research article

Prognostic value of plasma EBV DNA for nasopharyngeal cancer patients during treatment with intensity-modulated radiation therapy and concurrent chemotherapy

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Background. Plasma EBV DNA concentrations at the time of diagnosis (pre-EBV) and post treatment (post-EBV) have significant value for predicting the clinical outcome of nasopharyngeal cancer (NPC) patients. However, the prognostic value of the EBV concentration during radiation therapy (mid-EBV) has not been vigorously studied.

Patients and methods. This was a post hoc analysis of 105 detectable pre-EBV NPC patients from a phase II/III study comparing sequential (SEQ) *versus* simultaneous integrated boost (SIB) intensity-modulated radiation therapy (IMRT). Plasma EBV DNA concentrations were measured by PCR before commencement of IMRT, at the 5th week of radiation therapy and 3 months after the completion of IMRT. The objective was to identify the prognostic value of mid-EBV to predict overall survival (OS), progression-free survival (PFS) and distant metastasis-free survival (DMFS).

Results. A median pre-EBV was 6880 copies/ml. Mid-EBV and post-EBV were detectable in 14.3% and 6.7% of the patients, respectively. The median follow-up time was 45.3 months. The 3-year OS, PFS and DMFS rates were 86.0% *vs.* 66.7% (p = 0.043), 81.5% *vs.* 52.5% (p = 0.006), 86.1% *vs.* 76.6% (p = 0.150), respectively, for those with undetectable mid-EBV *vs.* persistently detectable mid-EBV. However, in the multivariate analysis, only persistently detectable post-EBV was significantly associated with a worse OS (hazard ratio (HR) = 6.881, 95% confident interval (CI) 1.699-27.867, p = 0.007), PFS (HR = 5.117, 95% CI 1.562–16.768, p = 0.007) and DMFS (HR = 129.071, 95%CI 19.031–875.364, p < 0.001).

Conclusions. Detectable post-EBV was the most powerful adverse prognostic factor for OS, PFS and DMFS; however, detectable mid-EBV was associated with worse OS, PFS especially Local-PFS (LPFS) and may facilitate adaptive treatment during the radiation treatment period.

Key words: plasma EBV; nasopharyngeal cancer; IMRT; Prognosis during treatment

Introduction

Epstein-Barr virus (EBV) is associated with nasopharyngeal carcinoma (NPC) in an endemic area. The plasma EBV DNA concentration at the time of diagnosis and after treatment can be used as a biomarker for screening, monitoring and predicting clinical outcomes in NPC.[1,2] Peng identified a pre-treatment plasma EBV DNA (pre-EBV) cut-off value of 2010 copies/ml in predicting disease-free survival, overall survival, loco-regional relapse-free survival and distant metastasis–free survival when NPC patients were treated with intensity-modulated radiation therapy (IMRT).[3] Others confirmed the prognostic value of pre-EBV in predicting clinical outcomes despite the different pre-EBV cut-off

values.[4-11] The persistent post-treatment EBV DNA concentration (post-EBV) has the strongest risk of disease relapse and distant metastasis[4,7,12-14] and may help in treatment modification, such as the intensification of adjuvant chemotherapy regimens.[15]

In recent decades, the use of concurrent chemotherapy with IMRT has shifted the pattern of recurrence from locoregional recurrence toward distant metastasis alone.[16-19] The mid-treatment plasma EBV-DNA may be useful in adaptive treatment, such as reducing treatment intensity in patients with early response or giving more intensified treatment to those without response. However, studies on the plasma EBV DNA during radiation treatment (mid-EBV) were scarce, and patients were not uniformly treated with IMRT or concurrent chemoradiation.[20,21] Our primary endpoint was to identify the prognostic value of mid-EBV to predict the overall survival, progression-free survival and distant metastasis-free survival rates.

Patients and methods

This study was a secondary analysis of a prospective randomized controlled trial that compared the utility of sequential (SEQ) or simultaneous integrated boost (SIB) IMRT in non-metastatic nasopharyngeal cancer.[22] This study was approved by the institutional review board. Informed consent was obtained from every patient before entry into the study. One hundred and twenty-three patients had detectable pre-EBV. After excluding 18 patients because of missing blood samples at any of the three time-points, there were 105 patients in this study.

