Annals of Surgery Publish Ahead of Print
DOI:10.1097/SLA.0000000000006920

ACCEPTED

Prognostic validation of perineural invasion severity score in pancreatic cancer: a prospective study

Giulia Gasparini MD, PhD[1,2*], Stefano Crippa MD, PhD[1,2*], Marco Schiavo Lena MD[3*], Giulio Belfiori MD[1], Francesca Aleotti MD[1], Francesca Di Salvo PhD[1], Marta Pellegatta PhD[4], Domenico Tamburrino MD, PhD[1], Stefano Partelli MD, PhD[1,2], Nicolò Pecorelli MD, MSc[1,2], Maria Giulia Cangi PhD[3], Carla Taveggia PhD[4], Michele Reni MD[2,5], Claudio Doglioni MD[2,3], Massimo Falconi MD[1,2].

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

*Gasparini, Crippa and Schiavo Lena share the first authorship

[1]*Division of Pancreatic Surgery, Pancreas Translational & Clinical Research Center, IRCCS San Raffaele Scientific Institute, Milan, Italy;* [2]*Vita Salute San Raffaele University, Milan, Italy;* [3]*Pathology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy;* [4] *Axo-Glial Interaction Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy;* [5]*Department of Medical Oncology, Pancreas Translational & Clinical Research Center, IRCCS San Raffaele Scientific Institute, Milan, Italy.*

Correspondence to: Massimo Falconi MD; Division of Pancreatic Surgery, Pancreas Translational and Clinical Research Center, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy. Electronic address: falconi.massimo@hsr.it

Conflicts of Interest and Source of Funding: This work was supported by a research grant from the Italian Association for Cancer Research Special Program in Metastatic Disease, AIRC I.D. 22737 (Falconi and Reni), by Italian Association for Cancer Research under IG 2020 I.D. 24774 (Taveggia) and by Fondazione Nadia Valsecchi (Crippa). The authors declare no conflicts of interest.

Running Head: PNI Severity Score in Resected PDAC

Authors contribution: Giulia Gasparini (Conceptualization, Data curation, Investigation, Writing—original draft), Stefano Crippa (Conceptualization, Investigation, Supervision, Writing—original draft), Marco Schiavo Lena (Data curation, Resources, Investigation, Writing—review & editing), Giulio Belfiori (Data curation, Methodology, Investigation, Writing—review & editing), Francesca Aleotti (Methodology, Writing—review & editing), Francesca Di Salvo (Methodology, Data curation, Writing—review & editing), Marta Pellegatta (Investigation, Writing—review & editing), Domenico Tamburrino (Investigation,

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

Writing—review & editing), Stefano Partelli (Investigation, Supervision, Writing—review & editing), Nicolò Pecorelli (Investigation, Writing—review & editing), Maria Giulia Cangi (Resources, Writing—review & editing), Carla Taveggia (Investigation, Supervision, Writing—review & editing), Michele Reni (Investigation, Supervision, Writing—review & editing), Claudio Doglioni (Methodology, Resources, Supervision, Writing—review & editing) and Massimo Falconi (Conceptualization, Supervision, Writing—review & editing)

ABSTRACT

Objective: This study aims to prospectively validate a perineural invasion (PNI) severity score in predicting recurrence and survival in resected PDAC.

Background PNI in pancreatic ductal adenocarcinoma (PDAC) is characterized by clinical and morphologic heterogeneity. Although a specific score for PNI severity has been proposed, it lacks validation.

Methods In this prospective registered (NCT04024358) monocentric study, 300 patients undergoing pancreatectomy between March 2019 and February 2022 were analyzed. PNI was scored as: 0 absent; 1 presence of neoplasia along nerves < 3 mm; 2 neoplastic infiltration of nerves ≥ 3 mm and/or massive PNI and/or necrosis of the infiltrated nerves. Association of PNI with disease recurrence and survival was evaluated.

Results Of 300 patients, 86% presented PNI, with 148 (49.3%) having PNI 1 and 110 (36.7%) PNI 2. Neoadjuvant treatment did not influence PNI (89.7% vs 83.9% after treatment, p= 0.332). PNI severity significantly correlated with worsening pathological features, shorter disease recurrence (24 months for PNI 0, 17 for PNI 1 and 15 for PNI 2, P <0.01) and survival (57 months for PNI 0, 51 for PNI 1 and 32 for PNI 2, P <0.01). PNI 2 independently predicted both recurrence and survival with HR of 2.082 (P= 0.006) and 3.304 (P= 0.014). PNI 2 benefitted most from adjuvant treatment, with longer disease recurrence

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

time (17 vs 12 months; P= 0.007) and survival (36 vs 17 months; P= 0.004).

Conclusions The PNI severity score improves prognostic stratification of resected PDAC and identifies patients who benefit the most from adjuvant treatment.

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

INTRODUCTION

Perineural invasion (PNI), defined as a growth pattern of neoplastic glands around a nerve fiber,[1,2] is mostly present in pancreatic ductal adenocarcinoma (PDAC) compared to other solid tumors and may serve as a route for neoplastic spread. [3]

The role of PNI as a prognostic factor in PDAC has been extensively discussed in the literature, focusing on overall (OS) or disease specific survival (DSS).[4–6] Its prognostic role is more debated when analyzing disease recurrence, both local and distant[7], as most studies are retrospective and heterogeneous. The results of previous studies on the role of neoadjuvant treatment on the rate of PNI are also conflicting. Some proved a lower rate of PNI in patients exposed to neoadjuvant chemotherapy,[8,9] while others did not show any difference.[7,10,11] Furthermore, no study specifically examined the association between different neoadjuvant treatments and PNI rates.

