Laryngoscope Investigative Otolaryngology

Perineural Invasion in Squamous Cell Carcinoma of the Oral Cavity: Histology, Tumor Stage, and Outcome

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Objectives/Hypothesis: To analyze the impact of different types of perineural invasion (PNI) in squamous cell carcinoma (SCC) of the oral cavity on overall survival and recurrence rate, with a special focus on histologic subtypes and tumor stage.

Study Design: Retrospective case-control study with clinicopathological analysis.

Methods: Seventeen patients who received primary surgical treatment for SCC of the oral cavity with PNI were matched to a control group. In a histologic review, PNI was classified into subtypes according to an adapted Liebig classification. The term type A was used to describe tumor invasion into the nerve, whereas type B was used to describe circumferential growth around the nerve. Clinical charts were reviewed, and a Kaplan-Meier survival analysis was performed.

Results: The recurrence-free survival rates were 47.1% versus 80.4% (PNI vs. matched control group, $P < 0.05$), 60.0% versus 94.1% (PNI in stage I and II disease vs. matched control group, $P < 0.05$) and 41.7% versus 73.5% (PNI in stage III and IV disease vs. matched control group, $P < 0.05$). In most cases ($n = 9$) of PNI, both histologic subtypes (type A and type B) were present. Five cases exclusively showed type A, and three cases exclusively showed type B.

Conclusions: Perineural invasion in early disease oral carcinoma has a particularly high impact on survival. Both histologic subtypes showed a significantly worse recurrence-free survival rate when compared to the control group.

Key Words: Perineural invasion, head and neck, squamous cell carcinoma, oral, histology.

Level of Evidence: 3.

INTRODUCTION

Perineural invasion (PNI)[1] describes a malignant tumor's affinity for neural tissue[2–4] and is associated with adverse outcome in many different types of cancer. In head and neck cancer, it is predominately found in adenoid cystic carcinoma[5] but also in squamous cell carcinoma (SCC).[6] Whereas in the first it is anticipated with the diagnosis, and a high local recurrence rate has to be expected, in the latter it is assumed to be an additional independent risk factor.[7] This is crucial in the case of early disease, for example, tumor-node-metastasis (TNM) tumor stage I in oral cavity carcinoma, for which the presence of PNI can alter the extent of treatment by adding adjuvant radiotherapy after surgical resection.[8] Although in the clinical setting PNI is treated as a homogenous entity, there are different histologic patterns of tumor cells interacting with nerve tissue that lead to the diagnosis of PNI. The sheer presence of tumor cells adjacent to a nerve does not ultimately qualify for PNI diagnosis. A widely accepted definition of PNI was provided by Liebig et al.[9] The authors described two histologic patterns that alone or in combination define PNI. One pattern is observed when clusters of tumor cells are located within the peripheral nerve sheath and infiltration to the epineurium, perineurium, and endoneurium can be distinguished. The criterion for the second pattern is met when tumor cells encircle at least 33% of the circumference of a nerve. The location of the affected part of the nerve, whether within the tumor or at a site outside the tumor margin, does not contribute to their definition of PNI.

Miller et al.[10] analyzed subcategories of PNI according to the location of PNI in comparison with the tumor margin and analyzed cohorts with noncutaneous head and neck SCCs. They observed a trend for a longer disease-free survival for patients with strictly intratumoral PNI when compared to patients with additional peripheral and extratumoral PNI.

The aim of the current study was to use a classification system based on the definitions by Liebig et al.[9] to analyze the recurrence-free survival of a homogenous group of patients with SCC of the oral cavity. To our knowledge, this is the first study analyzing the two different kinds of PNI patterns described by Liebig et. al in a comparing clinical context.

MATERIALS AND METHODS

Patients with SCC of the oral cavity who underwent primary surgery at a single tertiary referral center for head and

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Editor's Note: This Manuscript was accepted for publication 24 November 2015.

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The authors have no funding, financial relationships, or conflicts of interest to disclose.

