

# Epstein–Barr virus DNA level as a novel prognostic factor in nasopharyngeal carcinoma

## A meta-analysis

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### Abstract

**Background:** The plasma Epstein–Barr virus (EBV) DNA level in patients with nasopharyngeal carcinoma (NPC) performs as an appealing prognostic factor, but conclusions of its prognostic values from previous studies are inconsistent. In this study, we performed a comprehensive meta-analysis to evaluate the prognostic value of EBV DNA level in patients with NPC.

**Methods:** Published studies were searched in PubMed. The baseline characteristics of patients, overall survival (OS), and other survival outcomes were extracted. Pooled hazard ratio (HR), 95% confidence interval (CI), and *P* value were calculated to estimate the prognostic value of EBV DNA level. Each cut-off value mentioned in the studies was obtained. Kaplan–Meier curves were used to extract data, and graphical survival plots were extracted for calculating HR when the study did not describe the information directly.

**Results:** This meta-analysis pooled 23 eligible studies including 10,732 patients with NPC. The pooled HR (95% CI) of pretreatment plasma EBV DNA level (pre-DNA) for OS was 2.78 (2.19, 3.55), and the HR (95% CI) of posttreatment plasma EBV DNA level (post-DNA) for OS was 5.43 (2.72, 10.82), suggesting that EBV DNA level was significantly correlated to the outcomes of patients with NPC.

**Conclusion:** High expression levels of EBV DNA predicts poor prognosis in NPC.

**Abbreviations:** CI = confidence interval, DFS = disease-free survival, DMFS = distant metastasis-free survival, EBV = Epstein–Barr virus, HR = hazard ratio, NOS = Newcastle–Ottawa Scale, NPC = nasopharyngeal carcinoma, OS = overall survival, PFS = progression-free survival, RFS = recurrence-free survival, RT-PCR = real-time quantitative polymerase chain reaction.

**Keywords:** Epstein–Barr virus DNA, meta-analysis, nasopharyngeal carcinoma, prognosis

## 1. Introduction

Nasopharyngeal carcinoma (NPC) is a highly ethnic and geographical cancer that is relatively prevalent in South East Asia and mainland China.<sup>[1]</sup> The leading cause of death in patients with NPC is distant metastasis; thus early detection is of great importance in the prevention of metastasis in clinical practice.<sup>[2]</sup> However, the occurrence of occult primary tumors increases the difficulty of diagnosing the disease early and

accurately. Meanwhile, computed tomography (CT) and magnetic resonance imaging (MRI) are effective only when the diameter of the lesion is larger than 5 mm, which will delay the discovery of the tumor, and the use of imaging for diagnosis is prohibitive in some developing countries for its high cost. Furthermore, imageological examinations cannot accurately predict the prognosis and assess the effectiveness of the targeted drugs. Therefore, a noninvasive and cost-effective method for the early prediction of the outcome of NPC is urgently needed.

Nawroz et al<sup>[3]</sup> had developed a new method for detecting tumor-associated DNA levels in peripheral circulation. Also, the DNA level of the Epstein–Barr virus (EBV) was reported to be associated with NPC and could be used as an important tool to predict NPC progression.<sup>[1,4]</sup> Plasma EBV DNA level detected by real-time polymerase chain reaction (RT-PCR) has been widely used to determine tumor stage and monitoring NPC progression which has good sensitivity and specificity,<sup>[5,6]</sup> but controversy still exists in the clinical use of EBV DNA level. Previous study<sup>[7]</sup> also showed conflicting and heterogeneous results for the clinical use of EBV DNA level. Most of the previous studies focused on improving the efficiency of EBV DNA level in the prognosis of patients from high-incidence area of nasopharyngeal carcinoma, but few studies had investigated the prognostic value of EBV DNA level quantitatively. So there still lacks a standard that evaluates the effectiveness of using different cut-off values to predict the outcomes of patients. Our study aims to investigate the prognostic value of plasma EBV DNA level in NPC patients and to suggest an optimal cut-off value of the EBV DNA level to predict the survival outcome of NPC patients.

