European Journal of Radiology 114 (2019) 99–104

Contents lists available at ScienceDirect

European Journal of Radiology

journal homepage: www.elsevier.com/locate/ejrad

Research article

# The prognostic significance of intratumoral heterogeneity of 18F-FDG uptake in patients with oral cavity squamous cell carcinoma

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ARTICLE INFO

*Keywords:*
Oral squamous cell carcinoma
Positron emission tomography
Heterogeneity index
Heterogeneity factor
Prognostic marker

ABSTRACT

*Purpose:* This study aimed to evaluate the prognostic significance of two major indices of intratumoral heterogeneity of 18F-fluorodeoxyglucose uptake by positron emission tomography (PET)/computed tomography (CT), namely heterogeneity index (HI) and heterogeneity factor (HF), in patients with oral squamous cell carcinoma.

*Methods:* We performed a retrospective analysis of 62 patients who underwent resective surgery. HI, HF, maximum standardized uptake value ($SUV_{max}$), metabolic tumor volume (MTV), and total lesion glycolysis (TLG) were obtained from pretreatment PET. HI was obtained by dividing $SUV_{max}$ by $SUV_{mean}$ for the primary lesion; HF was obtained by taking the derivative (dV/dT) of the volume-threshold function from 30 to 70%. Univariate and multivariate analyses for the overall survival (OS) and disease-free survival (DFS) were performed using PET and clinicopathological parameters.

*Results:* Univariate and multivariate analyses of OS revealed that higher HI levels (threshold for the $SUV_{mean}$ is 30% of the $SUV_{max}$) were associated with poorer OS [hazard ratio (HR) = 11.57; 95% confidence interval (CI) = 1.45–92.28; $P = 0.021$]. Moreover, univariate and multivariate analyses of DFS revealed that higher TLG levels (threshold for the MTV and $SUV_{mean}$ is 4.0 of the SUV) were associated with poorer DFS (HR = 14.48; 95% CI = 1.27–164.78; $P = 0.031$).

*Conclusions:* HI and TLG may be statistically significant prognostic factors for OS and DFS, respectively.

## 1. Introduction

Surgery is the standard of care that provides initial definitive treatment for the majority of oral cavity squamous cell carcinomas (OSCC) [1,2]. However, various factors such as the size, location of the primary tumor, lymph node metastasis, and distant metastasis affect the treatment choice. Although many studies have reported various clinical, pathological, and radiographic prognostic factors in patients with OSCC, more accurate prognostic factors have not yet been identified for selecting the appropriate treatment for OSCC. Furthermore, these novel prognostic factors may improve risk stratification and promote the individualization of cancer treatment plans [3].

The Union for International Cancer Control (UICC) and the American Joint Committee on Cancer staging systems are generally accepted for staging. However, the prognostic value of these staging systems is limited as they are based on extent of primary tumor, regional lymph node metastasis, and distant metastasis and not on individual biological and molecular characteristics.

Positron emission tomography (PET)/computed tomography (CT) with the radioactive tracer 18F-fluorodeoxyglucose (FDG) has been used for initial diagnosis and staging workup in patients with OSCC. The maximum standardized uptake value ($SUV_{max}$), the most widely used PET parameter, is the highest SUV from a single voxel within a volume of interest (VOI). Although the $SUV_{max}$ has been reported as a prognostic factor in OSCC [4,5], it has some limitations as it reflects a single voxel value. It may not represent the overall metabolism of the tumor, particularly the tumor with heterogeneous features.

Recently, there has been an increasing interest in assessing the tumor heterogeneity and, specifically, intratumoral heterogeneity of 18F-FDG uptake. The heterogeneity index (HI) and heterogeneity factor

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https://doi.org/10.1016/j.ejrad.2019.03.004
Received 14 September 2018; Received in revised form 7 March 2019; Accepted 9 March 2019

(HF) are two quantitative measures of the intratumoral heterogeneity of 18F-FDG uptake [6–11]. HI can be easily calculated in clinical practice by dividing the $SUV_{max}$ with the $SUV_{mean}$ for the primary lesion [6–8]. HF is calculated as the slope from the linear regression of the threshold-volume curve [9–11]. Although these two major factors to measure the intratumoral heterogeneity demonstrated a prognostic value in various cancer types, no previous study has compared the potential prognostic values of these factors [6–12]. To the best of our knowledge, HI has not been examined to date; however, HF has been reported to be a prognostic factor for OS in patients with OSCC [11].

Volumetric PET parameters, such as metabolic tumor volume (MTV) and total lesion glycolysis (TLG), are gaining increasing interest in cancer research. MTV is defined as the total tumor volume segmented via the threshold SUV. TLG can be calculated by multiplying the MTV with the $SUV_{mean}$, which reflects both metabolic activity and volume [13]. Previous studies have suggested an association of these PET parameters with the clinical outcomes of patients with OSCC [3,14–16], however with conflicting results in some series [17].

All PET parameters, except $SUV_{max}$, are dependent on specific thresholds, such as voxel-wise SUV or percentage of $SUV_{max}$. However, the choice of SUV threshold levels for PET parameters is still under debate.

The aim of this study was to assess potential prognostic values of PET parameters, including $SUV_{max}$, HI, HF, MTV, and TLG in patients with OSCC.

## 2. Materials and methods

### 2.1. Patient selection

We performed a retrospective analysis of consecutive patients with pathologically proven OSCC who received treatment at our hospital between September 2008 and January 2017. A flow chart of the patient selection process is shown in Fig. 1. 62 patients who received treatment by undergoing resective surgery were enrolled and baseline PET examinations were performed on all patients prior to surgery. All patients with less than 6 months follow-up after surgery were excluded from the study.