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DOI: 10.1002/lio2.4

Modified Liebig Classification

Liebig Type A
Tumor infiltrating nerve
-Epineurium
-Perineurium
-Endoneurium

Liebig Type B
Tumor encircling nerve (at least 33%)

Fig. 1. Histologic subcategories.

neck surgery between 2000 and 2008, and whose histopathology reports mentioned PNI, were included. The time points were chosen to allow a follow-up period of at least 5 years. Seventeen patients were identified, and their histopathological slides were reviewed by a pathology resident (I.S.) and a head and neck pathologist (M.R.). In brief, histological slides of the cases were retrieved from the archives of the Institute of Surgical Pathology, University Hospital Zurich. Hematoxylin and eosin-stained slides were reviewed for presence, number of affected nerves, and pattern of PNI. In all cases, PNI was confirmed. The pattern of PNI was assessed according to a modified Liebig classification, as shown synoptically in Figure 1 and histologically in Figure 2. The term *type A* was used to describe the pattern when direct infiltration of tumor cells into a nerve was observed. The term *type B* describes tumor cells encircling at least 33% of a nerve.

The initial clinical presentation and the clinical course of disease in the 5-year follow-up period were reviewed retrospectively in the patient charts. Collected parameters included age at primary surgery; gender; tumor localization within the oral cavity; tumor stage with both TNM and general stage (I to IV) ; and whether they received adjuvant radiotherapy or not. At our

