Intraoperative frozen section histological analysis of resection samples is useful for the control of primary lesions in patients with oral squamous cell carcinoma

AKIHIKO MIYAWAKI[1], HIROSHI HIJIOKA[2], TAKAYUKI ISHIDA[2], ETSURO NOZOE[2], NORIFUMI NAKAMURA[2] and RYOICHI OYA[1]

[1]Department of Oral and Maxillofacial Surgery, University Hospital of Occupational and Environmental Health, Kitakyushu, Fukuoka 807-8556; [2]Department of Oral and Maxillofacial Surgery, Field of Oral and Maxillofacial Rehabilitation, Advanced Therapeutics Course, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Kagoshima 890-8520, Japan

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Abstract. To ensure reliable surgical margins, intraoperative frozen section histological analysis (FS) has been performed since October, 2005 as follows: i) the orientation at the anatomical position and extent of the tumor are shared between oral pathologists and oral surgeons using imaging evaluations and pathological pictures and the planned site of sampling for intraoperative FS is confirmed; ii) a tumor team is organized and the team marks the tumor area and sets the resection range to correct the setting errors of the resection range among operators; iii) vital Lugol staining is applied to the lesion prior to tumor resection, the surgical margin is set based on the non-stained region and the extent of the tumor is macroscopically confirmed in the maximum cross-sectional surface of the resected specimen; and iv) FS is performed using samples from resected specimens to confirm the mucoepithelium and safety margin of the deep stump. The aim of this study was to evaluate the usefulness of our FS method. The treatment outcomes of oral squamous cell carcinoma were retrospectively investigated in patients treated prior to (Group 1) and after (Group 2) the introduction of our FS method. The recurrence rate of the primary lesions was high (17.3%) in Group 1, but decreased significantly in Group 2 (6.9%). Regarding clinicopathological factors, the condition of the surgical margins was associated with recurrence of the primary lesion in Group 1, but not in Group 2. In conclusion, our FS method appears to be useful for resecting tumors with reliable safety margins.

Correspondence to: Dr Akihiko Miyawaki, Department of Oral and Maxillofacial Surgery, University Hospital of Occupational and Environmental Health, 1-1 Iseigaoka Yahatanishi-ku Kitakyushu, Fukuoka 807-8556, Japan
E-mail: makihiko@clnc.uoeh-u.ac.jp

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Introduction

Locoregional control and treatment outcomes for primary oral cancers and cervical lymph node metastases have improved markedly with improvements in imaging diagnosis, advances in multidisciplinary treatment applying surgical therapy, radiotherapy and chemotherapy and the development of supportive therapies for oral cancer treatment (1-3). However, despite these advances, the primary lesion recurs in several cases. Therefore, control of the primary lesion is a major concern for oral surgeons, as recurrent lesions are difficult to control and markedly compromise the quality of life of the patients. In surgical therapy for oral cancers, the resection range for the primary lesion is determined based on the TNM classification following evaluation of the clinical findings and images from contrast-enhanced computed tomography (CT), contrast-enhanced magnetic resonance imaging (MRI), positron emission tomography-CT and ultrasonography (1). The safety margins of the resected primary lesion are confirmed during surgery by palpation and from intraoperative frozen section histological analysis (FS). However, the resection range varies among operators, the usefulness of FS has not been verified and the primary lesion recurs in several cases. As regards the methods used for evaluating the safety margins of the resected primary lesions, the 2013 guidelines for the treatment of oral cancer (1) described vital Lugol staining as being useful for mucosal lesions in cancer of the tongue. The recurrence rate of the primary lesions was found to be lower among patients for whom the non-Lugol-stained region was included in the resection field compared to those for whom there was no vital Lugol staining in the resected lesions. Although the examination of all the surgical margins of the resected primary lesions in FS is difficult and the scope of evaluation is limited, investigating the presence or absence of residual tumor tissue in the resected margin appears to be useful. Although actual methods for FS are not frequently

