Value of Intratumoral Metabolic Heterogeneity and Quantitative ^{18}F-FDG PET/CT Parameters to Predict Prognosis in Patients With HPV-Positive Primary Oropharyngeal Squamous Cell Carcinoma

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Objective: The aim of this study was to evaluate the impact of intratumoral metabolic heterogeneity and quantitative FDG PET/CT imaging parameters for predicting patient outcomes in primary oropharyngeal squamous cell cancer (OPSCC).

Patients and Methods: We retrospectively investigated 105 patients with HPV-positive OPSCC. SUV_{max} and metabolic tumor volume (MTV) were measured for the primary tumors and when available for the metastatic sites. Primary tumor intratumoral metabolic heterogeneity was calculated as the area under a cumulative SUV volume histograms curve (AUC-CSH). The median follow-up time was 35.4 months (range, 3–92 months). Outcome end point was event-free survival (EFS). Kaplan-Meier survival plots and Cox regression analyses were performed.

Results: Of the 105 patients included, 19 patients relapsed and 11 deceased during the study period. AUC-CSH indexes were associated with EFS using PET gradient-based ($P = 0.034$) and 50% threshold ($P = 0.02$) segmentation methods, on multivariate analysis. Kaplan-Meier survival analysis using optimum cutoff of 16.7 SUV_{max} and 12.7 mL total MTV were significant predictors of EFS. Combining SUV_{max} and AUC-CSH index in 3 subgroups, patients with higher intratumoral heterogeneity and higher SUV_{max} were associated with worse outcome (log-rank, $P = 0.026$). Similarly, patients with higher intratumoral heterogeneity tumors and higher MTV had worse prognosis (log-rank, $P = 0.022$).

Conclusions: Intratumoral metabolic heterogeneity using FDG PET was a prognostic factor for EFS in patients with primary HPV (+) OPSCC. The combined predictive effect of FDG avidity, metabolic tumor burden, and intratumoral heterogeneity provided prognostic survival information in these patients.

Key Words: intratumoral heterogeneity, PET/CT, OPSCC, prognosis

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Head and neck (HN) cancer is the sixth most common cancer in the world, accounting for 650,000 new cancer cases and 350,000 cancer deaths worldwide, yearly.[1] More than 85% of HN cancers are squamous cell cancers (HNSCCs).[2] Tobacco and alcohol used to be the major risk factors for development of HNSCC; however, in the last decade, the infection with the human papillomavirus (HPV) has become the major risk factor for the subset of HNSCCs of the oropharynx,[3] arising from the tongue base and tonsils. HPV-positive oropharyngeal squamous cell carcinoma (OPSCC) represents an emerging disease that exhibits differences from HPV-negative OPSCC in natural history and prognosis. HPV-positive OPSCC has a predominance for middle age, nonsmoking, white men, and a strong association with sexual behaviors.[4] Patients with HPV-positive tumors usually respond better to therapy, with a higher life expectancy than those with HPV-negative tumors.[5,6]

At present, TNM staging system is the most important prognostic factor in these patients and the most commonly used parameter for guiding treatment decisions; however, TNM staging does not always provide satisfactory results, since population and tumors are heterogeneous at each stage with different propensities of relapse. Pretreatment selection of patients with poor prognosis is important in choosing candidates for aggressive therapy.[7–9] Therefore, identification of imaging-related prognostic factors that potentially predict long-term survival may allow for the development of individualized treatment strategies.

^{18}F-FDG PET/CT is useful for staging, management planning, monitoring treatment, early detection of recurrence, and outcome prediction for patients with HN cancers.[10] The most widely used PET-derived parameter to measure tracer accumulation in PET is the SUV_{max}, which quantifies tumor glucose metabolic uptake.[11,12] Recently, studies have supported the use of volumetric parameters such as metabolic tumor volume (MTV) as a potential marker for predicting outcome in patients with HN cancers.[9] Furthermore, recent interest has been raised in the development of new imaging strategies to assess for intratumoral metabolic heterogeneity using FDG PET imaging,[13,14] because intratumor heterogeneity has been reported to be implicated in treatment failure, higher chance of metastasis,[15] and may be used as a criterion to predict prognosis in various tumors types.[16–18]

Although the impact of pretreatment SUV_{max} and volume-based PET parameters in predicting patient's prognosis have been previously evaluated in various malignant tumors, including OPSCC, the usefulness of intratumoral metabolic heterogeneity for prediction of prognosis in patients with OPSCC is unexplored.