Another important issue is the grading of PNI, which is simply defined as 'present' or 'absent'. Of note, PNI is morphologically heterogeneous, ranging from infiltration of rare thin fibers to massive invasion and necrosis of large nerve bundles. We recently proposed a PNI severity score based on reproducible histological criteria, that integrates a twofold information, the caliber of the nerve fibers infiltrated by cancer cells and the extent (focal or diffuse) of the phenomenon.[12] The score was developed on a retrospective cohort of 356 patients with substantial agreement among pathologists, and it showed a strong correlation with histological features of aggressiveness. Nerve fiber infiltration is also one of the mechanisms at the basis of pain generation in PDAC, which is linked to poor prognosis. [13–15]

In this study, we sought to prospectively validate the PNI severity score focusing on cancer-

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

recurrence and patients' survival. Moreover, we aimed to perform a conclusive analysis to determine whether neoadjuvant therapy might reduce the incidence or severity of PNI.

METHODS

Study design and data collection

This monocentric prospective study included patients with histologically proven PDAC who underwent surgical resection at the Division of Pancreatic Surgery of San Raffaele Scientific Institute, Milan, Italy, between March 2019 and February 2022. The present study received the approval from the institutional review board (IRB) with the number 165/INT/2018. It represents the prospective cohort of the VANISSh study (ClinicalTrials.gov Identifier: NCT04024358) - the retrospective cohort has been previously published.[12]

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines were followed.

Adult patients (>18 years) with localized PDAC undergoing pancreatectomy were included. Exclusion criteria included: presence of synchronous distant metastases and of macroscopically positive surgical margins (R2 resection); patients with complete response after neoadjuvant treatment with no residual cancer cells at final pathological examination; patients who experienced post-operative mortality, defined as any in-hospital death or death within 90 days after resection due to surgery-related complications. At least 12 months of follow up were required unless patients developed recurrence and died before this period.

Data regarding demographics, clinical presentation, neoadjuvant treatment, pathological variables, intraoperative and postoperative course including complications, adjuvant treatment and survival were prospectively collected. The pathologic assessment of pancreatectomy specimens was performed in a systematic fashion as previously described.[12]

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

Neoadjuvant and adjuvant treatment were administered based on multidisciplinary tumor board discussion. As a general concept, neoadjuvant treatment was considered in the setting of biological borderline resectable (BR) tumors and strongly recommended for anatomical BR cancers (please read Supplementary Materials, Supplemental Digital Content 1, http://links.lww.com/SLA/F622 for the definitions).

Adjuvant treatment was administered within 3 months after surgery based on current guidelines.[16]

Patients' follow-up included the evaluation of CA19.9 levels and CT of chest and abdomen before adjuvant treatment, and then every 4 months for the first 2 years, every 6 months for the following three years and yearly thereafter.

An updated analysis was performed on the entire cohort of the VANISSh study, comprising both retrospective and prospective patients (n = 656), with the inclusion of the most recent follow-up data.

Outcome measures

The primary aim was to analyze if the severity of PNI score correlated with event-free survival (EFS), defined as cancer recurrence. Timing and pattern of recurrence were also of interest. Recurrence was considered as the first site of relapse, radiologically demonstrated, exemplified into local, systemic recurrence or both. Local recurrence was defined as recurrence in the pancreatic remnant or in the surgical bed, i.e. the soft tissue along the hepatic, celiac, superior mesenteric artery, portal or superior mesenteric vein or in the retroperitoneum.

Secondary aims included: i) a study on the association between neoadjuvant treatment and PNI, with a specific focus on assessing the impact of the various treatment regimens; ii) impact of PNI score on DSS.

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

Pathology and PNI score

Two experienced pancreatic pathologists (M.S.L and C.D.) examined all the pathological specimens. All tumor tissue and all the tissue that could be grossly recognized as tumor bed were submitted for histological examination. PNI was defined as the growth of neoplastic cells around nerve fibers, and it was determined by examination of the H&E stained histological slides. The abutment of the nerve fiber by the neoplastic glands was not considered as true PNI. All the sections in which the tumor was present were further evaluated to generate the PNI score that is: 0: absent; 1: presence of neoplastic gland(s) along nerves <3 mm in caliber; 2: massive perineural infiltration (≥20 distinct nerve fibers infiltrated by tumor) and/or neoplastic infiltration of nerve fibers ≥3 mm and/or the presence of necrosis of the nerve bundle invaded by tumor (please see Supplemental Digital Content 2, http://links.lww.com/SLA/F622).[12] Tumors were staged according to the American Joint Committee on Cancer classification, 8th edition.[17] The presence of cancer cells within 1 mm of any pancreatic margin defined a positive margin (R1).[18]

Statistical analysis and cohort size

Descriptive statistics were calculated to assess patients' demographic and prognostic factors. Comparison between categorical variables was performed using the Chi-square *t* test or Fisher's exact test, if appropriate. All tests were 2-tailed. Kendall's tau-b correlation coefficient was used to measure the strength and the direction of association between PNI and the other pathological variables. EFS was defined as the time (months) from diagnosis to the first evidence of disease recurrence. DSS was defined as the time (months) from diagnosis to disease-related death. EFS and DSS by PNI score were estimated using the Kaplan-Meier

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

method for cumulative probability. Multivariable Cox proportional hazard models with backward conditional analysis were used to study the impact of various factors on EFS and DSS. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistical Software, version 29 (IBM Corporation, Armonk, NY, USA).

The a priori sample size calculation for the whole cohort (300 patients in the prospective cohort, 80% power at the 5% significance level) was based on the expected distribution of patients in the different scoring class of the PNI score, as detailed in the Supplemental Digital Content 1, http://links.lww.com/SLA/F622.