reported, a survey of the American Head and Neck Society by Meier *et al* (4) stated that 76% of their members collected samples for FS from the surgical bed, 14% from the resected specimens and the remaining 10% from both sites. There were no differences in the findings of FS regardless of the sampling site. Black *et al* (5) reported the actual condition of FS from the viewpoint of the pathologists, stating that the evaluation of the margins was inaccurate, as the anatomical orientation was not labeled in the resected specimens submitted to pathologists, which requires cooperation with the surgeons. Another report stated that FS is inappropriate for routine investigation of the margins for resected oral cancers other than tongue cancer, as the anatomical structure is complicated and anatomical limits mean that surgical access to the tumor site is generally poor (6). However, Wang *et al* (7) histopathologically examined the surgical margins of resected tumor specimens in FS using samples obtained by excisional biopsy and reported that no patient required additional treatment following surgery. Kurita *et al* (8) observed cross-sectional preparations of resected tumor specimens under a digital light microscope and reported that evaluation of the deep margin of the tumor was useful. Therefore, although FS was reported to be useful, there is yet no established method. To achieve accurate FS, it is important to share patient information with the pathologists, indicate the anatomical orientation of the resected tumor specimens and prepare samples from appropriate sites (9,10). The advantages of FS using samples collected from resected tumor specimens are as follows: The anatomical orientation is readily determined; the distance between the surgical margin and tumor is macroscopically observed in the cross-sectional surface of the resected specimen; reliable sampling from an appropriate region is possible, as the anatomical orientation is readily determined; and the anatomical position of additional tumor resection is accurately reflected in the surgical field when the surgical margin is either close to the tumor or positive (9,10). Based on these advantages, we collected samples from resected tumor specimens for FS.

To evaluate the usefulness of our FS system in the control of primary lesions, using methods such as intraoperative vital Lugol staining and FS of surgical specimens, the outcomes of treatment for oral squamous cell carcinoma (OSCC) were retrospectively investigated in patients treated prior to and after the introduction of this FS method to Kagoshima University.

Materials and methods

Patient eligibility criteria. The subjects comprised 153 patients with OSCC who underwent radical surgery at the Department of Oral and Maxillofacial Surgery at Kagoshima University between January, 2000 and September, 2011. The patients were divided according to whether they underwent surgery prior to or after adopting FS for the control of primary lesions in October, 2005 as follows: Group 1 (52 patients), treated between January, 2001 and September, 2005; and Group 2 (101 patients), treated from October, 2005 onwards. The preservation of the morphological characteristics of the oral cavity and functions such as mastication, swallowing, speech and esthetics is crucial in the treatment of advanced OSCC (11). Several studies have reported the effect of preoperative chemoradiotherapy plus radical surgery for advanced squamous cell carcinoma of the oral cavity (11-14). As a result, surgery was performed as the main treatment and chemoradiotherapy was performed as preoperative treatment throughout this period. Surgery comprised en bloc resection of the primary site, with neck dissection in N1 or more advanced cases. Chemoradiotherapy included external beam radiotherapy with a total radiation dose of 30-40 Gy delivered in 10-20 fractions and concurrent chemotherapy using either platinum-containing agents, such as cisplatin or carboplatin, 5-fluorouracil, or oral S-1. The clinical characteristics of the patients are summarized in Table I. There were no significant differences according to gender, age, primary site, or distribution of T or stage classification between the groups. However, more patients were treated with surgery alone in Group 2 compared to Group 1, as Group 1 included a higher number of advanced cases. The duration of the follow-up ranged from 1 year to 10 years and 8 months (median, 2 years and 8 months).

This study was approved by the Ethics Committee of Kagoshima University and written informed consent was obtained from all the included patients.

FS. To ensure reliable surgical margins, we have been performing FS for the control of primary lesions since October, 2005 as follows: First, the orientation of the anatomical extent is determined by oral pathologists and oral surgeons based on images obtained by contrast-enhanced CT and MRI and pathological pictures and the planned sampling site for FS is confirmed. Second, a tumor team is organized and marks the tumor area, setting a reliable 1-cm resection range from the mark to correct the setting errors of the resection range by the operators. Third, only the presence or absence of tumor in tissues collected from the surgical bed of the tumor resection site is investigated in FS, but vital Lugol staining is applied (Fig. 1A) and the surgical margin is set based on the non-stained region. The distance from the tumor is macroscopically confirmed in the maximum cross-sectional surface of the resected specimen by oral surgeons and pathologists (Fig. 1B and C, white arrows). Finally, FS is performed using a sample collected from the resected specimen to confirm the mucoepithelium and safety margin of the deep stump (Fig. 1D).

