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Multimodal Prediction of Cervical Lymph Node Metastasis and Recurrence in Oral Squamous Cell Carcinoma

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Abstract. *Background/Aim: Oral squamous cell carcinoma (OSCC) is the most common malignancy in the head/neck region, and cervical lymph node (CLN) metastasis is a strong poor-prognosis factor. In addition, many patients with OSCC experience recurrence despite multidisciplinary treatment. We sought to identify factors associated with CLN metastasis and recurrence in patients with OSCC. Patients and Methods: We evaluated a total of 45 patients and 233 target CLNs. The longest diameter of the target CLN, the shortest diameter of the target CLN (LS), the area of the target CLN, and the relative computed tomography (CT) values of the target CLNs calculated based on the CT values of the internal jugular vein (LCT) were obtained from preoperative CT images, and the maximum standardized uptake values of the primary tumor (pSUV) and target CLN (nSUV) were obtained from preoperative ^{18}F-fluorodeoxyglucose-positron emission tomography/CT images. We performed immunohistochemical staining for cytokeratin 13 (CK13) and 17 (CK17) on neck dissection tissues. Results: A discrimination equation was used that can predict CLN metastasis with a 92.2% discrimination rate using LS, LCT, pSUV, and nSUV. The CLNs were divided into discrimination and non-discrimination groups based on discriminant equations and CK13 and CK17 were used as the objective variables. A significantly higher recurrence rate was observed in the non-discrimination group (CK13: 5-year recurrence rate 28.6% vs. 64.3%, $p<0.01$; CK17: 5-year recurrence rate 28.0% vs. 76.0%, $p<0.01$). Conclusion: CLN metastases in OSCC can be assessed by combining preoperative imaging. The combined use of CK13 and CK17 expression with imaging findings offers an integrated approach to predict OSCC recurrence.*

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Head and neck squamous cell carcinoma (HNSCC) is a cancer that originates from the mucosal epithelium of the oral cavity, pharynx, and larynx. Among the several types of HNSCC, oral squamous cell carcinoma (OSCC) is the most prevalent type, accounting for approximately half of all HNSCCs and arising primarily from the mucosal epithelium of the tongue, gingiva, buccal mucosa, or floor of the mouth (1). OSCC is characterized by its tendency to develop cervical lymph node (CLN) metastasis at an early timepoint. CLN metastasis is considered a strong poor-prognosis factor, as the presence of just one metastatic CLN is sufficient to classify OSCC as advanced Stage 3 or 4 (2, 3).

Modified radical neck dissection or radical neck dissection is generally favored by most surgeons as the primary management approach for local disease in patients with clinically node-positive (cN+) OSCC (4, 5). However, regarding patients with clinically node-negative (cN0) disease, there is no consensus on whether prophylactic neck dissection (such as supraomohyoid neck dissection or extended supraomohyoid neck dissection) should be performed or if strict follow-up without neck dissection is sufficient (5, 6). After the depth of invasion was added in the 8th edition of the UICC TNM classification, prophylactic neck dissection has been recommended for tongue cancer with a depth of invasion >4-5 mm (2, 5). Nonetheless, choosing the appropriate approach remains challenging due to the wide range of risk factors associated with CLN metastasis (7, 8).

CLNs are comprehensively evaluated through a combination of radiologic and histopathologic analyses. The integration of these examinations enhances the rate of positive findings and aids in the selection of appropriate treatment and management strategies (9). However, even experts in the field often encounter challenges in their evaluation, leading to diagnostic uncertainty. Our aim was thus to develop a statistical method that utilizes computed tomography (CT), which offers objectivity, reproducibility, and cost-effectiveness, plus ^{18}F-fluorodeoxyglucose positron emission tomography/CT (PET), which complements CT, to facilitate the objective and straightforward assessment of CLNs prior to surgery. We also designed the present study to create a method that effectively evaluates the risk of OSCC recurrence by integrating a postoperative histopathological evaluation.

