RT-elimination type), type Ⅳ (persistent-positivity type), and type Ⅴ (resurgence type).

† Adjusted for age, sex, tumor category, node category, histological subtype, and adjuvant chemotherapy.

Table 3 The characteristics and outcomes of 33 patients with NPC with "D-U-D" type

Patients	Age (y)	Sex	Tumor category	Node category	Stage	Pre-IC EBV DNA, copies/mL	Outcomes		
							Locoregional failure	Distant failure	Death
1	64	Male	3	1	III	178	No	No	No
2	58	Male	1	2	III	502	No	Lung	No
3	43	Male	3	1	III	10,600	No	Liver	No
4	44	Male	4	3	IV	57,400	No	Liver, bone	No
5	56	Male	3	2	III	3950	No	No	No
6	36	Male	1	2	III	47,900	No	No	No
7	61	Female	4	0	IV	36	No	No	No
8	44	Male	1	2	III	39.6	No	No	No
9	47	Male	3	2	III	13,000	No	Lung, bone	No
10	23	Female	1	3	IV	12,900	No	No	No
11	65	Male	2	2	III	29,300	No	Liver	Yes
12	38	Male	3	2	III	8080	No	Liver	No
13	54	Male	3	0	III	54.4	No	No	Yes
14	55	Male	2	2	III	4560	No	No	No
15	49	Female	3	1	III	16,700	No	No	No
16	48	Male	3	1	III	392	No	No	No
17	50	Male	4	1	IV	289	No	No	No
18	32	Male	3	1	III	3110	No	No	No
19	56	Male	2	2	III	142,000	No	No	No
20	63	Male	2	3	IV	3020	No	No	No
21	35	Male	3	1	III	13,400	No	No	No
22	42	Male	3	1	III	1330	Local failure	No	No
23	45	Male	2	2	III	3660	No	No	No
24	30	Female	3	1	III	229	No	No	No
25	47	Male	3	2	III	236,000	No	No	No
26	62	Male	1	3	IV	9290	No	Bone	Yes
27	58	Male	4	3	IV	297	No	No	No
28	42	Male	4	0	IV	369	No	No	No
29	51	Female	3	3	IV	738	No	No	No
30	54	Female	4	2	IV	1320	No	Bone	No
31	66	Male	3	3	IV	4030	No	No	No
32	57	Male	3	2	III	904	Local failure	No	No
33	40	Male	3	1	III	2190	Local and regional failure	No	Yes

Abbreviations: D = detectable; EBV = Epstein–Barr virus; IC = induction chemotherapy; NPC = nasopharyngeal carcinoma; U = undetectable.

offering insights into identifying patients who may benefit from AC.[24] A comprehensive review of longitudinal EBV DNA trajectory studies in NPC is presented in Table E3. In this study, we incorporated EBV DNA measurements at 3 critical time points during treatment to define EBV DNA clearance trajectories in patients undergoing IC followed by RT or chemoRT, identifying 5 distinct trajectory patterns. Despite variability in EBV DNA collection time points, analytical methodologies, and patient populations, our classification of trajectories types I to IV aligns with those reported by Lv et al[12] and Liu et al,[24] suggesting a potential shared biological pattern in EBV DNA

Table 4 The characteristics and outcomes of 26 patients with NPC with "U-D-U" type