Items analyzed in the two groups. First, the rates of positive surgical margins, recurrence of the primary lesion and disease-specific survival were compared. Second, the clinicopathological factors associated with recurrence of primary lesions were analyzed. The investigated clinicopathological factors included age, gender, tumor location, T classification, tumor properties, grade of differentiation, invasion pattern, presence or absence of lymphatic, vascular, or nerve invasion, condition of the surgical margins and histological therapeutic effect. The patients were divided by age into those aged ≥61 and those <60 years, by T classification into T2 or lower and T3 or more advanced cases, by grade of differentiation into moderately or poorly differentiated and well-differentiated cases and by condition of the surgical margins into cases with residual tumor (positive margins), without residual tumor but ≤3 mm from the tumor, or without residual tumor and >3 mm from the tumor (negative margins). The invasion pattern was classified as YK3 or lower and YK4C or more advanced, according to

Table I. Clinical characteristics of patients.

Characteristics	Group 1, no. (%) (n=52)	Group 2 , no. (%) (n=101)	Total patient no. (%) (n=153)
Gender			
Male	32 (38.5)	60 (40.6)	92 (60.1)
Female	20 (61.5)	41 (59.4)	61 (39.9)
Age (years)			
<60	13 (25.0)	33 (32.7)	46 (30.0)
≥61	39 (75.0)	68 (67.3)	107 (70.0)
Primary site			
Upper gingiva	6 (11.5)	10 (9.9)	16 (10.5)
Tongue	23 (44.2)	52 (51.5)	75 (49.0)
Lower gingiva	16 (30.8)	30 (29.7)	46 (30.0)
Other	7 (13.5)	9 (8.9)	16 (10.5)
Clinical T classification			
T1/2	37 (71.2)	83 (82.2)	120 (78.4)
T3/4	15 (28.8)	18 (17.8)	33 (21.6)
Stage			
I	10 (19.2)	18 (17.8)	28 (18.3)
II	12 (23.1)	40 (39.6)	52 (34.0)
III	19 (36.5)	24 (23.8)	43 (28.1)
IV	11 (21.2)	19 (18.8)	30 (19.6)
Treatment			
S	8 (15.4)	54 (53.4)	62 (40.5)
R→S	21 (40.4)	5 (5.0)	26 (17.0)
R+C→S	23 (44.2)	42 (41.6)	65 (42.5)

S, surgery; R, radiotherapy; C, chemotherapy.

the classification reported by Yamamoto *et al* (15). As regards the histological therapeutic effect, recurrence of the primary lesion was evaluated in patients who received preoperative therapy by dividing them into cases with Gr2a or lower and Gr2b or higher effects, according to the classification reported by Shimosato *et al* (16). Third, disease-specific survival rates were compared between the groups according to the condition of the surgical margins. Finally, the primary site, condition of the surgical margin, time of recurrence and prognosis were analyzed in cases with recurrence of the primary lesion in Groups 1 and 2.

Statistical analysis. Statistical analysis was performed using JMP® statistical analysis software, version 9 (SAS Institute, Tokyo, Japan). The associations between recurrence rate and clinicopathological factors were analyzed using the Pearson's χ^2 test. The survival rates were calculated using the Kaplan-Meier method and analyzed using the log-rank test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Comparison of surgical margin positivity, primary lesion recurrence and disease-specific survival by the Kaplan-Meier method. The surgical margin positivity rates were 9.6 and 3.9% in Groups 1 and 2, respectively, with a decreasing tendency, although the difference was not statistically significant (Table II). The recurrence rate for primary lesions was high (17.3%, 9/52) in Group 1, but improved significantly to 6.9% (7/101) in Group 2 (Table II). Disease-specific survival rates were 81.5 and 87.9% in Groups 1 and 2, respectively, showing a slight but non-significant tendency toward improvement (Fig. 2).

