RESEARCH ARTICLE

Different Prognostic Values of Plasma Epstein-Barr Virus DNA and Maximal Standardized Uptake Value of ^{18}F-FDG PET/CT for Nasopharyngeal Carcinoma Patients with Recurrence

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Abstract

Purpose

To evaluate and compare the prognostic value of Epstein-Barr virus (EBV) DNA and maximal standard uptake values (SUV_{max}) of ^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F-FDG-PET) in subgroups of nasopharyngeal carcinoma (NPC) patients with locoregional or distant recurrence.

Patients and Methods

A total of 194 patients with recurrent NPC (locoregional recurrence: 107, distant recurrence: 87) were enrolled. Patients took evidence of recurrence performed with ^{18}F-FDG-PET and an EBV DNA test before salvage treatment. Clinical parameters, the status of EBV DNA and the value of SUV_{max} were used for survival analysis using the Kaplan-Meier method and the Cox proportional hazards regression model.

Results

In the subgroup of patients with locoregional recurrence, patients with $SUV_{max} < 8.65$ had significantly better overall survival (OS) (P=0.005) compared with the patients with $SUV_{max} \geq 8.65$. However, both elevated EBV DNA load ($\geq 21,100$ copies/ml) and distant SUV_{max} (≥ 13.55) were significantly associated with worse OS compared with the patients with EBV DNA $< 21,100$ copies/ml or distant $SUV_{max} < 13.55$ for the subgroup with distant recurrence (P=0.015 and P=0.006, respectively). The predictive ability of EBV DNA was superior to that of SUV_{max} (P=0.062). Multivariate analysis showed that SUV_{max} was only an

independent prognostic factor for OS in patients with locoregional recurrence (P=0.042), whereas EBV DNA independently predicted OS for the patients with distant recurrence (P=0.007). For those patients with undetectable EBV DNA, SUV$_{max}$<8.65 was still an independent favorable prognostic factor (P=0.038).

Conclusions

SUV$_{max}$ is a useful biomarker for predicting OS in nasopharyngeal carcinoma patients with locoregional recurrence or with undetectable EBV DNA. Both distant SUV$_{max}$ and EBV DNA appear to be independent predictors of OS in patients with distant recurrence; however, the predictive ability of EBV DNA was superior to that of SUV$_{max}$.

Introduction

Nasopharyngeal carcinoma (NPC) is common in southern China, especially in the Guangdong province, where rates range from 20 to 30 cases per 100,000 population.[1, 2] Radiotherapy is the primary treatment modality for NPC, and concurrent chemo-radiotherapy (CCRT) with or without adjuvant chemotherapy is the standard treatment regimen for locoregional advanced disease.[3, 4] Despite significant improvements in local control due to advances in radiotherapy and combined modality treatments, local recurrence and distant metastasis remain the predominant mode of failure in patients with advanced NPC.[5, 6] Locoregional recurrent NPC re-irradiated by IMRT can still have an encouraging outcome; the 5-year local control rate (LCR) and overall survival rate (OS) for recurrent stage I, II, III and IV were 80.0%, 85.0%, 80.0% and 78.7% and 71.4%, 62.9%, 35.5% and 30.2%, respectively.[7] However, when distant metastases are present, the median survival for metastatic NPC is only 12–20 months.[8] There remains a mysterious heterogeneity of outcomes for patients with locoregional or distant recurrence. Therefore, the identification of effective prognostic factors that more accurately correlate with treatment outcome would be of great importance for determining which NPC patients might benefit from intense treatment.[9]

Currently, the most important and widely recognized prognostic factor identified to date is the AJCC tumor-node metastasis (TNM) staging system.[10–12] However, previous studies demonstrated that the clinical TNM staging for patients with recurrence and distant metastasis does not always provide a satisfactory prediction.[13] Accordingly, an increasing amount of effort is being devoted to identifying better prognostic markers to supplement the staging system. Epstein-Barr virus DNA is a marker that has long been intensively studied and is considered a useful tool to supplement the TNM system for prognostication in NPC.[14] The prognostication value of EBV DNA in recurrent NPC has been demonstrated in several studies.[15–17] However, in some cases of recurrent NPC, patients presented with undetectable levels of EBV DNA.[18] In addition, although EBV DNA levels were detectable, the amount of data on the EBV DNA load of patients with locoregional recurrent NPC is relatively low.[17]

Recent studies have addressed the role of ^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F-FDG-PET) maximal standard uptake value (SUV$_{max}$) as a risk stratification marker to predict therapeutic response or outcome in patients with head and neck cancers,[19, 20] lung cancer,[13] and NPC.[9]·[21]·[22] Notably, studies on the prognostic value of SUV$_{max}$ are mostly focused on the pretreatment SUV$_{max}$ of patients; however, data to evaluate the prognostic role of SUV$_{max}$ for patients with locoregional recurrence or distant recurrence are rare. Furthermore, the comparison of the role of EBV DNA load and SUV$_{max}$ in predicting the

outcome of patients with recurrent NPC has not yet been well defined. Therefore, in the present study, we aimed to determine and compare the prediction value of ^{18}F-FDG PET/CT scan SUV$_{max}$ and EBV DNA load in locoregional or distant recurrent NPC patients.

Materials and Methods

Patients

We retrospectively analyzed initial recurrent NPC patients who were referred to Sun Yat-sen University Cancer Center, Guangzhou, China between July 2007 and December 2013. All patients had received radiotherapy alone, concurrent chemo-radiotherapy (CCRT) or induction chemotherapy followed by CCRT, depending on the stage of disease on initial presentation. When patients clinically indicated with locoregional recurrence or distant recurrence, whole-body ^{18}F-FDG PET examination and EBV DNA measurement were performed on each patient before salvage treatment. In addition to ^{18}F-FDG PET, magnetic resonance imaging (MRI) of the head and neck and conventional workups, including physical examinations, endoscopy, chest radiography, whole-body bone scans, and abdominal ultrasonography, were performed depending on the status of each patient. These examinations were performed within 2 weeks of enrollment in the study. All enrolled patients received salvage treatment using platinum-based adjuvant chemotherapy. Patients were excluded who met the following criteria: (1) insulin-dependent diabetes or serum glucose levels > 200 mg/dl immediately before the ^{18}F-FDG was injected; (2) a history of previous or synchronous malignant tumors; (3) persistent residual NPC; (4) lost during follow-up; (5) incomplete medical records. Finally, a total of 194 patients were enrolled in our study. Eligible patients were divided into two subgroups, one with locoregional recurrence only and one with distant recurrence. The hospital records for each patient were reviewed for demographic and clinical data, including age, gender, family history of tumor, number of metastases, clinical tumor restage, the concentration of plasma EBV DNA and SUV$_{max}$ of ^{18}F-FDG PET/CT. The tumor of each patient was restaged according to the seventh American Joint Committee on Cancer (AJCC) TNM staging manual.[23] This study was approved by the independent Institute Research Ethics Committee at the Sun Yat-sen University Cancer Center (SYSUCC, Guangzhou, P. R. China), and written consents were obtained from all participants.

