Interobserver Variation Among Pathologists in Evaluating Perineural Invasion for Oral Squamous Cell Carcinoma

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Abstract The aims of this study are as follows: (1) to assess variations among pathologists in evaluating perineural invasion (PNI) in oral squamous cell carcinoma (OSCC), (2) to survey PNI criteria used by pathologists and how they came to adopt those criteria. An electronic survey was sent to 363 oral and/or surgical pathologists. Eligibility criteria included pathology board certification. The survey participants were asked to rate whether PNI was present, absent, or uncertain for 15 provided photomicrographs, which depicted various types of tumor-nerve relationships without excessive desmoplasia or lymphocytic host response. The survey obtained information regarding demographics, whether PNI criteria were taught during residency, criteria used by participants to evaluate PNI, how the participants developed their criteria, and agreement with six proposed PNI definitions. 88 pathologists completed the survey. The participants included 47 males and 41 females, with average age = 49 years and average practice experience = 17 years. Practice settings included dental school

(40 %), medical school (36 %), private pathology lab (13 %), and other (11 %). Agreement between participants in rating PNI status for the provided images was fair ($\kappa = .38$, 95 % CI .37–.39). 56 % of respondents indicated that they were taught PNI criteria during residency training. The basis for criteria currently used by participants included residency training ($n = 42$), published literature ($n = 29$), and own experience/views ($n = 32$). Agreement regarding six proposed PNI definitions was slight ($\kappa = .10$, 95 % CI .08–.11). In conclusion, interobserver agreement in assessing PNI status was fair. Our results suggest that more widely accepted, objective, and reproducible criteria are needed for evaluating PNI in OSCC.

Keywords Perineural invasion · Oral cavity · Squamous cell carcinoma

Introduction

Perineural invasion (PNI) is an important factor in determining both the treatment and prognosis of oral squamous cell carcinoma (OSCC). According to the National Comprehensive Cancer Network (NCCN) Guidelines for oral cavity cancer patients, PNI (as well as extracapsular nodal spread, positive surgical margins, pT3 or pT4 primary, N2 or N3 nodal disease, nodal disease in levels IV or V, and vascular embolism) is an adverse risk feature used in assessing the need for adjuvant treatment (i.e., radiation therapy, combined systemic and radiation therapy, or re-resection) [1]. Furthermore, several studies of OSCC have found PNI to be associated with increased risk for locoregional recurrence, increased incidence of cervical nodal metastasis regardless of tumor stage, and decreased overall patient survival [2–6].

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Despite the significance of PNI with respect to the treatment and prognosis of OSCC, proposed definitions of PNI vary considerably. Perhaps most frequently cited, Batsakis [7] defined PNI as tumor cell “invasion in, around, and through peripheral nerves” in his classical paper regarding neurotropic carcinomas of the head and neck. In addition, Batsakis commented that although the term PNI might be misinterpreted to represent tumor cell invasion of the perineurium, the terms *perineural* and *perineurial* are not interchangeable. That is, the *perineurium* refers to the connective tissue that lies just beyond the outermost nerve sheath, whereas the *perineurium* represents one of three connective tissue layers that comprise the nerve sheath. Specifically, the three nerve sheath layers include the following: (1) the innermost *endoneurium* [which surrounds individual nerve fibers (i.e., individual axons and their associated Schwann cells)], (2) the *perineurium* [which surrounds individual *nerve fascicles* (i.e., bundled nerve fibers) and is comprised of concentric laminae of endothelial cells vested by basal laminae], and (3) the outermost *epineurium* (which binds together several nerve fascicles to form larger nerves, and is comprised of an outer layer of loosely arranged connective tissue and an inner layer of more densely organized connective tissue) [8–10].

In order to provide further clarification of Batsakis' definition, Liebig et al. [9] specified that the presence of tumor cells within any of the three nerve sheath layers constitutes PNI. Furthermore, based on other frequently cited studies, these authors expanded their PNI definition to include “tumor in close proximity to nerve and involving at least 33 % of its circumference.” However, they conceded that there is wide variability among authors regarding the degree of tumor-nerve intimacy (e.g., mere contact versus tumor cells inside of the nerve sheath) and various tumor-nerve relationship patterns (e.g., complete vs. incomplete nerve encirclement, concentric lamination, tangential contact) that are considered to qualify as PNI.

Other investigators recognize that determination of PNI may be difficult in practice and, thus, offer guidelines for equivocal cases. For example, in addressing the issue of PNI identification in skin cancers, Dunn et al. [10] proposed the following: “In the presence of a malignancy, PNI may be diagnosed according to the observation of cytologically malignant cells within the perineural space of nerves. In equivocal cases, the observation of total or near-total circumferential involvement is supportive, as is the presence of perineural tracking in tangential sections and intraneural involvement.” Interestingly, although Batsakis [7], Liebig et al. [9], and Dunn et al. [10] regard intraneural involvement (or invasion of the endoneurium) as a subset of PNI, some head and neck cancer investigators make a distinction between perineural and intraneural invasion.

