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RESEARCH

Treatment strategy for cervical lymph node metastases from early-stage tongue and floor of the mouth squamous cell carcinoma using tumour budding and depth of invasion as predictors

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Abstract

Objectives This study aimed to determine whether elective neck dissection can help improve outcomes in early-stage tongue and floor squamous cell carcinoma (SCC) by statistically analysing the relationship between information obtained from biopsy specimens and the incidence and prognosis of cervical lymph node metastasis (CLM).

Materials and methods Biopsy specimens of 103 patients diagnosed with early cT1–T2 cancer of the tongue and floor of the mouth were included.

Results Multivariate analysis showed that the three parameters significantly correlated with CLM, and univariate analyses showed that budding score (BS) ≥ 5 and pathological depth of invasion (pDOI) ≥ 5 mm were independent risk factors for CLM. There were significant differences in the 5-year cumulative disease-specific survival between the BS < 5 and BS ≥ 5 groups, the pDOI < 5 mm and pDOI ≥ 5 mm groups, and the positive and negative budding and depth of invasion (BD) score groups.

Conclusion In early-stage tongue and floor of the mouth cancers with maximum tumour diameter ≤ 20 mm, it may be necessary to treat occult CLM during initial surgery based on the following preoperative criteria: pDOI ≥ 5 mm or BS ≥ 5 in biopsy specimens and DOI ≥ 8 mm on imaging. The BD model exhibited the highest specificity and proved helpful for CLM prediction.

Clinical relevance pDOI ≥ 5 mm and BS ≥ 5 were independent predictors of CLM and prognosis in early-stage tongue and floor of the mouth cancers with a maximum tumour diameter of 20 mm.

Keywords Early tongue and floor of the mouth squamous cell carcinoma · Cervical lymph node metastasis · Biopsy · Tumour budding · Depth of invasion

Background

Cervical lymph node metastasis (CLM) is a prognostic factor in oral squamous cell carcinoma (SCC) including the tongue and floor of the mouth. Clinically diagnosed early-stage oral SCC, including the tongue and floor of the mouth (T1/T2N0M0), shows occult CLM in 20%–40% of cases [1–5]. Therefore, it is important to manage occult CLM at the time of initial treatment, and elective neck dissection (END) is one such treatment option. In cases of primary tumour resection requiring reconstruction with free tissue transfer or pedicle flap transfer, there is less confusion in determining the indication for END in terms of the surgical field, as the extent of the reconstructive surgery extends to the neck. However, the primary site can often be directly closed after resection of the primary tumour in the early-stage tongue and floor of the mouth SCC. Even if the SCC is a high-grade type, there is often hesitation in performing END from the viewpoint of surgical invasion, and a wait-and-see policy is frequently adopted [3]. To further improve treatment

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outcomes for early-stage tongue and floor of mouth SCC, it may be helpful to predict the possibility of occult CLM based on preoperative information and only indicate END for high-risk cases.

Recently, the biological characteristics of the tumour invasion front have been reported to be valuable predictors of CLM in oral SCC [6, 7]. Tumour budding (TB) is defined as the presence of either isolated single cells or small-cell clusters scattered in the stroma ahead of the invasive tumour front [8, 9] and is clinically applied as a predictor of colorectal cancer lymph node metastasis [10]. A few studies have investigated the relationship between TB and CLM in oral cancer [6, 7, 11–14]. The concept of performing secondary END for cases judged to be high-risk based on resection specimens has also been reported [15, 16]; however, this requires multiple surgeries and is burdensome for the patient.

The depth of invasion (DOI) from the normal mucosal level correlates more strongly with CLM [17], which was introduced as one of the criteria for tumour classification (T-classification) by the 8th edition of the Union for International Cancer Control (UICC) [18]. Various imaging modalities, such as computed tomography (CT), magnetic resonance imaging (MRI), and ultrasonography (US), determine DOI from preoperative findings. However, accurate evaluation is difficult in early-stage tongue and floor of mouth SCC because of metal artefacts, body movements, and slice width problems.