PET/CT imaging

PET/CT imaging was performed with a combination PET/CT scanner (Discovery ST 16; GE Healthcare, Little Chalfont, United Kingdom) according to published guidelines for tumor imaging with PET/CT.[24] Helical CT was performed from the head to the proximal thigh before PET acquisition according to a standardized protocol. PET/CT scans from the head to the proximal thigh were begun 45 to 60 minutes after the injection of 5.55 MBq/kg of FDG. The PET images were reconstructed with the use of CT data for attenuation correction with an ordered-subset expectation maximization iterative reconstruction algorithm. The standardized FDG uptake value was calculated using the concentration of FDG in the region of interest, as measured by PET, divided by the injected FDG dose and multiplied by body weight as a normalization factor. The SUV$_{max}$ for each patient was used to minimize partial-volume effects. The SUV$_{max}$ values of locoregional and distant metastases were used as SUV$_{max}$ for the locoregional recurrent group and the distant recurrent group, respectively. ^{18}F-FDG PET/CT scans were performed on all patients before salvage treatment.

Plasma EBV DNA measurement

As previously described,[15, 25, 26] patient plasma EBV DNA (EBV DNA) concentrations were routinely measured by q-PCR before salvage treatment.

Statistical analysis

The Statistical Package for the Social Sciences software (SPSS v. 17, SPSS, Inc., Chicago, IL) was used for data analysis. The receiver operating characteristic (ROC) curve determined the optimal cutoff value for EBV-DNA and SUV$_{max}$ to predict outcome with the best trade-off between sensitivity and specificity. The distribution of the subgroups of locoregional recurrence and distant recurrence were skewed; hence, a Wilcoxon rank sum test of two independent samples was used to compare the value of EBV DNA and SUV$_{max}$ between the two subgroups. Overall survival (OS) was calculated from the date of recurrence diagnosis to the date of death from any cause or patient censoring at the date of the last follow-up. Median OS values were estimated using the Kaplan-Meier method, and groups of interest were compared using log-rank tests. The univariate and multivariate survival analyses were based on the Cox proportional hazards regression model. Variables in the model included sex, age, family history of tumor, restage, number of metastases, EBV DNA, and SUV$_{max}$. Spearman rank correlation was used to delineate the relationship between EBV-DNA, SUV$_{max}$ and clinical variables. A $P<0.05$ from a two-tailed test indicated statistical significance.

Results

Baseline characteristics and survival time in the subgroups of patients with locoregional or distant recurrence

A profile of this study is shown in Fig 1. Between July 2007 and December 2013, 208 patients were assessed and entered into this study. Fourteen cases were excluded: five due to diabetes or plasma glucose>200 mg/dl, two because there was not an initial failure after definitive radiotherapy, and the others due to attrition during follow-up or incomplete medical records. Therefore, 194 patients in all were eligible for analysis, among which 160 were men and 34 were women. Patient characteristics are described in Table 1. The primary histologic type of the 194 cases, 178 cases presented non-keratinizing undifferentiated carcinoma (formerly WHO type III), 4 cases presented non-keratinizing differentiated carcinoma (formerly WHO type II), only 1 presented keratinizing squamous cell carcinoma (type I) and 11 patients missed data. The mean age of all patients was 43.9 years (range, 10 to 70 years). Among the 194 patients, 107 had locoregional recurrence only, and 87 had distant failure with or without locoregional recurrence. 103 cases were diagnosed recurrence by pathology and 91 cases were diagnosed by imaging test. Based on whether the patients exhibited distant failure, all patients were divided into two subgroups, the locoregional recurrence group and the distant recurrence group. At the time of the final follow-up, 30 of 107 patients (28%) had died in the locoregional recurrence subgroup, whereas 31 of 87 patients (35.6%) had died in the distant recurrence subgroup. The median concentration of plasma EBV DNA was 4,000 copies/ml for patients with locoregional recurrence only, which was much lower than that of patients with distant metastasis (34,900 copies/ml, $P<0.001$). The median SUV$_{max}$ for patients with distant recurrence was much higher than that of the locoregional recurrent patients (13.3 vs. 11.50, $P<0.001$). We found that EBV DNA could not be detected in 37 of 107 patients (34.6%) with locoregional recurrence only and 12 of 87 patients (13.8%) with distant recurrence. Spearman correlation analysis demonstrated that EBV DNA levels positively correlated with number of metastases and that SUV$_{max}$ correlated with clinical restage and number of metastases (S1 Table).
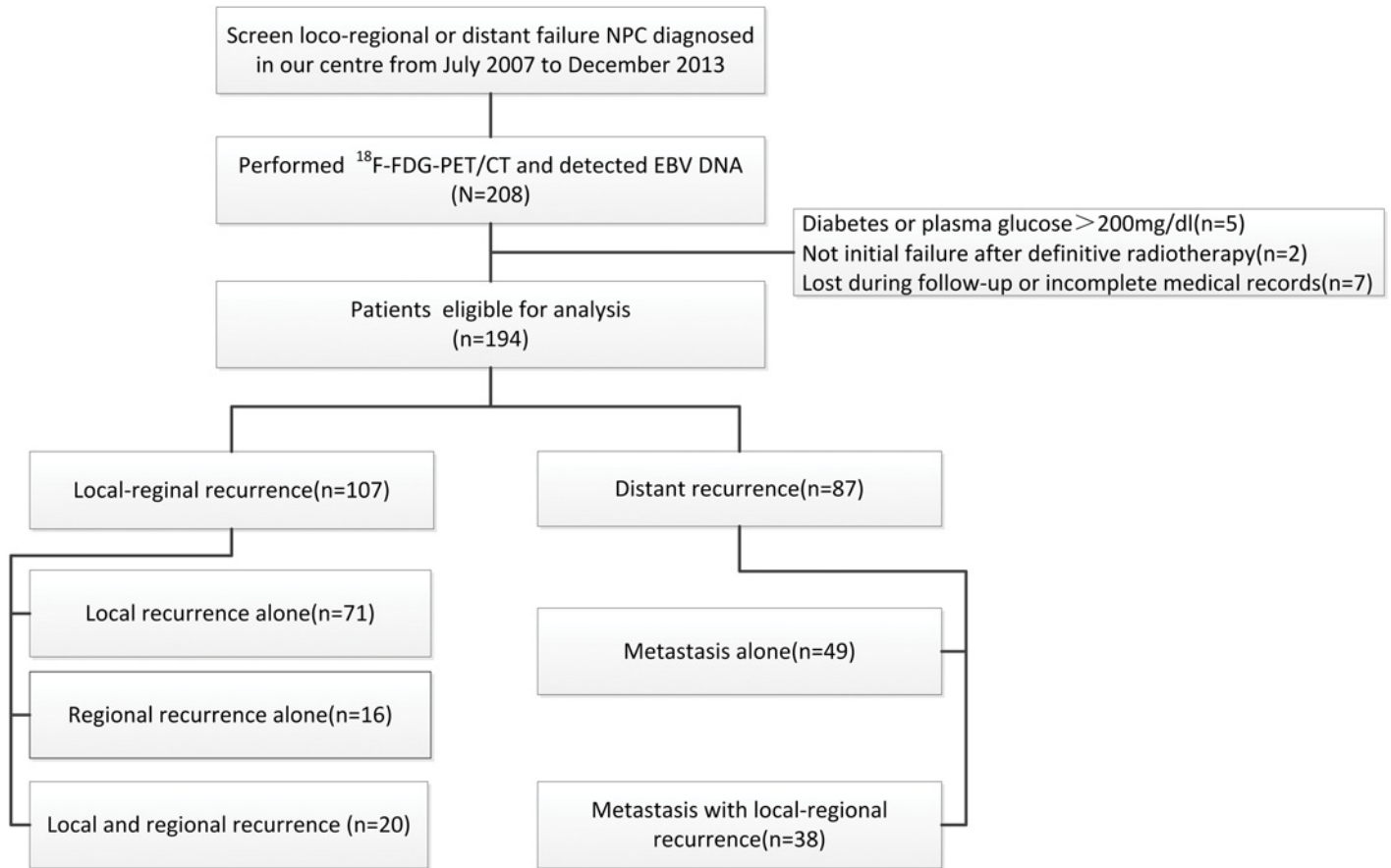
Fig 1. Flowchart of patients enrolled in this study.

