A clinicopathological study of perineural invasion and vascular invasion in oral tongue squamous cell carcinoma

Yuki Matsushita[1]*, Souichi Yanamoto[1], Hidenori Takahashi[1], Shin-ichi Yamada[1], Tomofumi Naruse[1], Yuki Sakamoto[1], Hisazumi Ikeda[2], Takeshi Shiraishi[2], Shuichi Fujita[3], Tohru Ikeda[3], Izumi Asahina[2], Masahiro Umeda[1]

1 Department of Clinical Oral Oncology, Nagasaki University Graduate School of Biomedical Sciences

2 Department of Regenerative Oral Surgery, Nagasaki University Graduate School of Biomedical Sciences

3 Department of Oral Pathology, Nagasaki University Graduate School of Biomedical Sciences

*Corresponding author:

Department of Clinical Oral Oncology, Unit of Translational Medicine, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki, Japan

Tel.: +81 95 819 7698; Fax: +81 95 819 7700; E-mail: matsushita@nagasaki-u.ac.jp

Key words: Oral tongue squamous cell carcinoma (OTSCC); Perineural invasion; Vascular invasion; intermediate risk,

Short title: Perineural/vascular invasions in OTSCC

ABSTRACT

The risk factors for the recurrence of head and neck cancer are classified as high or intermediate risk. Intermediate risks include multiple positive nodes without extracapsular nodal spread, perineural/vascular invasions, pT3/T4 primary tumors, and positive level IV/V nodes. However, little evidence is available to validate intermediate risk factors. We analyzed perineural/vascular invasions in 89 patients who underwent radical surgery for oral tongue squamous cell carcinoma, whose records were reviewed retrospectively. Perineural and vascular invasions were found in 27.0 and 23.6% of cases, respectively, and both had a strong relationship with histopathological nodal status ($P = 0.005$). The 5-year disease specific survival and overall survival rates of patients with perineural invasion were significantly lower than those of patients without perineural invasion ($P < 0.001$ and $P = 0.002$, respectively). The 5-year disease specific survival of UICC stage I and II cases with perineural/vascular invasion was significantly lower than those without ($P < 0.001$ and $P = 0.008$, respectively). Perineural/vascular invasions are risk factors for regional metastasis and poor prognosis. We recommend elective neck dissection when perineural/vascular invasions are found in clinical stage I and II cases. The accumulation of further evidence to consider intermediate risks is required.

INTRODUCTION

Extracapsular nodal spread and the presence of positive margins are major adverse prognostic factors for survival in head and neck cancer. Patients with these prognostic factors are considered to be at high risk of recurrence and have a survival benefit of postoperative adjuvant chemoradiotherapy in head and neck squamous cell carcinoma[1-3]. Moreover, intermediate risk factors (multiple positive nodes without extracapsular nodal spread, perineural/vascular invasions, pT3 or pT4 primary tumors, and oral cavity or oropharyngeal primary cancers with positive level IV/V nodes) are established as indications that patients should undergo postoperative radiation therapy (RT) and that adjuvant chemoradiotherapy should be considered[1-3]. The postoperative management of high-risk disease has been clarified by two multicenter randomized trials: The Radiation Therapy Oncology Group (RTOG) trial #9501[2], and The European Organization for Research and Treatment of Cancer (EORTC) trial #22931[3]. These two trials revealed common risk factors for the recurrence of oral cancer such as extracapsular nodal spread, positive surgical margins, and multiple positive nodes without extracapsular nodal spread[4]. However, the RTOG recently demonstrated that patients with two or more positive lymph nodes did not benefit from adding chemotherapy to RT[5]. Therefore, the criteria for high or intermediate risk factors that suggest postoperative adjuvant therapy are controversial and need to be studied further. In particular, not many studies have discussed the intermediate risk factors: only the RTOG trial #9501 and EORTC trial #22931[1-3]. Nevertheless, several studies have reported adverse effects associated with chemoradiotherapy[6,7]. Therefore, it is necessary to accumulate evidence regarding the truly effective treatments for patients with oral cancer in order to perform the correct postoperative adjuvant treatments. Perineural and vascular invasions are defined as

intermediate risk factors. Some reports revealed a relationship between perineural/vascular invasions and prognosis in oral tongue squamous cell carcinoma (OTSCC) patients[8-22]. However, the contribution of perineural and vascular invasions to prognosis remains unclear because of contradictory reports.

In this study, we reconsidered the high and intermediate risk factors for the recurrence of oral cancer, and particularly analyzed the relationship between perineural/vascular invasions and prognosis.

