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ORIGINAL ARTICLE

Value of early evaluation of treatment response using ^{18}F-FDG PET/CT parameters and the Epstein-Barr virus DNA load for prediction of outcome in patients with primary nasopharyngeal carcinoma

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Abstract

Purpose To determine the value of early evaluation of response to concurrent chemoradiotherapy (CCRT) using ^{18}F-FDG PET-derived parameters and the Epstein-Barr virus (EBV) DNA titre in outcome prediction in patients with primary nasopharyngeal carcinoma (NPC).

Methods Sixty patients with primary NPC were prospectively enrolled. All patients underwent ^{18}F-FDG PET/CT before and during CCRT. The plasma EBV DNA titre was measured along with the PET/CT-derived parameters. Changes in EBV DNA titre and PET/CT-derived parameters during CCRT were analysed in relation to response to treatment, recurrence-free survival (RFS) and overall survival (OS).

Results A total lesion glycolysis (TLG) reduction ratio of ≤0.6 and a detectable EBV DNA titre during CCRT were predictors of an unfavourable response to treatment, RFS and OS. In multivariate analysis, a TLG reduction ratio of ≤0.6 predicted incomplete remission ($p = 0.002$) and decreased RFS ($p = 0.003$). The proportion of patients with a TLG reduction ratio of >0.6 who achieved a complete response was more than twice that of patients with a TLG reduction ratio of ≤0.6. A detectable EBV DNA titre, a TLG reduction ratio of ≤0.6 and older age were independently associated with a poorer OS ($p = 0.037$, 0.009 and 0.016, respectively). A scoring system was developed based on these independent predictors of OS. Patients with a score of 1 and 2/3 had poorer survival outcomes than those with a score of 0 (hazard ratio 4.756, $p = 0.074$, and hazard ratio 18.973, $p = 0.001$, respectively). This scoring system appeared to be superior to the traditional TNM staging system ($p < 0.001$ versus $p = 0.175$).

Conclusion Early evaluation of response to CCRT using ^{18}F-FDG PET-derived parameters and the EBV DNA titre can predict outcome in patients with primary NPC. A combination of interim PET parameters and the EBV DNA titre enables better stratification of patients into subgroups with different survival rates.

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Keywords Nasopharyngeal carcinoma · ^{18}F-FDG PET · Epstein-Barr virus · Treatment response · Prognosis · Head and neck cancer

Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial carcinoma arising from the nasopharyngeal epithelium [1]. Although squamous in origin, NPC is considered to be different from squamous carcinoma in other head and neck regions in terms of geographic distribution and histopathological characteristics. The worldwide distribution of this cancer is quite heterogeneous, with particularly high incidences reported in south-eastern and south-central Asia and north-eastern Africa [2–4]. Furthermore, the histopathology of NPC is different from that of other head and neck squamous cell carcinomas. Nonkeratinizing carcinoma is the predominant type of NPC in endemic areas and is closely associated with Epstein-Barr virus (EBV) infection [2, 5].

The treatment for primary NPC is mainly radiotherapy, but this may be combined with chemotherapy for more advanced locoregional disease. Survival has been improved by the introduction of advanced radiotherapeutic techniques and their combination with chemotherapy [6], and several biomarkers have been proposed for the prediction of treatment outcome [2, 7–17]. The plasma EBV DNA titre has recently been recognized as a particularly promising prognostic biomarker. Survival in patients with NPC can be predicted by the pretreatment plasma EBV DNA titre and a reduction in the titre after treatment [7–11]. ^{18}F-Fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET), which identifies viable tumour by detecting enhanced tumour glycolysis, has long been used as an imaging modality for staging NPC and detecting recurrence of the disease [18–20]. In addition, semiquantitative parameters, including the standardized uptake value (SUV), as well as the volumetric parameters metabolic tumour volume (MTV) and total lesion glycolysis (TLG), are useful for risk stratification and evaluation of prognosis [12, 16, 17].

Pretreatment plasma EBV DNA titre and volumetric ^{18}F-FDG PET parameters have been shown to be useful for predicting survival in patients with NPC [13, 14, 21–24]. However, despite the promising results, the use of ^{18}F-FDG PET or the EBV DNA titre has so far been limited to the pretreatment and posttreatment settings. Moreover, very few studies have investigated the value of interim imaging or the EBV DNA titre during treatment for predicting outcome in patients with primary NPC [25–30]. Lin et al. investigated the prognostic value of ^{18}F-FDG PET performed during primary radiotherapy in patients with NPC and found that metabolic imaging biomarkers on interim PET were significant predictors of survival [27]. Leung et al. found that the EBV DNA load measured during radiotherapy predicted overall survival (OS) and progression-free survival in patients with primary NPC [28]. The biological features underlying EBV DNA and ^{18}F-FDG PET parameters in the pathogenesis of NPC are not the same. In this context, a combination of blood and imaging biomarkers may improve the prognostic stratification of patients with primary NPC. However, the value of combining interim PET imaging parameters with the EBV DNA titre for predicting the survival outcome in patients with NPC has not been well investigated.