Patients and Methods

Study setting and patients. This single-center, retrospective, observational study included patients who underwent neck dissection for a diagnosis of OSCC at the Department of Oral and Maxillofacial Surgery, Okayama University Hospital (Okayama, Japan) during the 10-year period from April 1, 2008 to March 31, 2018. Among them, the final study patients were selected from those without missing data who met the following criteria: (i) preoperative contrast CT and PET were conducted; (ii) no adjuvant therapy such as chemotherapy or radiation therapy was conducted before the neck dissection; and (iii) the neck dissection was performed on the affected side. We collected the patients' epidemiological information such as age, sex, blood test results, laterality, primary site, operative method, histopathological results of the resected specimen, presence or absence of recurrence, time to recurrence, and site of recurrence from the patients' medical records.

The primary sites of OSCC were defined as the tongue, maxillary gingiva, mandibular gingiva, buccal mucosa, and floor of the mouth. The CLN areas were defined as the submandibular lymph node (Level IA), submandibular lymph node (Level IB), upper jugular lymph node (Level II), middle jugular lymph node (Level III), lower jugular lymph node (Level IV), and posterior triangle lymph node (Level V). The evaluation of the resection specimens was performed by an oral pathologist from the Japanese Society of Oral Pathology. The patients' CT scans were performed using a multi-slice CT system at our hospital (Aquilion ONE manufactured by Canon Medical Systems Corporation, Otawara-shi, Tochigi, Japan, Discovery CT 750 by GE Healthcare, Chicago, IL, USA, or SOMATOM Definition Flash by Siemens, Erlangen, Bavaria, Germany), and nonionic iodine contrast agents were used. PET scans were performed using a Siemens Biograph 16 system at the Okayama Diagnostic Imaging Center. The maximum standardized uptake (SUVmax) values were measured using Fujifilm's Synapse medical imaging and information management system (Minato-ku, Tokyo, Japan). Recurrence was defined as local recurrence, cervical lymph node metastasis, or distant organ metastasis within 5 years after cervical lymph node resection. This retrospective study was approved by the Ethical Committee of the Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (Protocol No: 2009-003). The patients' data were anonymized, and the requirement for written informed consent was waived based on the retrospective design of the study.

Measurements using the preoperative CT and PET images. The target CLN was determined as the largest lymph node within each area on the preoperative CT axial image. The CLNs used for measuring SUVmax values in PET were confirmed to correspond to the target CLN determined in CT. An example measurement is shown in Figure 1. The measurement parameters and methods were as follows: LL: the longest diameter of the target CLN; LS: the shortest diameter of the target CLN; LA: the area of the target CLN; LCT: the relative CT values of the target CLNs calculated based on the CT values of the internal jugular vein; pSUV: the SUVmax value for the primary tumor, and nSUV: the SUVmax value for the target CLN.

Immunohistochemical analysis of CK13 and CK17 expressions. After excision, formalin-fixed CLNs were embedded in paraffin and sectioned at 4-μm thickness. The sections were deparaffinized, rehydrated, and incubated for 30 min in 1.25% hydrogen peroxide solution (Wako, Osaka, Japan) containing methanol (Wako) to inactivate endogenous peroxidase. For the purpose of antigen activation, slides were incubated in 10-fold diluted citrate buffer (Sigma, St. Louis, MO, USA) for 10 min using a pressure cooker. After the slides reached room temperature, blocking was performed with blocking reagent contained in ImmPRESS Universal Reagent, Anti-Mouse/Rabbit Ig (Vector, Burlingame, CA, USA) for 20 min. Anti-cytokeratin-13 (CK13, #ab16112, mouse IgG, Abcam, Cambridge, UK) and anti-cytokeratin-17 (CK17, #M7046, rabbit IgG, Dako, Glostrup, Denmark) antibodies were used for the immunohistochemical analysis.

Specimens were incubated with antibodies overnight at 4˚C and then washed with phosphate-buffered saline (PBS). Slides were then treated with ImmPRESS® Reagent HRP (Vector) for 20 min at room temperature. Immunoreactions were visualized using the ImmPACT DAB Substrate Kit, Peroxidase (HRP) (Vector) and counterstained using Hematoxylin QS (Vector). CK13− and CK17−positive and -negative cases are shown in Figure 2.

