Ting-bo Liu, PhD¹
Zhi-hong Zheng, PhD¹
Jie Pan, MD²
Li-li Pan, MD¹
Li-hong Chen, MD¹

¹Fujian Institute of Hematology, Fujian
Provincial Key Laboratory of Hematology,
Fujian Medical University Union Hospital,
Fuzhou 350001, China

²Department of Pathology, Medical University
Fuzhou, Fuzhou 350004, China

Prognostic role of plasma Epstein-Barr virus DNA load for nasopharyngeal carcinoma: a meta-analysis

Abstract

Purpose: Predicting prognosis and treatment outcomes for patients with for nasopharyngeal carcinoma (NPC) has been difficult due to the heterogeneous nature of the disease. This study aimed to evaluate pretreatment copy number of plasma Epstein-Barr virus (EBV) DNA as an outcome marker for survival in NPC.

Methods: MEDLINE, CENTRAL and Embase databases were searched until April 7, 2015. Included studies were randomized controlled trials, two-arm prospective studies, or retrospective studies in patients with newly diagnosed NPC. The primary outcome was overall survival and secondary outcomes were progression-free, relapse-free, disease-free and distant metastasis-free survival. Sensitivity, quality and publication bias assessments were performed.

Results: Sixteen studies were included in the meta-analysis, with a total of 7698 patients. For overall survival, pooled HR was 3.005 (95% confidence interval [CI] = 2.245–4.022; $P < 0.001$), indicating that higher levels of EBV DNA were associated with a greater risk of death. Pooled estimates for relapse-free, disease-free, progression-free and distant metastasis-free survival indicated that higher levels of EBV DNA were associated with an increased risk of relapse, disease recurrence, disease progression and distant metastasis in comparison with lower levels of EBV DNA (P values < 0.001).

Conclusion: This meta-analysis found that high EBV DNA levels indicate poor prognosis and reduced long-term survival in patients with newly diagnosed NPC; hence, EBV DNA levels are highly prognostic of survival in patients with NPC. None of the included studies used the WHO standard for EBV DNA measurement, indicating a greater need for harmonization in future studies.

Manuscript submitted 1st June, 2016
Manuscript accepted 21st October, 2016

Clin Invest Med 2017; 40 (1): E1-E12.

Correspondence to:

Ting-bo Liu
Fujian Institute of Hematology, Fujian Provincial Key Laboratory of Hematology,
Fujian Medical University Union Hospital
29 Xinquan Road, Fuzhou 350001, China
E-mail: liutb@medmail.com.cn

Nasopharyngeal carcinoma (NPC) has a significantly skewed geographic incidence; it is more prevalent in Southeast Asian countries and in certain populations of the Arctic region (Inuits and Aleuts), the Middle East and Northern Africa [1]. NPC occurs sporadically ($<1/100,000$) in other regions and populations of the world [2]. NPC is endemic in southern China and Sarawak, Malaysia with an incidence of $>20/100,000$ population [1,3-6]. Radiotherapy, plus different chemotherapy regimens, are the standard therapy for treating NPC [7]. Most patients (75-90%) present with locoregionally advanced disease; often with lymph node metastasis [2]. Patients with locoregional advanced NPC have poor prognoses and distant metastasis is the main cause of treatment failure [8]. Patients with distant metastasis (stage IV disease) have a five-year survival rate of $<10\%$ [2]. Another serious problem is the development of long term sequelae due to treatment with radiation and chemotherapy, including radiation-induced malignant tumors [2].

The etiology of NPC is not well understood and involves multiple components, including genetic, epigenetic, and environmental factors [1,9,10]. Infection by EBV can progress from a lytic to latent phase in nasal epithelial cells that have already achieved a premalignant state, as evidenced by mis-regulated expression of cell cycle proteins. Most cases of NPC are thought to result from clonal expansion of these cells prior to later events of transformation. Once a latent infection is established, viral proteins including Epstein-Barr nuclear antigen 1 (EBNA1), latent membrane protein 1 (LMP1), and LMP2 and viral micro RNAs alter the patterns of gene and protein expression in both nasopharyngeal cells and the neighboring stroma. The result is increased potential for proliferation, invasion and angiogenesis, decreased apoptotic potential and heightened immune activation. These extensive changes within the tumor niche promote the transformation to a malignant state.

Predicting the prognosis and treatment outcomes for patients with NPC has been difficult, likely due to the heterogeneous nature of the disease [1]. Although the TNM staging system is used as a prognostic indicator for NPC, a there is still a wide range treatment outcomes in patients with similarly staged tumors [11]. Various studies have evaluated potential molecular biomarkers to predict NPC progression. The quantification of the levels of Epstein-Barr virus (EBV) DNA prior to initiation of treatment has shown promise as a clinical tool for diagnosing NPC and for evaluating risk stratification, monitoring and prognosis [12-16]. EBV DNA levels correlate positively with disease stage and prognosis in NPC [16]. In addition, several groups have shown that plasma

EBV DNA load is significantly associated with tumor volume, measured by a treatment planning system, computed tomography (CT) or magnetic resonance imaging (MRI) [17-20]. The clinical relevance of this association is supported by findings that DNA load is correlated with apoptosis of tumor cells [21], and that viral DNA in circulation exists as fragments of free, non-encapsulated DNA [22]. Evaluation of EBV DNA levels is currently considered the strongest biomarker, which complements TNM classification in determining the prognosis of patients with NPC [23]. Quantification of EBV DNA levels using quantitative PCR (qPCR) shows good sensitivity and specificity at detecting NPC prior to therapy and may also be useful in diagnosing patients with early stage (stage I/II) NPC [24-26].