MATERIALS AND METHODS

Patients and pathological examinations

The records of 89 patients who underwent radical surgery for OTSCC, which was previously untreated, between January 2001 and December 2011 were reviewed retrospectively. The study cohort included patients with histologically confirmed OTSCC and a minimum follow-up of 12 months. All study patients underwent extensive pretreatment evaluations, including blood chemistry, complete blood cell count, chest X-ray, computed tomography (CT) and/or magnetic resonance imaging (MRI) of the head and neck area, ultrasonic echo (US), thoracoabdominal CT, and provided informed consent to participate in the study. In our institution, surgery alone was preferred for the initial treatment of patients with oral cancer. However, patients who hesitated to consent to surgical intervention or inoperable patients with unresectable cancer and/or severe systemic illness were selected for chemotherapy, radiation therapy and/or supportive palliation. All patients underwent glossectomy with curative intent. Neck dissection was performed for the cN positive cases and cN negative cases that need tongue reconstructive surgery because of the size of primary

tumor. No sentinel lymph node biopsy was performed. Postoperative adjuvant chemo/radiotherapy or radiation therapy was undergone in accordance with current the National Comprehensive Cancer Network (NCCN) guidelines[1]. Patients who had adverse features (high risk feature; extracapsular nodal spread and the presence of positive margins, intermediate risk feature; multiple positive nodes without extracapsular nodal spread, perineural/vascular invasions, pT3/T4 primary tumors, and positive level IV/V nodes.) were treated depending on the degree of the risk. Clinical staging was defined by palpation, inspection, CT, MRI, US, and so on according to the International Union against Cancer (UICC) TNM classification system[23]. Tumors were classified histopathologically as well-, moderately-, or poorly-differentiated according to their cellular differentiation, as defined by the World Health Organization criteria[23]. The pattern of invasion (POI) was examined at the host/tumor interface; POI types 1–4 were defined previously by Bryne et al.[24]. The depth of invasion (DOI) was measured as the infiltrative portion of the tumor that extended below the surface of the adjacent mucosa. Previous studies demonstrated that a DOI ≥4 mm had predictive value for cervical lymph node metastasis in patients with OTSCC[25-27]; therefore, DOI was classified as ≥4 and <4 mm in the current study. A previous large cohort study demonstrated that a pathological margin distance ≤4 mm was significantly associated with locoregional recurrence[28]; therefore, surgical margin status was classified as superficial (>4 mm) and deep (≤4 mm) in this study. Perineural invasion was defined as the presence of tumor cells within any of the three layers (the epineurium, perineurium, and endoneurium) of the nerve sheath. Vascular invasion was defined as the clear presence of tumor cells within a vascular space (lymphatic space or blood vessel), and the tumor was required to be adhered to the vessel endothelium or attached to a

thrombus in the vessel. Expert pathologists who were unaware of the clinical outcomes performed all pathological assessments. Disease-specific survival (DSS) was calculated from the time of initial examination to the time of death related to local, regional, or distant recurrence/metastasis of the disease or the time of last follow-up. Overall survival (OS) was calculated from the time of initial examination to the time of death or last follow-up.

Statistical analysis

Statistical analyses were performed using StatMate IV (Atms Co., Tokyo, Japan). Categorical data were assessed using the chi-squared or Fisher's exact tests, as appropriate. The clinicopathological information of perineural/vascular invasions were compared using chi-squared or Fisher's exact tests, as appropriate. The clinicopathological information included pT stages, histopathological nodal status, UICC stages, POI, local recurrence, and treatment. DSS and OS were calculated using the Kaplan–Meier method, and significance was evaluated using the log-rank test. A value of $P < 0.05$ was considered to be significant.

RESULTS

Patient characteristics

The patient demographics are summarized in Table 1. The male-to-female ratio was 1.28, and 50 subjects were male. The mean age at diagnosis was 63.4 years (range, 28–88 years). Perineural invasion was found in 24 of 89 (27.0%) patients, and vascular invasion was found in 21 (23.6%) individuals. Histopathological lymph node metastasis was found in 25 (28.1%) patients. Local recurrence developed in 11 patients (12.4%) during the follow-up period. Postoperative distant metastasis was occurred in 3 (3.3%)

patients. The mean follow-up period of the whole series was 49.4 months (range, 3–125 months).

Association of perineural invasion with clinicopathological factors and survival

Perineural invasion was associated significantly with T-classification, histopathological nodal status, POI, DOI, and distant metastasis, but not with local recurrence (Table 2). Univariate analysis using the two-tailed Fisher's exact tests revealed that perineural invasion had a strong relationship with T-classification ($P = 0.02$), histopathological nodal status ($P = 0.005$), POI ($P < 0.001$), DOI ($P < 0.001$), and distantmetastasis ($P = 0.02$). Kaplan–Meier analyses followed by log-rank tests showed that perineural invasion was significantly associated with 5-year DSS and OS (Figure 1A, B). The 5-year DSS and OS of patients with perineural invasion were significantly lower than those of patients without perineural invasion ($P < 0.001$ and $P = 0.002$, respectively). The 5-year DSS of individuals with perineural invasion was 60.9%, compared with 96.7% in those without. Similarly, the 5-year OS of patients with perineural invasion was 60.9%, compared with 90.2% in those without perineural invasion.