Patient records were analyzed for prognostic factors, such as gender, age, size and location of the primary tumor, clinical T and N classification, histological differentiation, perineural and lymphovascular invasion, and resection margin. Samples were staged according to the UICC staging system (7th edition).

Patient and tumor characteristics were collected until last follow-up, death of the patient, or cutoff date for data collection for this study (June 20, 2017).

This study protocol was approved by the clinical research ethics committee at our hospital (No. 20170427-6).

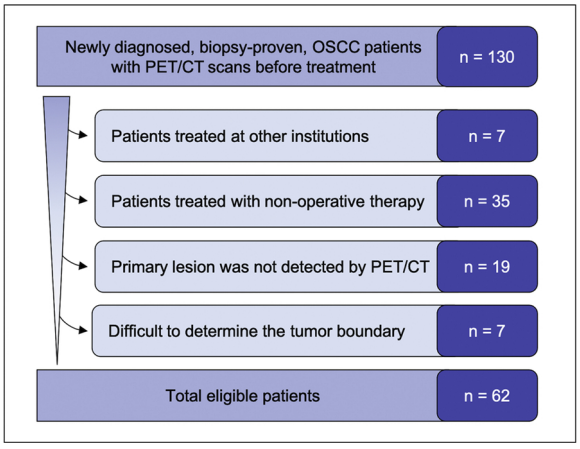


**Fig. 1.** Flow chart of the patient selection process for this study.

### 2.2. PET/CT imaging

PET/CT scans were performed using a Biograph 16 (Siemens Healthcare GmbH, Erlangen, Germany). All patients were requested to fast at least 5 h before undergoing PET/CT. A dose of 2.7–6.8 MBq/kg (median, 4.36) of FDG was injected intravenously. At the time of FDG injection, blood glucose level was 104 ± 25 mg/dl (mean ± standard deviation). Before undergoing PET, a non-contrast CT was performed at 60 min after FDG injection using a 16-slice helical CT scanner. Patients then underwent 3D-mode emission scans of the region between the thigh and head with the arms down. Scan duration was approximately 1.0–2.5 min per frame, depending on patient body weight. PET images were reconstructed iteratively with CT attenuation correction.

### 2.3. Image analysis

The FDG PET/CT data were reviewed by a board-certified nuclear medicine physician with more than 8 years of clinical experience in head and neck imaging using a dedicated workstation (syngo Multimodality Workplace, Siemens Healthcare GmbH, Erlangen, Germany). For each case, the observer read the images in random order blinded to the patient outcomes.

The FDG uptake was quantitatively assessed by calculating the SUV in a defined VOI. The tumor boundaries were identified and if necessary manually extended to include the entire tumor volume. Areas of physiological uptake were carefully excluded. Glucose metabolic activity was quantified using the SUV normalized by body weight. The $SUV_{max}$ and $SUV_{mean}$ of the primary tumor were calculated.

MTV was defined as the total tumor volume segmented by the threshold SUV (Fig. 2A–F). The threshold level for the SUV was selected using the cutoff values of 2.5, 3.0, 3.5, and 4.0, defined as $MTV_{2.5}$, $MTV_{3.0}$, $MTV_{3.5}$, and $MTV_{4.0}$, respectively. Additionally, $MTV_{30\%}$, $MTV_{40\%}$, $MTV_{50\%}$, $MTV_{60\%}$, and $MTV_{70\%}$ were calculated as the tumor volume, with 30%, 40%, 50%, 60%, and 70% of the $SUV_{max}$ as the threshold, respectively (Fig. 2D–G).

TLG was calculated by multiplying the threshold SUVs of the MTV with the $SUV_{mean}$ ($TLG_{2.5–4.0}$ and $TLG_{30\%–70\%}$):

$$TLG_x = MTV_x \times SUV_{meanx}$$

[values are dependent on chosen threshold (= $x$)]

HI was quantified by dividing the $SUV_{max}$ with the $SUV_{mean}$ of the primary lesion ($HI_{2.5–4.0}$ and $HI_{30\%–70\%}$) [6–8]:

$$HI_x = SUV_{max} / SUV_{meanx}$$

[values are dependent on chosen threshold (= $x$)]

Additionally, linear regression analysis was performed, and HF was calculated by finding the derivative (dV/dT) of the volume-threshold function for each tumor using Microsoft Excel for Mac, version 16.19. The MTV threshold range of 30%–70% was assigned by modifying a previous method [11] (Fig. 2D–G). HF had a more negative value that reflected a more heterogeneous tissue in the tumor.

### 2.4. Setting of cutoff values

For the assessment of patient outcomes, we calculated the overall survival (OS) and disease-free survival (DFS) using the Kaplan–Meier method. OS was calculated using the number of days from the initiation of treatment until death due to any cause for event case, and until date of censoring (June 20, 2017) or date of 5-year follow-up, whichever was earlier, for censored case.

