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Preoperative Elevated Neutrophil-to-Lymphocite Ratio Should Be an Indication for Neoadjuvant Chemotherapy in Patients with Periampullary and Pancreatic Cancers and Normal Levels of Preoperative CA 19.9.

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Preoperative Elevated Neutrophil-to-Lymphocite Ratio Should Be an Indication for Neoadjuvant Chemotherapy in Patients with Periampullary and Pancreatic Cancers and Normal Levels of Preoperative CA 19.9

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Abstract: Background. One of the most important prognostic factors in periampullary and pancreatic cancers is perineural infiltration, whose preoperative detection could be decisive in selecting which patients should not undergo upfront surgery. We evaluated CA 19.9 and the Neutrophil-to-lymphocyte ratio (NLR) as preoperative predictors of perineural invasion (PNI). **Methods**: Retrospective analysis including patients with periampullary and pancreatic cancers who underwent curative resection from January 2013 to August 2023 in our institution. A univariate analysis and multivariate analysis were performed to analyze the association between the CA 19.9 and NLR with the existence of PNI. **Results**: A total of 136 patients were included in the study. PNI was observed in 95 (69.9%) patients. The selected cut-off points were NLR: 2.2 and CA 19.9: 37 U/mL. In univariate analysis, NLR (p=0.001) and CA 19.9 (p=0.006) were statistically significantly associated with PNI. In multivariate analysis, baseline NLR levels (p=0.012; OR: 1.95; 95%CI: 1.16-3.29) as well as CA 19.9 levels (p=0.026; OR: 1.67; 95%CI: 1.06-2.64) remained independent prognostic factors for PNI. The area under de ROC curves was 0.67 for CA 19.9 (p=0.004) and 0.72 for NLR (p<0.001). A sensitivity of 65.9% with a positive predictive value of 81.7% were obtained for CA 19.9, and a sensitivity of 62.1% with a positive predictive value of 84.3% were achieved for NLR. **Conclusion**: Preoperative CA 19.9 and NLR levels seem to be good predictors of PNI. In patients with normal levels of CA 19.9, preoperative NLR above of 2.2 should be an indication for neoadjuvant therapy.

Keywords: neutrophil-to-lymphocyte ratio; CA 19.9; perineural invasion; periampullary cancer; pancreatic cancer

1. Introduction

Periampullary neoplasms are characterized as a heterogeneous group of tumors with different histological lineages but common features, such as anatomical location, diagnosis at advanced stages, and a very poor prognosis despite radical surgery[1]. This term includes tumors of the head of the pancreas, ampulla of Vater, distal bile duct, and duodenum adjacent to the ampullary area.

The incidence is 1,401,450 new cases diagnosed each year worldwide [2]. It is the fourth leading cause of cancer death in the USA and is predicted to become the second leading cause of cancer death by 2030[3]. This disease is associated with a poor prognosis, reflected by a 5-year survival of less than 5% when all stages are grouped together[4].

Several prognostic factors have been identified for periampullary and pancreatic cancer.

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Preoperative Carbohydrate-antigen 19-9 (CA 19.9) levels in the case of adenocarcinomas[5], the surgical margin after resection, venous and/or arterial vascular involvement, or histopathological features such as the existence of affected locoregional nodes or perineural or lymphovascular invasion, are the most relevant [6–8]. Among all these factors, perineural invasion may be the primary independent factor affecting the prognosis of these patients[9–12] particularly in patients with relatively favorable pathological features[13].

In this process, cancer cells spread along the nerves and their sheaths, using the perineural spaces as routes for tumor dissemination[11]. Although it is more frequent in patients with adenocarcinoma[14], perineural infiltration may also be present in tumors of other histological origin, such as neuroendocrine tumors [15].

The identification of perineural invasion prior to surgery may influence the clinical management of these patients. It could facilitate the stratification of risk, allowing for the identification of those patients who would most benefit from neoadjuvant treatments or more aggressive surgical procedures, such as vascular resections. However, perineural involvement is very difficult to detect preoperatively.

There are only a few studies that have analyzed the possible relationship between CA 19.9 and perineural invasion[16], and their results are inconsistent. Alternatively, some studies have demonstrated the usefulness of preoperative multidetector CT to detect extrapancreatic perineural invasion [17–19]. However, these radiological tests are not yet useful to directly detect microscopic perineural invasion, especially in early stages.