Association of vascular invasion with clinicopathological factors and survival

Vascular invasion was significantly associated with histopathological nodal status and DOI but not with local recurrence and distant metastasis (Table 2). Univariate analysis revealed that vascular invasion was a risk factor for histopathological nodal status ($P = 0.005$) and had a strong relationship with DOI ($P = 0.01$). The Kaplan–Meier analysis followed by log-rank tests revealed that vascular invasion was significantly associated with 5-year DSS (Figure 1C). The 5-year DSS of patients with vascular invasion was significantly lower than that of those without vascular invasion ($P = 0.03$). However, there was no relationship between vascular invasion and 5-year OS ($P = 0.12$; Figure

1D). The 5-year DSS of patients with vascular invasion was 70.9%, compared with 89.4% in those without. Similarly, the 5-year OS of individuals with and without vascular invasion was 70.9% and 89.2%, respectively.

Correlation between perineural and vascular invasion and UICC stage-specific survival rates

We next analyzed the prognosis of patients with different UICC stage tumors. The 5-year DSS of individuals with UICC stage I and II tumors with perineural invasion was significantly lower than those without perineural invasion ($P < 0.001$) (Figure 2A). In contrast, there was no difference in the 5-year DSS of UICC stage III and IV tumors with and without perineural invasion (Figure 2B). Similarly, the 5-year DSS of patients UICC stage I and II tumors with vascular invasion was significantly lower than those without vascular invasion ($P = 0.008$) (Figure 2C). In contrast, there was no difference in the 5-year DSS of vascular invasion-positive and -negative cases among those with UICC stage III and IV (Figure 2D). The 5-year DSS was 54.2% and 98.1% in stage I and II cancers that were positive and negative for perineural invasion, respectively. Similarly, the 5-year DSS was 64.7% in vascular invasion-positive cases compared with 92.9% in -negative cases among patients with stage I and II cancers. These TNM classifications were clinically defined. Thirteen of 72 cTNM stage I and II cancers were upstaged to stage III and IV after pathological findings because of occult positive lymph nodes. Compared the upstaged cases with no changed cases, there was no significant difference for DSS.

Correlation between perineural and vascular invasion and DOI-specific survival rates

Perineural and vascular invasion had strong relation with DOI, respectively. Because perineural/vascular invasion were possible to just be a surrogate marker for DOI, we

evaluated the relationship between perineural/vascular invasion and DSS in condition that each DOI groups (<4mm; n=55, ≥4mm; n=34) eliminating influence of DOI. In DOI <4mm group, only 2 patients were dead. It was difficult to dissert the tendency. Then, the relations were evaluated in DOI ≥4mm group. The results were shown in Table 3. Perineural invasion had significant relationship with DSS in DOI ≥4mm group (P = 0.04). On the other hand, vascular invasion had no relationship.

DISCUSSION

Perineural invasion is a well-known predictor of poor outcome in colorectal, pancreatic, and salivary gland cancers[8,9]. Although the perineural invasion of head and neck cancer was reported first by Liebig et al.[10], there are no unified perineural invasion classifications in oral cancer. The frequency of perineural invasion in oral squamous cell carcinoma was reported to be 2–82%[11,12]. In addition, some studies revealed a correlation between perineural invasion and prognostic factors[11-16]. Some reports suggested that perineural invasion had no effect on 5-year local control and OS[13,14]. In contrast, other studies demonstrated that perineural invasion was significantly related to local recurrence, regional metastasis, and survival[11,15]. In the present study, perineural invasion was unrelated to local recurrence, but had a strong relationship with regional metastasis and survival. Chatzistefanou et al.[16] also concluded that perineural invasion found to be an independent prognosticator for neck metastasis and regional recurrence. Consistent with this, some previous studies revealed that vascular invasion increased the risk of regional metastasis and poor prognosis[12]. In contrast, other reports demonstrated that vascular invasion was not related to any prognostic factors[8-19]. In the current study, vascular invasion-positive status was related to the occurrence of nodal metastasis and

had a strong relationship with 5-year DSS, but did not affect local recurrence and OS. Distant metastasis had a relation with perineural invasion and no relation with vascular invasion in present study. However, It is difficult to discuss about this point because distant metastasis were occurred only 3 cases in the current study. These results suggest that perineural/vascular invasions are effective predictors of regional metastasis. In addition, perineural invasion may be a clinical predictor of survival.

The current study also compared the relationship between perineural/vascular invasion and prognosis according to UICC stage. Perineural invasion-negative and vascular invasion-negative cases had a better prognosis than did perineural invasion-positive and vascular invasion-positive cases in UICC stage I and II patients. In contrast, there were no significant relationship between perineural/vascular invasions and prognosis in UICC stage III and IV patients. These results suggest that perineural and vascular invasion are important factors for predicting prognosis during the early stages of OTSCC. Thirteen cases of stage I and II cancers were upstaged to stage III and IV for a reason of occult lymph node metastasis. However, these upstaged 13 cases didn't show worse prognosis. These results suggest that perineural invasion and vascular invasion are acceptable for prognosticator for clinically defined early stage OTSCC.