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## 2. Materials and methods

### 2.1. Search strategy

Comprehensive search was conducted in PubMed (last update on January 2015), using a combination of the following keywords: “Epstein–Barr virus DNA,” “prognosis,” “prognostic,” and “nasopharyngeal carcinoma.” Because it is a meta-analysis, no ethics committee or institutional review board approval was necessary for this study.

### 2.2. Inclusion/exclusion criteria

Studies were considered eligible only when they met all of the following inclusion criteria: NPC patients were pooled; the EBV DNA level in the plasma was measured; the association between EBV DNA level and survival outcome (overall survival [OS], disease-free survival [DFS], distant metastasis-free survival [DMFS], progression-free survival [PFS], or recurrence-free survival [RFS]) was investigated; and the Kaplan–Meier survival curves of the survival outcomes and the log-rank *P* values were reported. Studies that did not directly report hazard ratios (HRs), 95% confidence intervals (CIs), or *P* values were also included when HR could be calculated by the methods developed by Tierney et al,<sup>[8]</sup> Williamson et al,<sup>[9]</sup> and Parmar et al.<sup>[10]</sup> Exclusion criteria were as follows: studies were published as review articles or letters; studies focused on analyzing other tumors and were not specific for NPC; and studies lacked vital information for analysis.

### 2.3. Data extraction

Two reviewers assessed the eligibility of each study independently. Disagreements were resolved through discussion within our research team. All the data of included studies were extracted by 2 independent reviewers and were carefully checked by a third author. Any disagreements were discussed until a final form was agreed upon. The data extracted from each study contained 3 parts:

- (1) Study characteristics: first author, country, year of publication, study design, and sample size.
- (2) Demographic characteristics of the studies: mean age, tumor grade, histological type, and other clinical characteristics.
- (3) Outcomes: HR, OS, DFS, DMFS, PFS, RFS, 95% CI, and *P* value. Each cut-off value mentioned in the studies was obtained.

Kaplan–Meier curves were used to extract data and graphical survival plots were extracted to calculate HR when the study did not directly describe the information.

### 2.4. Methodological assessment

To assess the quality of each study, 2 investigators independently scored the studies using the Newcastle–Ottawa Scale (NOS) criteria.<sup>[11]</sup> The score assessed 3 aspects of the study: subject selection: 0 to 4; comparability of subject: 0 to 2; and (3) clinical outcome: 0 to 3. The NOS scores ranged from 0 to 9, and the studies with 7 scores or more were graded as the high-quality ones in the scale.

### 2.5. Statistical analysis

To evaluate the prognostic value of the EBV DNA level, we used log HR and SE for aggregation of the survival results in the

meta-analysis. However, a number of studies did not directly provide vital information, such as HRs, 95% CIs, and *P* values. Therefore, we referred to the methods developed by Tierney et al,<sup>[8]</sup> Williamson et al,<sup>[9]</sup> and Parmar et al,<sup>[10]</sup> and calculated the necessary statistics on the basis of available numerical data with the Excel Tools developed by Tierney et al to calculate the log HR and SE. *Q* test and *I*<sup>2</sup> test were used to measure the heterogeneity among studies. A random-effect model (Der Simonian and Laird method) was applied if heterogeneity existed ( $P < 0.05$ ,  $I^2 > 50\%$ ), whereas the fixed-effect model was utilized in the absence of between-study heterogeneity ( $P \geq 0.05$ ,  $I^2 \leq 50\%$ ).<sup>[12]</sup> Sensitivity analysis was performed to examine the effect of variations in study quality excluding the studies with a NOS score of less than 6. Publication bias was assessed by Begg test ( $P < 0.05$  indicates statistically significant). All calculations cited above were performed using Stata 11.0 (Stata Corporation, College Station, TX).<sup>[13,14]</sup>

## 3. Results

### 3.1. Eligible studies

The initial search in PubMed yielded 146 studies. After review of titles and abstracts, 27 studies were excluded because they were letters, review articles, or written in non-English languages, or irrelevant to the current study. A total of 119 articles were further reviewed. Eighty-four studies were then excluded because they were not related to the topic. A detailed evaluation was performed for the remaining 35 studies. Four of them were laboratory research and 8 of them lacked necessary data for calculations. Finally, 23 potentially relevant studies<sup>[4–7,15–33]</sup> including 10,732 patients were eligible for the final analysis. The selection process was shown in Fig. 1.