A biopsy is essential for the diagnosis of oral cancer and is the most straight forward technique. Histopathological information, including TB and DOI, obtained using this very simple technique can be useful in determining treatment options for early-stage tongue and floor of mouth SCC.

This single-centre retrospective study aimed to determine whether END can help improve outcomes in early-stage tongue and floor SCC by statistically analysing the relationship between information obtained from biopsy specimens and the incidence and prognosis of CLM.

Patients and methods

Of 565 patients with oral SCC who underwent surgery at the Department of Oral and Maxillofacial Surgery, Gunma University Hospital, between 2010 and 2021, 311 had tongue and floor of mouth SCC. Among them, 103 cases of early T1–T2 (UICC 8th edition) with a maximum diameter of 20 mm were included.

Patient age and location at initial treatment were obtained from medical records. Histopathologial endpoints such as WHO tumour differentiation grade (Gr), mode of invasion (Yamamoto–Kohama mode of invasion [YK classification])

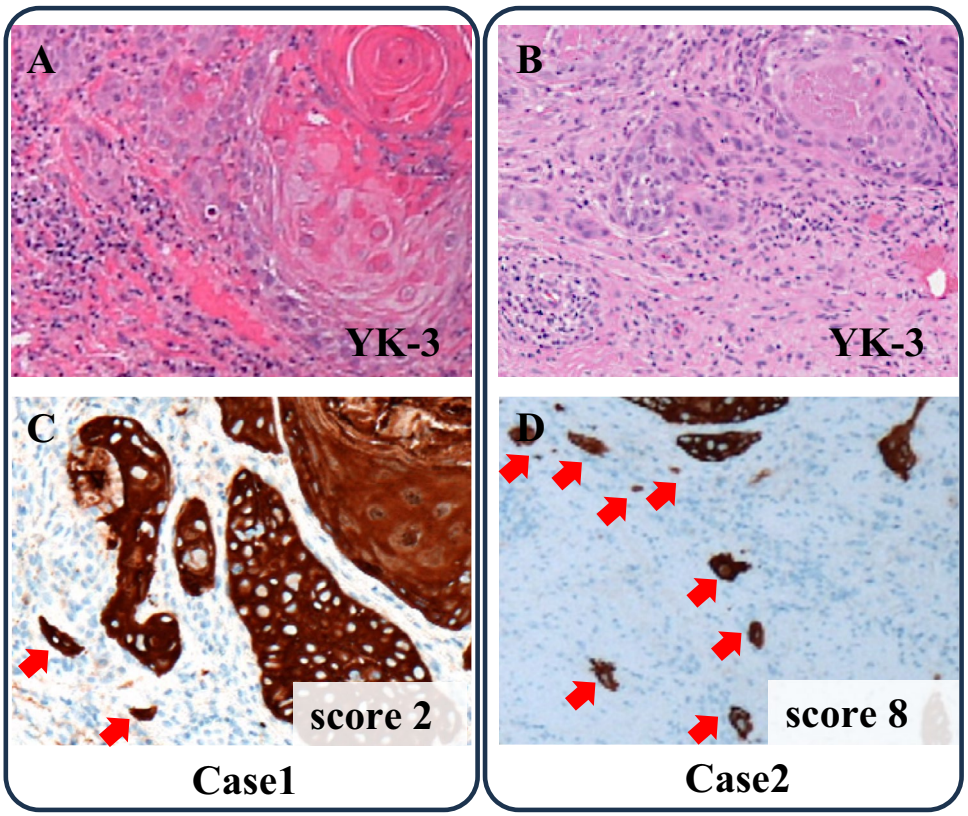


Fig. 1 Mode of invasion and tumour budding. TB seen in oral squamous cell carcinoma using haematoxylin–eosin staining (**A**, **B**) and immunostaining for cytokeratin (**C**, **D**). Both cases were classified as YK3 using haematoxylin–eosin staining, although BS was 2 and 8, respectively. TB: tumour budding, YK: Yamamoto–Kohama classification, BS: budding score

