Pancreatology 24 (2024) 553–561

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

Perineural invasion score system and clinical outcomes in resected pancreatic cancer patients

Filippo Nozzoli [a, *, 1], Martina Catalano [b, 1], Luca Messerini [a], Fabio Cianchi [c], Romina Nassini [b], Francesco De Logu [b], Luigi Francesco Iannone [b], Filippo Ugolini [a], Sara Simi [a], Daniela Massi [a], Pierangelo Geppetti [b], Giandomenico Roviello [b]

[a] Section of Anatomic Pathology, Department of Health Sciences, University of Florence, Florence, Italy
[b] Section of Clinical Pharmacology & Oncology, Department of Health Sciences, University of Florence, Florence, Italy
[c] Section of Surgery, Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

ARTICLE INFO

Article history:
Received 10 January 2024
Received in revised form
21 February 2024
Accepted 6 March 2024
Available online 15 March 2024

Keywords:
Pancreatic adenocarcinoma
Perineural invasion
PNI histopathological score system
Disease free survival
Overall survival

ABSTRACT

Background/objectives: Perineural invasion (PNI), classified according to its presence or absence in tumor specimens, is recognized as a poor prognostic factor in pancreatic ductal adenocarcinoma (PDAC) patients. Herein, we identified five histological features of PNI and investigated their impact on survival outcomes of PDAC resected patients.

Methods: Five histopathological features of PNI (diameter, number, site, sheath involvement, and mitotic figures within perineural invasion) were combined in an additional final score (ranging from 0 to 8), and clinical data of PDAC patients were retrospectively analyzed. PNI + patients were stratified in two categories according to the median score value (<6 and ≥ 6, respectively). Impact of PNI on disease-free survival (DFS) and overall survival (OS) were analyzed.

Results: Forty-five patients were enrolled, of whom 34 with PNI (PNI+) and 11 without PNI (PNI-). The DFS was 11 months *vs.* not reached (NR) ($p = 0.258$), while the OS was 19 months *vs.* NR ($p = 0.040$) in PNI+ and PNI- patients, respectively. A ≥6 PNI was identified as an independent predictor of worse OS *vs.* <6 PNI + patients (29 *vs.* 11 months, $p < 0.001$) and <6 PNI+ and PNI- patients (43 *vs.* 11 months, $p < 0.001$). PNI ≥6 was an independent negative prognostic factor of DFS *vs.* <6 PNI+ and PNI- patients (13 *vs.* 6 months, $p = 0.022$).

Conclusions: We report a PNI scoring system that stratifies surgically-treated PDAC patients in a graded manner that correlates with patient prognosis better than the current dichotomous (presence/absence) definition. However, further and larger studies are needed to support this PNI scoring system.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) has one of the poorest prognoses among malignant solid tumors, and it is expected to be the second leading cause of cancer-related deaths over the next decade [1]. Approximately 80% of patients with PDAC are diagnosed at a locally advanced or metastatic stage, which excludes a radical surgery approach. PDAC is also characterized by chemoresistance and lack of response to radiation treatments [2], further limiting therapeutic procedures. Improvements to systemic treatments have contributed to a limited increase in survival, with a 5-year survival rate of up to 8% [3,4].

Solid tumors disseminate locally and systemically through three major routes: direct invasion of surrounding tissues or spreading via vascular or lymphatic systems. An alternative route of dissemination is through perineural invasion (PNI), a process characterized by cancer cell migration along and around nerves [5]. PNI is defined by the histological identification of tumor cells in close proximity to nerves (involving at least 33% of its circumference) or tumor cells within any of the epineural, perineural, or endoneurial layers of the nerve sheath [6].

PNI is a dynamic process involving mutual tropism between the tumor and the nerve, and exhibiting different patterns across diverse tumors, which depend on anatomic location, density of

* Corresponding author. Viale Gaetano Pieraccini 6, 50139, Florence, Italy.
E-mail address: filippo.nozzoli@unifi.it (F. Nozzoli).
[1] Filippo Nozzoli and Martina Catalano equally contributed to the study.

https://doi.org/10.1016/j.pan.2024.03.004

innervation, and level of invasiveness. PDAC is characterized by a marked neurotropism as the presence of PNI, which, ranging from 76.2% to 97.8%, is the highest among most cancers, negatively impacting prognosis for both local recurrence and overall survival (OS) [7,8–13]. However, the underlying mechanisms of PNI are largely unknown and its therapy is unsatisfactory. Among the several factors responsible for the poor understanding and treatment of PDAC, the current staging system of PNI, which considers the presence or absence of PNI dichotomous variables without further classification according to the level of nerve involvement and severity of the invasion, may be included [12,13]. Herein, we evaluate PNI invasion according to a 5-level score, in an attempt to improve its assessment and evaluation in relation to prognosis.

2. Materials and methods

2.1. Case selection and tumor specimen collection

A total of 45 cases of localized PDAC, observed and treated at the Oncology Unit of Careggi University Hospital (Florence, Italy) between 2011 and 2021, were retrospectively identified. The hematoxylin and eosin (H&E) stained slides and formalin-fixed paraffin-embedded (FFPE) tissue specimens, obtained by surgical resection, were extracted from the archive of the Pathology Unit, Careggi University Hospital (Florence, Italy). We included patients with proven diagnosis of PDAC, obtained by all types of pancreatic surgical resection, with availability of at least one block of FFPE tumor tissue containing viable tumor cells and clinical follow-up data. Patients with other concomitant oncological diagnoses were excluded. Clinical data were obtained by chart review and correspondence with clinicians. This study was approved by the Local Ethics Committee (Comitato Etico Area Vasta Centro – Toscana) (22156_bio) and was performed in accordance with the principles of the Declaration of Helsinki.

2.2. Histopathologic review and PNI scoring system

Stained tissue sections were digitally scanned at × 400 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. The entire specimen collection was reviewed for morphology and immunoprofile to re-examine diagnosis and staging, according to the fifth edition of the World Health Organization (WHO) Classification of Digestive System Tumors and the eighth edition of American Joint Committee on Cancer (AJCC) Staging Manual, respectively. Other histologic features were considered, such as grade [14], lymph vascular invasion, and involvement of muscular vessels, as they have an impact on survival, although less than stage [15–17]. All H&E-stained slides from each tumor were then reviewed to assess PNI. Reproducibility was assessed using an inter-observer variability method. Specifically, H&E-stained sections were revised for diagnosis and PNI score assessment by two independent pathologists, and cases of difficult interpretation were discussed until full consensus was achieved.