Clinicopathological factors associated with recurrence of the primary lesions. The Pearson's χ^2 test was performed regarding the presence or absence of recurrence of the primary lesion as a response variable and gender, age, location, T classification, tumor properties, grade of differentiation, invasion pattern, presence or absence of lymphatic, vascular, or nerve invasions, condition of the surgical margins and histological therapeutic effect as explanatory variables. In Group 1, factors associated with recurrence of the primary lesion were the presence or absence of nerve invasion and the condition of the surgical margins; recurrence rate was found to be significantly higher among cases with surgical margins close to the tumor or residual tumor in the surgical margins (positive margins). In Group 2, none of the explanatory factors were significantly associated with the presence or absence of recurrence of the primary lesion. Regarding the association between primary

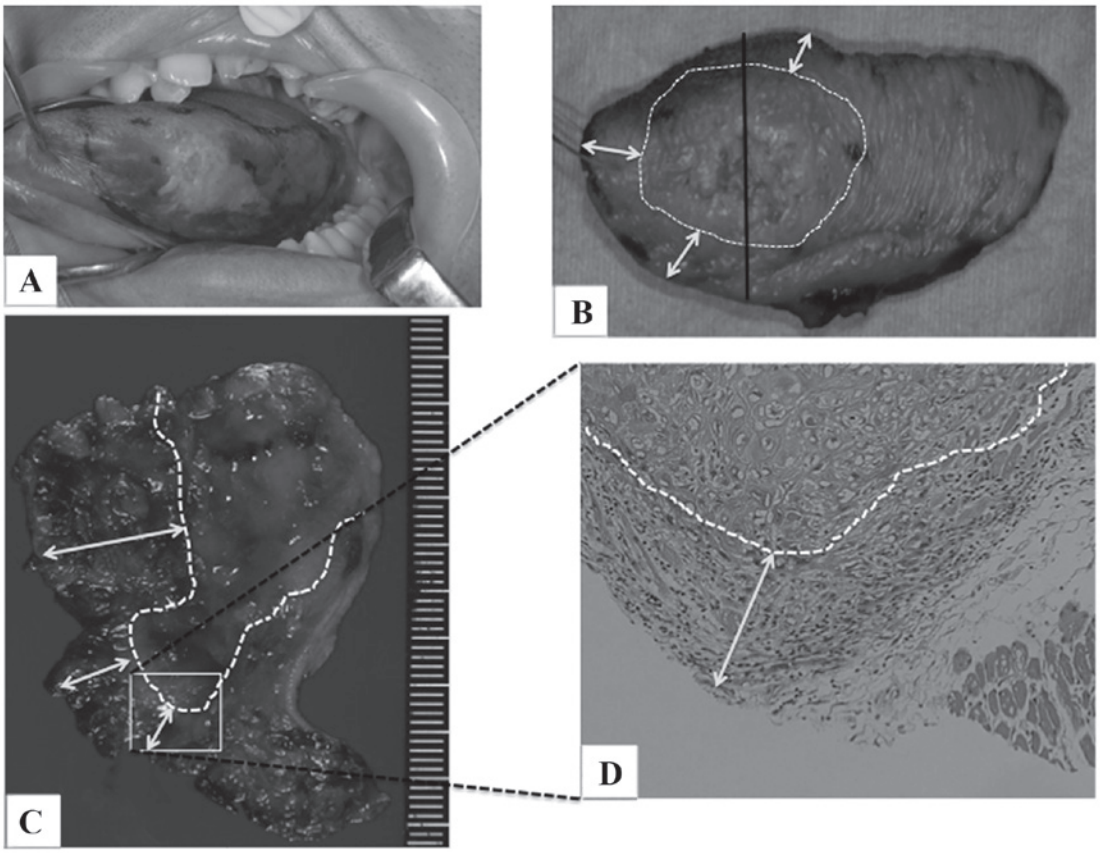

Figure 1. Intraoperative frozen section histological analysis method. (A) A case of T1 cancer of the tongue. Vital Lugol staining was applied during surgery and the surgical margins 10 mm from the tumor were determined. (B) Resected tumor specimen. The specimen was cut in cross-section (black line) in the center of the tumor (region circled with white dotted line) with a palpable induration. The white arrows show the distance between the surgical margins and the tumor macroscopically. (C) The cross-sectional surface of the tumor was observed macroscopically to evaluate the surgical margins (the white dotted line represents the tumor margin). The white arrows show the distance between the surgical margins and the tumor. (D) Hematoxylin and eosin staining (magnification, x200). A sample was collected from the deepest region close to the macroscopic tumor and subjected to intraoperative rapid pathological examination. The white arrows shows the distance between the region demarcated by the white dotted line and the surgical margins microscopically.

Table II. Rates of negative surgical margins and recurrence at primary site in Groups 1 and 2.

Variables	Group 1	Group 2	P-value
Margins			
Positive	47	97	