DFS was calculated as the number of days from the initiation of treatment to locoregional recurrence, new onset of distant metastasis, or death due to any cause, whichever was earlier. Patients without

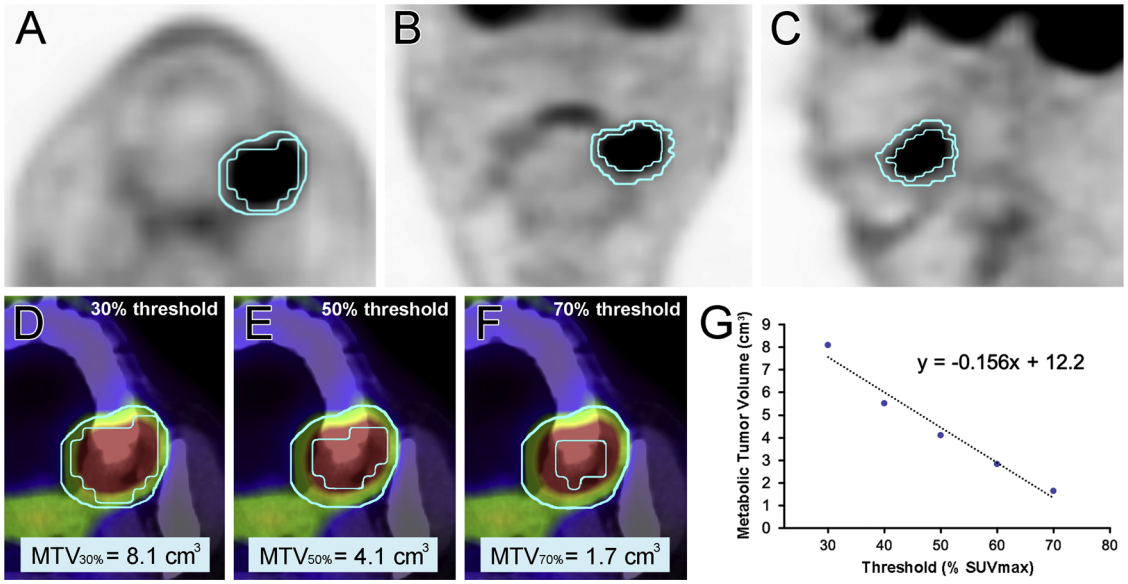


**Fig. 2.** Methods for calculating indices of intratumoral heterogeneity.
PET/CT images from 73-year-old male patient with squamous cell carcinoma of the left upper gingiva who was treated with partial maxillectomy. Postoperative course was uneventful. PET images depicting manually drawn MTV on three planes are shown: axial plane (A), coronal plane (B), and sagittal plane (C). MTV was obtained by delineating the volume of interest using different $SUV_{max}$ thresholds. Decreasing tumor volumes, defined by 30% to 70% thresholds of $SUV_{max}$, are shown in (D, E and F). Decreasing tumor volumes are plotted graphically; HF was calculated as the slope of the threshold-volume curve (HF = −0.1558) (G). $HI_{30\%}$ was calculated by dividing $SUV_{max}$ (11.5) by $SUV_{mean}$ (6.2), resulting in a $HI_{30\%}$ of 1.85.

disease recurrence or death were censored at the date of their last tumor assessment or 5-year follow-up, whichever was earlier.

Receiver operating characteristic (ROC) curve analyses were performed to determine the cutoff value for PET parameters, including SUVmax, MTV, TLG, HI, and HF. The cutoff value with optimum balance of sensitivity and specificity was used for prediction of each endpoint (OS and DFS). Furthermore, the area under the ROC curve (AUC) was used as a measure of how well the PET parameters can distinguish between two groups as prognostic factors.

### 2.5. Statistical analysis

The OS and DFS curves were calculated using the Kaplan–Meier method. A univariate analysis was performed using the log–rank test, and the survival times for all prognostic factors were compared. Variables with probability ($P$) value < 0.05 (two-tailed) were further selected for multivariate analyses, which were performed using the Cox proportional hazards model with forced-entry method. In the multivariate analyses, an alpha level for indication of statistical significance was set at a $P$ value < 0.05 (two-tailed). All statistical analyses were performed using IBM SPSS Statistics (Version 20; IBM Corporation, Armonk, New York, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan). EZR is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [18].

## 3. Results

The patient characteristics are summarized in Table 1 (n = 62). The median follow-up duration was 42 months (range, 6.5–100.7 months). All patients underwent wide resection of the primary tumor with curative intent. Unilateral neck dissection was performed in 20 patients, and bilateral dissection in 4 patients. Nodal metastases were identified and defined according to physical examination, contrast-enhanced computed tomography (CECT) and/or magnetic resonance imaging of the neck, ultrasound, and PET/CT.

At the end of the follow-up, 46 of the 62 patients had no evidence of

**Table 1**
Clinicopathological characteristics of the study patients.

| Characteristics | Number (n = 62) | % |
|---|---|---|
| Age | | |
| Median (year) | 67.3 | |
| Range (year) | 32-88 | |
| Gender | | |
| Male | 36 | 58.1 |
| Female | 26 | 41.9 |
| Site of primary tumor | | |
| Tongue | 27 | 43.5 |
| Gingiva | 24 | 38.7 |
| Floor of mouth | 6 | 9.7 |
| Buccal mucosa | 5 | 8.1 |
| cT classification | | |
| T1 | 21 | 33.9 |
| T2 | 23 | 37.1 |
| T3 | 5 | 8.1 |
| T4 | 13 | 21.0 |
| cN classification | | |
| N0 | 45 | 72.6 |
| N1 | 5 | 8.1 |
| N2 | 12 | 19.4 |
| TNM stage | | |
| I | 21 | 33.9 |
| II | 16 | 25.8 |
| III | 6 | 9.7 |
| IV | 19 | 30.6 |
| Differentiation | | |
| Well differentiated | 34 | 54.8 |
| Moderately differentiated | 23 | 37.1 |
| Poorly differentiated | 5 | 8.1 |
| Positive margin | | |
| No | 58 | 93.5 |
| Yes | 4 | 6.5 |
| Lymphovascular invasion | | |
| No | 51 | 82.3 |
| Yes | 11 | 17.7 |
| Perineural invasion | | |
| No | 57 | 91.9 |
| Yes | 5 | 8.1 |

