The Epstein-Barr Virus (EBV) DNA Test as a Predictor of The Course of Nasopharyngeal Cancer

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ABSTRACT

Background: Nasopharyngeal cancer (NPC) incidence has been largely found in Southern China and Southeast Asia and was associated with Epstein-Barr Virus (EBV). Some advanced-stage NPC may still rise to local recurrence or distant metastasis and higher plasma EBV DNA was still found in locally advanced nasopharyngeal cancer (LA-NPC) at 1 month or even 3 years after completing radiotherapy (RT). Even though EBV DNA has not been widely used in clinical practice, it could be an important value for determining treatment outcomes and risk of disease relapse.

Methods: This review article gathered studies from the PubMed database from 2021 to 2022. Using various searching terms 434 articles were found and were narrowed down to 7 according to the inclusion criteria. The individual review was made for each article and endpoints such as overall survival (OS), progression-free survival (PFS), distant metastasis-free survival (DMFS), disease-free survival (DFS), and local recurrence-free survival (LRFS) were drawn.

Results: Overall subjects for these studies ranged up to 2,354 LA-NPC patients (median of 1,073 subjects). All studies observed the pre-treatment and post-treatment EBV DNA and only two studies observed post-neoadjuvant chemotherapy (post-NAc). EBV DNA currently is the most reliable biomarker available for clinical purposes and its versatility can be useful, especially to value prognosis and to determine the course of treatment.

Conclusions: Apart from survival outcomes, pre-treatment EBV DNA is considered good for predicting the overall prognosis. Meanwhile, post-induction chemotherapy (post-IC) or post-NAc EBV DNA is suitable for adjuvant therapy indicators, especially in LA-NPC. Even though the cut-off value for the tests was still varied across laboratories (ranging from 1,500 to 4,000), post-NAc and post-treatment might have some benefit to help predict any locoregional recurrence and distant metastasis, considering pre-treatment will not change the therapeutic course completely.

INTRODUCTION

Nasopharyngeal cancer (NPC) incidence has been largely found in Southern China and Southeast Asia, noticeable as an endemic area compared to most Caucasian countries. From early 2D radiotherapy modality to advanced modern therapy, the use of intensity-modulated radiation therapy (IMRT) become the choice of treatment with better results and less toxicity. A large study from Hong Kong (HKN PCSG 1301) showed excellent locoregional control rates of up to 90% in stage T1-3 NPC patients [1]. However, these findings were different in locally advanced NPC (LA-NPC). With varied local control rates and even great progression-free survival (PFS) up to 66% with chemoradiation (CRT), 30% of patients with advanced-stage NPC eventually will emerge to local recurrence or distant metastasis with poorer outcomes [2–5].

Therefore, many studies are conducted in search of the use of specific biomarkers to predict treatment outcomes and disease relapse. Epstein-Barr virus (EBV) was associated with NPC, with a special feature of nucleic acid shedding in harbored EBV in cancer cells, making traces of EBV DNA and RNA properties can be found. Although EBV viral load did not predict NPC extensiveness, multiple findings showed, that higher plasma EBV DNA could still be found in LA-NPC at 1 month or even 3 years after completing RT, showing the risk of recurrence [6–8]. The detectable EBV DNA
post-RT also influences the overall survival (OS) rate up to 97% compared to undetectable ones [8]. Therefore, persistently elevated post-treatment plasma EBV could be used as a prognostic biomarker to predict recurrences and were also correlated to locoregional failure, distant metastasis, and even death [9]. Another useful finding is the use of post-chemotherapy clearance EBV levels, Wang et al. [10] showed poorer outcomes from patients with a slower EBV clearance rate. The prognostic information then could lead clinicians to evaluate treatment response and consider intensification. Even though Guidelines from the National Comprehensive Cancer Network (NCCN) and the European Society of Medical Oncology (ESMO) have their recommendation regarding the clinical use of EBV DNA, EBV utilization has not been extensively used in clinical application [11,12]. Thus, the purpose of this study was mainly to gain more information about the role of the EBV-DNA test in NPC patients. Objectively considering its potential for clinical use, for the de-escalation or escalation option throughout the course of treatment and encouraging DNA testing in tertiary hospitals.

