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Factors predicting perineural invasion in hypopharyngeal squamous cell carcinoma

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Introduction

The spread of tumours through nerves is known as perineural invasion (PNI).[1] The prevailing definition of PNI is "tumour in close proximity to a nerve and involving at least 33% of its circumference or tumour cells within any of the three layers of the nerve sheath".[2] PNI is an important pathological feature of many malignant neoplasms, including head and neck cancer, gastrointestinal tumour, breast cancer, pancreatic cancer, prostate cancer, and bile duct cancer. Positive PNI often indicates poor prognosis and decreased survival rate.[2]

Hypopharyngeal cancer (HPC) is the least common type of cancer of the head and neck, but has the worst prognosis. Morbidity and mortality rates for HPC have continued to increase in the past decades. [3] PNI in hypopharyngeal squamous cell carcinoma (HPSCC), a subtype of HPC, is also associated with poor prognosis, namely decreased survival and increased risk of metastasis.[4] However, the correlation between PNI and clinicopathological parameters of HNSCC remains unclear.[4-7] This study aimed to investigate the positivity rate of PNI in HPSCC and determine its association with clinicopathological parameters of HPSCC. We anticipate that our findings will potentially provide new insights about PNI and further guide clinical decisions.

Materials and methods

Patients

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Complying with good clinical practice guidelines in China and the Declaration of Helsinki (2013), the institutional review board of Shandong Provincial ENT Hospital approved this retrospective study (approval number 2024-18-01) and waived the requirement for informed consent. We enrolled patients with hypopharyngeal tumours between September 2019 and March 2024. The criteria for exclusion were: (1) non-SCC postoperative pathology; (2) patients with primary HPSCC who received radiotherapy or neoadjuvant chemotherapy prior to surgery; (3) recurrent or metastatic disease; (4) laser surgery; and (5) incomplete postoperative histopathology reports or no report of PNI or lymphovascular invasion (LVI) status. Figure 1 shows the detailed process for patient recruitment. Ultimately, 182 consecutive patients were identified and included in the final analysis. The clinical and histopathological parameters of all participants were retrospectively analyzed, including sex, age, primary tumour sites, tumour thickness (TT, the total thickness of the tumour along its invasive axis), tumour diameter (TD, the longest diameter in the resected specimen), histologic differentiation, tumour–node–metastasis classification (the 2017 edition of the tumour–node–metastasis classification of the American Joint Committee on Cancer), stage, lymph node metastasis (LNM), number of positive nodes (pN), lymph node density (LND, the ratio of the number of metastatic lymph nodes to the total number of resected lymph nodes), extranodal extension (ENE), PNI, LVI, and Ki-67 index. We divided patients with HPSCC into two groups: the PNI-positive and PNI-negative groups, according to PNI status on postoperative pathological reports.

Pathological evaluation

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Pathological specimens of all participants' primary tumours were carefully identified by a head and neck pathologist (with 8 years of work experience) to check the PNI status of the pathological reports. Histopathological morphology on hematoxylin and eosin stain and S-100 protein immunohistochemical staining were used to detect PNI within the primary tumours. Positive PNI was defined as: "tumour in close proximity to a nerve and involving at least 33% of its circumference or tumour cells within any of the three layers of the nerve sheath".[2]

Statistical analysis

IBM SPSS 27.0 software (Chicago, IL, USA) was used for statistical analyses. Inter-group comparisons of quantitative data were conducted using an independent sample t-test or Wilcoxon–Mann–Whitney U. Two-group comparisons of qualitative data were analyzed using chi-square or Fisher's exact tests. A binary logistic regression model was used for multivariate analysis of meaningful variables in univariate analysis. A $p < 0.05$ was considered statistically significant.

Results

Patient characteristics

Table 1 provides an overview of the clinicopathological features of patients with HPSCC. The mean age was 62 years (range, 42–81 years), with 178 males and 4 females. The location of the primary tumour was as follows: 137 cases involving the pyriform fossa, 29 cases involving the posterior pharyngeal wall, and 16 cases involving the postcricoid area.

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PNI was noted in 68 of the 182 patients (37.4%). In the PNI-positive group, the majority of patients (37; 54.4%) were at the T3 stage, while only one (1.5%) patient was at the T1 stage. In the PNI-positive group, most patients were at the N2 stage (41.2%), whereas N0 stage was the least common, accounting for only 13.2%. In the PNI-positive group, 58 (85.3%) patients had stage IV disease, 10 (14.7%) had stage III disease, and none had stage I or II disease. In the PNI-positive group, 46 (67.6%) patients had moderately differentiated tumours, while only two patients (2.9%) had well-differentiated tumours.