The purpose of this prospective study was to determine the value of interim ^{18}F-FDG PET and the EBV DNA titre for assessing treatment response and survival in patients with primary NPC.

Materials and methods

Study population and definitive treatment

The protocol used in this prospective study was approved by the local institutional review board and ethics committee. Written informed consent was obtained from all study participants. The study was performed between August 2011 and July 2014. All participants in the study had newly diagnosed NPC and were consecutively enrolled at the Chang Gung Memorial Hospital, Lin-Kou, Taiwan. Patients with other malignancies, those with distant metastatic disease at presentation, and those who had been treated previously at another hospital were excluded.

All the study subjects had biopsy-proven NPC that was staged according to the 7th edition of the American Joint Committee on Cancer staging manual [31]. All received definitive external beam radiotherapy with a cumulative dose of at least 64.8 Gy and concurrent cisplatin-based chemotherapy consisting of intravenous cisplatin 50 mg/m^2 on day 1 and oral tegafur 800 mg/day plus oral leucovorin 60 mg/day from day 1 to day 14. This schedule was repeated every 2 weeks throughout the course of radiotherapy [32]. None of the patients received induction chemotherapy during the study period. Each patient underwent a comprehensive examination before and 3 months after treatment. The examinations included magnetic resonance imaging (MRI) of the head and neck, chest radiography, bone scan and abdominal ultrasonography. ^{18}F-FDG PET/CT scans were performed and plasma EBV DNA titre measured in all patients before treatment and while receiving 35.0–40.0 Gy of radiotherapy.

Plasma EBV DNA analysis

A 10-mL blood sample was collected by venepuncture from each patient. The samples were collected into tubes containing ethylenediaminetetraacetic acid and immediately centrifuged at 2,000 *g* to obtain the plasma. The plasma samples were stored in polypropylene tubes at −80 °C until analysis, and were not exposed to more than one freeze–thaw cycle. Plasma EBV DNA extraction and quantification were performed according to a previously described method [13, 33, 34].

^{18}F-FDG PET imaging protocol

All patients fasted for at least 6 h before ^{18}F-FDG PET/CT imaging. The scans were performed using a Biograph mCT system (Siemens Medical Solutions, Malvern, PA, USA) that consists of a four-ring PET scanner with an axial field of view (FOV) of 22.1 cm and a transaxial FOV of 70 cm, and a 40-section CT scanner. PET emission images were obtained between 50 and 70 min after injection of ^{18}F-FDG (370 MBq) from the vertex to the mid-thigh, with 1.5 min of scanning time per table position (matrix size 200 × 200). The PET scanner has a reported spatial resolution of 4.4 mm (full-width at half-maximum) at 1 cm and 5.2 mm at 10 cm from the transverse FOV and a sensitivity of 9.7 kcps/MBq at the centre of the FOV. Before PET acquisition, a standard helical CT scan was acquired from the head to the proximal thighs using the manufacturer's dose reduction software (CARE kV and CARE Dose 4D). The preset was 120 kV with a collimation of 40 × 0.6 mm, a pitch of 1.5, and a slice thickness of 2 mm. No intravenous iodinated contrast agent was used. The PET images were reconstructed using the CT data for attenuation correction and an ordered subsets expectation maximization iterative reconstruction algorithm (two iterations and 21 subsets).

Follow-up schedule and clinical outcomes

The findings of the imaging study were discussed at a conference convened by our NPC research group. Image-guided biopsies were obtained whenever possible for lesions with suspicion of malignancy. If biopsy of a suspicious lesion was not feasible or yielded a negative result in a patient with equivocal or positive imaging findings, the patient was kept under close clinical and imaging follow-up. The patients were followed weekly during treatment, at 3-monthly intervals for 2 years, at 4-monthly intervals for the following 2 years, and every 6 months thereafter. Flexible fibre-optic nasopharyngoscopy was performed at each visit. A conventional work-up that included MRI of the head and neck, chest radiography, abdominal ultrasonography, and a bone scan was performed 3 months after completion of treatment and on a yearly basis or whenever clinically indicated thereafter. Treatment responses were quantified by physical examination and other imaging studies performed 3 months after completion of chemoradiotherapy. The responses at the primary site and in the regional nodes were scored separately and the worse of the two responses was recorded as the overall response. A complete response was defined as disappearance of all clinical evidence of tumour.