The mean follow-up time was 18.09 months (range, 0.62 to 55.88 months). Survival time was analyzed using the Kaplan-Meier method and compared with log-rank tests between the locoregional recurrence and distant recurrence subgroups. The results are shown in Fig 2. The median survival time for NPC patients with locoregional recurrence or distant recurrence was 42.45 months (95%CI, 26.11 to 58.79 months) and 23.20 months (95%CI, 18.49 to 27.90 months), respectively (P = 0.001, as shown in Fig 2).

Prognostic value of EBV DNA and SUV_{max} for patients with locoregional recurrence

Next, we constructed ROC curves for death events to separately identify the impact of SUV_{max} and EBV DNA levels on the survival of locoregional recurrent and metastatic NPC patients. We used a series of cutoff points and finally selected the best cutoff values as the threshold for the subsequent binary variable analysis (Tables 2 and 3). With the value of 8.65 as the optimal cutoff point for patients with locoregional recurrence, patients with $SUV_{max} \geq 8.65$ were found to have the most discriminate significance for OS (P = 0.005; Fig 3). The median OS was 31.05 months (95%CI, 22.76 to 39.33 months) for the patients with $SUV_{max} \geq 8.65$, which was much shorter than that of the patients with $SUV_{max} < 8.65$, who had a value of 55.88 months (95%CI, 39.77 to 58.70 months). Selecting the optimal cutoff point of 4000 copies/ml for EBV DNA, the Kaplan-Meier method demonstrated that there was no significant difference for OS between

Table 1. Characteristics of patients with recurrent or metastatic nasopharyngeal carcinoma after definitive radiotherapy.

Characteristic	Number of patients	
	Locoregional Recurrence (%) (107)	Distant recurrence (%) (87)
Gender		
Female	21 (19.6)	13 (14.9)
Male	86 (80.4)	74 (85.1)
Age		
<46	58 (54.2)	56 (35.6)
≥46	49 (45.8)	31 (64.4)
Family history of tumor		
Negative	87 (79.8)	75 (86.2)
Positive	20 (20.2)	12 (13.8)
EBV DNA[※1]		
mean (copies/ml)	2.67×10^4	2.58×10^6
median (copies/ml)	4.00×10^3	3.49×10^4
Restage		
I	10 (9.3)	0
II	10 (9.3)	0
III	49 (45.9)	0
IV	38 (35.5)	87 (100)
SUV$_{max}$[※2]		
mean	12.48	14.39
median	11.5	13.3
Histopathology		
WHO[※3] 1	0	1
WHO 2	4	0
WHO 3	97	81

Abbreviations:

[※1]. EBV DNA, Epstein-Barr virus DNA;

[※2]. SUV$_{max}$, the maximal standardized uptake value. For metastasis, SUV$_{max}$ represents distant recurrent SUV$_{max}$; for locoregional recurrence, SUV$_{max}$ represents locoregional recurrent SUV$_{max}$;

[※3] WHO, world health organization.

doi:10.1371/journal.pone.0122756.t001

the low and high EBV DNA groups (P = 0.541; Fig 3). Univariate analysis showed that a locoregional SUV$_{max}$≥8.65 was an unfavorable prognostic factor (P = 0.013; Table 2). To adjust for SUV$_{max}$, the potentially associated parameters were introduced into the multivariable Cox regression model. The multivariate results confirmed that an SUV$_{max}$≥8.65 was a highly significant predictor for OS for locoregional recurrent patients (HR = 4.882; 95%CI = 1.055–22.590; P = 0.042; Table 2), independent of restage, EBV DNA level, family history of tumor, gender and age. These results indicated that the predictive ability of SUV$_{max}$ was superior to that of EBV DNA for predicting survival for patients with locoregional recurrence.

Prognostic value of EBV DNA levels and SUV$_{max}$ for patients with distant recurrence

We found that ROC curves, which identified the impact of SUV$_{max}$ and EBV DNA levels on the survival of metastatic NPC patients, had significantly different areas under the curve
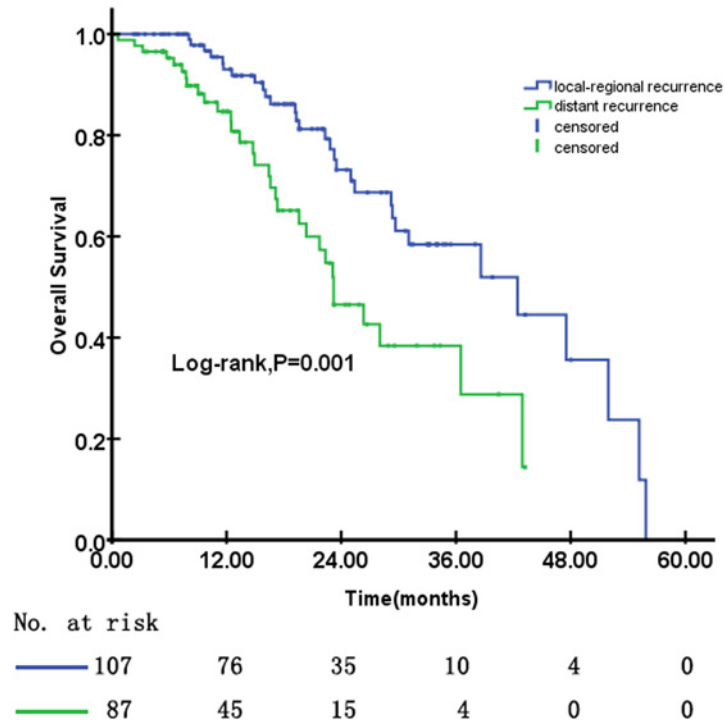
Fig 2. Kaplan–Meier estimates of overall survival between patients with locoregional recurrence and those with distant recurrence.