Data analysis. After confirming that the target CLN corresponded to the postoperative histopathological diagnosis, we divided the patients into two groups: N+ for the group that was positive for metastasis and N− for the group that was negative for metastasis. Continuous variables are presented as the median and interquartile range (IQR), and nominal variables are presented as numbers and percentages. To examine factors associated with metastasis, we used Fisher's exact test for nominal variables and the Mann–Whitney *U*-test for continuous variables. For the continuous variables, we performed a receiver operating curve analysis to calculate cutoff values. We computed a discriminant equation by using a discriminant analysis to predict metastasis or recurrence. The log-rank test and Kaplan–Meier curves were used to determine the recurrence rate. A probability value <0.05 was considered significant. Package R ver. 4.0.5 (R Core Team, Vienna, Austria) and Excel (Microsoft, Redmond, WA, USA) were used for the statistical analyses.

Results

Patient clinical characteristics. In total, 45 patients with OSCC and 233 target CLNs were included in this retrospective analysis. The patients' clinicopathological features are summarized in Table I. The patient age at the first visit ranged from 28 to 89 years, with a median age of

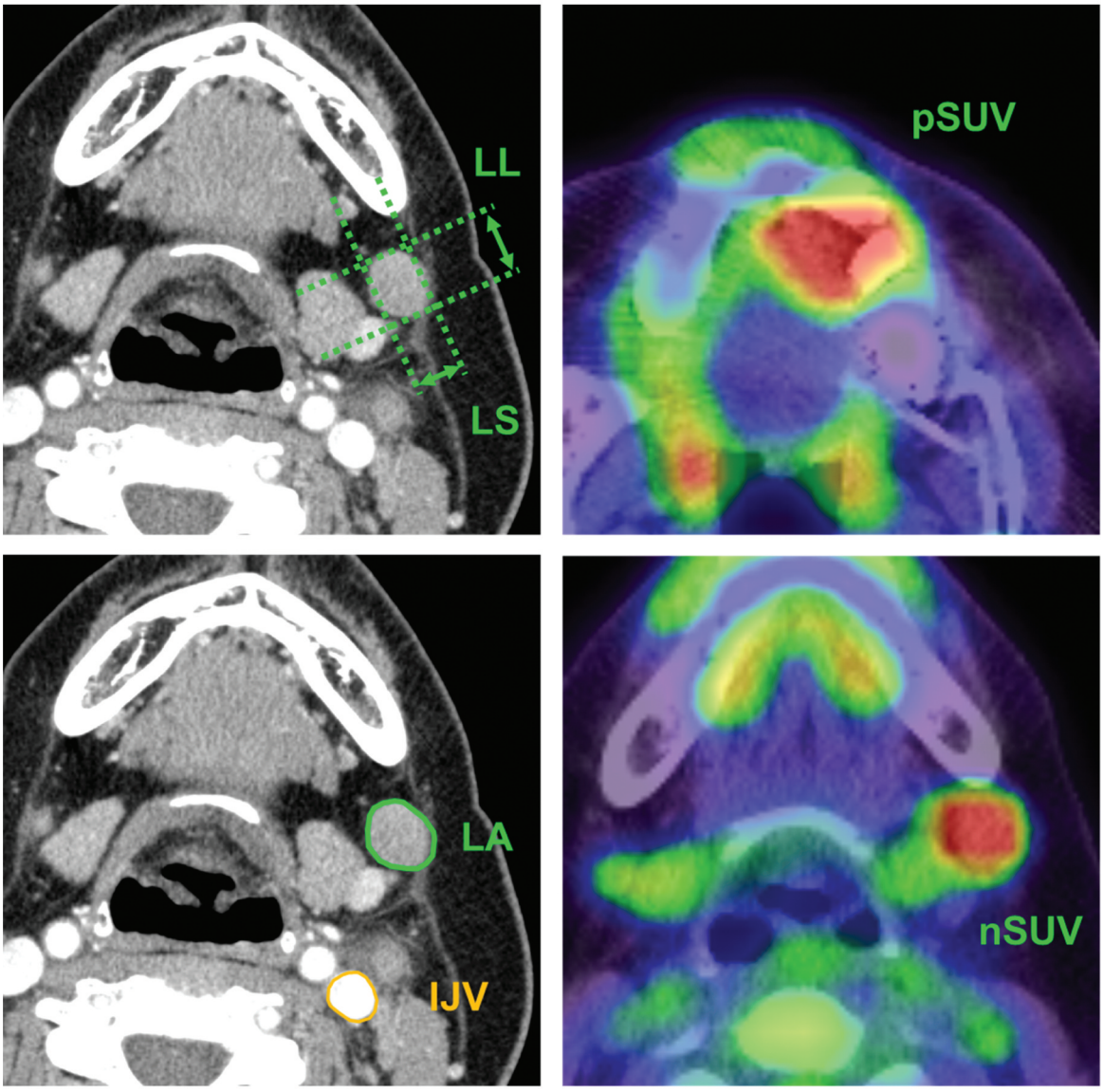