The presence of PNI was further characterized by a score that included five histopathological features derived from multiparametric scores used in cutaneous squamous cell carcinoma and adapted for PDAC [18]. The evaluation was performed on the most representative slide for PNI (Fig. 1). The features included were: a) nerve diameter; b) number of distinct nerve structures involved *per* tumor; c) intratumoral *vs.* extratumoral nerve involvement (namely if located either within the malignancy or separated at least 1 mm from the main lesion); d) localization as focal (tumor cells surrounding ≤50% of the nerve sheath), circumferential (≥50% of the nerve sheath or intraneuronal), or intraneural (if different nerves demonstrated different extents of involvement, the most severe grade of involvement was considered); and e) presence or absence of mitotic figures within the perineural invasion area.

A three-level score (0, 1, or 2) was assigned to three of five features (a, b, d), and a binary score of 0 or 1 was assigned to the remaining two (c, e). Finally, these five PNI histological features were combined in an additional PNI score ranging from 0 to 8, and patients were stratified in two categories (low or high) according to the median score value (Supplementary Table 1).

2.3. Statistical analysis

Variables were summarized descriptively, namely median (range) for continuous variables and number (percentage) for categorical data. For independent variables, the *t*-test for continuous variables and the chi-square test of independence or the Fisher's exact test for categorical variables were applied. The Kaplan-Meier method was used to estimate median disease-free survival (DFS) and overall survival (OS), and differences were assessed using the log rank test. DFS refers to time from treatment until the recurrence of disease (or death) after undergoing curative-intent treatment. OS was defined as the time between diagnosis and death from any cause. Patients who had not died were censored at the date of the last follow-up visit.

Continuous variables, such as age, TNM staging, number of nerves, nerve diameter, and nerve sheath involvement, were analyzed both as continuous variables and categorical variables to optimize the PNI scoring system. Sex, tumor site, aggressive histology, lymphovascular invasion, presence of PNI, intratumoral versus extratumoral PNI, and presence versus absence of mitotic figures within the perineural invasion area were analyzed as categorical dichotomous variables. All the data were analyzed using SPSS software version 26.0 (IBM Corp. SPSS Statistics, Armonk, NY, USA). No imputation was performed for missing data; variables presenting missing data are reported in the tables or in the manuscript. All statistical tests were two-sided, and $p < 0.05$ was considered significant.

3. Results

3.1. Clinical and demographic characteristics

Forty-five surgically treated PDCA patients were included in the study (22 males [48.8%]), with a median age at diagnosis of 68 years (range 42–88). Twenty-eight patients (62.2%) had pathological tumor (pT) ≥ 3 disease and 25 (57.6%) had a nodal disease (pN+). Thirty-five primary tumors (77.8%) were in the head of the pancreas, and 53.3% showed moderate grade differentiation (G2). Lymphovascular invasion was documented in 17 patients (37.8%). Thirty-four (75.6%) patients were classified as PNI positive (+). No patients had received neoadjuvant therapy. Full details of the overall cohort and of patients according to PNI (+ or -) are reported in Table 1. Comparing PNI+ and PNI- cohorts, only median age at diagnosis and lymph node involvement (N ≥ 1) were significantly different ($p = 0.017$ and $p = 0.020$, respectively) (Table 1).

The median PNI score was 6 (range 1–8; standard deviation 1.793) in 34 patients. Eighteen (52.9%) had a score ≥6, and no significant difference was observed between patients with PNI score <6 (n = 16) or ≥6 in any of the evaluated parameters (Supplementary Table 2). Regarding histopathological features, in 28 cases (82.3%) nerve diameter was ≥2 mm, and in 24 cases (70.5%) the nerves involved were ≥5. Nerve sheath involvement was <50% in 10 (29.4%) cases, 50–100% in 13 (38.2%) cases, whereas