Chemotherapy consisted of weekly treatments of 40 mg/m^2 cisplatin given concurrently with 70 Gy IMRT in 33-35 fractions to those with more than T1 or positive nodal disease for a maximum of 7 cycles. Adjuvant chemotherapy, consisting of 80 mg/m^2 cisplatin and 1000 mg/m^2/per day 5-fluorouracil (5-FU) over a 96-hour continuous infusion, was given at 4-week intervals for 3 cycles.

Quantitative measurement of plasma EBV DNA level

Plasma EBV DNA concentrations were evaluated before treatment (pre-EBV), at the 5th week of the radiation course (mid-EBV) and 3 months after the completion of radiation treatment (post-EBV). We elected to test the mid-EBV in the 5th week of IMRT because it was the best time to perform re-simulation and in accordance with Leung's study which tested mid-EBV at completion of 4 weeks of radiation therapy.[21] The EBV nucleic acids were purified from the plasma samples using the QIAsymphony SP in combination with the QIAsymphony DSP Virus/Pathogen Midi Kit (QIAGEN, Germany) using the manufacturer's recommended protocol. After extraction, the eluates in the 96-microwell plates were transferred to the module for assembly with the master mix (QIAGEN artus EBV QS RGQ kit) by the instrument. The aliquoted reactions were subsequently put in a Rotor-Gene Q. The amplification parameters were as follows: 95°C for 10 min and 45 cycles of 95°C for 15 s, 65°C for 30 s, and 72°C for 20 s. The plasma DNA samples were quantified for EBV DNA using an RTQ-PCR system targeting the BamHI-W fragment region of the EBV genome. A plasma EBV DNA concentration of < 316 copies/ml was defined as an undetectable level in our institution. Note that in the following section, values of 0 represent an undetectable plasma EBV DNA concentration.

Statistical analysis

Local progression-free survival (LPFS), regional progression-free survival (RPFS), distant metastasis-free survival (DMFS), progression-free survival (PFS) and overall survival (OS) were analyzed using the Kaplan–Meier method and the log-rank test. Cox proportional hazard models with univariate and multivariate analyses were performed to identify the predictors for OS, PFS and DMFS. The factors, including age, sex, stage, pre-EBV, mid-EBV, post-EBV, WHO subtypes, and IMRT techniques, were included as covariates in this exploratory analysis. Factors with a P-value of < 0.25 in the univariate analysis were entered into the multivariate Cox regression model. All tests were two-sided, and a P-value of < 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS statistics (version 22.0, SPSS Inc., Chicago, Ill).

Results

Between October 2010 and September 2015, 105 NPC patients who had detectable pre-EBV and completed blood sampling between RT and 3 months after RT were included. The median age was 50 years. Patient characteristics are outlined in Table 1. The median follow-up time was 45.3 months. Most patients were stage III-IVb. The ma-

TABLE 1. Patient characteristics

	N = 105
Age < 45	34 (32.4%)
Age ≥ 45	71 (67.6%)
Sex	
Male	82 (78.1%)
Female	23 (21.9%)
T-stage	
1	30 (28.6%)
2	28 (26.7%)
3	29 (27.6%)
4	18 (17.1%)
N-stage	
0	1 (1.0%)
1	26 (24.8%)
2	57 (54.3%)
3	21 (20.0%)
Stage grouping (AJCC 2010)	
II	14 (13.3%)
III	54 (51.4%)
IVa-b	37 (35.2%)
WHO subtypes	
2A	11 (10.5%)
2B	94 (89.5%)
Mid-EBV	
undetectable	90 (85.7%)
detectable	15 (14.3%)
Post-EBV	
undetectable	98 (93.3%)
detectable	7 (6.7%)

jority had undifferentiated squamous cell carcinoma and male gender. Patients received a median of 6 cycles of concurrent weekly cisplatin (88.6% received ≥ 5 cycles) and a median of 3 cycles of adjuvant chemotherapy (76.2% completed 3 cycles).