Published data evaluating the use EBV DNA for predicting survival is mostly limited to studies with small patient populations. The objective of this study was to evaluate pre-treatment copy number of plasma EBV DNA as an outcome marker for survival in NPC.

Materials and Methods

Search strategy

The meta-analysis was performed in accordance with the PRISMA guidelines. MEDLINE, CENTRAL and Embase databases were searched until April 7, 2015 using the following search terms: (plasma Epstein-Barr virus DNA) AND (nasopharyngeal carcinoma), (Epstein Barr virus OR EBV) AND (nasopharyngeal carcinoma). Included studies were randomized controlled trials, two-arm prospective studies or retrospective studies in patients with newly diagnosed NPC. All included studies had to quantitatively report survival data. Reviews, letters, comments, editorials, case reports, proceedings and personal communications were excluded. Two reviewers determined the appropriateness of a study for inclusion and a third reviewer was consulted to resolve any discrepancies.

Data extraction and quality assessment

The following data was extracted: name of first author, study design, number of patients, gender, method of measuring EBV DNA, length of follow-up and cut-off of EBV DNA for a given outcome assessment.

The quality of the included studies was evaluated using the method of Hayden *et al.* [27]. This analysis assesses six sources of bias related to study participation, study attrition, prognostic factor measurement, confounding measurement and account, outcome measurement and analysis. The data

TABLE 1. Summary of basic characteristics of included studies for meta-analysis

Study	Study design	Number of patients	Age	Male (%)	WHO pathology (n)	EBV DNA measurement	Length of follow up (months)	Cut-off point of EBV DNA (copies/mL)
Tang, 2015 [28]	Prospective	3113	47.15	73.0%	Type I: 6 Type II: 129 Type III: 2978		51 (40-62) ^b	4000
Tang, 2014 [29]	Retrospective	2563	46.91	73.6%	Type I: 8 Type II: 93 Type III: 2462	qPCR	37 (28-46) ^b	Tertile (326 and 11333) ^c
Wei, 2014 [30]	Retrospective	214	49.77	76.2%	Differentiated: 16 Undifferentiated: 200	Fluorescence qPCR	60.1 (9-80) ^a	1500
Leung, 2014 [31]	Prospective	107			NR	qPCR	73 (4.8-89.6) ^b	4000
Wang, 2013 [32]	Prospective	210	45.6	74.3%	Type I: 5 Type II: 170 Type III: 35	RT-PCR	at least 6 years	1500
Li, 2013 [34]	Prospective	210	≤ 46 yr: 62.9% A 46 yr: 37.1%	78.1%	Type I: 10 Type II: 13 Type III: 187	PCR	73 (10-104) ^a	
Chai, 2012 [33]	Retrospective	390	≤ 50 yr: 50.1% A 50 yr: 49.9%		Type I: 6 Type II: 79 Type III: 305	qPCR		8000
Chang, 2012 [35]	Prospective	108		77.7%	NR			307
Hsu, 2012 [36]	Prospective	73	≤ 55 yr: 53.4% A 55 yr: 46.6%	83.6%	NR	RT-qPCR	31 (20-40) ^a	1000, 5000 and 50000 ^d
An, 2011 [37]	Prospective	127	45 (24-70) ^a	80.3%	NR	RT-PCR	21 (3-88) ^a	Median (2.33x10 ⁵)
Wang, 2010 [38]	Prospective	34	≤ 50 yr: 52.9% A 50 yr: 47.1%	91.2%	NR	RT-qPCR	30 (18-50) ^a	1000, 1500, 5000, 10000 and 50000 ^d
Twu, 2007 [39]	Prospective	114	46 (24-74) ^a	76.3%	Type I: 4 Type II: 90 Type III: 20	RT-qPCR	46 (22-67) ^a	1500
Hou, 2006 [40]	Prospective	69	48 (28-73) ^a	41.0%	Type I: 0 Type II: 17 Type III: 52	RT-PCR	Median: 30	20000
Lin, 2004 [13]	Prospective	99	47 (24-74) ^a	74.0%	Type I: 4 Type II: 80 Type III: 15	RT-qPCR	30 (14-48) ^a	1500
Chan, 2002 [12]	Prospective	170	46 (18-80) ^a	84.1%	NR	RT-qPCR	116 weeks (37-239) ^a	4000
Lin, 2001 [41]	Prospective	124	48.55	72.6%	Type I: 0 Type II: 101 Type III: 22	PCR	38 (24-56) ^a	N

Note: Age was presented by mean or percentage for age groups if not specified.

^aData were presented by median and range. ^bData were presented by median and inter-quartile range ^cTo determine the hazard ratio (HR), the cut-off point was ≥ 11333 copies/mL versus < 326 copies/mL. ^dTo determine the HR, the cut-off points were > 5000 versus ≤ 5000.