Previous studies revealed that patients with high-risk factors (extracapsular nodal spread and/or positive surgical margin) require adjuvant chemoradiotherapy[1-3]. However, the amplifying effect of chemotherapy to RT is not elucidate for the cases of presence of intermediate risk factors (multiple positive nodes without extracapsular nodal spread, perineural/vascular invasions, pT3 or pT4 primary tumors, and oral cavity or oropharyngeal primary cancers with positive level IV or V nodes). The criteria for the use of adjuvant therapy in intermediate risk patients are unclear. Moreover, it remains

unclear which intermediate risk factor has the strongest relationship with prognosis. In the current study, the relationship between the intermediate risk factors of perineural and vascular invasion and prognosis was evaluated. Although both were related to regional metastasis and DSS, only a perineural invasion-positive status decreased OS. These results suggest that various intermediate risk factors have different relationships with prognosis.

Generally, risk factors are given scores or rankings[29], and the diagnosis and treatment strategies of various diseases are decided according to these scores. Evaluating the priority of each intermediate risk is needed. Finally, criteria need to be defined to determine the optimal postoperative treatment of patients with OTSCC. Therefore it is important that more studies are performed that consider intermediate risks, similar to the present study.

DOI is currently the best predictor of occult metastasis; therefore, it should be used as a guide for elective neck dissection. For tumors with a depth >4 mm, elective neck dissection should be considered if RT is not planned. For those with a depth <2 mm, elective neck dissection is only considered in highly selective situations. For those with a depth of 2–4 mm, clinical judgment (regarding the reliability of follow-up, clinical suspicions, and other factors) must be used to determine the suitability of elective dissection[1,25-27]. The present study demonstrated the strong relation between perineural/vascular invasion and DOI. There was capability that perineural/vascular invasion was just a surrogate marker for DOI. Evaluating the independent role of perineural/vascular invasion eliminating the influence of DOI, perineural invasion was suggested the strong prognosticator. The current study suggested that perineural and vascular invasions are related to neck metastasis and survival in patients with early

stage OTSCC. It is possible that cases of OTSCC with perineural/vascular invasion may have already metastasized regionally. Some previous reports suggested that perineural invasion should be considered when making the decision whether to perform elective neck dissection and which postoperative treatment to use[16,20-22]. The results of the current study suggest that elective neck dissection should be considered if perineural or vascular invasion is observed. Until recent years, the effectiveness of sentinel lymph node biopsy had been obscure. Therefore, our institution had not performed sentinel lymph node biopsy. Actually in this study, we didn't undergo sentinel lymph node biopsy. However, recent review concluded the high detection rate of sentinel lymph node and the high sensitivity of the test justify an important role of sentinel lymph node biopsy in the diagnostic pathway of cT1/T2 oral cavity squamous cell carcinoma patients[31]. Latest NCCN guidelines also added sentinel lymph node biopsy in treatment algorithm about T1-2N0 oral cavity cancer. We should consider performing sentinel lymph node biopsy in the future. It is important to decide the neck dissection comprehensively by perineural/vascular invasion, DOI, sentinel lymph node biopsy, and so on.

On the other hand, the present study did not evaluate the effect of preventive chemoradiotherapy and RT (with irradiation extending to the neck region) because of our no experiences. The appropriate extension of the irradiating range for OTSCC cases with perineural/vascular invasion should be analyzed further.

In conclusion, perineural and vascular invasion are risk factors for regional metastasis and adverse prognosis. In particular, perineural invasion has a strong relationship with prognosis. We recommend that elective neck dissection should be considered when

perineural or vascular invasion is found in tumor samples obtained during preoperative incisional biopsy in clinical stage I and II cases.

Competing interests

None declared.

Funding

None.

Ethics approval

This study was approved by the ethics committees of the Nagasaki University Hospital.

Patient consent

Consent obtained.

Statement to confirm

All authors have viewed and agreed to the submission

CAPTIONS TO ILLUSTRATIONS

Figure 1. Comparison of the Kaplan–Meier curves for 5-year disease-specific survival (DSS) and overall survival (OS) in cases with different perineural and vascular invasion statuses. A, DSS according to perineural invasion status; B, OS according to perineural invasion status; C, DSS according to vascular invasion status; D, OS according to vascular invasion status.

Figure 2. Comparison of Kaplan–Meier curves for 5-year disease-specific survival (DSS) according to UICC stage-specific perineural and vascular invasion. A, DSS according to perineural invasion status in UICC stage I and II cases; B, DSS according to perineural invasion status in UICC stage II and IV cases; C, DSS according to vascular invasion status in UICC stage I and II cases; D, DSS according to perineural invasion status in UICC stage III and IV cases.