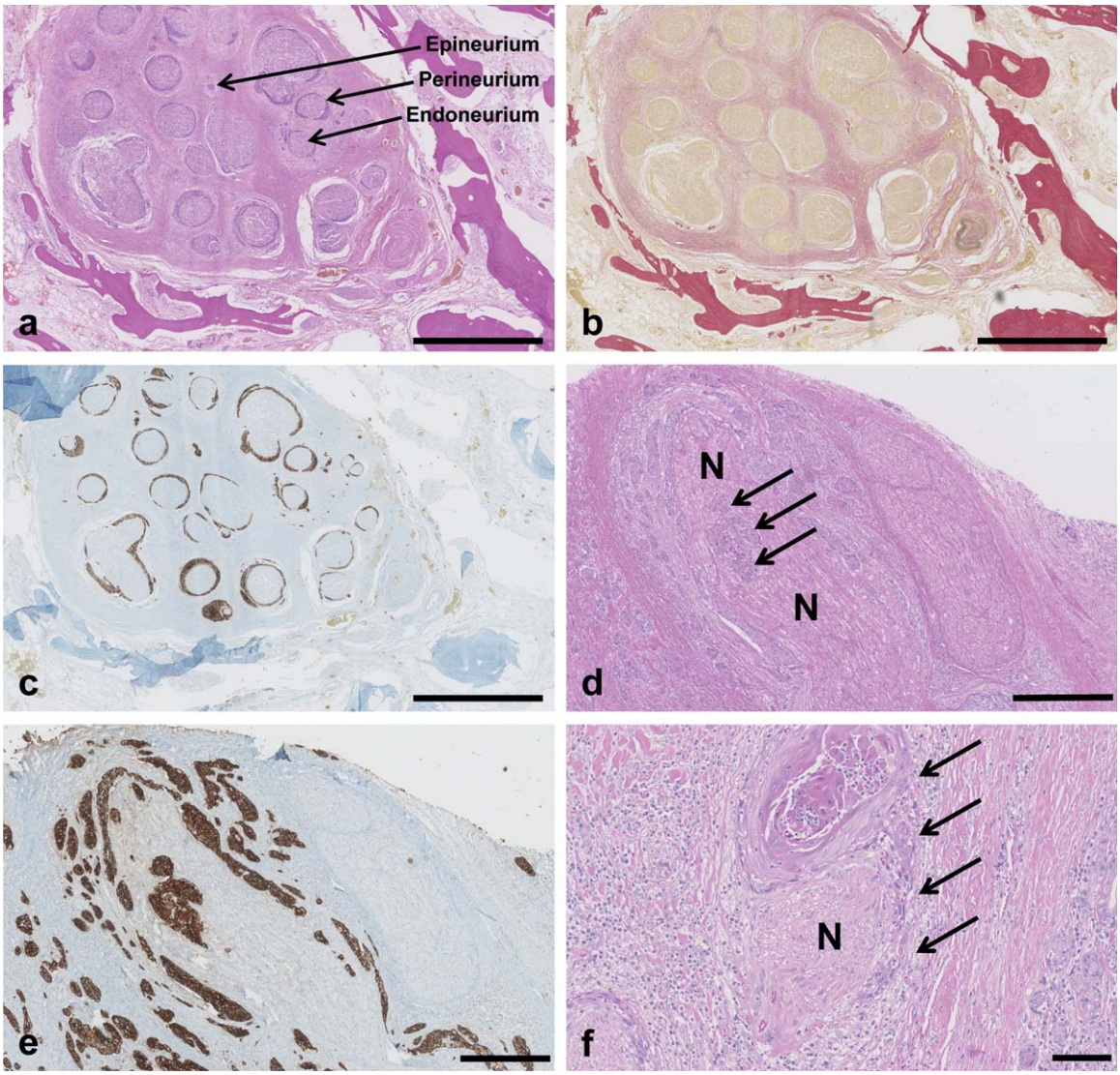

Fig. 2. Representative histological sections demonstrating perineural invasion (PNI) of squamous cell carcinoma: (a–c) Infiltration of the epineurium and perineurium or (d–e) endoneurium representing type A according to the modified Liebig classification. (f) Involvement of at least 33% of the perineural circumference represents classification as Liebig type B. Arrows = carcinoma, N = nerve. (a,d,f) hematoxylin and eosin stains; (b) Elastica van Gieson stain; and (c + e) immunohistochemical pancytokeratin staining labeling carcinomatous infiltrates. Scale bars: a–c 1 mm, d–e 500 μm, f 100 μm.

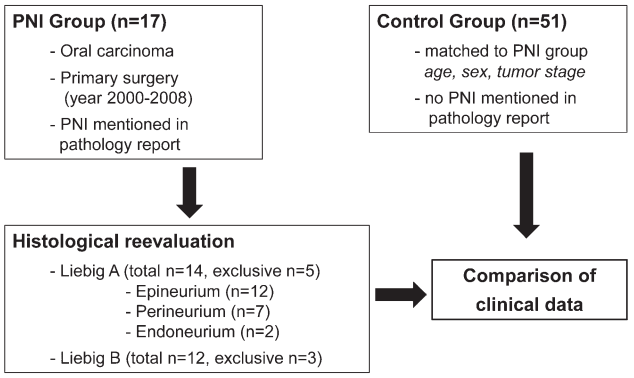

Fig. 3. Study setup. PNI = perineural invasion.

institution, the decision on adjuvant radiotherapy with or without concomitant chemotherapy is made at an interdisciplinary tumor board based on the patient's individual medical history. Generally, PNI, lymphangiosis, N+ stage, and a T stage of 3 or higher lead to recommending the patient adjuvant radiotherapy. Accordingly, concomitant chemotherapy is added for patients with adequate renal function and age below 70 years.

Data were compared to a matched control group of patients who also underwent primary surgery for oral SCC, but with no PNI mentioned in the histopathology report. Each PNI patient was matched with three no PNI patients, resulting in 51 patients in the no PNI control group. Matching was performed according to age at primary therapy, gender, and tumor stage (I to IV). Figure 3 gives a schematic overview of the study setup.

Kaplan-Meier survival analysis (IBM SPSS Statistics for Windows, Version 22.0; IBM Corp., Armonk, NY) was used to calculate recurrence-free survival and overall survival rates and the log-rank test to identify significant differences.

The study was performed according to the rules of ethics committee of the local authority.

RESULTS

Demographic and clinical data is shown in Table I and Table II. The male-to-female ratio was 10:7 and the average age was 56 ± 9 years (range 39–72). At time of primary surgery, five patients of the study group suffered from early disease (TNM stage I and II) and 12 patients had already regionally advanced disease (TNM stage III and IV). Within the oral cavity, the primary site of cancer was the floor of mouth in eight cases, the tongue in eight cases, and the hard palate in one case. Three of the eight patients with tongue cancer suffered from regional advanced disease, in contrast to only one of the eight patients with the floor of mouth as primary site.