Figure 1. *Measurement technique using preoperative computed tomography (CT) and 18-F fluorodeoxyglucose-positron emission tomography/CT images. IJV: Internal jugular vein; LA: the area of the target cervical lymph node (CLN); LCT: the relative CT value of the target CLN calculated based on the CT value of the IJV; LL: the longest diameter of the target CLN; LS: the shortest diameter of the target CLN; nSUV: the SUVmax of the target CLN; pSUV: the SUVmax of the primary tumor.*

69 years. Among the patients, 109 (46.6%) were male, with a median age of 65 years (range=28-87 years); the median age of the 124 female patients (53.4%) was 74 years (range=48-89 years) and tended to be higher than that of the male patients ($p<0.01$). The most frequent histology was mandibular gingiva (n=18, 40%), followed by 15 cases (33.3%) at the tongue and 14 cases (31.1%) in the maxillary gingiva. In the males, the maxillary gingiva cases were the most frequent (n=12, 85.7%), and in the females, mandibular gingiva cases were the most frequent (n=12, 66.7%). Twenty-two patients were assigned to the N+ group, and the remaining 23 patients were assigned to the N– group.

Regarding the N+ rate by primary tumor, nine cases (50.0%) of mandibular gingiva were the most frequent, followed by seven cases (46.7%) at the tongue and two cases (33.3%) in the mouth floor. Concerning the number of N+ per case, 13 cases (28.9%) were limited to one area, six cases (13.3%) appeared in two areas, one case (2%) appeared in three areas, and the remaining case (2%)

