Clinical Significance of Perineural Invasion in T1-2 Oral Cavity Squamous Cell Carcinoma

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Perineural invasion (PNI) is a pathologic feature associated with a poor prognosis in many human malignancies, including oral squamous cell carcinoma (OSCC). OSCC is a surgically treated cancer in which the PNI status is routinely reported and is regarded as an adverse feature according to treatment guidelines. Despite the wide implications of PNI in the clinical management of OSCC, controversies exist regarding the diagnosis, prevalence and clinical significance of PNI, especially for early T1-2 OSCC. The objectives of this review are to discuss the available evidence and limitations regarding the clinical significance and application of PNI. This review focuses on the roles of PNI in T1-2 OSCC for the prognostication of oncologic outcomes and for guiding clinical management including neck dissection and postoperative adjuvant therapy. Current evidences suggest that PNI is an important adverse pathologic feature of T1-2 OSCC, and that it is primarily associated with cervical lymph node metastasis and poor survival. Most reported data support the idea that T1-2N0 OSCC with PNI requires aggressive elective neck dissection. However, evidence is lacking for the role of postoperative adjuvant therapy in the improvement of locoregional control or survival of T1-2 OSCC with PNI as the only indication. Patient with low-risk T1-2 OSCC in which PNI is the only adverse feature should be informed about the risks, long-term toxicities and benefits regarding postoperative adjuvant therapy so that they can participate in individual decision making.

Key words: elective neck dissection, observation, oral squamous cell carcinoma, perineural invasion, postoperative adjuvant therapy

Oral Squamous Cell Carcinoma (OSCC)

Oral squamous cell carcinoma (OSCC) is a common type of cancer worldwide. For early T1-2 OSCC, the mainstay of treatment is surgery. Postoperative adjuvant therapy is needed in patients with high-risk prognostic factors, such as positive surgical margin, advanced N2-3 neck disease, or extranodal extension (ENE). Excellent treatment results and functional outcomes can be achieved for T1-2 OSCC, and the presence of cervical lymph node metastasis (LN+) is a major poor prognostic factor that is associated with decreased survival. In patients with clinical N0 (cN0) neck at initial diagnosis, 15 ~ 40% of them actually exhibit occult neck metastases beyond the detection ability of current imaging modalities, including CT and MRI. Therefore, the management of cN0 neck continues to be an issue of extensive debates because elective neck dissection (END) does
not always demonstrate survival benefits in the literature and can lead to potential sequelae.\(^7\text{-}11\) Another controversial issue is whether postoperative adjuvant chemoradiotherapy improves oncologic outcomes at the presence of worrisome pathologic features such as perineural invasion (PNI).

**Perineural Invasion (PNI)**

PNI is the process of tumor invasion of nerves and has been an important pathologic feature associated with poor prognosis in many human malignancies, including prostatic, pancreatic, colorectal, skin and head and neck cancers (HNCs).\(^12\) PNI is a distinct route of cancer spread and metastasis that is different from hematologic or lymphatic invasion,\(^13\) and is also not simply spillage of tumor cells into a low resistant plane.\(^14\) Although still poorly understood, crosstalk between tumors and the peripheral nervous system is now considered to occur during the pathogenesis of PNI.\(^15,16\) OSCC is a common form of HNCs in which PNI is a pathologic feature that should be reported according to the protocol published by the College of American Pathologists (CAP).\(^17\) In treatment guidelines of the National Comprehensive Cancer Network (NCCN), PNI is an adverse feature indicating the need for postoperative radiotherapy or even chemoradiotherapy.\(^18\) However, controversies exist despite the wide clinical implications of PNI in OSCC. The pathologic criteria as well as the definition of PNI are still not standardized. Clinical data regarding PNI in the literature frequently include early and late cancers of various head and neck subsites.\(^19,20\) Moreover, few studies have focused specifically on the role of PNI in OSCC. Despite the above caveats, substantial agreement has been observed in the poor prognostic impacts of PNI and has paradoxically enforced its importance in OSCC. We herein reviewed reported data and data from our recent studies for more clearly defining the clinical significance of PNI in early T-2 OSCC.