**Table 2**
Univariate and multivariate analysis of PET parameters for overall survival.

| | | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|---|
| Variables | Number | HR | 95 % CI | *P* value | HR | 95 % CI | *P* value |
| $SUV_{max}$ | | | | | | | |
| < 8.12 | 30 | 1 | | | 1 | | |
| ≥8.12 | 32 | 10.79 | 1.38-84.48 | 0.005 | 6.15 | 0.34-7.83 | 0.089 |
| $MTV_{4.0}$ | | | | | | | |
| < 2.05 | 28 | -[a] | | | – | | |
| ≥2.05 | 34 | -[a] | -[a] | 0.001 | – | – | -[b] |
| $TLG_{4.0}$ | | | | | | | |
| < 10.84 | 29 | -[a] | | | – | | |
| ≥10.84 | 33 | -[a] | -[a] | 0.0005 | – | – | -[b] |
| $HI_{30\%}$ | | | | | | | |
| < 1.96 | 38 | 1 | | | 1 | | |
| ≥1.96 | 24 | 17.21 | 2.20-134.50 | 0.0002 | 11.57 | 1.45-92.28 | 0.021 |
| HF | | | | | | | |
| ≥-0.145 | 29 | 1 | | | 1 | | |
| < -0.145 | 33 | 4.17 | 0.90-19.31 | 0.047 | 1.64 | 0.34-7.83 | 0.534 |

$SUV_{max}$, maximum standardized uptake value; MTV, metabolic tumor volume; TLG, total lesion glycolysis; HI, heterogeneity index; HF, heterogeneity factor; HR, hazard ratio; CI, confidence interval.

[a] HRs and 95% CIs could not be calculated using $MTV_{4.0}$ and $TLG_{4.0}$ values, because their calculations did not converge.

[b] $MTV_{4.0}$ and $TLG_{4.0}$ were excluded from the multivariate analysis, because they showed a large standard error.

**Table 3**
Univariate and multivariate analysis of PET parameters for disease-free survival.

| | | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|---|
| Variables | Number | HR | 95 % CI | *P* value | HR | 95 % CI | *P* value |
| $SUV_{max}$ | | | | | | | |
| < 8.12 | 30 | 1 | | | 1 | | |
| ≥8.12 | 32 | 3.78 | 1.38-10.37 | 0.006 | 0.66 | 0.11-4.13 | 0.661 |
| $MTV_{2.5}$ | | | | | | | |
| < 10.80 | 32 | 1 | | | 1 | | |
| ≥10.80 | 30 | 3.03 | 1.17-7.84 | 0.016 | 0.47 | 0.11-1.99 | 0.305 |
| $TLG_{4.0}$ | | | | | | | |
| < 10.84 | 29 | 1 | | | 1 | | |
| ≥10.84 | 33 | 5.11 | 1.71-15.29 | 0.001 | 14.48 | 1.27-164.78 | 0.031 |
| $HI_{2.5}$ | | | | | | | |
| < 2.21 | 33 | 1 | | | – | | |
| ≥2.21 | 29 | 2.51 | 1.01-6.24 | 0.041 | – | – | -[a] |
| HF | | | | | | | |
| ≥-0.101 | 15 | 1 | | | – | | |
| < -0.101 | 47 | 3.72 | 0.87-15.99 | 0.058[b] | – | – | -[b] |

$SUV_{max}$, maximum standardized uptake value; MTV, metabolic tumor volume; TLG, total lesion glycolysis; HI, heterogeneity index; HF, heterogeneity factor; HR, hazard ratio; CI, confidence interval.

[a] $HI_{2.5}$ was excluded from multivariate analysis due to multicollinearity.

[b] HF was excluded from the multivariate analysis, because it was not a significant predictor in univariate analysis ($P > 0.05$).

disease, two patients were alive with the disease, 10 patients died due to the disease, and four patients died due to unrelated causes. Additionally, five patients experienced local recurrences; neck and distant metastasis occurred in 14 and two patients, respectively.

The cutoff values for $SUV_{max}$ for the prediction of OS and DFS were 8.12 with the AUC being 0.697 and 0.660, respectively. We used the $MTV_{4.0}$, $TLG_{4.0}$, and $HI_{30\%}$ to show the highest AUC for prediction of the OS and $MTV_{2.5}$, $TLG_{4.0}$, and $HI_{2.5}$ to predict the DFS. The cutoff values for HF for the prediction of OS and DFS were −0.145 and −0.101, with the AUC being 0.665 and 0.587, respectively.

Univariate analysis for OS revealed that PET parameters, including the $SUV_{max}$, $MTV_{4.0}$, $TLG_{4.0}$, $HI_{30\%}$, and HF, were significant predictors ($P < 0.05$), as shown in Table 2. Univariate analysis for DFS revealed that the $SUV_{max}$, $MTV_{2.5}$, $TLG_{4.0}$, and $HI_{2.5}$ were significant predictors ($P < 0.05$), as shown in Table 3.

No other factors were significantly associated with OS and DFS ($P > 0.05$; data not shown).

PET parameters with $P < 0.05$ including $SUV_{max}$, $MTV_{4.0}$, $TLG_{4.0}$, $HI_{30\%}$ and HF were selected for multivariate analyses of OS using the Cox proportional hazard model. As a result of multivariate analyses, $MTV_{4.0}$ and $TLG_{4.0}$ were not independent prognostic factors for OS (data not shown). Additionally, these PET parameters showed a large standard error and were therefore excluded from the multivariate analysis of OS. Finally, higher $HI_{30\%}$ (≥1.96) was associated with worse OS [hazard ratio (HR) = 11.57; 95% confidence interval (CI) = 1.45–92.28; $P = 0.021$; Table 2]. A representative case of high $HI_{30\%}$ is shown in Fig. 3.