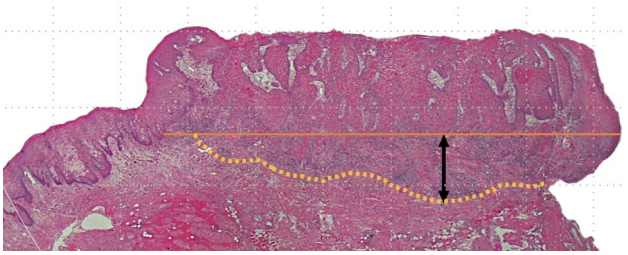


Fig. 2 Pathological depth of invasion. The pDOI (mm) is measured as the perpendicular distance from the basement membrane region to the deepest point of the infiltrative front of the tumour in the biopsy specimens. pDOI: pathological tumour depth of invasion

[19], budding score (BS), pathological DOI (pDOI), and mitotic count were evaluated in the biopsy specimens. The relationship between occult CLM and prognosis was examined retrospectively.

The YK classification as a mode of invasion was based on the general rules for clinical and pathological studies of oral cancer [20] and was classified into five types: YK1 to YK 4D. TB is described as a cancer cell cluster comprising < 5 cells or isolated single cells without a distinct structure, appearing as a bud near a large cancer nest (Fig. 1). The number of tumour buds in all biopsy specimens was evaluated using slides immunostained for pan-cytokeratin (AE1/AE3; 1:100 dilution; DAKO). As in Seki et al. [21, 22], the field with the largest number of budding foci was selected within a slide, and number of budding foci was counted in one field using a 20 × objective lens. Budding was assessed along the edge of the tumour invasion front, and a BS for each case was reported (Fig. 1). The pDOI (mm) was measured in the biopsy specimens as the perpendicular distance from the basement membrane region to the deepest point of the infiltrative front of the tumour according to the general rules for clinical and pathological studies on oral cancer in the 2nd edition [20] (Fig. 2). Mitosis was determined by observing 10 fields of view under an optical microscope at a high magnification of 400 × and using the total number of divisions (per 10 high-power fields). Two oral and maxillofacial surgeons and two pathologists analysed all histopathological variables.

The six factors (age, Gr, YK classification, BS, pDOI, mitosis) were compared between the groups using univariate analysis with a cross-table with Fisher's exact test used to compare CLM positivity and negativity. Items with significant differences between the two groups were analysed using logistic regression with the forward selection method to identify the clinical and pathological factors influencing CLM. The extracted explanatory variables had variance inflation factors of ≤ 10, confirming the absence of multicollinearity. Gr was divided into Gr 1 and 2/3, and YK classifications into YK1/2/3 and 4C/4D, each converted to a binary value. The cut-off point for age was set as the median. The cut-off value of the BS was set at 5 based on reports by Seki et al. [21, 22]. Discriminant analysis was performed on the extracted factors for the risk of CLM, and the sensitivity, specificity, and positive and negative predictive values were calculated. The 5-year cumulative overall survival (5y-OS) and the 5-year cumulative disease-specific

Table 1 Patients' background

Factors	Number of cases
Sex	
Male	48
Female	55
Location	
Tongue	96
floor of mouth	7
Tumor differentiation grade (WHO)	
Gr3	3
Gr2	24
Gr1	76
YK classification	
1	9
2	21
3	45
4C	25
4D	3
BS	
< 5	74
≥ 5	29
pDOI	
< 5 mm	80
≥ 5 mm	23
Mitotic count (/10 HPF)	
< 5	83
≥ 5	20
Cervical lymph node metastasis	
Negative	84
Positive	19
(Late lymph node metastasis)	15
Outcome	
Survival	94
Death of cancer	4
Death of other disease	5
Factors	**Median (range)**
Age	67 (24–94)
Budding score	1 (0–16)
Mitotic count (/10HPF)	2 (0–12)
Follow-up (months)	47 (2–140)