(AUCs). As S1 Fig shows, the AUC of EBV DNA was 0.664 (95%CI, 0.543 to 0.784), which is larger than that of SUV$_{max}$, which had an AUC of 0.595 (95%CI, 0.455 to 0.735). The best cut-off was selected for metastatic NPC, and patients were categorized into two groups for separate Kaplan-Meier analysis according to their cutoff values of EBV DNA and SUV$_{max}$. Fig 4 shows the Kaplan-Meier estimates for OS based on EBV DNA and SUV$_{max}$. SUV$_{max} \geq 13.55$ and EBV DNA$\geq 21,100$ copies/ml were two factors that significantly associated with shorter OS ($P = 0.006$ and $P = 0.015$, respectively; Fig 4). For the patients with SUV$_{max} \geq 13.55$, the median OS was much shorter compared with the patients with SUV$_{max} < 13.55$, with values of 17.12 months (95%CI, 12.95–21.29 months) vs. 36.50 months (95%CI, 9.21–63.79 months), respectively ($P = 0.006$). Similarly, patients with EBV DNA$\geq 21,100$ copies/ml had a shorter median OS of 21.72 months (95%CI, 14.15–29.29 months) compared with 31.65 months (95%CI, 25.41–37.89 months, $P = 0.015$) in the group with EBV DNA$< 21,100$ copies/ml. Univariate Cox proportional hazards regression analyses revealed that being male and having an age≥ 46, EBV DNA$\geq 21,100$ copies/ml, number of metastases≥ 3 and an SUV$_{max} \geq 13.55$ was strongly associated with an unfavorable OS (Table 3). Multivariate Cox proportional hazards regression analyses further confirmed that age (HR = 2.687; 95%CI = 1.156–6.244; $P = 0.022$) and EBV DNA (HR = 3.269; 95%CI = 1.383–7.728; $P = 0.007$) were highly significant prognostic factors for OS, and SUV$_{max}$ of patients with distant recurrence was borderline significant (HR = 2.415; 95%CI = 0.956–6.101; $P = 0.062$). These results showed that the predictive ability of EBV DNA was superior to that of SUV$_{max}$ for predicting survival for patients with distant metastasis.

Table 2. Univariate and multivariate analyses of factors associated with survival in a cohort of 107 recurrent nasopharyngeal carcinoma patients after definitive radiotherapy.

Factors	Univariate	Multivariate	
	HR (95%CI) P value	HR (95%CI)	P value
Gender			
Female	Baseline		
Male	1.321(0.557–3.135) 0.528	0.779 (0.276–2.196)	0.636
Age			
<46	Baseline		
≥46	1.777 (0.844–3.740) 0.130	1.445 (0.617–3.387)	0.397
Family history of tumor			
Negative	Baseline		
Positive	1.060(0.399–2.814) 0.907	1.145 (0.392–3.343)	0.804
EBV DNA[※1]			
<4000 copiem/ml	Baseline		
≥4000 (copies/ml)	1.273(0.586–2.763) 0.542	1.793 (0.749–4.291)	0.19
Restage			
I-II	Baseline		
III-IV	6.642(0.893–49.416) 0.064	2.931 (0.354–24.283)	0.319
Locoregional SUV$_{max}$[※2]			
<8.65	Baseline		
≥8.65	6.220 (1.470–26.31) 0.013	4.882 (1.055–22.590)	0.042

Abbreviations:

※1. EBV DNA, Epstein-Barr virus DNA;

※2. SUV$_{max}$, the maximal standardized uptake value.

doi:10.1371/journal.pone.0122756.t002

Prognostic value of SUV$_{max}$ in patients with undetectable EBV DNA

A total of 49 patients (37 of 107 with locoregional recurrences and 12 of 87 with distant recurrences) had undetectable EBV DNA levels. To assess the prognostic value of SUV$_{max}$ in patients with undetectable EBV DNA levels, this special population was further analyzed using the Kaplan-Meier method and compared with log-rank tests. Because the analysis was limited by a small number of samples, patients with locoregional or distant recurrence with undetectable EBV DNA levels were analyzed together. A cutoff SUV$_{max}$ of 8.65 was used for the analysis. The results are presented in S2 Fig. A higher SUV$_{max}$ (SUVmax≥8.65) was strongly correlated with poor OS in recurrent patients with undetectable EBV DNA (P = 0.011). Multivariate analysis demonstrated that a level of SUV$_{max}$≥8.65 was still a significant predictor for OS for recurrent patients with undetectable EBV DNA (HR = 5.093; 95%CI = 1.093–23.721; P = 0.038; S2 Table). These results illustrated that SUV$_{max}$ is still an effective prognostic indicator for those with undetectable levels of EBV DNA.

Discussion

Prognostic factors, which can be assayed noninvasively, are extraordinarily vital for cancer treatment stratification and potentially improving treatment outcome. The recent demonstration of the use of ^{18}F-FDG-PET SUV$_{max}$ and EBV DNA levels in NPC patients has opened up new possibilities for detecting and monitoring recurrent NPC patients in southern China and

Table 3. Univariate and multivariate analyses of factors associated with survival in a cohort of 87 metastatic nasopharyngeal carcinoma patients with or without recurrence after definitive radiotherapy.

Factors	Univariate		Multivariate	
	HR (95%CI)	P value	HR (95%CI)	P value
Gender				
Female	Baseline			
Male	1.742 (0.602–5.043)	0.036	2.375 (0.678–8.315)	0.176
Age				
<46	Baseline			
≥46	2.117 (0.844–3.740)	0.046	2.687 (1.156–6.244)	0.022
Family history of tumor				
Negative	Baseline			
Positive	1.901 (0.771–4.685)	0.163	0.687 (0.262–1.802)	0.446
EBV DNA[※1]				
<21,100 (copies/ml)	Baselie			
≥21,100 (copies/ml)	2.608 (1.165–5.836)	0.020	3.269 (1.383–7.728)	0.007
Number of metastases				
<3 (n = 50)	Baseline			
≥3 (n = 37)	2.077 (1.021–4.224)	0.044	0.760 (0.297–1.944)	0.567
Distant SUV$_{max}$[※2]				
<13.55	Baseline			
≥13.55	2.818 (1.302–6.098)	0.009	2.415 (0.956–6.101)	0.062

Abbreviations:

[※1]. EBV DNA, Epstein-Barr virus DNA;

[※2]. SUV$_{max}$, the maximal standardized uptake value.

doi:10.1371/journal.pone.0122756.t003

Southeast Asia.[9, 21] A study by Chang suggested that tumor TLG, nodal TLG, total TLG, tumor SUV$_{max}$, and nodal SUV$_{max}$ were all significantly associated with plasma EBV DNA load.[27] However, this correlation of SUV$_{max}$ and EBV DNA was not found in this study of patients with recurrent NPC. We explored and compared the prognostic implication of SUV$_{max}$ and EBV DNA in subgroups of locoregional recurrent or distant recurrent NPC patients in the present study. Intriguingly, we found that the value of SUV$_{max}$ was an effective prognostic biomarker for overall survival in patients with locoregional recurrence and patients with undetectable EBV DNA levels. According to our findings, the predictive power of EBV DNA load was superior to that of SUV$_{max}$, although both distant SUV$_{max}$ and EBV DNA load were independent prognostic factors that predicted survival for patients with distant recurrence.