Plasma EBV DNA level correlated with disease and treatment outcomes

Median pre-EBV was 6880 copies/ml. (interquartile range, IQR, 2555–14600 copies/ml). The corresponding values for stage II, III and IV were 3690 copies/ml (Interquartile range (IQR), 1462–8885), 6880 copies/ml. (IQR, 2407–16475) and 5620 copies/ml (IQR, 3735–17200), respectively. Fifteen patients (14.3%) had persistent mid-EBV, 4 of whom had residual post-EBV. Among the remaining 90 patients who had undetectable mid-EBV, 3 patients had rebound detectable post-EBV. A total of 7 patients (6.7%) had residual post-EBV.

Survival outcomes

During the follow-up period, a total of 24 (22.9%) patients died, 39 (37.1%) had progressive disease, and 27 (25.7%) developed distant metastases. The 3-year OS, PFS and DMFS for the patients were 83.2%, 77.4%, 84.7%, respectively. The overall survival rates for stages II, III, and IV were 85.1%, 88.7% and 75.0%, respectively (p = 0.253), while the PFS was 85.1%, 83% and 67.3%, respectively (p = 0.070). The corresponding DMFS was 100%, 88.5% and 74.2%, respectively (p = 0.102) (Figure 1).

Using a pre-EBV cut-off of 2010 copies/ml[3], the 3-year OS, PFS and DMFS rates were 88.4% *vs.* 82.1% (p = 0.360), 82.9% *vs.* 76.2% (p = 0.114), and 82.9% *vs.* 85.2% (p=0.390), respectively, for those with pre-EBV < 2010 copies/ml *vs.* ≥ 2010 copies/ml (Figure 1). The 3-year OS, PFS and DMFS rates were 86.0% *vs.* 66.7% (p = 0.043), 81.5% *vs.* 52.5% (p = 0.006), and 86.1% *vs.* 76.6% (p = 0.150), respectively, for those with undetectable mid-EBV *vs.* persis-

TABLE 2. Overall survival (OS), progression-free survival (PFS), distant metastasis-free survival (DMFS), local progression-free survival (LPFS) and regional progression-free survival (RPFS) rates among different plasma EBV time points

	3-year OS (95%CI)	p-value	3-year PFS (95%CI)	p-value	3-year DMFS (95%CI)	p-value	3-year LPFS (95%CI)	p-value	3-year RPFS (95%CI)	p-value
Pre-EBV < 2010	88.4 (73.1–103.7)	0.360	82.9 (65.3–100.5)	0.114	82.9 (62.3–100.5)	0.386	100	0.328	100	0.475
Pre-EBV ≥ 2010	82.1 (73.9–30.3)		76.2 (67.0–85.4)		85.2 (77.0–93.4)		94.4 (89.1–99.6)		96.6 (91.9–101.3)	
Undetectable mid-EBV	86.0 (78.5–93.4)	0.040	81.5 (73.3–89.7)	0.006	86.1 (78.5–93.7)	0.15	97.5 (94.0–101.0)	0.01	98.6 (95.9–101.3)	0.113
Detectable mid-EBV	66.7 (42.8–90.6)		52.5 (26.8–78.2)		76.6 (52.9–100.3)		79.5 (53.8–105.2)		87.5 (64.6–110.4)	
Undetectable post-EBV	86.1 (79.0–93.2)	< 0.001	79.9 (71.9–87.9)	< 0.001	88.2 (81.3–95.1)	< 0.001	97.6 (94.3–100.9)	< 0.001	97.2 (93.5–100.9)	0.841
Detectable post-EBV	42.9 (6.3–79.6)		42.9 (6.3–79.6)		22.9 (15.7–61.5)		33.3 (20.0–86.6)		100	