REFERENCES

1. The National Comprehensive Cancer Network; NCCN Clinical Practice Guidelines in Oncology: Head and Neck Cancers Version 2.2014. Available from: www.nccn.org.
2. Cooper JS, Pajak TF, Forastiere AA, Jacobs J, Campbell BH, Saxman SB, Kish JA, Kim HE, Cmelak AJ, Rotman M, Machtay M, Ensley JF, Chao KS, Schultz CJ, Lee N, Fu KK; Radiation Therapy Oncology Group 9501/Intergroup. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. N Engl J Med 2004: 350: 1937-1944.
3. Bernier J, Domenge C, Ozsahin M, Matuszewska K, Lefèbvre JL, Greiner RH, Giralt J, Maingon P, Rolland F, Bolla M, Cognetti F, Bourhis J, Kirkpatrick A, van Glabbeke M; European Organization for Research and Treatment of Cancer Trial 22931. Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. N Engl J Med 2004: 350: 1945-1952.
4. Bernier J, Cooper JS, Pajak TF, van Glabbeke M, Bourhis J, Forastiere A, Ozsahin EM, Jacobs JR, Jassem J, Ang KK, Lefèbvre JL. Defining risk levels in locally advanced head and neck cancers: a comparative analysis of concurrent postoperative radiation plus chemotherapy trials of the EORTC (#22931) and RTOG (# 9501). Head Neck 2005: 27: 843-850.
5. Cooper JS, Zhang Q, Pajak TF, Forastiere AA, Jacobs J, Saxman SB, Kish JA, Kim HE, Cmelak AJ, Rotman M, Lustig R, Ensley JF, Thorstad W, Schultz CJ, Yom SS, Ang KK. Long-term follow-up of the RTOG 9501/intergroup phase III trial: postoperative concurrent radiation therapy and chemotherapy in high-risk squamous

cell carcinoma of the head and neck. Int J Radiat Oncol Biol Phys 2012: 84: 1198-1205.

6. Langerman A, Maccracken E, Kasza K, Haraf DJ, Vokes EE, Stenson KM. Aspiration in chemoradiated patients with head and neck cancer. Arch Otolaryngol Head Neck Surg. 2007: 133: 1289-1295.
7. Langius JA, Zandbergen MC, Eerenstein SE, van Tulder MW, Leemans CR, Kramer MH, Weijs PJ. Effect of nutritional interventions on nutritional status, quality of life and mortality in patients with head and neck cancer receiving (chemo)radiotherapy: a systematic review. Clin Nutr. 2013: 32: 671-678.
8. Bapat AA, Hostetter G, Von Hoff DD, Han H. Perineural invasion and associated pain in pancreatic cancer. Nat Rev Cancer. 2011: 11: 695-707.
9. Speight PM, Barrett AW. Prognostic factors in malignant tumours of the salivary glands. Br J Oral Maxillofac Surg. 2009: 47: 587-593.
10. Liebig C, Ayala G, Wilks JA, Berger DH, Albo D. Perineural invasion in cancer: a review of the literature. Cancer. 2009: 115: 3379-3391.
11. Fagan JJ, Collins B, Barnes L, D'Amico F, Myers EN, Johnson JT. Perineural invasion in squamous cell carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg. 1998: 124: 637-640.
12. Kurtz KA, Hoffman HT, Zimmerman MB, Robinson RA. Perineural and vascular invasion in oral cavity squamous carcinoma: increased incidence on re-review of slides and by using immunohistochemical enhancement. Arch Pathol Lab Med. 2005: 129: 354-359.
13. Liao CT, Chang JT, Wang HM, Ng SH, Hsueh C, Lee LY, Lin CH, Chen IH, Huang SF, Cheng AJ, See LC, Yen TC. Does adjuvant radiation therapy improve outcomes

in pT1-3N0 oral cavity cancer with tumor-free margins and perineural invasion? Int J Radiat Oncol Biol Phys. 2008: 71: 371-376.

14. Magnano M 1, Bongioannini G, Lerda W, Canale G, Tondolo E, Bona M, Viora L, Gabini A, Gabriele P. Lymphnode metastasis in head and neck squamous cells carcinoma: multivariate analysis of prognostic variables. J Exp Clin Cancer Res. 1999: 18: 79-83.
15. Woolgar JA, Scott J. Prediction of cervical lymph node metastasis in squamous cell carcinoma of the tongue/floor of mouth. Head Neck. 1995: 17: 463–472.
16. Chatzistefanou I, Lubek J, Markou K, Ord RA. The role of neck dissection and postoperative adjuvant radiotherapy in cN0 patients with PNI-positive squamous cell carcinoma of the oral cavity. Oral Oncol. 2014: 50: 753-758.
17. Fagan JJ, Collins B, Barnes L, D'Amico F, Myers EN, Johnson JT. Perineural invasion in squamous cell carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg. 1998: 124: 637–640.
18. Parsons JT, Mendenhall WM, Stringer SP, Cassisi NJ, Million RR. An analysis of factors influencing the outcome of postoperative irradiation for squamous cell carcinoma of the oral cavity. Int J Radiat Oncol Biol Phys. 1997: 39: 137–148.
19. Magnano M, Bongioannini G, Lerda W, et al. Lymph node metastasis in head and neck squamous cell carcinoma: multivariate analysis of prognostic variables. J Exp Clin Cancer Res. 1999: 18: 79–83.
20. McMahon JD, Robertson GA, Liew C, McManners J, Mackenzie FR, Hislop WS, Morley SE, Devine J, Carton AT, Harvey S, Hunter K, Robertson AG. Oral and oropharyngeal cancer in the West of Scotland-long-term outcome data of a prospective audit 1999–2001. Br J Oral Maxillofac Surg. 2011: 49: 92–98.