Studies are lacking on prognostic markers for patients with locoregional recurrence. Chan explored the value of SUV$_{max}$ and EBV DNA in predicting the outcome of nasopharyngectomy and cervical lymphadenectomy for patients with locoregional recurrent NPC.[17] However, the study was limited as it was unable to predict prognosis, although it found that preoperative EBV DNA and SUV$_{max}$ levels of tumors had a predictive value for surgical resection margin status. In this study of a subgroup of 107 locoregional recurrent NPC patients, we noted that a higher SUV$_{max}$, rather than EBV DNA level, had significant clinical value for predicting poor OS. To our knowledge, previous studies indicated that EBV DNA was associated with the TNM stage and reflected the tumor burden. Although there are controversial opinions regarding from where the plasma/serum circulating EBV DNA is derived, an increasing number of studies indicate that EBV DNA originates from tumor lesions and correlates with tumor load.
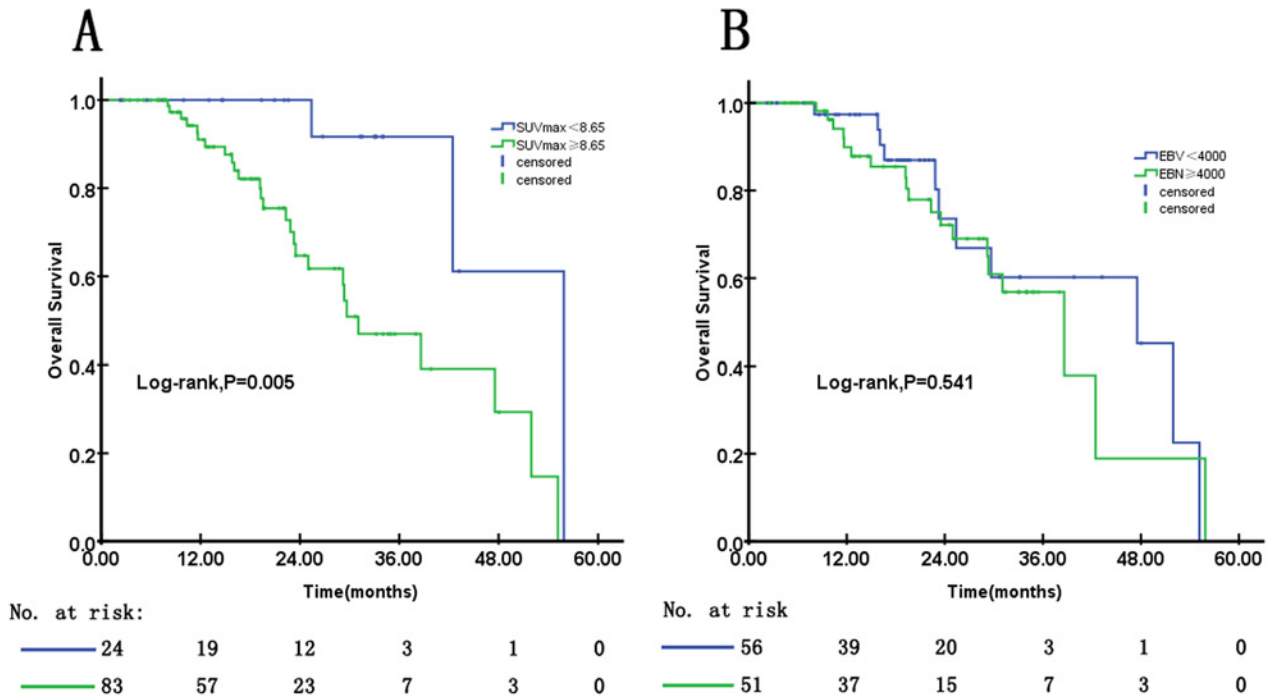
Fig 3. Kaplan–Meier estimates of overall survival according to the locoregional SUV$_{max}$ or the plasma EBV DNA concentration in locoregional recurrent patients.

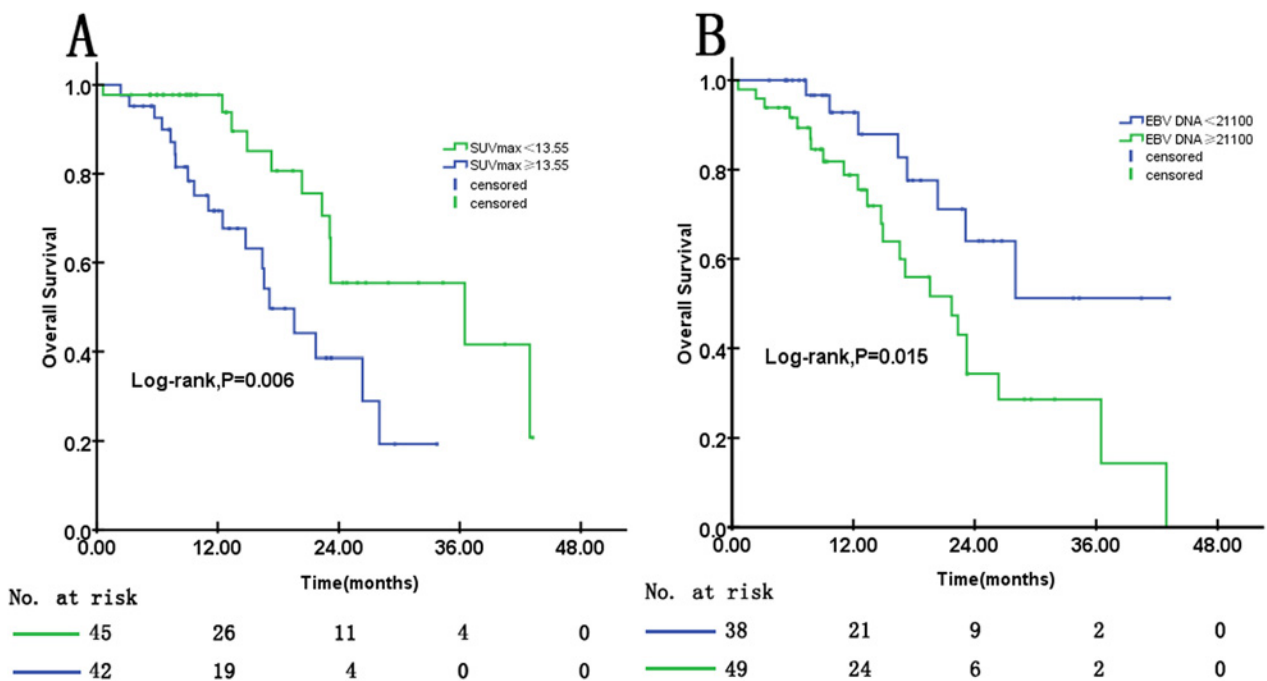doi:10.1371/journal.pone.0122756.g003

Fig 4. Kaplan–Meier estimates of overall survival according to the distant SUV$_{max}$ or the plasma EBV DNA concentration in distant recurrent patients.