The objective of the current study was to assess the predictive value of FDG PET imaging parameters, including MTV, TLG, and intratumoral metabolic heterogeneity, extracted from initial staging scans of patients with HPV-positive OPSCC.

PATIENTS AND METHODS

Eligible Patients and Follow-up

This is an institutional review board–approved, retrospective study, performed under a waiver of informed consent in accordance with the Health Insurance Portability and Accountability Act guidelines. Patients with baseline FDG PET/CT scans being

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assessed for primary OPSCC were identified from our PET center database and included in the study. Patients with history of a secondary primary malignancy were excluded. Patients were followed till death or their last day of follow-up at our center. The median follow-up time was 35.4 months (range, 3–92 months). Most of the patients, 81 (77.1%) of the 105 patients were treated with chemoradiation, 17 patients (16.2%) were treated with combination of surgery and chemoradiation, 3 patients were treated with surgery only, 2 patients with chemotherapy only, 1 patient with radiation only, and 1 patient with combination of radiation and surgery (Table 1). Outcome end point was event-free survival (EFS), including recurrence-free survival and overall survival (OS). Patients who were alive were censored at the last date of follow-up, whereas the date of death was used for the patients who expired.

PET/CT Imaging Protocol

FDG PET/CT was performed according to our institutional clinical protocol. Patients were instructed to fast for at least 4 hours before scanning, having blood glucose levels lower than 200 mg/dL at the time of ^{18}F-FDG injection. PET/CT was acquired 60 minutes after FDG administration (dose of 5.55 MBq/kg [0.068 mCi/lb]). Patients were scanned using a Discovery VCT (GE Healthcare) or a Biograph mCT Scanner (Siemens Healthcare). Images were reconstructed using the ordered subset expectation maximization algorithm, with 128 × 128 matrix, 2 iterations, 21 subsets, 3-mm postreconstruction Gaussian filter, and standard Z filter, 4.7-mm pixel, and 3.27-mm slice thickness. An unenhanced CT was acquired for attenuation correction and anatomical coregistration. CT parameters were 50 cm axial dynamic FOV, weight-based amperage 20 to 200 mA, 120 to 140 kVp, 3.75-mm slice thickness, pitch of 0.984, 0.5-second gantry rotation speed, and 512 × 512 matrix.[19]

TABLE 1. Patients' Clinical Characteristics

Patient Characteristics	n	%
Age, y		
40–50	20	19.0
51–70 y	73	69.5
>70	12	11.5
Sex		
Male	89	84.8
Female	16	15.2
Tumor location		
Base of the tongue	54	51.4
Tonsils	51	48.6
Tumor stage		
Stage I	0	0
Stage II	4	3.8
Stage III	13	12.4
Stage IVA	81	77.1
Stage IVB	4	3.8
Stage V	3	2.9
Treatment received		
Chemotherapy	2	1.9
Radiotherapy	1	0.95
Surgery	3	2.9
Chemoradiation	81	77.1
Surgery + chemotherapy	1	0.95
Surgery + chemoradiation	17	16.2
Recurrence		
No	86	82
Yes	19	18
Survival status		
Alive	94	89.5
Dead	11	10.5

PET/CT Image Analysis

PET/CT scans were reviewed on an MIM workstation (version 6.3.2; MIM Software Inc, Cleveland, Ohio).[20–22] A board-certified nuclear medicine physician, blinded to the outcome data, reviewed the PET/CT images. Axial, coronal, and sagittal PET, CT, and PET/CT images were used for the identification of the primary lesions (n = 105), lymph nodes (n = 87, in 73 patients), and metastatic sites (n = 6, in 3 patients). The automated semiquantitative PET parameters included the SUV_{max}, reflecting a maximum single-pixel uptake value adjusted for lean body mass; the peak SUV (SUV_{peak}) calculated using an automated computed maximal average SUV in a 1.0 cm^3 spherical volume within the tumor[23]; the MTV expressed as FDG-avid tumor volume, and the tumor glycolytic activity (TLG) representing the tumor metabolic volume multiplied by average SUVs of included voxels.[24] The SUV_{max}, SUV_{peak}, MTV, and TLG were measured using 2 validated PET segmentation methods: a gradient-based and a 50% SUV_{max} threshold. The gradient-based segmentation consisted of an edge-detection tool, generating an automated volume of interest, outlined based on the boundaries of the FDG-avid lesion, avoiding adjacent structures. For the threshold segmentation technique, a 50% SUV_{max} threshold was applied using a spherical volume of interest, predefined by MIM software tool.[19]

The quantitative index of intratumoral metabolic heterogeneity (AUC-CSH index) was calculated as the area under the curve (AUC) of a cumulative SUV volume histogram (CSH) obtained by plotting the percent volume greater than the percentage of SUV_{max} (calculated for gradient-based and for 50% SUV_{max} threshold), with lower AUC corresponding to higher degrees of heterogeneity.[25] AUC-CSH indexes were extracted from MIM software for the 2 PET segmentation methods outlining the primary tumor.