YK: Yamamoto–Kohama, BS: budding score, pDOI: pathological depth of invasion, HPF: high-power field

Table 2 Summary of patients who died due to distant metastasis

Patients	1	2	3	4
Sex	Man	Man	Man	Man
Age	74	77	83	79
Location	Tongue	Tongue	Tongue	Floor of mouth
Maximum diameter(mm)	12	14	18	16
Tumor differentiation grade	3	3	2	2
pDOI (mm)	5.0	6.0	6.5	5.0
YK classification	4D	4D	4C	4C
Budding score	7	10	5	15
Mitosis (10HPF)	2	1	2	2
CLM (Post initial treatment period, month)	Late-CLM(9 M)	Late-CLM(6 M)	Late-CLM(4 M)	Late-CLM(4 M)
Response to CLM	ND + CRT	ND + RT	ND	ND
Distant metastasis	Lung Mediastinal lymph node Hilar lymph node	Mediastinal lymph node Paravalvular lymph node	Lung	Peripharyngeal lymph node
Survival period (month)	20 M	55 M	17 M	24 M

pDOI: pathological depth of invasion, CLM: cervical lymph node metastasis

YK: Yamamoto–Kohama, HPF: high-power field, ND: no disease, CRT: chemoradiotherapy, RT: radiotherapy

survival (5y-DSS) were determined using Kaplan–Meier and log-rank tests.

Statistical analyses were performed using SPSS software (ver. 28 IBM Corp; Armonk, NY, USA). *P*-values < 0.05 were considered statistically significant. This study conformed to the Declaration of Helsinki and was approved by the Institutional Research Committee of Gunma University (IRB number: HS2023-043). Written informed consent was obtained from all enrolled patients using the opt-out method.

Table 3 Univariate and multivariate analyses of clinical and pathological variables for lymph node metastasis

	Lymph node metastasis		Univariate analysis	Multivariate analysis		
	Positive ($n=19$)	Negative ($n=84$)	*p*-value	Odds ratio	*p*-value	95% CI
Age						
< 67	6	43	0.169			
≧67	13	41				
Tumor differentiation grade						
Gr2/3	9	18	0.024			
Gr1	10	66				
YK classification						
YK-4C/-4D	7	21	0.295			
YK-1/-2/-3	12	63				
Budding score						
≧ 5	13	16	< 0.001	4.900	0.011	1.436–16.717
< 5	6	68				
pDOI (mm)						
≧ 5	12	11	< 0.001	6.294	0.003	1.840–21.535
< 5	7	73				
Mitotic count (10HPF)						
≧ 5	6	14	0.195			
< 5	13	70				

YK: Yamamoto–Kohama, pDOI: pathological depth of invasion HPF: high-power field

Table 4 Comparison of diagnostic accuracy of each indicator for cervical lymph node metastasis

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
BS ≥ 5	68.4	81.0	44.8	91.9
pDOI ≥ 5 mm	63.2	86.9	52.2	91.2
BD score positive	47.4	92.9	60.0	88.6

BS: budding score, BD: budding and depth of invasion model, PPV: positive predictive value, NPV: negative predictive value

Results

Patient background

The patient backgrounds are presented in Table 1. The age range was 24–94 years, and the median was 67. Forty-eight patients were men and 55 were women. Gr 1 was seen in 76 patients (73.8%), Gr 2 in 24 (23.3%), and Gr 3 in 3 (2.9%). YK1–3 and YK4C/4D were seen in 75 (72.8%) and 28 (27.2%) patients, respectively. BS < 5 and BS ≥ 5 were seen in 74 (71.8%) and 29 (28.2%) patients, respectively, with a maximum of 16 and a median of 1. pDOI < 5 mm was seen in 80 (77.7%) patients, while pDOI ≥ 5 mm was seen in 23 (22.3%) patients. The median mitosis count per 10 high-powered fields was 2, with 20 patients showing 5 or more mitoses. The postoperative observation period ranged from 2–140 months, with a median of 47.