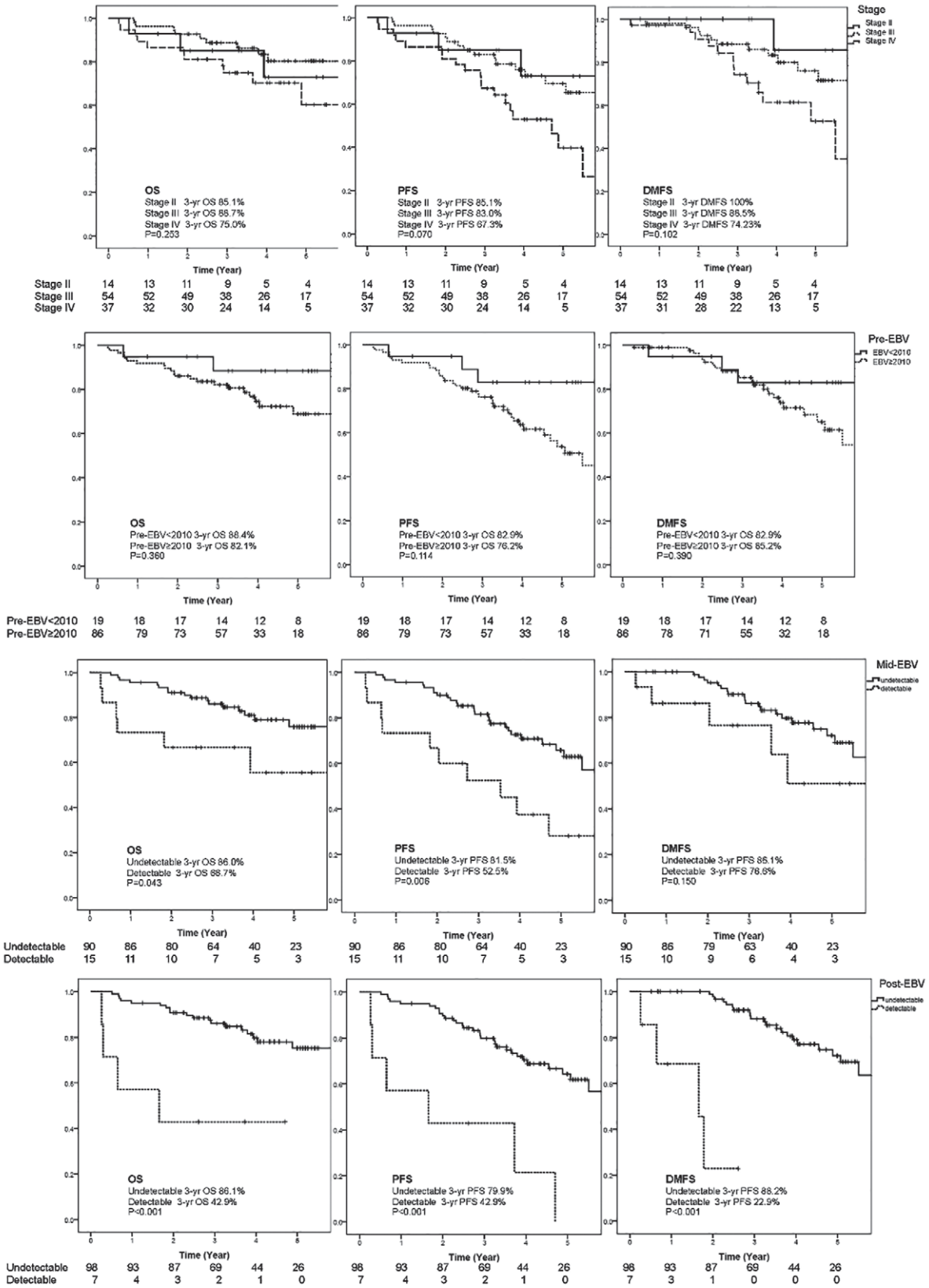

FIGURE 1. Kaplan-Meier curves for the overall survival (OS), progression-free survival (PFS), distant metastasis-free survival (DMFS) stratified by stage and plasma EBV DNA at different time points.

TABLE 3. Univariate analyses for the clinical parameters and EBV values associated with the overall survival (OS), progression-free survival (PFS), distant metastasis-free survival (DMFS)