METHODS

The data searching for this study uses the PubMed database, finding the latest articles within the last 2 years (from 2021 until 2022). Strategic keywords are used; “nasopharyngeal cancer”, “Epstein Barr Virus”, “analysis survival”, and “prognosis” with search terms: ((prognosis [MeSH Terms]) OR (analysis, survival [MeSH Terms])) AND (epstein barr virus [MeSH Terms]) AND (nasopharyngeal cancer [MeSH Terms]). Inclusion criteria are; 1) Nasopharyngeal cancer patients (regardless of stage), 2) tested for Epstein Barr Virus examination (EBV DNA with or without any EBV Serology), 3) had overall survival (OS), progression-free survival (PFS), distant metastasis-free survival (DMFS), locoregional free survival (LRFS) as the endpoint outcome, 4) study design from a clinical trial, retrospective or prospective trial, systematic review, meta-analysis within 2 years of publication in English language, available as full text and categorized in human study. Exclusion criteria consist of metastatic NPC with a patient under 18 years old.

After data searching in the PubMed database leads to 434 articles based on the strategic keywords used previously. English-written articles are reselected within the last 2 years. The inclusion and exclusion criteria managed to narrow down the search to 10 articles. Seven articles from 2020 to 2022 were reviewed individually to find the clinical points and association between the EBV DNA test for NPC prognostication (Figure 1). Subjects for these studies ranged from 254 to 2,354 LA-NPC patients with a median of 1,073. All studies observed pre-treatment and post-treatment EBV DNA with only 2 studies observed for post-neoadjuvant chemotherapy. The endpoints for these studies are OS, PFS, DMFS, DFS, and LRFS.
EBV DNA Test for Nasopharyngeal Cancer
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Table 1. Summary of selected articles on EBV testing in nasopharyngeal cancer for prognostication purposes

<table>
<thead>
<tr>
<th>No</th>
<th>Study</th>
<th>Subject</th>
<th>Method</th>
<th>EBV DNA cut-off*</th>
<th>Pre-Treatment (PRE)</th>
<th>Post Neoadjuvant Chemotherapy (Post NAc)/Post Induction Chemotherapy (Post IC)</th>
<th>Post-Treatment (POST)</th>
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<tbody>
<tr>
<td></td>
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<td></td>
<td>Patients with EBV DNA &gt;6,170 had less favorable 3 year of all outcomes</td>
<td>Patients with detectable Post accr DNA had worse 3 year of all outcomes</td>
<td>Detectable 1 week POST had worse 3-year of all outcomes</td>
</tr>
<tr>
<td>1</td>
<td>Zong J et al. (2022/Retrospective) [20]</td>
<td>Plasma EBV DNA samples in stage III-IVA NPC</td>
<td>&lt;6,710 (lower) vs. ≥6,710 (higher)</td>
<td>81.3 vs. 96%; P&lt;0.001</td>
<td>84.9 vs. 87.0%; P&lt;0.001</td>
<td>55.8 vs. 85.1%; P&lt;0.001</td>
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<td>72.8 vs. 87%; P&lt;0.001</td>
<td>72.8 vs. 87.0%; P&lt;0.001</td>
<td>81.3 vs. 87.0%; P&lt;0.001</td>
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<td></td>
<td>84.6 vs. 94.5%; P&lt;0.001</td>
<td>84.6 vs. 94.5%; P&lt;0.001</td>
<td>81.4 vs. 94.5%; P&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>Lv J et al. (2022/Population based retrospective cohort study) [21]</td>
<td>LA-NPC treated with NAc followed by CRT</td>
<td>cfEBV&gt; 2,000 copies/mL positively correlated with node stage P&lt;0.05</td>
<td>71.4% 14% 50% 18% 83.6% (95% CI = 78.0–88.1%)</td>
<td>71.4% 14% 50% 18%</td>
<td>83.6% (95% CI = 78.0–88.1%)</td>
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<td></td>
<td>cBR Post IC strongly predicts long-term prognosis. Non-cBR Post NAc shows more failure</td>
<td>cEBV DNA reflects as a decent indicator (predict the whole POST)</td>
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</tbody>
</table>

Table 1. Summary of selected articles on EBV testing in nasopharyngeal cancer for prognostication purposes (cont.)