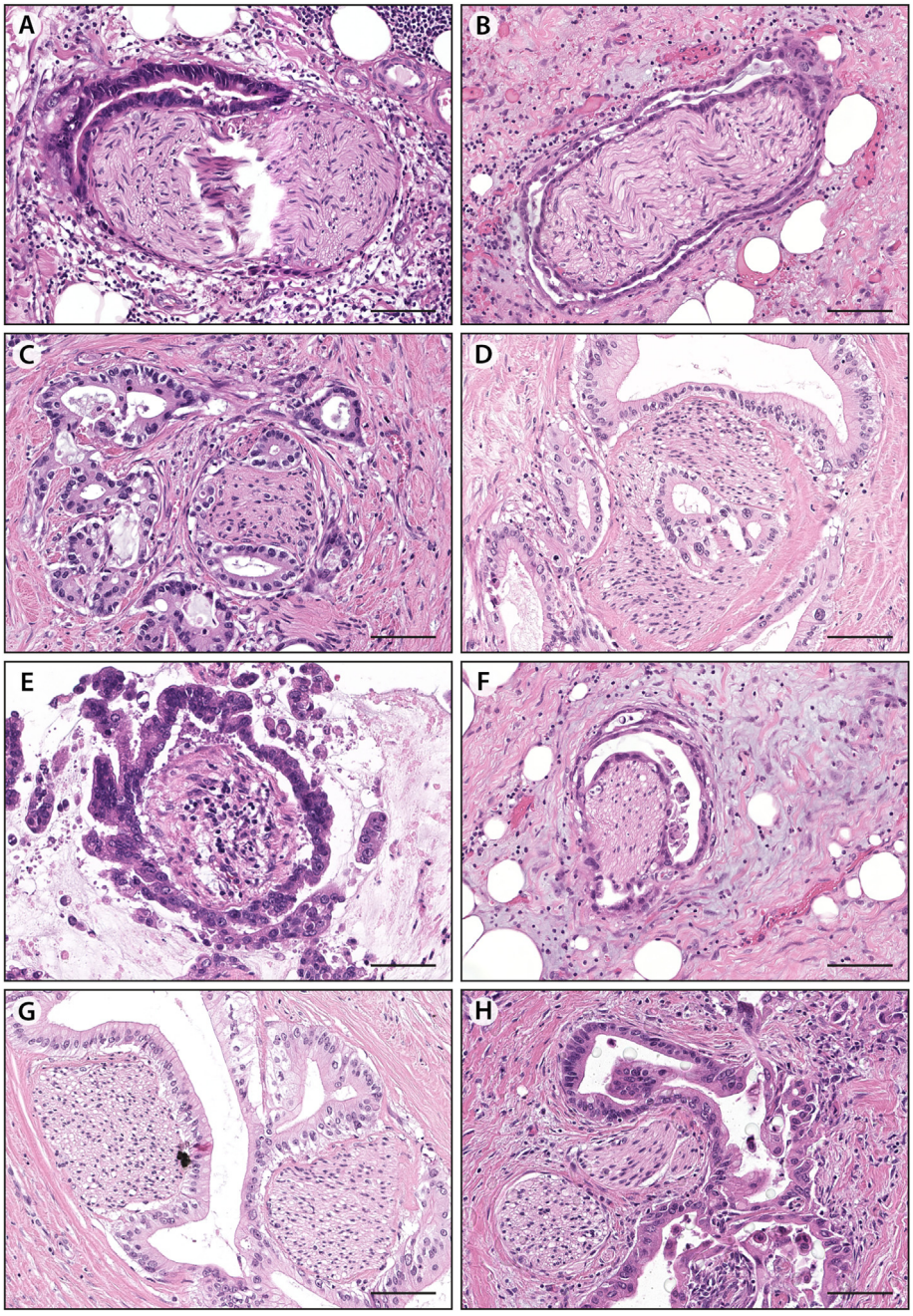

Fig. 1. Peripheral nerves in PDAC microenvironment stained with H&E, showing different patterns of tumor cell interaction with nerves. (A) Single nerve involvement with focal to 50% PNI (magnification 20x, scale bar 100 μm). (B) Single nerve surrounded by a thin layer of tumor cells with total sheath encirclement (magnification 20x, scale bar 100 μm). (C–D) Representative images of nerves with both peri- and intraneural involvement (magnification 20x, scale bar 100 μm). (E–F) Extratumoral nerves with total sheath encirclement. (G) Two intratumoral nerves with more than 50% of sheath involvement (magnification 20x, scale bar 100 μm). (H) Intratumoral multiple nerves with focal encirclement (magnification 20x, scale bar 100 μm).

intraneural involvement was found in 11 (32.3%) cases. Finally, 30 cases (88.2%) had intratumoral PNI, and 24 cases (70.5%) presented mitotic figures within perineural invasion (Supplementary Table 3).

3.2. PNI and outcome correlations

3.2.1. Disease-free survival

DFS analysis was performed on the 39 patients with available data. In the overall population, median DFS was 11 months (95%CI 8.6–13.8). DFS was 11 months (95% CI 7.8–14.1) in PNI + patients and not reached (NR) in PNI- patients, with no differences between groups (Table 2 and Fig. 2A). PNI + patients with a score ≥6 showed a significant correlation with worse DFS compared to patients with a score <6 (6 *vs.* 13 months, respectively, $p = 0.040$) (Fig. 2B). However, this trend was not confirmed by the Cox regression analysis ($p = 0.059$), as shown in Table 2. No statistical difference was observed in PNI- *vs.* <6 PNI + patients (NR *vs.* 13, $p = 0.071$), whereas a significantly worse DFS was recorded in ≥6 PNI + patients *vs.* all remaining patients (PNI- plus <6 PNI+) (6 *vs.* 13, $p = 0.022$) (Table 2 and Fig. 2C–E).

The univariate Cox regression analysis to assess the associations between clinical-pathological variables and DFS was performed.

Table 1
Patients' baseline characteristics.

	All patients (**N=45**)	PNI+ (**N=34**)	PNI-(**N=11**)	*p*
Age				
Median (range)	68 (42–88)	67.5 (42–88)	74 (64–82)	**0.017**
Gender, n (%)				
Male	22 (48.9)	16 (47.05)	6 (54.54)	0.666
Site, n (%)				
Head	35 (77.8)	27 (79.41)	8 (72.72)	0.687
Lymphovascular invasion, n (%)				
Yes	17 (37.8)	15 (62.2)	2 (18.18)	0.165
ECOG, n (%)				
0	22 (48.9)	15 (44.11)	7 (63.63)	0.188
1	21 (46.7)	17 (50.0)	4 (36.36)	
2	2 (4.4)	2 (5.88)	0	
pT, **n (%)**				
1	4 (8.9)	2 (5.88)	2 (18.18)1	0.623
2	13 (28.9)	10 (29.41)	3 (27.27)	
3	27 (60)	21 (61.76)	6 (54.54)	
4	1 (2.2)	1 (2.94)	0	
pN, **n (%)**				
0	20 (44.4)	11 (32.35)	9 (81.81)	**0.020**
1	21 (46.7)	19 (55.88)	2 (18.18)	
2	4 (8.9)	4 (11.76)	0	
Grading				
1	11(24.4)	7(20.58)	4 (36.36)	0.647
2	24 (53.3)	19 (55.88)	5 (45.45)	
3	10 (22.2)	8 (23.52)	2 (18.18)	
Staging				
I	6 (13.3)	3 (8.82)	3 (27.27)	0.468
II	27 (60)	21 (61.76)	6 (54.54)	
III	2 (4.4)	2 (5.88)	0 (0)	
IV	10 (22.2)	8 (23.52)	2 (18.18)	
Biliary Stent[a]				
Yes	12(26.7)	10 (29.41)	2 (18.18)	0.693
Radiotherapy[b]				
Yes	6 (13.3)	4 (11.76)	2 (18.18)	0.613
Adjuvant Therapy				
Yes	25 (55.6)	20 (58.82)	5 (45.45)	0.500
PNI				
Yes	34 (75.6)	_	_	_

ECOG, Eastern Cooperative Oncology Group; pT, pathological Tumot; pN, pathological lymph node; PNI, perineural invasion. Percentages are calculated on column total if not otherwise specified.
[a] Calculated on 39 patients.
[b] Calculated on 38 patients.

Among all variables, pN+ ($p = 0.041$), number of involved nerves ($p = 0.041$) and stage ≥ III ($p = 0.044$) correlated with DFS at the univariate analysis. DFS was not different between PNI + *vs*. PNI- and between ≥6 PNI + *vs*. <6 PNI+. No difference was found when the five PNI histopathological features were assessed separately. On the contrary, a worse DFS was observed in ≥6 PNI patients *vs*. PNI- plus <6 PNI + patients (p=0.035). At the multivariate Cox analysis, stage ≥ III ($p = 0.025$) and ≥6 PNI+ (*vs*. PNI- plus <6 PNI+) ($p = 0.029$) resulted as independent predictors of tumor recurrence.

3.2.1.1. Overall survival. OS analysis was performed on the whole cohort of 45 patients. The median follow-up was 18 months (range, 1–86 months). Median OS, which was 23 months (95% CI 15.6–30.3) for the entire population, decreased to 19 months in PNI + patients (95% CI 14.5–23.4), and was NR in PNI- patients ($p = 0.040$) (Fig. 3A). A PNI score <6 correlated with longer OS than PNI score ≥6 (29 *vs*. 11 months, respectively, $p < 0.001$) (Fig. 3B), while no significant difference was observed between <6 PNI + patients and PNI- patients (29 *vs*. NR months, $p = 0.062$). Likewise, a PNI score ≥6 compared with PNI- and <6 PNI + patients correlated with worse OS (11 *vs*. 43 months, $p < 0.001$) (Fig. 3C–E).