The number of patients in the studies ranged from 34 to 6287 (mean 466). These patients came from 2 countries (22 from China and 1 from Malaysia). The studies were published between 2001 and 2015. Nineteen studies directly provided HRs and CIs, and the remaining 4 studies required additional calculations to get HRs and CIs.<sup>[4,21,23,25]</sup> We listed the articles containing different cut-off values, tumor grades, and histological types, and discussed them separately. Eighteen of the 23 included studies got 6 scores or more in methodological assessment. The characteristics of the included studies were shown in Table 1.

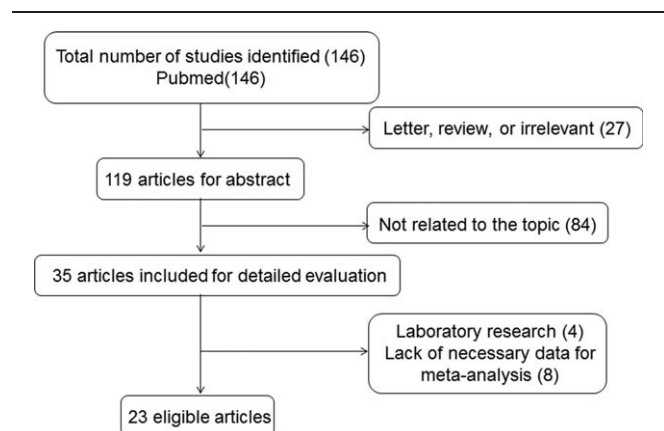


Figure 1. Selection of studies.

**Table 1**

**Characteristics of all identified studies.**

First author	Date	Location	Study design	N	Age (median)	Tumor grade (I-III/III-IV)	Sampling site	Histological differentiation (differentiated/undifferentiated)	Method	Attitude	Survival outcome	Cut-off (copies/mL)	Quality score
An	2011	China	Prospective	127	45	NA	Plasma	NA	RT-PCR	pos	OS PFS	Pre: median (233,000) Post: 0	8
Chai Chan	2012	Malaysia	Retrospective	390	50.5	104, 286	Plasma	85, 305	RT-PCR	pos	OS	8000	7
	2002	China	Prospective	170	46	70, 100	Plasma	0, 170	RT-PCR	pos	OS DMFS RFS PFS	Pre: 4000 Post: 500	5
Chang	2010	China	Prospective	132	NA	34, 97	Plasma	65, 67	RT-PCR	pos	OS	335	8
Chang	2012	China	Prospective	108	NA	29, 78	Plasma	43, 63	RT-PCR	pos	OS DMFS DFS	307	8
Chen	2014	China	Retrospective	717	47	96, 621	Plasma	NA	RT-PCR	pos	OS DMFS DFS	Pre: 4000 Post: /	7
Hou	2006	China	Prospective	69	48	25, 44	Plasma	17, 52	RT-PCR	pos	DMFS	Pre: 20000 Post: 0	7
Hou	2011	China	Retrospective	69	48	28, 41	Plasma	17, 52	RT-PCR	pos	OS DMFS	0	5
Hsu	2011	China	Prospective	73	55	NA	Plasma	10, 63	RT-PCR	pos	OS	1500, 5000, 50000	6
Jin	2011	China	Retrospective	799	NA	NA	Plasma	NA	RT-PCR	pos	OS	1000	6
Leung	2003	China	Prospective	90	NA	90, 0	Plasma	NA	RT-PCR	pos	DMFS	4000	5
Leung	2006	China	Retrospective	376	47	155, 221	Plasma	NA	RT-PCR	pos	OS	4000	4
Leung	2014	China	Prospective	107	NA	25, 82	Plasma	NA	RT-PCR	pos	OS DMFS RFS PFS	Pre: 4000	5
Li	2013	China	Prospective	210	47	0, 210	Plasma	NA	RT-PCR	pos	OS DMFS PFS	Post: 0 Pre: median (4154) Post: /	6
Lin	2001	China	Prospective	124	NA	I-III 31, IV 93	Plasma	NA	RT-PCR	pos	OS DMFS PFS	Pre: 0 Post: /	7
Lin	2004	China	Prospective	99	47	0, 99	Plasma	84, 15	RT-PCR	pos	OS RFS	Pre: 1500 Post: 0	6
Lin	2007	China	Retrospective	152	46	50, 102	Plasma	119, 33	RT-PCR	pos	OS RFS	Pre: 1500 Post: 0	7
Ma	2004	China	Prospective	160	46	64, 96	Plasma	NA	RT-PCR	pos	OS PFS DMFS	500	7
Tang	2015	China	Retrospective and prospective	6287	NA	1069, 5218	Plasma	NA	RT-PCR	pos	OS DMFS DFS	Pre: 4000	8
Twu	2006	China	Prospective	114	46	1, 113	Plasma	94, 20	RT-PCR	pos	OS RFS	Post: / Pre: 1500 Post: 0	7
Wang	2010	China	Prospective	34	50	7, 27	Plasma	26, 8	RT-PCR	neg	OS	1000, 1500, 5000, 10000, 50000	8
Wang	2013	China	Prospective	111	NA	IIb-III 52/IV 59	Plasma	NA	RT-PCR	pos	OS RFS	Pre: 1500 Post: 0	7
Wei	2014	China	Retrospective	214	NA	7, 207	Plasma	14, 200	RT-PCR	pos	OS DMFS RFS PFS	Pre: 1500 Post: /	7