$HI_{2.5}$ was excluded from multivariate analysis of DFS due to multicollinearity. Higher $TLG_{4.0}$ (≥10.84) was associated with worse DFS (HR = 14.48; 95% CI = 1.27–164.78; $P = 0.031$; Table 3).

Kaplan–Meier curves of OS rates for patients with $HI_{30\%}$ lower than (solid line) and greater than or equal to the cutoff value (dashed line) are presented in Fig. 4A. The 5-year OS rates were 95.8% and 45.8%, respectively ($P = 0.0002$; Fig. 4A). The Kaplan–Meier curves for DFS in patients with a $TLG_{4.0}$ < 10.84 or ≥10.84 are presented in Fig. 4B.

## 4. Discussion

OSCC treatment strategies have evolved in recent years, resulting in reduced rates of disease progression and increased survival rates [19]. Clinical and pathological factors, such as TNM classification, tumor thickness, volume, differentiation of the primary tumor, perineural and lymphovascular invasion, and resection margin have been reported as prognostic factors in patients with OSCC [19–25]. Furthermore, among the PET parameters, the $SUV_{max}$ of the primary tumor site was the most common prognostic factor for OS or DFS [4,5]. In the present study, multivariate analysis revealed that $SUV_{max}$ is not a significant prognostic factor for OS and DFS.

The $SUV_{max}$ is a simple and reproducible 18F-FDG PET parameter because it is less affected by physician-dependent differences in the VOI setting [26]. However, the $SUV_{max}$ is highly sensitive to noise and may not be representative of the metabolic activity of the entire tumor, although it reflects the part of the tumor with the highest metabolic activity [26]. HI may be significantly influenced by $SUV_{max}$. However, the HI value reflects not only the part of the tumor area with the highest metabolic activity but also the metabolic activity of the entire tumor represented by $SUV_{mean}$.

Although HI is simple, can accurately predict prognosis, and has been reported as a prognostic factor in locally advanced nasopharyngeal carcinoma [8], to the best of our knowledge, no previous study has evaluated the prognostic value of HI in patients with OSCC. Our findings showed that HI is a statistically significant prognostic factor for OS in patients with OSCC treated with primary surgery.

Obtaining the HF is a more complicated process than obtaining the HI. Kwon et al. reported that HF may be a significant prognostic factor for OS, in addition to cervical lymph node metastasis, in patients with OSCC [11]. However, the study sample included patients with OSCC who had received only radiotherapy treatment [11]. Our study selected only patients with OSCC who underwent curative intent surgery as the first treatment modality and excluded those who had been treated by chemotherapy, radiotherapy, or both as the first treatment. In the present study, the threshold range for calculating HF of 30%–70% was assigned by modifying a previous method [11]. It has previously been

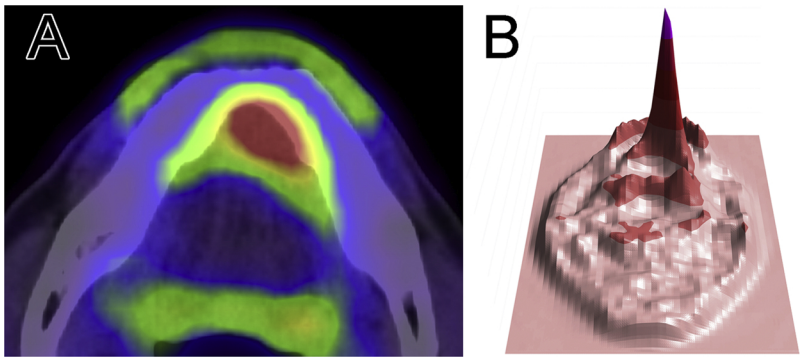


**Fig. 3.** Representative case with a high $HI_{30\%}$ value.
PET/CT image (A) and 3D plot of SUV (B) are shown, which were taken from a 51-year-old male patient with squamous cell carcinoma of the floor of mouth measuring 2.4 × 1.9 cm in diameter. The patient was treated with tumor resection combined with segmental mandibulectomy and bilateral neck dissection. The patient developed a recurrence 8 months after surgery and died from the disease 30 months after surgery. This case presented with a high $HI_{30\%}$ ($SUV_{max}$ = 11.9, $SUV_{mean}$ = 5.5, and $HI_{30\%}$ = 2.16).

reported that the minimum threshold representing actual tumor volume is 40%, and that values of < 40% include too much background activity from normal tissue in patients with advanced cervical cancer [27]. However, optimal threshold range to calculate HF in patients with OSCC has not yet been reported. Therefore, we used a threshold range of 30%–70% because it was possible to evaluate the tumor boundaries with this threshold range in the present study.

The main advantage of our study is that this is the first study to use the HI in a patient with OSCC. Additionally, no study has reported a direct comparison between the HI and HF. As a result, our findings suggest that HI is a better statistically significant prognostic factor for OS than HF. The size of the primary tumor may affect both HI and HF values. In the present study, the minimum primary tumor size outlined by PET was 1 cm in diameter. Kwon et al. also previously excluded patients with tumors smaller than 1 cm when evaluating HF in OSCC patients [11]. Thus, we considered that reliable evaluation of primary lesions requires a minimum lesion size of approximately 1 cm in diameter.