Statistical Analysis

SPSS 15.0 (SPSS Inc, Chicago, Ill) software was used for statistical analysis. Descriptive values were expressed as the mean ± standard deviation (SD) or median and interquartile range. Overall survival was the primary outcome measure. Univariate and multivariate Cox regression models were utilized including age, sex, race, primary site, grade, primary cancer stage, SUV_{max}, SUV_{peak}, MTV_{total}, or TLG_{total} and AUC-CSH to adjust for important prognostic factors. Outcome end point was event-free survival (EFS), including recurrence-free survival and OS. Outcome data were recorded from the review of patients' medical records and a public registry of death database[26] and was defined as the time between the baseline PET/CT scan and the date of last follow-up or date of death. Kaplan-Meier survival curves and the Mantel-Cox log-rank test were performed. Statistical significance was set at 2-tail $P = 0.05$ for all tests.[27,28]

RESULTS

Patients' Characteristics

From a total of 135 patients collected for the study, 105 patients with biopsy-proven newly diagnosed OPSCC were included. At least 1 FDG-avid primary lesion was identified per patient. Four patients had stage II, 13 patients stage III, 81 patients

TABLE 2. Multivariate Cox Regression Analysis

	Multivariate		
Parameter	**HR**	**95% CI**	***P***
Age	1.03	0.96–1.06	0.6
Stage	2.38	0.96–5.89	0.6
SUV_{max}	16.0	3.3–77.5	<0.001*
SUV_{peak}	5.38	1.52–19.08	0.009*
MTV	1.2	0.3–3.9	0.07
TLG	4.55	1.52–13.6	0.007*
AUC-CSH (by gradient-based)	4.2	1.41–12.6	0.034*
AUC-CSH (by 50% threshold)	8.9	2.2–36.1	0.022*

**P* values of statistical significance.

stage IVA, 4 patients stage IVB, and 3 patients stage IVC OPSCC. Table 1 summarizes patients' clinical characteristics. Of the 105 HPV-positive patients, 19 patients (18%) relapsed and 11 (10.4%) deceased with a median follow-up time of 35.4 months from the date of baseline PET/CT scan (range, 3–92 months).

Cox Regression Analysis and Patient Outcome

Univariate and multivariate Cox regression models were performed including clinical covariates: age, sex, race, cancer TNM stage, tumor location, treatment modality, optimum SUV_{max}, SUV_{peak}, TLG_{total}, MTV_{total}, and primary tumor AUC-CSH index. Univariate analysis demonstrated that SUV_{max} ($P = 0.006$; hazards ratio [HR], 5.8; 95% CI, 1.6–20.5), SUV_{peak} ($P = 0.025$; HR, 3.3; 95% CI, 1.1–9.4), total MTV ($P = 0.004$; HR, 3.1; 95% CI, 1.1–9.0), and total TLG ($P = 0.033$; HR, 2.9; 95% CI, 1.1–7.7) were associated with EFS, and most of the PET parameters remained significant in multivariate analysis (Table 2); AUC-CSH indexes were associated with EFS using either PET gradient-based ($P = 0.034$) and 50% threshold ($P = 0.02$) segmentations methods, on multivariate analysis.

Kaplan-Meier Survival Analysis

Mantel-Cox log-rank test was performed to compare the OS. There was a significant difference in OS when optimum SUV_{max} of 16.75 (log-rank test, $P = 0.02$), and optimum SUV_{peak} of 12.15 (log-rank test, $P = 0.005$) were used as cutoff. Similarly, significant differences were seen in OS when using optimum total TLG of 107.12 g (log-rank test, $P = 0.025$) and when using total MTV of 12.73 mL (log-rank test, $P = 0.005$). Optimum cutoff point values for AUC-CHS also showed significant differences in OS when using PET gradient-based segmentation (log-rank test, $P = 0.045$) and for PET 50% threshold method (log-rank test, $P = 0.04$; Fig. 1).