	Hazard ratio for OS	Univariate 95.0% CI		p-value	Hazard ratio for PFS	Univariate 95.0% CI		p-value	Hazard ratio for DMFS	Univariate 95.0% CI		p-value
		Lower	Upper			Lower	Upper			Lower	Upper	
Age <45 vs. ≥45	0.497	0.185	1.331	0.164	0.655	0.319	1.346	0.250	0.807	0.353	1.845	0.611
Sex Male vs. Female	1.442	0.493	4.222	0.504	1.623	0.679	3.879	0.276	1.078	0.434	2.677	0.872
WHO type IIA vs. IIB	1.344	0.399	4.532	0.633	1.795	0.748	4.31	0.190	2.101	0.719	6.141	0.175
T Stage				0.152				0.026				0.119
T1 vs. T4	0.155	0.031	0.773	0.023	0.176	0.055	0.564	0.003	0.212	0.05	0.89	0.034
T2 vs. T4	0.73	0.257	2.073	0.555	0.523	0.224	1.222	0.134	0.617	0.204	1.862	0.391
T3 vs. T4	0.667	0.223	1.991	0.468	0.73	0.319	1.671	0.457	0.93	0.316	2.743	0.896
N Stage				0.740				0.501				0.188
N1 vs. N3	1.222	0.386	3.865	0.733	0.887	0.368	2.138	0.789	0.587	0.208	1.657	0.314
N2 vs. N3	0.844	0.297	2.4	0.750	0.651	0.302	1.406	0.275	0.445	0.187	1.063	0.068
Stage				0.253				0.070				0.102
Stage II vs. IVa-b	0.888	0.281	2.8	0.839	0.491	0.166	1.449	0.198	0.326	0.073	1.451	0.141
Stage III vs. IVa-b	0.484	0.2	1.17	0.107	0.472	0.242	0.92	0.028	0.47	0.214	1.034	0.061
Tech SIB vs. SEQ	1.619	0.707	3.71	0.255	1.309	0.693	2.472	0.407	1.461	0.674	3.167	0.336
Concurrent chemotherapy 0-5 vs. 6-7 cycles	1.172	0.525	2.616	0.699	0.795	0.418	1.514	0.486	0.632	0.287	1.393	0.255
Adjuvant chemotherapy 0-2 vs. 3 cycles	1.089	0.432	2.748	0.856	1.101	0.536	2.263	0.793	0.937	0.377	2.327	0.888
Pre-EBV ≥ 2010 vs. < 2010	1.760	0.524	5.915	0.360	2.309	0.819	6.512	0.114	1.595	0.550	4.625	0.390
Mid-EBV detectable vs undetectable	2.600	1.031	6.556	0.043	2.746	1.337	5.640	0.006	2.041	0.772	5.397	0.15
Post-EBV detectable vs. undetectable	5.923	1.989	17.638	0.001	5.961	2.457	14.465	<0.001	29.758	8.155	108.593	<0.001

tently detectable mid-EBV (Figure 1). In comparing the patients with residual post-EBV *vs.* undetectable post-EBV, the OS, PFS and DMFS are demonstrated in Figure 1. Details on the OS, PFS, DMFS, LPFS and RPFS regarding the different pre-EBV, mid-EBV and post-EBV subgroups are presented in Table 2.

Among the 39 patients who had progressive disease, persistent mid-EBV and post-EBV were observed in 25.6% and 15.4% of these patients, respectively. There were 4 patients who had both persistent mid-EBV and post-EBV, and their 3-year OS, PFS and DMFS rates were 25%, 25% and 37.5%, respectively, which were significantly worse than those of the 11 patients who had detectable mid-EBV but undetectable post-EBV. The 3-year OS, PFS and DMFS rates in the latter group were 81.8%, 62.3% and 88.9%, respectively. Among the 3 patients who had undetectable mid-EBV but had rebound detectable post-EBV, the 3-year OS, PFS and DMFS rates were 66.7%, 66.7% and 33.3%, respectively. The best prognostic group was the patients who had both undetectable mid-EBV and post-EBV, and their 3-year OS, PFS and DMFS rates were 86.7%, 82.1% and 88.2%, respectively.