CLM occurred in 19 of the 103 patients (18.4%). Fifteen of these 19 patients (78.9%) had late CLM. Four patients with END required tongue reconstruction at the primary site of resection due to tongue size and safety margins, combined with neck dissection, and the results revealed a CLM positivity. Of the 103 patients, 94 survived without recurrence, 4 died of distant metastasis, and 5 died of other diseases during follow-up.

Table 2 shows the background of the four patients with poor prognosis due to distant metastasis. All patients had late CLM after primary tumour resection and had a Gr 2 or 3, BS ≥ 5 mm, and pDOI ≥ 5 mm. Survival ranged from 17–55 months, and three patients died within 2 years. Neck dissection controlled neck metastases in all four patients.

Analysis of histopathological predictive factors of CLM

For each clinical and pathological factor, the results of the two-group comparisons between the CLM-negative and CLM-positive groups are shown in Table 3. Gr. 2/3, BS ≥ 5, and pDOI ≥ 5 mm differed significantly between two groups (Gr. 2/3: $P = 0.024$, BS ≥ 5: $P < 0.001$, pDOI ≥ 5 mm: $P < 0.001$). Multivariate analysis using the three parameters significantly correlated with CLM in the univariate analyses showed that BS ≥ 5 and pDOI ≥ 5 mm were independent risk factors for CLM (BS ≥ 5: $P = 0.011$, pDOI ≥ 5 mm: $P = 0.003$). Additionally, a BD model combining tumour budding and depth of invasion was created [23] and a positive diagnosis was made if BS was ≥ 5 and pDOI was ≥ 5 mm. The sensitivities of the BS (BS ≥ 5), pDOI (pDOI ≥ 5 mm), and BD models were 68.4%, 63.2%, and 47.4%, respectively, with specificities of 81.0%, 86.9%, and 92.9%, respectively. The corresponding positive predictive values were 44.8%, 52.2%, and 60.0%, and the corresponding negative predictive values were 91.9%, 91.2%, and 88.6%, respectively (Table 4).

