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Review

Plasma Epstein-Barr virus DNA as a biomarker for nasopharyngeal carcinoma

K.C. Allen Chan[1,2,3]

Abstract

Nasopharyngeal carcinoma (NPC) is common in southern China and Southeast Asia. Epstein-Barr virus (EBV) infection is an important etiology for NPC, and EBV genome can be detected in almost all tumor tissues of NPC in this region. Plasma EBV DNA, when quantitatively analyzed using real-time polymerase chain reaction (PCR), has been developed as a biomarker for NPC. In this review, the different clinical applications of plasma EBV DNA in the management of NPC, including screening, monitoring, and prognostication, are discussed. In addition, the biological issues of circulating EBV DNA, including the molecular nature and clearance kinetics, are also explored.

Key words Plasma Epstein-Barr virus EBV DNA, tumor markers, nasopharyngeal carcinoma, cancer screening

Nasopharyngeal carcinoma (NPC) is a common cancer in southern China and Southeast Asia. The peak incidence of NPC occurs in males between the ages of 40 and 60 years and is as high as 40 cases per 100,000 in southern China[1]. Epstein-Barr virus (EBV) infection is an important etiological factor for NPC. In Asia, almost all cases of NPC are undifferentiated cancer that harbor the EBV genome[2]. Because of the close relationship between EBV infection and NPC, EBV serological tests, including IgA for early antigen (EA) and viral capsid antigen (VCA), have been used for NPC detection since the 1970s. Although EBV serology is used extensively as a surrogate marker for NPC, the sensitivity and specificity of these serological tests have room for improvement. In addition, these serological tests have a limited role in treatment monitoring because antibody titers generally exhibit little change, even after curative therapies. In 1998, Mutirangura *et al*.[3] explored if circulating EBV DNA would be a more accurate biomarker for NPC. Using conventional polymerase chain reaction (PCR), EBV DNA was detected in the serum of 31% (13 of 42) of patients with NPC[3]. In contrast, none of the 82 healthy subjects had a positive result. The high specificity of this test was surprising because over 95% of the healthy individuals would previously have contracted EBV. Their results suggest that EBV associated with latently infected lymphocytes would not release cell-free EBV DNA into the serum and thereby would not cause false-positive results[4]. However, the relatively low sensitivity for NPC detection was inadequate for clinical application of this approach. Lo *et al*.[5] applied real-time PCR analysis for more sensitive detection of circulating, cell-free EBV DNA. EBV DNA was detected in 96% (55 of 57) of patients with NPC and 7% (3 of 43) of healthy control subjects. In addition to the much improved sensitivity over conventional PCR for detecting NPC, real-time PCR was also capable for quantifying EBV DNA in patient plasma. Thus, Lo *et al*.[5] showed that the concentration of plasma EBV DNA was 8-fold higher in patients with advanced NPC (stages III/IV) compared with patients with early disease (stages I/II). Subsequent analyses showed that the sensitivity for detecting NPC was related to the clinical stage of the disease[6,7]. Leung *et al*.[6] demonstrated that the sensitivity for detecting early and advanced NPC was 90% and 98%, respectively; for the same cohort of patients, the sensitivities of VCA-IgA serology were 72% and 85%, respectively. The superior diagnostic accuracy of plasma EBV DNA over VCA-IgA serology was independently confirmed by other research groups[8,9]. Because of the remarkable sensitivity and specificity of plasma EBV DNA for NPC, this marker has rapidly been adopted for research and clinical management of patients with NPC[10-12]. In this review, the clinical applications of plasma EBV DNA as a tumor marker for NPC and the biological characteristics of this marker are discussed.

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Staging and Tumor Load

Because higher concentrations of EBV DNA in the plasma

correspond to more advanced NPC, plasma EBV DNA levels may reflect a patient's tumor load[5]. Ma *et al*.[13] studied the relationship between the pretreatment concentration of plasma EBV DNA and the tumor volume as measured with magnetic resonance imaging (MRI). They demonstrated a positive correlation between the plasma EBV DNA concentration and the tumor volume in 57 patients with advanced NPC[13]. Using a mouse model, Chan *et al*.[14] further demonstrated a linear relationship between the concentration of tumor-derived EBV DNA in the plasma and the weight of implanted NPC tumor tissues. Hence, given that plasma EBV DNA concentrations reflect the tumor load in NPC patients, the prognostic value of pretreatment plasma EBV DNA levels was explored[7,15-17]. Lo *et al*.[17] demonstrated that patients with high plasma EBV DNA concentrations at diagnosis were more likely to develop distant metastasis within 1 year after treatment with curative intent. The prognostic value of plasma EBV DNA was also shown to be independent of tumor stage[17]. In another cohort of 139 patients with advanced NPC who received curative-intent treatment, each 10-fold increase in circulating EBV DNA level was associated with a 1.6-fold increase in the risk of NPC-related death after accounting for clinical stage and other patient factors[17]. For patients with early disease, plasma EBV DNA was even more powerful than the Union for International Cancer Control (UICC) stage in predicting survival probability (**Figure 1**)[7]. Thus, plasma EBV DNA concentrations would be particularly useful for identifying the subgroup of patients with stage II disease who actually have higher risk for disease recurrence, so that more aggressive treatment can be given. Furthermore, given its high prognostic value, pretreatment plasma EBV DNA concentrations can be usefully incorporated into the staging system for NPC[7].