The unadjusted univariate analyses for the clinical parameters and the EBV-value associated with the OS, DMFS and PFS are shown in Table 3. Undetectable mid-EBV was significantly associated with better OS and PFS but not DMFS, while undetectable post-EBV was significantly associated with better OS, PFS and DMFS. T-stage was a significant prognostic factor for PFS only (p = 0.026). In the multivariate analysis (Table 4), only detectable post-EBV was significantly associated with a worse OS (hazard ratio (HR) = 6.881, 95% confident interval (CI) 1.699–27.867, p = 0.007), PFS (HR = 5.117, 95% CI 1.562–16.768, p = 0.007) and DMFS (HR = 129.071, 95%CI 19.031–875.364, p < 0.001)

Zhang[1] proposed the risk stratification into 4 groups based on the stage and mid-EBV as follows: (1) patients who had stage I-II with undetectable mid-EBV; (2) patients who had stage III-IV with undetectable mid-EBV; (3) patients who had stage

TABLE 4. Multivariate analyses for the clinical parameters and EBV values associated with the overall survival (OS), progression-free survival (PFS), distant metastasis-free survival (DMFS)

	Hazard ratio for OS	95.0% CI for OS		P-value
		Lower	Upper	
Age < 45 *vs.* ≥ 45	0.409	0.140	1.190	0.101
T				0.305
T1 *vs.* T4	0.286	0.052	1.570	0.150
T2 *vs.* T4	1.214	0.361	4.078	0.754
T3 *vs.* T4	1.177	0.341	4.069	0.796
Mid-EBV undetectable vs detectable	1.620	0.617	4.251	0.327
Post-EBV undetectable *vs.* detectable	6.881	1.699	27.867	0.007
	Hazard ratio for PFS	**95.0% CI for PFS**		
		Lower	**Upper**	
Age <45 *vs.* ≥ 45	0.422	0.180	0.988	0.047
WHO type IIA *vs.* IIB	1.354	0.441	4.159	0.597
T Stage				0.101
T1 *vs.* T4	0.497	0.104	2.375	0.381
T2 *vs.* T4	1.660	0.450	6.125	0.446
T3 *vs.* T4	2.099	0.646	6.825	0.218
Stage				0.099
Stage II *vs.* IVa-b	1.016	0.256	4.035	0.982
Stage III *vs.* IVa-b	0.416	0.170	1.021	0.055
EBV < 2010 *vs.* ≥ 2010	0.370	0.113	1.211	0.100
Mid-EBV undetectable *vs.* detectable	1.427	0.630	3.234	0.394
Post-EBV undetectable *vs.* detectable	5.117	1.562	16.768	0.007
	Hazard ratio for DMFS	**95.0% CI for DMFS**		
		Lower	**Upper**	
WHO type IIA *vs.* IIB	1.653	0.470	5.810	0.433
T Stage				0.113
T1 *vs.* T4	0.945	0.068	13.158	0.966
T2 *vs.* T4	5.632	0.388	81.647	0.205
T3 *vs.* T4	4.628	0.384	55.737	0.228
N Stage				0.410
N1 *vs.* N3	4.484	0.305	65.955	0.274
N2 *vs.* N3	2.018	0.151	26.935	0.595
Stage				0.340
Stage II *vs.* IVa-b	0.090	0.003	2.435	0.153
Stage III *vs.* IVa-b	0.150	0.009	2.474	0.185
Mid-EBV undetectable vs detectable	1.583	0.517	4.843	0.421
Post-EBV undetectable *vs.* detectable	129.071	19.031	875.364	0.000