On the other hand, in recent years, evidence has been supporting that the systemic inflammatory response could play an important role in relation to the prognosis of different neoplasms[20]. Within these inflammatory reactants, the neutrophil-lymphocyte ratio (NLR) has been gaining prominence[21]. Regarding pancreatic cancer, there are many studies that suggest that the presence of high preoperative levels of the NLR is related to a worse prognosis [21–25]. However, no studies have been previously published in which a possible relationship between this inflammatory marker and perineural infiltration has been analyzed.

The aim of this study was to investigate whether preoperative levels of NLR and CA 19.9 marker could predict the presence of histopathologically objectified perineural invasion after surgery. We hypothesize that these two simple preoperative laboratory tests could behave as good markers of perineural invasion in patients with periampullary and pancreatic cancer.

2. Methods

2.1. Study Design and Participants

A retrospective, cross-sectional, observational study was conducted on all patients who were operated on for neoplasia of the biliopancreatic junction and pancreatic cancer between January 2013 and August 2023 in our institution. The setting was a tertiary referral center that serves a population of approximately 400,000 inhabitants.

Inclusion criteria: patients with a histopathological diagnosis of malignancy, in whom the neoplasia was resected and who had a pre-surgical complete blood count that made it possible to calculate the RNL index.

Exclusion criteria: Patients with non-malignant neoplasms, non-operated patients, and those with incomplete or unavailable clinical records were excluded. The number and characteristics of the excluded patients were not collected.

This study was approved by the Ethics Committee of the hospital (Code 2024-260-1). The work has been reported in line with the STROBE Statement.

2.2. Management of the Patient

After completing a thorough medical history and physical examination, blood tests including a complete blood count, blood biochemistry, liver function tests, and tumor markers CEA and CA 19.9 were requested. In recent years, a nutritional study of the patient has also been added. All patients

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underwent preoperative thoraco-abdominal tomography and/or magnetic resonance imaging to exclude distant metastasis, and to determine the local extent and resectability of the neoplasm. Electrocardiogram, and additional tests were also performed based on each patient's underlying condition.

The patients were then classified according to NCCN guidelines[26] as resectable, borderline or locally advanced and evaluated by a multidisciplinary tumor committee. In cases of diagnostic doubt and/or for patients referred for neoadjuvant therapy, a fine-needle aspiration cytology was performed under conventional ultrasound or endoscopic ultrasonography guidance.

All patients with resectable neoplasms and no risk factors were submitted for upfront surgery. Neoadjuvant treatment was proposed for those patients whose tumor was classified as borderline, locally advanced or resectable but with biological criteria of poor prognosis. Among the latter, CA 19.9 levels of over 200 U/mL, decision that was implemented for patients operated on in the last years.

The systemic treatment administered was FOLFIRINOX or gemcitabine/abraxane depending on the characteristics of the patient after evaluation by an expert oncologist. In some patients, radiotherapy was recommended after chemotherapy.

In patients initially referred for upfront surgery, those who responded or in whom the disease did not progress after neoadjuvant surgery, a cephalic duodenopancreatectomy with Child type reconstruction was performed. In some patients, total pancreaticoduodenectomy was carried out.

The resection specimens were analyzed by a pathologist to determine the histologic nature of the tumors according to a previously established protocol[27].

2.3. Variables, Data Collection and Definitions

Data were retrospectively collected from the computerized database of the hospital and all medical records were reviewed. The following variables were analyzed:

Demographic and clinical data: age, sex, body mass index (BMI), comorbidity measured by Charlson Comorbidity Index, and surgical risk according to ASA classification.

Data laboratory: pre-therapy blood count and Neutrophil-to-Lymphocyte ratio. The NLR was obtained by dividing the absolute number of neutrophils by the absolute number of lymphocytes in the blood count. This variable was categorized according to a cut-off point of 2.2 points obtained from the calculation of the Youden index[28] in our series. Tumoral markers Carbohydrate-antigen 19-9 and Carcinoembryonic antigen (CEA) also were recorded. The reference values for CA 19.9 were 0.00-37 U/mL, and for CEA, they were 0.00-5.00 ng/mL.