**Diagnosis and Prevalence of PNI**

Liebig et al.\(^12\) advocated for a more specific and widely used definition of PNI as the presence of tumor cells in any layer of the nerve sheath, or tumor in close proximity to the nerve involving more than one-third of its circumference. Other authors have also defined PNI broadly as tumor cell invasion in, around and through the nerves.\(^21\) The lack of a standardized definition can lead to major limitations with respect to reported PNI data.\(^22\) This also explains why PNI can be easily underestimated. Most peripheral nerve fibers in head and neck area are myelinated containing myelin sheath surrounding axons. The morphology of myelination allows the identification of peripheral nerves by microscopic examination (Fig. 1A and 1B). In regular pathologic examination, PNI is identified by hematoxylin and eosin (H & E)-staining of resected tumor specimens (Fig. 1C and 1D). Ueno et al.\(^23\) accessed the inter-observer reproducibility using unified criteria for PNI in colorectal cancers. They demonstrated that the identification of PNI is reproducible and is better than the identification of vascular invasion.

In addition to the lack of a standardized definition, detailed procedures to identify PNI during pathologic examination are also not clearly defined. Therefore, the prevalence of PNI in OSCC according to the literature varies between 20% and 40%. A higher rate of PNI up to 50% to 80% has been reported by immunohistochemical (IHC) staining for nerve-specific molecules, such as S-100 protein, because IHC aids in the identification of small nerve fibers.\(^24,25\) However, the higher PNI rates with IHC technique might not always contribute to greater clinical significance, as reported by Kurtz et al.\(^25\) The optimal technique and detection threshold with optimal clinical significance for PNI evaluation therefore still require further investigation. According to the CAP protocol, PNI is evaluated by standard H&E staining and is reported dichotomously as positive or negative.\(^17\) Some argue that simple dichotomization in the reporting of PNI might result in the loss relevant information. Efforts in the classification or quantification of PNI have been investigated in prostate cancer according to PNI diameter\(^26\) or PNI location as intraglandular or extraglandular.\(^27\) In non-cutaneous HNCs, Miller et al. classified PNI as intratumoral, peripheral or extratumoral.\(^28\) However, not all classification methods demonstrate valid clinical significance under significantly increased workload of pathologists, and further research efforts are needed before wide application in routine practice.

We have uniformly investigated PNI in over 300 cases of early T1-2 OSCC by standard H&E staining and used the criteria for PNI advocated by Liebig et
al. described above. In addition, the pathologists performed the examination according to a standardized process. Each whole tumor section was scanned serially under low magnification (Fig. 1C and 1E) (40X or 100X), followed by confirmation of any identified PNI focus under high magnification (Fig. 1D and 1F) (200X or 400X). In our experience, the limit of identified nerve fibers is approximately 0.05 mm (Fig. 1E and 1F), and the prevalence of PNI in T1-2 OSCC is 27% (17% for T1 and 36% for T2).

**Association between PNI and Other Pathologic Features**

The presence of PNI frequently correlates with the presence of many other clinicopathologic features. Since most studies did not primarily focus on PNI, few data have been reported that clarify these relationships in HNSCC or OSCC. Rahima et al. analyzed 101 patients with oral and oropharyngeal carcinoma, 73% of whom were diagnosed with T1-2 disease. They found that PNI was present in 25.7% of the patients and was significantly associated with poor differentiation, LN+, and increased depth of invasion.
We specifically evaluated a cohort of 307 patients with T1-2 OSCC and the presence of PNI was found in 27.4% of the patients. In consistent, PNI was associated with many other pathologic features, including higher T2 classification, increased tumor thickness, poor differentiation, lymphovascular invasion (LVI), and ENE. Defining high-risk patients as those with positive margins, N2-3 disease or ENE, we found that the PNI-positive rates were nearly three-fold higher (55.9 vs. 19.2%) in high-risk T1-2 OSCC patients. These high-risk patients, regardless of PNI status, would have been recommended to receive more intensified postoperative adjuvant therapy. Given the strong association of PNI with other pathologic features and the higher rate of PNI in high-risk patients, the clinical significance of PNI needs to be further clarified.

T1-2 OSCC with or without PNI

Since a majority of patients with T1-2 OSCC (> 75%) are low-risk patients who have negative margins, N0-1 disease and no ENE, the presence of adverse pathologic features can potentially change the treatment decision for T1-2 OSCC. Two major controversies in the treatment for T1-2 OSCC are whether or not to undergo elective neck dissection (END) or postoperative adjuvant therapy. Reported data most consistently indicate that PNI predicts LN+ in HNCs due to a significantly higher LN+ rate in patients with PNI compared with patients without PNI. It is not surprising that PNI correlates with poor survival in most reported data. The LN+ rates in patients with PNI+ T1-2 OSCC were approximately 60% in our study and in the study by Sparano et al. In our series, the poor survival impact of PNI was most obvious in those patients with T1-2 OSCC who underwent neck observation (Fig. 2A). We further demonstrated that PNI remains independently predictive for LN+ rate and poor survival in the subgroup of 146 patients with early T1 OSCC. Chen et al. and Liao et al. reported that PNI is not a risk factor for poor survival in patients with pT1-2N0 OSCC, which seems to be contradictory. Notably, both series include only low-risk patients (pN0 and margin free) who underwent neck dissection. In our series, we actually also found that PNI had no impact on disease-specific survival in the subgroup of low-risk patients who underwent neck dissection (Fig. 2B). All these results indicate that PNI is an important poor prognostic factor that is associated with high LN+ rates and poor survival of patients with T1-2 OSCC and that its impacts on survival can be influenced or rescued after appropriate treatment.

