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Original research

Evaluating perineural invasion severity in head and neck cutaneous squamous cell carcinomas to refine prognostic accuracy: A retrospective case-control study

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ABSTRACT

Aims: Perineural invasion (PNI) is a well-recognized risk factor for head and neck cutaneous squamous cell carcinoma (HNCSCC). This study investigates whether a detailed scoring system for PNI can improve prognostic accuracy in HNCSCC, beyond the traditional PNI binary assessment.
Methods: Among the Emilia-Romagna Cancer Registry, we analysed 61 patients with tumour progression and 61 without, using six histological PNI features: number of nerves involved, nerve diameter and depth, intra- *vs.* extratumoral location, nerve sheath involvement, and presence of mitotic figures within PNI. These parameters were compiled into a cumulative PNI score, categorizing patients as high- or low-risk based on the median score. An additional 20 patients treated with immunotherapy and 25 external PNI-positive cases were included for validation. Prognostic correlations were then analyzed by Cox proportional hazards regression model.
Results: Twenty-one patients (17.2 %) had PNI and 81 % of them were cases with recurrence. A significantly higher proportion of cases (81.0 %) exhibited perineural invasion compared to controls (19.0 %) ($p = 0.005$). High-risk PNI scores were significantly associated with worse outcomes: five-year overall survival was lower in high-risk patients (10.1 % vs. 23.3 %), and disease-specific survival was also reduced (75.0 % vs. 88.9 %). High-risk PNI scores conferred a four-fold increased risk of recurrence and nearly eight-fold higher risk of death.

Abbreviations: PNI, perineural invasion; HNCSCC, head and neck cutaneous squamous cell carcinoma.

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Among immunotherapy-treated patients, the risk of death was nineteen times higher for those in the high-risk PNI group.
Conclusions: Our findings suggest that a multi-parameter PNI scoring system can refine prognostic stratification, potentially guiding more personalized treatment decisions and informing updates to the current staging guidelines.

1. Introduction

Head and neck cutaneous squamous cell carcinoma (HNCSCC) represents the second most common histotype belonging to non-melanoma skin cancers (NMSC) [1,2]. Despite its generally favorable prognosis, a recurrence rate of 3.0–6.7 % [3,4] and a metastatic rate of 3.7–5.8 % [5] have been reported, with 1.5–2.1 % of cases resulting in disease-specific death [6,7].

Among the currently recognized high-risk histological prognostic features [3,6,8–14], perineural invasion (PNI) with nerves $\geq$ 0.1 mm in diameter lying deeper than the dermis is included [6,8,15,16]. In fact, the subset of HNCSCC patients exhibiting significant neurotropism, resulting in PNI and neural spread, can be divided into two categories: incidental PNI (iPNI) and clinical PNI (cPNI) [17]. iPNI has an incidence ranging from 2.5 % to 14 % in HNCSCC [18]. It is more frequently associated with male patients, recurrent tumours, tumours located on the face, poorly differentiated histology, deep extension and desmoplasia [19], and it is strongly correlated with recurrence and with higher disease-specific death rates [20].

According to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 8th Edition [21] and the Brigham and Women's Hospital (BWH) [22] staging systems, iPNI is assessed as a binary variable (presence *vs.* absence). In this context, growing interest has risen around the histological evaluation of PNI, in particular whether the deepening of additional features could lead to a better prognostic stratification of patients [23,24] and potentially enhance selection for personalized treatments.

The primary aim of our study was to apply a scoring system of PNI in HNCSCC patients, based on additional histological measures, in a population-based case-control study nested in a cancer registry cohort to verify whether it may better stratify patients with disease progression and help to identify those deserving more aggressive treatments. Furthermore, we attempted to consolidate the assessment method by testing its reproducibility with an external cohort of PNI-positive cases. The secondary objectives were: (a) to evaluate whether there was a difference in disease-free and disease-specific survival between HNCSCC with a PNI score below or above the median value; (b) to identify histopathological features that predict the likelihood of a PNI score below or above the median value and (c) to verify the prognostic significance of the PNI score among immunotherapy-based treated HNCSCC patients.

2. Materials and methods

2.1. Ethics approval

The study was approved by the Local Ethics Committee of Emilia - Romagna (C.E. ROM.: ID L3P2552; protocol number: 0007570/2021), Local Ethics Committee of Tuscany (C.E. AVC.: ID PNI; protocol number: 22156_bio) and was performed in accordance with the principles of the Declaration of Helsinki.

2.2. Case selection and tumour specimen collection

The Emilia-Romagna Cancer Registry (ERCR) is located in northern Italy, and it covers a population of nearly four and half million. A matched case-control study, nested within this cancer registry cohort, was designed. Cases were identified as those with disease progression. Specifically, progression was considered as the first site of relapse exemplified into local, nodal, systemic recurrence or both, according to the sources of information available at the ERCR (hospital discharge notes, death certificates, pathology reports).

We identified n = 115 cases (patients with recurrence), and we selected the same number of controls from non-disseminated HNCSCC in the same population of the cases. Cases and controls were matched 1:1 with respect to age (5-year age groups), sex, and period of diagnosis (5-year time intervals: 2006–2010, 2011–2015). The hematoxylin and eosin (H&E) stained slides and formalin-fixed paraffin-embedded (FFPE) tissue specimens, obtained by excisional biopsy, were retrieved from the archives of the Pathology Units parts of the Local Health Authority of Romagna (Forlì, Cesena, Ravenna, Rimini). Clinical data were obtained by chart review.