In the Cox regression analysis, features associated with OS were pT ≥ 3 ($p = 0.042$), pN+ ($p = 0.042$), grading ≥2 ($p = 0.005$), lymph vascular metastasis ($p = 0.048$), adjuvant therapy ($p = 0.006$), PNI+ ($p = 0.012$), and PNI score ≥6 ($p < 0.001$). Among 34 PNI + patients, nerve diameter ≥2 ($p = 0.040$), number of involved nerves ≥5 ($p = 0.017$), extratumoral nerve site ($p = 0.007$), presence of mitotic figures within the perineural invasion ($p = 0.005$), and a PNI score ≥6 (p < 0.001), were associated with worse OS (Table 3). At multivariate analysis, grading ≥2 ($p = 0.048$), adjuvant therapy ($p = 0.001$), PNI+ ($p = 0.001$), and PNI score ≥6 ($p < 0.001$), were confirmed as independent predictors of OS in the total population (Table 4). Accordingly, a score of PNI ≥6 ($p < 0.001$) was an independent prognostic factor within the PNI + population (Table 4).

4. Discussion

PNI is a recognized negative prognostic factor of pancreatic

Table 2
Univariate and multivariate analysis for DFS.

Variable	Univariate		Multivariate	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Age (>70)	0.991 (0.958–1.024)	0.586	_	_
Gender (male)	0.644 (0.306–1.357)	0.248	_	_
pT ≥ 3[a]	2.172 (0.950–4.961)	0.066	_	_
pN +[a]	**2.281 (1.035–5.030)**	**0.041**	_	_
ECOG ≥1	1.695 (0.812–3.538)	0.160	_	_
Grading ≥2[a]	1.471 (0.624–3.468)	0.377	_	_
Lymphovascular invasion	1.687(0.643–4.426)	0.288	_	_
Stage ≥ III	**2.317 (1.023–5.246)**	**0.044**	2.699 (1.134–6.427)	**0.025**
Adjuvant therapy	0.582 (0.270–1.256)	0.168	_	_
Site (head)	0.699 (0.241–2.022)	0.508	_	_
PNI+	1.687 (0.643–4.426)	0.288	_	_
PNI ≥6 (*vs*. PNI<6)	2.231 (0.971–5.126)	0.059	_	_
PNI <6 (*vs*. PNI-)	1.211 (0.419–3.495)	0.724	_	_
PNI≥6 (*vs*. PNI-)	2.273 (0.803–6.493)	0.122	_	_
PNI ≥6 (*vs*. PNI- and PNI<6)	2.237 (1.056–4.738)	**0.035**	2.413 (1.093–5.330)	**0.029**
Nerve diameter ≥2 mm[a]	2.486 (0.732–8.444)	0.144	_	_
Number of involved nerves ≥5 [a]	**2.586 (1.039–6.431)**	**0.041**	_	_
Site (extratumoral)	2.112 (0.614–7.263)	0.236	_	_
Nerve sheath involvement [a]	1.234 (0.510–2.986)	0.642	_	_
Mitotic figures within PNI	2.279 (0.915–5.679)	0.077	_	_

ECOG, Eastern Cooperative Oncology Group; pT, pathological Tumor; pN, pathological node; PNI, perineural invasion.
[a] *Considered as categorical variables.*

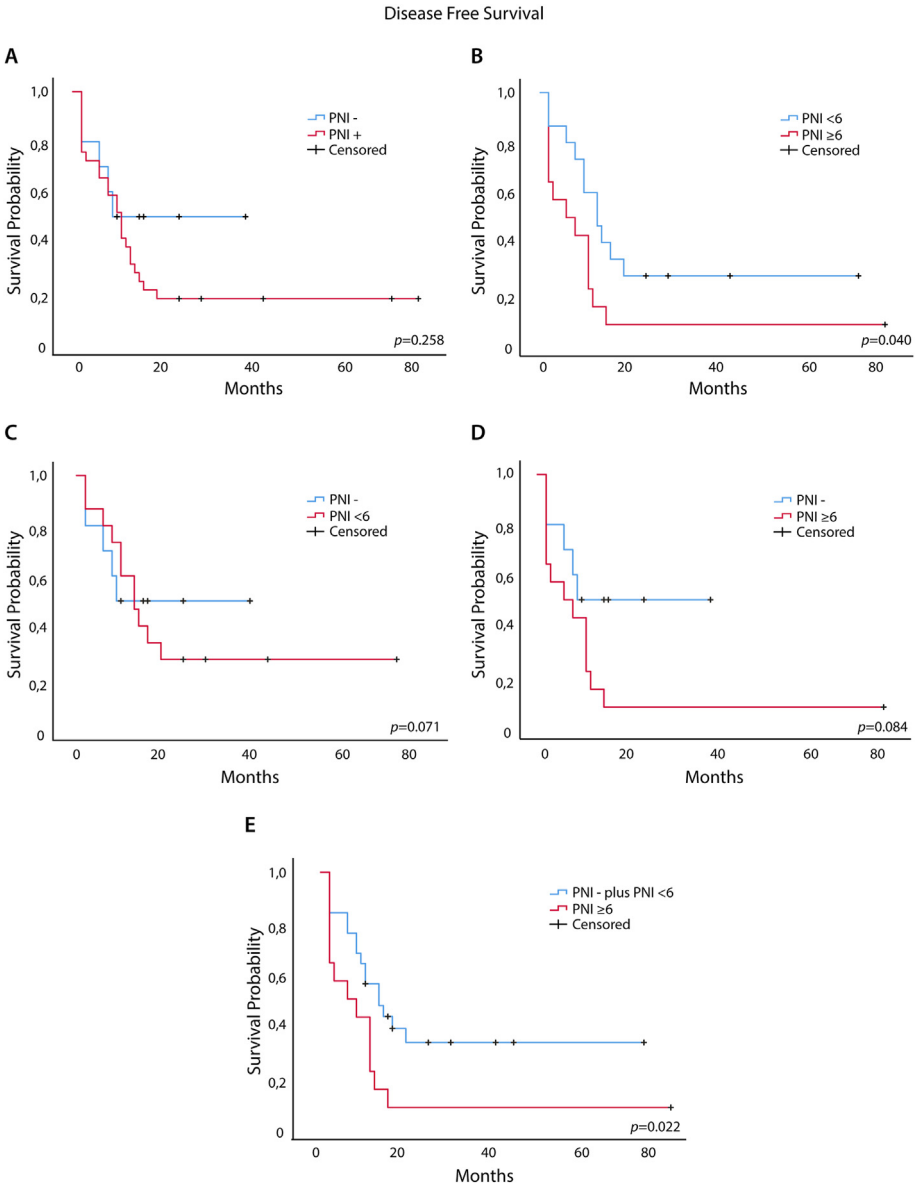

Fig. 2. Kaplan-Meier for disease free survival according to: (A) PNI + *vs.* PNI-, (B) PNI <6 *vs.* PNI ≥6, (C) PNI– *vs.* PNI <6, (D) PNI– *vs.* PNI ≥6, (E) PNI ≥6 *vs.* PNI– plus PNI <6.