RESULTS

Study population and PNI score

In the study period, 312 patients met the inclusion criteria and were enrolled. Of these, ten patients were excluded due to screening failure for complete response to neoadjuvant treatment at final pathological examination. Two patients were also excluded due to surgery-related mortality (study flowchart, Supplemental Digital Content 3, http://links.lww.com/SLA/F622). Collectively, 300 patients (45,7% female, median age 68 years, 60-73 IQR) constituted the study population. **Table 1** shows the clinico-pathological and surgical data of the entire population. Overall, 256 (86%) of patients presented with PNI, of which 148 (49.3%) had PNI 1 and 110 (36.7%) PNI 2. PNI was present in 26 out of 37 patients (70.3%, P= 0.006, 95%CI 0.142-0.702) presenting with severe pain at diagnosis, requiring opioid medications.

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

There were no differences in terms of PNI score neither between tumors of the head and body-tail of the pancreas (Supplemental Digital Content 4, http://links.lww.com/SLA/F622), nor between patients undergoing neoadjuvant therapy or upfront surgery **(**Supplemental Digital Content 5, http://links.lww.com/SLA/F622). The type of neoadjuvant treatment, divided as platinum-based chemotherapy (mFOLFIRINOX - fluorouracil, leucovorin, oxaliplatin, and irinotecan; PAXG - cisplatin, nab-paclitaxel, gemcitabine, capecitabine) versus non-platinum-based (Gemcitabine-Nab-paclitaxel), did not significantly impact on PNI score, even when stratified according to resectability status at diagnosis (Supplemental Digital Content 6A and 6B, http://links.lww.com/SLA/F622, respectively).

The univariable analysis of the association between pathological factors and PNI score is displayed in **Table 2**. More aggressive pathological features are associated with higher severity of PNI score, with a significant worsening difference observed among all three severity grades. Only one patient with PNI 2 had T1 tumors, and only 12.7% (14) of them did not have lymph node metastases. Patients without PNI were radically resected in 88.1% (37) of the cases, compared to 56.1% (83) in PNI 1 patients and 39.1% (43) in PNI 2 patients.

Cancer Recurrence

None of the patients with PNI 0 experienced early recurrence (defined as recurrence in less than 12 months). Early recurrence was significantly higher in patients with PNI 2 compared to PNI 1 (37 patients, 43.5%, vs 21 patients, 25.6% respectively, p= 0.022). Examining the site of recurrence, 52.3% (45 patients) of recurrences were localized at the liver in patients with PNI 2. This was significantly higher compared to the other classes of PNI (see **Table 2**). No significant association between PNI severity and local recurrence was found (PNI 0 vs PNI 1, 95%CI 0.523-1.947, OR 1.01, p=0.979; PNI 1 vs PNI 2, 95%CI 0.530-1.220, OR 0.804, p=0.305).

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

Survival analysis

Median follow-up was 36 months (IQR, 27-44). Univariable analysis of factors associated with EFS and DSS in the entire cohort is shown in **Table 3**. PNI 0 patients had the longest EFS (24 months versus 17 months of PNI 1 and 15 months of PNI 2, P <0.01; IQR 12-22), and the longest DSS (57 months versus 51 months of PNI 1 and 32 months of PNI 2, P <0.01; IQR 21-39.75), as depicted in **Figure 1**.

A Cox multivariable model (**Table 3**) showed tumor differentiation grade (HR, 1.387; CI95%, 1.020-1,886; P= 0.037), neoadjuvant treatment (HR 0.579; 95%CI 0.419-0.799; P <0.001) and PNI 2 (HR 2.082, 95%CI 1.229-3.528; P= 0.006) to be independent predictors of recurrence. Regarding DSS, neoadjuvant treatment (HR, 0.667; 95%CI, 0.446-0.996; P= 0.048), presence of lymph node metastases (HR, 2.449; 95%CI, 1.359-4.413; P= 0.003), G3 grading (HR, 2.397; 95%CI 1.613-3.562; P <0.01), PNI 2 (HR 3.304; 95%CI, 1.275-8.563, P= 0.014) were significantly associated with shorter survival.

The effect of adjuvant treatment on cancer recurrence in patients with different scores of PNI was analyzed (**Figure 2**). Adjuvant treatment was associated with increased EFS in PNI 2 patients (17 months [95%CI 15.164-18.836] vs 12 months [95%CI 10.700-13.300]; P = 0.007) and with an increased DSS in PNI 2 patients (36 months [95%CI 29.820-42.180] vs 17 months [95%CI 8.394-25.606]; P= 0.004). In patients who underwent neoadjuvant treatment, the same trend was observed although not statistically significant (EFS: 18 months for patients who received adjuvant treatment [95%CI 16.068-19.932] vs 14 months for patients who did not [95%CI 11.228-16.772], P= 0.138; DSS: 38 months [95%CI 32.124-43.876] vs

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

27 months [95%CI 21.416-32.584], P= 0.201; Supplemental Digital Content 7, http://links.lww.com/SLA/F622).

Finally, the analysis was performed on the entire cohort (n = 656) using updated follow-up data. **(**Supplemental Digital Content 8, http://links.lww.com/SLA/F622). At Cox multivariable model, PNI 2 significantly shortened EFS (HR, 1.511; 95%CI 1.097-2.082; P= 0.011), together with tumor grading, lymph node metastases and severe complications. Regarding DSS, PNI 2 (HR, 1.554; 95%CI 1.043-2.315; P= 0.030), tumor grading, lymph node metastases and ASA score >3 were independent predictors of survival at multivariable analysis.