Abbreviations: PCR=polymerase chain reaction; qPCR=quantitative PCR; RT-PCR=real-time PCR. citing no one study overly; NR=Not reported

TABLE 2. Results of the meta-analysis for prognostic role of plasma Epstein-Barr virus for survival status of nasopharyngeal carcinoma

Outcomes	Number of studies	Heterogeneity test	Model	HR (95% CI)	P-value
Overall survival	13 [12,28-33, 35-39, 41]	Q=31.3 P=0.002 I ² =61.6%	Random	3.005 (2.245–4.022)	<0.001
Progression-free survival	6 [12, 30, 31, 34, 37, 41]	Q=3.4 P=0.643 I ² =0%	Fixed	2.420 (1.806–3.244)	<0.001
Relapse-free survival	3[13, 32, 39]	Q=0.6 P=0.758 I ² =0%	Fixed	2.202 (1.462–3.317)	<0.001
Disease-free survival	3 [28, 29, 35]	Q=5.6 P=0.060 I ² =64.4%	Random	3.121 (2.273–4.285)	<0.001
Distant metastasis-free survival	9 [12, 28-31, 34, 35, 40, 41]	Q=8.2 P=0.412 I ² =2.8%	Fixed	3.684 (3.055–4.444)	<0.001

TABLE 3. Subgroup analysis of the prognostic role of plasma Epstein-Barr virus for survival status of nasopharyngeal carcinoma

Outcomes	Subgroup	Number of studies	Heterogeneity test	HR (95% CI)	P-value
Overall survival	EBV-positive patients $\geq$ 90%	7 [12,13,31,36-39]	Q=11.5 P=0.074 I-square=47.9%	3.218 (2.332–4.440)	<0.001
Progression-free survival	EBV-positive patients $\geq$ 90%	3 [30, 34, 41]	Q=2.0 P=0.364 I-square=1.1%	3.011 (1.820–4.981)	<0.001
Disease-free survival	EBV-positive patients $\geq$ 90%	2 [28, 29]	Q=1.7 P=0.191 I-square=41.5%	3.391 (2.889–3.980)	<0.001
Distant metastasis-free survival	EBV-positive patients $\geq$ 90%	6 [28-30, 34, 40, 41]	Q=6.6 P=0.252 I-square=24.2%	3.826 (3.138–4.665)	<0.001

were extracted and the quality of the included studies assessed by two independent reviewers and a third reviewer was consulted for resolution of disagreement.

Data analysis

The primary outcome was overall survival and the secondary outcomes were progression-free survival, relapse-free survival, disease-free survival and distant metastasis-free survival. Hazard ratio (HR) and 95% confidence interval (95% CI) were used as the index of effect size; HR > 1 indicates higher risk for the occurrence of events (e.g., death, progression, relapse, distant metastasis, etc.) in patients with higher levels of EBV DNA. Heterogeneity among the studies was assessed by the Cochran Q and the I² statistic. The Q statistic was defined

as the weighted sum of the squared deviations of the estimates of all studies; P < 0.10 was considered statistically significant for heterogeneity. For the I² statistic, which indicated the percentage of the observed between-study variability due to heterogeneity, the suggested ranges are as follows: no heterogeneity (I² = 0-25%); moderate heterogeneity (I² = 25-50%); large heterogeneity (I² = 50-75%); and, extreme heterogeneity (I² = 75-100%). If either P < 0.1 for the Q statistics or the I² statistic was >50%, a random-effect model was used (DerSimonian–Laird method); otherwise, a fixed-effect model (Mantel-Haenszel method) was used. A 2-sided P-value < 0.05 was considered statistically significant. The leave-one-out approach was used to assess sensitivity of meta-analysis for overall survival. To evaluate whether EBV

FIGURE 1. Flow diagram of study selection

status may have affected the pooled results, a subgroup analysis was performed that included only those studies with EBV-positive patients comprising $\geq 90\%$ of the total. Publication bias was assessed by constructing funnel plots for the primary outcome. The absence of publication bias was indicated by the data points forming a symmetric funnel-shaped distribution. Egger's test was performed to examine the symmetry of funnel plot; a one-tailed $P > 0.05$ indicated there was no publication bias. All statistical analyses were performed using the statistical software Comprehensive Meta-Analysis, version 2.0 (Biostat, Englewood, NJ, USA).

Results

Literature search

Of the 123 studies initially identified, 66 were excluded for being irrelevant (Figure 1). The remaining 57 were fully evaluated for inclusion and 41 were eliminated for not reporting the outcomes of interest, reporting data that could not be pooled for a meta-analysis, not being relevant or reporting findings from a study that was already included.

Sixteen studies were included in the study with a total of 7698 patients [12,13,28-41]. Three of the studies were retrospective in design and the remaining 13 studies were prospective (Table 1). No randomized controlled studies were included. The sample size ranged 69 to 3113 patients, with two

studies having >2000 patients. The mean or median age ranged from 45-49.77 years. Except for one study [40], the majority of patients were male (72.6%–91.2%). The median follow-up duration ranged from 21 months to 6 years. The methods for measuring levels of EBV virus included quantitative PCR (qPCR), fluorescent qPCR and real-time (RT)-PCR. The cut-off points for EBV DNA varied across studies and ranged from 307-50,000 copies/mL. Ten studies reported the percentage of EBV-positive patients (70%-100%). Ten studies also reported data for the distribution of tumors among the three WHO types (I: keratinizing squamous cell carcinoma, II: differentiated non-keratinizing carcinoma, and III: undifferentiated carcinoma) with the proportion of patients with WHO Type I disease ranging from 0% to 7.5%). Eight studies reported data for clinical or disease stage; however, none of the studies reported the proportion of EBV-positive patients for each WHO tumor type or clinical stage.