21. Rodolico V, Barresi E, Di Lorenzo R, Leonardi V, Napoli P, Rappa F, Di Bernardo C. Lymph node metastasis tumour size, histologic variables and p27Kip1 protein expression. Oral Oncol. 2004; 40: 92–8.
22. Tai SK, Li WY, Chu PY, Chang SY, Tsai TL, Wang YF, Huang JL. Risks and clinical implications of perineural invasion in T1-2 oral tongue squamous cell carcinoma. Head Neck. 2012: 34: 994-1001.
23. Sobin LH, Gospodarowicz MK, Wittekind C, eds. TNM classification of malignant tumors, 7th edition. New York: Wiley-Blackwell; 2009.
24. Bryne M, Koppang HS, Lilleng R, Kjaerheim A. Malignancy grading of the deep invasive margins of oral squamous cell carcinomas has high prognostic value. J Pathol. 1992: 166: 375-381.
25. Asakage T, Yokose T, Mukai K, Tsugane S, Tsubono Y, Asai M, Ebihara S. Tumor thickness predicts cervical metastasis in patients with stage I/II carcinoma of the tongue. Cancer. 1998: 82: 1443-1448.
26. Lim SC, Zhang S, Ishii G, EndohY, Kodama K, Miyamoto S, Hayashi R, Ebihara S, Cho JS, Ochiai A. Predictive markers for late cervical metastasis in stage I and II invasive squamous cell carcinoma of the oral tongue. Clin Cancer Res. 2004: 10: 166-172.
27. Huang SH, Hwang D, Lockwood G, Goldstein DP, O'Sullivan B. Predictive value of tumor thickness for cervical lymph-node involvement in squamous cell carcinoma of the oral cavity: a meta-analysis of reported studies. Cancer. 2009: 115: 1489-1497.

28. Ganly I, Goldstein D, Carlson DL, Patel SG, O'Sullivan B, Lee N, Gullane P, Shah JP. Long-term regional control and survival in patients with "low-risk," early stage oral tongue cancer managed by partial glossectomy and neck dissection without postoperative radiation: the importance of tumor thickness. Cancer. 2013: 119: 1168-1176.
29. Gage BF, Waterman AD, Shannon W, Boechler M, Rich MW, Radford MJ. Validation of Clinical Classification Schemes for Predicting Stroke. JAMA. 2001: 285: 2864-2870.
30. Govers TM, Hannink G, Merkx MA, Takes RP, Rovers MM. Sentinel node biopsy for squamous cell carcinoma of the oral cavity and oropharynx: a diagnostic meta-analysis. Oral Oncol. 2013: 49: 726-732.