DISCUSSION

In this study we prospectively validated a PNI severity score[12] proposed for patients undergoing pancreatectomy for pancreatic cancer. PNI is an extremely frequent feature in PDAC, as demonstrated by an incidence of 86% in this cohort, in line with literature data (75-90%).[5] PNI is a heterogeneous phenomenon and the morphological stratification into different categories provided by the score has the advantage of highlighting the prognostic impact of PNI severity. Specifically, PNI 2 was a negative independent predictor for both EFS and DSS compared with PNI 0 and 1. The increasing severity of the score correlated with worsening pathological features such as larger neoplasms, higher rates of lymph nodes metastases and R1 resections. This increase was significant among the different classes of PNI, suggesting a different biological role depending on the severity of the infiltration.

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

Neoadjuvant therapy improves different pathological features of PDAC, but its role on PNI is debated. In a recent multicenter study, no significant differences regarding PNI between patients who received neoadjuvant treatment or upfront surgery were noted (84.1% vs 88.6% respectively, p= 0.008).[7] Other retrospective studies that included more heterogeneous patients' populations showed a possible benefit in reducing PNI after neoadjuvant treatment.[8,19]

In this prospective study, neoadjuvant therapy did not decrease the rate of PNI (89.7% vs 83.9% after treatment, p= 0.332). Even when analyzed according to the type of chemotherapy (i.e. platinum-based regimens vs gemcitabine-nab-paclitaxel) and the resectability status at diagnosis, no impact on PNI was found.

The proposed score is focused on the size of the invaded nerve fiber, which is not altered by neoadjuvant treatment in our experience, evaluated in a homogeneous patient population at baseline; these two aspects might explain the different result compared with other recent studies[20,21] focused on histological response after neoadjuvant treatment in borderline-resectable and locally advanced PDAC. Neoadjuvant treatment can induce significant alterations in the tumor microenvironment, including fibrosis and necrosis, which may alter the appearance of residual viable tumor cells and their relationship with nerves, making accurate PNI quantification highly sensitive to these changes. Prospective targeted studies are needed to conclusively evaluate the association between neoadjuvant therapy and PNI.

PNI 2, along with neoadjuvant treatment, less differentiated tumor (G3) and N+ disease, emerged as an independent prognostic factor for both disease recurrence and survival. PNI severity score allows differentiating groups of patients with substantially different prognosis. This stratification would be lost if PNI was stratified only as 0 vs 1, as is currently done in clinical practice.

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

Patients without PNI showed a remarkable DSS of 57 months compared to the 32 months of PNI 2. This applies also to EFS, with a median of 24 months for PNI 0 patients compared to 15 months for those with PNI 2 score. These data were where confirmed by the analysis of the overall cohort of 656 patients enrolled into the VANISSh trial, where PNI 2 was an independent predictor of poor EFS and DSS.

Regarding a potential correlation between PNI score and site of recurrence, our data revealed that about 50% of patients with more severe PNI (2) experienced hepatic recurrence, known to be associated with worse prognosis.[22] This finding corroborates the observation by Crippa et al. [7], that reported a higher risk of distant recurrence if PNI was present in patients with N0/R0 tumors.

Another relevant result regards EFS. None of the patients without PNI developed early recurrence (<12 months [23]), whereas nearly half of those (43.5%) with PNI 2 and 25.6% of those with PNI 1 experienced early recurrence. This might reflect the fact that the presence of PNI itself is associated with a systemic disease. This is in line with recent literature, where the absence of PNI becomes a predictor of long-term survival.[24–29]

Finally, patients with PNI 2 are the ones who benefit the most from adjuvant treatment, with an increased EFS (17 months vs 12 months; P= 0.007) and DSS (36 months vs 17 months; P= 0.004). Although it is well known that adjuvant treatment improves survival after pancreatectomy, different chemotherapy regimens are available, and nowadays mFOLFIRINOX represents the standard of care.[30] The final decision to carry out adjuvant therapy (and which type) also takes into consideration other factors such as patients' age and their clinical conditions after surgery. In this setting PNI 2 could be considered as a "high

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

risk" feature and its presence would not only represent an indication for treatment but could push the use of more aggressive schemes with multi-agents chemotherapy.

The role of adjuvant therapy in patients who previously underwent neoadjuvant treatment is even more debated. Despite not reaching statistical significance, our data showed an increased EFS and DSS for patients with PNI 2 who underwent adjuvant therapy after neoadjuvant treatment (median EFS: 18 vs 14 months; median DSS: 38 months vs 27 months). This data aligns with recent literature, where in a subgroup of N0 patients who underwent neoadjuvant therapy and surgery, those who benefit the most in terms of prolonged survival from adjuvant treatment are precisely patients presenting with PNI.[31]

Compared to other PNI scoring systems, the proposed score offers a few advantages. It is specifically designed for pancreatic cancer, a key differentiation from one of the first PNI score proposed by Liebl et al.[3] that encompassed all gastrointestinal malignancies. Furthermore, its reliance on a dimensional criterion and standard H&E staining facilitates its routine use, in contrast with multifactorial scores[32,33] that often introduce advanced morphological assessments and increase evaluation time. On the other hand, for an accurate PNI severity evaluation, the examination of the whole tumor specimen by dedicated pancreatic pathologists is mandatory. Soon, this time-consuming aspect will likely be overcome by advancements in artificial intelligence and automated systems, leading to enhanced efficiency and standardization.

LIMITATIONS

This study has some limitations. Despite it is a prospective study, the validation is carried out within a single Institution involving two pathologists. In a previous study we found that Cohen's K was 0.61 (CI 95% 0.33–0.89) suggesting that the interobserver agreement between

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

the pathologists was substantial – of note the entire set of slides of each case was reviewed and the entire tumor was sampled. However, an external multi-institutional validation will be necessary to confirm our findings. Potential extra costs or additional time for PNI score evaluation were not evaluated as well. Another limitation may be sample size. The number of 300 patients (80% power at the 5% significance level) was determined based on the expected distribution of patients across the PNI scoring groups. While this sample size was sufficient to address the primary endpoint, it is possible that certain secondary analyses did not reach statistical significance due to the relatively small size of specific subgroups (i.e. effect of different neoadjuvant chemotherapy regimens on PNI score, correlation between PNI score and recurrence sites, prognostic relevance of adjuvant therapy following neoadjuvant treatment in patients with PNI 2).