Preoperative local staging according to NCCN classification[26]: Based on radiological findings, tumors were classified preoperatively into resectable, borderline and locally advanced tumors[26].

Neoadjuvant therapy: The number of patients who received neoadjuvant therapy (chemo and/or radiotherapy) was collected.

Surgical data: Surgical procedure, surgical approach, postoperative complications according to Clavien-Dindo classification[29], and operative mortality were recorded. Operative mortality was defined as any death occurring within 90 days of surgery or any subsequent death that was determined to be a direct consequence of a postoperative complication.

Histopathological features: The histological type, resection margins, and existence of perineural and/or lymphovascular invasion were recorded. The tumors were classified into four histopathologic stages according to the American Joint Committee on Cancer (AJCC) TNM Staging of Pancreatic Cancer (8th ed.)[30], regardless of the histological type of tumor.

Output measure: Perineural invasion was the variable output. It was defined as the presence of cancer cells along the nerves and/or in the epineural, perineural or endoneural space of the neural sheaths[31] .

2.4. Statistical Analysis

The statistical software package SPSS 29.0 for Windows (IBM Corporation, Armonk, NY, USA) was used to analyze the data.

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Categorical variables were expressed as frequencies and percentages. Continuous quantitative variables were expressed as means and standard deviations (±SD) when the data followed a normal distribution, or medians and interquartile ranges (IQR = 25–75th percentile) when they did not.

Next, a univariate analysis was performed to assess the association between the different predictor variables and perineural infiltration as the output variable. For univariate analysis, the $\chi 2$ test or Fisher's exact test was used in the case of categorical variables. For normally distributed numerical variables, Student's t-test was used to compare means. Mann–Whitney U test was used for non-normal distribution.

Multivariate analysis (logistic regression) was performed to detect independent prognostic factors related to perineural infiltration. The most clinically revealing variables that were statistically significant ($p<0.05$) in the univariate analysis were introduced into the model.

Receiver operative characteristic (ROC) curve analyses were performed to test the discriminatory ability of the NLR and CA 19.9 variables in order to predict perineural infiltration. ROC curves were constructed and the areas under the curve (AUROC) were compared.

For basal NLR, the Youden index (sensitivity + specificity-1)[28] was used to select a threshold for estimating sensitivity and specificity. In the case of CA 19.9, the cut-off point used was 37 U/mL, the laboratory's reference value. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (PPN) were calculated for both variables.

A significance level of $p<0.05$ was considered and the odds ratio (CI 95%) was used as measure of the magnitude of the association.

3. Results

3.1. Patient Characteristics

A total of 136 patients were included in the study, 76 (55.9%) men and 60 (44.1%) women, with a mean age of 64.7 years (SD ±9.9). Mean Body Mass Index was 25.2 (SD ±4.8). Ninety-nine patients (72.8%) presented with medical comorbidities. The most common was essential hypertension, affecting 80 patients (58.8%), followed by diabetes mellitus, affecting 43 patients (31.6%). Most patients (79.4%) were considered as high-risk comorbidity (Charlson index >3). Regarding surgical risk, the most frequent score was ASA III (55.5%).

3.2. Laboratory Values

Preoperative NLR had a wide distribution with a median of 2.22 (IQR: 1.60-3.15). The median tumor marker CA 19.9 was 54.0 U/mL (IQR:8.9-338.4) and the median CEA marker was 2.76 ng/mL (IQR: 1.7-4.7).

We highlight that of the 52 (42.3%) patients with CA 19.9 ≤ 37 U/mL, 24 (46.2%) had NLR levels above 2.2. Additionally, of these 24 patients with normal CA 19.9 levels and NLR > 2.2, 18 (75%) had perineural infiltration.

3.3. Neo and Adjuvant Therapy

During the study period, the use of neoadjuvant therapy (NAT) for PDAC was not utilized routinely at our institution. The majority of patients underwent upfront duodenopancreatectomy. A total of 16 (11.8%) patients received neoadjuvant therapy: 10 only chemotherapy and 6 chemoradiotherapy. Of the total number of operated patients, 89 (65%) patients received adjuvant treatment.