cancer [19]. However, its prognostic value is currently limited to the presence *vs.* absence of PNI, without considering the specific contribution of different PNI features to worsen the prognosis. We used an objective histopathologic scoring system for PNI in PDAC that significantly correlated with adverse outcomes of recurrence and survival. To the best of our knowledge, this is the first study using a histological PNI scoring system that stratifies patients with surgically resected PDAC. In line with a previous meta-analysis [10], we found an elevated PNI rate of 75.6% in the present cohort, which is an independent prognostic factor for OS but not for DFS. Of the five histopathologic features related to PNI, we identified four that were significantly associated with OS. However, only the presence of mitosis within perineural invasion was confirmed as an independent factor at multivariate analysis. By combining the five histopathological features into a PNI score and stratifying for medium value, we observed a significant correlation with OS and a trend of correlation with DFS, suggesting its potential role for a better stratification of risk. Furthermore, we compared PNI- patients with <6 PNI + patients, but failed to find any differences in OS and DFS. In contrast, when analyzing ≥6 PNI + patients *vs.* PNI- patients, we observed a statistically significant difference in OS. Similarly, when comparing ≥6 PNI + patients with the remaining population (including PNI- patients and <6 PNI + patients), we found statistically significant differences in both OS and DFS (Fig. 4).

Pancreatic cancer is characterized by genomic complexity, including chromosomal instability, telomere dysfunction, aneuploidy, nuclear atypia, and abnormal mitosis [20]. Previous evidence has reported a high mitotic index and the presence of multipolar mitosis as independent histopathological prognostic markers for pancreatic cancer [20]. However, no data regarding the correlation between mitotic figures within perineural invasion and survival outcomes are available to date. The choice to use the nerve diameter as a histological parameter included in the scoring system to assess the number of nerves was derived from the recently reported association between “extensive” involvement (greater than 2 nerves on the histological field) and clinical outcome in both head and neck cutaneous squamous cell carcinoma (HNcSCC) [21,22] and PDAC [23]. A recent study that analyzed nerve infiltration in the

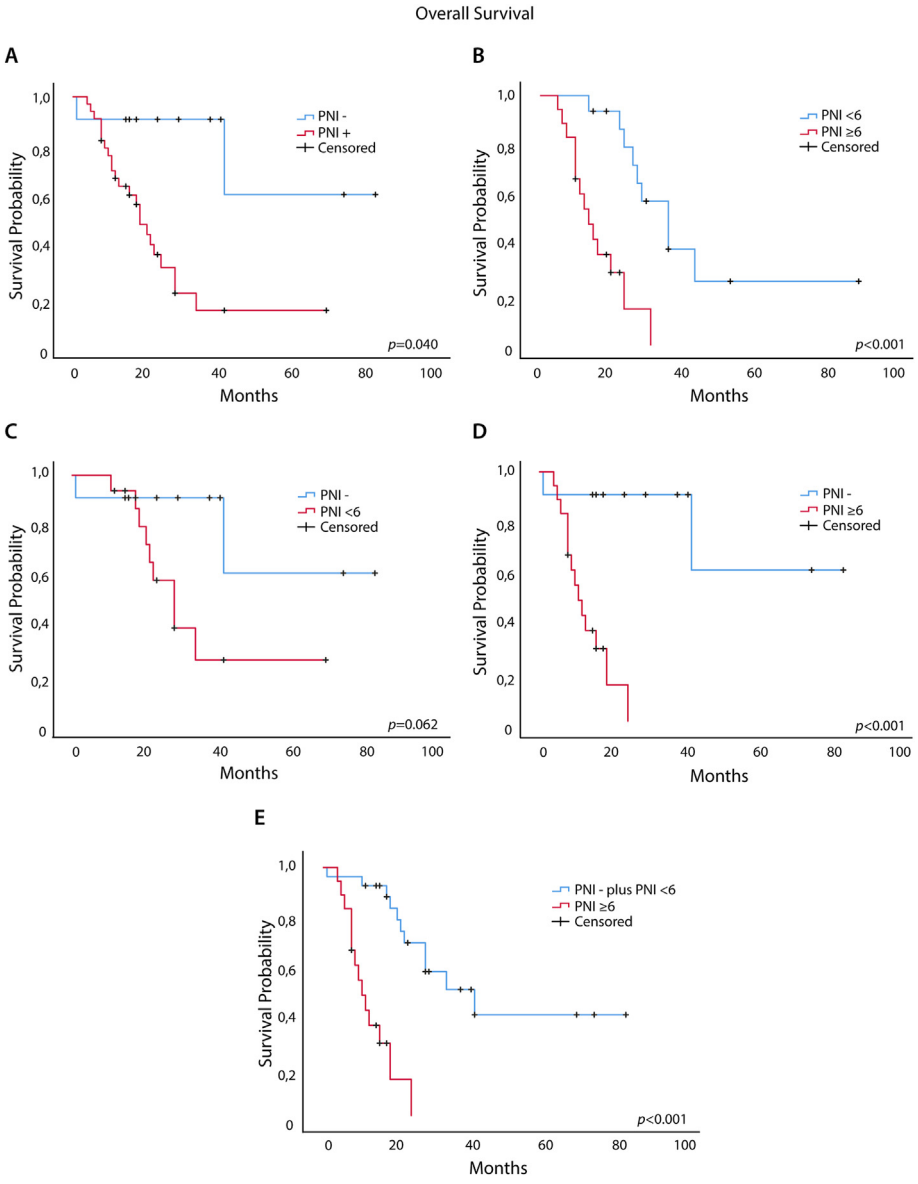

Fig. 3. Kaplan-Meier for overall survival according to: (A) PNI + *vs.* PNI- (B) PNI <6 *vs.* PNI ≥6, (C) PNI− *vs.* PNI <6, (D) PNI− *vs.* PNI ≥6, (E) PNI ≥6 *vs.* PNI− plus PNI <6.

tumor microenvironment of patients affected by PC revealed that presence and size (as measured by cross-sectional area) of nerves within the pancreatic cancer microenvironment were associated with tumor aggressiveness [23]. However, it is unclear how different degrees of nerve involvement affect prognosis, and further data will be required to establish a standard dimensional cut-off definition of enlarged nerve, considering the wide variability of the measurement system used (maximum cross-sectional area or diameter) [23,24]. While the impact of extratumoral PNI on adverse outcome is a consolidated finding in HNcSCC [22], poor information is available in PDAC. Extratumoral PNI seems to be related to the invasion of the retroperitoneal neural plexus, thus influencing postoperative survival of patients with PDAC [21].