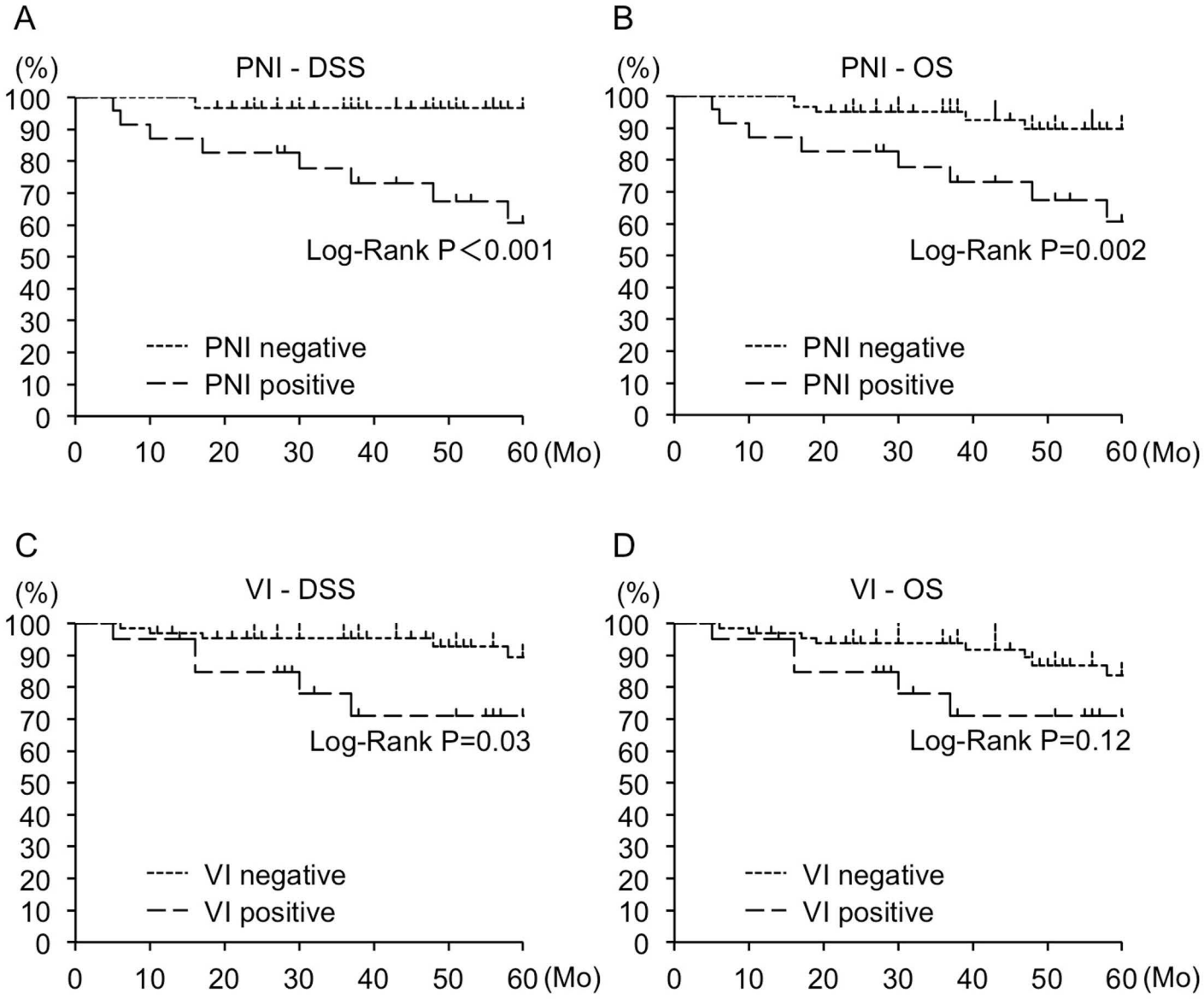
A
PNI - DSS
(%)
100
90
80
70
60
50
40
30
20
10
0
Log-Rank P＜0.001
PNI negative
PNI positive
0 10 20 30 40 50 60 (Mo)
B
PNI - OS
(%)
Log-Rank P=0.002
PNI negative
PNI positive
0 10 20 30 40 50 60 (Mo)
C
VI - DSS
(%)
Log-Rank P=0.03
VI negative
VI positive
0 10 20 30 40 50 60 (Mo)
D
VI - OS
(%)
Log-Rank P=0.12
VI negative
VI positive
0 10 20 30 40 50 60 (Mo)

Figure 1

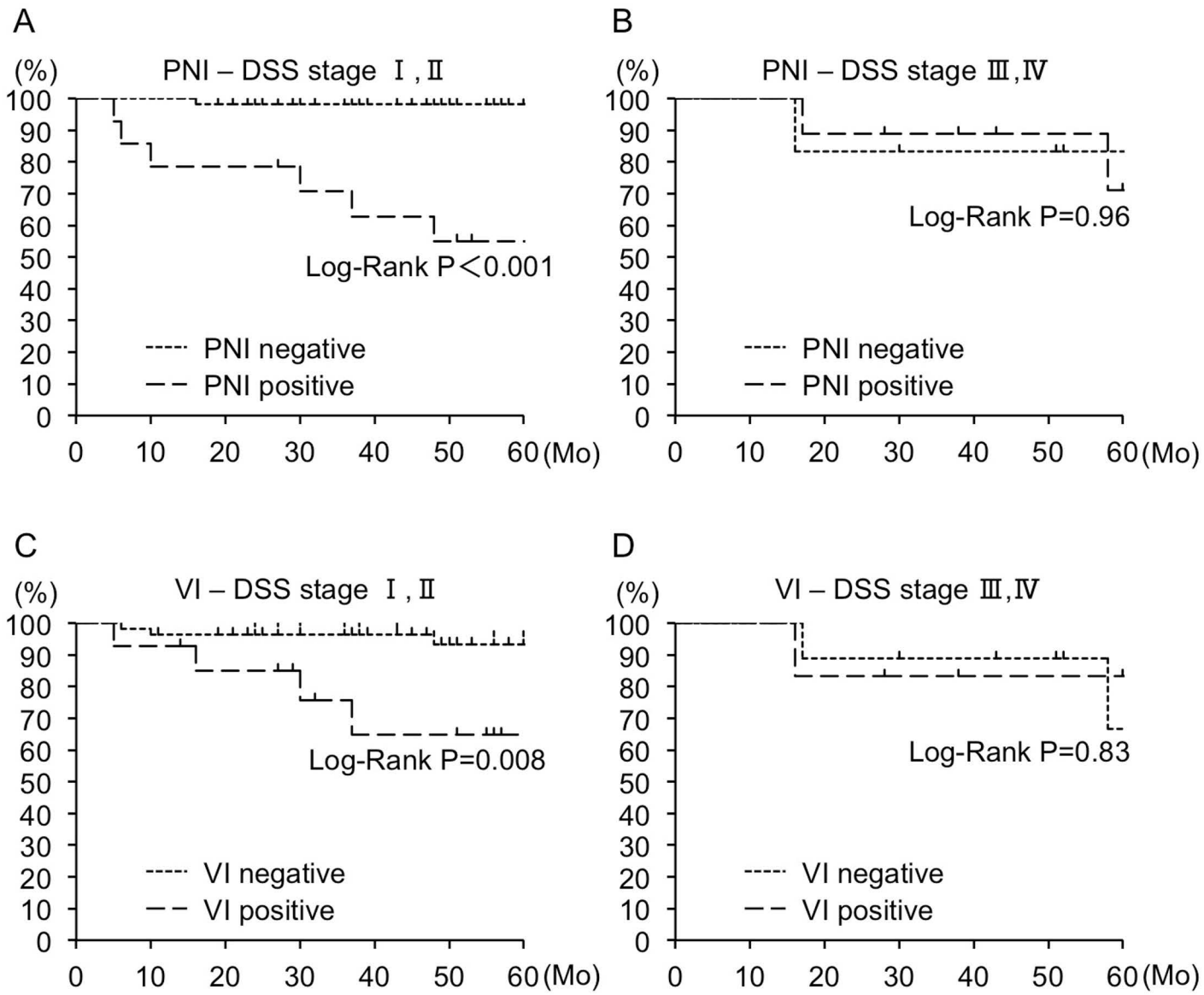
A
(%)
PNI – DSS stage Ⅰ, Ⅱ
100
90
80
70
60
50
40
30
20
10
0
Log-Rank P＜0.001
PNI negative
PNI positive
0 10 20 30 40 50 60 (Mo)
B
(%)
PNI – DSS stage Ⅲ, Ⅳ
Log-Rank P=0.96
PNI negative
PNI positive
0 10 20 30 40 50 60 (Mo)
C
(%)
VI – DSS stage Ⅰ, Ⅱ
Log-Rank P=0.008
VI negative
VI positive
0 10 20 30 40 50 60 (Mo)
D
(%)
VI – DSS stage Ⅲ, Ⅳ
Log-Rank P=0.83
VI negative
VI positive
0 10 20 30 40 50 60 (Mo)