3.4. Tumor Characteristics

The most frequently observed histological type was ductal adenocarcinoma (79 patients, 58.1%), followed by cholangiocarcinoma (22 patients, 16.2%), neuroendocrine tumors (15 patients, 11.0%), intestinal adenocarcinomas 12 (8.8%), and a group of 8 (5.9%) patients labeled as miscellaneous,

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which included 2 pancreatic metastases (colon cancer and breast cancer), and poorly undifferentiated carcinomas (6 cases, 4.4%) in which their exact origin could not be determined.

The most common tumor stage was IIB (37.5%), followed by III (19.9%), IA (18.4), IB (11.8%), IIA (11.0%) and stage IV (1.5%). A patient with ductal adenocarcinoma stage IV was diagnosed after a focus of carcinomatosis appeared in the final specimen, which had not been detected during the surgical procedure. The other case was a patient with a neuroendocrine tumor with a liver metastasis that was resected in the same surgical procedure.

In the definitive histopathological study of the specimen, perineural invasion was observed in 95 patients (69.9%), which varied significantly depending on the type of tumor ($p<0.001$) (Figure 1).

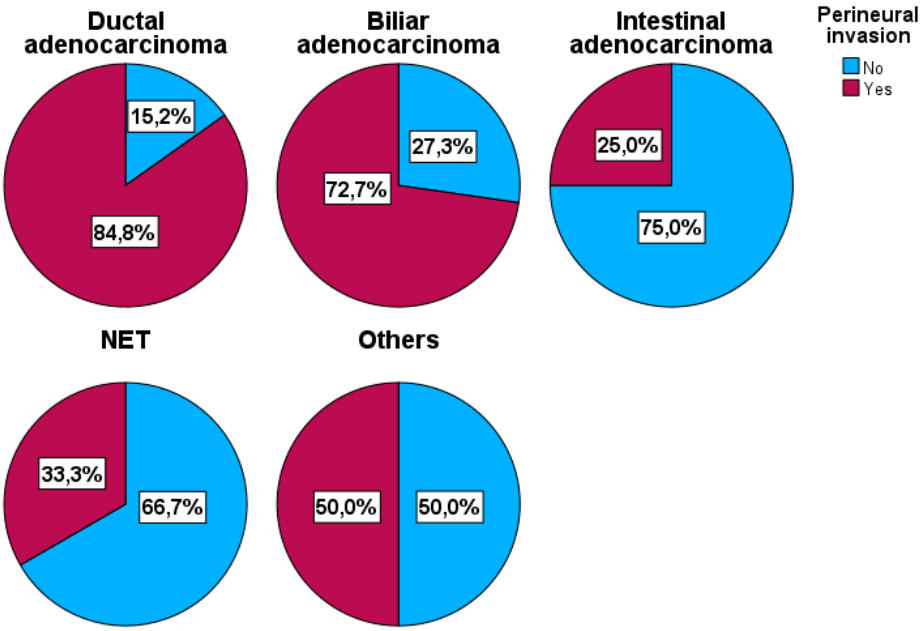

Figure 1. - Perineural invasion depending on the type of tumor.

3.5. Surgical Data

Most patients underwent cephalic duodenopancreatectomy (110 patients, 80.9%), followed by total duodenopancreatectomy (13 patients, 9.6%) and corporocaudal splenopancreatectomy (13 patients, 9.6%). A laparoscopic approach was performed in 24 (17.7%) patients and a robotic approach in 4 (2.9%). The R0 rate was 67.9%.

Postoperative major complications according to the Clavien-Dindo classification system occurred in 50 patients (36.5%). Seven patients (5.1%) died within 90 days from surgery.

3.6. Univariate Analysis

The univariate analysis between the different predictor variables and the presence or not of perineural infiltration are summarized in Table 1. Male patients ($p=0.028$), with higher preoperative tumor markers, both CEA ($p=0.04$) and CA 19.9 ($p=0.006$) (figure 2), elevated baseline NLR levels ($p=0.001$) (figure 3), ductal adenocarcinoma and stages III-IV ($p=0.003$) had statistically significantly more frequent perineural infiltration. In the subgroup of patients with neuroendocrine tumors, NLR values were higher in patients with PNI, but this relationship did not reach statistical significance ($p=0.138$).