Image analysis

The ^{18}F-FDG PET findings were evaluated semiquantitatively using the PMOD 3.2 software package (PMOD Technologies Ltd., Zurich, Switzerland). The regions of interest used to calculate SUVs were drawn by an experienced nuclear medicine physician. The SUV was calculated as follows: SUV = (decay-corrected activity in kilobecquerels per millilitre of tissue volume)/(injected FDG activity in kilobecquerels/body weight in grams). The MTV was obtained from the attenuation-corrected ^{18}F-FDG PET images. The boundaries were drawn large enough to include the primary tumour in the nasopharynx. An SUV cut-off value of 2.5 was used to generate the contour margin around the target inside the boundary [14, 23]. The contour margin was automatically produced, and the voxels with SUV >2.5 within the contour margin were taken together to define the MTV. The mean SUV within the contour margin was also generated. The TLG was calculated according to the following formula: TLG = mean SUV × MTV. The TLG value and the SUVmax of the primary tumour were termed the tumour TLG and the tumour SUVmax, respectively.

Data analysis

All of the patients were followed up until March 2018 or death if that occurred earlier. The demographic data are expressed as frequencies or means and standard deviations as appropriate. The study endpoints were tumour treatment response, OS and RFS. Associations between tumour response at 3 months after completion of treatment and clinical, ^{18}F-FDG PET and EBV DNA variables were evaluated using the chi-squared test or Fisher's exact test. Multivariate logistic regression analysis was then used to identify independent predictors of survival. OS was calculated from the date of diagnosis to the date of death or censored at the date of the last follow-up for surviving patients. RFS was defined as the time between the end of treatment and the date of recurrence or censored at the date of the last follow-up. Univariate survival analysis was performed using the Kaplan-Meier method (log-rank test). The cut-off values for the study variables were determined using the log-rank test based on the OS rates in the entire study cohort [14]. An example of the application of the cut-off value is shown in the figure in Online Resource 1. During chemoradiotherapy the EBV

DNA titre (the interim EBV DNA value) decreased to zero, so we used "detectable" versus "undetectable" instead of a cut-off value to dichotomize the patients.

The effect of the study variables on survival outcomes was investigated using Cox regression models. The effect of each individual variable on the study outcomes was first tested in a univariate analysis. The multivariate regression model was then used to identify independent predictors of survival after allowing for potential confounders. A two-tailed p value <0.05 was considered statistically significant.

Results

Patient demographics

The general characteristics of the study participants are summarized in Table 1. The median follow-up period was 4.32 years (range 5–77 months). Response evaluation results at 3 months after completion of treatment were available in 59 patients. Of these patients, 53 (89.8%) achieved a complete response after definitive treatment. At the time of the last follow-up, there had been 11 deaths, one of which was not related to NPC. Disease recurrence was seen in 13 patients, and the sites of failure were as follows: regional failure alone ($n = 4$), distant failure alone ($n = 1$), local and regional failure ($n = 5$), and failure at locoregional and distant sites ($n = 3$). At 3 years the OS and RFS rates were 86.7% and 79.6%, respectively. The median TLG and EBV DNA titre before treatment were 59.63 g/mL × mL (range 1.46–387.23 g/mL × mL) and 2,037.19 copies/mL (range 0–138,365.89 copies/mL), respectively. At the time of the interim study, the median TLG reduction ratio and EBV DNA titre were 0.94 (range 0.14–1.00) and 0 copies/mL (range 0–21,045.72 copies/mL), respectively.

Table 1 Patient characteristics at baseline

Variable	Value
Age, years, mean ± SD	45.7 ± 14.25
Sex, *n* (%)	
Male	42 (70.0)
Female	18 (30.0)
Histology, *n* (%)	
Nonkeratinizing carcinoma	1 (1.7)
Undifferentiated carcinoma	59 (98.3)
T classification, *n* (%)	
T1	15 (25)
T2	4 (6.7)
T3	16 (26.7)
T4	25 (41.6)
N classification, *n* (%)	
N0	2 (3.3)
N1	22 (36.7)
N2	26 (43.3)
N3	10 (16.7)
Overall stage, *n* (%)	
II	3 (5.0)
III	27 (45.0)
IV	30 (50.0)