All samples were collected, registered, and analyzed at Section of Anatomic Pathology, Department of Health Sciences, University of Florence (Florence, Italy). Patients were followed until 31 December 2022. Overall, 122 patients were available for the analysis: n = 61 cases (patients with tumour progression) and n = 61 controls (patients without tumour progression). Case selection procedure is illustrated in Fig. 1.

The study was also enriched by an external cohort of n = 20 immunotherapy-based treated HNCSCC patients with histologically proven diagnosis of PNI during the period 2018–2023 and followed-up until December 2024 at Careggi University Hospital (Florence, Italy) and at Santa Maria Annunziata Hospital (Florence, Italy). All of the immunotherapy-based treated patients received cemiplimab, an anti-programmed cell-death protein 1 inhibitor, as adjuvant therapy.

2.3. Histopathologic review and PNI scoring system

H&E stained tissue sections were digitally scanned at × 400

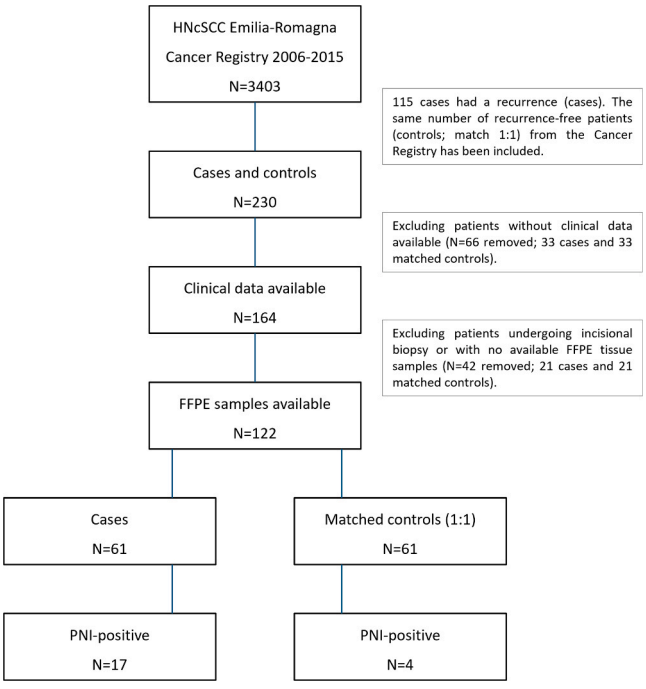


Fig. 1. Flowchart of the analysed group populations and selection criteria.

magnification with Aperio AT2 platform (Leica Biosystems, Wetzlar, Germany) into whole slide digital images (WSI). Each SVS format file was imported into the HALO Link® (Indica Labs, Albuquerque, NM) image management system. A systematic histopathologic review of all available H&E slides for each case, blinded to patient outcome was performed to re-examine diagnosis and staging, according to the 5th edition of the World Health Organization (WHO) Classification of Skin Tumours and the AJCC Cancer Staging Manual, 8th Edition, respectively. Perineural invasion (PNI) was first classified as negative (PNI−) or positive (PNI+), then further characterized using a modified scoring system [25,26], based on six histopathological features: (a) number of nerves involved, (b) nerve diameter (transverse section), (c) nerve depth, (d) location (intratumoral vs. extratumoral), (e) extent of nerve sheath involvement, and (f) presence of mitotic figures in the PNI area. Features (a), (b), and (e) were scored from 0 to 2; (c), (d), and (f) were binary (0 or 1). When multiple characteristics were present for a parameter, the most severe was scored. These scores were summed into a cumulative PNI score (0–9), with patients stratified into low or high PNI groups based on the median value. PNI was evaluated on all tumour slides, with the most severe findings used when multiple slides showed differing PNI features. The histological scoring method is illustrated in Fig. 2.

2.4. Reproducibility assessment

Reproducibility was at first assessed using an inter-observer variability method. Specifically, H&E stained sections were reviewed for diagnosis and PNI score assessment by four independent board-certified pathologists (FN, DM, CA, AC), and cases of difficult interpretation were then discussed under a multi-head microscope, until full consensus was achieved.

In order to analyze methodological pitfalls and to additionally evaluate the reproducibility of the score assessment, an external validation sample case cohort of PNI-positive patients with proven histological diagnosis of HNCSCC (n = 25) was selected from the archive of the Anatomic Pathology Unit of Florence (Azienda USL Toscana Centro, Florence, Italy) and analyzed. The complete set of slides of each surgical specimen was independently reviewed by four board-certified pathologists (FN, DM, CA, AC) blinded to all clinical, treatment-related and outcome data. Cases of discordant interpretation were further discussed together at a multi-head microscope, and a final agreement was reached in each case. The concordance percentage for each parameter and the total score were recorded.