The impact of PNI on the dissemination of cancer is well known [25–28]. However, only four studies have evaluated the correlation between PNI and DFS in PDAC (see the meta-analysis [10]) reported PNI as an independent predictor of tumor recurrence in PDAC. This correlation has recently been confirmed with a median DFS of 26 *vs.* 12.9 months in the PNI- *vs.* PNI + group ($p < 0.001$) [29]. However, another study found PNI to be an independent predictor of DFS in early-stage PDAC (namely patients with R0/N0 disease and tumor size ≤20 mm), but not in the overall population [30]. In this study, the rate of PNI was significantly higher in patients with exclusively systemic recurrence compared with local or local/systemic recurrence, suggesting that PNI may represent the determinant factor of recurrence in earlier stages of PDAC progression. Contrary to previous studies, we did not find a correlation between the presence of PNI and tumor recurrence. Also, in PNI + patients, a PNI score ≥6 showed only a trend of correlation with worse DFS ($p = 0.040$), not confirmed at the Cox regression analysis ($p = 0.059$). Instead, in the entire population, a PNI score ≥6 has been confirmed as an independent prognostic factor of worse DFS ($p = 0.022$). However, no statistically significant difference emerged comparing <6 PNI + patients with PNI- ($p = 0.071$) or ≥6 PNI+ with PNI- patients ($p = 0.084$). Finally, although the number of involved nerves ≥5 was significantly associated with DFS at univariate analysis ($p = 0.041$), it failed to result as an independent factor of DFS ($p = 0.507$).

In comparison, the association between PNI and OS in PDAC has

Table 3
Univariate analysis for OS.

Variable	HR (95%CI)	*p*
Age (>70)	1.013 (0.974–1.053)	0.519
Gender (male)	0.536 (0.233–1.234)	0.143
pT ≥ 3[a]	2.765 (1.036–7.381)	**0.042**
pN+[a]	2.594 (1.033–6.512)	**0.042**
Grading ≥2[a]	8.182 (1.897–35.290)	**0.005**
ECOG ≥1	2.043 (0.917–4.594)	0.080
Lymphovascular invasion	2.232 (1.008–4.942)	**0.048**
Site (head)	1.544 (0.609–3.911)	0.360
Stage ≥ III	1.776 (0.740–4.262)	0.198
Adjuvant therapy	0.321 (0.143–0.721)	**0.006**
PNI +	6.532 (1.513–28.209)	**0.012**
PNI ≥6 (*vs.* PNI<6)	6.524 (2.322–18.322)	**<0.001**
PNI<6 (*vs.* PNI-)	3.896 (0.828–18.332)	0.085
PNI ≥6 (*vs.* PNI-)	18.738 (2.332–150.573)	**0.006**
PNI ≥6 (*vs.* PNI- and PNI<6)	8.640 (3.215–21.218)	**<0.001**
Nerve diameter ≥2 mm[a]	4.651 (1.074–20.131)	**0.040**
Number of involved nerves ≥5[a]	3.316 (1.237–8.891)	**0.017**
Site (extratumoral)	5.060 (1.568–16.324)	**0.007**
Nerve sheath involvement[a]	1.634 (0.634–4.090)	0.294
Mitotic figures within PNI	5.176 (1.656–16.184)	**0.005**

ECOG, Eastern Cooperative Oncology Group; pT, pathological Tumor; pN, pathological lymph node; PNI, perineural invasion.
[a] *Considered as categorical variables.*

Table 4
Multivariate analysis for OS.

Variable	Multivariate	
All population	**HR (CI 95%)**	***p***
Adjuvant therapy	0.156 (0.054–0.451)	**0.001**
Grading ≥3[a]	6.145 (1.019–37.176)	**0.048**
PNI + (*vs.* PNI-)	39.681 (4.927–319.580)	**0.001**
	HR (CI 95%)	***p***
Adjuvant therapy	0.296 (0.115–0.760)	**0.011**
Grading ≥3[a]	5.743 (1.049–31.435)	**0.044**
PNI ≥6 (*vs.* PNI– and PNI<6)	10.836 (3.390–34.639)	**<0.001**
	HR (CI 95%)	***p***
Adjuvant therapy	0.068 (0.012–0.384)	**0.002**
PNI ≥ 6 (*vs.* PNI-)	67.826 (4.748–968.877)	**0.002**
PNI + patients	**HR (CI 95%)**	***p***
Adjuvant therapy	0.088 (0.023–0.341)	**<0.001**
PNI ≥6 (*vs.* PNI <6)	11.175 (2.639–47.321)	**<0.001**

pT, pathological Tumor; pN, pathological lymph nodes; PNI, perineural invasion. [a]*Multivariate analysis performed on PNI- and PNI≥ 6 population.* [a]*Multivariate analysis performed on PNI + patients considering histopathological features that reached a significance on multivariate analysis.*
[a] *Considered as categorical variables.*

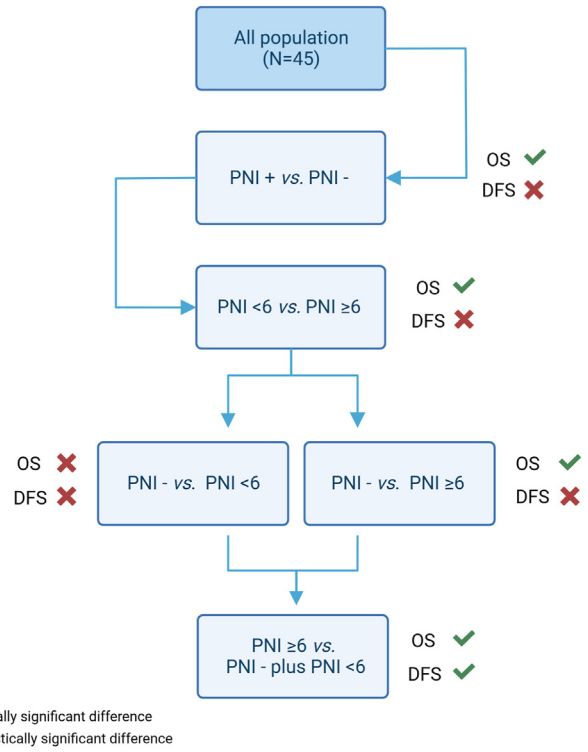

Fig. 4. Flowchart of the analyzed group populations. *Created with BioRender.com.*

been extensively evaluated. The metanalysis mentioned above included 36 studies evaluating the impact of PNI on OS. PNI was identified as an independent negative prognostic factor for OS in patients with resected PDCA [10]. These data have been subsequently confirmed by two recent studies reporting a median OS of 50 *vs.* 27 months and 64.9 *vs.* 18.1 months in PNI- and PNI + patients, respectively [29,30]. Our results confirm the correlation between PNI+ and worse OS (19 *vs.* NR, $p = 0.040$), along with grading and adjuvant therapy. The novel finding reported in the present study is that, in PNI + patients, OS was higher in patients with <6 score compared with ≥6 score (11 *vs.* 29 months, $p < 0.001$). In PNI + patients, a PNI score ≥6 ($p = 0.001$), and the presence of mitotic figures within perineural invasion ($p = 0.009$), were independent negative prognostic factors for survival. Likewise, PNI + patients with score ≥6 had lower OS compared with PNI- patients ($p < 0.001$) and PNI- plus PNI + score <6 patients ($p < 0.001$), confirming that a PNI score of ≥6 is an independent prognostic factor of worse OS in the entire PDAC population.