DFS = disease-free survival, DMFS = distant metastasis-free survival, N = number of patients, NA = not available, OS = overall survival, PFS = progression-free survival, post = posttreatment, pre = pretreatment, RFS = recurrence-free survival, RT-PCR = reverse transcription-polymerase chain reaction, pos = positive, neg = negative.

**Table 2**  
Summary of meta-analysis results.

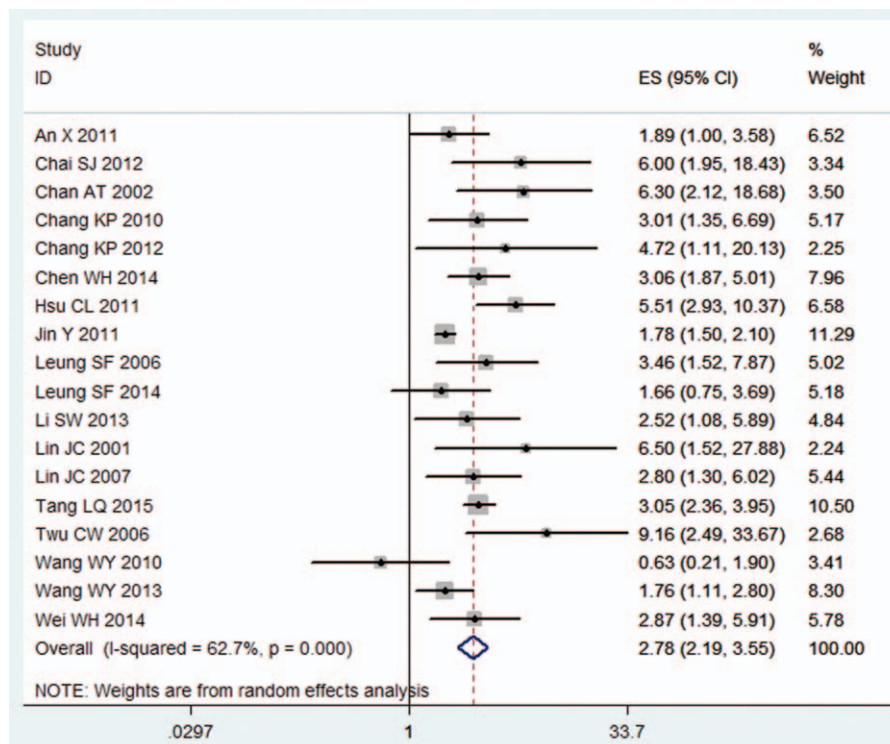
Outcomes	No. of studies	Model	Hazard ratio (95% CI)	P	Heterogeneity (I <sup>2</sup> , P)
Pre-DNA					
OS	18	Random	2.78 (2.19, 3.55)	<0.001	62.7%, <0.001
DFS	3	Fixed	2.84 (2.39, 3.39)	<0.001	45.8%, 0.158
DMFS	10	Fixed	3.26 (2.67, 3.98)	<0.001	19.6%, 0.262
PFS	6	Fixed	2.42 (1.81, 3.24)	<0.001	0.0%, 0.643
RFS	7	Fixed	2.07 (1.51, 2.85)	<0.001	0.0%, 0.907
Post-DNA					
OS	9	Random	5.43 (2.72, 10.82)	<0.001	90.5%, <0.001
DMFS	5	Random	8.19 (1.96, 34.31)	0.004	93.1%, <0.001
PFS	4	Random	3.55 (1.46, 8.61)	0.005	93.2%, <0.001
RFS	6	Random	7.63 (4.06, 14.35)	<0.001	57.5%, 0.038
Cut-off value (pre-DNA/OS)					
<1500	4	Fixed	1.86 (1.59, 2.19)	<0.001	50.9%, 0.106
1500	5	Random	2.29 (1.26, 4.17)	0.007	64.5%, 0.024
4000	5	Fixed	3.03 (2.46, 3.73)	<0.001	0.6%, 0.403