Another widely-used method for evaluating the heterogeneity using PET is texture analysis [28]. However, no PET textural analysis parameters are widely accepted for measuring the tumor heterogeneity [12]. In addition, the textural analysis is difficult to assess in clinical practice due to difficulty in acquiring the measurement (another software should be used, not including the standard workstation) [12]. Therefore, we used the two methods mentioned above to measure the intratumoral heterogeneity because of their simplicity.

The volumetric PET parameters MTV and TLG have also been reported to be superior to the $SUV_{max}$ as prognostic parameters [3,14–16]. The measurement of tumor burden in terms of MTV on PET was adopted to complement the limitations of $SUV_{max}$ [29]. However, MTV cannot reflect the part of the tumor with the highest metabolic activity. For this reason, the prognostic value of MTV is limited [29].

In our study, multivariate analysis revealed that TLG is the only statistically significant prognostic factor for DFS. TLG was initially suggested by Larson et al. and is defined as the MTV multiplied with the $SUV_{mean}$ within that volume [13]. TLG reflects both metabolic activity and volume and was proposed as a quantitative index of tumor metabolism [13]. Several studies have reported that TLG is a reliable prognostic factor even when compared with common prognostic factors such as $SUV_{max}$, histological differentiation, and resection margin [3,15]. Our results support these studies. In contrast, Kendi et al. reported that there is no association between the PET parameters, including MTV and TLG, and the clinical outcome in patients with OSCC [17]. They also suggested that the CECT ring/heterogeneous enhancement pattern of the primary tumor is an indicator of poor prognosis [17]. Therefore, we thought that it was better to use both PET/CT and CECT to predict patient's outcome.

The choice of the threshold level of SUV is controversial. In routine clinical practice, a certain SUV such as 2.5, 3.0, or percentages of $SUV_{max}$ such as 30%–50% are widely used to determine the MTV [30]. Van de Wiele et al. suggested that while the choice of the threshold for either method may affect the absolute value of the MTV [31], if used within a reasonable range, the consistency of the measurements will not be affected. This suggests that MTV is a potential biomarker that should be evaluated for prognosis assessment [31].

Our study had several limitations, including a relatively short follow-up period and a small number of samples. Furthermore, treatment modalities were heterogeneous, particularly those of the chemotherapy regimen for patients with recurrent or metastatic diseases. Heterogeneous treatment modalities may have influenced the patient's outcome. Although our results showed that HI was the statistically significant prognostic factor for OS, there was a wide confidence

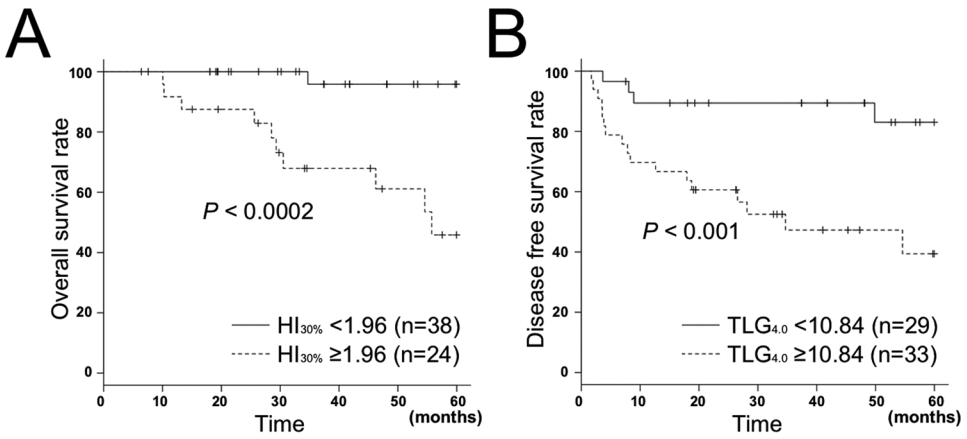


**Fig. 4.** Kaplan–Meier survival curves according to significant prognostic factors.
(A) Kaplan–Meier curves of OS according to $HI_{30\%}$ (n = 62). Upper line $HI_{30\%}$ < 1.96; lower line $HI_{30\%}$ ≥1.96, $P$ = 0.0002. (B) Kaplan–Meier curves of DFS according to $TLG_{4.0}$ (n = 62). Upper line $TLG_{4.0}$ < 10.84; lower line $TLG_{4.0}$ ≥10.84, $P$ = 0.001.

interval, which may indicate an inadequate sample size and/or higher dispersion of the samples. Therefore, a large-scale prospective study with a unified treatment modality will be required to confirm the prognostic factors in patients with OSCC. Additionally, interpretation of FDG PET/CT studies can be difficult because of the unusual patterns of high FDG uptake in the head and neck because FDG is not a tumor-specific tracer [32]. In particular, some patients with oral cavity cancers show significant FDG uptake because of inflammatory conditions (including periodontal disease and dental infection). Furthermore, physiological uptake of FDG is commonly observed in the lymphoid tissue of the Waldeyer's ring, major and minor salivary glands [32]. FDG uptake in these areas may obscure tumor boundaries and make diagnosis difficult. In the present study, we excluded seven cases showing unclear tumor boundaries because of these reasons (Fig.1). This process and the exclusion of cases not depicted by PET/CT (n = 19) may have influenced the negative result from univariate analysis on the ability of clinicopathological parameters, including clinical T and N classification and histological differentiation, to predict patient outcome.

## 5. Conclusions

Our findings revealed that HI is a statistically significant prognostic factor for OS in patients with OSCC than other PET parameters, including $SUV_{max}$ and HF. Additionally, our study suggested that TLG is the only statistically significant prognostic factor for DFS. These results highlighted the potential of using these parameters together to predict patient's outcome.