Meta-analysis

Across the 13 studies that reported findings for overall survival, there was evidence of heterogeneity ($Q = 31.3$, $P = 0.002$, $I^2 = 61.6\%$); therefore, a random-effects model was used to generate pooled estimate. The pooled HR was 3.005 (95% confidence interval [CI] = 2.245–4.022; $P < 0.001$), indicating that higher levels of EBV DNA were associated with higher risk of death (Figure 2). Sensitivity analysis using the

FIGURE 2. Forest plot of prognosis role of re-treatment plasma EBV DNA for overall survival in patients with nasopharyngeal carcinoma.

FIGURE 3. Sensitivity analysis of prognostic role of pre-treatment plasma EBV DNA for overall survival in patients with nasopharyngeal carcinoma.

FIGURE 4. Funnel plot for publication bias.

leave-one-out approach found that the direction and magnitude of the combined estimates and the hazard ratios (HR) did not change when individual studies were removed one at a time (Figure 3), indicating no one study overly influenced the findings.

Results of meta-analysis for all survival outcomes are summarized in Table 2. Six studies provided data pertaining to progression-free survival. A fixed-effect model was used as there was no sign of heterogeneity among the studies ($Q = 3.4$, $P = 0.643$, $I^2 = 0\%$). The meta-analysis showed that the pooled HR was 2.420 (95% CI = 1.806–3.244, $P < 0.001$), indicating patients with high levels of pre-treatment EBV DNA had higher risk of disease progression compared with those with low levels of EBV DNA. Similarly, additional analyses indicated that high levels of EBV DNA prior to therapy were associated with a higher risk of disease relapse and recurrence of disease (HR = 2.202, 95% CI = 1.462–3.317, $P < 0.001$ for relapse-free survival; HR = 3.121, 95% CI = 2.273–4.285, $P < 0.001$ for disease-free survival). In addition, high pre-treatment EBV DNA levels were associated with higher risk of the occurrence of distant metastasis (HR = 3.684, 95% CI = 3.055–4.444, $P < 0.001$).

The results of a subgroup analysis showed that our pooled results for each outcome were not affected by the patients' EBV status. The direction and magnitude of the association in a subgroup of studies whose patient populations were at least

90% EBV-positive did not deviate from the pooled results of all included studies (Table 3).

Publication bias and quality assessment

Analysis of publication bias found there was no evidence by Egger's test for publication bias ($P = 0.109$) (Figure 4). Quality assessment found that for most of the included studies there was low risk of bias for study participation, study attrition, measurement of prognostic factors, outcome measurements, confounding measurements and accounts, and analysis (Table 4), indicating the studies were generally of moderate to high quality.

Discussion

Sixteen studies were included in the meta-analysis with a total of 7698 patients. For overall survival, the pooled HR was 3.005 (95% CI = 2.245–4.022; $P < 0.001$), indicating that higher levels of EBV DNA prior to therapy were associated with higher risk of death. The pooled estimates also indicated that higher levels of pre-treatment EBV DNA was associated with an increased risk of relapse and disease recurrence, disease progression, and distant metastasis than low levels of pre-treatment EBV DNA (P values < 0.001). The meta-analysis found that high pre-treatment levels of EBV DNA had good value as a prognostic indicator of survival and disease outcomes in patients with NPC.

TABLE 4. Summary of basic characteristics of included studies for meta-analysis

Study	Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Confounding measurement and account	Analysis
Tang, 2015	Low	UN	Low	Low	Low	Low
Tang, 2014	Low	UN	Low	Low	Low	Low
Wei, 2014	Low	Low	Low	Low	Low	Low
Leung, 2014	Low	Low	Low	Low	Low	Low
Wang, 2013	Low	UN	Low	Low	Low	Low
Li, 2013	Low	Low	Low	Low	UN	Low
Chai, 2012	Low	Low	Low	Low	Low	Low
Chang, 2012	Low	UN	Low	UN	Low	Low
Hsu, 2012	Low	UN	Low	Low	Low	Low
An, 2011	Low	UN	Low	Low	Low	Low
Wang, 2010	Low	UN	Low	Low	UN	Low
Twu, 2007	Low	UN	Low	Low	Low	Low
Hou, 2006	Low	UN	Low	Low	Low	Low
Lin, 2004	Low	Low	Low	Low	Low	Low
Chan, 2002	Low	Low	Low	Low	Low	Low
Lin, 2001	Low	Low	Low	Low	Low	Low