Negative (%)	5 (9.6)	4 (3.9)	0.16
Recurrence			
No	43	94	
Yes (%)	9 (17.3)	7 (6.9)	0.047[a]

[a]P<0.05 (Pearson's χ^2 test).

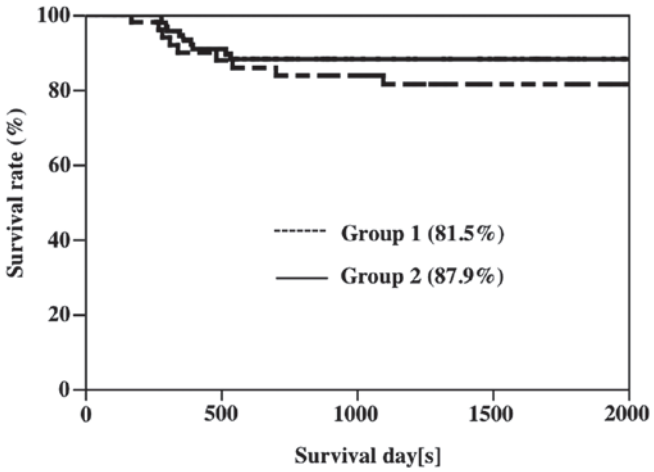

Figure 2. Disease-specific survival rates in Groups 1 and 2.

site and recurrence of the primary lesion, primary lesions in the upper and lower gingiva frequently recurred in both groups, but the incidence decreased in Group 2 and cancer of the tongue recurred in only 1 patient (Table III).

Disease-specific survival rate by condition of the surgical margins in Groups 1 and 2. In Group 1, the survival rate was 87.8% in cases with negative surgical margins, 72.8% in cases with margins close to the tumor and 60.0% in cases with positive margins. In Group 2, the survival rates of cases with negative margins and cases with margins close to the tumor were 93.3 and 78.3%, respectively, exhibiting a tendency toward higher rates compared to those in Group 1, although the differences were not significant. The disease-specific survival rate in positive-margin cases was 50.0%, which was

Table III. Clinicopathological factors associated with recurrence at primary site.

	Group 1			Group 2		
Variables	No recurrence	Recurrence no. (%)	P-value	No recurrence	Recurrence no. (%)	P-value
Gender						
Male	28	4		57	4	
Female	15	5	0.25	37	3	0.36
Age (years)						
≥61	12	1		32	1	
<60	31	8	0.29	62	6	0.28
Primary site						
Upper gingiva	4	2 (33.3)		8	2 (20.0)	
Tongue	20	3 (13.0)		51	1 (1.9)	
Lower gingiva	13	3 (18.8)		26	4 (13.3)	
Other	6	1	0.56	9	0	0.12
Clinical T classification						
T1/2	32	5		78	5	
T3/4	11	4	0.26	16	2	0.44
Pattern of tumor growth						
Superficial spreading	6	0		22	3	
Outgrowing	2	0		24	1	
Ingrowing	35	9	0.37	48	3	0.49
Differentiation						
Moderate/poor	29	7		81	7	
High	14	2	0.54	13	0	0.29
Mode of invasion[b]						
≤YK3	36	6		76	4	
YK4C/4D	7	3	0.24	18	3	0.16
Lymphatic invasion						
Negative	39	7		82	6	
Positive	4	2	0.27	11	1	0.85
Vascular invasion						
Negative	37	7		76	6	
Positive	6	2	0.53	17	1	0.79
Nerve invasion						
Negative	42	7		86	7	
Positive	1	2	0.02[a]	7	0	0.45
Surgical margin						
Negative	32	3		73	4	
Close (<3 mm)	9	3		17	3	
Positive	2	3	0.01[a]	4	0	0.21
Chemoradiation effect[c]						
≤Gr2a	11	5		12	2	
≥Gr2b	22	3	0.13	30	1	0.17