In addition to LN+ and survival, PNI does not demonstrate consistent impacts on local or regional disease control according to the literature. Most reported data do not support the idea that PNI predicts

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Fig. 2. Kaplan-Meier curves of disease-specific survival according to perineural invasion in 227 low-risk T1-2 OSCC patients treated by surgery alone. Low-risk indicates negative margins, N0-1 disease and no extranodal extension. (A) A subgroup of low-risk patients who underwent neck observation (n = 84). (B) A subgroup of low-risk patients who underwent neck dissection (n = 143). PNI, perineural invasion.
increased local recurrence (LR). In our cohort of 307 patients with T1-2 OSCC, the rates of positive margins (9.5 vs. 6.3%, \( p = 0.326 \)) and LR (19.0 vs. 13.5%, \( p = 0.221 \)) in PNI+ patients were not significantly higher than those in PNI- patients.\(^{29}\) In regards to the issue of regional control, many reports demonstrate a positive impact of PNI on neck recurrence (NR),\(^{8,19,31}\) but conflicting results have also been reported.\(^{37,41}\) In our study, observation of cN0 neck was done in 94 (30.6%) of the 307 patients with T1-2 OSCC, and was more frequent in those with T1 OSCC (47.4%, 65/146). We found that the NR rate was significantly higher in PNI+ patients than in PNI- patients (26.2 vs. 10.3%, \( p < 0.001 \)).\(^{29,30}\) However, in the 143 low-risk patients who underwent neck dissection, PNI positivity did not correlate with a significantly higher NR rate, although a trend was observed (15.6 vs. 6.3%, \( p = 0.14 \)).\(^{29}\) Given that PNI predicts LN+, we speculate that the influence of PNI on NR can be modulated by the policy of cN0 neck management.

The way in which PNI leads to LN+ remains poorly understood. Since PNI and tumor thickness both correlate with LN+ and poor survival in patients with T1-2 OSCC, we analyzed 212 patients with T1-2 buccal and tongue SCC to determine the possibility of hierarchical impacts between PNI and tumor thickness.\(^{42}\) Higher LN+ (27.4 vs. 16.4%, \( p = 0.035 \)) and poor 5-year disease-specific survival (DSS) rates (86.4 vs. 93.7%, \( p = 0.054 \)) were found in patients with thick tumors (> 6 mm) when they were compared with patients with thin tumors (≤ 6 mm). However, using patients with thin tumors as a reference, patients with thick tumor demonstrated a higher LN+ rate (41.9% vs. 16.4%, \( p = 0.001 \)) (Table 1) and a lower 5-year DSS rate (77.5% vs. 93.7%, \( p = 0.006 \)) (Fig. 3) only when PNI was present. We speculate that, compared with tumor thickness or depth of invasion, which serves as simple dimensional parameters, PNI represents a more direct consequence of the interactions of OSCC with tumor microenvironment, and

### Table 1. Cervical LN metastasis according to tumor thickness and PNI status of 212 patients with T1-2, cN0 oral tongue and buccal SCC\(^{42}\)

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. (%)</th>
<th>HR</th>
<th>95% CI</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical LN metastasis(^{1})</td>
<td></td>
<td></td>
<td>--------------</td>
<td>--------</td>
</tr>
<tr>
<td>Thickness ≤ 6 mm (110)</td>
<td>18 (16.4)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness &gt; 6 mm, PNI- (59)</td>
<td>11 (18.6)</td>
<td>1.17</td>
<td>0.51 ~ 2.68</td>
<td>0.708</td>
</tr>
<tr>
<td>Thickness &gt; 6 mm, PNI+ (43)</td>
<td>18 (41.9)</td>
<td>3.68</td>
<td>1.67 ~ 8.10</td>
<td>0.001</td>
</tr>
</tbody>
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*LN lymph node, PNI perineural invasion, LVI lymphovascular invasion, OSCC oral squamous cell carcinoma, HR hazard ratio, CI confidence interval.*

\(^{1}\)Binary logistic regression analysis.