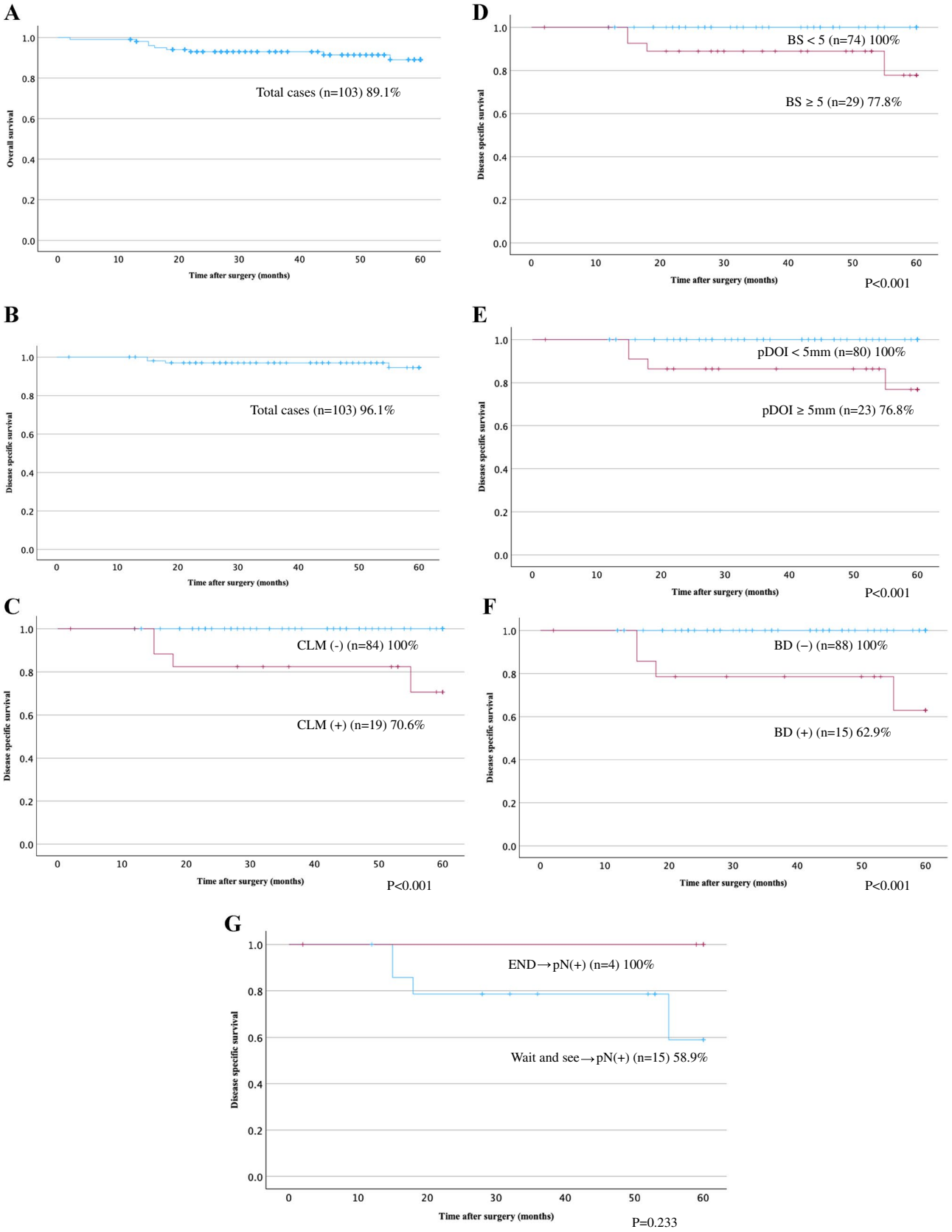
Five-year cumulative survival rate

The 5y-OS of all 103 patients was 89.1%, and the 5y-DSS was 96.1% (Fig. 3A, B). The 5y-DSS of the CLM-negative group was 100%, and that of the CLM-positive group was 70.6%, showing a significant difference ($P < 0.001$) (Fig. 3C). There were significant differences in 5y-DSS between the BS < 5 (100%) and BS ≥ 5 (77.8%) groups ($P < 0.001$) (Fig. 3D), between the pDOI < 5 mm (100%) and pDOI ≥ 5 mm (76.8%) groups ($P < 0.001$) (Fig. 3E), and between the negative (100%) and positive (62.9%) BD score groups ($P < 0.001$) (Fig. 3F). In the END group and the neck dissection group after late CLM, the 5y-DSS of the END group was 100% better than that of the late CLM group (58.9%), but the difference was not significant ($P = 0.233$) (Fig. 3G).

Discussion

This study made two important clinical observations. First, the 5y-OS and 5y-DSS of patients with early-stage tongue and floor of the mouth SCC that could be directly closed at the primary site were favourable, but the prognostic factor was CLM. Patients who underwent END for CLM tended to have a better prognosis than those who underwent neck dissection for late CLM. Second, the BS and pDOI of biopsy specimens were both predictors and prognostic factors for CLM. This single-centre retrospective study included 103 patients treated with a uniform treatment strategy and surgeon. Therefore, the data were considered more accurate than those of previous multicentre studies because of the absence of inter-institutional bias.