Low indicates low risk of bias and UN indicates unclear risk of bias

Most of the studies included in this meta-analysis found that increased levels of EBV DNA prior to treatment was associated with poor overall survival [13,28-30,32-37,39,40]. Of the studies that evaluated the other outcomes, high pre-treatment EBV DNA levels was also associated with increased risk for disease progression, relapse, recurrence, and distant metastasis. Leung *et al.* [31] did not find an association between pre-treatment EBV DNA levels and overall survival ($P = 0.2135$); however, they also evaluated the association of EBV DNA levels with survival outcomes mid-way through treatment and found at this time point high levels of EBV DNA were associated with poor overall survival ($P = 0.0077$). Both pre-treatment and mid-treatment high EBV DNA levels were not associated with locoregional failure (P values ≥ 0.1378). The differences among Leung's, ours and other studies are not clear and may include differences in study design and assay conditions. Wang *et al.* [38] also found that the overall two-year survival rate was similar between patients with high pre-treatment EBV DNA (>5000 copies/mL) and low

pre-treatment EBV DNA (≤ 5000 copies/mL); however, interpretation of the results of the Wang *et al.* study is confounded by the fact that it included only 34 patients.

One point that should be emphasized is that the relationship between EBV DNA load and overall survival is extremely robust. The majority of studies identified a negative impact of EBV DNA load, even though they differed in study design and assay cut-off values. Moreover, methods to quantitate DNA have evolved over the period of the review (15 years) and institutional protocols are not standardized [42,43]. Use of real-time PCR to measure EBV DNA was first reported in 1999 [16,44], and since then, the availability and diversity of reagents and protocols have expanded considerably [45]. Not unexpectedly, several studies have documented substantial variability in quantitative assays [46-48]. The World Health Organization (WHO) introduced an EBV DNA standard in 2011 [49], providing an opportunity for institutions to reduce the inter-institutional variability of their data. None of the studies included in this review used the

WHO standard, indicating a greater need for harmonization in future studies. This crucial optimization will strengthen meta-analyses such as this one and allow more in-depth study of the effects of EBV burden on additional outcomes beyond mortality.

Our findings are similar to a recent meta-analysis by Zhang *et al.* [25], where the objective was to assess the potential of plasma EBV DNA assays to predict clinical outcomes in patients with NPC [25]. Specifically, their meta-analysis evaluated the correlation between pre-treatment, mid-treatment and post-treatment EBV DNA and half-life value of plasma EBV DNA clearance rate ($t_{1/2}$) with clinical outcomes. Their meta-analysis included 14 studies with a total of 7836 patients. They found that high levels of pre-treatment, mid-treatment and post-treatment EBV DNA and slow EBV DNA clearance were all significantly associated with poorer overall survival (HRs ranged from 2.81-3.58 across endpoints). They also found that high levels of pre-treatment, mid-treatment and post-treatment EBV DNA were associated with a higher risk of disease progression, distant metastasis and increased locoregional failure. We did not evaluate locoregional failure survival in our study. Furthermore, we did not analyze the association of mid-treatment and post-treatment EBV DNA levels with survival outcomes because the primary objective of this study was to assess the pre-treatment DNA level. The use of pre-treatment EBV DNA levels can not only predict patient prognosis but also support diagnosis and treatment decisions.

While our meta-analysis was not focused on comparing detection rates for different clinical stages of NPC, it is an important issue that must be considered by institutions that currently use or in the future will use qPCR of EBV DNA to screen patients for NPC. In a recent paper published, Yip *et al.* discussed the limitations of EBV qPCR to detect stage I NPC [24]. Recent findings indicate that the sensitivity for detection of stage I NPC is substantially lower than that of stages II, III, and IV (50%–86% versus 94%–95%, 91%–100% and 94%–98%, respectively); however, these statistics may not influence clinicians' research efforts, as we identified only one trial that reported detection rates for patients with stage I disease [33]. The lack of data on use of EBV testing to detect stage I NPC may be expected, given that the majority of patients with NPC present at later stages [2]; regardless, improving detection of early stage NPC would most likely lead to improved survival, and this should be a goal of future randomized trials.

There are several limitations to our meta-analysis. First, we did not perform subgroup analysis due to the cut-off values for copies of EBV DNA used varied across studies. Second, we did

not have individual patient data but only aggregate data. The use and analysis of individual patient data, particularly with NPC which shows diverse disease outcomes even in patients with similar staged tumors, may give important insight into what patient characteristics influence the prognostic characteristics of pre-treatment EBV DNA levels in NPC. Third, of the included studies were randomized controlled studies raising the possibility that findings were influenced by selection and/or treatment allocation bias. Lastly, low enrollment of patients with WHO Type I tumors, coupled with incomplete reporting by the included studies, precluded a subgroup analysis of the prognosis of patients with each type of WHO tumor. Large studies should be conducted in low-incidence regions such as the United States and Japan, as these are known to have a higher proportion of patients with WHO type I NPC [24].

In conclusion, our meta-analysis supports prior work that indicates pre-treatment plasma EBV DNA levels are highly prognostic of long-term survival and distant metastasis in patients with NPC. None of the included studies were randomized controlled trials and many had small patient populations, indicating the need for well-designed clinical trials to further test the use of EBV DNA in diagnosing and treating NPC.

Acknowledgments

The authors would like to acknowledge the contributions of the following individuals for literature research (Lili Pan, MD and Lihong Chen, MD), data acquisition (Jie Pan, MD and Lihong Chen, MD) and statistical analysis (Zhihong Zheng, PhD, Jie Pan, MD and Lihong Chen, MD).