2.5. Statistical analysis

A descriptive analysis was performed to compare cases and controls using McNemar's and Stuart-Maxwell tests for categorical variables and the Wilcoxon signed-rank test for continuous variables Conditional logistic regression estimated odds ratios (ORs) and 95 % confidence intervals (CIs) for recurrence likelihood, accounting for perineural invasion (PNI) and confounders. Kaplan-Meier curves assessed disease-free and disease-specific survival, with censoring for unrelated deaths or loss to follow-up. Cox proportional hazards models determined the prognostic impact of PNI, adjusting for demographic and histopathological factors, providing univariable and multivariable hazard ratios (HRs). Disease-free survival was also evaluated in an external cohort of 20 HNCSCC patients treated with immunotherapy, but only overall survival was reported due to unknown cause of death. Among patients with PNI, demographic and histopathological characteristics were compared across PNI score groups using Pearson's chi-squared and median tests. An unconditional logistic regression predicted likelihood of high vs. low PNI score. In the multivariable models, variables were selected using a backward stepwise selection method. The statistical significance was set at the 90 % confidence level (p-value<0.10). For each covariate, we reported the p-value for the Wald test. Analysis using Stata statistical package, version 15 (StataCorp, TX, USA).

3. Results

3.1. Patients' baseline and histopathological characteristics

Table 1 shows the distribution of the 122 eligible patients (n = 61 cases and n = 61 controls) according to the main clinical characteristics of the primary HNCSCC. The median age was 81 and 82 years for cases and controls, respectively. The most common tumour localization among the HN anatomic subsites was the auricular (n = 27, 22.1 %), followed by cheek (n = 23, 18.8 %), temple (n = 19, 15.6 %), forehead (n = 15, 12.3 %), scalp (n = 14, 11.4 %), perilabial (n = 13, 10.6 %), nose (n = 7, 5.8 %) and eyelid (n = 4, 3.4 %). A greater proportion of cases had positive surgical margins (81.5 %) compared to controls (18.5 %) ($p < 0.001$). Regarding the grade of differentiation, a significantly higher percentage of cases had poorly differentiated tumours (G3: 77.3 %) compared to controls (G3: 22.7 %) ($p < 0.001$), while a greater proportion of controls had well-differentiated tumours (G1: 76.2 %). In terms of DoI, the cases showed a significantly greater median depth of invasion (4.2 mm) compared to the controls (1.8 mm) ($p < 0.001$). Similarly, tumour size was larger in cases (median 1.2 cm) than in controls (median 0.8 cm) ($p < 0.001$). The LoI also differed significantly between groups. A higher proportion of cases had a higher level of invasion (IV-V: 65.2 %) compared to controls (IV-V: 34.8 %) ($p < 0.001$). Regarding desmoplasia, a significantly higher percentage of cases exhibited desmoplasia (79.2 %) compared to controls (20.8 %) ($p = 0.002$). A notable finding was the lymphovascular invasion, which was observed in 100 % of cases but in none of the controls ($p = 0.001$). Finally, for pathologic T stage, a higher proportion of cases had advanced T stage (T3), with 77.8 % of cases classified as T3 compared to 22.2 % of controls ($p < 0.001$).

Twenty-one patients (17.2 %) had PNI and 81 % of them were cases with recurrent disease. The median value of the PNI score was 4 and according to the median value, the patients were stratified in two classes: (a) low-risk, with a score < 4 and (b) high-risk, with a score ≥ 4. In the high-risk class, ten out of eleven patients were cases. A significantly higher proportion of cases (81.0 %) exhibited perineural invasion compared to controls (19.0 %) ($p = 0.005$). When stratified by perineural invasion score, a similar trend was observed, with a higher prevalence of high-risk perineural invasion in the cases (90.9 %) versus the controls (9.1 %) ($p = 0.011$). Supplementary Table 1 shows the frequency distribution of the histopathological features used to define the PNI scoring system, by disease recurrence (cases versus controls). None of the variables were distributed differently between cases and controls.

3.2. Prognostic factors for disease recurrence, disease-free and disease-specific survival

The median follow-up time was 1.5 years for the 61 patients with recurrence (mean: 2.0 years; range: 0.08–7.7 years); 5.5 years for the 61 patients without recurrence (mean: 6.2 years; range: 0.03–16.8 years) and 1.9 years for the 20 patients undergoing immunotherapy (mean: 2.0 years; range: 0.27–4.5 years). Table 2 shows the prognostic factors for disease recurrence based on the conditional logistic regression model. Multivariable analysis confirmed the univariable significance of three characteristics of cancer, namely: status of surgical margins ($p = 0.005$), grade of differentiation ($p = 0.008$) and presence of desmoplasia ($p = 0.021$).

Fig. 3A shows the disease-free survival curve by PNI score classes for the PNI-positive patients (n = 21). Patients with a high-risk PNI score had lower survival rates compared to the patients with a low-risk PNI score, with a 5-year survival of 10.1 % (95 % CI, 0.6–36.1 %) versus 23.3 % (95 % CI, 3.6–52.9 %). Also, for disease-specific survival (Fig. 3B), the higher the PNI score the lower the survival: 5-year survival