CONCLUSIONS

This prospective study validates a proposed PNI score as an independent predictor of recurrence and survival in patients undergoing pancreatectomy for pancreatic cancer.
This score improves the prognostic stratification of resected PDAC and it is useful in the selection of patients who may benefit the most from adjuvant treatment.

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

References

1. Amit M, Na'ara S, Gil Z. Mechanisms of cancer dissemination along nerves. *Nat Rev Cancer*. 2016;16:399–408.
2. Boilly B, Faulkner S, Jobling P, et al. Nerve Dependence: From Regeneration to Cancer. *Cancer Cell*. 2017;31:342–354.
3. Liebl F, Demir IE, Mayer K, et al. The impact of neural invasion severity in gastrointestinal malignancies: A Clinicopathological Study. In: Annals of Surgery:900–908.
4. Belfiori G, Crippa S, Francesca A, et al. Long-Term Survivors after Upfront Resection for Pancreatic Ductal Adenocarcinoma: An Actual 5-Year Analysis of Disease-Specific and Post-Recurrence Survival. *Ann Surg Oncol*. . Epub ahead of print 2021. DOI: 10.1245/s10434-021-10401-7.
5. 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;26:105–115.
6. Schouten TJ, Kroon VJ, Besselink MG, et al. Perineural Invasion is an Important Prognostic Factor in Patients With Radically Resected (R0) and Node-negative (pN0) Pancreatic Cancer. *Ann Surg*. . Epub ahead of print May 6, 2024. DOI: 10.1097/SLA.0000000000006320.
7. 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*.;Publish Ah . Epub ahead of print October 19, 2020. DOI: 10.1097/SLA.0000000000004464.
8. Ferrone CR, Marchegiani G, Hong TS, et al. Radiological and surgical implications of neoadjuvant treatment with FOLFIRINOX for locally advanced and borderline resectable pancreatic cancer. *Ann Surg*. 2015;261:12–7.
9. Chatterjee D, Katz MH, Rashid A, et al. Perineural and Intraneural Invasion in Posttherapy Pancreaticoduodenectomy Specimens Predicts Poor Prognosis in Patients With Pancreatic Ductal Adenocarcinoma. *Am J Surg Pathol*. 2012;36:409–417.
10. 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.
11. Timmermann L, Rosumeck N, Klein F, Pratschke J, Pelzer U, Bahra M MT. Neoadjuvant Chemotherapy Enhances Local Postoperative Histopathological Tumour Stage in Borderline Resectable Pancreatic Cancer – A Matched-Pair Analysis. *Anticancer Res*. 2019;39:5781–5787.
12. Schiavo Lena M, Gasparini G, Crippa S, et al. Quantification of perineural invasion in pancreatic ductal adenocarcinoma: proposal of a severity score system. *Virchows Archiv*. 2023;483:225–235.
13. Bapat AA, Hostetter G, Von Hoff DD, et al. Perineural invasion and associated pain in pancreatic cancer. *Nat Rev Cancer*. 2011;11:695–707.
14. Carr RA, Roch AM, Zhong X, et al. Prospective Evaluation of Associations between Cancer-Related Pain and Perineural Invasion in Patients with Resectable Pancreatic Adenocarcinoma. *Journal of Gastrointestinal Surgery*. 2017;21:1658–1665.
15. Gasparini G, Pellegatta M, Crippa S, et al. Nerves and Pancreatic Cancer: New Insights into A Dangerous Relationship. *Cancers (Basel)*. 2019;11:893.

ACCEPTED

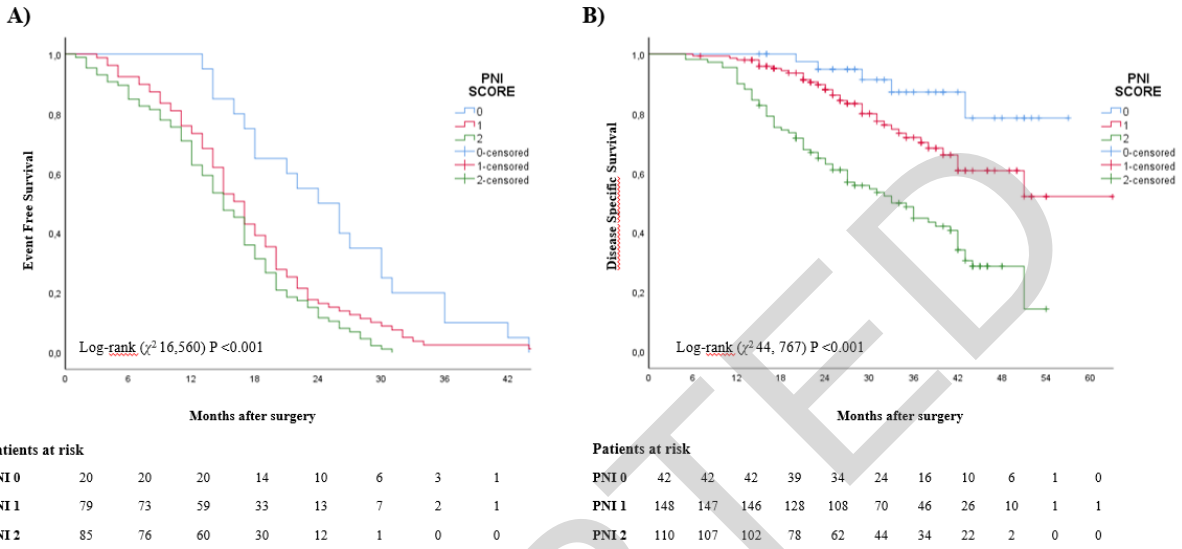
Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