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Table 1. - Univariate analysis. NLR: Neutrophil-to-Lymphocite Ratio. BMI: Body Mass Index.

	Total N (%) 136 (100)	**Perineural Invasion**		**P OR (95% CI)**
		No N (%) 41 (30.1)	**Yes N (%) 95 (69.9)**	
Age Mean ± SD	64.7 (±9,9)	62.7 (±10.4)	65.6 (±9.6)	0.120 1.03 (0.99-1.07)
Sex: Men Women	 76 (55.9) 60 (44.1)	 17 (41.5) 24 (58.5)	 59 (62.1) 36 (37.9)	 **0.028** 0.43 (0.21-0.91)
Charlson score Median (IQR)	4.0 (4.0 – 6.0)	4.0 (4.0-6.0)	5.0 (4.0-5.3)	0.460 1.10 (0.86-1.41)
BMI Mean ± SD	25.2 (±4.8)	26.4 (±5.2)	24.6 (±4.6)	0.074 0.93 (0.85-1.01)
CEA ng/ml Median (IQR)	2.76 (1.7-4.7)	2.05 (1.4 -4.0)	2.90 (1.7-5.4)	**0.040** 1.22 (1.01-1.48)
CA 19.9 U/mL Median (IQR)	54.0 (8.9-338.4)	13.4 (4.3-102.5)	86.4 (22.9-458.7)	**0.006** 1.67 (1.10-2.54)
Basal NLR Median (IQR)	2.23 (1.6-3.2)	1.81 (1.32-2.29)	2.47 (1.79-3.57)	**0.001** 2.07 (1.33-3.20)
Preoperative resectabilty: Resectable Borderline/Locally advanced	 122 (89.7) 14 (10.3)	 35 (85.4) 6 (14.6)	 87 (91.6) 8 (8.4)	 0.279 0.54 (0.17-1.66)
Neoadjuvant therapy No Yes	 120 (88.2) 16 (11.8)	 34 (82.9) 7 (17.1)	 86 (90.5) 9 (9.5)	 0.213 0.51 (0.18-1.47)
Histopathological type: Ductal Biliary Intestinal Neuroendocrine Others	 79 (58.1) 22 (16.2) 12 (8.8) 15 (11.0) 8 (5.9)	 12 (29.3) 6 (14.6) 9 (22.0) 10 (24.4) 4 (9.8)	 67 (70.5) 16 (16.8) 3 (3.2) 5 (5.3) 4 (4.2)	 **<0.001** 0.64 (0.51-0.78)
Histopathological stage: I-II III-IV	 56 (41.2) 80 (58.8)	 25 (61.0) 16 (39.0)	 31 (32.6) 64 (67.4)	 **0.003** 3.23 (1.51-6.90)
Nodal involvement No Yes	 60 (44.1) 76 (55.9)	 28 (68.3) 13 (31.7)	 32 (33.7) 63 (66.3)	 **<0.001** 4.24 (1.94-9.28)
Resection margin R0 R1	 94 (69.1) 42 (30.9)	 39 /95.1) 2 (4.9)	 55 (57.9) 40 (42.1)	 <0.001 14.18 (3.23-62.19)