<table>
<thead>
<tr>
<th>No</th>
<th>Study</th>
<th>Subject</th>
<th>Method</th>
<th>EBV DNA cut-off*</th>
<th>Pre-Treatment (PRE)</th>
<th>Post-Treatment (POST)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Lin C et al. (2022/Retrospective) [22]</td>
<td>Plasma EBV-encoded micro RNA BART8-3p in M0 NPC</td>
<td>1,668</td>
<td>HR 3.82, 95% CI 1.77–8.24; P=0.001</td>
<td>HR 2.82, 95% CI 1.36–5.85; P=0.005</td>
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<td>HR 1.94, 95% CI 1.12–3.35; P=0.018</td>
<td>HR 2.74, 95% CI 1.27–5.91; P=0.010</td>
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<td></td>
<td>HR 2.69, 95% CI 1.27–5.91; P=0.010</td>
<td>HR 3.27, 95% CI 1.57–6.81; P=0.002</td>
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<td></td>
<td></td>
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<td></td>
<td>High cut-off POST was an independent unfavorable predictor for all outcomes</td>
<td>Publication bias was discovered in PRE OS and PFS (p=0.03983, p=0.006583 respectively)</td>
</tr>
<tr>
<td>4</td>
<td>El Alami et al. (2022/Meta-analysis) [18]</td>
<td>Meta-analysis of 26 studies to evaluate pre and post EBV DNA test to predict the prognostic value of NPC</td>
<td>PRE EBV DNA: High level strongly correlates to high risk of death and DM (early phase) of NPC (HR 95% CI) Cut-off: 1,500 relate to all outcomes except DFS, and LRFS; 1,500 relates to all outcomes except OS and DFS</td>
<td>2.09</td>
<td>2.53</td>
<td>1.78</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td>POST EBV DNA: High level strongly correlates to 8-fold risk of metastasis (HR 95% 8.88) Publication bias was discovered in PRE OS and PFS (p=0.03983, p=0.006583 respectively)</td>
<td>4.52</td>
</tr>
<tr>
<td>5</td>
<td>Li WZ et al. (2021/Multi-center prognostic study) [17]</td>
<td>Analyzing prognostic value comparing (+) vs (-) EBV DNA using refined staging* on non metastatic NPC</td>
<td>EBV DNA range stage: I (0–0); II (0–350); III (0–4,520); IVa (0–17,875)</td>
<td>86.3% [95% CI, 84.2–88.3]</td>
<td>93.3% [95% CI, 91.2–95.4]</td>
<td>75.4% [95% CI, 84.2–88.3]</td>
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<tr>
<td></td>
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<td>PFS Progression at 5 years</td>
<td>87.7% [95% CI, 91.2–95.4]</td>
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<td></td>
<td>10%</td>
<td>20%</td>
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<td></td>
<td>78.9%</td>
<td>77.1%</td>
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<td>64.4%</td>
<td>64.5%</td>
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</table>

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Table 1. Summary of selected articles on EBV testing in nasopharyngeal cancer for prognostication purposes (cont.)

<table>
<thead>
<tr>
<th>No</th>
<th>Study</th>
<th>Subject</th>
<th>Method</th>
<th>EBV DNA cut-off</th>
<th>Post-Treatment (POST)</th>
<th>5-year OS by Post RT EBV DNA5-year</th>
<th>5-year OS by RPA</th>
<th>5-year OS by Stage #</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Hui EP et al. (2020/prospective)</td>
<td>745, 340, 837 (n=1,922) 3 group cohorts</td>
<td>Evaluate OS using RPA of patients post-RT/ CRT EBV DNA from 3 perspective cohorts. OS are divided into 3 groups; low, intermediate, and high-risk patients.</td>
<td>0, 1–49, 50–499, 500 copies/mL</td>
<td>I (1–49 copies/mL)</td>
<td>87.3% Low</td>
<td>89.4%</td>
<td>Stage II 88.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>II (50–49 copies/mL)</td>
<td>83.2% Inter-</td>
<td>78.5%</td>
<td>Stage III 81%</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td>III (50–499 copies/mL)</td>
<td>50.5% Stage</td>
<td>69.4%</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Liu LT et al. (2021/Prospective)</td>
<td>307</td>
<td>Evaluate the prognostic patients of LA-NPC patient stage III-IVb (AJCC 7th) using imaging assessment from MRI’s ADC (ΔADC%) And evaluate outcomes; OS, PFS, DMFS, LRFS</td>
<td>=0 vs. &gt;0 (Post IC) 70.7% vs 59.3%, p =0.037 (Post RT) 92.7% vs 97.5%, p =0.093;</td>
<td>Response</td>
<td>ΔΔADC% high EBV DNA</td>
<td>OS</td>
<td>PFS</td>
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<td></td>
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<td></td>
<td>Early (p&lt;0.001) ΔΔADC% high Undetectable</td>
<td>95.6%</td>
<td>90.6%</td>
<td>96%</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Inter-mediate (p&lt;0.001) ΔΔADC% high Detectable or ΔΔADC% low undetectable</td>
<td>86.7%</td>
<td>71.5%</td>
<td>84.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None (p&lt;0.001) ΔΔADC% low detectable</td>
<td>70.1%</td>
<td>42.6%</td>
<td>69.4%</td>
</tr>
</tbody>
</table>