Relationship between PNI and clinicopathological features

We observed a definite correlation between PNI and TT ($p = 0.003$), LVI ($p = 0.049$), LNM ($p = 0.037$), LND ($p = 0.011$), and pN value ($p = 0.039$), as shown in Table 1. The median value of TT in the PNI-positive and PNI-negative group was 1.7 cm (1.3 cm, 2.075 cm) and 1.3 cm (1 cm, 1.8 cm), respectively. Fifty-nine (86.8%) patients had LNM in the PNI-positive group, and only nine (13.2%) patients had negative lymph nodes. No significant relationship was observed between positive PNI and other clinicopathological characteristics.

Risk factors of PNI in HPSCC

TT remained a significant risk factor for PNI in HPSCC (odds ratio = 1.614; 95% confidence interval: 1.019–2.557; $p = 0.041$) in the multivariate analysis, as shown in Table 1.

Discussion

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Although HNSCCs are similar in microstructure, they are heterogeneous in terms of location, etiology, and clinical characteristics. The prevalence of PNI in HNSCC is highly variable, with reported incidence rates ranging from 5.2% to 90%.[7] This is because PNI incidence is related to the location and staging of the primary tumour. Oral cavity cancer, especially tongue cancer, has a high prevalence of PNI.[8] The occurrence of PNI increases in a stepwise fashion according to pathological tumour status,[9] and the incidence of PNI in advanced tumours is high, seemingly unrelated to location.[10] A previous study reported that 35.6% of laryngeal and hypopharyngeal cancers were PNI-positive, including patients with stage III-IV.[11] In a study by Joo et al.,[4] 25.7% (27/105) of patients with HPSCC were PNI-positive. In the present study, we also exclusively included patients with HPSCC and the PNI rate was 37.4% (68/182), higher than that reported by Joo et al. This may have been because we included more patients with advanced disease (174/182 versus 95/105).

There is a global consensus that LNM is an important prognostic factor and has a significant impact on survival rate. Consistently, LNM significantly negatively affects the prognosis and survival of patients with HPSCC: patients with HPSCC with LNM have worse disease-specific survival than those without LNM.[12, 13] The impact of LND and pN value on prognosis and survival of HNSCC has also been investigated in some studies. An LND $\geqslant$ 0.07 was related to poorer overall survival and disease-specific survival.[14] pN value demonstrated superior prognostic value compared to LND and the American Joint Committee on Cancer node staging in surgical patients.[13] ENE is a well-known indicator of poor prognosis and a clear

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indication for the escalation of adjuvant therapy in patients with HNSCC.[15] The association between PNI and LNM in HPSCC was unclear in previous reports, and hence remains controversial. One study suggested that PNI was significantly related to node classification in HPSCC.[4] However, other studies have indicated that no association exists between PNI and LNM.[5, 6] Our results showed that the PNI was related to LNM, but had no association with node classification. We also verified the relationship between pN value, LND, ENE, and PNI. In the present study, PNI was significantly correlated with LND and pN value in HPSCC. However, in the multivariate analysis, we found that neither LND nor pN value were independent predictors of PNI in HPSCC. Consistent with the results of Joo et al.,[4] our results suggest that PNI is not related to ENE.

LVI refers to "the presence of tumour cells within definite endothelial-lined spaces, either lymphatic or blood vessels, as detected by hematoxylin and eosin staining or immunohistochemistry".[16] Similar to PNI, LVI is also an adverse pathological feature of HPSCC, associated with poor prognosis. LVI-positive patients with HPSCC experience an increased local and regional recurrence, and a decreased 5-year overall survival, disease-free survival, and disease-specific survival.[17] However, due to a lack of literature, the relationship between LVI and PNI remains unclear. Joo et al.[4] reported that PNI was not related to LVI. Our results indicate that LVI is associated with PNI, which is consistent with Samanta et al.[18] In the present study, LVI positivity in the PNI-positive group was 47.1%, higher than in the PNI-negative group (32.5%).

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Researchers have focused on the relationship between tumour size, tumour differentiation, and PNI in HNSCC. One study suggested PNI was significantly associated with tumour differentiation and invasion depth in oral and oropharyngeal carcinoma.[19] Other studies have suggested no relationship between the PNI and TD (the longest diameter in the resected specimen) or tumour differentiation in HNSCC. [4, 6] Samanta et al.[18] reported that PNI was related to maximum tumour size and depth of invasion, but was not correlated with tumour differentiation in oral cavity carcinoma. Additionally, they did not clarify the method used for measuring depth of invasion. Our results showed that PNI in HPSCC was significantly associated with TT, but not with TD or tumour differentiation. TT remained the only independent risk factor for PNI of patients with HPSCC in the multivariate analysis. To our knowledge, this is the first study to investigate the correlation between PNI status and TT in HPSCC. TT reflects the ability of tumours to infiltrate deeper structures. As TT increases, the propensity of the tumour to extend to adjacent blood vessels, lymphatic vessels, and nerves increases, and accordingly, the risk of PNI increases.