Fig. 1 Photomicrographs for Questions in Survey Section I (image question numbers indicated in brackets): **a** question 1 (original magnification $\times 400$), **b** question 2 (original magnification $\times 400$), **c** question 3 (original magnification $\times 400$), **d** question 4 (original magnification $\times 400$), **e** question 5 (original magnification $\times 200$), **f** question 6 (original magnification $\times 400$), **g** question 7 (original magnification $\times 600$), **h** question 8 (original magnification $\times 400$), **i** question 9 (original magnification $\times 400$), **j** question 10 (original magnification $\times 400$), **k** question 11 (original magnification $\times 400$), **l** question 12 (original magnification $\times 400$), **m** question 13 (original magnification $\times 200$), **n** question 14 (original magnification $\times 400$), **o** question 15 (original magnification $\times 400$)

Nevertheless, there is currently insufficient evidence to determine whether tumors that have invaded the endoneurium exhibit more biologically aggressive behavior and poorer clinical outcomes compared to those that have infiltrated or surrounded the outermost nerve sheath only.

In addition to these varying views in the literature, we have found differences of opinion regarding the definition of PNI during informal discussions with our pathology colleagues. Therefore, in the present study, we aim to assess variations among pathologists in evaluating PNI in OSCC. In addition, we aim to survey PNI criteria used by pathologists and how they came to adopt those criteria.

Materials and Methods

A web-based survey was conducted using the Research Electronic Data Capture (REDCapTM) system, with survey invitations sent to 363 oral and maxillofacial pathologists and/or surgical pathologists with an interest in head and neck pathology [11]. Eligibility criteria included pathology board certification. The study was approved by the Institutional Review Boards of the Medical University of South Carolina, Texas A&M University-Baylor College of Dentistry, Memorial Sloan-Kettering Cancer Center, and the University of Toledo. The survey was pretested to confirm functionality and clarity. E-mail invitations included a study description, consent information, and a link to the survey. Consent was indicated by submission of completed survey responses. A reminder e-mail invitation was sent to those who did not respond to the initial invitation after 2 weeks.

The survey questions are exhibited in the Appendix, and the full survey can be viewed online at <https://redcap.musc.edu/surveys/index.php?s=Rh2nHb5iwL>. Briefly, the survey consisted of two sections. Section I included 15 photomicrographs of OSCC (see Fig. 1a–o), for which participants were asked to assess PNI as they would in their pathology practice and to indicate whether PNI was present, absent, or uncertain. The photomicrographs were chosen by the three pathologist investigators (A.C.C., N.K., and Y.L.C.),

who verified image quality (i.e., in focus with tumor and nerve not unduly obscured by the lymphocytic host response or desmoplasia) and selected images to depict the following types of scenarios: (a) tumor close to, but not touching, a nerve (questions 1, 5, 9), (b) tumor surrounding the entire circumference of a nerve (questions 3, 10, 12, 15; with 3 and 10 showing the tumor cells assuming the shape of a nerve, 12 showing a small nerve surrounded by a sheet of tumor, and 15 depicting an “onion-skinning” pattern), (c) tumor partially surrounding and assuming the shape of a nerve (question 4), (d) tumor focally touching the perineurium and/or focally causing blurring of the perineurium (questions 2, 6, 7, 11, 13), and (e) intraneural invasion (questions 8, 14). Section II included questions regarding participant demographics (i.e., age, gender, number of years in practice, practice setting), whether or not participants have ever indicated that PNI is present in pathology reports they have issued for OSCC, whether or not participants distinguish between PNI and intraneural invasion in their pathologic reporting of OSCC, whether or not histologic criteria for PNI were learned during residency training, and how participants formulated the histologic PNI criteria that they currently use. In addition, participants were asked if they agree or disagree with six proposed PNI definitions.

Statistical analysis was performed using SPSS software (version 21.0, IBM Corp., Armonk, NY). Descriptive statistics were calculated for each parameter. In addition, Fleiss kappa with 95 % confidence interval (CI) was used to analyze interobserver agreement for the following: (a) PNI ratings for the 15 microscopic images, (b) 6 proposed PNI definitions. By convention, relative strength of agreement was determined as per Landis and Koch [12] (i.e., $\kappa < 0$ poor, $\kappa = 0\text{--}0.20$ slight, $\kappa = .21\text{--}0.40$ fair, $\kappa = .41\text{--}0.60$ moderate, $\kappa = .61\text{--}0.80$ substantial, $\kappa = .81\text{--}1.00$ almost perfect). Percentage of agreement for each of the 15 microscopic images and each of the 6 proposed PNI definitions also was calculated. In addition, we compared survey results between those practicing in a dental school and those who practicing in a medical school by performing a two-sided *t* test (for age and years of practice) and Chi square analysis (for each of the other survey parameters), with significance set at $p \leq 0.05$.