SD standard deviation

Association between study variables and treatment response

The final tumour treatment response was evaluated at 3 months after completion of chemoradiotherapy. The associations between clinical variables, ^{18}F-FDG PET parameters and EBV DNA titre and treatment response are summarized in Table 2. An initial T4 tumour, an initial TLG of ≥125 g/mL × mL, a TLG reduction ratio of ≤0.6, and detectable EBV DNA during the course of treatment were significantly associated with incomplete disease remission. The variables for which the association was statistically significant were examined further by multivariate logistic regression, and a TLG reduction ratio of ≤0.6 was the only independent predictor of an unfavourable response (odds ratio 53.742, confidence interval 4.199–687.885; $p = 0.002$). The proportion of patients with a TLG reduction ratio of >0.6 who achieved a complete response was more than twice that of patients with a TLG reduction ratio of ≤0.6. Of the nine patients with a TLG reduction ratio of ≤0.6, only four (44.4%) achieved a complete response after definitive treatment. In contrast, 98% of the 50 patients with the reduction ratio of >0.6 had a complete response. Figures 1 and 2 show the ability of the TLG reduction ratio to predict response to CCRT in patients with primary NPC.

Univariate and multivariate survival analyses for survival endpoints

The results of the univariate survival analysis for the various study parameters are summarized in the table in Online Resource 2. At the time of the pretreatment evaluation, age 55 years or older, a T4 tumour, and an initial TLG of ≥125 g/mL × mL were significant risk factors for a poor OS, whereas a T4 tumour, stage IV disease, an initial TLG of ≥125 g/mL × mL, and an initial EBV DNA titre ≥19,000 copies/mL were significantly risk factors for a shorter RFS. A TLG reduction ratio of ≤0.6 and detectable EBV DNA on the interim study were significant predictors of both a poor OS and a poor RFS (see figure in Online Resource 3).

The variables that were significantly associated with survival in the univariate analysis were selected for inclusion in the multivariate analysis using a Cox regression model. In the multivariate analysis, age 55 years or older, a detectable interim EBV DNA, and a TLG reduction ratio of ≤0.6 were

Table 2 Association between demographic and clinical variables and tumour response at 3 months after completion of treatment

Variable	Number of patients	Complete response (%)	*p* value	
			Univariate analysis	Multivariate analysis
Age, years				
≥55	14	78.6	0.139	
<55	45	93.3		
Sex				
Male	41	87.8	0.656	
Female	18	94.4		
T classification				
1–3	35	97.1	0.036*	NS
4	24	79.2		
N classification				
0/2	50	92.0	0.224	
3	9	77.8		
Initial stage				
II/III	30	96.7	0.103	
IVa/IVb	29	82.6		
Initial SUVmax				
≥13	18	100.0	0.164	
<13	41	95.6		
Initial TLG (g/mL × mL)				
≥125	14	71.4	0.024*	NS
<125	45	95.6		
Initial EBV DNA (copies/mL)[a]				
≥19,000	5	60.0	0.056	
<19,000	52	94.2		
TLG reduction ratio				
>0.6	50	98.0	<0.001*	0.002*
≤0.6	9	44.4		
Interim EBV DNA[b]				
Undetectable	48	93.8	0.032*	NS
Detectable	8	62.5		

EBV Epstein-Barr virus, *NS* not significant, *SUVmax* maximum standardized uptake value, *TLG* total lesion glycolysis

*$p < 0.05$

[a] Data for the initial EBV DNA copy number were not available in two patients who refused a blood test

[b] Data for interim EBV DNA copy number were not available in three patients who refused to take the test

independently associated with a shorter OS, and only a TLG reduction ratio of ≤0.6 was independently associated with a poor RFS (Table 3). The statistical power of these independent risk factors exceeded 0.99 in all cases (see table in Online Resource 4).

We developed a scoring system for predicting OS according to the number of independent risk factors present (age 55 years or older, a detectable interim EBV DNA, and a TLG reduction ratio of ≤0.6). The presence or absence of each risk factor was assigned a score of 1 or 0, respectively, resulting in scores ranging from 0 to 3. Because only one patient had a score of 3, score 2 and score 3 were considered as the same risk group (score 2/3). This system allowed stratification of the patients into risk groups with distinct survival outcomes (see table in Online Resource 5). Categorizing patients with a score of 0 as the reference category in the multivariate analysis, OS was poorer in patients with a score of 1 (hazard ratio, HR, 4.756, $p = 0.074$) or 2/3 (HR 18.973, $p = 0.001$). The 3-year survival rate in patients with a score of 2/3 was significantly lower than in those with a score of 1 or 0 (55.6% vs. 77.8% vs. 96.2%, $p < 0.001$). Figure 3 shows the value of this scoring system for stratifying OS in our patients.