[a]P<0.05 (Pearson's χ^2 test). [b]Classification reported by Yamamoto *et al* (15). [c]Classification reported by Shimosato *et al* (16).

lower compared to that in Group 1. Significant differences according to the condition of the surgical margins were noted in the survival rates of both groups (Fig. 3).

Patients with recurrence of primary lesions in Groups 1 and 2 and outcome. In Group 1, the primary tumors recurred in 9 of the 52 patients (17.3%). By primary site, recurrence

Table IV. Cases of recurrence at primary site and prognosis.

Case	Age (years)	Gender	Primary site	TN stage	Surgical margins	Site of recurrence	Time to recurrence	Salvage treatment	Outcome
Group 1									
1	52	Female	Upper gingiva	T2N1	Close	Skin	3y 2m	Excision	Alive
2	63	Male	Upper gingiva	T3N0	Close	Buccal mucosa	5m	Excision	Alive
3	70	Female	Tongue	T1N0	Negative	Tongue	3y 11m	Excision	Alive
4	67	Male	Tongue	T2N0	Negative	Tongue	1y 5m	Excision	Deceased
5	71	Male	Tongue	T2N1	Negative	Skin	3y	Excision	Alive
6	62	Male	Lower gingiva	T4N2b	Positive	Retromolar	3m	Chemotherapy	Alive
7	68	Female	Lower gingiva	T2N0	Positive	Skin	1m	-	Deceased
8	86	Female	Lower gingiva	T2N1	Close	Gingiva	5m	Chemotherapy	Deceased
9	84	Female	Buccal mucosa	T3N0	Positive	Buccal mucosa	1m	Excision	Deceased
Group 2									
10	66	Male	Upper gingiva	T2N2b	Negative	Buccal mucosa	1y	Radiotherapy	Alive
11	84	Female	Upper gingiva	T3N0	Close	Skin	7m	Excision	Deceased
12	81	Female	Tongue	T4N0	Negative	Tongue	1y	Radiotherapy	Deceased
13	72	Male	Lower gingiva	T4N1	Close	Skin	4m	Excision	Deceased
14	81	Female	Lower gingiva	T2N0	Negative	Skin	3m	Excision	Alive
15	84	Female	Lower gingiva	T4N0	Close	Gingiva	5m	-	Deceased
16	60	Female	Lower gingiva	T2N0	Negative	Gingiva	1y 9m	Excision	Alive

Y, years; m, months.