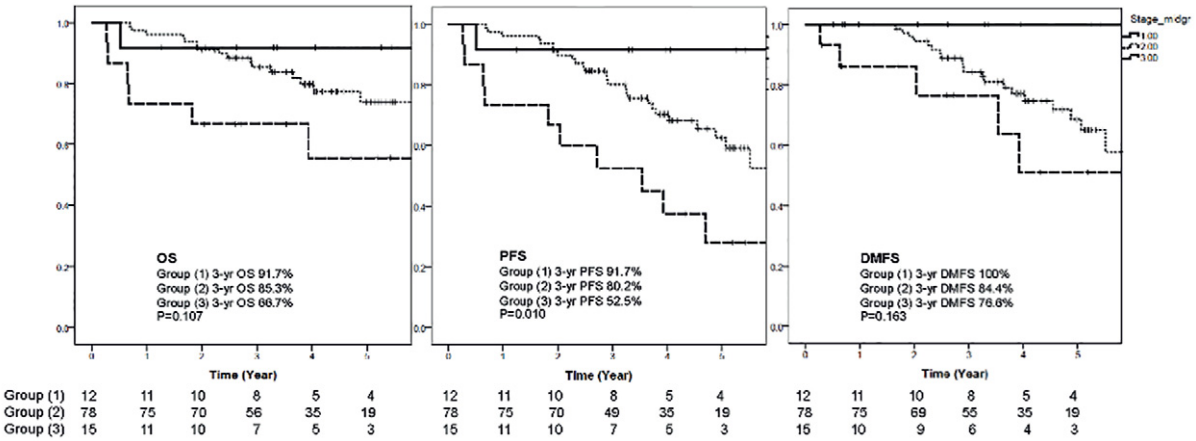

FIGURE 2. Kaplan-Meier curves for the overall survival (OS), progression-free survival (PFS), distant metastasis-free survival (DMFS) stratified by a combination of stage and mid-EBV concentration.

II-IV with detectable mid-EBV; and (4) patients who had stage I with detectable mid-EBV. In our study, there were no patients in group (4). Thus, we analyzed the survival in group (1)-(3). The overall survival rates for group (1), (2), and (3) were 91.7%, 85.3% and 66.7%, respectively (p = 0.107), while the PFS rates were 91.7%, 80.2% and 52.5%, respectively (p = 0.010). The corresponding DMFS rates were 100%, 84.4% and 76.6%, respectively (p = 0.163) (Figure 2).

Discussion

The plasma EBV DNA concentration has been identified as a prognostic biomarker and is correlated with the overall survival in NPC. Pre-EBV concentration has been evaluated in many studies. A higher concentration was associated with a higher mortality, poorer PFS and poorer DMFS.[1,4-10,12,23] Leung *et al*.[5] retrospectively reviewed 376 NPC patients who were treated with conventional RT. In their multivariate analysis, pre-EBV was an independent prognostic factor for OS (p = 0.0053) and DMFS (p = 0.0002). Note that there was no mid-EBV or post-EBV testing in their study. Two recent meta-analyses reported pooled HRs for an OS of 3.01 (95%CI = 2.25–4.02; P < 0.001)[23] and 2.81 (95%CI 2.44–3.24, p < 0.00001)[1], indicating that the higher levels of pre-EBV were associated with higher mortality, while our study revealed a corresponding HR of 1.76 (95%CI 0.52-5.92).

Other than the pre-EBV, mid-EBV may provide additional information on the risk of disease failure. To our knowledge, there is only one prospective trial that studied the prognostic value of mid-EBV. Leung *et al*. found that persistent mid-EBV at the completion of the 4th week of chemoradiation was associated with a worse OS (HR 3.29, 95% CI 1.37–7.89), worse PFS (HR 4.05, 95%CI 1.89–8.67) and more distant failure (HR 12.02, 95%CI 2.78–51.93).[21] Although Leung *et al*. study had a long median follow-up time and involved stage IIB-IVB NPC (AJCC 1997 edition), only 78 of the 107 patients received concurrent chemotherapy. No patient received adjuvant chemotherapy, and an unknown proportion of patients were treated with conventional 2D RT or IMRT. In accordance with Leung *et al*. study, we also found that the detectable mid-EBV value was a predictor for OS (HR 2.60, 95%CI 1.03–6.56), PFS (HR 2.75 95%CI 1.34–5.64) and DMFS (HR 2.01 95%CI 0.77–5.40). In our study, the number of concurrent or adjuvant chemotherapy cycles did not affect OS, PFS or DMFS. However, in a multivariate analysis, the prognostic value of mid-EBV was lower compared to the persistent post-EBV. It was noted that post-EBV was not incorporated into the multivariate analysis in Leung *et al*. study.[21] Mid-EBV was not a predictive factor for locoregional failure in Leung's study, but mid-EBV was a significant factor in predicting LPFS in our study (p = 0.01). Among the 4 patients who had local progression, 3 patients were T4 and 2 patients had detectable mid-EBV. We hypothesized that those patients who had detectable mid-EBV might benefit from the adaptive radiotherapy, such as dose escalation for better local control. The high locoregional failures of 18% in the patients who had detectable mid-EBV, but undetectable post-EBV, and 11% in the patients who had both undetectable mid-EBV and post-EBV in Leung *et al*. study[21] reflected the pattern of recurrence in

the pre-IMRT era. In contrast, we uniformly used IMRT and concurrent cisplatin-based chemotherapy followed by adjuvant cisplatin and 5FU which gave very high rates of LPFS and RPFS.[22] In our previous report of phase III study comparing SEQ and SIB IMRT, both IMRT techniques provided excellent survival outcomes with few late toxicities. There were no statistically significant differences in the cumulative incidence of grade 3–4 acute and late toxicities.[24] We acknowledged that the effect of mid-EBV evaluation in adaptive treatment during chemoradiation still has to be investigated in the future.