Results

88 board-certified pathologists completed the survey. The participants included 47 males and 41 females, with a mean age of 49 years (range 31–78). The mean number of years in pathology practice was 17 (range 1–50). Practice settings included the following: dental school ($n = 35$, 40 %), medical school ($n = 32$, 36 %), private pathology lab

($n = 11$, 13 %), other ($n = 10$, 11 %). “Other” practice settings included hospital (community, private, mixed private/academic, Veteran’s Administration, or not otherwise specified) ($n = 5$), “specialized academic cancer practice” or “academic cancer center not associated with a medical school” ($n = 2$), military lab ($n = 1$), private oral and maxillofacial surgery group ($n = 1$), and pathology fellow with previous treatment and practice experience ($n = 1$). Most participants were located in North America ($n = 77$, 88 %), with a smaller number from the Middle East/Asia ($n = 8$, 9 %) and Europe ($n = 3$, 3 %). The vast majority of respondents ($n = 85$, 97 %) answered “yes” to section II/question #5 (“In pathology reports that you have issued for OSCC, have you ever indicated that PNI is present?”).

For the 15 provided photomicrographs, overall agreement among respondents in rating PNI status was fair ($\kappa = .38$, 95 % CI .37–.39). Responses for each image are summarized in Table 1. Percentage of agreement ranged from 43 to 100 %; agreement was greatest for image question #3, #4, #10, and #15 (showing partial to complete encirclement with tumor assuming the shape of the nerve), with 99–100 % of respondents rating PNI as present. Agreement was also high for image question #8 and #14 (depicting intraneural involvement), with 97 and 96 % of respondents rating PNI present, respectively. More than 50 % of participants rated PNI as absent for image question #1, #5, #6, and #9. The questions for which the greatest proportion of participants rated PNI as uncertain were #2, #7, #11, and #13.

Table 1 Responses regarding perineural invasion status for 15 provided photomicrographs

Image question #	PNI present n (%)	PNI absent n (%)	PNI uncertain n (%)
1	23 (26.1 %)	54 (61.4 %)	11 (12.5 %)
2	34 (38.6 %)	38 (43.2 %)	16 (18.2 %)
3	88 (100.0 %)	0 (0 %)	0 (0 %)
4	87 (98.9 %)	0 (0 %)	1 (1.1 %)
5	13 (14.8 %)	64 (72.7 %)	11 (12.5 %)
6	30 (34.1 %)	50 (56.8 %)	8 (9.1 %)
7	41 (46.6 %)	26 (29.5 %)	21 (23.9 %)
8	85 (96.6 %)	3 (3.4 %)	0 (0 %)
9	5 (5.7 %)	72 (81.8 %)	11 (12.5 %)
10	88 (100.0 %)	0 (0 %)	0 (0 %)
11	40 (45.5 %)	28 (31.8 %)	20 (22.7 %)
12	73 (83.0 %)	6 (6.8 %)	9 (10.2 %)
13	29 (33.0 %)	41 (46.6 %)	18 (20.5 %)
14	84 (95.5 %)	2 (2.3 %)	2 (2.3 %)
15	87 (98.9 %)	0 (0 %)	1 (1.1 %)

PNI perineural invasion

Comments submitted for the image questions are summarized as follows:

1. Among the 65 individuals who submitted comments for any of the 15 image questions, 24 (37 %) considered the need to examine additional levels/deeper sections/recuts/serial sections/more tissue or “to look around more”/“look elsewhere,” and 15 (23 %) considered immunohistochemistry [e.g., for cytokeratins, S-100 protein, epithelial membrane antigen (EMA), glucose transporter 1 (GLUT1)].
2. Among the 37 individuals who rated PNI as uncertain or absent for image question #1, #5 and/or #9 with accompanying comments, 14 (38 %) used the following descriptors: “close approximation,” “proximity,” “approximation rather than penetration,” “abut,” “adjacent,” “immediately adjacent,” or other comparable terms.
3. Among the 47 individuals who rated PNI as uncertain for image question #2, #7, #11, and/or #13 with accompanying comments, 14 (30 %) used the following descriptors: “suspicious,” “strongly suggestive,” “suggestive but not conclusive,” “questionable,” “significance...uncertain,” “equivocal,” “possible,” or other comparable terms. Among the 55 individuals (regardless of PNI rating) who commented on any of these four questions, 26 (47 %) used the following descriptors: “adjacent,” “immediately adjacent,” “focally in contact,” “abutting,” “proximity,” “close proximity,” “close but not within,” “juxtaposition,” or other similar terms. For question #13, one individual who rated PNI as uncertain stated “leaning to calling it,” whereas another stated, “favor reporting as negative.”
4. Among the 15 individuals who rated PNI as uncertain or absent for image question #12 (showing complete encirclement of a nerve by a solid sheet of tumor), 6 (40 %) used the terms “engulfed,” “engulfment,” “encased,” “entrapped,” “trapped tumor not clearly in nerve sheath,” or “difficult to say whether it is actual PNI or nerve tissue caught in the centre of an invading mass of cells.”
5. Among the 81 individuals who rated PNI as present for question #8 and/or #14, 20 (25 %) submitted comments mentioning “intraneural invasion” or “endoneural invasion.” In contrast, 2 individuals who rated PNI as absent for both these questions explained that they distinguish perineural from intraneural invasion (“this is intraneural invasion, which I report separately from perineural”; “intraneural, not perineural”).