>4000	4	Random	3.40 (1.88, 6.13)	<0.001	56.7%, 0.074
Ratio of tumor grade (I–II/III–IV) (pre-DNA/OS)					
<50%	12	Fixed	2.95 (2.46, 3.54)	<0.001	29.9%, 0.153
>50%	2	Fixed	4.30 (2.24, 8.29)	<0.001	0.0%, 0.389
Ratio of histological differentiation (differentiated/undifferentiated) (pre-DNA/OS)					
<50%	4	Fixed	4.62 (3.08, 6.93)	<0.001	0.0%, 0.476
>50%	5	Random	2.81 (1.32, 5.99)	0.007	62.6%, 0.030

DFS=disease-free survival, DMFS=distant metastasis-free survival, OS=overall survival, PFS=progression-free survival, post-DNA=psottreatment DNA level, pre-DNA=pretreatment DNA level, RFS=recurrence-free survival.

**3.2. Overall analyses**

The meta-analysis showed that the EBV DNA levels in the plasma collected before (pre-DNA) and after treatment (post-DNA) both exhibited significant prognostic value. The HR (95% CI) of pre-DNA was 2.78 (2.19, 3.55) for OS, 2.84 (2.39, 3.39) for DFS,

3.26 (2.67, 3.98) for DMFS, 2.42 (1.81, 3.24) for PFS, and 2.07 (1.51, 2.85) for RFS. Also, the HR (95% CI) of post-DNA was 5.43 (2.72, 10.82) for OS, 8.19 (1.96, 34.31) for DMFS, 3.55 (1.46, 8.61) for PFS, and 7.63 (4.06, 14.35) for RFS (Table 2, Figs. 2 and 3).