![Fig. 3.](https://example.com/fig3.png) **Fig. 3.** Kaplan-Meier curves of disease-specific survival of 212 T1-2, cN0 tongue and buccal SCC patients.\(^{42}\) (A) DSS between patients with thick tumors (> 6 μm) and patients with thin tumors (≤ 6 μm). (B) DSS of patients with thick tumors (> 6 μm) was analyzed according to PNI status using the group of patients with thin tumors (≤ 6 μm) as the reference.
this in turn contributes to a poor prognosis. Our data therefore suggest that PNI is one key determinant for the aggressiveness associated with increased tumor thickness. The unclear interactions between tumor and the neural microenvironment, however, warrant further mechanistic research.

**Role of PNI in cN0 Neck Management of T1-2 OSCC**

Whether or not to perform END in cN0 patients with T1-2 OSCC has been long debated. Aggressive END has been widely adopted to provide precise pathologic examination and to optimize neck control at the expense that 70 to 80% of the patients who are truly LN- will be overtreated. However, END can still result in possible sequela such as scarring and shoulder disability despite low morbidity, and many studies including RCT trials did not demonstrate a survival benefit by END. Although a recent RCT study by D’Cruz et al. showed that END resulted in higher rates of survival compared with therapeutic neck dissection, some authors still questioned whether END accounted for the observed difference.

Since neck metastasis is one major factor associated with poor prognosis in T1-2 OSCC, neck dissection will no doubt improve both neck control and survival if performed in LN+ patients. The lack of survival benefit from END in previously reported data including RCT studies may partly result from the inclusion of LN- patients for whom END definitely plays no role. In the era of individualized cancer treatment, ideal targeted cN0 neck management should be END specifically for LN+ patients and observation for LN- patients. A similar concept has been reported by Ebrahimi et al. who demonstrated that END improved survival of 153 patients with thick (> 4 mm) T1-2 OSCC who were likely to be LN+. However, they did not analyze whether cN0 neck of patients with thin tumors can be safely observed. According to the policy of aggressive END at our institution, observation has been applied mostly in patients with early T1, cN0, thin tumors. We found that such clinical judgment and pretreatment imaging did not successfully lead to the recommendation of END specifically for LN+ patients. Given that PNI is associated with LN+ and is one key determinant for the aggressiveness associated with increased tumor thickness, it is reasonable to investigate how PNI status might be used to guide neck management for patients with T1-2 OSCC.

The clinical contribution of LN+ predictors in modifying neck treatment planning is limited under the policy of aggressive END. In contrast, predictors of LN- (high negative predictive value, NPV) will be more valuable for the selection of appropriate patients for cN0 observation. In addition to PNI, LVI has also been shown to predict LN+ in reported data. The combination of multiple pathologic features such as PNI/LVI has been shown to be promising for the prognostication of OSCC. We have studied 253 patients with T1-2, cN0 OSCC and demonstrated that PNI/LVI- (double negative) exhibited the best NPV (85.5%) for LN- status among other clinicopathologic features. In a hypothetical model to test the efficacy of guiding neck management by PNI/LVI status, we found a 50% decrease in the number of patients for END and more than doubled the number of cases for observation. Importantly, this hypothetical modification in neck management achieved a 2.5-fold decrease in the overtreatment rate (54.2% to 20.2%) while a low undertreatment rate was maintained (6.3% to 9.9%). These data indicate that PNI combined with LVI can potentially improve precise, targeted neck management in patients with T1-2 OSCC.

However, all pathologic parameters can be determined only after excision of primary tumors. A second general anesthesia would be needed if a treatment decision for END is made at the presence of PNI. To overcome this limitation, strategies for pretreatment prediction of PNI are necessary. We recently reported the role of pretreatment clinical and quality of life parameters in the prediction of PNI in a prospective cohort of 102 patients with newly diagnosed OSCC (71.9% T1-2). We found that PNI can be predicted by more severe pretreatment pain (a higher visual analogue scale pain score), as well as more advanced clinical T classification. These results therefore suggest that careful evaluation of pretreatment pain should be emphasized in patients with T1-2 OSCC. Increased pretreatment pain with pain killer use and interference of social eating and social contact may indicate the possibility of the presence of PNI and the need for aggressive END.