In survey section II, 60 % of participants indicated that they do not make a distinction between perineural and intraneural invasion in pathologic reporting of OSCC.

56 % of participants stated that they learned histologic criteria for PNI during residency training. Descriptions of these criteria considered the following:

1. Tumor relationship to the perineurium, perineural space, or any nerve sheath layers (n = 24) (e.g., “tumor invasion into the nerve sheath, penetrating the perineurium”; “present within perineurium”; “invasion of the perineurium”; “breaking through the perineural sheath”; “tumor within the perineural sheath”; “inside perineurium”; “between perineurium and neural tissue”; “malignant cells within perineurium”; “tumor cells need to damage the perineurium”; “distorting shape of the nerve, or around or closely approximating the perineurium”; “if it (tumor) does not actually breach the perineurium then it is not true PNI”; “tumor invasion into the nerve sheath”; “tumor cells within any of the 3 layers of the nerve sheath”).
2. Tumor surrounding or wrapping around the nerve (n = 16).
3. Tumor surrounding at least 1/4 or 1/3 of the circumference of the nerve (n = 4).
4. PNI location relative to the tumor mass or tumor margin (n = 5) [“PNI should be away from the main tumor mass”; “invasion along peripheral nerves which are isolated from or not circumscribed by cancer cell mass”; “...when it’s present beyond cancer margin”; “often stipulate location within tumor (advancing margin);” “outside the tumor”].
5. The literature (authors mentioned: Batsakis, Cotel, Fagan) (n = 3).
6. “Tumors grow along the nerve bundles, follow the contour of the nerve sheath” (n = 1).
7. Nerve size (“separate between > or <1 mm nerves”) (n = 1).

The basis for formulation of PNI criteria currently used by survey participants is summarized in Table 2. Among the 29 respondents who indicated that they use criteria published in the literature, 12 (41 %) submitted descriptions of these criteria. These descriptions included references to works by Liebig et al. [9], Batsakis [7], Dunn et al. [10], and/or Fagan et al. [6]. In addition, five individuals described criteria for which we could not identify a specific source (e.g., “malignant cells inside perineurium,” “malignant cells in the perineural space,” “invading or touching nerves,” “evidence of neurotropism to a significant degree,” “tumor between branches of a nerve bundle or in extremely close proximity to a nerve without wrapping around it”). Among the 32 respondents who indicated that they base their criteria on their own experience or views, 10 (31 %) provided various descriptions of the tumor-nerve relationship [i.e., tumor invading any of the nerve sheath layers (n = 1); tumor invading perineurium

Table 2 Formulation of histologic PNI criteria currently used by respondents

How did you formulate the histologic criteria for PNI that you currently use? ^a	n (%)	Summary of submitted comments
By criteria taught during residency training	42 (48 %)	Tumor relationship to the perineurium/perineural space/nerve sheath layers (n = 24) Tumor surrounding/encircling/wrapping around nerve (n = 16) ≥1/4 or 1/3 of nerve circumference surrounded by tumor (n = 4) PNI located outside of the main primary tumor mass (n = 4) Literature (Batasakis, Cottel, and/or Fagan) (n = 3) “Tumors grow along the nerve bundles, follow the contour of the nerve sheath” (n = 1) Nerve size (n = 1)
By adopting criteria published in the literature	29 (33 %)	Published works cited: Liebig et al. (n = 5), Batsakis (n = 3), Dunn et al. (n = 2), Fagan et al. (n = 2), “multiple sources” (n = 1) PNI criteria described without an identifiable source (n = 5)
Based on my own experience and/or views	32 (36 %)	Various descriptions of the tumor-nerve relationship (see text) (n = 10) Neurotropism/“cancer cell affinity to the basement membrane” (n = 4) Discussions with other pathologists (n = 3), experience working with other pathologists trained at various institutions (n = 1), general consensus among pathologists (n = 1) Practical experience (n = 1), reading (n = 1), gestalt (n = 1), selective use of levels/deepers and immunohistochemistry (n = 1)
Other	7 (8 %)	Tumor board discussions of this finding on treatment decisions and follow-up recommendations (n = 1) Direct observation (n = 1) Slide seminar presentations (n = 1) Residency training, experience, work with other pathologists, research experience, and/or published works (n = 4)

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^a Respondents were able to select more than one answer

(n = 1); tumor invading the perineurium and wrapping around at least 1/3 of the nerve (n = 1); tumor invading the nerve sheath layers and surrounding the nerve (n = 1); tumor intimately surrounding or encircling a nerve (n = 2); tumor cells contact and disturb the perineural cells (n = 1); tumor touches the nerve or nerve capsule (n = 2), tumor touches the perineurium of a small to medium nerve or epineurium of a large nerve with curvilinear but not focal nerve contact (n = 1)].