RESULTS

EBV DNA superiority

There are many types of EBV-related detection towards the prognosis of NPC from EBV, many studies mostly use EBV DNA or in combination with other types of EBV serology tests. As discovered by Lo et al. [7] in 1999, EBV DNA components came from short fragments of EBV virome, and its presence could be found in the blood circulation when apoptosis developed, also can be detected by assays such as polymerase chain reaction (PCR) [7,13]. Therefore, the versatile use of this biomarker not only correlates with clinical staging but also gives additional predictive value for tumor recurrence [13,14]. In a previous study by Liu et al. [15], the sensitivity and specificity of EBV DNA were more than 90% (91.4%, and 93.2% for the plasma group post-IC and RT respectively). Two studies prove the high sensitivity and specificity to support the EBV DNA use, increasing positive predictive value (PPV) of 11% to 19.6% and high value of area under the curve (AUC) of >90% (AUC = 0.96), shows the higher value of EBV DNA than other serology test, proven high accuracy in detecting NPC [9,16].

EBV DNA pre-treatment vs post-treatment

The theory behind detecting EBV DNA comes from the latent EBV form in plasmids, which thereafter infect cells and then replicate aggressively, producing cancer cells. NPC cells then release EBV DNA during apoptosis, which occurs in such unpredictable timing. Therefore, it was hypothesized, that the different time frames of detecting EBV DNA either before, during, or after treatment could give a piece of high-value prognostic information. According to the course of treatment of NPC, pre-treatment, post-neoadjuvant/induction chemotherapy, and post-treatment (CRT or RT only) were most yearned after. The addition of testing the pre-treatment EBV DNA into staging could depict a better prediction [17]. Li et al. [17], proved in comparison with negative patients, positive pre-treatment EBV DNA showed a worse 5-year outcome (PFS 75.4% vs 87.7%; OS 86.3% vs 93.3%; P<0.001), which might progress to tumor progression (10% of stage III). This finding also matches with a study from El Alami et al. [18], which showed high pre-EBV DNA (>1,500) correlates with a high-rise probability of death and distant metastasis. Moreover, Prayongrat et al. [19] concluded the correlation between pre-treatment EBV DNA with NPC staging, showed LA-NPC with detectable pre-treatment
EBV DNA leads to poor 3-year PFS (66.9%) compared to undetectable subjects (83.6%). The prediction given by pre-treatment EBV DNA testing could give a pointer for clinicians to manage high-risk patients with treatment intensification and close monitoring during the course of treatment.

Compared to pre-treatment findings, high levels of post-treatment EBV DNA have their role in predicting clinical remission. Besides evaluating after CRT or RT-only response but also to reanalyze false positive patients with previous negative EBV DNA results. This might envision ongoing tumor apoptosis, not a growing mass [25,26]. Thus, this finding prevents the use of unnecessary adjuvant systemic therapies. However, assessing the need to add adjuvant therapy post-CRT cannot solely be decided by post-treatment EBV DNA levels alone. A study by Chan et al. [27] showed, that even though post-treatment EBV DNA is superior in detecting the risk of subclinical disease, the combination of post-RT plasma EBV DNA with TNM staging was still preferable and more significant for prognostication, eyeing the HR intersection between undetectable with low level (1-49 copies/mL) of post-RT EBV DNA. This finding is also in line with a study from Hui et al. [23], which used RPA risk groups, and combined molecular information from post-RT EBV DNA with clinical TNM staging (UICC) to predict OS. Thus, the low to the intermediate stage still achieved the best OS up to 75%, while even lower to higher LA-NPC stage with more up to 50 to 500 copies/mL of post-RT EBV DNA showed the lowest OS [23].

A high level of post-treatment EBV DNA strongly correlates up to 5 and 8-fold risk respectively for mortality and metastasis (HR 95%), which showed a non-invasive way to predict locoregional and distant recurrences after CRT [18]. From a local experiment, there was a 27-fold reduction in post-treatment (two months after CRT) EBV DNA taken by whole blood, with a decrease in patient’s circulating EBV DNA (previously >2,000 copies/mL) from 51% to 8.8%, which might reflect reduced tumor activity [25]. Even though EBV DNA has great results in detecting NPC, the findings of high EBV DNA are much more prominent in positive distant metastatic patients, rather than locoregional failure (65-100%, 20-100% respectively). The theory lies between this finding was the difficulties of recurrent NPC cells from fibrotic tissue (from previously irradiated sites) to rise into plasma [9]. Another technique of post-NAC ctDNA (on-treatment liquid biopsy: analyzing the circulating EBV DNA through withdrawal of blood plasma) had better specificity (86% vs 41%) and lower sensitivity (42.3% vs 81.3%) compared to EBV DNA for predicting distant metastasis [9,28]. For that reason, high EBV DNA post-therapy could give us more alarm to be cautious about distant metastatic risk, while locoregional recurrence should be proven with imaging and pathological exams.