Effect of FDG Avidity, Metabolic Tumor Burden, and Intratumoral Metabolic Heterogeneity on Survival

We investigated the combined predictive effect of FDG avidity (SUV_{max}) and metabolic tumor burden, by stratifying the

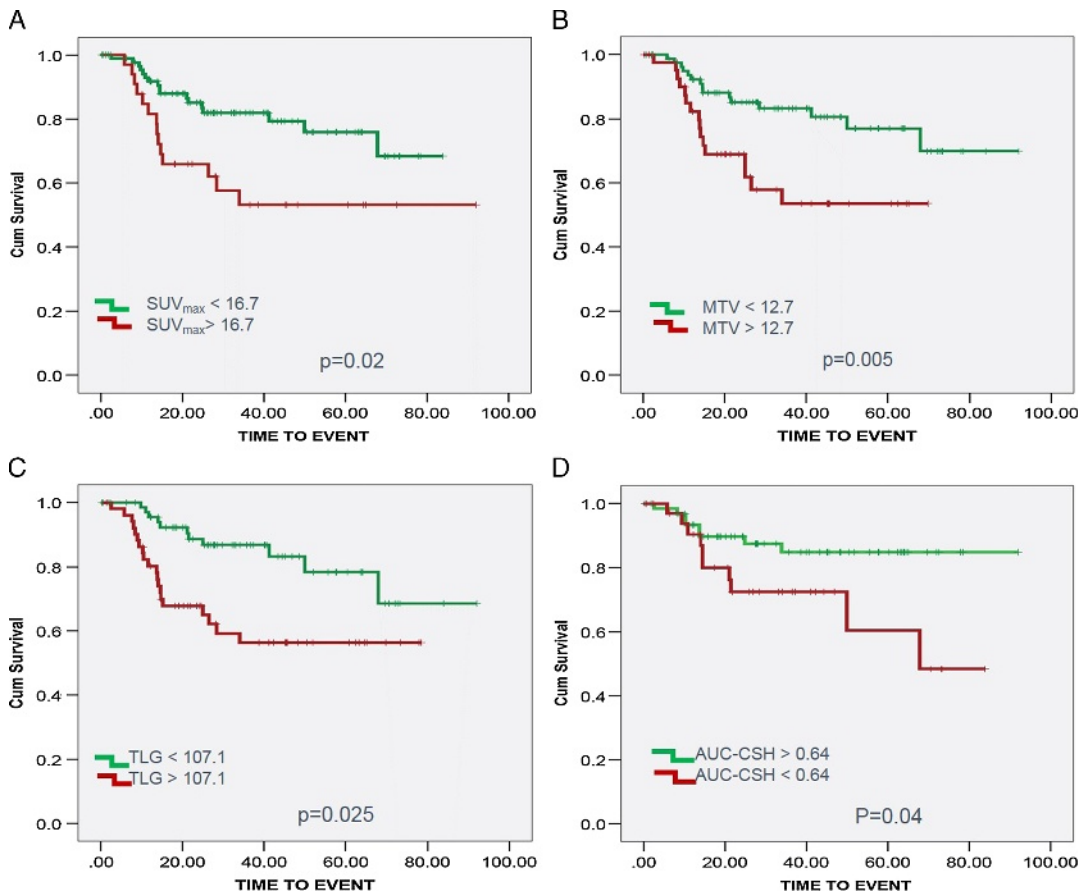

FIGURE 1. Survival curves based on optimum cutoff points for SUV_{max} (**A**) ($P = 0.02$); MTV_{Total} (**B**) ($P = 0.005$); TLGTotal (**C**) ($P = 0.025$); and gradient-based AUC-CHS index (**D**) ($P = 0.04$).

patients into groups based on the optimum cutoff of SUV_{max} and MTV values. Group A (score 0) included all patients who had both SUV_{max} and MTV_{total} lower than optimal cutoff; group B (score 1) included patients who had either SUV_{max} or MTV_{total} higher than optimal cutoff; and group C (score 2) included patients who had both SUV_{max} and MTV_{total} above optimal cutoff. As expected, the Kaplan-Meier survival analyses showed that patients with higher SUV_{max} and MTV values had worse outcome (log-rank, $P = 0.006$).