This study showed that CLM is the most important prognostic factor even in early-stage tongue and floor of mouth SCCs ≤ 20 mm diameter that can be closed directly after primary cancer resection. CLM was found in 19 (18%) of 103 patients in this study. In previous studies, 20%–40% of such cases already have occult CLM. The 5y-DSS of 70.6%


A
Overall survival
Total cases (n=103) 89.1%
Time after surgery (months)
B
Disease specific survival
Total cases (n=103) 96.1%
Time after surgery (months)
C
Disease specific survival
CLM (-) (n=84) 100%
CLM (+) (n=19) 70.6%
Time after surgery (months)
P<0.001
D
Disease specific survival
BS < 5 (n=74) 100%
BS ≥ 5 (n=29) 77.8%
Time after surgery (months)
P<0.001
E
Disease specific survival
pDOI < 5mm (n=80) 100%
pDOI ≥ 5mm (n=23) 76.8%
Time after surgery (months)
P<0.001
F
Disease specific survival
BD (−) (n=88) 100%
BD (+) (n=15) 62.9%
Time after surgery (months)
P<0.001
G
Disease specific survival
END→pN(+) (n=4) 100%
Wait and see→pN(+) (n=15) 58.9%
Time after surgery (months)
P=0.233

◂**Fig. 3** Five-year cumulative survival rate. The 5y-OS of all 103 patients was 89.1%, and the 5y-DSS was 96.1% (**A**, **B**). The 5y-DSS of 84 CLM-negative patients was 100%, and that of 19 CLM-positive patients was 70.6%, showing a significant difference between the two groups ($P<0.001$) (**C**). There were significant differences in the 5y-DSS between the BS < 5 (100%) and BS ≥ 5 (77.8%) groups ($P<0.001$) (**D**), between the pDOI < 5 mm (100%) and pDOI ≥ 5 mm (76.8%) groups ($P<0.001$) (**E**), and between the BD score negative groups (100%) and BD model score positive group (62.9%) ($P<0.001$) (**F**). In the END group ($n=4$) and the neck dissection group after late CLM ($n=15$), the 5y-DSS of the END group was 100% better than that of the late metastasis group (58.9%), but the difference was not significant ($P=0.233$) (**G**). 5y-OS: 5-year cumulative overall survival, 5y-DSS: 5-year cumulative disease-specific survival, CLM: cervical lymph node metastasis, pDOI: pathological depth of invasion, BS: budding score, BD model: budding and depth of invasion model, END: elective neck dissection

in the CLM-positive group was significantly lower than that in the CLM-negative group (100%). It must be recognised that the occurrence of CLM despite the cancer's early stage indicates that such cancers are oncologically more invasive, i.e. more aggressive.

This study compared the 5y-DSS of a group with CLM detected by END and a group that underwent neck dissection for late CLM. The results showed better 5y-DSS in the END group (100% versus 58.9%), but the difference was insignificant. D'cruz et al. [24] conducted a prospective randomised trial of 496 patients with T1–T2 oral cancer and reported a significantly better 3y-OS of 67.5% for wait-and-see versus 80.0% for END treatment. To the best of our knowledge, this is the first study to demonstrate the superiority of END over the wait-and-see approach in early-stage oral cancer. However, due to the possibility of inadequate postoperative follow-up and differences in healthcare systems, it is dangerous to apply these results directly to the Japanese healthcare system. The problem with END is that it is unnecessary in approximately 80% of cases without occult CLM. Recently, supraomohyoid neck dissection has been recommended for END. Although postoperative functional disability is reduced compared to radical neck dissection, postoperative sequelae such as whistling deformity of the lower lip due to facial nerve injury, cervical cutaneous sensory abnormality, and pain due to complex regional pain syndrome (CRPS) may occur even in the 80% of cases who do not require surgery. Several studies have compared END with observation with early-stage cN0 tongue cancer, showing no significant difference in survival rates between END and observation [25–28], and some reports recommend a wait-and-see policy [29]. However, the wait-and-see approach may delay treatment with possible CLM. For these problems, there is no consensus on the indications for END. Further improvements in the accuracy of occult CLM diagnosis are essential for functional preservation and improved survival.