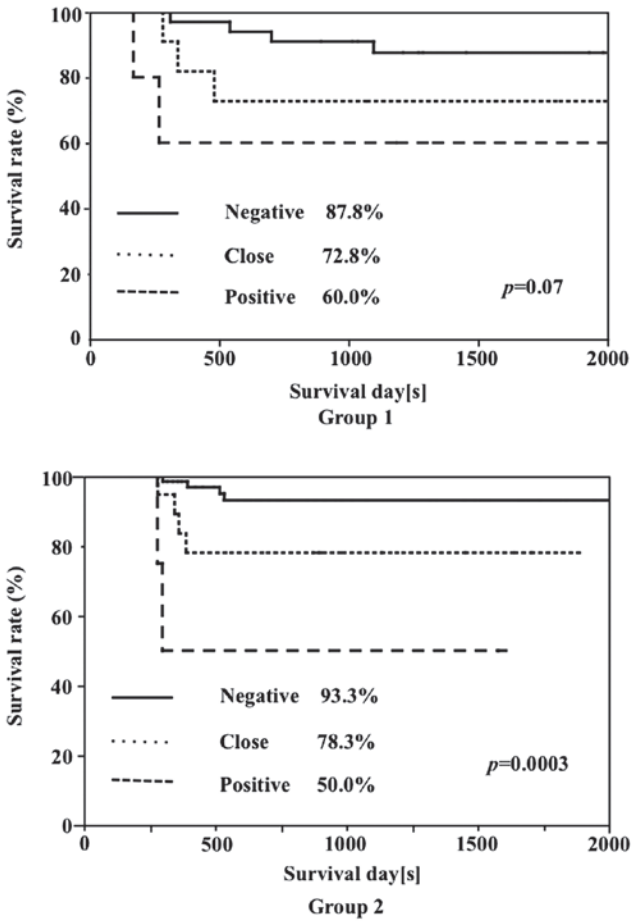

Figure 3. Disease-specific survival rate according to the surgical margin in Groups 1 and 2.

occurred in the upper gingiva in 2 patients, tongue in 3, lower gingiva in 3 and buccal mucosa in 1 patient. The T classification varied between T1 and T4 and the surgical margins were negative, close to the tumor and positive in 3 patients each. The recurrence site was the tongue, gingiva, buccal mucosa and retromolar mucosa around the primary site in 6 patients and the tumor advanced into the skin and recurred in 3 patients. The time to recurrence was between 1 and 3 months in cases with positive margins, after 5 months in 2 cases with close margins and significantly later in cases with negative margins (range, 1 year and 5 months to 3 years and 11 months).

The treatment comprised tumor resection or chemotherapy in 8 patients and 5 patients (62.5%) survived, but the outcomes were poor and 4 patients (37.5%) succumbed to the primary tumor.

In Group 2, the primary lesions recurred in 7 of the 101 patients (6.9%). The primary site was located in the upper and lower gingiva in 6 cases and in the tongue in 1 case. The T classification was late T2 or more advanced and the surgical margins were negative in 4 and close to the tumor in 3 cases; however, no positive cases were recorded. The site of recurrence was the tongue, gingiva and buccal mucosa around the primary lesion in 4 patients and the skin in 3 patients. The time to tumor recurrence was 4-7 months in cases with close margins, >1 year in 2 cases with negative margins, but only 3 months after surgery in 1 case with negative margins. The treatment comprised radiotherapy or resection in 6 patients, of whom 3 (50%) survived and 3 succumbed to the primary lesion. One patient with lower gingival cancer was untreatable and eventually succumbed to the disease. The characteristics of the cases with recurrence of the primary tumor are summarized in Table IV.

Discussion

The major clinical factor determining the prognosis of patients with OSCC is cervical lymph node metastasis, whereas the depth and pattern of invasion are important factors associated with recurrence of the primary lesion and lymph node metastasis (1). In addition to the depth and invasion pattern of the tumor, the presence or absence of tumor cells in the surgical margins is crucial for the surgical treatment of OSCC (17,18). Setting a safety margin ≥10 mm is considered as appropriate for the resection of oral cancers, although a clear basis for this distance is currently lacking (19). We have attempted to control primary lesions by following this criterion (10-mm safety margin), confirming that the region remains unstained on vital Lugol staining during surgery and including this region in the resection field, confirming the macroscopic tumor extent in the cross-sectional surface of the resected specimen and performing FS for a sample collected from the resected specimen. Although the disease-specific survival rate was not significantly affected, the rate of positive surgical margins was decreased. The rate of primary lesion recurrence was high (17.3%, 9/52) in Group 1, but improved significantly to 6.9% (7/101) in Group 2. Among the clinicopathological factors, the condition of the surgical margins and the presence or absence of nerve invasion were associated with recurrence of the primary lesion in Group 1, but no significant association between the surgical margin status and recurrence of the primary lesion was observed in Group 2. However, the prognosis of patients with positive margins was poor in both groups and, although the incidence of recurrent cancer of the tongue tended to decrease, upper and lower gingival cancers recurred in a number of patients, reflecting the limitations to our approach for the control of primary lesions.