Adjuvant radiotherapy was performed in 59% of the patients in the PNI group and in 45% of the patients in the control group. Of the seven patients in the PNI

TABLE I.
Overall Demographic and Clinical Data

No.	Gender	Age	Location	T	N	Tumor Stage	Type A	Type A Epineurium	Type A Perineurium	Type A Endoneurium	Type B	Recurrence-Free Survival (months)	Recurrence Site	Overall Survival (months)
1	M	54	tongue	1	0	I	1	1	1	0	1	47	regional	47
2	F	44	tongue	2	0	II	1	1	0	0	1	25	regional	40
3	M	39	tongue	2	0	II	1	1	1	0	0	no recurrence	–	alive
4	F	61	hard palate	2	0	II	1	1	1	0	1	4	local	16
5	F	49	floor of mouth	2	0	II	1	0	1	0	1	no recurrence	–	alive
6	M	60	tongue	2	1	III	1	1	0	0	1	no recurrence	–	alive
7	F	62	tongue	1	1	III	1	1	0	0	1	no recurrence	–	alive
8	M	46	floor of mouth	2	1	III	0	0	0	0	1	3	local	22
9	M	49	floor of mouth	2	1	III	0	0	0	0	1	7	local	28
10	M	72	tongue	2	1	III	1	1	0	0	1	8	regional	13
11	F	65	tongue	2	1	III	1	0	1	1	0	8	regional	9
12	M	48	floor of mouth	2	2b	IV	1	1	0	0	0	no recurrence	–	alive
13	F	62	floor of mouth	4a	1	IV	1	1	1	0	0	0	local	5
14	M	60	tongue	2	2b	IV	0	0	0	0	1	3	local and regional	18
15	M	59	floor of mouth	4	2c	IV	1	1	1	1	0	2	local	5
16	F	60	floor of mouth	4a	2c	IV	1	1	0	0	1	no recurrence	–	alive
17	M	69	floor of mouth	4a	0	IV	1	1	0	0	1	no recurrence	–	alive

f = female; m = male; T and N according TNM.

TABLE II.
Demographic and Clinical Data of Subgroups

Stage I–IV	PNI Group	Control Group	*P* Value
n =	17	51	
Gender (m:f)	10:7	30:21	1.00
Age (mean)	56 ± 9 years	56 ± 9 years	1.00
Location	tongue 8	tongue 32	0.27
	floor of mouth 8	floor of mouth 18	0.40
	hard palate 1	buccal mucosa 1	–
Adjuvant radiotherapy	10 (59%)	23 (45%)	0.41
Concomittant chemotherapy	10 (59%)	9 (18%)	0.004
Stage I and II	**PNI Group**	**Control Group**	***P* Value**
n =	5	17	
Stage (I:II)	1:4	3:14	1.00
Gender (m:f)	2:3	6:11	1.00
Age (mean)	49 ± 8 y	50 ± 10 y	0.89
Location	tongue 3	tongue 13	0.59
	floor of mouth 1	floor of mouth 4	1.00
	hard palate 1		–
Adjuvant radiotherapy	2 (40%)	1 (6%)	0.12
Concomittant chemotherapy	0 (0%)	0 (0%)	1.00
Stage III and IV	**PNI Group**	**Control Group**	***P* Value**
n =	12	34	
Stage (III:IV)	6:6	9:25	0.16
Gender (m:f)	8:4	24:10	1.00
Age (mean)	59 ± 8 y	58 ± 8 y	0.68
Location	tongue 5	tongue 19	0.51
	floor of mouth 7	floor of mouth 14	0.34
		buccal mucosa 1	–
Adjuvant radiotherapy	8 (67%)	22 (65%)	1.00
Concomittant chemotherapy	8 (67%)	9 (26%)	0.02

f = female; m = male.

group who did not receive adjuvant radiotherapy, reasons include patient's primary decision (n = 2); early termination of already started radiotherapy on patient's demand (n = 2); and a sentinel lymph node result, pN0(i+) and pN1(mi), for which a neck dissection was chosen over radiotherapy (n = 3). Postoperative radiation therapy prescription dose was 60 Gy to 66 Gy applied in 30 to 33 fractions, five times a week, administered to the tumor bed area, whereas elective treatment of the nodal pathways of 54 Gy (low risk) or 60 Gy (higher risk) was given. All treatments were performed using simultaneously integrated boost technique. In patients with residual gross tumor, 70 Gy were applied. Concomitant chemotherapy consisted of cisplatin in all cases, except for two cases in the PNI group for which cetuximab was used. As shown in Table II, concomitant chemotherapy was expectedly not added equally in both groups.