**Figure 2.** Hazard ratio (HR) of pretreatment EBV DNA level for overall survival. EBV=Epstein-Barr virus.

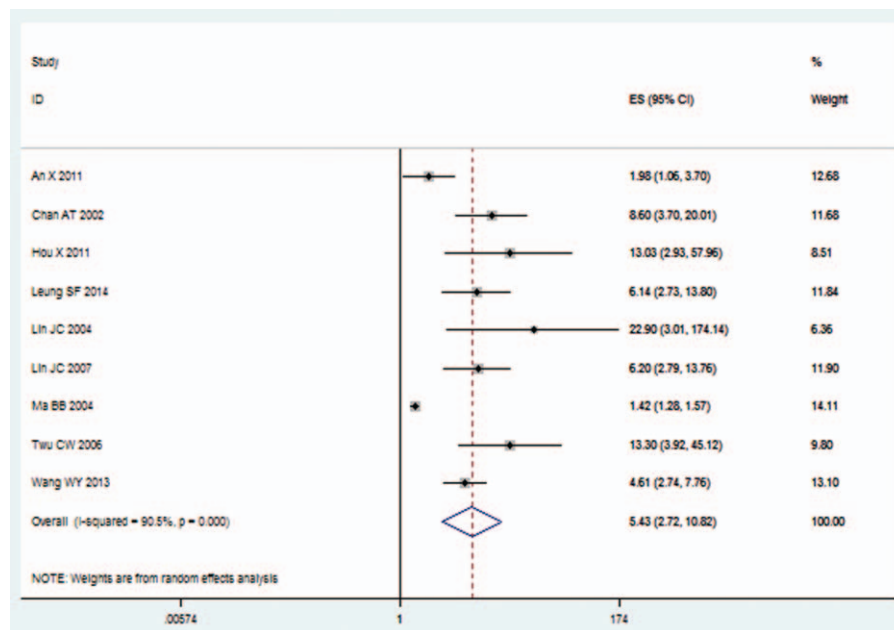


Figure 3. Hazard ratio (HR) of posttreatment EBV DNA level for overall survival. EBV=Epstein-Barr virus.

### 3.3. Subgroup analysis

We used the studies that examined the prognostic value of pretreatment plasma EBV DNA level for OS to do subgroup analysis. We summarized the results of the subgroup analyses in Table 2. The prognostic significance of the cut-off values (ie, <1500, 1500, 4000, and >4000 copies/mL) was first evaluated. EBV DNA level with a cut-off value <1500 copies/mL (including 0) was correlated with poor OS (HR 1.86, 95% CI 1.59–2.19, n=4). EBV DNA level with the cut-off value of 1500 copies/mL was correlated with poorer OS (HR 2.29, 95% CI 1.26, 4.17, n=5). The EBV DNA level with the cut-off value at 4000 copies/mL was associated with even poorer OS (HR 3.03, 95% CI 2.46, 3.73, n=5). Also, the EBV DNA level with cut-off value >4000 copies/mL showed the worst OS (HR 3.40, 95% CI 1.88, 6.13), n=4).

In the subgroup analysis of the tumor grade, tumor grade I to II/III to IV <50% or >50% was used to represent that the ratio of patient number in grade I to II to the number in grade III to IV is

less than or larger than 50%. Likewise, as to the histological differentiation, differentiated/undifferentiated <50% or >50% was used to represent that the ratio of differentiated patient number to the undifferentiated number is less than or larger than 50%. For tumor grade and histological differentiation, the above numbers in grade I to II or III to IV, differentiated or undifferentiated refer to groups of individuals rather than values for a person. The HR (95% CI) in the tumor grade I to II/III to IV <50% group for OS was 2.95 (2.46, 3.54) (n=12). The HR (95% CI) in the tumor grade I to II/III to IV >50% group for OS was 4.30 (2.24, 8.29) (n=2). In the subgroup of histological differentiation, poor prognostic effects were observed in >50% group (OS: HR 2.81, 95% CI 1.32, 5.99, n=5) and poorer in <50% group (OS: HR 4.62, 95% CI 3.08, 6.93, n=4) (Table 2).

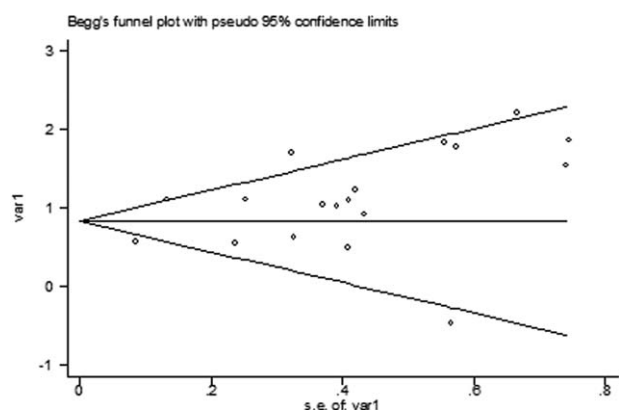
### 3.4. Sensitivity analysis

Eighteen studies which scored 6 or more on the NOS were included in the sensitivity analysis (Table 3). Except for