The number of studies reporting the recurrence rate of primary lesions in detail is limited. Although the rates vary depending on the primary site, Yamamoto *et al* (18) reported a rate of 10.3% in patients with T1/2 cancer of the tongue, whereas that of oral cancers of other regions, including the tongue, was reported to be 9-18% by other studies (18,20-22). Although a simple comparison with these reports is not feasible due to the differences in patient background and treatment strategy, the rate of primary lesion recurrence was 17.3% in Group 1, which was similar to the previously reported rates, and decreased to 6.9% in Group 2, which was lower compared to the rates reported elsewhere. In addition, among the clinicopathological factors, the condition of the surgical margins and nerve invasion were associated with recurrence of the primary lesion in Group 1, while no significant correlation was noted between surgical margin status and recurrence of the primary lesion in Group 2. Surgical margin positivity represents a significant factor associated with decreased survival rate and a high risk of postoperative recurrence (1,22). The condition of the surgical margins was significantly associated with survival rate in both groups (Fig. 3), suggesting that our approach for the control of primary lesions contributes to decreasing the risk of recurrence and our FS method appears to be useful for the evaluation of the surgical margins. However, the survival rate did not significantly improve in Group 2 compared to that in Group 1, although a tendency towards an increase was observed. The poor prognosis of patients with cervical lymph node metastasis, including secondary cervical lymph node metastasis in Group 2 (data not shown), may have affected our results.

The recurrence rate of the primary lesions varies depending on the primary site. The oral cavity has a complex structure, comprising mixed hard and soft tissues and the invasion pattern varies depending on the direction of tumor advancement. Such factors may contribute to the difficulties in the determination of the resection range with adequate safety margins (1). Recurrence of the primary lesion was frequently noted in the upper and lower gingiva in both groups. This tendency persisted in Group 2, but the incidence was decreased in all the primary sites. As regards cancer of the tongue, a low rate of primary lesion recurrence (3.8%) has been reported (15). In our patients with cancer of the tongue, the rate of primary site recurrence was 13.0% in Group 1, but decreased to 1.9% in Group 2. In Group 2, recurrence occurred in the upper and lower gingiva in 2 and 4 patients, respectively (Table IV), but recurrence in the tongue occurred in only 1 case. The advances in imaging diagnosis may also be a decisive factor when determining the resection range, but the advantages of our FS method (i.e., the cross-sectional surface of tumors is readily observed macroscopically, the distance between the surgical margin and tumor is readily determined and the anatomical orientation is readily identified) is evident in tissues retaining anatomical continuity, such as the tongue, which may facilitate determining a reliable resection range for cancer of the tongue. In Group 2, although recurrence was negative on intraoperative rapid pathological diagnosis, upper and lower gingival cancers recurred in the surrounding tissue relatively early after surgery (3-7 months) in 4 of the 6 patients. These cases reflect the limitations of our FS method in assisting with determining a reliable tumor resection range, in addition to the difficulties involved in imaging diagnosis of tumors located in regions with a complex anatomical structure, such as advanced upper and lower gingival cancers containing hard as well as soft tissues. The prognosis for cases with recurrence is very poor (23,24). To determine the resection range for the primary lesion in such cases, further improvements are required in the imaging evaluation of jaw bone infiltration, tumor invasion pattern and infiltration into the surrounding soft tissues in consideration of the direction of tumor advancement (25).

In conclusion, our FS method appears to be useful for resecting tumors with reliable safety margins for tissues retaining anatomical continuity, such as the tongue. The macroscopic observation of cross-sections of the resected tumor specimens is easy and the surgical margins may be readily investigated. However, this method is insufficient for determining a resection range in tissues containing soft tissue and jaw bone, such as upper and lower gingival tumors, and other methods to control primary lesions must be investigated.

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