A recently introduced scoring system for PNI was presented by Schiavo Lena et al. [31]. In this system, PNI was assessed based only on one single parameter (nerve caliber) as follows: 0 indicated its absence, 1 indicated the presence of neoplasia along nerves with a caliber of less than 3 mm, and 2 indicated neoplastic infiltration of nerve fibers with a caliber of 3 mm or more, massive perineural infiltration, or the presence of necrosis within the infiltrated nerve bundle. The severity score for PNI exhibited a significant correlation with decreased DFS and Disease-Specific Survival in univariate analysis, but this was not confirmed in the multivariate analysis. Thus, although PNI score demonstrated a potential prognostic role, it was found to be less robust compared to lymph node metastases and tumor differentiation grade. To the best of our knowledge, there are no studies on scoring systems using the combination of obvious multiple histological features of PNI in PDAC. In the present study, we adapted a 5-histological parameter system previously tested with encouraging results in head and neck squamous cell carcinoma (HNcSCC) [18]. However, further investigation is needed to refine the histological parameters of PNI, based on the specific characteristics of pancreatic cancer neural microenvironment. Among the other variables considered in our analysis, we identified a significant difference between PNI+ and younger age, which could be partially explained by the higher disease aggressiveness reported in younger patients [32] Moreover, according to previous evidence [29,30,33], PNI + patients had significantly higher rates of lymph node metastasis (67.6%) than PNI- patients (18.1%) ($p = 0.020$). The correlation between PNI and lymph node status is consistent with previous findings indicating PNI as a solid predictor of shorter disease-free survival, overall survival, and lymph node metastases, compared to no PNI [34]. Moreover, data reported that the perineural sheath likely acts as a consecutive route for tumor spread to surrounding solid and lymphatic tissues [29]. However,

the relationship between PNI and lymph node metastases is still controversial, and deserves further evaluation [33,35].

Altogether, our results indicate that a histopathologic classification of PNI that stratifies the presence of PNI according to several variables identifies features associated with recurrence and survival outcome. Furthermore, we show that a graded score of PNI based on several histopathological parameters appears to be better associated with patient prognosis than the currently used PNI definition. The main limitations of the study are the small sample size and the incomplete data on disease-free survival. However, our aim to find a way to overcome the limitations of the current dichotomous patient stratification is supported by these initial results, although obtained with a relatively small sample size. While consistent with a recent study [31], present data need to be validated with larger, multicenter case cohorts. Other limitations are primarily due to the retrospective and monocentric design. Furthermore, although we recognize the potential usefulness of applying the scoring system preoperatively, the histopathological assessment is normally practicable in resection specimens. However, biopsy sample dimensions, usually small, are rarely representative of the lesion in pancreatic cancer and do not allow the assessment of PNI. In addition, of our PNI cohort, only a small fraction of cases had a preoperative biopsy, and PNI was not documented in any of those cases. In addition, prognostic biomarkers, such as carcinoembryonic antigen and carbohydrate antigen 19.9, comorbidities, and recurrence disease management might be confounding elements not evaluated in this study. Thus, further prospective cohort studies with a larger sample size are needed to better define the relative prognostic significance of each PNI histopathologic feature to refine risk assessment in PNI + PDAC. In this regard, a clinical trial to validate a PNI and vascular invasion scoring system, aimed to obtain a detailed stratification of PNI and its correlation with DFS, is currently ongoing (NCT04024358). A validated PNI scoring system and its incorporation into staging systems may be useful for stratifying patients at higher risk, refining prognostic accuracy, and targeting patients appropriately for additional monitoring, diagnostic imaging, or adjuvant therapy. The strength of our study is that it is the first to identify an objective histopathological scoring system for PNI in PDAC patients, advancing from the presence or absence stratification. We also performed different and specific analyses for subgroups based on PNI features.

5. Conclusion

PDAC has one of the highest incidences of PNI of all types of cancer, which correlates with poor prognosis and decreased survival. However, the evaluation is limited to describe PNI as a present/absent dichotomous variable. In this study, we introduced a PNI scoring system based on five histopathological features specifically developed for pancreatic surgical specimens. Our results show that a higher PNI score ($\geq$6) significantly correlates with DFS and OS in patients with surgically resected PDAC, suggesting that the grade of PNI may enable a better prognostic risk stratification compared to its simple presence/absence. Additional larger prospective studies are needed to validate the scoring system and to explore its potential use in improving prognosis and risk stratification. In addition, a better understanding of the molecular mechanisms of PNI could be useful for the development of novel therapeutic strategies.

Declaration of competing interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pan.2024.03.004.