YK4C and YK4D levels were not significant predictors of CLM in this study. This is because the YK classification is largely based on subjective factors, resulting in variations between diagnosticians and institutions [30]. Other possible histopathological reasons are: 1) some cases diagnosed as YK2 or YK3 contain a high-grade pathology similar to 4C/4D; 2) some cases are YK3 but have a BS > 5 (Fig. 1); and 3) some cases are YK4C with diffuse infiltration but a BS < 5. Combining such histopathological malignancy indices with numerical scores, such as the BS, allows for more accurate histopathological malignancy grading. In cases with marked pericancerous lymphocytic infiltration, it is difficult to evaluate TB using haematoxylin and eosin staining [15, 31, 32], and immunostaining is essential. Immunostaining can facilitate diagnosis, reduce inter-diagnostic variation, and provide a more objective evaluation of malignancy. Differences in histopathological grading between biopsy and resection specimens have also been reported [33], and these differences need to be reduced to accurately evaluate the mode of invasion and BS from biopsy specimens. This means that the deepest part of the cancer must be sampled in the biopsy. The deepest part of the tumour can be easily sampled in early-stage tongue and floor of the mouth SCC. Yamamoto et al. [19] emphasised the importance of sampling the deepest part of the tumour during biopsy. Sampling the deepest part of the tumour based on US and MRI findings is necessary to accurately evaluate the mode of invasion and BS in biopsy specimens.

pDOI ≥ 5 mm, a predictor of CLM in this study, correlates to DOI ≥ 8 mm before contraction, considering a contraction rate of 50%–60% with formalin [34]. These results are consistent with a report of an increased rate of cervical lymph node metastasis in tongue cancer with DOI ≥ 8 mm on MRI/US [35]. END should be considered for histopathological DOIs ≥ 5 mm in biopsy specimens from early-stage tongue and oral floor SCC and in cases with DOI ≥ 8 mm on imaging when evaluation of biopsy specimens is difficult.

The BD score, a composite factor of BS and pDOI, may be a more accurate predictor of CLM and prognosis than either factor alone because of its high specificity and negative predictive value. Furthermore, the positive BD score group had a significantly lower 5y-DSS than the negative BD score group.

Recently, new biomarkers have been applied to determine malignancy and prognosis in addition to TB and DOI [36]. In addition, new tumour–stromal relationships and tumour–mesenchymal states have been identified [7, 13, 37–40]. In future studies, histopathological information sampled from biopsy materials should be synthesised clearly and concisely to facilitate appropriate oral cancer treatment decisions.

The four patients with tumour-related death underwent neck dissection for late CLM, and primary cancer and neck metastasis were controlled, but distant metastases

were not. This is a problem with this treatment that this study identified. Further research is necessary to determine whether END can effectively control distant metastases.

In conclusion, in early-stage tongue and floor of the mouth SCC with a maximum tumour diameter of 20 mm, it may be necessary to treat occult CLM during initial surgery (e.g. primary resection + direct closure + END) based on the following preoperative criteria: pDOI ≥ 5 mm or BS ≥ 5 in biopsy specimens, cDOI ≥ 8 mm on imaging. In addition, the BD model exhibited the highest specificity and proved helpful in confirming CLM predictions.

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Author contributions MO: conceived and designed the study; acquired, analysed, and interpreted the data. TY, KS, TS, and TM: analysed the patient's data/findings. MS: performed immunoassays and immunohistochemical staining. SY: conceived the study, participated in its design and coordination, and helped in drafting the manuscript. All authors read and approved the final manuscript.

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Declarations

Ethics approval The study was approved by the Institutional Review Board for Clinical Research of Gunma University Hospital and conforms to the Declaration of Helsinki.

Competing interests The authors declare no competing interests.

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