Discussion

Despite modern treatments and prognostic advances in primary NPC, a considerable proportion of patients still experience

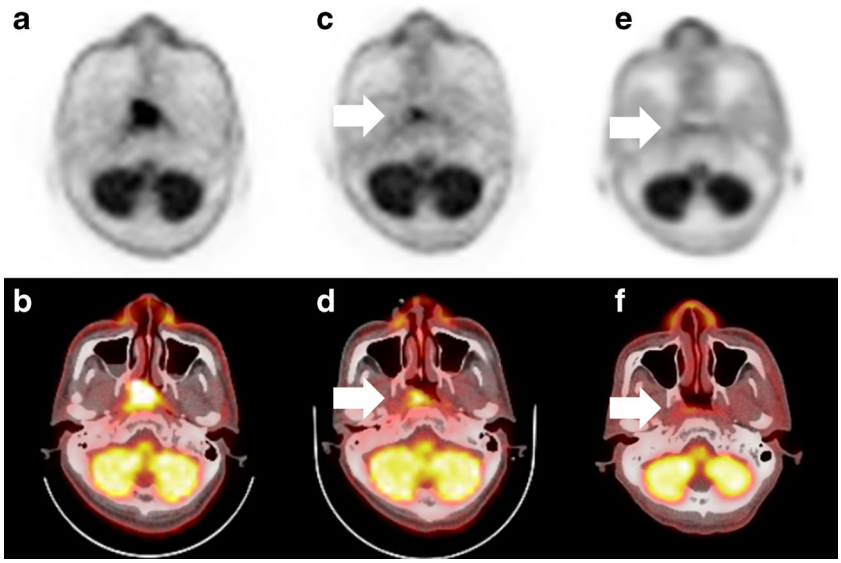

Fig. 1 Axial ^{18}F-FDG PET and PET/CT images in a 32-year-old man with T3 nasopharyngeal carcinoma. **a**, **b** Pretreatment images show an ^{18}F-FDG-avid primary tumour over the nasopharynx on the right. **c**, **d** Mid-treatment images show a marked reduction in ^{18}F-FDG uptake in the primary tumour (*arrow*, TLG reduction ratio 0.88). The interim EBV DNA titre was 945.8 copies/mL. **e**, **f** Images acquired at 3 months after chemoradiotherapy show a complete response (*arrow*). The patient was still alive without recurrence 3.3 years after treatment

recurrence of the disease [35–38]. Survival in patients with primary NPC is not only related to pretreatment prognosticators but is also closely associated with the response to chemotherapy or radiotherapy [39, 40]. In this context, the availability of predictive biomarkers during radiotherapy/chemoradiotherapy may provide an additional dimension for prognostic stratification and individualized therapy. This concept has already been endorsed by the Lugano classification in treating selected types of lymphoma [41]. To the best of our knowledge, this is the first study to investigate and compare the prognostic value of ^{18}F-FDG PET parameters and the EBV DNA titre during radiotherapy in patients with primary M0 NPC.

In this study, a lower TLG reduction ratio was the only independent risk factor for a poor treatment response. Only 44% of patients with a TLG reduction ratio of ≤0.6 achieved a complete response. Recently, changes in PET parameters between pretreatment and interim scans have been found to be correlated with response to treatment in several types of cancer other than NPC, including lymphoma, oesophageal cancer, lung cancer, and head and neck cancer [42–49]. However, there has been no investigation of the ability of the metabolic parameters seen on ^{18}F-FDG PET to differentiate patients with primary NPC who will achieve a complete response after radiotherapy from those who will have residual disease [22]. It has been reported that up to 17% of patients with advanced NPC do not achieve a complete response after therapy [36, 38, 50, 51]. Furthermore, the 5-year OS rate in patients with incomplete disease remission after chemoradiation or radiotherapy is only around 65% [52, 53].

The early identification of patients at high risk of developing persistent NPC is attractive because it may lead to an