More importantly, we investigated the predictive effect of combining SUV_{max} and AUC-CSH values (using PET gradient and 50% threshold segmentation) stratifying the patients according to the optimum cutoff of MTV and AUC-CSH values. Group A included homogeneous tumors and lower SUV_{max} cutoff (Fig. 2); group B included patients with heterogeneous tumors and lower SUV_{max} values; and group C included patients with heterogeneous tumors and higher SUV_{max} above optimal cutoffs (Fig. 3). Kaplan-Meier

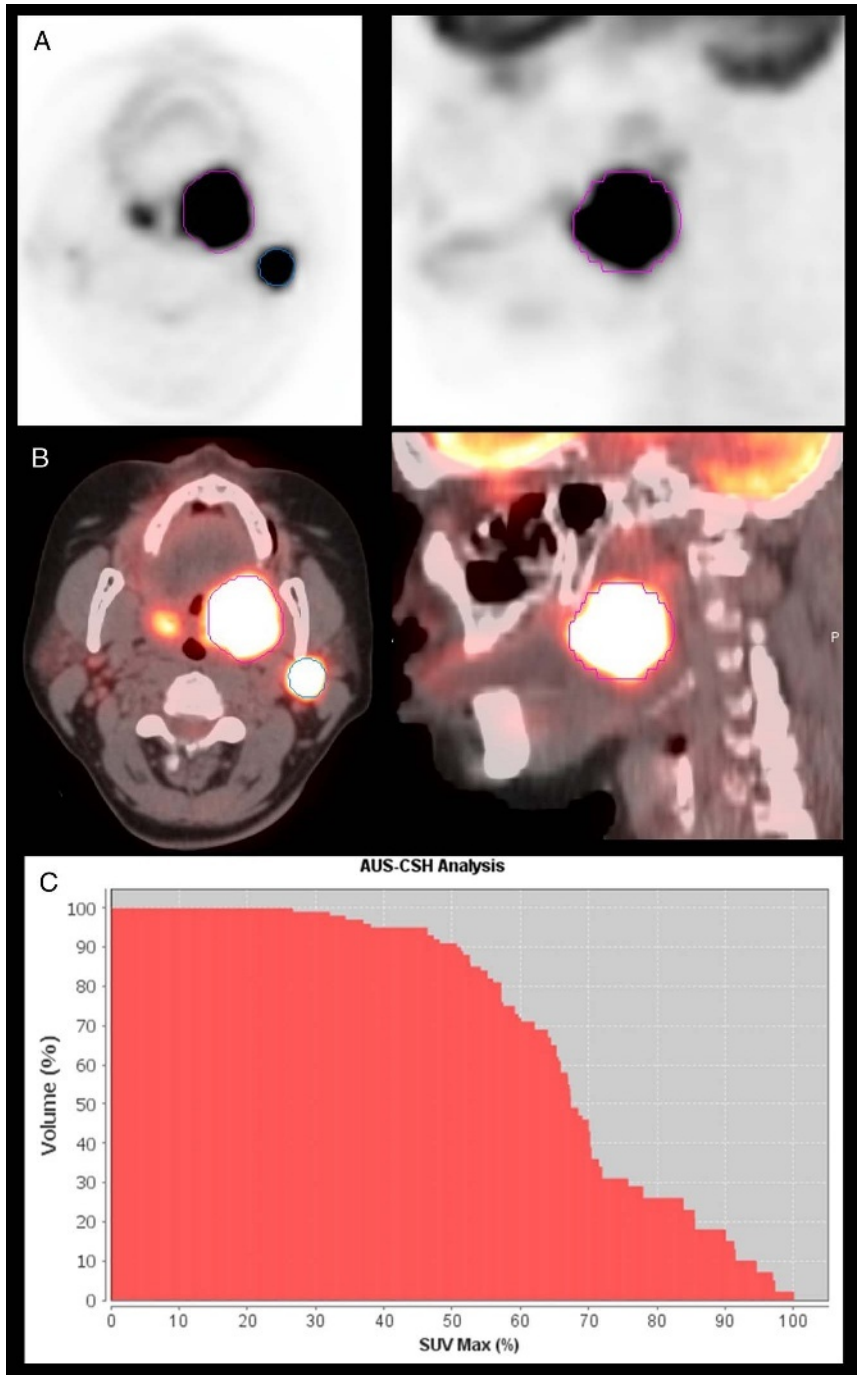

FIGURE 2. A 53-year-old man with HPV-positive tonsil SCC, stage IVa (T2 N2b M0). Axial and sagittal PET (**A**), axial and sagittal fused PET/CT (**B**), and axial and sagittal fused PET/CT images demonstrate intense FDG-avid primary tumor and left level II node, with the highest SUV_{max} of 16.4, MTV_{Total} of 21.7 mL, and TLG_{Total} of 221.7 g, by gradient segmentation. The calculated AUC-CSH index was 0.68 (**C**). Patient underwent chemoradiation therapy, being free of disease 5 years postdiagnosis.