Histologically, the reevaluation of the histological slides revealed five patients (29%) with exclusively Liebig type A pattern, three patients (18%) with exclusively Liebig type B pattern, and nine patients (53%) with a mixed Liebig type A and B pattern. That is, of the 17 patients with PNI, 14 (82%) displayed a type A pattern and 12 (71%) a type B pattern. The corresponding data is shown in Figure 3 and Table I.

The results from the survival analysis are shown in Figure 4 (a–f). The 5-year overall survival of the PNI group (n = 17) was 41.2%, compared to 72.5% in the control group (n = 51, $P < 0.05$, log-rank test). The 5-year recurrence-free survival for the same groups was 47.1% and 80.4%, respectively ($P < 0.05$). The analysis of the histologic subcategories showed a different 5-year recurrence-free survival rate for the PNI patients with a type A pattern (n = 14) of 57.1% versus 81.0% in their matched controls, with no PNI (n = 42) as a trend ($P = 0.05$). Patients whose histology showed the presence of type B pattern (n = 12) had a 5-year recurrence-free survival of 50.0% versus 80.6% in their matched controls (n = 36, $P < 0.05$). The nine patients who had a mixed type A and B pattern are a part of both mentioned PNI subgroups. Survival analysis of subcategories within the PNI group, for example, patients with