doi:10.1371/journal.pone.0122756.g004

[28–30] In contrast to other head and neck cancer and epithelial malignancy in general, a unique feature of NPC is its strong association with EBV. The life cycle of EBV includes latent and lytic stages. In NPC tumor cells, EBV hides in a latent status and latent viral genomes are periodically reactivated to the lytic cycle.[31, 32] For the patients with advanced stage disease, more NPC cells were reactivated into lytic stage, and released quantities of EBV DNA fragment into the circulation by lysis death. [33]Patients with locoregional recurrence showed a relatively low tumor load and therefore displayed low EBV DNA levels, partially explaining why EBV DNA was not an effective prognostic indicator for locoregional recurrence. In contrast, SUV$_{max}$ of a lesion tends to show its metabolic activity and thus the aggressiveness of the tumor.[34, 35] In addition, a systematic review of 1813 patients from the published literature showed that ^{18}F-FDG PET/CT imaging was significantly superior to MRI and CT for the diagnosis of recurrent locoregional disease,[36] and according to our findings, the SUV$_{max}$ of ^{18}F-FDG PET/CT also serves as a valuable predictive factor. Furthermore, the overexpression of glucose transporter 1 (GLUT1) has been shown to be closely related to ^{18}F-FDG uptake in human cancer, [37] and it has been widely reported that GLUT1 correlates with poor prognosis and tumor aggressiveness in lung[38] and colorectal[39] carcinomas and in squamous cell carcinoma of the head and neck[40]. Together, these results suggest a possible explanation for why the predictive ability of SUV$_{max}$ was superior to that of EBV DNA levels in patients with locoregional recurrence. However, there were still some NPC cases accompanied concurrently by locoregional recurrence and subclinical distant metastasis. In this circumstance, the plasma EBV DNA test should be routinely performed to monitor disease progression during the retreatment and follow up.

Several studies have also been published concerning the predictive value of SUV$_{max}$ and EBV DNA load for distant recurrence after treatment. These studies demonstrated that EBV DNA levels in metastatic/recurrent NPC could predict tumor response and patient survival. [15, 18]' However, the two studies failed to reveal the definite pattern of recurrence (i.e., locoregional recurrence or distant recurrence). Chan revealed that SUV$_{max}$ was an effective biomarker for predicting prognosis in metastatic NPC.[41] Consistent with these results, we found that patients with higher distant SUV$_{max}$ values presented a worse overall survival compared with patients with lower distant SUV$_{max}$ values. It has been reported that EBV DNA load is a better prognosticator for distant metastatic recurrence than locoregional recurrence in NPC.[42] Consistent with this previous report, we found that EBV DNA load is an independent prognostic factor for overall survival in patients with distant recurrence but not in patients with locoregional recurrence. It is also logical to suggest that the higher tumor burden in patients with distant recurrence accounts for the better predictive ability of the level of EBV DNA. Another alternative explanation for the better predictive ability of EBV DNA load in patients with distant recurrence compared with patients with locoregional recurrence is that distant recurrences originate from subclinical micro-metastases in the circulation that are seeded from the primary tumor before the commencement of radiation therapy.[42] We also found that the predictive ability of SUV$_{max}$ is inferior to that of the level of EBV DNA for patients with distant recurrence. It is worth noting that SUV$_{max}$ is only the reflection of metabolism activity of a locoregional site and cannot sufficiently reflect tumor cells in the circulation, which are known as micro-metastases. Thus, it is reasonable to hypothesize that for those with distant metastasis, there may be numerous micro-metastases in the circulation. Present imaging modalities are not sufficient to discover this micro-metastasis. However, the higher EBV DNA concentration in patients with distant recurrent NPC, as reflected in our results, might closely correlate with the degree of micro-metastasis.

In our analysis of 194 recurrent patients, 49 presented with undetectable EBV DNA levels, and further study showed that SUV$_{max}$ was also predictive of survival for these patients. NPC

patients with undetectable EBV DNA levels have been reported in previous studies. Yip et al. summarized previous studies and found that an EBV DNA qPCR achieved sensitivities of tumor detection of 22–86%, 48–95%, 74–100% and 79–100% in stage I, II, III and IV patients, respectively.[43] They further reviewed 6 studies from 2003 to 2013 that compared the ability of post-treatment EBV DNA loads to detect distant metastasis (DM) and local recurrence (LR) or locoregional recurrence (LRR). The detection rate for DM ranged from 55% to 96% and varied from 0% to 67% for LR/LRR. It is noteworthy that a low or undetectable EBV DNA load does not necessarily predict good prognosis. Nevertheless, SUV$_{max}$ is an independent determinant to EBV DNA load in predicting survival for patients with recurrent NPC and undetectable EBV DNA.

However, some limitations remain in the present study. First, our study was limited by the relatively small sample size and short follow-up time. Second, the measurements were recorded from a single center. Third, the clinical application of quantitative plasma EBV DNA results has been limited by varying sensitivities when different segments of viral gene or different viral genes are assayed in different study group[44–46]. Hence, a larger, multicenter cohort study with unified standardized procedures is warranted.

In conclusion, we clearly demonstrated that pretreatment locoregional SUV$_{max}$ level was the only independent predictive factor of OS for patients with locoregional recurrence, but for patients with distant recurrence, the predictive ability of EBV DNA load was superior to that of SUV$_{max}$. In addition, SUVmax remains an effective indicator for patients with undetectable amounts of EBV DNA. This study may guide individualized treatment for recurrent NPC patients. Further prospective, multicenter investigations to evaluate the feasibility and role of SUV$_{max}$ and EBV DNA concentration for prognostication in recurrent NPC are warranted.

Supporting Information

S1 Fig. Receiver operating characteristic (ROC) curves for EBV DNA and SUV$_{max}$.
(TIF)

S2 Fig. Kaplan–Meier estimates of overall survival in 49 recurrent patients with undetectable EBV DNA levels.
(TIF)

S1 Table. Relationship between EBV DNA and SUV$_{max}$
(PDF)

S2 Table. Multivariate analyses of factors associated with survival in a cohort of 49 recurrent nasopharyngeal carcinoma patients with undetectable EBV DNA loads.
(PDF)

S1 Dataset. The origin dataset of the manuscript.
(SAV)

Acknowledgments

Author Contributions

Conceived and designed the experiments: TS LQT HQM HYM. Performed the experiments: TS LQT. Analyzed the data: TS LQT DHL QYC. Contributed reagents/materials/analysis tools:

TS LQT DHL QYC PJL DMM SSG LTL CNQ XG MSZ. Wrote the paper: TS LQT HQM HYM.