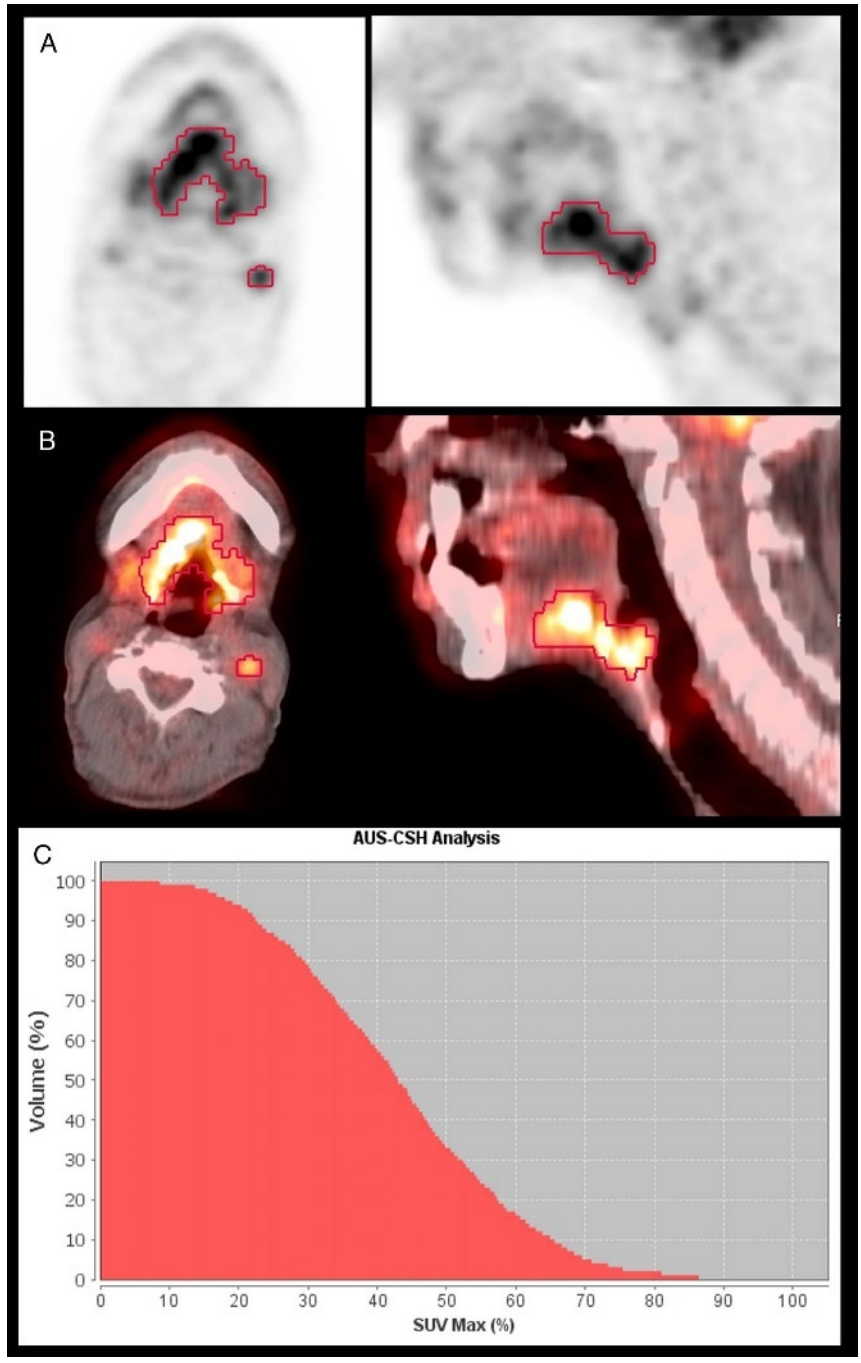

FIGURE 3. A 54-year-old woman with HPV-positive base of the tongue SCC stage IVa (T4a N2b M0). Axial and sagittal PET (**A**), axial and sagittal fused PET/CT (**B**), and axial and sagittal PET/CT images demonstrate intense FDG-avid primary tumor, and left level IIb lymph nodes, with the highest SUV_{max} of 7.7, MTV_{Total} of 65.2 mL, and TLG_{Total} of 619.7 g, by gradient-based segmentation. The calculated AUC-CSH index was 0.43 (**C**). Patient underwent chemoradiation therapy developing locoregional recurrence 15 months later.