Posttreatment Monitoring

In contrast to EBV serology, which only reflects the body's reaction to the cancer, plasma EBV DNA is directly derived from the tumor cells. Therefore, plasma EBV DNA can provide a noninvasive and convenient way for monitoring disease progression and treatment efficacy. To *et al*.[18] showed that after the surgical resection of NPC, EBV DNA was rapidly cleared from the circulation, demonstrating a half-life of only 2 hours. There are two important implications from this interesting observation. First, the finding implies that NPC tumor tissues release a large amount of EBV DNA into the patient's circulation to achieve and maintain an equilibrium level. For example, to maintain a plasma EBV DNA concentration of 5,000 copies/mL, which is the median level for patients with early NPC, the tumor tissue would need to release 125 million copies of EBV DNA into the circulation everyday. Yet, tumor tissue is not the only possible source of EBV DNA in the circulation. Viral replication could also produce intact virions that enter the circulation. However, Chan *et al*.[19] demonstrated that most of the circulating EBV DNA is short fragments of less than 200 bp, suggesting that the DNA is released by tumor cells upon apoptosis rather than through viral replication. This observation is consistent with the findings of other studies showing that circulating DNA in patients with other cancers was likely derived from apoptotic cancer cells[20,21].

The second implication of the rapid clearance of circulating EBV DNA is that almost all the EBV DNA would be recently derived from the tumor tissues. Therefore, quantitative measurement of plasma EBV DNA could reflect the residual tumor load for patients undergoing radiotherapy and chemotherapy almost in a real-time manner. Interestingly, in patients undergoing radiotherapy, an initial

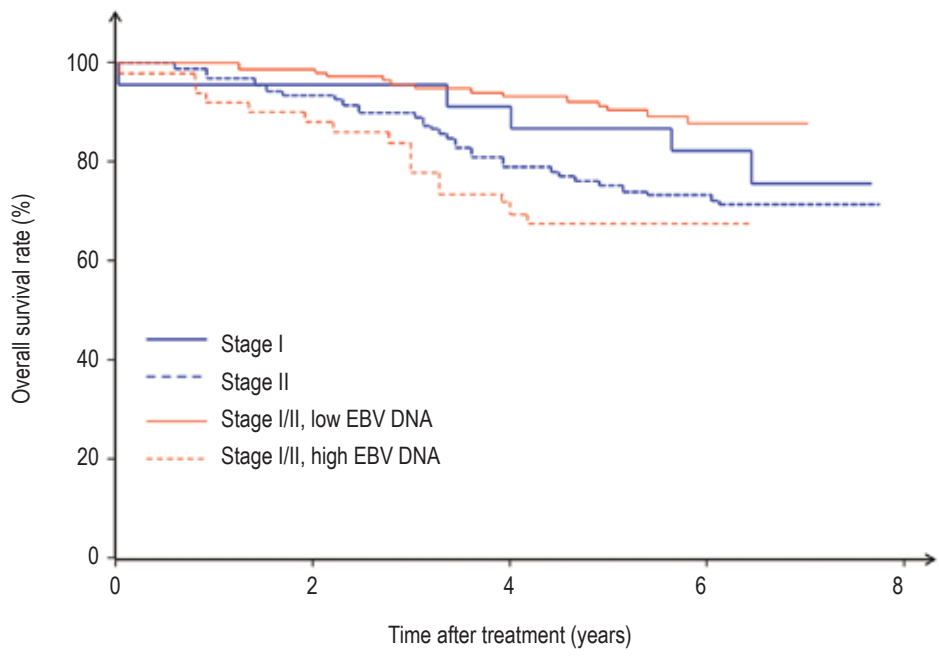

Figure 1. **Overall survival curves for patients with different clinical stages of nasopharyngeal carcinoma (NPC), and with different concentrations of plasma Epstein-Barr virus (EBV) DNA. Plasma EBV DNA concentration is useful to supplement clinical staging for predicting patient survival.** The patients with stages I and II NPC and with low plasma EBV DNA concentration have better survival than those with stage I disease. The patients with stages I and II NPC and with high plasma EBV DNA concentration have poor survival than those with stage II disease. The staging is according to the AJCC/UICC system.

surge in plasma EBV DNA concentration could be observed within the first two weeks of starting treatment, followed by a gradual decline[22]. The initial surge is due to the rapid release of the dying tumor cell's nuclear content into the circulation, and the decline reflects the reduction in tumor load upon treatment[22]. Subsequent studies showed that the rate at which EBV DNA level declined also had prognostic value; patients with more rapid clearance of EBV DNA from the circulation responded better to treatment and had a better survival probability compared with patients with a slower clearance[23,24].