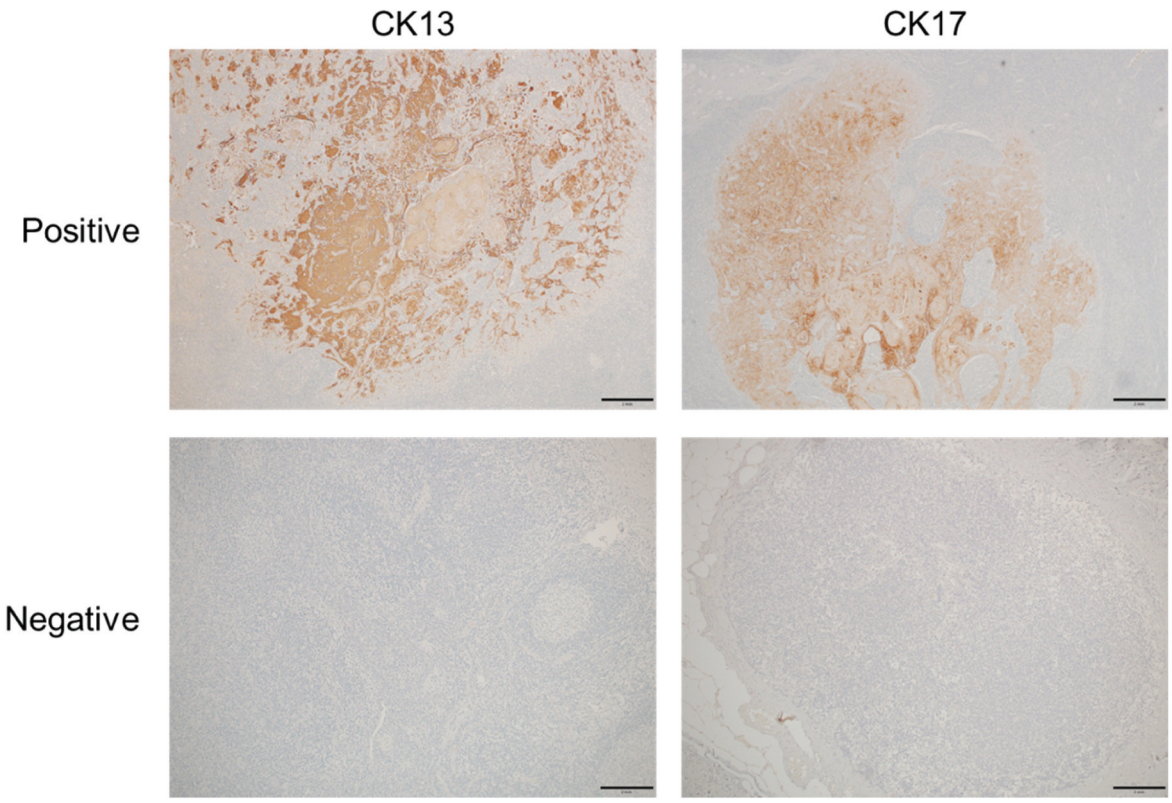

Figure 2. *Positive and negative cases of CK13 and CK17 in immunohistochemical staining Scale bar: 2 mm.*

appeared in four areas. The number of N+ lymph nodes was 35 (15%), and that of N− lymph nodes was 198 (85%). Regarding the sites of N+, the upper internal jugular lymph nodes were the most frequent site with 15 cases (42.8%), followed by of submandibular lymph nodes with 11 cases (31.4%) and middle internal jugular lymph nodes with four cases (11.4%). The immunohistochemical staining of the target lymph nodes for CK13 and CK17 revealed 19 (8.1%) CK13-positive nodes and 32 (13.7%) CK17-positive nodes.

Statistical evaluation obtained from the preoperative CT and PET images. The results of the univariate analysis of radiological measurements from the patients' preoperative CT and PET images are shown in Table II. The values of LL, LS, LA, and nSUV were significantly higher in the N+ group compared to the N− group (LL: 9.69 *vs*. 6.36, $p<0.01$; LS: 7.02 *vs*. 4.13, $p<0.01$; LA: 52.87 *vs*. 20.97, $p<0.01$; nSUV: 3.79 *vs*. 1.94, $p<0.01$). The LCT and pSUV values were not significantly different between the N+ and N− groups (LCT: 148.34 *vs*. 147.55, $p=0.41$; pSUV: 13.41 *vs*. 12.46, $p=0.84$). As a result of the discriminant analysis, a discriminant equation, i.e., Eq. (1) was derived with a discrimination rate of 92.2% (Figure 3).

Statistical evaluation obtained from the immunohistochemical staining of target CLNs. When CK13 and CK17 were used as the objective variables, the cutoff values were as follows: LL=7.89, LS=6.72, LCT=158.97, pSUV=14.3, and nSUV=3.03. The discriminant analysis derived two equations as depicted in Figure 3; the discriminant equation for CK13 is Eq. (2), and the discriminant equation for CK17 is Eq. (3). Among the CLNs, 23 could not be distinguished by Eq. (1), 16 could not be distinguished by Eq. (2), and 17 could not be distinguished by Eq. (3). The respective Kaplan–Meier curves are shown in Figure 4.

The log-rank test results indicated that the CLNs that could not be discriminated by Eq. (2) or Eq. (3) had a significantly higher recurrence rate compared to the CLNs that could be discriminated (CK13 1-year recurrence rate: 18.9% *vs*. 43.8%, $p<0.01$, 5-year recurrence rate: 28.6% *vs*. 64.3%, $p<0.01$; CK17 1-year recurrence rate: 18.8% *vs*. 46.7%, $p<0.01$, 5-year recurrence rate: 28.0% *vs*. 76.0%, $p<0.01$). However, there was no significant difference in the recurrence rate between the CLNs that could not or could be discriminated by Eq. (1): 19.2% *vs*. 32.0%, $p=0.18$, 5-year recurrence rate: 29.4% *vs*. 44.0%, $p=0.18$).

Table I. *Clinical characteristics of the 45 patients with oral squamous cell carcinoma.*