References

1. Hutajulu SH, Kurnianda J, Tan IB, Middeldorp JM. Therapeutic implications of Epstein-Barr virus infection for the treatment of nasopharyngeal carcinoma. *Ther Clin Risk Manag* 2014;10: 721-736.
2. Petersson F. Nasopharyngeal carcinoma: a review. *Semin Diagn Pathol*. 2015;32: 54-73.
3. Chen CJ, You SL, Lin LH, Hsu WL, Yang YW. Cancer epidemiology and control in Taiwan: a brief review. *Jpn J Clin Oncol* 2002;32 Suppl: S66-81.
4. Devi BC, Pisani P, Tang TS, Parkin DM. High incidence of nasopharyngeal carcinoma in native people of Sarawak, Borneo Island. *Cancer Epidemiol Biomarkers Prev* 2004;13: 482-486.
5. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015;65: 87-108.

6. Chang ET, Adami HO. The enigmatic epidemiology of nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev* 2006;15: 1765-1777.
7. Pfister DG, Ang KK, Brizel DM, Burtress BA, Busse PM, Caudell JJ, Cmelak AJ, Colevas AD, Dunphy F, Eisele DW, Gilbert J, Gillison ML, Haddad RI, Haughey BH, Hicks WL Jr, Hitchcock YJ, Kies MS, Lydiatt WM, Maghami E, Martins R, McCaffrey T, Mittal BB, Pinto HA, Ridge JA, Samant S, Schuller DE, Shah JP, Spencer S, Weber RS, Wolf GT, Worden F, Yom SS, McMillian NR, Hughes M; National Comprehensive Cancer Network. Head and neck cancers, version 2.2013. Featured updates to the NCCN guidelines. *J Natl Compr Canc Netw* 2013;11: 917-923.
8. Cheng SH, Yen KL, Jian JJ, Tsai SY, Chu NM, Leu SY, Chan KY, Tan TD, Cheng JC, Hsieh CY, Huang AT. Examining prognostic factors and patterns of failure in nasopharyngeal carcinoma following concomitant radiotherapy and chemotherapy: impact on future clinical trials. *Int J Radiat Oncol Biol Phys* 2001;50: 717-726.
9. Raab-Traub N. Nasopharyngeal Carcinoma: An Evolving Role for the Epstein-Barr Virus. *Curr Top Microbiol Immunol* 2015;390: 339-363.
10. Tsang CM, Tsao SW. The role of Epstein-Barr virus infection in the pathogenesis of nasopharyngeal carcinoma. *Virol Sin* 2015;30: 107-121.
11. Ludwig JA, Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. *Nat Rev Cancer* 2005;5: 845-856.
12. Chan AT, Lo YM, Zee B, Chan LY, Ma BB, Leung SF, Lai M, Ho S, Huang DP, Johnson PJ. Plasma Epstein-Barr virus DNA and residual disease after radiotherapy for undifferentiated nasopharyngeal carcinoma. *J Natl Cancer Inst* 2002;94: 1614-1619.
13. Lin JC, Wang WY, Chen KY, Wei YH, Liang WM, Jan JS, Jiang RS. Quantification of plasma Epstein-Barr virus DNA in patients with advanced nasopharyngeal carcinoma. *N Engl J Med* 2004;350: 2461-2470.
14. Leung SF, Chan AT, Zee B, Ma B, Chan LY, Johnson PJ, Lo YM. Pretherapy quantitative measurement of circulating Epstein-Barr virus DNA is predictive of posttherapy distant failure in patients with early-stage nasopharyngeal carcinoma of undifferentiated type. *Cancer* 2003;98: 288-291.
15. Leung SF, Zee B, Ma BB, Hui EP, Mo F, Lai M, Chan KC, Chan LY, Kwan WH, Lo YM, Chan AT. Plasma Epstein-Barr viral deoxyribonucleic acid quantitation complements tumor-node-metastasis staging prognostication in nasopharyngeal carcinoma. *J Clin Oncol* 2006;24: 5414-5418.