Serial plasma EBV DNA analysis is also a useful tool for monitoring treatment and for early detection of disease recurrence. Lo *et al*.[25] demonstrated that in all NPC patients with serial monitoring of plasma EBV DNA after treatment, an initial drop of plasma EBV DNA concentrations could be observed. For patients in continuous clinical remission, plasma EBV DNA would continue to be undetectable or at very low concentrations. On the contrary, significant increases in circulating EBV DNA levels were observed in patients who subsequently developed locoregional recurrence or distant metastasis[25]. The surge in plasma EBV DNA concentration could occur up to 6 months before clinical deterioration[25]. Thus, serial monitoring of plasma EBV DNA would be useful for monitoring the treatment and disease progression for NPC patients.

In a later study, Leung *et al*.[6] showed that the sensitivity of plasma EBV DNA analysis is lower for detecting tumors regrown from an irradiated site compared with detecting tumor growth at a radiation-naïve or distant metastasis site. In their study, the detection rate was only 42% for the 12 tumors regrown at the irradiated site compared with 92% for the 51 treatment-naïve cancers. Plasma EBV DNA can also be used to select patients for receiving more costly or invasive investigations to detect posttreatment failures. In this regard, Wang *et al*.[26] followed up 245 patients with NPC who received curative-intent treatment with plasma EBV DNA concentration every 3 to 6 months. Patients with either a positive plasma EBV DNA result or clinical suspicion for recurrence underwent positron emission tomography (PET) scan. All 36 patients who had detectable plasma EBV DNA were later confirmed to have disease recurrence[26]. In contrast, the 5 patients who had negative plasma EBV DNA but signs suggestive of recurrence were later shown to be recurrence-free[26]. These results suggest that plasma EBV DNA analysis would be useful to identify the patients most likely to benefit from more extensive treatment and to spare other patients from unnecessary procedures and undue economic burden.

Posttreatment Prognostication

Among different prognosticators, posttreatment plasma EBV DNA level is the most powerful single predictor for disease recurrence and long-term survival[27-31]. After treatment with curative intent, residual cancer cells can be sensitively detected with plasma EBV DNA analysis. Chan *et al*.[27] followed up a cohort of 170 patients with NPC, without metastatic disease at presentation, for a median of 116 weeks. Plasma EBV DNA concentrations were measured at 6 to 8 weeks after the completion of radiotherapy. All patients with plasma EBV DNA concentrations $\geqslant$ 500 copies/mL developed disease progression within 2 years, whereas patients with undetectable plasma EBV DNA after treatment had a progression-free survival rate >95% at 2 years (**Figure 2**)[27]. Patients with detectable plasma EBV DNA but <500 copies/mL had a moderate prognosis[27]. These results were confirmed by different groups in patients with different ethnic