## Conflict of interest

All authors have no conflict of interest to disclose.

## Acknowledgement

We thank Shigeaki Ohtsuki, PhD, President of Japan Institute of Statistical Technology, for technical support and advice on statistical analyses.

## References

[1] P.H. Montero, S.G. Patel, Cancer of the oral cavity, Surg. Oncol. Clin. N. Am. 24 (3) (2015) 491.
[2] D.G. Pfister, S. Spencer, D.M. Brizel, B. Burtness, P.M. Busse, J.J. Caudell, A.J. Cmelak, A.D. Colevas, F. Dunphy, D.W. Eisele, Head and neck cancers, version 1.2015, J. Natl. Compr. Cancer Netw. 13 (7) (2015) 847–856.
[3] Y.G. Abd El-Hafez, H.M. Moustafa, H.F. Khalil, C.T. Liao, T.C. Yen, Total lesion glycolysis: a possible new prognostic parameter in oral cavity squamous cell carcinoma, Oral Oncol. 49 (3) (2013) 261–268.
[4] S.Y. Kim, J.L. Roh, J.S. Kim, C.H. Ryu, J.H. Lee, K.J. Cho, S.H. Choi, S.Y. Nam, Utility of FDG PET in patients with squamous cell carcinomas of the oral cavity, Eur. J. Surg. Oncol. 34 (2) (2008) 208–215.
[5] H. Suzuki, Y. Hasegawa, A. Terada, I. Hyodo, T. Nakashima, M. Nishio, T. Tamaki, FDG-PET predicts survival and distant metastasis in oral squamous cell carcinoma, Oral Oncol. 45 (7) (2009) 569–573.
[6] J. Salamon, T. Derlin, P. Bannas, J.D. Busch, J. Herrmann, M. Bockhorn, C. Hagel, R.E. Friedrich, G. Adam, V.F. Mautner, Evaluation of intratumoural heterogeneity on (1)(8)F-FDG PET/CT for characterization of peripheral nerve sheath tumours in neurofibromatosis type 1, Eur. J. Nucl. Med. Mol. Imaging 40 (5) (2013) 685–692.
[7] A.K. Tahari, K.C. Alluri, H. Quon, W. Koch, R.L. Wahl, R.M. Subramaniam, FDG PET/CT imaging of oropharyngeal squamous cell carcinoma: characteristics of human papillomavirus-positive and -negative tumors, Clin. Nucl. Med. 39 (3) (2014) 225–231.
[8] Z. Yang, Q. Shi, Y. Zhang, H. Pan, Z. Yao, S. Hu, W. Shi, B. Zhu, Y. Zhang, C. Hu, Pretreatment (18)F-FDG uptake heterogeneity can predict survival in patients with locally advanced nasopharyngeal carcinoma–a retrospective study, Radiat. Oncol. 10 (2015) 4.
[9] B. Huang, T. Chan, D.L. Kwong, W.K. Chan, P.L. Khong, Nasopharyngeal carcinoma: investigation of intratumoral heterogeneity with FDG PET/CT, AJR Am. J. Roentgenol. 199 (1) (2012) 169–174.
[10] E.A. Kidd, P.W. Grigsby, Intratumoral metabolic heterogeneity of cervical cancer, Clin. Cancer Res. 14 (16) (2008) 5236–5241.
[11] S.H. Kwon, J.K. Yoon, Y.S. An, Y.S. Shin, C.H. Kim, D.H. Lee, K.S. Jo, S.J. Lee, Prognostic significance of the intratumoral heterogeneity of (18) F-FDG uptake in oral cavity cancer, J. Surg. Oncol. 110 (6) (2014) 702–706.
[12] Y.I. Kim, Y.J. Kim, J.C. Paeng, G.J. Cheon, D.S. Lee, J.K. Chung, K.W. Kang, Heterogeneity index evaluated by slope of linear regression on (18)F-FDG PET/CT as a prognostic marker for predicting tumor recurrence in pancreatic ductal adenocarcinoma, Eur. J. Nucl. Med. Mol. Imaging 44 (12) (2017) 1995–2003.
[13] S.M. Larson, Y. Erdi, T. Akhurst, M. Mazumdar, H.A. Macapinlac, R.D. Finn, C. Casilla, M. Fazzari, N. Srivastava, H.W. Yeung, J.L. Humm, J. Guillem, R. Downey, M. Karpeh, A.E. Cohen, R. Ginsberg, Tumor treatment response based on visual and quantitative changes in global tumor glycolysis using PET-FDG imaging. The visual response score and the change in total lesion glycolysis, Clin. Positron Imaging 2 (3) (1999) 159–171.
[14] S.J. Lee, J.Y. Choi, H.J. Lee, C.H. Baek, Y.I. Son, S.H. Hyun, S.H. Moon, B.T. Kim, Prognostic value of volume-based (18)F-fluorodeoxyglucose PET/CT parameters in patients with clinically node-negative oral tongue squamous cell carcinoma, Korean J. Radiol. 13 (6) (2012) 752–759.
[15] I.S. Ryu, J.S. Kim, J.L. Roh, K.J. Cho, S.H. Choi, S.Y. Nam, S.Y. Kim, Prognostic significance of preoperative metabolic tumour volume and total lesion glycolysis measured by (18)F-FDG PET/CT in squamous cell carcinoma of the oral cavity, Eur. J. Nucl. Med. Mol. Imaging 41 (3) (2014) 452–461.
[16] H. Zhang, H. Seikaly, N.T. Nguyen, J.T. Abele, P.T. Dziegielewski, J.R. Harris, D.A. O'Connell, Validation of metabolic tumor volume as a prognostic factor for oral cavity squamous cell carcinoma treated with primary surgery, Oral Oncol. 57 (2016) 6–14.