**PNI and Postoperative Adjuvant Therapy in T1-2 OSCC**

According to NCCN guidelines, PNI has been one of the adverse features of HNCs that suggest
adjuvant postoperative radiotherapy (PORT) or concurrent chemoradiotherapy (POCCRT). Of note, the list of adverse features is universal for cancers of the oral cavity, oropharynx and hypopharynx. It is reasonable to speculate that the impacts of these adverse features are not the same across different subsites and tumor stages. In our study, over 75% of patients with T1-2 OSCC patients were low-risk who achieved negative surgical margins, N0-1 disease and no ENE after surgery. However, the presence of PNI in pathologic reports commonly raises a sense of insecurity after surgery owing to the higher prevalence of PNI in high-risk patients who have poor prognosis. The decision of whether or not to arrange postoperative adjuvant therapy in early T1-2 OSCC when PNI (or LVI) is the only indication remains a topic of significant debate. Further concerns include the negative impacts on the quality of life that result from late adverse effects of PORT or POCCRT, such as xerostomia, dysphagia, soft tissue fibrosis, radionecrosis or even radiation-induced malignancies after long-term follow-up.

Whether the risk of recurrence is high and can be effectively lowered should both be considered in the arrangement of postoperative adjuvant therapy in order to avoid overtreatment and long-term sequelae. However, most studies do not support or provide evidence that PORT or POCCRT improves the outcome of T1-2 OSCC with PNI. As has been discussed, most reported data for PNI+, T1-2 OSCC do not demonstrate a higher rate of LR after radical primary tumor excision. In addition, the problem of increased NR associated with PNI can actually be nearly rescued by END. In fact, in low-risk T1-2 OSCC patients after neck dissection, PNI was not found to be a risk factor for poor survival by our study or by other reported series (Fig. 2B). These results indicate that the residual risk of PNI in T1-2 OSCC can be minimal after radical excision and neck dissection. These results also indicate that postoperative adjuvant therapy in cases where PNI (or LVI) is the only indication can be an overtreatment that exposes patients to unnecessary risks of radiation toxicity. The lack of improvement in locoregional control and survival by PORT or POCCRT in patients with PNI (or LVI) was also demonstrated by Chatzistefanou et al. and Chen et al.

Notably, a non-significant trend of higher NR (15.6 vs. 6.3%) was still demonstrated after neck dissection in PNI+ patients compared with PNI- patients in our study and the study by Chatzistefanou et al. (10.5 vs. 5.2%). We therefore consider that it remains an open question whether or not to arrange adjuvant therapy when PNI is the only indication. With current best retrospective evidence, low-risk T1-2 OSCC patients should be informed about the risks and benefits of PORT or POCCRT and participate with clinical professions in individual decision making regarding adjuvant therapy, as suggested by Bur et al.

**Conclusion and Future Perspective**

Current evidence suggests that PNI is an important adverse pathologic feature in T1-2 OSCC and that it is mainly associated with LN+ and poor survival. Its impacts on local and neck recurrences may be rescued or diminished by adequate surgery including neck dissection. Most reported data support the idea that T1-2 OSCC with PNI requires END which both reduces neck recurrence and improves survival. Observation can be performed in cN0 patients without PNI and LVI, but close follow-up is still required. Pain that necessitates the use of pain killers and that interferes with social eating and social contact at presentation can be used as pretreatment surrogate clinical signs that indicate the possibility of the presence of PNI and the need of END. However, current evidence does not support a role of adjuvant PORT or POCCRT in improving local, regional controls or survival of patients with T1-2 PNI+ OSCC. Importantly, available evidence for PNI in T1-2 OSCC from studies including ours is all limited by the retrospective design, the smaller sample sizes of PNI+ or LN+ patients, and the difficulty to control for all other risk factors to avoid confounding data. Prospective randomized study of known adverse pathologic features such as PNI might not be feasible for ethical reasons. It would be the responsibility of clinicians to make informed, individual recommendations based on current available evidence for any interventions including neck dissection and postoperative adjuvant therapy.

The way how PNI contributes to metastasis in patients with T1-2 OSCC remains a key question to be investigated, as mechanistic research for PNI-related metastasis remains largely preliminary. The association of PNI and the expression of neurotrophic factors in clinical tissue samples has been reported. Nerve-tumor interactions have been hypothesized and the role of different cell types, including Schwann cell, has attracted research efforts in recent years.
Further investigations are needed to improve our understanding of the molecular pathways involved in OSCC and perineural microenvironment. These efforts may hopefully lead to the development of innovative strategies against the acquisition of the metastatic phenotype via PNI in OSCC.

References