Overall, among the 74 individuals who submitted any comments regarding PNI criteria learned during residency and/or currently used, the most frequently mentioned criteria were the following: (1) tumor within/inside/invading/penetrating/disturbing the perineurium or perineural space (n = 25 respondents, 34 %) and (2) tumor surrounding/encircling/partially surrounding a nerve (n = 18 respondents, 24 %).

For the six proposed definitions of PNI, overall agreement among participants was slight ($\kappa = .10$, 95 % CI .08–.11). A summary of responses to each of the proposed definitions is provided in Table 3. Agreement was greatest (89 %) with “penetration of the perineurium by tumor

cells” and least (51 %) with “tumor cells closely approximate at least 33 % of the circumference of a nerve or are present within any of the nerve sheath layers...” (with the latter definition proposed by Liebig et al.) [9]. In particular, 11 respondents disagreed with the stated percentage (e.g., “may vary by plane or level of section,” “10 % or less is enough,” “requires >50 %,” “arbitrary”/“random”/“not reproducible”/needs statistical validation). In addition, six respondents commented that they disagreed with “closely approximate” or found this phrase unclear.

Comparison of survey responses between those practicing in a dental school setting versus those practicing in a medical school setting revealed no significant differences, except for the following: image question #7 ($p = 0.01$) [dental school participant PNI ratings: 21 (60 %) present, 6 (17 %) absent, 8 (23 %) uncertain; medical school participant PNI ratings: 9 (28 %) present, 15 (47 %) absent, 8 (25 %) uncertain]; image question #9 ($p = 0.03$) [dental school participant PNI ratings: 3 (8 %) present, 24 (69 %) absent, 8 (23 %) uncertain; medical school participant PNI ratings: 0 present, 30 (94 %) absent, 2 (6 %) uncertain]; and image question #11 ($p = 0.01$) [dental school

Table 3 Agreement among participants regarding proposed definitions of perineural invasion

Definition	Agree n (%)	Disagree n (%)	No response n (%)	Summary of submitted comments
Tumor cell invasion in, around, and through the nerves [7]	67 (76.1 %)	21 (23.9 %)	0 (0 %)	<p>“Around”: disagree or find unclear (n = 9)</p> <p>Tumor around a nerve = PNI versus in and through a nerve = intraneural invasion (n = 2)</p> <p>Ambiguous, not specific or precise enough (n = 3)</p> <p>Too stringent/change “and” to “or” (n = 1)</p> <p>Would cause one to “overcall” PNI (n = 1)</p>
Penetration of the perineurium by tumor cells	78 (88.6 %)	10 (11.4 %)	0 (0 %)	<p>Disagree with “penetration” (n = 2)</p> <p>Too vague or not practical (n = 2)</p> <p>Does not address large nerves with epineural involvement (n = 1)</p>
Tumor cells present within any of the nerve sheath layers (i.e., epineurium, perineurium, endoneurium) [9]	74 (84.1 %)	14 (15.9 %)	0 (0 %)	<p>Not practical (n = 2)</p> <p>“One of the most practical” (n = 1)</p> <p>Does not address intraneural invasion</p> <p>Neurotropism (“there should be selective growth along these layers”) (n = 1)</p> <p>Need to clarify “nerve” versus “axon”</p>
Tumor cells either touch a nerve or invade the nerve sheath [6]	48 (54.5 %)	39 (44.3 %)	1 (1.1 %)	<p>“Touch”: disagree or find unclear (n = 19)</p> <p>Not specific enough</p> <p>“Could be peri(neural) or intra(neural)” (n = 1)</p>
Tumor cells closely approximate at least 33 % of the circumference of a nerve or are present within any of the nerve sheath layers (i.e., epineurium, perineurium, endoneurium) [9]	45 (51.1 %)	41 (46.6 %)	2 (2.3 %)	<p>Disagree with “tumor cells closely approximate at least 33 % of the nerve” (n = 19)</p> <p>Does not address neurotropism (n = 3)</p> <p>“Could be peri(neural) or intra(neural)” (n = 1)</p> <p>Too restrictive (n = 1)</p> <p>“I generally follow this” (n = 1)</p>
Cytologically malignant cells are present in the perineural space. In equivocal cases, the following are supportive of perineural invasion: total or near-total circumferential involvement of the nerve, perineural tracking in tangential sections, and intraneural involvement. [10]	75 (85.2 %)	12 (13.6 %)	1 (1.1 %)	<p>Agree or use this definition (n = 4)</p> <p>Unclear, complicated, or too restrictive (n = 4)</p> <p>Does not distinguish between PNI and intraneural invasion (n = 2)</p> <p>Not helpful in equivocal cases (n = 1)</p> <p>Disagree with 1st sentence without accompanying images (n = 1)</p> <p>Need to clarify “nerve” versus “axon” (n = 1)</p>

PNI perineural invasion

participant PNI ratings: 29 (83 %) present, 4 (11 %) absent, 2 (6 %) uncertain; medical school participant PNI ratings: 26 (81 %) present, 0 absent, 6 (19 %) uncertain].