Patients	Age (y)	Sex	Tumor category	Node category	Stage	Pre-RT EBV DNA, copies/mL	Outcomes		
							Locoregional failure	Distant failure	Death
1	50	Female	1	1	Ⅱ	1140	No	No	No
2	39	Male	1	1	Ⅱ	347	Regional failure	No	No
3	27	Male	2	2	Ⅲ	452	No	No	No
4	43	Male	1	2	Ⅲ	105	No	No	No
5	61	Male	1	3	Ⅳ	180	Regional failure	No	Yes
6	49	Male	4	2	Ⅳ	478	No	No	No
7	51	Male	3	1	Ⅲ	70.4	Local failure	No	Yes
8	43	Male	3	2	Ⅲ	1060	No	No	No
9	65	Male	4	1	Ⅳ	182	No	No	Yes
10	53	Female	3	1	Ⅲ	21,100	No	No	No
11	53	Male	1	1	Ⅱ	1160	No	No	No
12	63	Male	1	2	Ⅲ	232	No	No	No
13	60	Male	3	2	Ⅲ	458	No	No	No
14	65	Male	3	1	Ⅲ	5370	No	No	No
15	64	Female	3	2	Ⅲ	214	No	No	No
16	38	Male	4	2	Ⅳ	167	No	No	No
17	44	Female	3	1	Ⅲ	114	No	No	No
18	33	Male	4	1	Ⅳ	204	No	No	No
19	73	Male	3	3	Ⅳ	65.6	No	No	No
20	58	Female	3	0	Ⅲ	262	No	No	No
21	42	Male	3	1	Ⅲ	73.2	Regional failure	No	Yes
22	25	Female	3	1	Ⅲ	115	No	No	No
23	28	Male	3	3	Ⅳ	1930	No	No	No
24	62	Male	3	3	Ⅳ	2310	No	No	No
25	65	Male	3	1	Ⅲ	275	No	No	No
26	46	Male	2	2	Ⅲ	83.6	No	No	No

Abbreviations: D = detectable; EBV = Epstein–Barr virus; NPC = nasopharyngeal carcinoma; RT = radiation therapy; U = undetectable.

production, clearance, and disease evolution in NPC, warranting further investigation.[23]

Studies have demonstrated that the dynamic monitoring of EBV DNA clearance rates is of significant importance.[7,13,25] Plasma EBV DNA levels serve as sensitive indicators of tumor regression during treatment, reflecting responsiveness to IC and RT.[13] In the type Ⅱ trajectory group, patients exhibited complete clearance of EBV DNA after IC, with levels remaining U throughout RT, indicating sensitivity to chemotherapy and predicting a favorable prognosis. Prior research supports that EBV DNA status post-IC effectively reflects RT and chemotherapy sensitivity, with a higher likelihood of complete tumor regression in patients achieving EBV DNA negativity after IC.[13,26] Conversely, delayed clearance or persistently elevated EBV DNA levels are associated with inferior survival and increased recurrence risk.[13,27] This was also partially demonstrated in our study by lower PFS and LRFS in type Ⅲ than in type Ⅱ. Additionally, 3.3% of patients exhibited EBV DNA resurgence (type V) despite aggressive treatment, correlating with poorer prognosis. Within type Ⅴ, patients with the "D-U-D" pattern experienced significantly inferior PFS and distant DMFS compared with those with the "D-U-U" pattern (type Ⅱ). All patients with the "D-U-D" trajectory presented with advanced-stage disease, suggesting that a high tumor burden may contribute to micrometastasis. Evidence indicates that the redetection of EBV DNA during treatment, after its rapid initial clearance, may signify the presence of residual refractory tumor clones with a predisposition for dissemination, leading to a simultaneous decline in long-term tumor control, especially for the control of distant metastatic lesions.[23] This provides a plausible explanation for