Ki-67 index also reflects the invasiveness of tumours. In the current study, the correlation between PNI and Ki-67 index was explored for the first time. We found a median Ki-67 index in the PNI-positive group of 50% (35%, 60%), which appeared to be slightly higher than that in the PNI-negative group (40% (35%, 53.75%)); however, the difference was not significant ($p = 0.278$).

The present study had certain limitations, such as its retrospective design, which was subject to selection bias, and its small sample size. Therefore, a larger,

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prospective study should be conducted in the future to verify our findings. Lastly, because our study employed only one pathologist, inter-observer variability was not taken into consideration and this could have affected the results.

Summary

- PNI is an unfavorable pathological characteristic of HPSCC associated with poor prognosis. The relationship between PNI and clinicopathological features of HPSCC remains unclear.
- We retrospectively collected the clinicopathological data of 182 patients with HPSCC and investigated the relationships between PNI and clinicopathological features.
- PNI was correlated with TT, LVI, LNM, LND, and pN value. TT emerged as the only independent risk factor. This suggested a connection between deeper tumour invasion and a heightened risk of PNI in HPSCC.

Conclusion

The main modes of tumour cell dissemination in HPSCC are lymphatic and hematogenous metastasis, and PNI can potentially represent a third pathway. In the current study, 37.4% of patients with HPSCC were PNI-positive, which was identified to be significantly associated with TT, LVI, LNM, pN value, and LND. Multivariate analysis showed that TT was an independent risk factor for PNI, with the incidence of PNI increasing with TT. Therefore, it is crucial to include PNI analysis in postoperative histopathology reports for HPSCC to guide clinicians in choosing

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adjuvant therapy. Future investigations with larger sample sizes are needed to confirm the results of this study.

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None

Conflict of interests

None declared

Founding

None declared

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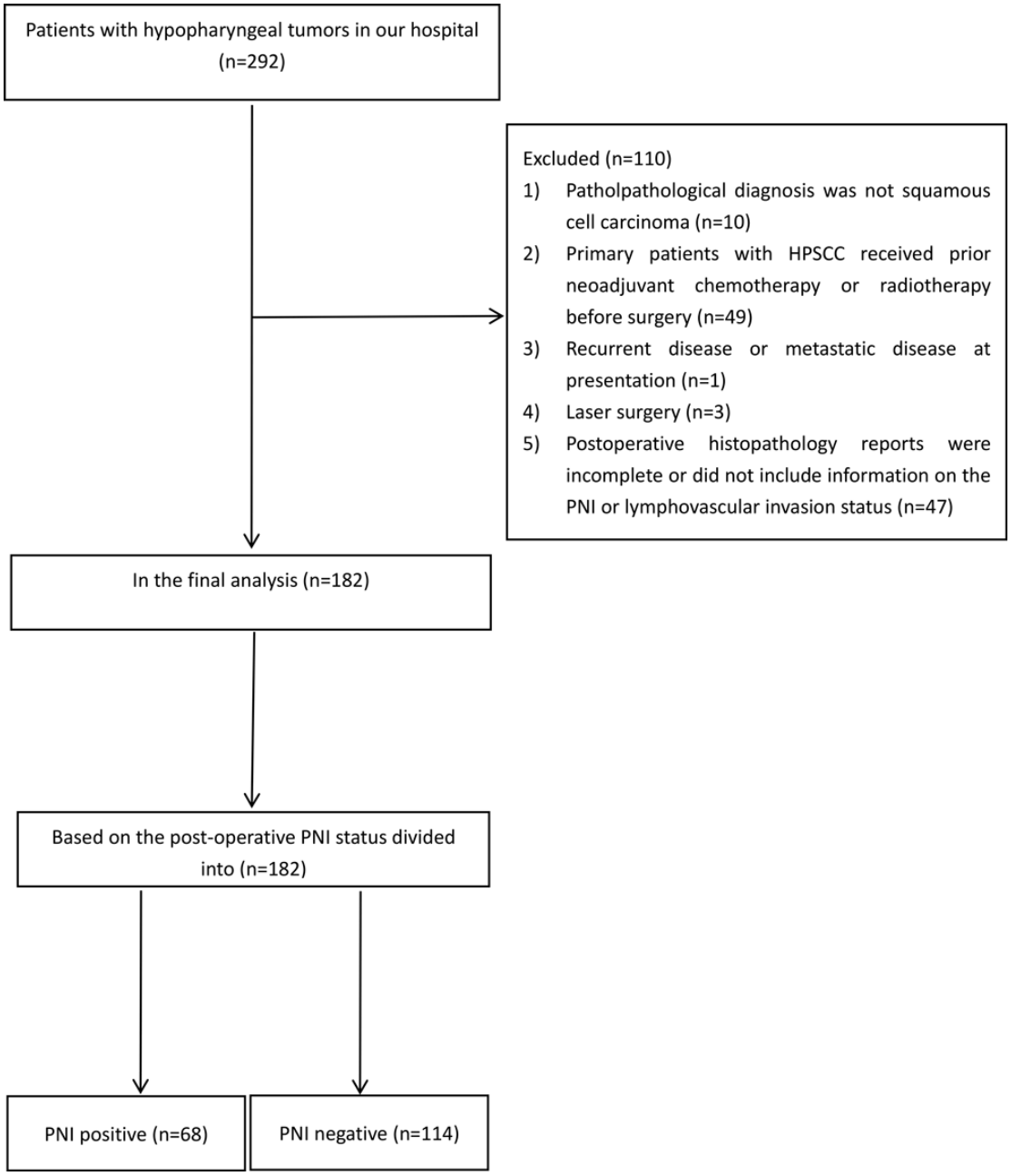