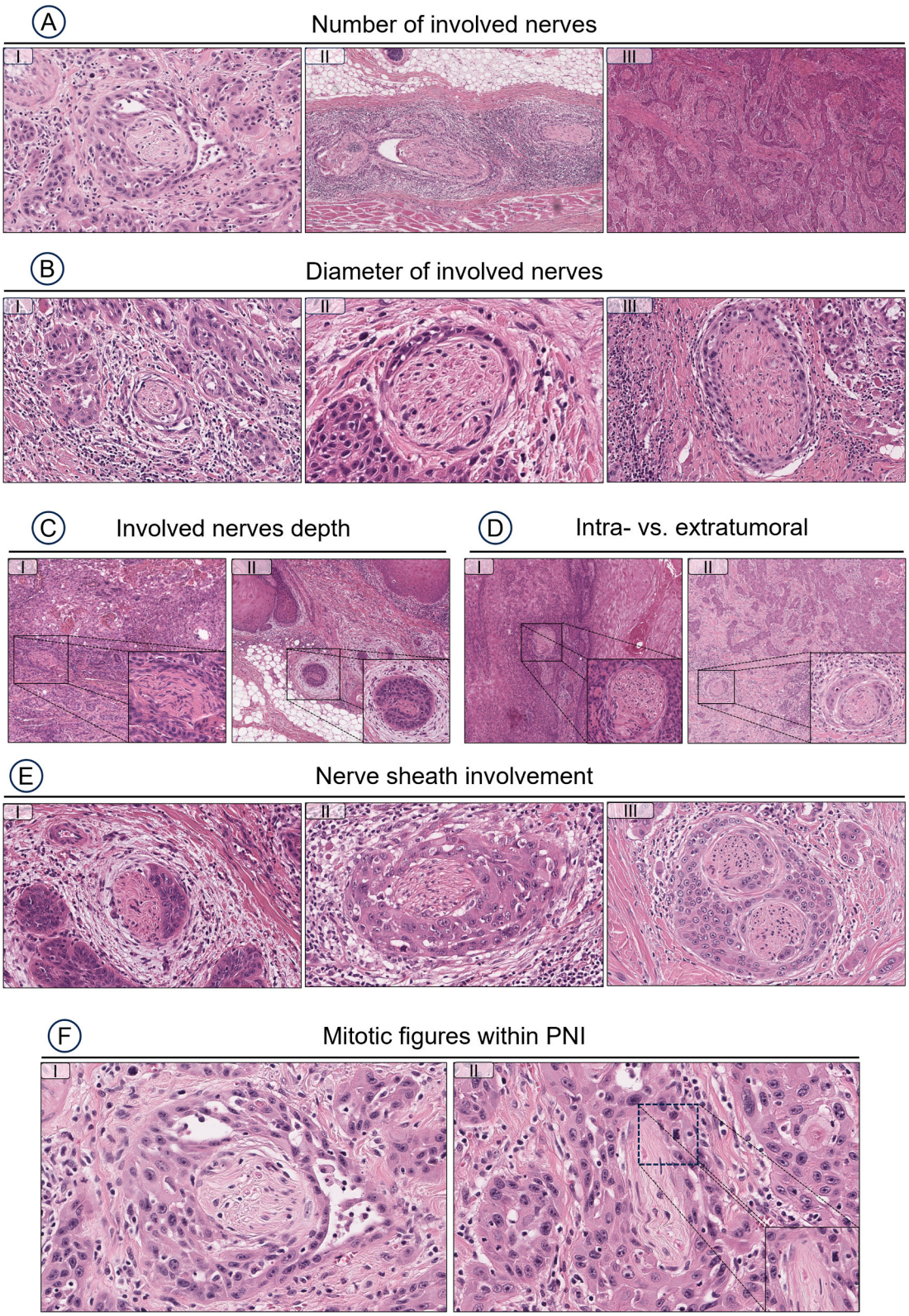


Fig. 2. Representative histological images of each additional measure of PNI included in the multiparametric scoring system applied in the cohort. A) Number of distinct nerve structures involved per tumour: 1, score 0 (AI, magnification 200x), 3, score 1 (AII, magnification 100x), 5, score 2 (AIII, magnification 100x); B) Nerve diameter: 0.06 mm, score 0 (BI, magnification 100x), 1.22 mm, score 1 (BII, magnification 200x), 2.31, score 2 (BIII, magnification 200x). C) Nerve depth: dermis, score 0 (CI, magnification 100x, inset 200x) vs. hypodermis, score 1 (CII, magnification 100x, inset 200x); D) Location of nerve involved with regard to tumour mass: intratumoral, score 0 (DI, magnification 50x, inset 200x) vs. extratumoral, score 1 (DII, magnification 50x, inset 200x); E) Severity of nerve sheath involvement: $\leq$ 50 % of the nerve sheath, score 0 (EI, magnification 400x), nearby total encirclement, score 1 (EII, magnification 400x), intraneural, score 2 (EIII, magnification 400x); F) Absence of mitotic figures, score 0 (FI, magnification 400x) vs. presence of mitotic figures within the perineural invasion area (FII, magnification 400x, inset 400x). A three-level score (0, 1, or 2) was assigned to three of five features (A, B, E), and a binary score of 0 or 1 was assigned to the remaining three (C, D, F).

Table 1
Patient and tumour characteristics by disease recurrence (N = 122 patients, 61 cases and 61 controls). Romagna cohort, 2006–2015.