16. Conroy T, Pfeiffer P, Vilgrain V, et al. Pancreatic cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Annals of Oncology*. 2023;34:987–1002.
17. Amin MB, Greene FL, Edge SB, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA Cancer J Clin*. 2017;67:93–99.
18. Verbeke C, Webster F, Brosens L, et al. Dataset for the reporting of carcinoma of the exocrine pancreas: recommendations from the International Collaboration on Cancer Reporting (ICCR). *Histopathology*. 2021;79:902–912.
19. Barnes CA, Chavez MI, Tsai S, et al. Survival of patients with borderline resectable pancreatic cancer who received neoadjuvant therapy and surgery. *Surgery (United States)*. 2019;166:277–285.
20. Mota Reyes C, Teller S, Muckenhuber A, et al. Neoadjuvant Therapy Remodels the Pancreatic Cancer Microenvironment via Depletion of Protumorigenic Immune Cells. *Clin Cancer Res*. 2020;26:220–231.
21. Çifcibaşı K, Goess R, Munkhbaatar E, et al. Pancreatic cancer cells survive in the neural niche during neoadjuvant therapy. *Cancer Lett*. 2025;630:217894.
22. van Oosten AF, Daamen LA, Groot VP, et al. Predicting post-recurrence survival for patients with pancreatic cancer recurrence after primary resection: A Bi-institutional validated risk classification. *Eur J Surg Oncol*. 2023;49:106910.
23. Groot VP, Gemenetzis G, Blair AB, et al. Defining and Predicting Early Recurrence in 957 Patients With Resected Pancreatic Ductal Adenocarcinoma. *Ann Surg*. 2019;269:1154–1162.
24. Cha DE, Yu AT, Khajoueinejad N, et al. Perineural Invasion of Pancreatic Ductal Adenocarcinoma is Associated with Early Recurrence after Neoadjuvant Therapy Followed by Resection. *World J Surg*. 2023;47:1801–1808.
25. Tummers WS, Groen J V., Sibinga Mulder BG, et al. Impact of resection margin status on recurrence and survival in pancreatic cancer surgery. *BJS*. 2019;bjs.11115.
26. Javed AA, Mahmud O, Fatimi AS, et al. Predictors for Long-Term Survival After Resection of Pancreatic Ductal Adenocarcinoma: A Systematic Review and Meta-Analysis. *Ann Surg Oncol*. 2024;31:4673–4687.
27. Belfiori G, Crippa S, Pagnanelli M, et al. Very Early Recurrence After Curative Resection for Pancreatic Ductal Adenocarcinoma: Proof of Concept for a "Biological R2 Definition." *Ann Surg Oncol*. 2024;1–12.
28. Stark AP, Sacks GD, Rochefort MM, et al. Long-term survival in patients with pancreatic ductal adenocarcinoma. *Surgery*. 2016;159:1520–1527.
29. Belfiori G, Crippa S, Aleotti F, et al. Long-Term Survivors after Upfront Resection for Pancreatic Ductal Adenocarcinoma: An Actual 5-Year Analysis of Disease-Specific and Post-Recurrence Survival. *Ann Surg Oncol*. 2021;28:8249–8260.
30. Nießen A, Hackert T. State-of-the-art surgery for pancreatic cancer. *Langenbecks Arch Surg*. 2022;407:443–450.
31. Hammad AY, Hodges JC, Almasri S, et al. Evaluation of Adjuvant Chemotherapy Survival Outcomes among Patients with Surgically Resected Pancreatic Carcinoma with Node-Negative Disease after Neoadjuvant Therapy. *JAMA Surg*. 2023;158:55–62.
32. Ceyhan GO, Bergmann F, Kadihasanoglu M, et al. Pancreatic Neuropathy and Neuropathic Pain—A Comprehensive Pathomorphological Study of 546 Cases. *Gastroenterology*. 2009;136:177-186.e1.
33. Nozzoli F, Catalano M, Messerini L, et al. Perineural invasion score system and clinical outcomes in resected pancreatic cancer patients. *Pancreatology*. 2024;24:553–561.

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

Figure 1. Kaplan-Meier Curves for Event Free Survival (**A**) and Disease Specific Survival (**B**) according to PNI score.