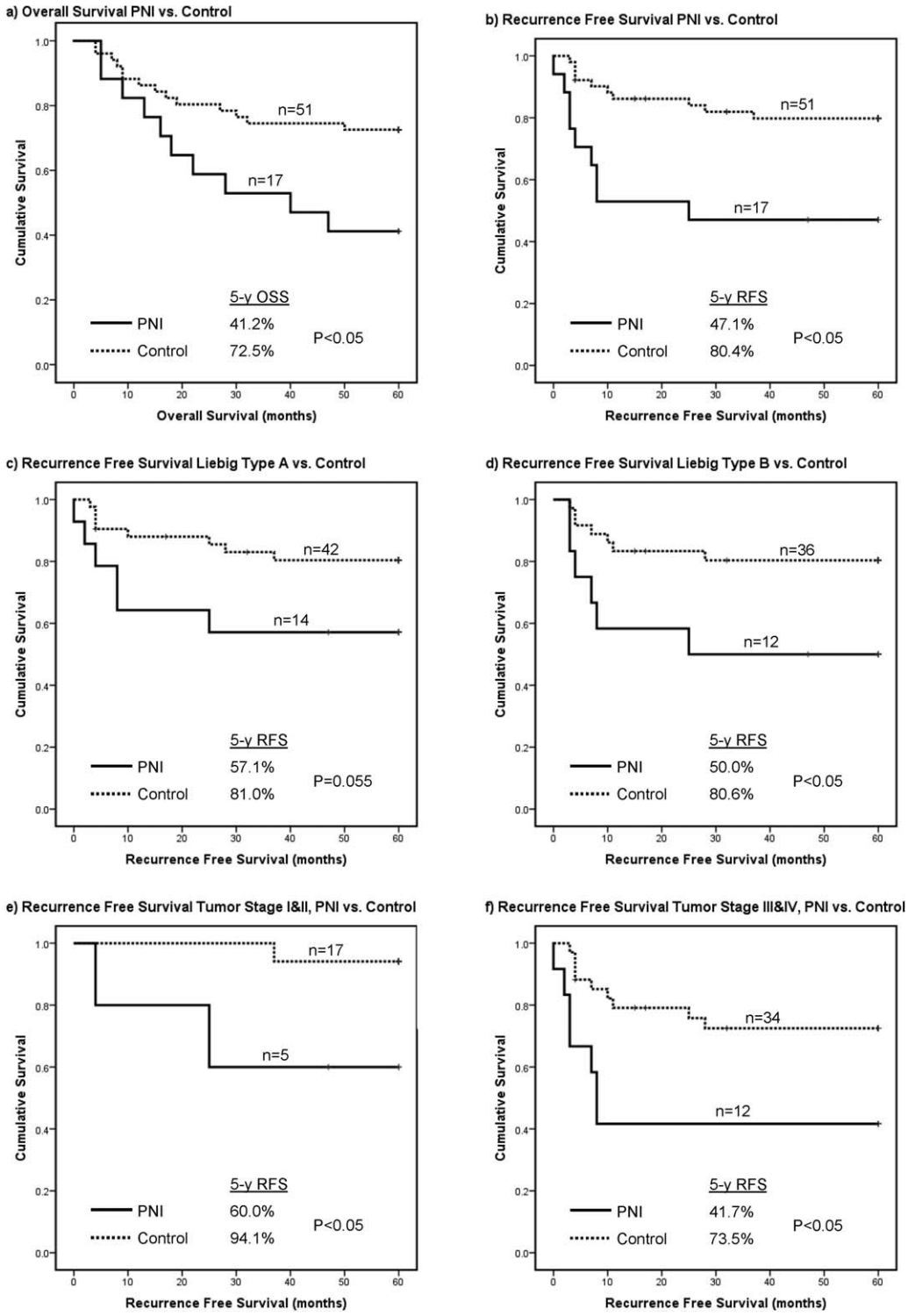

Fig. 4. Kaplan-Meier curves of the OS and RFS of the total group in (a) and (b), of RFS of the histologic subtypes Liebig type A and Liebig type B in (c) and (d), and of the RFS of early disease and late disease in (e) and (f). OS = overall survival; PNI = perineural invasion; RFS = recurrence-free survival.

exclusively type A (n = 5) versus patients with exclusively type B (n = 3) showed no significant difference (data not shown).

Patients with PNI and local disease (TNM stage I and II) (n = 5) had a 5-year recurrence-free survival of 60% versus 94.1% in the matched control group (n = 17, $P < 0.05$). The 5-year recurrence-free survival rate of patients with PNI and regional advanced disease (TNM stage III and IV) (n = 12) was 41.7% versus 73.5 in their matched control group (n = 34, $P < 0.05$).

DISCUSSION

The clinical, histological, and biological features of PNI have been studied extensively in different kinds of cancer. It is well known that PNI in oral carcinoma is associated with higher recurrence rates and a lower disease-free survival.[11,12] Remarkably, not only the risk of local recurrence, but also the risk of regional recurrence is increased, which indicates that PNI is not only a question of tumor-free margins and a local further-than-expected spread along the nerves.[13] This feature could be observed in our cohort as well as with five cases of local recurrence, four cases of regional recurrence, and one case with local and regional recurrence. Although described in studies as early as 1963,[1] a standardized definition of PNI among pathologists has not been established in total accordance.[9,14] The definition of Liebig et al.[9] allows different patterns of histologic findings to conclude with the diagnosis of PNI. In our study, most patients with PNI showed both infiltration and encircling of the nerve (53%). Infiltration without encircling of the nerve only was observed in three cases (18%). The histology of five patients (29%) showed exclusively encircling of the nerves without infiltration. The histologic reevaluation was performed with a focus on identifying these histologic patterns. Because most cases showed a mixed pattern, it seems possible that once one pattern is present, it is just a question of time and tumor growth until the other pattern develops as well. This supports the Liebig classification in summarizing both patterns independently as PNI, also because there was no difference in the recurrence-free survival of the histologic subgroups. This is in accordance with results of Gil et al., who examined paranasal sinus tumors.[15] However, comparison was limited due to small sample sizes.

The overall survival rates, the recurrence-free rates, and the demographic data of our total PNI and control group are in accordance with reported data in literature.[16] The demographic data of the stage I and II subgroup showed more affected women, a younger mean age, and predominantly the tongue as the primary tumor site when compared to the stages III and IV subgroup. For example, this could indicate that older men with the floor of the mouth as the primary site are more likely to notice their disease with delay, or that this tumor site predisposes a more rapid tumor progress into a higher tumor stage.

It is known that PNI has an impact on early stage oral SCC.[17] In our study, stage I and II patients without PNI had an excellent recurrence-free survival rate of 94.1%. This rate dropped to 60% if PNI was present, which is lower than the recurrence-free survival rate of stage III and IV patients without PNI. And even in extended disease, PNI had a significant additional impact on recurrence-free survival.

CONCLUSION

In the majority of PNI cases, both histologic patterns (encircling and infiltration of the nerve) were present. The different histologic patterns qualifying for PNI, according to Liebig et al., showed no different clinical outcome.[9] The presence of PNI in early disease (stage I and II) has a particularly serious impact on outcome in oral SCC and warrants a more aggressive primary treatment.

Acknowledgments

Authors R.D.L. and I.S. contributed equally to this work.

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