Figure 1: The flow diagram showing the selection process of study population

PNI: Perineural invasion

HPSCC: Hypopharyngeal Squamous Cell Carcinoma

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Table 1 Univariate analysis and multiple logistic regression analysis of predictive factors for PNI

Category	Univariate analysis				Multiple logistic regression analysis	
	PNI−	PNI+	*p* value	t/χ2/Z	OR (95% CI)	*p* value
Age(years)	63±8	62±9	0.591[a]	0.538		
Sex			1.000[b]	-		
Male	111(97.4%)	67(98.5%)				
Female	3(2.6%)	1 (1.5%)				
Tobacco smoking			0.580[b]	0.306		
Yes	105(92.1%)	61(89.7%)				
No	9(7.9%)	7(10.3%)				
Alcohol consumption			0.888[b]	0.020		
Yes	103(90.4%)	61(89.7%)				
No	11(9.6%)	7(10.3%)				
Primary tumor site			0.186[b]	3.362		
Pyriform	84(73.7%)	53(77.9%)				

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sinus						
Posterior pharyngeal wall	22(19.3%)	7(10.3%)				
Postcricoid	8(7.0%)	8(11.8%)				
TD (cm)	3.7(3.0,4.5)	3.8(3,4.9)	0.671[c]	−0.424		
TT (cm)	1.3(1.0,1.8)	1.7(1.3,2.1)	**0.003**[c]	−2.993	1.614(1.019–2.557)	**0.041**
T classification			0.117[b]	-		
T1	3(2.6%)	1(1.5%)				
T2	19(16.7%)	4(5.9%)				
T3	60(52.6%)	37(54.4%)				
T4	32(28.1%)	26(38.2%)				
N classification			0.199[b]	4.656		
N0	30(26.3%)	9(13.2%)				
N1	14(12.3%)	12(17.6%)				
N2	42(36.8%)	28(41.2%)				
N3	28(24.6%)	19(27.9%)				
Stage			0.050[c]	-		

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I	1(0.9%)	0		
II	7(6.1%)	0		
III	24(21.1%)	10 (14.7%)		
IV	82(71.9%)	58(85.3%)		
Tumor differentiation			0.658[b]	1.198
Well	1(0.9%)	2(2.9%)		
Moderate	81(71.1%)	46(67.6%)		
Poor	32(28.1%)	20(29.4%)		
LNM			**0.037[b]**	4.328
Yes	84(73.4%)	59(86.8%)		
No	30(26.3%)	9(13.2%)		
pN	2(0,4)	2.5(1,5)	**0.039[c]**	−2.068
LND	0.027(0.000,0.057)	0.037(0.021,0.109)	**0.011[c]**	−2.558
LVI			**0.049[b]**	3.859
Yes	37(32.5%)	32(47.1%)		
No	77(67.5%)	36(52.9%)		
ENE			0.523[b]	0.409
Yes	27(23.7%)	19(27.9%)		

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No	87(76.3%)	49(72.1%)		
Ki-67 index	40%(35%,54%)	50%(35%,60%)	0.278[c]	−1.084

Quantitative data were expressed as mean ± standard deviation or median(q1,q3), qualitative data were described by n(%)

PNI−, perineural invasion negative; PNI+, perineural invasion positive; OR, odds ratio; CI, confidence interval; TD, tumour diameter; TT, tumour thickness; LNM, lymph node metastasis; pN, number of positive lymph nodes; LND, lymph node density; LVI, lymphovascular invasion; ENE, extranodal extension

Bold values denote *p*-value < 0.05

[a] Independent sample t-test

[b] Chi-square test or Fisher's exact test

[c] Mann–Whitney U test

-No statistical value

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