**Table 3**  
Sensitivity analysis excluding studies with <6 score on the Newcastle–Ottawa Scale.

Outcomes	No. of studies	Model	Hazard ratio (95% CI)	P	Heterogeneity (I <sup>2</sup> , P)
Pre-DNA					
OS	15	Random	2.75 (2.11, 3.57)	<0.001	65.5%, <0.001
DFS	3	Fixed	2.84 (2.39, 3.39)	<0.001	45.8%, 0.158
DMFS	7	Fixed	3.20 (2.60, 3.96)	<0.001	0.0%, 0.728
PFS	4	Fixed	2.45 (1.71, 3.49)	<0.001	10.0%, 0.343
RFS	5	Fixed	2.14 (1.50, 3.05)	<0.001	0.0%, 0.949
Post-DNA					
OS	6	Random	4.28 (1.98, 9.25)	<0.001	90.1%, <0.001
DMFS	2	Random	3.68 (0.50, 27.15)	0.202	86.9%, 0.006
PFS	2	Random	1.70 (1.06, 2.72)	0.028	72.6%, 0.056
RFS	4	Fixed	9.38 (6.30, 13.98)	<0.001	38.1%, 0.183

DFS=disease-free survival, DMFS=distant metastasis free-survival, OS=overall survival, PFS=progression-free survival, post-DNA=psottreatment DNA level, pre-DNA=pretreatment DNA level, RFS=recurrence-free survival.



**Figure 4.** The Begg publication bias plot of 18 studies that reported pre-DNA for OS. OS=overall survival.

the post-DNA meta-analysis for DMFS, there was no change in the significance of the other outcomes. The degree of between-study heterogeneity decreased slightly for DMFS data of pre-DNA, and DMFS, PFS, and RFS data of post-DNA.

### 3.5. Assessment of publication bias

Begg test was performed to evaluate publication bias. No publication bias was observed in the overall analyses or subgroup analyses. The Begg publication bias plot of 18 studies that reported pre-DNA for OS was provided in Fig. 4.

## 4. Discussion

Our study was based on a large pool of clinical studies and was conducted to evaluate the effectiveness and to identify the value of EBV DNA level as a tool to predict the survival outcomes of NPC. Twenty-three studies, including 10,732 patients, were combined to evaluate the prognostic value of EBV DNA level. According to Hayes criterion,<sup>[34]</sup> a prognostic factor with relative risk  $>2$  was a useful value. In this study, the prognostic value of EBV DNA level in NPC was revealed (Table 2, Figs. 2 and 3). The prognostic ratio was obtained from multivariate analysis, and if not, we used univariate ratio instead. Our result provided evidence that both pretreatment and posttreatment plasma EBV DNA levels were significantly associated with poor survival of patients with NPC. This result was consistent with previous studies.<sup>[5,35]</sup> In summary, EBV DNA expression level can be used as a promising prognostic factor to predict the outcomes of patients with NPC.

Previous studies revealed that EBV DNA level had a good prognostic value of OS and PFS in patients with nonmetastatic NPC treated with radiotherapy and in those with locally advanced NPC treated with concurrent chemoradiotherapy.<sup>[4]</sup> From clinical perspective,<sup>[36]</sup> the application of EBV DNA level detection in serum exhibits many advantages such as time-saving and cost-effective. Previous studies<sup>[37,38]</sup> also showed that this technique yielded results with high accuracy and effectiveness.