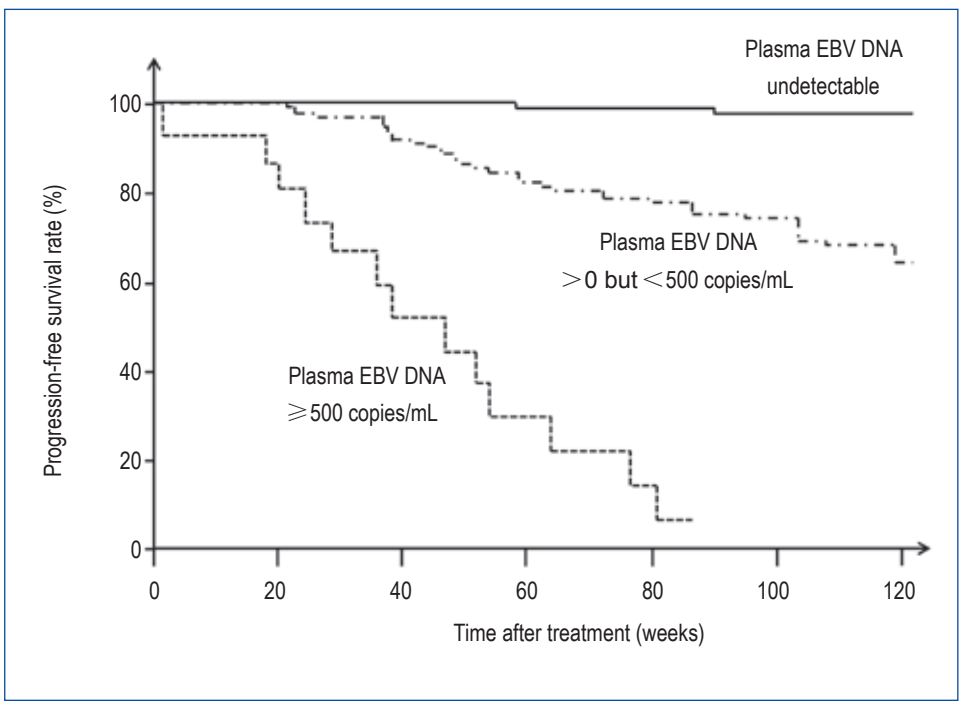

Figure 2. **Progression-free survival curves for patients with NPC and with different concentrations of plasma EBV DNA after curative-intent treatment. Posttreatment plasma EBV DNA is a powerful predictor for patient survival.** All patients with plasma EBV DNA concentrations of $\geqslant$ 500 copies/mL had developed clinical relapse within 2 years. In contrast, patients with undetectable plasma EBV DNA after treatment had very good survival. The staging is according to the AJCC/UICC system.

origins and different clinical stages of diseases[28-31]. Because patients with significant levels of plasma EBV DNA after treatment have a high chance of disease progression, further treatment before clinical progression may be useful for improving treatment outcome. To this end, an international, multicenter, randomized clinical trial has been initiated to investigate if preemptive chemotherapy given to patients with detectable plasma EBV DNA after treatment can improve overall and progression-free survival. A harmonization program has been undertaken to standardize EBV DNA assays to ensure that the quantitative plasma EBV DNA results from different centers are comparable[32].

Screening for Early Disease

The extent of disease involvement at presentation is a key factor affecting the treatment outcome for patients with NPC. In patients with localized disease, the 5-year survival rate is up to 90%[33]. This figure drops below 50% in patients with metastatic disease. In addition, patients with more advanced disease are subject to higher treatment-associated morbidities[33]. Unfortunately, 75%–90% of patients with NPC have already developed local or regional metastasis at presentation[7,33]. Therefore, screening for early disease in asymptomatic individuals may potentially improve the treatment outcomes of patients with NPC. Chan *et al*.[34] investigated if plasma EBV DNA analysis is sensitive enough for the detection of early, asymptomatic NPC. In the study, over 1,300 asymptomatic subjects were screened for NPC using plasma EBV DNA and VCA IgA serology. All subjects showing a positive result in either examination underwent nasal endoscopy. Through this screening, 3 cases of early NPC (1 stage I and 2 stage II) were identified. All 3 patients showed persistently positive plasma EBV DNA results, but only 1 showed a positive VCA IgA serology result[34]. Positive plasma EBV DNA results were also observed in 5%–9% of subjects without NPC. However, in two-thirds of these subjects, the presence of detectable EBV DNA in plasma was only transient and lasted for less than 2 weeks. Therefore, repeating the detection of plasma EBV DNA in the individuals with initial positive results is a useful strategy for discriminating the patients with NPC from the ivdividuals without NPC (false positives). The patients with true NPC would have persistent levels of circulating EBV DNA, whereas the individuals with false-positive in the first test would have negative results on the following examinations. Interestingly, in approximately 0.2% of subjects without NPC, EBV DNA was persistently detectable in their plasma over a period of more than 1 year. The clinical significance of this observation remains unclear. In a previous study, subjects with positive IgA serology were found to have a 4- to 10-fold increased risk of developing NPC[35]. It is possible that subjects with persistently positive plasma EBV DNA results may also have a higher risk for future NPC development.

Detection of EBV DNA in Other Sample Types

In addition to plasma, EBV DNA can also be detected in other bodily fluids or tissues as a biomarker for NPC. For example, EBV DNA was detected in the urine of patients with NPC[36]. The detection rate in urine was only 55% compared with the 99% detection rate for plasma EBV DNA. Moreover, the size of EBV DNA was shorter in the urine than in the plasma, possibly because the smaller DNA fragments are more likely to pass through the glomerular membrane[36]. EBV DNA was also detected in the cerebrospinal fluid in a patient with leptomeningeal metastasis of NPC[37].

Conclusions

Quantitative analysis of EBV DNA is an excellent example of how a molecular tumor marker can contribute to different aspects of patient management, including early cancer detection, staging and risk stratification, posttreatment monitoring, and prognostication. Although the analysis of viral DNA as a tumor marker is simple and relatively inexpensive, this method cannot be generalized to all types of cancers because most cancers do not harbor any viral genome. In this regard, the detection of other cancer-associated aberrations, including single nucleotide mutation[38,39], translocation[40], chromosome copy aberrations[41,42], and methylation changes[43], have been explored. Given the example of plasma EBV DNA in NPC management, the analysis of cancer-derived DNA is expected to become an important tool in the management of cancers in the near future[41-43].

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