References

1. Yu MC, Yuan JM. Epidemiology of nasopharyngeal carcinoma. Seminars in cancer biology. 2002; 12(6):421–9. PMID: 12450728

2. Wei WI, Sham JS. Nasopharyngeal carcinoma. Lancet. 2005; 365(9476):2041–54. PMID: 15950718

3. Al-Sarraf M, LeBlanc M, Giri PG, Fu KK, Cooper J, Vuong T, et al. Chemoradiotherapy versus radiotherapy in patients with advanced nasopharyngeal cancer: phase III randomized Intergroup study 0099. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 1998; 16(4):1310–7. PMID: 9552031.

4. Wee J, Tan EH, Tai BC, Wong HB, Leong SS, Tan T, et al. Randomized trial of radiotherapy versus concurrent chemoradiotherapy followed by adjuvant chemotherapy in patients with American Joint Committee on Cancer/International Union against cancer stage III and IV nasopharyngeal cancer of the endemic variety. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2005; 23(27):6730–8.

5. Kam MK, Teo PM, Chau RM, Cheung KY, Choi PH, Kwan WH, et al. Treatment of nasopharyngeal carcinoma with intensity-modulated radiotherapy: the Hong Kong experience. International journal of radiation oncology, biology, physics. 2004; 60(5):1440–50. PMID: 15590175

6. Lee AW, Sze WM, Au JS, Leung SF, Leung TW, Chua DT, et al. Treatment results for nasopharyngeal carcinoma in the modern era: the Hong Kong experience. International journal of radiation oncology, biology, physics. 2005; 61(4):1107–16. PMID: 15752890

7. Hua YJ, Han F, Lu LX, Mai HQ, Guo X, Hong MH, et al. Long-term treatment outcome of recurrent nasopharyngeal carcinoma treated with salvage intensity modulated radiotherapy. European journal of cancer. 2012; 48(18):3422–8. doi: 10.1016/j.ejca.2012.06.016 PMID: 22835782

8. Chan AT. Nasopharyngeal carcinoma. Annals of oncology: official journal of the European Society for Medical Oncology / ESMO. 2010; 21 Suppl 7:vii308–12.

9. Lee SW, Nam SY, Im KC, Kim JS, Choi EK, Ahn SD, et al. Prediction of prognosis using standardized uptake value of 2-[(18)F] fluoro-2-deoxy-d-glucose positron emission tomography for nasopharyngeal carcinomas. Radiotherapy and oncology: journal of the European Society for Therapeutic Radiology and Oncology. 2008; 87(2):211–6.

10. Fleming ID, Cooper JS, Henson DE. AJCC cancer staging manual, 1997, 31–39. Lippincott-Raven, Philadelphia.

11. Ho JH. Stage classification of nasopharyngeal carcinoma: a review. IARC scientific publications. 1978; (20:):99–113. PMID: 365707

12. Min H, Hong M, Ma J, Zhang E, Zheng Q, Zhang J, et al. A new staging system for nasopharyngeal carcinoma in China. International journal of radiation oncology, biology, physics. 1994; 30(5):1037–42. PMID: 7961009

13. Higashi K, Ueda Y, Arisaka Y, Sakuma T, Nambu Y, Oguchi M, et al. 18F-FDG uptake as a biologic prognostic factor for recurrence in patients with surgically resected non–small cell lung cancer. Journal of Nuclear Medicine. 2002; 43(1):39–45. PMID: 11801701

14. Leung SF, Zee B, Ma BB, Hui EP, Mo F, Lai M, et al. Plasma Epstein-Barr viral deoxyribonucleic acid quantitation complements tumor-node-metastasis staging prognostication in nasopharyngeal carcinoma. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2006; 24(34):5414–8. doi: 10.1200/JCO.2006.07.7982

15. An X, Wang FH, Ding PR, Deng L, Jiang WQ, Zhang L, et al. Plasma Epstein-Barr virus DNA level strongly predicts survival in metastatic/recurrent nasopharyngeal carcinoma treated with palliative chemotherapy. Cancer. 2011; 117(16):3750–7. doi: 10.1002/cncr.25932 PMID: 21319149

16. Wang W-Y, Twu C-W, Chen H-H, Jan J-S, Jiang R-S, Chao JYC, et al. Plasma EBV DNA clearance rate as a novel prognostic marker for metastatic/recurrent nasopharyngeal carcinoma. Clinical Cancer Research. 2010; 16(3):1016–24. doi: 10.1158/1078-0432.CCR-09-2796 PMID: 20103659

17. Chan JY, Chow VL, Mok VW, Ho AC, Wei WI. Prediction of surgical outcome using plasma Epstein-Barr virus dna and (18)F-FDG PET-CT scan in recurrent nasopharyngeal carcinoma. Head & neck. 2012; 34(4):541–5.

18. Shotelersuk K, Khorprasert C, Sakdikul S, Pornthanakasem W, Voravud N, Mutirangura A. Epstein-Barr virus DNA in serum/plasma as a tumor marker for nasopharyngeal cancer. Clinical Cancer Research. 2000; 6(3):1046–51. PMID: 10741733

19. Allal AS, Slosman DO, Kebdani T, Allaoua M, Lehmann W, Dulguerov P. Prediction of outcome in head-and-neck cancer patients using the standardized uptake value of 2-[18F]fluoro-2-deoxy-D-glucose. International journal of radiation oncology, biology, physics. 2004; 59(5):1295–300. doi: 10.1016/j.ijrobp.2003.12.039 PMID: 15275712

20. Seol YM, Kwon BR, Song MK, Choi YJ, Shin HJ, Chung JS, et al. Measurement of tumor volume by PET to evaluate prognosis in patients with head and neck cancer treated by chemo-radiation therapy. Acta Oncol. 2010; 49(2):201–8. doi: 10.3109/02841860903440270 PMID: 20100156

21. Chan SC, Chang JT, Wang HM, Lin CY, Ng SH, Fan KH, et al. Prediction for distant failure in patients with stage M0 nasopharyngeal carcinoma: the role of standardized uptake value. Oral oncology. 2009; 45(1):52–8. doi: 10.1016/j.oraloncology.2008.03.010 PMID: 18487079

22. Yen TC, Lin CY, Wang HM, Huang SF, Liao CT, Kang CJ, et al. 18F-FDG-PET for evaluation of the response to concurrent chemoradiation therapy with intensity-modulated radiation technique for Stage T4 nasopharyngeal carcinoma. International journal of radiation oncology, biology, physics. 2006; 65(5):1307–14. PMID: 16863922

23. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Annals of surgical oncology. 2010; 17(6):1471–4. doi: 10.1245/s10434-010-0985-4 PMID: 20180029

24. Delbeke D, Coleman RE, Guiberteau MJ, Brown ML, Royal HD, Siegel BA, et al. Procedure guideline for tumor imaging with 18F-FDG PET/CT 1.0. Journal of nuclear medicine: official publication, Society of Nuclear Medicine. 2006; 47(5):885–95. PMID: 16644760

25. Shao JY, Li YH, Gao HY, Wu QL, Cui NJ, Zhang L, et al. Comparison of plasma Epstein-Barr virus (EBV) DNA levels and serum EBV immunoglobulin A/virus capsid antigen antibody titers in patients with nasopharyngeal carcinoma. Cancer. 2004; 100(6):1162–70. PMID: 15022282

26. Lo YM, Chan LY, Lo KW, Leung SF, Zhang J, Chan AT, et al. Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma. Cancer research. 1999; 59(6):1188–91. PMID: 10096545

27. Chang KP, Tsang NM, Liao CT, Hsu CL, Chung MJ, Lo CW, et al. Prognostic significance of 18F-FDG PET parameters and plasma Epstein-Barr virus DNA load in patients with nasopharyngeal carcinoma. Journal of nuclear medicine: official publication, Society of Nuclear Medicine. 2012; 53(1):21–8.