	All	Metastasis lymph nodes		*p*-Value
		Positive	Negative	
n	233	35	198	
Age, yrs, median (IQR)	70 (28-89)	73 (69-81)	69 (28-89)	0.05
Sex, n (%)				0.46
Male	109 (46.7%)	14 (40.0%)	95 (47.9%)	
Female	124 (53.7%)	21 (60.0%)	103 (52.1%)	
Left and right, n (%)				0.11
Left	160 (68.6%)	20 (57.1%)	140 (70.7%)	
Right	73 (31.4%)	15 (42.9%)	58 (29.3%)	
Lymph nodes, n (%)				<0.01**
Level IA	43 (18.5%)	3 (8.5%)	40 (20.2%)	
Level IB	45 (19.3%)	12 (34.3%)	33 (16.7%)	
Level II	41 (17.6%)	14 (40.2%)	27 (13.6%)	
Level III	36 (15.4%)	4 (11.4%)	32 (16.1%)	
Level IV	34 (14.6%)	1 (2.8%)	33 (16.7%)	
Level V	34 (14.6%)	1 (2.8%)	33 (16.7%)	
Primary site, n (%)				0.32
Tongue	85 (36.4%)	11 (31.4%)	74 (37.4%)	
Mandibular gingiva	87 (37.3%)	13 (37.1%)	74 (37.4%)	
Maxillary gingiva	24 (10.3%)	5 (14.2%)	19 (9.6%)	
Buccal mucosa	9 (3.8%)	0 (0.0%)	9 (4.5%)	
Mouth floor	28 (12.2%)	6 (17.1%)	22 (11.1%)	
Immunohistochemistry, n (%)				
CK13				<0.01**
Positive	29 (12.4%)	25 (71.4%)	4 (2.0%)	
Negative	204 (87.6%)	10 (28.6%)	194 (98.0%)	
CK17				<0.01**
Positive	32 (13.7%)	28 (80.0%)	4 (2.0%)	
Negative	201 (86.3%)	7 (20.0%)	194 (98.0%)	

**$p<0.01$.

Table II. *Univariate analysis of radiological measurements, median (IQR).*

	All	Metastasis lymph nodes		*p*-Value	Cutoff value
		Positive	Negative		
CT					
LL, mm	7.84 (2.15-51.26)	9.69 (3.40-26.37)	6.36 (2.15-51.26)	<0.01**	7.05
LS, mm	5.28 (1.21-39.71)	7.02 (2.70-17.25)	4.13 (1.21-39.71)	<0.01**	6.42
LA, mm^2	45.59 (2.75-1,598.71)	52.87 (8.54-329.92)	20.97 (2.75-1,598.71)	<0.01**	35.8
LCT, HU	151.43 (47.33-293.42)	148.34 (92.24-293.08)	147.55 (47.33-293.42)	0.41	187.2
(18)F-FDG PET/CT					
pSUV	13.3 (3.69-37.99)	13.41 (5.48-37.99)	12.46 (3.69-37.99)	0.84	6.49
nSUV	2.54 (0.34-20.50)	3.79 (1.41-20.50)	1.94 (0.34-6.39)	<0.01**	3.03

LA: Area of the target cervical lymph node (CLN); LCT: computed tomography value of the target CLN; LL: longest diameter of the target CLN; LS: shortest diameter of the target CLN; nSUV: SUV max value for the target CLN; pSUV: SUV max value of the primary tumor. **$p<0.01$.

Discussion

Oral cancer is the most common malignant tumor in the head and neck region, with an estimated 377,000 cases occurring worldwide in 2020 (10). The incidence of oral cancer has been increasing, and it is projected to accelerate rapidly in the coming years in terms of the average annual percentage change (11, 12). OSCC is the most common malignancy in

$$y = 0.118 \times LS + 0.090 \times LCT + 0.251 \times pSUV + 0.572 \times nSUV + 0.009 \quad \text{Eq.(1)}$$

$$y = 0.062 \times LL + 0.286 \times LS + 0.139 \times LCT - 0.120 \times pSUV + 0.171 \times nSUV - 0.0006 \quad \text{Eq.(2)}$$

$$y = 0.060 \times LL + 0.313 \times LS + 0.120 \times LCT - 0.170 \times pSUV + 0.217 \times nSUV + 0.003 \quad \text{Eq.(3)}$$

Figure 3. *The discriminant equations. The objective variables are N+ or N− for Eq. (1), CK13+ or CK13− for Eq. (2), and CK17+ or CK17− for Eq (3).*