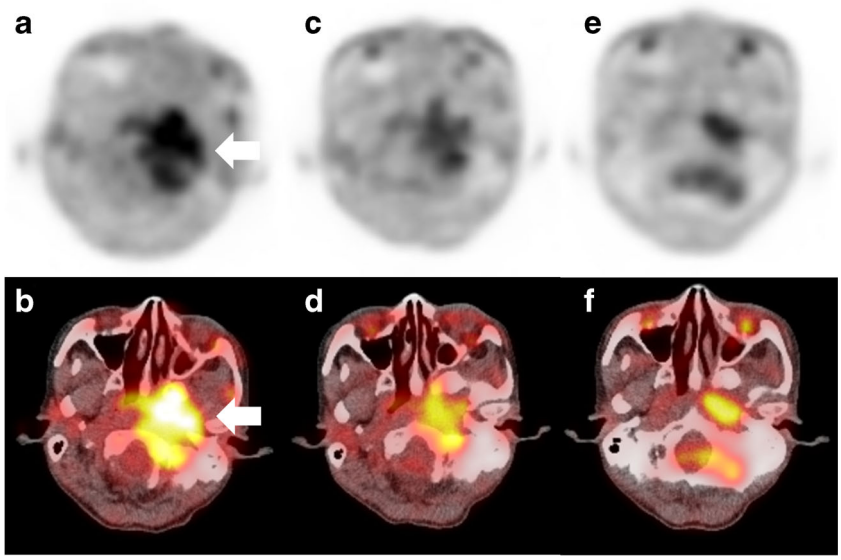

Fig. 2 Axial ^{18}F-FDG PET and PET/CT images in a 65-year-old man with nasopharyngeal carcinoma. **a**, **b** Pretreatment images show an ^{18}F-FDG-avid tumour over the left nasopharyngeal area (*arrow*). **c**, **d** Mid-treatment axial ^{18}F-FDG PET and PET/CT images show an ^{18}F-FDG-avid residual tumour (TLG reduction ratio of 0.39). The interim EBV DNA was undetectable (the initial EBV DNA titre was 28,442.8 copies/mL). **e**, **f** The patient failed to achieve complete response after chemoradiotherapy and died of nasopharyngeal carcinoma after an overall survival time of only 0.58 years

Table 3 Multivariate analysis of risk factors associated with survival in patients with nasopharyngeal carcinoma

Variable	Recurrence-free survival		Overall survival	
	Hazard ratio (95% confidence interval)	*p* value	Hazard ratio (95% confidence interval)	*p* value
Age (years)		N/A		0.016*
≥55			5.460 (1.377–21.653)	
<55			Reference	
T classification		NS		NS
1–3				
4				
Initial stage		NS		N/A
II/III				
IVa/IVb				
Initial TLG (g/mL × mL)		NS		NS
≥125				
<125				
Initial EBV DNA (copies/mL)		NS		N/A
≥19,000				
<19,000				
TLG reduction ratio >0.6	Reference	0.007*	6.477 (1.593–26.334) Reference	0.009*
≤0.6	6.011 (1.645–21.961)		6.477 (1.593–26.334)	
Undetectable Interim EBV DNA		NS	4.173 (1.090–15.970) Reference	0.037*
Detectable			4.173 (1.090–15.970)	

EBV Epstein-Barr virus, *N/A* not applicable, *NS* not significant,*TLG* total lesion glycolysis

*$p < 0.05$)

increase in the disease control rate by allowing modification of treatment, such as adjuvant therapy. However, a reliable predictor that identifies patients with a high risk of persistent NPC has yet to be identified and controversy persists regarding whether patients with primary NPC and specific adverse prognostic factors would benefit from adjuvant treatment [6,