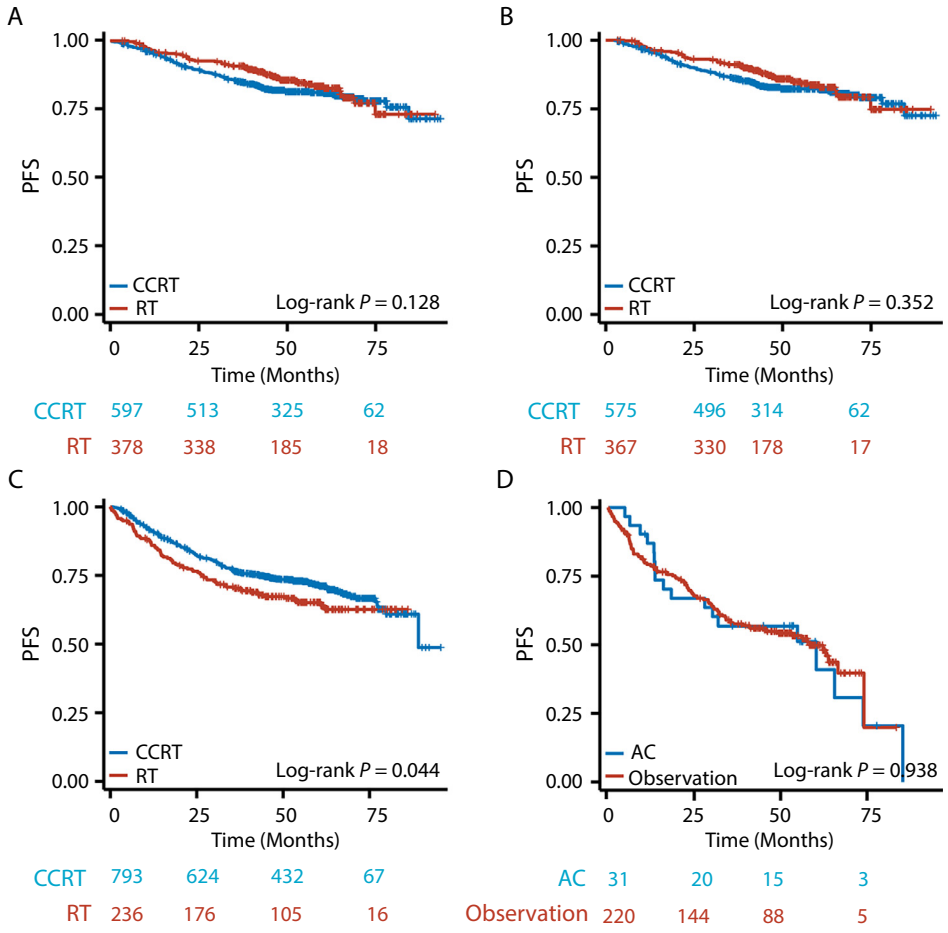

Fig. 2. Survival outcomes of EBV DNA trajectory patterns based on chemotherapy intensity. (A) PFS for patients who achieved the first clearance of EBV DNA prior to RT (including type Ⅱ [ie, "D-U-U" type] and "D-U-D" types), stratified by RT versus CCRT. (B) PFS for patients in type Ⅱ (ie, "D-U-U" type), stratified by RT versus CCRT. (C) PFS for patients whose EBV DNA remained detectable prior to RT (including type Ⅲ [ie, "D-D-U" type] and type Ⅳ [ie, "D-D-D" type]), stratified by RT versus CCRT. (D) PFS for patients whose EBV DNA remained detectable after RT (type Ⅳ [ie, "D-D-D" type]), stratified by AC versus observation. *Abbreviations:* AC = adjuvant chemotherapy; CCRT = concurrent chemoRT; EBV = Epstein−Barr virus; PFS = progression-free survival; RT = radiation therapy.

our findings. In contrast, in our cohort, patients with "U-D-U" pattern exhibited 5-year PFS rates comparable to those with sustained EBV DNA negative ("U-U-U" pattern) and demonstrated significantly improved prognosis compared with patients with delayed adverse rebound ("D-U-D" pattern). The early benign bounce phenomenon is likely attributable to the release of viral DNA from tumor cells undergoing therapy-induced lysis, rather than representing true disease progression. It underscores the importance of differentiating early benign bounce from true treatment failure because premature escalation of therapy in these patients may lead to unnecessary toxicity and limited clinical benefit.

Patients in the persistent-positivity group exhibited a markedly higher risk of disease progression in our study. Chan et al[28] also noted that in radical RT, persistent EBV DNA positivity suggests a high likelihood of residual tumor, which may partially account for the poorer prognosis observed in patients with the type Ⅳ trajectory in our study. In addition, type Ⅰ patients, characterized by consistently U EBV DNA throughout treatment, exhibited outcomes comparable to those in the IC and RT-elimination groups. However, given the paucity p16 and EBV-encoded RNA staining data for most patients within this subtype, the potential association with EBV or human papillomavirus infection remains unclear. Although EBV infection is predominantly associated with nonkeratinizing NPC, limited studies have proposed a potential link between EBV and keratinizing NPC.[29,30] Our findings align with this pattern, identifying only 15 keratinizing cases, 13 of which exhibited D EBV DNA at 1 or more time points. Consequently, keratinizing NPC cases were also stratified into different trajectory subtypes based on their EBV DNA profiles in this study.