[17] A.T. Kendi, A. Corey, K.R. Magliocca, D.C. Nickleach, J. Galt, J.M. Switchenko, M.W. El-Deiry, J.T. Wadsworth, P.A. Hudgins, N.F. Saba, D.M. Schuster, 18F-FDG-PET/CT parameters as imaging biomarkers in oral cavity squamous cell carcinoma, is visual analysis of PET and contrast enhanced CT better than the numbers? Eur. J. Radiol. 84 (6) (2015) 1171–1176.
[18] Y. Kanda, Investigation of the freely available easy-to-use software' EZR' for medical statistics, Bone Marrow Transplant. 48 (3) (2013) 452–458.
[19] P. Garzino-Demo, E. Zavattero, P. Franco, M. Fasolis, G. Tanteri, A. Mettus, P. Tosco, L. Chiusa, M. Airoldi, O. Ostellino, M. Schena, M. Rampino, U. Ricardi, A. Evangelista, F. Merletti, S. Berrone, G. Ramieri, Parameters and outcomes in 525 patients operated on for oral squamous cell carcinoma, J. Craniomaxillofac. Surg. 44 (9) (2016) 1414–1421.
[20] J.S. Cooper, T.F. Pajak, A.A. Forastiere, J. Jacobs, B.H. Campbell, S.B. Saxman, J.A. Kish, H.E. Kim, A.J. Cmelak, M. Rotman, Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck, N. Engl. J. Med. 350 (19) (2004) 1937–1944.
[21] M.J. Zelefsky, L.B. Harrison, D.E. Fass, J.G. Armstrong, J.P. Shah, E.W. Strong, Postoperative radiation therapy for squamous cell carcinomas of the oral cavity and oropharynx: impact of therapy on patients with positive surgical margins, Int. J. Radiat. Oncol. Biol. Phys. 25 (1) (1993) 17–21.
[22] C.S. Lin, A.B. de Oliveira Santos, E.L. Silva, L.L. de Matos, R.A. Moyses, M.A. Kulcsar, F.R. Pinto, L.G. Brandao, C.R. Cernea, Tumor volume as an independent predictive factor of worse survival in patients with oral cavity squamous cell carcinoma, Head Neck 39 (5) (2017) 960–964.
[23] A.P. Yuen, K.Y. Lam, W.I. Wei, K.Y. Lam, C.M. Ho, T.L. Chow, W.F. Yuen, A comparison of the prognostic significance of tumor diameter, length, width, thickness, area, volume, and clinicopathological features of oral tongue carcinoma, Am. J. Surg. 180 (2) (2000) 139–143.
[24] M. Grimm, Prognostic value of clinicopathological parameters and outcome in 484 patients with oral squamous cell carcinoma: microvascular invasion (V+) is an independent prognostic factor for OSCC, Clin. Transl. Oncol. 14 (11) (2012) 870–880.
[25] Q.S. Xu, C. Wang, B. Li, J.Z. Li, M.H. Mao, L.Z. Qin, H. Li, X. Huang, Z. Han, Z. Feng, Prognostic value of pathologic grade for patients with oral squamous cell carcinoma, Oral Dis. (2017).
[26] Y. Jeong, S. Baek, J.W. Park, J.H. Joo, J.S. Kim, S.W. Lee, Lymph node standardized uptake values at pre-treatment 18F-fluorodeoxyglucose positron emission tomography as a valuable prognostic factor for distant metastasis in nasopharyngeal carcinoma, Br. J. Radiol. 90 (1071) (2017) 20160239.
[27] T.R. Miller, P.W. Grigsby, Measurement of tumor volume by PET to evaluate prognosis in patients with advanced cervical cancer treated by radiation therapy, Int. J. Radiat. Oncol. Biol. Phys. 53 (2) (2002) 353–359.
[28] M. Hatt, M. Majdoub, M. Vallieres, F. Tixier, C.C. Le Rest, D. Groheux, E. Hindie, A. Martineau, O. Pradier, R. Hustinx, R. Perdrisot, R. Guillevin, I. El Naqa, D. Visvikis, 18F-FDG PET uptake characterization through texture analysis: investigating the complementary nature of heterogeneity and functional tumor volume in a multi-cancer site patient cohort, J. Nucl. Med. 56 (1) (2015) 38–44.
[29] E.S. Choi, S.G. Ha, H.S. Kim, J.H. Ha, J.C. Paeng, I. Han, Total lesion glycolysis by 18F-FDG PET/CT is a reliable predictor of prognosis in soft-tissue sarcoma, Eur. J. Nucl. Med. Mol. Imaging 40 (12) (2013) 1836–1842.
[30] K. Pak, G.J. Cheon, H.Y. Nam, S.J. Kim, K.W. Kang, J.K. Chung, E.E. Kim, D.S. Lee, Prognostic value of metabolic tumor volume and total lesion glycolysis in head and neck cancer: a systematic review and meta-analysis, J. Nucl. Med. 55 (6) (2014) 884–890.
[31] C. Van de Wiele, V. Kruse, P. Smeets, M. Sathekge, A. Maes, Predictive and prognostic value of metabolic tumour volume and total lesion glycolysis in solid tumours, Eur. J. Nucl. Med. Mol. Imaging 40 (2) (2013) 290–301.
[32] B.S. Purohit, A. Ailianou, N. Dulguerov, C.D. Becker, O. Ratib, M. Becker, FDG-PET/CT pitfalls in oncological head and neck imaging, Insights Imaging 5 (5) (2014) 585–602.