In the past decades, EBV DNA level was considered as a prognostic factor of NPC. Considering that needle biopsy is rarely applied in NPC for its invasiveness, RT-PCR is a quite safe and convenient alternative because the sampling site is the serum. RT-PCR is applied as the main approach for the detection of EBV DNA level. Previous study<sup>[39]</sup> has found that RT-PCR is limited to polymorphisms that may occur at the 3' end of miRNAs

because these miRNAs display a single-stranded structure, and the significance of these variations remains unclear. Compared with miRNA, EBV DNA level exhibits a more stable expression and can be easily evaluated by RT-PCR with high analytical sensitivity and specificity. Although the use of EBV DNA level for prediction is very promising for patients with NPC and is applied in most relevant studies, a universal cut-off value of EBV DNA level is hard to define for lack of sufficient EBV DNA expression level data. For this reason, our study is trying to evaluate different cut-off values and to determine a universal cut-off value.

We compared the results from studies using different cut-off values. Some researchers<sup>[4,22]</sup> thought that 0 copy/mL (detectable vs undetectable) was the best cut-off value in predicting prognosis. Our results suggested that HR of OS was the higher with the increase of the cut-off value, and that the highest HR occurred in the cut-off value  $>4000$  copies/mL group (Table 2). In most studies, the measurement of plasma EBV DNA level was conducted via RT-PCR. The result of RT-PCR could be affected by many factors. Compared with  $>1500$  copies/mL, it was obvious that 0 copy/mL as a cut-off value might induce many false-positive errors. Our results suggested that it might be better to set the cut-off value between 1500 and 4000 copies/mL. We recommend 1500 copies/mL as the cut-off value, because the cut-off value of 4000 copies/mL would decrease the sensitivity of the analysis. However, the results still need to be further confirmed by multicenter prospective trials.

In the subgroup analysis, tumor types might affect the HR. The HR in the grade I to II/III to IV  $<50\%$  group (4.30 [2.24, 8.29]) was higher than that in the I to II/III to IV  $>50\%$  group (2.98 [1.43, 6.21]). Whereas in the subgroup analysis of histological type, the HR in the differentiated/undifferentiated  $<50\%$  group (4.62 [3.08, 6.93]) was higher than that in the  $>50\%$  group (2.81 [1.32, 5.99]). This result could be attributed to the poor outcomes of undifferentiated malignant tumor compared with differentiated tumor. These factors possibly affected the prognostic value of EBV DNA level to some extent, and further studies should be conducted to clarify the inner association.

As shown in Table 2, there was significant between-study heterogeneity in our meta-analysis. Heterogeneity was found in the overall analyses of post-DNA and OS analysis of pre-DNA. To minimize heterogeneity, we performed subgroup analysis and sensitivity analysis. In the subgroup of tumor grade, no heterogeneity was observed in both groups, suggesting that tumor grade might be the source of heterogeneity. In the cut-off value subgroup, significant heterogeneity was found when analysis was limited in 5 studies with an EBV DNA level cut-off value at 1500 copies/mL and in 4 studies with the cut-off value at  $>4000$  copies/mL. In the histological differentiation group, the 5 studies in the  $>50\%$  group showed heterogeneity. And as mentioned above, the degree of some between-study heterogeneity decreased slightly after we introduce sensitivity analysis. So, the heterogeneity in our study may be attributed to the tumor grade, the cut-off value, the histological differentiation, and the variations in study quality.

There are several limitations in our study. Firstly, this study was based on a small number of studies (23). Secondly, significant heterogeneity was observed in our study, which might come from the differences between studies, such as cut-off values, technical characteristics, histological differences, patient sources, tumor stages, and statistical analysis tools. But we addressed the issue of heterogeneity by using a random-effects model for more conservative estimates. Thirdly, we have included available data, but possibilities were that some studies with negative results were left out, thus influencing the prognostic value of EBV DNA level.

Lastly, publication bias was a major concern for the results of all meta-analyses. In our meta-analysis, no publication bias was found, but it should be noted that any meta-analysis could not completely exclude biases.

In conclusion, our present results showed that EBV DNA level exhibits good accuracy as a prognostic factor of NPC. The detection of EBV DNA level at different cut-off values may be used as an effective method to guide the treatment of patients with NPC. We suggest >1500 copies/mL to be a considerable cut-off value in EBV DNA level. Clinical trials with a larger sample size may aid in defining the cut-off value and further evaluating the clinical application of EBV DNA level.

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