**Post-neoadjuvant or induction chemotherapy EBV DNA**

Not satisfied with pre-treatment and post-treatment EBV DNA findings, researchers also discovered more about post-induction chemotherapy EBV DNA levels. Pre-treatment EBV DNA was known to have information on the prognosis of the disease, but this evaluation, unfortunately, cannot reflect the therapy response. In contrast with post-treatment, which could evaluate the whole treatment response, it was questioned what the risk of excessive treatment. Especially for low-risk or unresponsive patients alongside treatment. Therefore, the idea of finding more evidence about post-induction chemotherapy EBV DNA arises. An example of the risk stratification comparison could be seen eye to eye [20]. In intermediate NPC (T1-2N1 or T2-3N0M0) treated with RT only have comparable failure-free-survival (FFS) to CCRT with less toxicity (90.7% vs. 92.1% respectively) [29]. Meanwhile, in LA-NPC, patients treated with induction chemotherapy of 3 cycles of cisplatin, have better 3-year recurrence-free survival compared to the CRT group alone (85.3% vs. 76.5% respectively), even with a higher grade of side effects (9.2%) [28]. With the risk of late effects in the induction chemotherapy group, there’s room for other supporting data, to prevent unnecessary use of IC in LA-NPC, especially in non-high-risk groups using EBV DNA levels.

Zong et al. [20] identified the role of post-two cycles of induction chemotherapy EBV DNA test (Post2CICT-DNA), which had beneficial value in predicting poor prognosis or risk for recurrence, especially in high-risk locally advanced NPC patients (Post2CICT-DNA >0, stage N3). With a positive detection rate of 29.6%, Post2CICT-DNA (≥ 6,710 copies/mL) had worse 3-year outcomes (OS 97.1%; PFS 87%; DMFS 93.1%) and therefore can be used as an indicator for early treatment modification [20]. Another finding comes from Lv et al. [21] mentioned, that even though 2-4 cycles of NAC contribute to the cfEBV DNA drop to zero copies/mL (95% CR patients and 75% PR patients), there’s still 5.3% and 24.7% (CR, PR respectively) had detectable cfEBV DNA (non-complete biological response = non-CBR). Even PR patients had worse DFS with (HRDFS = 3.17). On the opposite, zero cfEBV DNA post-NAC (Complete Biological Response = cBR) strongly predicts a better long-term prognosis (HRDFS = 3.28) [21].

**Various cut-off values for EBV DNA**

Regarding the cut-off value of EBV DNA, there is still a lot of work to do, since variability between laboratories does exist, and even the Food and Drug Administration (FDA) hasn’t had any standardization for this assay [9,30]. Besides diversity in the institution’s approaches in search of the optimal cut-off, there is also stage distribution and different periods as contributing variation. Many studies use the receiving
operator characteristic (ROC) to determine the EBV DNA cut-off, with most studies having a range as low as 500 to 1,500-4,000 copies/mL, which positively correlates with declining OS and increasing risk of relapses [8,31].

Liu et al. [24] findings had a cut-off value of 1668 with sensitivity and specificity of 87.8%, and 89.6% respectively. While detecting a biochemical state in post-induction chemotherapy, patients with undetectable EBV DNA (= 0 copies/mL) had a better prognosis (favorable). Meanwhile, EBV DNA levels of ≤1,500-2,000 copies/mL were studied as a stable cut-off point in the pre-treatment setting, and 1,500-4,000 being the most common value used in most studies [18,21].

CONCLUSIONS

EBV DNA was the most reliable one for clinical purposes. The versatility of the EBV DNA test can be useful, especially to value prognosis and to determine the course of treatment. Certain benefits for predicting a patient’s prognosis can be found through pre-treatment, post-NAc, or post-treatment. Apart from survival outcomes, pre-treatment EBV DNA is good for predicting the overall prognosis. Meanwhile, post-induction chemotherapy EBV DNA is quite suitable for therapy modifications, especially in LA-NPC. Even though the value for the EBV DNA test is still varied across laboratories, 1,500-4,000 were the most common cut-off in most studies. Post-NAc and post-treatment combined with TNM staging might be beneficial to predict any locoregional recurrence and distant metastasis, considering pre-treatment will not change the therapeutic course completely. Therefore, EBV DNA could be used to predict treatment outcomes, nevertheless narrowing to necessary tests should also be done to save up any redundant costs for NPC patients.

DECLARATIONS

Competing interest
The authors declare no competing interest in this study.

Acknowledgment
None

REFERENCES