Patients at risk

PNI 0	20	20	20	14	10	6	3	1
PNI 1	79	73	59	33	13	7	2	1
PNI 2	85	76	60	30	12	1	0	0

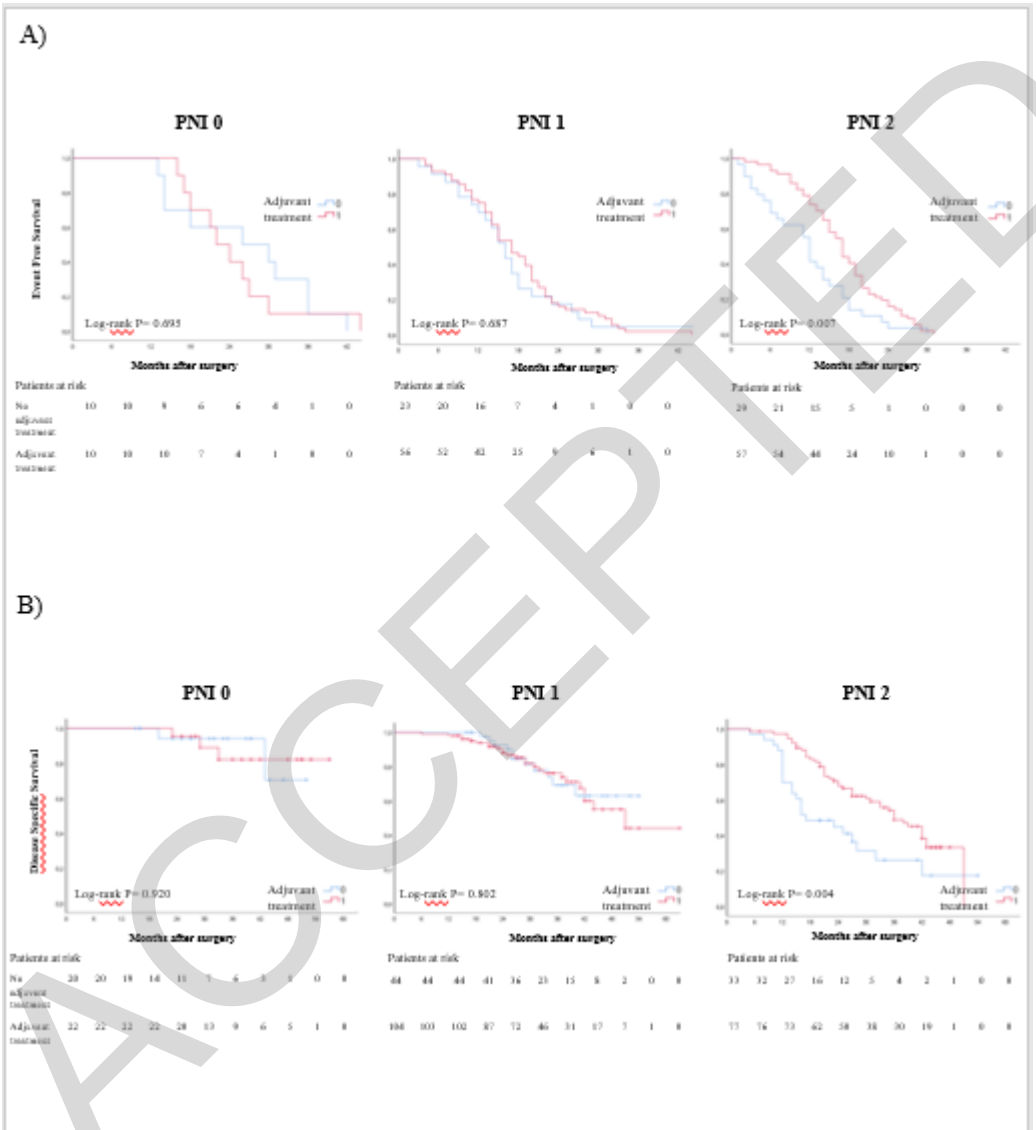
Patients at risk

PNI 0	42	42	42	39	34	24	16	10	6	1	0
PNI 1	148	147	146	128	108	70	46	26	10	1	1
PNI 2	110	107	102	78	62	44	34	22	2	0	0

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

Figure 2. Kaplan-Meier Curves stratified by receipt of adjuvant treatment and PNI score for Event Free Survival (**A**) and for Disease Specific Survival (**B**).



ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

Table 1. Clinical and pathological characteristics of the entire cohort of 300 patients undergoing surgical resection for pancreatic ductal adenocarcinoma.

Variables		**All patients n= 300 (%)**
Sex	*Female*	137 (45.7)
	Male	163 (54.3)
Age, median (interquartile range)		68 (60-73)
Diabetes	*No*	225 (75)
	Yes	75 (25)
Pain at diagnosis	*No*	263 (87.7)
	Yes	37 (12.3)
Jaundice	*No*	164 (54.7)
	Yes	136 (45.3)
Weight Loss	*No*	229 (76.3)
	Yes	71 (23.7)
Tumor location	*Head*	218 (72.7)
	Body-tail	82 (27.3)
CA19.9 at diagnosis (IU/ml)	*≤200*	162 (54)
	>200	93 (31)
	Patients not expressing CA19.9	45 (15)
Neoadjuvant Treatment	*No*	107 (35.7)
	Yes	193 (64.3)
Type of surgical resection	*Pancreaticoduodenectomy*	202 (67.3)
	Distal pancreatectomy	65 (21.7)
	Total pancreatectomy	33 (11)
Preoperative Tumor size (mm)	*≤10*	11 (3.7)
	>10 and ≤20	74 (24.7)
	>20 and ≤30	152 (50.6)
	> 30	63 (21)
Grading	*1*	9 (3)
	2	163 (54.3)
	3	128 (42.7)
pT status	*T1a*	4 (1.3)
	T1b	6 (2)
	T1c	71 (23.7)
	T2	199 (66.3)
	T3	20 (6.7)
pN status	*N0*	86 (28.7)
	N1	113 (37.7)

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

	N2	101 (33.7)
Margin status [18]	*R0*	163 (54.3)
	R1	137 (45.7)
Perineural Invasion	*No*	42 (14)
	Yes	258 (86)
Perineural Invasion Score	*0*	42 (14)
	1	148 (49.3)
	2	110 (36.7)
Vascular invasion	*No*	78 (26)
	Yes	222 (74)
Adjuvant Treatment	*No adjuvant treatment*	97 (32.3)
	Chemotherapy/radiotherapy	77 (25.7)
	Neoadjuvant plus adjuvant treatment	126 (42)

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

Table 2. Association between PNI severity and pathological features.