Characteristics	Cases n (%)	Controls n (%)	*p*-value
Age (years)			matched
Median (SD)	81 (7.2)	82 (6.9)	
Sex			matched
Male	49 (50.0)	49 (50.0)	
Female	12 (50.0)	12 (50.0)	
Years of diagnosis			matched
2006–2010	24 (50.0)	24 (50.0)	
2011–2015	37 (50.0)	37 (50.0)	
Perineural Invasion			**0.005***
No	44 (43.6)	57 (56.4)	
Yes	17 (81.0)	4 (19.0)	
Perineural Invasion (score)			**0.011****
No	44 (43.6)	57 (56.4)	
Low-risk	7 (70.0)	3 (30.0)	
High-risk	10 (90.9)	1 (9.1)	
Status of surgical margins			**0.000***
Negative	39 (41.1)	56 (58.9)	
Positive	22 (81.5)	5 (18.5)	
Grade of differentiation			**0.000****
G1	10 (23.8)	32 (76.2)	
G2	34 (58.6)	24 (41.4)	
G3	17 (77.3)	5 (22.7)	
Depth of invasion (mm)			**0.000******
Median (SD)	4.2 (3.6)	1.8 (2.2)	
Tumour size (cm)			**0.000******
Median (SD)	1.2 (3.6)	0.8 (2.2)	
Level of invasion			**0.000***
I-II-III	18 (32.1)	38 (67.9)	
IV-V	43 (65.2)	23 (34.8)	
Desmoplasia			**0.002***
No	42 (42.9)	56 (57.1)	
Yes	19 (79.2)	5 (20.8)	
Lymphovascular invasion			**0.001***
No	49 (44.5)	61 (55.5)	
Yes	12 (100.0)	0 (0.0)	
Pathologic T Stage			**0.000***
T1-T2	33 (38.4)	53 (61.6)	
T3+	28 (77.8)	8 (22.2)	

* p-value for McNemar's chi-squared test; ** p-value for Stuart-Maxwell test; *** p-value for Wilcoxon signed-rank test.

Table 2
Conditional logistic regression analysis (matching 1:1) to estimate the likelihood of recurrence by PNI and histopathological features (N = 122 patients, 61 cases and 61 controls). Romagna cohort, 2006–2015.

Characteristics	Univariable model		Multivariable model	
	OR (95 % CI)	*p*-value*	OR (95 % CI)	*p*-value*
Perineural Invasion (score)		**0.042**		0.800
No	1.00 (Ref.)		1.00 (Ref.)	
Low-risk	2.33 (0.60; 9.02)		0.57 (0.10; 3.11)	
High -risk	10.00 (1.28; 78.12)		1.12 (0.09; 13.86)	
Status of surgical margins		**0.003**		**0.005**
Negative	1.00 (Ref.)		1.00 (Ref.)	
Positive	9.50 (2.21; 40.78)		24.02 (3.20; 180.28)	
Grade of differentiation		**0.001**		**0.008**
G1	1.00 (Ref.)		1.00 (Ref.)	
G2	5.39 (1.84; 15.77)		12.78 (2.19; 74.38)	
G3	13.39 (3.17; 56.60)		47.49 (4.25; 530.20)	
Depth of invasion (mm)	1.38 (1.14; 1.67)	**0.001**		
Tumour size (cm)	2.73 (1.42; 5.26)	**0.003**		
Level of invasion		**0.001**		
I-II-III	1.00 (Ref.)			
IV-V	4.33 (1.78; 10.53)			
Desmoplasia		**0.006**		**0.021**
No	1.00 (Ref.)		1.00 (Ref.)	
Yes	5.67 (1.66; 19.34)		5.92 (1.30; 26.87)	
Pathologic T Stage		**0.001**		
T1-T2	1.00 (Ref.)			
T3+	4.33 (1.78; 10.53)			

* p-value for the Wald test. Lymphovascular invasion not assessable. In the multivariable model, not all variables will be presented, but only the significant ones (except for the variable "Perineural Invasion"). Abbreviations: Ref., reference category.

of 75.0 % (95 % CI, 29.8–93.4 %) versus 88.9 % (95 % CI, 43.3–98.4 %).

Significant prognostic factors for disease-free and disease-specific survival were identified with a Cox regression analysis. Table 3 shows univariable and multivariable hazard-ratios of disease-free survival and disease-specific survival. In the multivariable model, with respect to disease-free survival, the risk of recurrence was approximately four-fold higher for the lesions with high-risk PNI score compared to low-risk PNI score class with HR: 3.86 (95 % CI, 1.03; 14.47) and for lesions with desmoplasia, HR: 3.99 (95 % CI, 0.95; 16.77). Disease-free survival diminished with increased age and decreased tumour size. With respect to the disease-specific survival, the risk of death for HNCSCC was almost eight-fold higher for high-risk PNI score, HR: 7.86 (95 % CI, 0.53; 116.54). The disease-specific survival decreased also with increased age, HR: 1.21 (95 %, CI 0.97; 1.51).