Discussion

To the best of our knowledge, this study represents the first to examine interobserver variation in evaluating PNI in OSCC. Interobserver agreement was fair ($\kappa = .38$) with

regard to PNI status ratings for provided photomicrographs and slight ($\kappa = .10$) with regard to proposed definitions of PNI. By convention, we adhered to qualitative categories proposed by Landis and Koch [12] for relative strength of agreement. Using these categories, one might conclude that $\kappa = .38$ is within the upper range of fair or “nearly moderate.” Although there is a tendency for investigators to regard moderate agreement as sufficient, McHugh [13] cautioned that such interpretation may be problematic for health-related studies. Particularly in a clinical laboratory

setting, standards for evaluating patient samples are high, with some authorities recommending .80 as the minimum acceptable interrater agreement [13]. Although our survey study is not an assessment of interobserver agreement in evaluating PNI in actual clinical practice, our results suggest that there is a need for calibration among pathologists in evaluating PNI in OSCC.

This study also provides valuable insights that may assist in the formulation of a widely accepted and objective definition of PNI. In survey section I, 99–100 % of respondents agreed that PNI was present for image question #3, #4, #10, and #15. These findings indicate that nearly all participating pathologists considered tumor completely or partially surrounding the circumference of the nerve—while assuming the shape or contour of the nerve—to be PNI. However, this feature was not the PNI criterion most frequently described by respondents in survey section II. Among those who submitted comments regarding PNI criteria learned during residency and/or currently used, invasion of the perineurium was mentioned most often (25 of 74 respondents, 34 %), followed by tumor surrounding, encircling, or partially surrounding a nerve (18 of 74 respondents, 24 %). Similarly, among proposed PNI definitions in survey section II, “penetration of the perineurium by tumor cells” exhibited the highest percentage of agreement (89 %).

There seems to be no consensus regarding a minimum numerical value for partial circumferential involvement in PNI. With regard to the PNI definition proposed by Liebig et al. [9], many participants ($n = 19$) commented that they disagree with the stipulation that “tumor cells closely approximate at least 33 % of the circumference of a nerve,” because they deemed this cutoff to be arbitrary, not reproducible, or lacking statistical validation. Indeed, only five individuals mentioned a requirement for at least 1/4–1/3 circumferential involvement when describing PNI criteria that they learned during residency or currently use.

Although a minimum percentage of circumferential involvement seems to be inherently difficult to establish, the majority of participants did not consider only focal tumor-nerve contact to represent PNI. In particular, there was <50 % agreement in rating PNI status for image question #2, #7, #11, and #13. For these questions, the photomicrographs showed tumor focally touching and/or blurring the perineurium. In such cases, it may be difficult to determine whether tumor invades or penetrates the perineurium. Indeed, 30 % of individuals who commented on these cases used descriptors, such as “suspicious,” “suggestive,” “questionable,” or other similar terms. Submitted comments also revealed that when faced with uncertain cases, some individuals chose to rate PNI “absent” or “present,” whereas others elected to report it as “uncertain.” Along similar lines, in survey section II, definition #4 by Fagan et al. [6] and definition #5 by Liebig et al. [9] elicited the lowest levels of agreement (55 and 51 %,

respectively), with 19 participants indicating disagreement with the word “touch” and 6 indicating disagreement with “closely approximate.” Thus, many participants did not consider tumor contact with a nerve or proximity to a nerve to be sufficient evidence of PNI. In short, it appears to be especially difficult to resolve among pathologists whether tumor focally invading or touching the perineurium represents PNI.