survival analyses (Fig. 4) demonstrated that patients with higher SUV_{max} and heterogeneous tumors had worse outcome (log-rank, $P < 0.001$), by using a gradient-based segmentation method. When investigating the predictive effect of combining MTV and AUC-CSH values, Kaplan-Meier survival curves (Fig. 4) demonstrated that patients with heterogeneous tumors (AUC-CSH < 0.64) had worse outcome, independently of the MTV values (log-rank, $P = 0.022$). By selecting a sample of patients with only stage IVA tumors, which were about 77% of our cohort (81/105 patients), the Kaplan-Meier survival curves also demonstrated that the combination of heterogeneous tumors with higher SUV_{max} had significant predictive outcome (log-rank

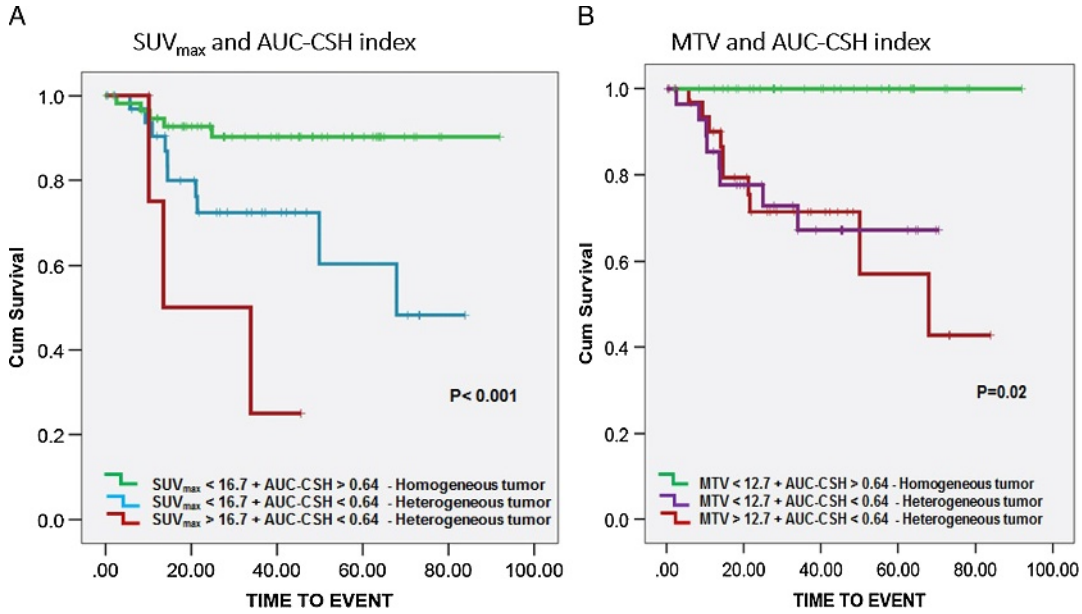

FIGURE 4. Kaplan-Meier survival curves illustrating that patients with SUV_{max} greater than 16.7 and heterogeneous tumors (AUC-CSH lower than 0.64) had worse outcome (**A**) ($P < 0.001$); similarly, patients with MTV greater than 12.7 mL and heterogeneous tumors (AUC-CSH lower than 0.64) showed worse outcome (**B**) ($P = 0.022$).

$P = 0.0228$); and similarly, heterogeneous tumors with higher MTV values significantly showed worse outcome (log-rank, $P = 0.032$).

DISCUSSION

The aim of this study was to determine the usefulness of quantitative PET-derived parameters in predicting survival outcomes for pretreated patients with HPV-positive OPSCC. Our results revealed that intratumoral metabolic heterogeneity, using AUC-CSH indexes, can predict EFS. The survival analysis showed that those patients with tumors having higher intratumoral heterogeneity, that is, lower AUC-CSH indexes, with an optimum cutoff of 0.64, had worse outcome. The intratumoral heterogeneity can serve as a novel, independent predictor of outcome. It is recognized that intratumoral heterogeneity in gene mutations and expression can contribute to treatment failure and drug resistance.[29,30] There is a growing interest in evaluating intratumoral heterogeneity by using FDG PET with different parameters, such as textural analysis,[31] coefficient of variance,[32] CSHs,[33] AUC-CSH,[25] fractal analysis,[34,35] or heterogeneity factors (HFs).[17] To date, several studies have investigated the association between metabolic intratumoral heterogeneity of FDG uptake and patient's outcomes in several malignant tumors[36–38] including HN cancers.[17,39]