In the therapeutic context, plasma EBV DNA has been extensively studied both pretreatment and posttreatment for risk stratification, guiding decisions on treatment

deintensification or intensification.[26,31-34] Our findings underscore the utility of EBV DNA dynamics in optimizing treatment selection for NPC. For type II patients who achieved the first clearance of EBV DNA prior to RT (ie, the "D-U-U" subtype), the prognostic impact of chemotherapy intensity appears diminished. These results align with previous studies, including Jin et al,[35] who reported comparable survival outcomes in patients with stage II NPC with U pretreatment EBV DNA receiving RT alone versus CCRT. Similarly, Xu et al[36] showed that IC combined with RT is equivalent to CCRT in low-risk locally advanced NPC, highlighting opportunities for treatment deintensification in select cases without compromising outcomes. Prospective studies, such as those by Guo et al,[19] further support this notion, showing that low-dose radiation (60 Gy) yielded favorable survival outcomes and reduced treatment-related toxicity in patients with low-risk stage III NPC responsive to IC. However, our study identified EBV DNA reactivation in approximately 1.5% of patients (33 of 2203), a group characterized by a higher risk of distant metastases. Notably, there were also 3 patients who experienced local or regional recurrences during follow-up in this cohort. Although some relapses were partially attributed to suboptimal dosing intended to protect normal tissues, additional factors, such as RT resistance and central tumor hypoxia, likely played a contributory role.[37] These findings suggest that although reduced-intensity therapy may be appropriate for patients sensitive to IC, caution remains essential, particularly in those with locoregionally advanced disease.

Conversely, patients with D EBV DNA after IC may derive greater benefit from CCRT than RT alone, likely due to synergistic effects, suggesting that suboptimal IC response does not preclude CCRT efficacy.[38,39] However, AC using the same regimen as IC showed limited benefit in type IV patients, particularly when tumors remained EBV DNA-positive ("D") post-IC, indicating that repeating the same regimen seems unwise and underscoring the need for novel systemic therapies. In addition, we observed both pre-RT (P = .003) and post-RT EBV DNA levels (P = .014) were higher in these type IV patients receiving AC compared with those under observation. These findings suggest that patient stratification and EBV DNA burden may influence treatment decisions and outcomes. Given the small sample sizes and inherent selection biases, these results should be interpreted with caution. Persistent EBV DNA positivity may indicate aggressive, treatment-resistant disease, warranting exploration of alternative strategies such as immunotherapy or targeted therapy rather than chemotherapy intensification.[40,41] Recent advances, including the CONTINUUM trial, demonstrate that adding sintilimab to standard chemoRT significantly improves event-free survival in high-risk, locally advanced NPC, with manageable safety and inclusion in clinical guidelines.[42] Ongoing trials (eg, NCT04782765, NCT03700476, NCT04453826, NCT04910347) are further evaluating immunotherapy in combination with IC, CCRT, or as maintenance therapy, potentially reshaping the therapeutic landscape for NPC.[43–46]

Unlike previous studies that focused on single time point measurements of EBV DNA, our study leveraged longitudinal EBV DNA measurements taken regularly during treatment allowing us to define distinct EBV DNA trajectory patterns, thereby highlighting the prognostic value of dynamic EBV DNA changes. The relatively large sample size and adjustment for confounding factors enhance the robustness of our findings. Nonetheless, being an observational retrospective study, it is subject to inherent limitations and biases. First, we excluded patients who did not undergo IC, which introduced selection bias. However, it is necessary to ensure the comparability and integrity of longitudinal data for each patient. Next, differences in treatment intensity and patient stratification may affect clinical outcomes, and our unplanned subgroup analyses require further investigation, particularly regarding the role of AC in specific subgroups. Lastly, it is generally accepted that the EBV DNA PCR-based tests are prone to significant differences between diverse laboratories.[47] Nonetheless, our study consisted of individuals treated at a single center, thereby mitigating this concern. The identified EBV DNA trajectories need validation in multiple hospitals nationwide to confirm their generalizability and broader clinical applicability.

Conclusions

In conclusion, the temporal clearance pattern EBV DNA can offer additional prognostic insights beyond single time point measurements in NPC. Persistent EBV DNA positivity and resurgence during treatment are associated with poorer outcomes, whereas rapid clearance after IC is indicative of a favorable prognosis. Caution is warranted when considering treatment de-escalation in patients demonstrating sensitivity to IC. Our findings underscore the importance of dynamic EBV DNA monitoring for individualized treatment of patients with NPC.

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