28. Wei WI, Yuen AP, Ng RW, Ho WK, Kwong DL, Sham JS. Quantitative analysis of plasma cell-free Epstein-Barr virus DNA in nasopharyngeal carcinoma after salvage nasopharyngectomy: a prospective study. Head & neck. 2004; 26(10):878–83.

29. Lo YMD, Chan LYS, Chan ATC, Leung S-F, Lo K-W, Zhang J, et al. Quantitative and temporal correlation between circulating cell-free Epstein-Barr virus DNA and tumor recurrence in nasopharyngeal carcinoma. Cancer research. 1999; 59(21):5452–5. PMID: 10554016

30. Chan JY, Wong ST. The role of plasma Epstein-Barr virus DNA in the management of recurrent nasopharyngeal carcinoma. The Laryngoscope. 2014; 124(1):126–30. doi: 10.1002/lary.24193 PMID: 23686740

31. Wildeman MA, Novalic Z, Verkuijlen SAWM, Juwana H, Huitema ADR, Tan IB, et al. Cytolytic Virus Activation Therapy for Epstein-Barr Virus-Driven Tumors. Clinical Cancer Research. 2012; 18(18):5061–70. doi: 10.1158/1078-0432.CCR-12-0574 PMID: 22761471

32. Liu S, Li H, Chen L, Yang L, Li L, Tao Y, et al. (-)-Epigallocatechin-3-gallate inhibition of Epstein-Barr virus spontaneous lytic infection involves ERK1/2 and PI3-K/Akt signaling in EBV-positive cells. Carcinogenesis. 2013; 34(3):627–37. doi: 10.1093/carcin/bgs364 PMID: 23180656

33. Raab-Traub N. Epstein–Barr virus in the pathogenesis of NPC. Seminals in Cancer Biology. 2002; 12(6):431–41. PMID: 12450729

34. Kitagawa Y, Sano K, Nishizawa S, Nakamura M, Ogasawara T, Sadato N, et al. FDG-PET for prediction of tumour aggressiveness and response to intra-arterial chemotherapy and radiotherapy in head and neck cancer. European journal of nuclear medicine and molecular imaging. 2003; 30(1):63–71. PMID: 12483411

35. Eronen E, Haaparanta M, Joensuu H. Prediction of survival with fluorine-18-fluoro-deoxyglucose and PET in head and neck cancer. Journal of Nuclear Medicine. 1997; 38(12):1907–11. PMID: 9430467

36. Liu T, Xu W, Yan W-L, Ye M, Bai Y-R, Huang G. FDG-PET, CT, MRI for diagnosis of local residual or recurrent nasopharyngeal carcinoma, which one is the best? A systematic review. Radiotherapy and Oncology. 2007; 85(3):327–35. PMID: 18037523

37. Kaira K, Endo M, Abe M, Nakagawa K, Ohde Y, Okumura T, et al. Biologic correlation of 2-[18F]-fluoro-2-deoxy-D-glucose uptake on positron emission tomography in thymic epithelial tumors. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2010; 28(23):3746–53.

38. Younes M, Brown RW, Stephenson M, Gondo M, Cagle PT. Overexpression of Glut1 and Glut3 in stage I nonsmall cell lung carcinoma is associated with poor survival. Cancer. 1997; 80(6):1046–51. PMID: 9305704

39. Haber RS, Rathan A, Weiser KR, Pritsker A, Itzkowitz SH, Bodian C, et al. Glut1 glucose transporter expression in colorectal carcinoma. Cancer. 1998; 83: 34–40. PMID: 9655290.

40. Ayala FR, Rocha RM, Carvalho KC, Carvalho AL, da Cunha IW, Lourenco SV, et al. GLUT1 and GLUT3 as potential prognostic markers for Oral Squamous Cell Carcinoma. Molecules. 2010; 15(4): 2374–87. doi: 10.3390/molecules15042374 PMID: 20428049

41. Chan SC, Hsu CL, Yen TC, Ng SH, Liao CT, Wang HM. The role of 18F-FDG PET/CT metabolic tumour volume in predicting survival in patients with metastatic nasopharyngeal carcinoma. Oral oncology. 2013; 49(1):71–8. doi: 10.1016/j.oraloncology.2012.07.016 PMID: 22959277

42. Leung S-f, Lo YMD, Chan ATC, To K-f, To E, Chan LYS, et al. Disparity of sensitivities in detection of radiation-naive and postirradiation recurrent nasopharyngeal carcinoma of the undifferentiated type by quantitative analysis of circulating Epstein-Barr virus DNA1, 2. Clinical cancer research. 2003; 9(9): 3431–4. PMID: 12960133

43. Yip TT, Ngan RK, Fong AH, Law SC. Application of circulating plasma/serum EBV DNA in the clinical management of nasopharyngeal carcinoma. Oral oncology. 2014; 50(6):527–38. doi: 10.1016/j. oraloncology.2013.12.011 PMID: 24440146

44. Lin J-C, Wang W-Y, Liang W-M, Chou H-Y, Jan J-S, Jiang R-S, et al. Long-term prognostic effects of plasma epstein-barr virus DNA by minor groove binder-probe real-time quantitative PCR on nasopharyngeal carcinoma patients receiving concurrent chemoradiotherapy. International Journal of Radiation Oncology* Biology* Physics. 2007; 68(5):1342–8.

45. Le QT, Zhang Q, Cao H, Cheng AJ, Pinsky BA, Hong RL, et al. An international collaboration to harmonize the quantitative plasma Epstein-Barr virus DNA assay for future biomarker-guided trials in nasopharyngeal carcinoma. Clinical cancer research: an official journal of the American Association for Cancer Research. 2013; 19(8):2208–15. doi: 10.1158/1078-0432.CCR-12-3702 PMID: 23459720

46. Yang X, Dai W, Kwong DLw, Szeto CYY, Wong EHw, Ng WT, et al. Epigenetic markers for noninvasive early detection of nasopharyngeal carcinoma by methylation-sensitive high resolution melting. International Journal of Cancer. 2015; 136(4):E127–E35. doi: 10.1002/ijc.29192 PMID: 25196065