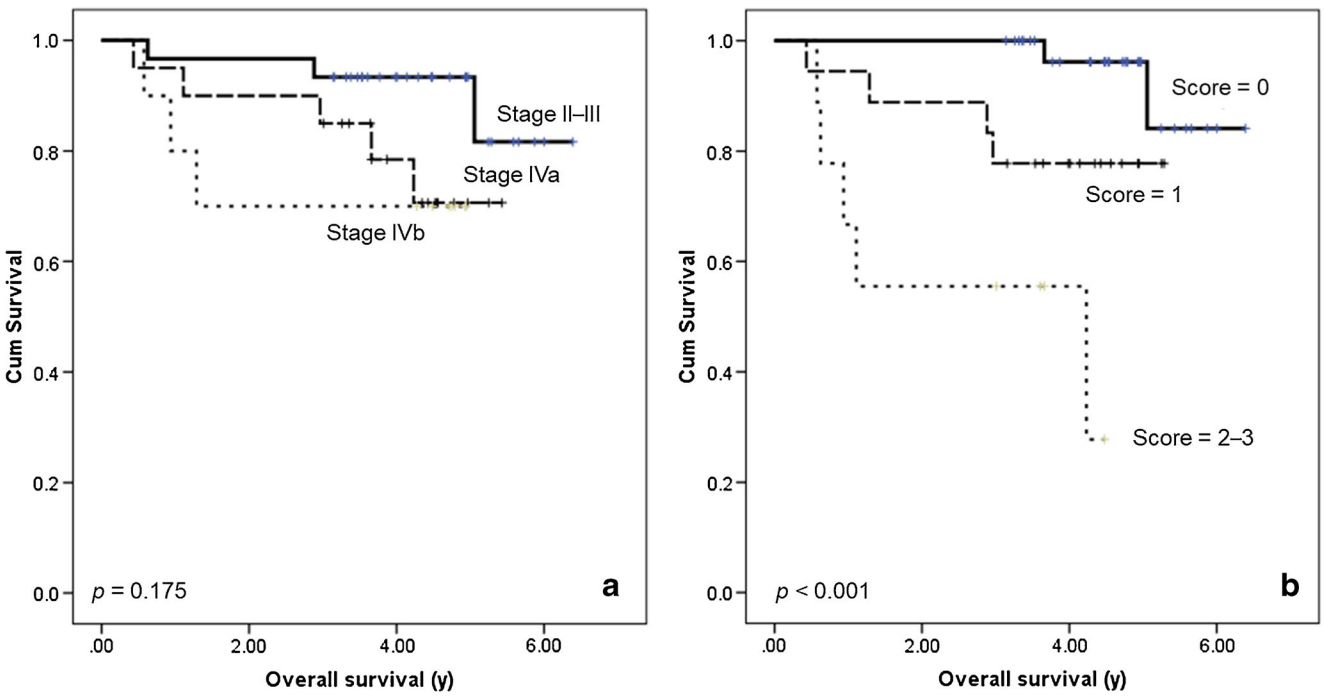

Fig. 3 Kaplan-Meier curves showing overall survival in patients with primary nasopharyngeal carcinoma stratified according to TNM stage (**a**) and the combination score for the independent risk factors (age, reduction of total lesion glycolysis, and interim EBV titre) identified in the study (**b**). The combination score enabled better stratification of patients in terms of survival than the TNM staging system ($p < 0.001$ versus $p = 0.175$)