A meta-analysis confirmed the significant value of mid-EBV in terms of the OS, PFS and DMFS[1]; however, detectable post-EBV was a stronger prognostic factor than pre-EBV and mid-EBV. The HRs for the OS, PFS and DMFS of post-EBV were 4.26 (95%CI 3.26–5.57), 5.21 (95%CI 3.29–8.27) and 7.54 (95%CI 3.39–16.77), respectively. Peng *et al*.[3] retrospectively reviewed 584 NPC patients treated with IMRT. Overall, 77.7% of patients had detectable pre-EBV, and 8.6% of patients had detectable post-EBV. Among patients who had pre-EBV ≥ 2010 copies/ml, the 3-year OS, PFS and DMFS rates were 92.3%, 78.1% and 85.5% in Peng *et al*. study, which were slightly better than the corresponding percentages of 82.1% 76.2% and 85.2%, respectively, in our patient population. In contrast to Peng *et al*. study, which demonstrated statistically significant values of pre-EBV and post-EBV to OS, PFS and DMFS in the multivariate analysis, we found that only the post-EBV was an independent predictor for OS, PFS and DMFS. By comparing between pre-EBV ≥ 4000 copies/ml and post-EBV > 500 copies/ml in 170 NPC patients treated with conventional RT, Chan *et al*.[4] found that only post-EBV > 500 copies was associated with the DMFS (RR 2.1 95% CI 0.70–6.58, p = 0.182) in their multivariate analysis, while the HR for DMFS was 129.07, with a 95% CI from 19.03–875.36, p < 0.001 in our study. The HRs predicting the DMFS were 3.89, 12.02 and 7.54 in high pre-EBV, detectable mid-EBV and detectable post-EBV, respectively[1], in a meta-analysis. The corresponding HRs for the DMFS in our patient population were 1.60, 2.04 and 29.7, respectively.

We further compared the patient groups according to the risk stratification proposed by Zhang *et al*.[1] Zhang *et al*. regarded patients in group (1) as having modified stage I. In concordance with Zhang *et al*., this group had the best 3-year OS, PFS and DMFS at approximately 91.7%–100% in our study. The survival rates were also comparable to the 5-year disease-specific survival and DMFS rates of 97.3% and 100%, respectively, in early stage NPC reported by Su *et al*.[25] and the 5-year OS and DMFS rates for those in stage T1N0 of 96.6% and 94.9% as reported by Xiao *et al*.[26] This group might not need adjuvant chemotherapy. Patients in group (2) were regarded as having modified stage II by Zhang *et al*.; however, the OS, PFS and DMFS in group (2) were closer to the stage III survivals in our study. The OS, PFS and DMFS in group (3) were 66.7%, 52.5% and 76.6%, respectively, which compared unfavorably to stage IV patients [OS, PFS and DMFS of 75%, 67.3% and 74.2% in stage IV patients]. The above results suggested that patients in group (2) and (3) should be regarded as higher risk than those proposed by Zhang *et al*. Moreover, the combination of stage and mid-EBV was more powerful in discriminating PFS than stage alone.

A limitation of our study is that the follow-up time was relatively short. The prognostic value of mid-EBV was not our primary objective in our randomized controlled trial comparing the different IMRT techniques, which may not have adequate power to detect the prognostic significance of mid-EBV on DMFS. However, the strength of our study was the uniform use of the IMRT technique and concurrent cisplatin-based chemotherapy followed by adjuvant cisplatin and 5FU in a prospective randomized trial.

In conclusion, detectable EBV during chemoradiation was an adverse prognostic factor for OS and PFS; however, it was not as a strong prognostic factor as post-EBV. Prospective clinical trials are warranted to allow evidence-based recommendations for adaptive treatment based on mid-EBV.

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