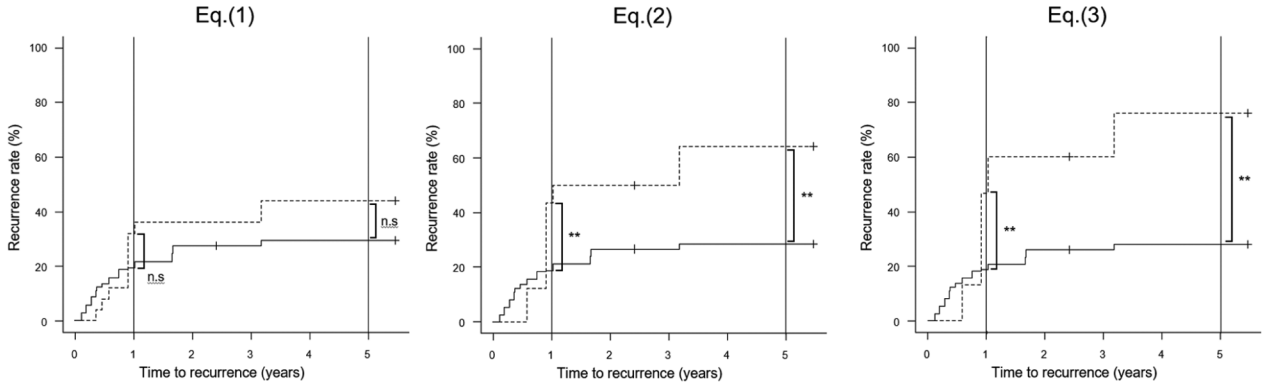

Figure 4. *Comparison of clinical outcomes by the discriminant equations. Solid lines: the group that can be discriminated. Dashed lines: the group that cannot be discriminated. The 1-year recurrence rate and 5-year recurrence rate were not significantly different using Eq. (1), but significant differences of p<0.01 were observed using Eq. (2) and Eq. (3).*

the head and neck region. Several factors contribute to the development of OSCC, including individual lifestyle, genetic factors, tobacco smoking, and excessive alcohol consumption (13). CLN metastasis in OSCC significantly impacts the prognosis, leading to a reported 50% reduction in survival when positive (3). In addition, due to the high rate of CLN metastasis in OSCC (approx. 20%-40%), prophylactic neck dissection is recommended even in Stage 1 and Stage 2 cases, considering the tumor thickness and other clinical findings (5, 14-16). However, neck dissection can result in complications such as the paralysis of various nerves, cervico-omo-brachial syndrome, and facial edema (17). A preoperative evaluation of CLN metastasis should thus be performed appropriately to determine the necessity of neck dissection.

Palpation is the basic diagnostic method for evaluating CLNs in OSCC. However, accurate diagnoses through palpation require extensive clinical experience, heavily relying on the skill of the examining physician. It is thus now recommended that imaging tests be combined with palpation to enhance diagnostic accuracy (18, 19). CT is as accurate as MRI for evaluating cervical lymph node and extranodal metastases (20, 21). In particular, CT is recognized as a straightforward and rapid imaging technique, offering high examination efficiency and exceptional temporal and spatial resolution. The most important feature indicative of CLN metastasis on CT is necrosis (22). However, not all metastases exhibit macroscopic necrosis, and their evaluation relies on factors such as the short diameter value, the ratio of long to short diameter values, and irregular contours of CLNs. The most frequently used short diameter cutoffs among them are 10-15 mm, but the majority of occult CLN metastases are <10 mm, and in most cases central necrosis is not present (21-25). In the present study, the cutoff value for LS was 6.42 mm (<10 mm), which may be useful in predicting occult CLN metastases.

Although it is challenging to evaluate PET findings qualitatively, PET has proven valuable for identifying the presence or absence of CLN metastases, and particularly for detecting small metastases. The cutoff value of the SUVmax for CLNs is approx. 3.5, and in our present analyses, the cutoff value was low (26); however, it is important to note that (18)F-FDG PET/CT uptake is not specific to tumors and can occur at all sites of elevated glucose metabolism, including inflammation, infection, and other benign conditions (27, 28).

This characteristic may lead to false-positive PET/CT results, making PET an imaging test complementary to other modalities, including CT, rather than a standalone diagnostic tool (29). Each imaging modality thus possesses its own strengths and limitations, and studies have reported no significant difference in diagnostic performance among these modalities, especially in N0 cases (22, 30). It is therefore crucial to evaluate the findings obtained by a combination of imaging modalities, as demonstrated in this study.