3.3. Prognostic factors for a high-risk PNI score

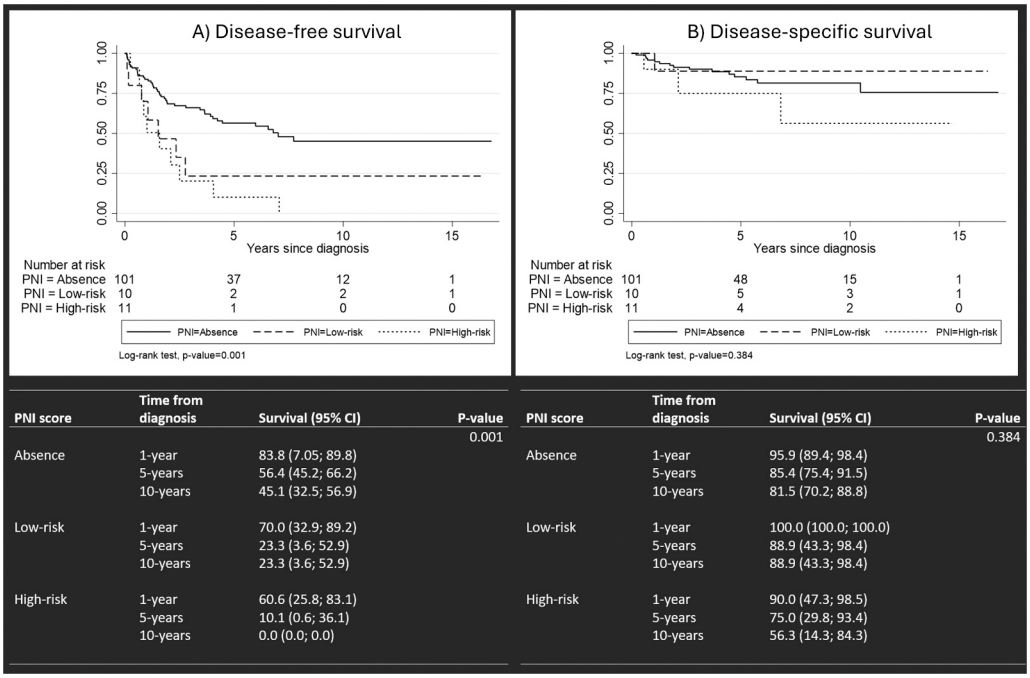
Supplementary Table S2 shows the frequency distribution of demographic and histopathological factors by PNI score class. All the variables were distributed in the two classes in an analogous way as confirmed by the univariable models (Supplementary Table S3). Supplementary Table S4 shows the frequency distribution of each histological variable used to define the PNI score by low-risk versus high-risk score (according to the median value). The following factors were distributed differently across the PNI score classes: number of involved nerves ($p = 0.035$), diameter of the affected nerves ($p = 0.007$), nerve sheath involvement ($p = 0.017$) and mitotic figures within the PNI area ($p = 0.011$).

3.4. Score assessment reproducibility

In the external sample case cohort of 25 PNI-positive patients with proven histological diagnosis of HNCSCC, the assessment of the number of distinct nerve structures involved was concordant in 24/25 cases (96 %), nerve diameter in 23/25 cases (92 %), nerve depth (dermis *vs.* hypodermis or deeper) in 23/25 cases (92 %), intratumoral *vs.* extratumoral nerve involvement in 24/25 cases (96 %), location of the tumour in relation to the nerve as focal, circumferential or intraneural in 21/25 cases (84 %), and presence or absence of mitotic figures within the PNI in 22/25 cases (88 %). The total score was concordant in 22/25 cases (88 %).

3.5. Immunotherapy-based treated HNCSCC patients with histologically proven diagnosis of PNI

Clinical and pathological features of immunotherapy-based treated patients are summarised in Supplementary Table S5. Table 4 shows univariable HRs of disease-free survival and univariable and multivariable overall survival. For disease-free survival, multivariable models



PNI score	Time from diagnosis	Survival (95% CI)	P-value
			0.001
Absence	1-year	83.8 (7.05; 89.8)	
	5-years	56.4 (45.2; 66.2)	
	10-years	45.1 (32.5; 56.9)	
Low-risk	1-year	70.0 (32.9; 89.2)	
	5-years	23.3 (3.6; 52.9)	
	10-years	23.3 (3.6; 52.9)	
High-risk	1-year	60.6 (25.8; 83.1)	
	5-years	10.1 (0.6; 36.1)	
	10-years	0.0 (0.0; 0.0)	

PNI score	Time from diagnosis	Survival (95% CI)	P-value
			0.384
Absence	1-year	95.9 (89.4; 98.4)	
	5-years	85.4 (75.4; 91.5)	
	10-years	81.5 (70.2; 88.8)	
Low-risk	1-year	100.0 (100.0; 100.0)	
	5-years	88.9 (43.3; 98.4)	
	10-years	88.9 (43.3; 98.4)	
High-risk	1-year	90.0 (47.3; 98.5)	
	5-years	75.0 (29.8; 93.4)	
	10-years	56.3 (14.3; 84.3)	

Fig. 3. Disease-free (A) and disease-specific (B) survival curve by perineural invasion status and score (n = 21 patients with the presence of perineural invasion; *p*-value for the log-rank test) confirm a lower survival in the high-risk PNI score class. Romagna, 2006–2015.

Table 3
Cox regression analysis of prognostic factors for disease-free and disease-specific survival. Patients with perineural invasion (N = 21). Romagna cohort, 2006–2015.