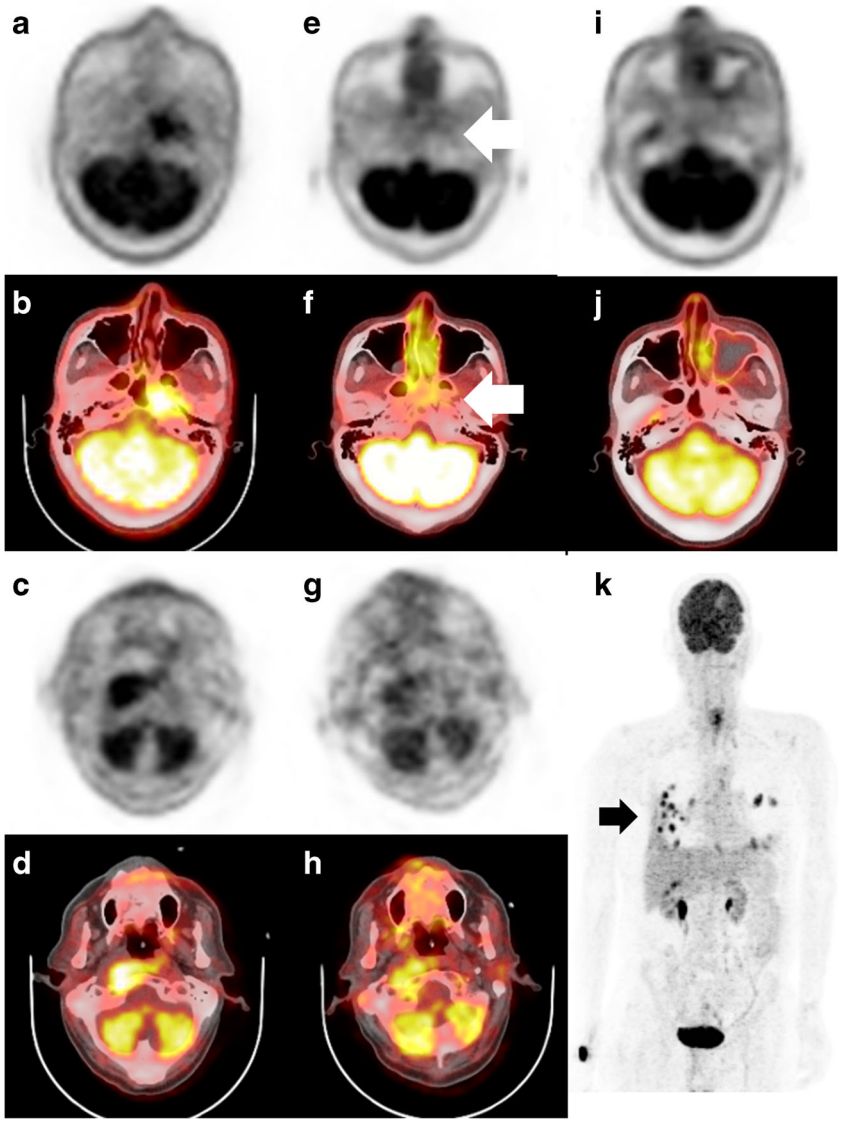

Fig. 4 ^{18}F-FDG PET and PET/CT imaging in two representative patients with primary nasopharyngeal carcinoma with different prognostic scores for overall survival (*top rows* a 43-year-old man; *bottom rows* a 64-year-old man). **a–d** Pretreatment images indicated the same stage in the two patients (cT4N3aM0). **e–h** Mid-treatment images. The first patient (**e**, **f**) shows good tumour regression (*arrows*), with a TLG reduction ratio of 0.95. Only increased ^{18}F-FDG uptake due to nasal mucositis is noted. A score of 0 was assigned to this patient. In contrast, the second patient (**g**, **h**) shows an incomplete tumour response, with a TLG reduction ratio of 0.5. A score of 3 was assigned to this patient. **I–k** Follow-up images at 3 months after definitive chemoradiation. The first patient (**I**, **j**) shows a complete response. and the OS in this patient was 4.8 years. The second patient (**k**) developed multiple pleural metastases (*arrow*) after definitive chemoradiation, and the OS in this patient was only 0.9 years. CT, computed tomography

54–57]. A recent meta-analysis has indicated that there is no significant improvement in survival following CCRT with adjuvant chemotherapy when compared with CCRT alone in patients with primary NPC [58]. This finding highlights the importance of identifying patients suitable for adjuvant therapy before they are entered into clinical trials. Therefore, subjects identified as having an unfavourable response (TLG reduction ratio of ≤0.6) in this study may be suitable candidates for adjuvant therapy in a future clinical trial. For example, adjuvant immunotherapy has emerged as a promising therapeutic strategy in patients with NPC who have failed definitive treatment [59].

In the present study, patient age, TLG reduction ratio, and EBV DNA titre measured during radiotherapy were independent predictors of OS. Metabolic parameters on pretreatment PET have been confirmed to be prognostically superior to traditional risk factors in patients with NPC [13, 23]. Recently, imaging parameters based on ^{18}F-FDG PET measured during therapy have also been shown to be independent predictors of survival [27]. Other studies have demonstrated that the pretherapy EBV DNA load has prognostic importance in NPC, which might also be interpreted as the baseline tumour burden and radiosensitivity [2, 7, 8, 13, 60–62]. In our study, a higher pretreatment EBV DNA titre also tended to predict a worse OS ($p = 0.086$). Patients with a detectable EBV DNA load during CCRT had an even lower OS (HR 4.173, $p < 0.001$). This finding is supported by the work of He et al. [29] who investigated the prognostic value of the dynamic change in EBV DNA load during treatment for NPC. Their results highlight the value of identifying patients with poorer OS by assessing EBV DNA load at later time points. Taking the results of previous studies and those of the present study into account, it appears that the ^{18}F-FDG PET parameters and EBV DNA titre measured during

radiotherapy predict OS in patients with primary NPC better than pretreatment values.

Although the metabolic parameters assessed on ^{18}F-FDG PET and the EBV DNA titre are considered important prognostic biomarkers for primary NPC, they reflect different biological features and therefore may have complementary roles in predicting outcomes. It has been reported that up to 15% of patients with primary NPC and undetectable EBV DNA before treatment will eventually relapse [63]. Therefore, the ^{18}F-FDG PET parameter would be more useful in this situation (see figure in Online Resource 6). In this study, the EBV DNA load and reduction in TLG during radiotherapy combined with patient age enabled better stratification of survival. We devised a scoring system that had a range from 0 to 2/3 for the prediction of OS according to the number of independent risk factors present. Survival was poorer in patients with a score of 1 (HR 4.756, $p = 0.074$) and was much poorer in those with a score of 2/3 (HR 18.973, $p = 0.001$; Fig. 4). Using this proposed scoring system, it would be possible to restratify patients and modify their treatment strategies on an individual basis; for example, add-on treatment in those with a higher score and a reduction in treatment intensity in those with a favourable score. Patients with a high score would be good candidates for a strict surveillance protocol to detect early tumour recurrence or to monitor their health status after completion of chemoradiotherapy.

Our study had several limitations. First, the study population was relatively small, which might have adversely affected the statistical analysis. Second, the cut-off value used for the baseline EBV DNA titre was different from that used in previous studies. This might reflect the fact that most patients in our study were initially diagnosed at an advanced stage of NPC. These issues require clarification by future research in a larger patient population.

Conclusion

In summary, our preliminary data indicate that the TLG reduction ratio and EBV DNA titre obtained during radiotherapy are early biomarkers predicting survival in patients with primary NPC after definitive treatment. These biomarkers may enable early identification of patients at risk of a persistent tumour or mortality, and may facilitate the design of more biomarker-integrated clinical trials to optimize individualized treatment strategies.

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Compliance with ethical standards

Conflicts of interest None.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All patients whose data are included in this manuscript signed a written informed consent.

Informed consent Informed consent was obtained from all the study participants.

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