CK13 is an intermediate filament protein typically found in the normal oral mucosa's suprabasal cell layer. In OSCC, CK13 is down-regulated due to stratification loss. In contrast, CK17 is rarely expressed in normal oral mucosa but increases in advancing epithelial dysplasia and is over-expressed in OSCC. Immunohistochemical staining for CK13/CK17 is recommended for OSCC and the diagnosis of epithelial dysplasia (31-33). Some research groups consider CK17 one of the OSCC diagnostic markers (34-36). The expression and intensity of CK17 are highly correlated with malignancy, differentiation, and prognosis in various cancers such as gastric, papillary thyroid, non-small cell lung, epithelial ovarian cancers, and triple-negative breast cancer (36-41). It has been reported that CLN metastasis is closely associated with prognosis even in OSCC (34, 36); however, these reports were limited to multivariate analyses examining the correlation with CK13/CK17 expression in resected primary tissue specimens.

Renault *et al*. conducted a quantitative reverse transcription-polymerase chain reaction (RT-PCR) using CLN tissues from oral and oropharyngeal carcinoma, and they observed that CK17 was significantly up-regulated in both macrometastases and micrometastases (42). We have found no published investigation of CK13/CK17 expression in CLN tissue from OSCC. Our present results demonstrated that the findings obtained by the immunohistochemical staining of CK13/CK17, along with the values obtained from preoperative imaging, can be integrated into a discriminant equation that proves useful for predicting OSCC recurrence.

Numerous studies have evaluated the recurrence and prognosis of OSCC from various perspectives, considering multiple factors including tumor location and differentiation, T stage, the presence of positive CLN metastasis, the ratio of positive CLNs to the total number of excised CLNs, and more (3, 36, 43, 44). The following factors were identified as predictors of CLN metastasis: the degree of differentiation, the pathological grade, the growth pattern, the depth of invasion, and the neutrophil/lymphocyte ratio (3, 36, 43, 44). In addition, like this study, several investigations have endeavored to characterize metastatic lymph nodes by amalgamating diverse factors and specific markers. Arimoto *et al*. and Sugimoto *et al*. reported significantly higher positivity for podoplanin, lymphatic vessel endothelial hyaluronan receptor 1, and CD11b in cases exhibiting lower differentiation or higher rates of lymph node metastasis (45, 46). However, the use of many of these factors requires expertise in radiology and histology, making their evaluation complex. In contrast, we used CT and PET images, which are commonly obtained in OSCC patients, thus providing a straightforward method to predict CLN metastasis. This method can offer an objective and convenient tool for determining whether to perform a neck dissection.

In addition, the risk of recurrence can be assessed by combining radiological measurements with the expressions of CK13 and CK17. Postoperative treatment decisions are currently based on the number of positive CLNs and the presence of extranodal extension (47), but considering the status of CK13 and CK17 can further contribute to the selection of appropriate postoperative treatment strategies. Moreover, patients who are identified by this method as having a high risk of recurrence can be monitored more closely than usual after surgery in order to detect recurrence earlier.

Study limitations. First, it might not be possible to generalize our findings because this was a single-center study of Japanese patients. Second, the factors examined in this study were limited, and the addition of MRI, ultrasound, and other immunohistological factors may change the results. Third, due to the limited number of patients (n=45), the results may change if additional cases are added such as the cases of patients who have undergone preoperative adjuvant chemotherapy or postoperative chemoradiotherapy, cases with recurrence, and/or cases with contralateral CLN metastases.

Conclusion

The results of our analyses demonstrated that the discriminant equation using values obtained from preoperative CT and PET images can be used to predict CLN metastasis in OSCC. Additionally, the discriminant equation using CK13 and CK17 can be used to predict recurrence. These findings may help determine treatment strategies and improve patient outcomes. However, further studies are needed to determine whether these discrimination equations are applicable to all patients with OSCC.

Conflicts of Interest

The Authors have no conflicts of interest in relation to this study.

Authors' Contributions

H. Kanemoto collected and analyzed the data, conducted experiments including immunohistochemistry staining, and prepared the manuscript. K. Obata analyzed the data and contributed to the final draft of the manuscript. K. Hasegawa and S. Ono collected the data and assisted in writing the manuscript. K. Ono prepared the clinical sections. S. Ibaragi corrected and approved the manuscript. The final manuscript was read and approved by all Authors.

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