Characteristics	Disease-free survival				Disease-specific survival			
	Univariable model		Multivariable model		Univariable model		Multivariable model	
	HR (95 % CI)	*p*-value*	HR (95 % CI)	*p*-value*	HR (95 % CI)	*p*-value*	HR (95 % CI)	*p*-value*
Perineural Invasion (score)		0.405		**0.045**		0.289		0.134
Low-risk	1.00 (Ref.)		1.00 (Ref.)		1.00 (Ref.)		1.00 (Ref.)	
High-risk	1.51 (0.57; 4.00)		3.86 (1.03; 14.47)		3.4 (0.35; 32.74)		7.86 (0.53; 116.54)	
Age (years)	1.06 (1.00; 1.12)	0.066	1.16 (1.04; 1.29)	**0.009**	1.06 (0.93; 1.22)	0.365	1.21 (0.97; 1.51)	0.097
Sex		0.496				na		na
Male	1.00 (Ref.)							
Female	1.69 (0.37; 7.69)							
Years of diagnosis		0.455				0.643		
2006–2010	1.00 (Ref.)				1.00 (Ref.)			
2011–2015	1.47 (0.54; 4.01)				1.71 (0.18; 16.74)			
Status of surgical margins		0.859				0.628		
Negative	1.00 (Ref.)				1.00 (Ref.)			
Positive	1.11 (0.35; 3.48)				1.75 (0.18; 17.09)			
Grade of differentiation		0.473				na		na
G1	1.00 (Ref.)							
G2	1.15 (0.14; 9.47)							
G3	2.06 (0.25; 16.92)							
Depth of invasion (mm)	1.05 (0.95; 1.16)	0.313			0.97 (0.75; 1.25)	0.796	0.84 (0.60; 1.17)	0.296
Tumour size (cm)	1.30 (0.66; 2.54)	0.446	0.37 (0.12; 1.11)	0.076	2.35 (0.59; 9.35)	0.226		
Level of invasion		na		na		na		na
I-II-III								
IV-V								
Desmoplasia		0.577		0.059		0.950		
No	1.00 (Ref.)		1.00 (Ref.)		1.00 (Ref.)			
Yes	1.32 (0.50; 3.46)		3.99 (0.95; 16.77)		1.06 (0.15; 7.61)			
Pathologic T Stage		0.571				na		na
T1-T2	1.00 (Ref.)							
T3+	0.55 (0.07; 4.40)							

* p-value for the Wald test; na: not assessable. Abbreviations: Ref., reference category.

were not presented because there were no covariates with a *p*-value < 0.10 in the univariable analysis. In the univariable analysis, the risk of recurrence was almost eight-fold higher for the lesions with desmoplasia, HR: 7.83 (95 % CI, 0.71; 86.38). With respect to the disease-specific survival, the multivariable model shows that the risk of death was approximately nineteen-fold higher for high-risk PNI score, HR: 19.42 (95 % CI, 2.16; 174.66) and seven-fold higher for cases diagnosed in the last calendar period, 2021–2023, HR: 7.12 (95 % CI, 1.05; 48.37).

Supplementary Table S6 shows the frequency distribution of demographic and histopathologic factors by PNI score class. The status of

Table 4
Cox regression analysis of prognostic factors for disease-free and overall survival in immunotherapy-based treated HNCSCC patients (n = 20), 2018–2023.

Characteristics	Disease-free survival		Overall survival			
	Univariable model		Univariable model		Multivariable model	
	HR (95 % CI)	*p*-value*	HR (95 % CI)	*p*-value*	HR (95 % CI)	*p*-value*
Perineural Invasion (score)		0.452		**0.039**		**0.008**
Low-risk	1.00		1.00		1.00	
High-risk	2.40 (0.24; 23.56)		8.94 (1.12; 71.29)		19.42 (2.16; 174.66)	
Age (years)	0.92 (0.84; 1.02)	0.128	0.98 (0.93; 1.03)	0.332		
Sex		na		0.971		
Men			1.00			
Women			0.97 (0.25; 3.78)			
Years of diagnosis		0.590		0.441		**0.045**
2018–2020	1.00		1.00		1.00	
2021–2023	2.12 (0.14; 32.75)		1.9 (0.37; 9.78)		7.12 (1.05; 48.37)	
Status of surgical margins		na		0.401		
Negative			1.00			
Positive			1.71 (0.49; 5.98)			
Grade of differentiation		na		0.619		
G1			1.00			
G2			0.38 (0.06; 2.63)			
G3			0.54 (0.10; 3.01)			
Depth of invasion (mm)	1.03 (0.80; 1.33)	0.811	1.12 (0.94; 1.33)	0.219		
Tumour size (cm)	0.96 (0.55; 1.68)	0.898	1.12 (0.91; 1.37)	0.280		
Level of invasion		0.338		0.773		
II-III	1.00		1.00			
IV-V	0.30 (0.03; 3.48)		1.36 (0.17; 11.03)			
Desmoplasia		0.093		0.741		
No	1.00		1.00			
Yes	7.83 (0.71; 86.38)		1.31 (0.27; 6.36)			

ˆ Multivariable models were not presented because there were no covariates with a p-value < 0.10; *P-values for the Wald test; na: not assessable

surgical margins was distributed differently across the PNI score classes and the statistical significance of this variable was confirmed in the univariable model's analysis of the likelihood of a high-risk PNI score, based on the calculation of the odds-ratios, as shown in Supplementary Table S7.

Supplementary Table S8 shows the frequency distribution of each histological variable used to define the PNI score by low-risk versus high-risk score (according to the median value). Number, diameter and depth of involved nerves were differently distributed across the PNI score classes.