Image question #12 posed a somewhat different scenario—a small nerve completely surrounded by a solid sheet of tumor cells; 83 % of participants rated PNI present. Among the 17 % of participant who rated PNI absent or uncertain, the most frequently mentioned issue was that a nerve “encased,” “engulfed,” or “entrapped” by a mass of tumor cells does not necessarily constitute PNI. Therefore, although the majority of survey participants interpreted this case to represent PNI, some might question whether there was true neurotropism or selective tumor growth with nerve invasion. Similarly, among descriptions of PNI criteria currently used by participants, the need to demonstrate PNI in an area away from the main primary tumor mass or the need to demonstrate neurotropism was mentioned 8 times. Previously published studies have considered similar issues. For example, in a retrospective study of noncutaneous head and neck SCC, Miller et al. [14] subcategorized PNI extent as intratumoral, peripheral, or extratumoral; these investigators found that PNI extent was significantly correlated with disease-free survival and that there was a trend toward increased disease-free survival among patients with negative/peripheral/intratumoral PNI compared to those with extratumoral PNI. We recognize that the photomicrographs provided in the image questions in survey section I did not allow for participants to determine the location of PNI with respect to the main tumor mass. However, this was because the focus of our study was to assess the varying views of pathologists with respect to defining and evaluating PNI itself, rather than to evaluate methods for further subclassifying PNI. Also of interest, in a study of rectal carcinoma, Seefeld and Barga [15] excluded cases of so-called “secondary involvement of nerves” from their definition of PNI; that is, a nerve completely surrounded by carcinoma but with an intact perineural space devoid of tumor cells was not considered to represent PNI. These investigators typically observed this phenomenon within or close to the primary tumor mass. Alternatively, some authors use the term *perineural spread* to indicate tumor spread along a nerve beyond the main tumor mass; however, this term implies grossly or radiographically evident tumor spread, whereas PNI is considered by many authorities to be a microscopic finding, not necessarily indicative of perineural spread [2, 16]. Other investigators use the term *clinical PNI* in reference to clinically or radiographically evident invasion versus *incidental PNI* for asymptomatic but microscopically evident invasion [17].

Among the six PNI definitions that we presented to survey participants, “penetration of perineurium by tumor cells” exhibited the highest level of agreement (89 %), followed by “tumor cells present within any of the nerve sheath layers (i.e., epineurium, perineurium, endoneurium)” with 84 % agreement. Likewise, tumor within/inside/in-vading/penetrating/disturbing the perineurium/perineural space was the most frequently mentioned PNI criterion among those who submitted comments regarding criteria learned during residency and/or currently used (25 of 74 respondents); however, only four participants mentioned all 3 nerve sheath layers in their descriptions of PNI criteria for these questions. The high frequency of comments focusing on the perineurium likely reflects the fact that most nerves encountered during histopathologic examination of OSCC specimens are relatively small, with an outer sheath of perineurium but no epineurium. With light microscopic examination, it can be challenging at times to visualize the perineurium and to delineate the epineurium. Therefore, although most pathologists in our study agreed with the concept that tumor invasion of the perineurium or any of the nerve sheath layers constitutes PNI, practical application of this definition may be difficult.

There are inconsistencies in the literature regarding whether intraneural invasion (or invasion of the endoneurium) should be distinguished from PNI [7, 9, 10, 18, 19]. In our survey, most participants (60 %) answered “no” when asked if they make a distinction between perineural and intraneural invasion in their pathologic reporting of OSCC. We designed image question #8 and #14 to depict intraneural invasion, with 97 and 96 % of respondents rating PNI present, respectively. Many individuals who rated PNI as present also submitted optional comments mentioning intraneural or endoneural invasion, whereas only two individuals commented that they rated PNI as absent because they separate intraneural invasion from PNI. Our findings suggest that recommendations regarding appropriate reporting of intraneural invasion in OSCC are needed; such recommendations would require further studies to determine whether distinction between invasion of the endoneurium versus invasion of (or growth surrounding) the outer nerve sheath is of clinical significance.

In practice, additional sections and/or immunohistochemistry may be helpful in determination of PNI, as frequently suggested by comments in response to the survey image questions. We concede that providing only a single photomicrograph for each case in our survey does not reflect the reality that multiple sections or tumor fields typically are examined during pathologic examination of a case. Thus, an equivocal focus of PNI may not present a quandary if more definitive evidence is found elsewhere; indeed, one participant commented that PNI “is generally not an isolated event and there should be other more convincing foci.” With regard

to immunohistochemistry as a useful adjunct, Kurtz et al. [20] identified PNI in 30 % of OSCC cases as per original pathology reports versus 62 % upon review of slides and 82 % upon review with the aid of S-100 protein immunohistochemistry. These investigators found S-100 protein immunohistochemistry to be especially helpful for highlighting small nerves and distinguishing nerves from desmoplastic stroma. Similarly, other investigators have reported increased detection of PNI in nonmelanoma skin cancers with dual immunohistochemistry for S-100 protein and AE1/AE3 or p63 (highlighting nerve and tumor, respectively) compared to hematoxylin and eosin staining alone [21, 22]. S-100 protein is expressed by Schwann cells, whereas markers such as EMA, GLUT1, and claudin-1 are expressed by perineurial cells [23]. Although S-100 protein antibodies are widely available and may aid in identification of peripheral nerves, we surmise that antibodies targeting the perineurium may be most helpful when evaluating the relationship between the perineurium and adjacent tumor. However, EMA, GLUT1, and claudin-1 may be expressed by not only perineurium but also OSCC [24–26]. Also of interest, in a study of PNI in colorectal cancer staging by Ueno et al. [27], GLUT1 immunohistochemistry showed tumor outside the perineurium even in a case regarded as a typical example of PNI based on hematoxylin and eosin staining (i.e., tumor surrounding most of the circumference of a nerve). Although 89 % of participants in our study regarded invasion of the perineurium to be a PNI criterion, this immunohistochemical finding by Ueno et al. suggests that PNI may include tumor surrounding a nerve—even in the absence of invasion of the perineurium. In addition, a growing understanding of the mechanisms underlying PNI may lead to the discovery of additional immunohistochemical markers for PNI evaluation [28, 29]. In short, further studies investigating the utility of such immunohistochemical markers for detection of PNI in OSCC are needed.