References

[1] Rahib L, Smith BD, Aizenberg R, et al. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 2014;74:2913–21. https://doi.org/10.1158/0008-5472.CAN-14-0155.
[2] Adamska A, Elaskalani O, Emmanouilidi A, et al. Molecular and cellular mechanisms of chemoresistance in pancreatic cancer. Adv. Biologic.Regulat. 2018;68:77–87. https://doi.org/10.1016/j.jbior.2017.11.007.
[3] Siegel RL, Miller KD, Jemal A, Cancer statistics. CA: a cancer journal for clinicians, vol. 67; 2017. p. 7–30. https://doi.org/10.3322/caac.21387. 2017.
[4] Zakharova OP. Pancreatic adenocarcinoma: outstanding problems. World J Gastrointest Surg 2012;4:104. https://doi.org/10.4240/wjgs.v4.i5.104.
[5] Amit M, Na'Ara S, Gil Z. Mechanisms of cancer dissemination along nerves. Nat Rev Cancer 2016;16:399–408. https://doi.org/10.1038/nrc.2016.38.
[6] Liebig C, Ayala G, Wilks JA, Berger DH, Albo D. Perineural invasion in cancer: a review of the literature. Cancer 2009;115(15):3379–91. https://doi.org/10.1002/cncr.24396.
[7] Mitsunaga S, Hasebe T, Kinoshita T, et al. Detail histologic analysis of nerve plexus invasion in invasive ductal carcinoma of the pancreas and its prognostic impact. Am J Surg Pathol 2007;31:1636–44. https://doi.org/10.1097/PAS.0b013e318065bfe6.
[8] Ozaki H, Hiraoka T, Mizumoto R, et al. The prognostic significance of lymph node metastasis and intrapancreatic perineural invasion in pancreatic cancer after curative resection. Surg Today 1999;29:16–22. https://doi.org/10.1007/BF02482964.
[9] Nakao A, Harada A, Nonami T, et al. Clinical significance of carcinoma invasion of the extrapancreatic nerve plexus in pancreatic cancer. Pancreas 1996;12:357–61. https://doi.org/10.1097/00006676-199605000-00006.
[10] Schorn S, Demir IE, Haller B, et al. The influence of neural invasion on survival and tumor recurrence in pancreatic ductal adenocarcinoma – a systematic review and meta-analysis. Surg. Oncol. 2017;26:105–15. https://doi.org/10.1016/j.suronc.2017.01.007.
[11] Bapat AA, Hostetter G, Von Hoff DD, et al. Perineural invasion and associated pain in pancreatic cancer. Nat Rev Cancer 2011;11:695–707. https://doi.org/10.1038/nrc3131.
[12] Selvaggi F, Melchiorre E, Casari I, et al. Perineural invasion in pancreatic ductal adenocarcinoma: from molecules towards drugs of clinical relevance. Cancers 2022;14:5793. https://doi.org/10.3390/cancers14235793.
[13] Chun YS, Pawlik TM, Vauthey JN. 8th edition of the AJCC cancer staging manual: pancreas and hepatobiliary cancers. Ann Surg Oncol 2018;25:845–7. https://doi.org/10.1245/s10434-017-6025-x.
[14] Giulianotti PC, Boggi U, Fornaciari G, et al. Prognostic value of histological grading in ductal adenocarcinoma of the pancreas - klöppel vs TNM grading. Int J Pancreatol 1995;17:279–89. https://doi.org/10.1007/BF02785825.
[15] Garcea G, Dennison AR, Ong SL, et al. Tumour characteristics predictive of survival following resection for ductal adenocarcinoma of the head of pancreas. Eur J Surg Oncol 2007;33:892–7. https://doi.org/10.1016/j.ejso.2007.02.024.
[16] Chen JWC, Bhandari M, Astill DS, et al. Predicting patient survival after pancreaticoduodenectomy for malignancy: histopathological criteria based on perineural infiltration and lymphovascular invasion. HPB 2010;12:101–8. https://doi.org/10.1111/j.1477-2574.2009.00140.x.
[17] Wang H, Chatterjee D, Katz MH, et al. Perineural and intraneural invasion in posttherapy pancreaticoduodenectomy specimens predicts poor prognosis in patients with pancreatic ductal adenocarcinoma. Am J Surg Pathol 2012;36:409–17. https://doi.org/10.1097/PAS.0b013e31824104c5.
[18] Totonchy MB, McNiff JM, Suozzi KC, et al. A histopathologic scoring system for perineural invasion correlates with adverse outcomes in patients with cutaneous squamous cell carcinoma, vol. 47. Dermatologic Surgery : Official Publication for American Society for Dermatologic Surgery [et Al; 2021. p. 445–51. https://doi.org/10.1097/DSS.0000000000002923.
[19] Hirai I, Kimura W, Ozawa K, et al. Perineural invasion in pancreatic cancer. Pancreas 2002;24:15–25. https://doi.org/10.1097/00006676-200201000-00003.
[20] Matsuda Y, Yoshimura H, Ishiwata T, et al. Mitotic index and multipolar mitosis in routine histologic sections as prognostic markers of pancreatic cancers: a clinicopathological study. Pancreatology 2016;16:127–32. https://doi.org/10.1016/j.pan.2015.10.005.
[21] Lin C, Tripcony L, Keller J, et al. Perineural infiltration of cutaneous squamous cell carcinoma and basal cell carcinoma without clinical features. Int J Radiat Oncol Biol Phys 2012;82:334–40. https://doi.org/10.1016/j.ijrobp.2010.09.040.
[22] Sapir E, Tolpadi A, McHugh J, et al. Skin cancer of the head and neck with gross or microscopic perineural involvement: patterns of failure. Radiother Oncol 2016;120:81–6. https://doi.org/10.1016/j.radonc.2016.06.011.
[23] Ferdoushi A, Griffin N, Marsland M, et al. Tumor innervation and clinical outcome in pancreatic cancer. Sci Rep 2021;11:7390. https://doi.org/10.1038/

s41598-021-86831-w.
[24] Ross AS, Miller Whalen F, Elenitsas R, et al. Diameter of involved nerves predicts outcomes in cutaneous squamous cell carcinoma with perineural invasion: an investigator-blinded retrospective cohort study. Dermatol Surg 2009;35:1859–66. https://doi.org/10.1111/j.1524-4725.2009.01354.x.
[25] Magnon C, Hall SJ, Lin J, et al. Autonomic nerve development contributes to prostate cancer progression. Science 2013;341:1236361. https://doi.org/10.1126/science.1236361.
[26] Schmitd LB, Scanlon CS, D'Silva NJ. Perineural invasion in head and neck cancer. J Dent Res 2018;97:742–50. https://doi.org/10.1177/0022034518756297.
[27] Duraker N, Şişman S, Can G. The significance of perineural invasion as a prognostic factor in patients with gastric carcinoma. Surg Today 2003;33:95–100. https://doi.org/10.1007/s005950300020.
[28] Niu Y, Förster S, Muders M. The role of perineural invasion in prostate cancer and its prognostic significance. Cancers 2022;14:4065. https://doi.org/10.3390/cancers14174065.
[29] Felsenstein M, Lindhammer F, Feist M, et al. Perineural invasion in pancreatic ductal adenocarcinoma (PDAC): a saboteur of curative intended therapies? J Clin Med 2022;11:2367. https://doi.org/10.3390/jcm11092367.
[30] Crippa S, Pergolini I, Javed AA, et al. Implications of perineural invasion on disease recurrence and survival after pancreatectomy for pancreatic head ductal adenocarcinoma. Ann Surg 2022;276:378–85. https://doi.org/10.1097/SLA.0000000000004464.
[31] Schiavo Lena M, Gasparini G, Crippa S, et al. Quantification of perineural invasion in pancreatic ductal adenocarcinoma: proposal of a severity score system. Virchows Arch 2023;483(2):225–35. https://doi.org/10.1007/s00428-023-03574-x.
[32] Kasbi AA, Ashary MA, Baksh M, et al. Age-related survival outcomes for pancreatic cancer by age. Cancer Diagn. Progn. 2022;2:71–7. https://doi.org/10.21873/cdp.10078.
[33] Kayahara M, Nakagawara H, Kitagawa H, et al. The nature of neural invasion by pancreatic cancer. Pancreas 2007;35:218–23. https://doi.org/10.1097/mpa.0b013e3180619677.
[34] Chatterjee D, Katz MH, Rashid A, Wang H, Iuga AC, Varadhachary GR, Wolff RA, Lee JE, Pisters PW, Crane CH, Gomez HF, Abbruzzese JL, Fleming JB, Wang H. Perineural and intraneural invasion in posttherapy pancreaticoduodenectomy specimens predicts poor prognosis in patients with pancreatic ductal adenocarcinoma. Am J Surg Pathol 2012 Mar;36(3):409–17. https://doi.org/10.1097/PAS.0b013e31824104c5.
[35] Gasparini G, Pellegatta M, Crippa S, et al. Nerves and pancreatic cancer: new insights into a dangerous relationship. Cancers 2019;11:893. https://doi.org/10.3390/cancers11070893.