16. Lo YM, Chan LY, Lo KW, Leung SF, Zhang J, Chan AT, Lee JC, Hjelm NM, Johnson PJ, Huang DP. Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma. *Cancer Res* 1999;59: 1188-1191.
17. Chen M, Yin L, Wu J, Gu JJ, Jiang XS, Wang DJ, Zong D, Guo C, Zhu HF, Wu JF, He X, Guo WJ. Impact of plasma Epstein-Barr virus-DNA and tumor volume on prognosis of locally advanced nasopharyngeal carcinoma. *Biomed Res Int* 2015;2015: 617949.
18. Hou X, Zhao C, Guo Y, Han F, Lu LX, Wu SX, Li S, Huang PY, Huang H, Zhang L. Different clinical significance of pre- and post-treatment plasma Epstein-Barr virus DNA load in nasopharyngeal carcinoma treated with radiotherapy. *Clin Oncol (R Coll Radiol)* 2011;23: 128-133.
19. Lu L, Li J, Zhao C, Xue W, Han F, Tao T, Chang H, Jia W, Lu T. Prognostic efficacy of combining tumor volume with Epstein-Barr virus DNA in patients treated with intensity-modulated radiotherapy for nasopharyngeal carcinoma. *Oral Oncol* 2016;60: 18-24.
20. Ma BB, King A, Lo YM, Yau YY, Zee B, Hui EP, Leung SF, Mo F, Kam MK, Ahuja A, Kwan WH, Chan AT. Relationship between pretreatment level of plasma Epstein-Barr virus DNA, tumor burden, and metabolic activity in advanced nasopharyngeal carcinoma. *Int J Radiat Oncol Biol Phys* 2006;66: 714-720.
21. Mutirangura A, Pornthanakasem W, Theamboonlers A, Sriuranpong V, Lertsanguansinchi P, Yenrudi S, Voravud N, Supiyaphun P, Poovorawan Y. Epstein-Barr viral DNA in serum of patients with nasopharyngeal carcinoma. *Clin Cancer Res* 1998;4: 665-669.
22. Chan KC, Zhang J, Chan AT, Lei KI, Leung SF, Chan LY, Chow KC, Lo YM. Molecular characterization of circulating EBV DNA in the plasma of nasopharyngeal carcinoma and lymphoma patients. *Cancer Res* 2003;63: 2028-2032.
23. Ng WT, Yuen KT, Au KH, Chan OS, Lee AW. Staging of nasopharyngeal carcinoma--the past, the present and the future. *Oral Oncol* 2014;50: 549-554.
24. Yip TT, Ngan RK, Fong AH, Law SC. Application of circulating plasma/serum EBV DNA in the clinical management of nasopharyngeal carcinoma. *Oral Oncol* 2014;50: 527-538.
25. Zhang W, Chen Y, Chen L, Guo R, Zhou G, Tang L, Mao Y, Li W, Liu X, Du X, Sun Y, Ma J. The clinical utility of plasma Epstein-Barr virus DNA assays in nasopharyngeal carcinoma: the dawn of a new era?: a systematic review and meta-analysis of 7836 cases. *Medicine (Baltimore)* 2015;94: e845.
26. Sun D, Yang Z, Fu Y, Chen Y, Wang S, Zhang Y, Ma Y, Zhang X. Clinical value of serum Epstein-Barr virus DNA assay in the diagnosis of nasopharyngeal carcinoma. *Tumour Biol* 2014;35: 8787-8793.
27. Hayden JA, Cote P, Bombardier C. Evaluation of the quality of prognosis studies in systematic reviews. *Ann Intern Med* 2006;144: 427-437.
28. Tang LQ, Li CF, Chen QY, Zhang L, Lai XP, He Y, Xu YX, Hu DP, Wen SH, Peng YT, Chen WH, Liu H, Guo SS, Liu LT, Li J, Zhang JP, Guo L, Zhao C, Cao KJ, Qian CN, Zeng YX,