In conclusion, our survey of board-certified oral and/or surgical pathologists showed interobserver agreement to be fair in evaluating PNI status for selected microscopic images of OSCC and slight in considering various proposed definitions of PNI. Our results suggest that among pathologists there is an urgent need for calibration and widely accepted criteria that will produce more consistent determination of PNI in OSCC. In addition, our study provides insights regarding specific challenges in evaluating PNI, and points to the need for further studies. We believe that our study findings are important to raise awareness and stimulate interest regarding the challenges of defining PNI—an issue that seems to be taken for granted. In addition, our study findings may aid in the difficult task of formulating a widely accepted and objective definition of PNI, which subsequently could be used in clinical studies assessing the prognostic significance of PNI.

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Appendix: Survey Questions

Please confirm that you are a board-certified pathologist.

- ☐ Yes, I am a board-certified pathologist.
☐ No, I am not a board-certified pathologist.

SECTION I: For each of the following questions, a photomicrograph is provided. Please assess the presence or absence of perineural invasion just as you would in your pathology practice.

Questions 1 through 15: For the photomicrograph shown below, how would you assess perineural invasion status?

- ☐ Present
☐ Absent
☐ Uncertain

If uncertain, how would you address that in your pathology report?

Additional comments (optional):

SECTION II:

1. What is your age? (in years)

2. What is your gender?

- ☐ male
☐ female

3. How many years have you practiced surgical pathology?

4. What is the nature of your pathology practice?

- ☐ associated with a dental school
☐ associated with a medical school
☐ private pathology lab
☐ none of the above

If the nature of your practice is none of the above, please specify type:

5. In pathology reports that you have issued for oral squamous cell carcinoma, have you ever indicated that perineural invasion is present?

- ☐ Yes
☐ No

6. In pathologic reporting of oral squamous cell carcinoma, do you make a distinction between perineural and intraneural invasion?

- ☐ Yes
☐ No

7. During your residency training, did you learn histologic criteria for perineural invasion?

- ☐ Yes
☐ No

If yes, please list or describe the histologic criteria that you learned.

8. How did you formulate the histologic criteria for perineural invasion that you currently use?

- ☐ By criteria taught during residency training (as I have described in the previous question)
☐ By adopting criteria published in the literature as follows:

- ☐ Based on my own experience or views as follows:

- ☐ Other (please specify below):

9. Below are some definitions of perineural invasion previously published in the literature or commonly suggested by pathologists. For each definition, please indicate if you agree or disagree.

a) Tumor cell invasion in, around, and through the nerves [Batsakis JG. Nerves and neurotropic carcinomas. *Ann Otol Rhinol Laryngol* 94(4 Pt 1):427-7, 1985]]

- ☐ agree
☐ disagree

comment (optional):

b) Penetration of the perineurium by tumor cells

- ☐ agree
☐ disagree

comment (optional):

c) Tumor cells present within any of the nerve sheath layers (i.e., epineurium, perineurium, endoneurium) (Liebig C et al. Perineural invasion in cancer. *Cancer* 115:3379-91, 2009)]

- ☐ agree
☐ disagree

comment (optional):

d) Tumor cells either touch a nerve or invade the nerve sheath (Fagan JJ et al. Perineural invasion in squamous cell carcinoma of the head and neck. *Arch Otolaryngol Head Neck Surg* 124:637-40,1998)

- ☐ agree
☐ disagree

comment (optional):

e) Tumor cells closely approximate at least 33% of the circumference of a nerve or are present within any of the nerve sheath layers (i.e., epineurium, perineurium, endoneurium) (Liebig C et al. Perineural invasion in cancer. *Cancer* 115:3379-91, 2009)

- ☐ agree
☐ disagree

comment (optional):

f) Cytologically malignant cells are present in the perineural space. In equivocal cases, the following are supportive of perineural invasion: total or near-total circumferential involvement of the nerve, perineural tracking in tangential sections, and intraneural involvement. (Dunn et al. Perineural invasion: identification, significance, and a standardized definition. *Dermatol Surg* 35:214–21, 2009.)

☐ agree

☐ disagree

comment (optional):

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