Figure 2

Table 1. Demographic characteristics of 89 patients.

Characteristics	No. of cases (%)
Gender	
Male	50 (56.2)
Female	39 (43.8)
Age	
≥64	48 (53.9)
≤63	41 (46.1)
Disease stage	
I	37 (41.6)
II	35 (39.3)
III	9 (10.1)
IV	8 (9.0)
Histological grade	
Well	82 (92.1)
Moderately	5 (5.6)
Poorly	2 (2.3)
Pattern of invasion	
1	6 (6.7)
2	25 (28.2)
3	40 (44.9)
4	18 (20.2)
Depth of invasion	
<4mm	55 (61.8)
≥4mm	34 (38.2)
Surgical margin	
>4mm	68 (76.4)
≤4mm	21 (23.6)
Perineural invasion	
No	65 (73.0)
Yes	24 (27.0)
Vascular invasion	
No	68 (71.9)
Yes	21 (23.6)
Nodal status	
No metastasis	64 (71.9)
Metastasis	25 (28.1)
Local recurrence	
No	78 (87.6)
Yes	11 (12.4)

Distant metastasis	
No	86 (96.7)
Yes	3 (3.3)

Table 2. Association of perineural/vascular invasions with clinicopathological factors.

	PNI +	PNI -	P value	VI +	VI -	P value
Gender						
Male	12	38	NS	13	37	NS
Female	12	27		8	31	
Age						
≥64	12	36	NS	11	37	NS
≤63	12	29		10	31	
pT stage						
T1+T2	19	63	0.02	18	64	NS
T3+T4	5	2		3	4	
Histopathological nodal status						
No metastasis	12	52	0.005	10	54	0.005
Metastasis	12	13		11	14	
UICC stage						
I, II	14	59	NS	14	58	NS
III, IV	5	6		7	10	
Pattern of invasion						
1+2+3	12	59	＜0.001	14	57	NS
4	12	6		7	11	
Depth of invasion						
<4mm	4	51	＜0.001	8	47	0.01
≥4mm	20	14		13	21	
Local recurrence						
No	19	59	NS	18	60	NS
Yes	5	6		3	8	
Distant metastasis						
No	21	65	0.02	21	65	NS
Yes	3	0		0	3	
Disease specific survival						
Alive	17	62	0.004	16	62	NS
Dead	7	3		5	6	
Overall survival						

Alive	16	60	0.007	16	59	NS
Dead	8	5		5	9	

PNI: perineural invasion VI: vascular invasion NS: Not significant

Table 3. Association of perineural/vascular invasions with DSS in DOI ≥4mm group.

	PNI +	PNI -	P value	VI +	VI -	P value
Disease specific survival						
Alive	12	13	0.04	10	15	NS
Dead	8	1		3	6	

PNI: perineural invasion VI: vascular invasion NS: Not significant