Kwon et al evaluated the prognostic significance of intratumoral heterogeneity in oral cavity cancers, by using an HF, obtained as the derivative (dV/dT) of a tumor volume threshold function of a series of SUV thresholds from 40% to 80% for a primary tumor. Investigators found that HF was an independent predictor of OS ($P = 0.002$); patients with heterogeneity index less than −0.13 showed a worse prognosis than those with HF −0.13 or greater ($P = 0.005$).[39] Similarly, Yang et al explored the utility of pretreatment FDG PET tumor heterogeneity in 40 patients with locally advanced nasopharyngeal carcinoma, concluding that lower tumor heterogeneity indexes, calculated by dividing SUV_{max} by SUV_{mean}, significantly predicted progression-free survival and OS.[17] FDG PET textural features was successfully used for predicting clinical outcome and treatment response by Cheng et al[40] in 88 patients with advanced T3 or T4 OPSCC, concluding that zone-size nonuniformity was an independent predictor factor of progression-free survival and disease-specific survival.

Moreover, our results revealed that SUV_{max}, SUV_{peak}, and TLG parameters of FDG PET were significantly associated with EFS on univariate and multivariate analysis. Several investigators have evaluated the prognostic significance of pretreatment SUV_{max} and FDG PET-derived volumetric parameters, including MTV and TLG, in variable stage HN cancers.[9,21,41–50] Pak et al[9] conducted a large systematic meta-analysis, exploring the prognostic value of using MTV and TLG in pretreated patients with several HN cancers, comprising 13 publications and 1180 patients. Despite the variability in segmentation methods used between studies, higher volumetric parameters, that is, MTV and TLG, were found to significantly predict a worse prognosis. The cutoff values for MTV predicting worse prognosis ranged between 7.7 and 45 cm^3 and those of TLG ranged from 55 to 330 g.[9] In a retrospective study including 221 patients, Kim et al[51] assessed the prognostic values of pretreatment FDG PET markers, as SUV_{max}, MTV, and TLG in a uniform sample of only OPSCC patients, although the HPV status was obtained for a limited sample of patients. Using univariate Cox proportional hazards regression analysis, investigators concluded that age older than 60 years, advanced tumor stage, primary tumor SUV_{max} greater than 7.55, SUV_{peak} greater than 6.80, MTV greater than 11.06 mL, and TLG greater than 78.56 g were significantly associated with decreased OS and disease-free survival. Alluri et al[52] investigated 70 patients with stage III and IV HPV-positive OPSCC, concluding that total MTV and primary lesion MTV were associated with survival outcomes; however, the Kaplan-Meier survival curves using optimum cutoff of 41 mL for total MTV were not significant. Including all type of stage HPV-positive OPSCC tumors (n = 47), Kikuchi et al[50] reported that all volume-based PET parameters were significant prognostic factors for disease-free survival, disease-specific survival, and OS.

Establishing quantitative PET parameters as independent prognostic indicators in our study, we further studied the value of combining these functions. An integrated risk stratification

score with FDG avidity (SUV_{max}), total tumor burden, and intratumoral heterogeneity provided prognostic survival information for these patients.

We acknowledge some limitations in our study, including the possibility of inherent biases related to the retrospective nature of the study. The patients included in the study underwent primary surgery, RT, or CRT with/without induction chemotherapy or postoperative RT/CRT; these various treatment modalities might have affected the clinical outcomes. Patients were scanned by 2 different types of PET scanners over a long period of longitudinal time. Also, our results are derived from a single reader segmenting the tumor volumes and a single vendor's commercial segmentation software. Finally, the patients' dates of events were collected using the patient medical records at our hospital and a public registry, and there may be a lag time between the actual time of event and the update of the information in the public registry, which could result in loss of accurate mortality data. Similarly, for patients who were alive, the OS was censored to their last date of follow-up at our institution, which could affect the accuracy of the survival data.

CONCLUSIONS

The present study included a relatively large cohort of patients with HPV-positive OPSCC. We demonstrated that pretreatment primary intratumoral metabolic heterogeneity using AUC-CSH index FDG PET/CT imaging was a prognostic factor for EFS in patients with HPV (+) OPSCC. More importantly, the combined predictive effect of FDG avidity, metabolic tumor burden, and intratumoral heterogeneity provided prognostic survival information in these patients.

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