	PNI severity score n (%)			**0 vs 1 (95%CI)**	**1 vs 2 (95%CI)**
Tumor size	**0**	**1**	**2**	**P <0.001**	**P <0.001**
≤10 [11]	7 (16.7)	3 (2)	1 (0.9)		
>10 and ≤20 [74]	20 (47.6)	44 (29.7)	10 (9.1)	OR 2.416 (1.473-3.963)	OR 2.791 (1.850-4.210)
>20 and ≤30 [152]	10 (23.8)	82 (55.5)	60 (54.5)		
> 30 [63]	5 (11.9)	19 (12.8)	39 (35.5)	ζ 0.243	ζ 0.309
Tumor size				**P <0.001**	**P <0.001**
≤ 20 *[85]*	27 (64.3)	47 (31.8)	11 (10)	OR 3.868 (1.883-7.945)	OR 4.188 (2.054-8.541)
>20 [215]	15 (35.7)	101 (68.2)	99 (90)	ζ 0.277	ζ 0.258
N status				**P <0.001**	**p= 0.002**
***N0** [86]*	27 (64.3)	45 (30.4)	14 (12.7)	OR 2.761 (1.636-4.662)	OR 1.798 (1.279-2.527)
N1[113]	11 (26.2)	57 (38.5)	45 (40.9)	ζ 0.277	ζ 0.198
N2 [101]	4 (9.5)	46 (31.1)	51 (46.4)		
Grading				p= 0.052	p= 0.080
G2 [163]	27 (77.1)	85 (57.8)	51 (46.8)	OR 2.462 (1.048-5.784)	OR 1.559 (0.947-2.567)
G3 [128]	8 (22.9)	62 (42.2)	58 (53.2)	ζ 0.157	ζ 0.109
Margin status				**P <0.001**	**p= 0.015**
R0 [163]	37 (88.1)	83 (56.1)	43 (39.1)	OR 5.956 (2.216-16.006)	OR 1.864 (1.130-3.074)
R1[137]	5 (11.9)	65 (43.9)	67 (60.9)	ζ 0.280	ζ 0.152
Early recurrence				**P <0.001**	**P=0.022**
No [129]	20 (100)	61 (74.4)	48 (56.5)		
*Yes (≤ **12 *months***)[58]*	0	21 (25.6)	37 (43.5)	ζ 0.459	OR 2.461 (1.347-1.534) ζ 0.188
Recurrence site				p= 0.370	**p= 0.012**
Liver [72]	3 (15)	24 (29.6)	45 (52.3)		
Lung [38]	7 (35)	22 (27.2)	9 (10.5)		
Local [51]	8 (40)	21 (25.9)	22 (25.6)		OR 0.738 (0.558-0.977)
Else [26]	2 (10)	14 (17.3)	10 (11.6)		ζ -0.164
Vascular invasion					
No [78]	36 (85.7)	31 (20.9)	11 (10)	**P <0.001**	**p= 0.021**
Yes [222]	6 (14.3)	117 (79.1)	99 (90)	OR 22.645 (8.752-58.591) ζ 0.280	OR 2.385 (1.140-4.988) ζ 0.152

Between []: number of patients; OR: Odds ratio; CI: confidence interval; ζ: Kendall's tau-b correlation coefficient

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

Table 3. Univariable and multivariable analysis for Event-Free Survival (EFS, **A**) and Disease Specific Survival (DSS, **B**).

A)	**Univariable Analysis**			**Multivariable Analysis**		
		Median EFS (months)	P Value	HR	95%CI	P Value
Sex	Male Female	15 17	0.166			
ASA score	1-2 3	18 15	0.147			
Neoadjuvant Treatment	No Yes	13 18	**<0.001**	0.579	0.419-0.799	**<0.001**
Tumor size	≤2 cm >2 cm	17 16	0.245			
N status	0 ≥1	18 15	**0.002**	1.212	0.803 - 1.829	0.360
Grading	1-2 ≥3	16 15	**0.003**	1.387	1.020-1.886	**0.037**
Perineural Invasion	0 1 2	24 17 15	**<0.001**	 1.602 2.082	 0.961-2.671 1.229-3.528	**0.019** 0.071 **0.006**
Vascular Invasion	No Yes	18 16	0.092			
Margin Status	R0 R1	17 17	0.238			
Clavien-Dindo ≥3[30]	No Yes	17 15	0.390			
Adjuvant Treatment	No Yes	14 17	0.138			

B)	**Univariable Analysis**			**Multivariable Analysis**		
		Median DSS (months)	P Value	HR	95% CI	P Value
Sex	Male Female	51 42	0.942			
ASA score	1-2 ≥3	51 42	**0.032**	1.166	0.963-1.412	0.116
Neoadjuvant treatment	No yes	36 44	**0.017**	0.667	0.446-0.996	**0.048**
Tumor size	≤2 cm > 2cm	52 42	**<0.001**	1.377	0.803-2.359	0.245

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.

N status	0 ≥1	54 40	**<0.001**	2.449	1.359-4.413	**0.003**
Grading	1-2 3	45 35	**<0.001**	2.397	1.613-3.562	**<0.001**
Perineural Invasion	0 1 2	57 51 32	**<0.001**	 1.262 3.304	 0.478-3.300 1.275-8.563	**<0.001** 0.639 **0.014**
Vascular Invasion	No Yes	53 42	**<0.001**	1.300	0.604-2.798	0.503
Margin status	R0 R1	54 42	**<0.001**	1.203	0.802-1.805	0.372
Clavien-Dindo ≥3[30]	No Yes	43 42	0.813			
Adjuvant treatment	No Yes	43 43	0.400			

Abbreviations: ASA, American Society of Anesthesiologists; HR, Hazard Ratio.

ACCEPTED

Copyright © 2025 Wolters Kluwer Health, Inc. All rights reserved. Unauthorized reproduction of the article is prohibited.