4. Discussion

In this retrospective population-based case-control study, an objective histopathologic scoring system for PNI in HNCSCC was applied and it showed significant correlations with adverse outcomes such as recurrence, metastases, and disease-specific death. Patients with a PNI score $\geq$ 4 (median value) showed lower survival rates compared to the patients with PNI score $<$ 4. Also, for disease-specific survival, the higher the PNI score the lower the survival. With respect to disease-free survival, the risk of recurrence was approximately four-fold higher in cases with a PNI score $\geq$ 4 compared to the lower score class. As regards disease-specific survival, the risk of HNCSCC-specific death was almost eight-fold higher for the PNI score $\geq$ 4, and it was found similarly higher for high-risk PNI score among immunotherapy-treated patients. Since we aimed at estimating the likelihood of overall recurrence (loco-regional or distant metastasis) in HNCSCC patients by PNI scoring classes, multivariable analysis showed the presence of a PNI score $\geq$ 4 (median) to indicate an OR approximately 12 % greater than the absence of PNI, all other factors being equal. These results are consistent with prior literature [25,27–31] demonstrating poorer outcomes with PNI-related adverse histological additional features, beyond the ordinary assessment as a dichotomous variable (presence *vs.* absence).

The overall incidence rate of PNI found in our cohort is 17.2 %, in accordance with the current literature [18] and not surprisingly, PNI displayed a significantly higher distribution in the case (27.9 %) compared to the control group (6.5 %), confirming its high-risk significance. Furthermore, 4 out of 21 cases with PNI showed extensive nerve involvement, defined as the involvement of more than 5 distinct nerve structures, and all of these cases belonged to the case group. This evidence supports previous findings [25,31] demonstrating poorer patient outcomes with extensive PNI (ePNI).

In our matched case-control cohort, clinicopathological features such as age at diagnosis, positive margins, poor differentiation, lesion diameter, and lymphovascular invasion were distributed differently among the score classes in a statistically significant way, emerging as risk factors for a PNI score $\geq$ 4. This evidence supports the association of multiple high-risk factors in determining the unfavourable prognostic course of these neoplasms, and it fits in the context of previous results [17,32] revealing characteristics such as male sex, recurrent tumours, midface location, poorly differentiated histology, deep subclinical extension, large tumour size, lymphovascular invasion and chronic immunosuppression as the most frequently PNI-associated variables. Furthermore, PNI specific features such as large-calibre nerve invasion emerged as associated with an elevated risk of nodal metastasis and death, but this could be partly attributed to multiple other risk factors associated with large-calibre nerve invasion [33]. Both poorly differentiated histology and desmoplasia showed a significant recurrence risk value in our cohort, contrary to the reported results in a recent HNCSCC and PNI study [25].

Recent retrospective studies [25,27] identified nerve diameter as significantly associated with recurrence and survival. The association between more than 2 nerves involved and clinical outcome in HNCSCC was also reported [27–30]. More recently, Massey et al. [31] explored PNI measurement comparing 4 features of PNI in HNCSCC, such as nerve caliber, number of involved nerves per section, PNI maximal depth, and PNI location with poor outcomes: of these features, only involvement of 5 or more distinct nerves, called ePNI, was found to be independently associated with local recurrence, disease-specific death, and any poor outcome.

Among the six parameters used to define the score, number of involved nerves, nerve diameter, severity of sheath involvement, and

mitotic figures showed the most significant discriminatory value. No extensive perineural invasion (PNI) or large nerve involvement appeared in controls, according to previous findings [25]. Distinguishing intratumoral from extratumoral PNI was based on evidence linking extratumoral PNI to poorer disease-free survival [34,35]. Deep nerve involvement was also associated with lymphatic spread and disease-specific mortality [28].

PNI is a well-established adverse prognostic marker which confers a higher risk of local recurrence and regional and distant metastasis [36]. However, the implementation of additional measures of PNI in a combined score system would be most effective to determine high-risk HNCSCC with PNI of stage ≥T2 of the AJCC8 and stage ≥T2b of the BWH staging systems [23,24].

To the best of our knowledge, this is the first matched case-control study in which PNI additional measures were explored. Strengths of our study also include the population-based setting, an extended follow-up period, strict definitions of PNI characteristics, the presence of an external validation case cohort to test the assessment reproducibility of each parameter considered in the score, and the participation of multiple reviewers, including four board-certified dermatopathologists. This study has also some limitations, including the relatively low event-rate, limiting the statistical power. Furthermore, despite the assessment of multiple PNI-related features could add accuracy in PNI evaluation, it is a time-consuming practice which may limit its adoption in routine practice.

In conclusion, our study supports the evaluation of additional morphological, dimensional, and spatial features related to the interaction between tumour cells and neural microenvironment in HNCSCC to enhance the prognostic stratification of patients, as it may optimize the current staging guidelines in terms of recurrence risk assessment and potentially improve patient selection for personalised adjuvant treatments.

Contributors

D.M, I.S. and E.C. performed study concept and design; F.N., S.M., R. N., and P.G. performed development of methodology and writing, review and revision of the paper; A.A.S, E.C., L.R., G.D.L., A.L., F.F., R.V., F.Z., L.B., S.S., V.D.G., L.D., I.V., A.C. and C.A. provided acquisition, analysis and interpretation of data, and statistical analysis; M.D. provided technical and material support. All authors read and approved the final paper.

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Declaration of Competing Interest

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejcskn.2025.100743.

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