- Guo X, Mai HQ, Zeng MS. High-sensitivity C-reactive protein complements plasma Epstein-Barr virus deoxyribonucleic acid prognostication in nasopharyngeal carcinoma: a large-scale retrospective and prospective cohort study. *Int J Radiat Oncol Biol Phys* 2015;91: 325-336.
29. Tang LQ, Chen QY, Guo SS, Chen WH, Li CF, Zhang L, Lai XP, He Y, Xu YX, Hu DP, Wen SH, Peng YT, Liu H, Liu LT, Yan SM, Guo L, Zhao C, Cao KJ, Liu Q, Qian CN, Ma J, Guo X, Zeng MS, Mai HQ. The impact of plasma Epstein-Barr virus DNA and fibrinogen on nasopharyngeal carcinoma prognosis: an observational study. *Br J Cancer* 2014;111: 1102-1111.
 30. Wei W, Huang Z, Li S, Chen H, Zhang G, Li S, Hu W, Xu T. Pretreatment Epstein-Barr virus DNA load and cumulative cisplatin dose intensity affect long-term outcome of nasopharyngeal carcinoma treated with concurrent chemotherapy: experience of an institute in an endemic area. *Oncol Res Treat* 2014;37: 88-95.
 31. Leung SF, Chan KC, Ma BB, Hui EP, Mo F, Chow KC, Leung L, Chu KW, Zee B, Lo YM, Chan AT. Plasma Epstein-Barr viral DNA load at midpoint of radiotherapy course predicts outcome in advanced-stage nasopharyngeal carcinoma. *Ann Oncol* 2014;25: 1204-1208.
 32. Wang WY, Twu CW, Chen HH, Jiang RS, Wu CT, Liang KL, Shih YT, Chen CC, Lin PJ, Liu YC, Lin JC. Long-term survival analysis of nasopharyngeal carcinoma by plasma Epstein-Barr virus DNA levels. *Cancer* 2013;119: 963-970.
 33. Chai SJ, Pua KC, Saleh A, Yap YY, Lim PV, Subramaniam SK, Lum CL, Krishnan G, Mahiyuddin WR; Malaysian NPC Study Group, Teo SH, Khoo AS, Yap LF. Clinical significance of plasma Epstein-Barr Virus DNA loads in a large cohort of Malaysian patients with nasopharyngeal carcinoma. *J Clin Virol* 2012;55: 34-39.
 34. Li SW, Wang H, Xiang YQ, Zhang HB, Lv X, Xia WX, Zeng MS, Mai HQ, Hong MH, Guo X. Prospective study of prognostic value of Raf kinase inhibitory protein and pretreatment plasma Epstein-Barr virus DNA for distant metastasis in locoregionally advanced nasopharyngeal carcinoma. *Head Neck* 2013;35: 579-591.
 35. Chang KP, Tsang NM, Liao CT, Hsu CL, Chung MJ, Lo CW, Chan SC, Ng SH, Wang HM, Yen TC. Prospective study of prognostic value of Raf kinase inhibitory and plasma Epstein-Barr virus DNA load in patients with nasopharyngeal carcinoma. *J Nucl Med* 2012;53: 21-28.
 36. Hsu CL, Chang KP, Lin CY, Chang HK, Wang CH, Lin TL, Liao CT, Tsang NM, Lee LY, Chan SC, Ng SH, Li HP, Chang YS, Wang HM. Plasma Epstein-Barr virus DNA concentration and clearance rate as novel prognostic factors for metastatic nasopharyngeal carcinoma. *Head Neck* 2012;34: 1064-1070.
 37. An X, Wang FH, Ding PR, Deng L, Jiang WQ, Zhang L, Shao JY, Li YH. Plasma Epstein-Barr virus DNA level strongly predicts survival in metastatic/recurrent nasopharyngeal carcinoma treated with palliative chemotherapy. *Cancer* 2011;117: 3750-3757.
 38. Wang WY, Twu CW, Chen HH, Jan JS, Jiang RS, Chao JY, Liang KL, Chen KW, Wu CT, Lin JC. Plasma EBV DNA clearance rate as a novel prognostic marker for metastatic/recurrent nasopharyngeal carcinoma. *Clin Cancer Res* 2010;16: 1016-1024.
 39. Twu CW, Wang WY, Liang WM, Jan JS, Jiang RS, Chao J, Jin YT, Lin JC. Comparison of the prognostic impact of serum anti-EBV antibody and plasma EBV DNA assays in nasopharyngeal carcinoma. *Int J Radiat Oncol Biol Phys* 2007;67: 130-137.
 40. Hou X, Zhang L, Zhao C, Li S, Lu LX, Han F, Shao JY, Huang PY. [Prognostic impact of plasma Epstein-Barr virus DNA concentration on distant metastasis in nasopharyngeal carcinoma]. *Ai Zheng* 2006;25: 785-792.
 41. Lin JC, Chen KY, Wang WY, Jan JS, Liang WM, Tsai CS, Wei YH. Detection of Epstein-Barr virus DNA in the peripheral-blood cells of patients with nasopharyngeal carcinoma: relationship to distant metastasis and survival. *J Clin Oncol* 2001;19: 2607-2615.
 42. Ruf S, Wagner HJ. Determining EBV load: current best practice and future requirements. *Expert Rev Clin Immunol* 2013;9: 139-151.
 43. De Paoli P, Pratesi C, Bortolin MT. The Epstein Barr virus DNA levels as a tumor marker in EBV-associated cancers. *J Cancer Res Clin Oncol* 2007;133: 809-815.
 44. Kimura H, Morita M, Yabuta Y, Kuzushima K, Kato K, Kojima S, Matsuyama T, Morishima T. Quantitative analysis of Epstein-Barr virus load by using a real-time PCR assay. *J Clin Microbiol* 1999;37: 132-136.
 45. Gulley ML, Tang W. Using Epstein-Barr viral load assays to diagnose, monitor, and prevent posttransplant lymphoproliferative disorder. *Clin Microbiol Rev* 2010;23: 350-366.
 46. Hayden RT, Hokanson KM, Pounds SB, Bankowski MJ, Belzer SW, Carr J, Diorio D, Forman MS, Joshi Y, Hillyard D, Hodinka RL, Nikiforova MN, Romain CA, Stevenson J, Valsamakis A, Balfour HH Jr; U.S. EBV Working Group. Multicenter comparison of different real-time PCR assays for quantitative detection of Epstein-Barr virus. *J Clin Microbiol* 2008;46: 157-163.
 47. Le QT, Zhang Q, Cao H, Cheng AJ, Pinsky BA, Hong RL, Chang JT, Wang CW, Tsao KC, Lo YD, Lee N, Ang KK, Chan AT, Chan KC. An international collaboration to harmonize the quantitative plasma Epstein-Barr virus DNA assay for future biomarker-guided trials in nasopharyngeal carcinoma. *Clin Cancer Res* 2013;19: 2208-2215.
 48. Preiksaitis JK, Pang XL, Fox JD, Fenton JM, Caliendo AM, Miller GG; American Society of Transplantation Infectious Diseases Community of Practice. Interlaboratory comparison of

epstein-barr virus viral load assays. *Am J Transplant* 2009;9: 269-279.

49. Fryer JF, Heath A, Wilkinson DE, Minor PD, Unit. WHO, WHO Expert Committee on Biological Standardization (2011 : Geneva, Switzerland). Collaborative study to evaluate the proposed 1st WHO international standards for Epstein-Barr Virus (EBV) for Nucleic Acid Amplification Technology (NAT)-Based Assays. WHO/BS/2011.2172, English only, 2011; 43 p.