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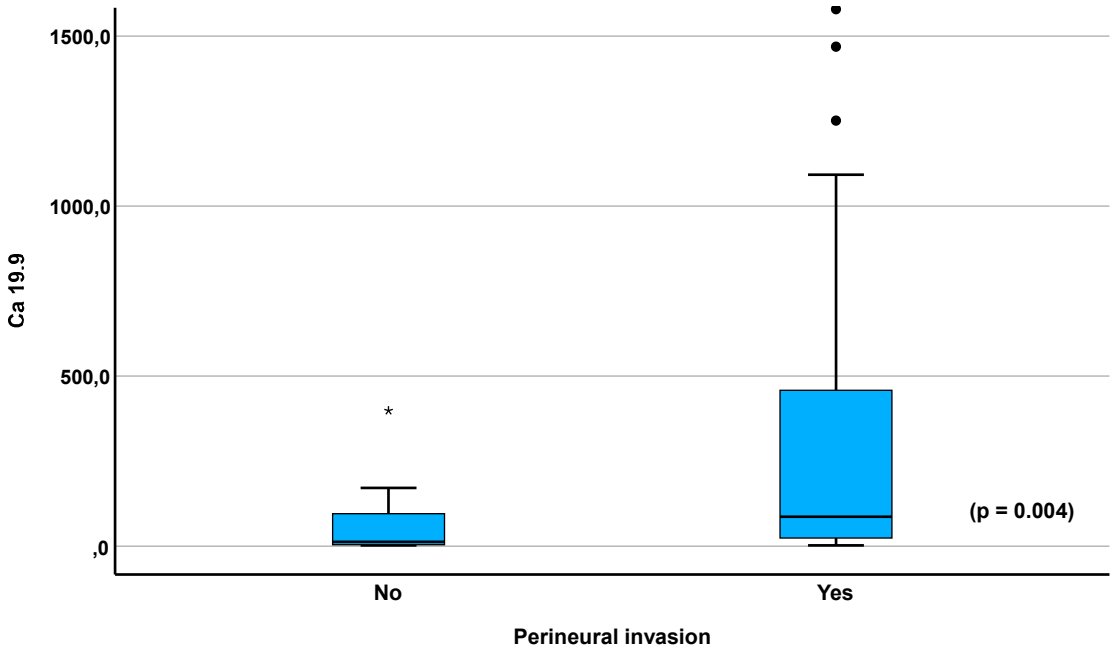

Figure 2. - Boxplot CA 19.9 and perineural invasion.

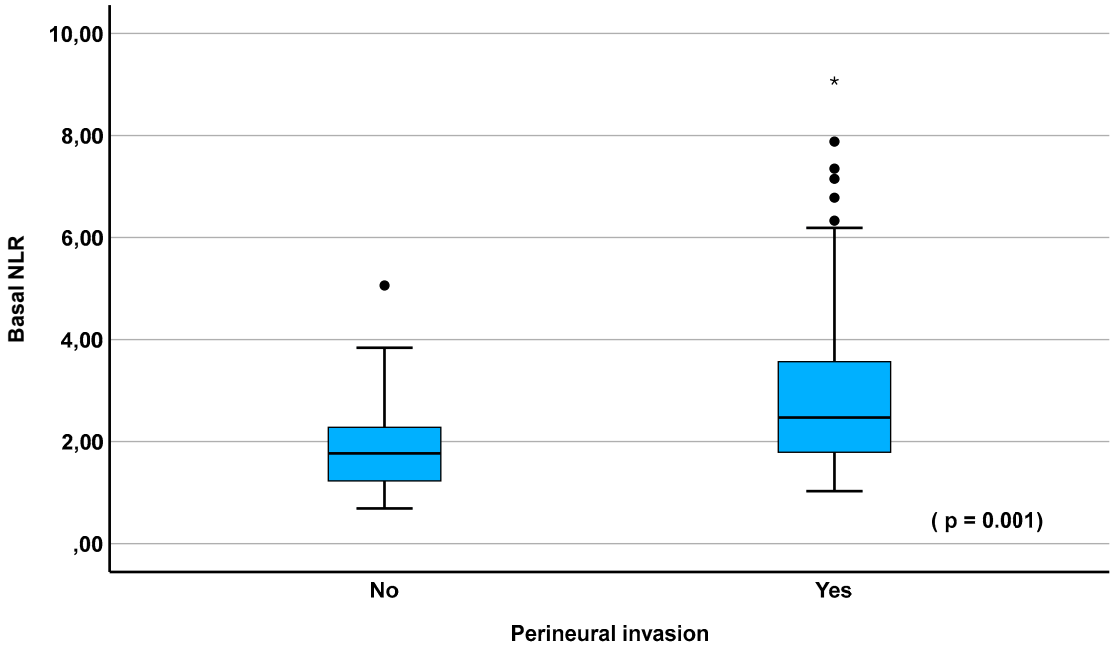

Figure 3. - Boxplot basal NLR and perineural invasion.

3.7. ROC Curves

The ROC curves and Area Under the ROC Curves (AUC) obtained are shown in the figure 4.

Table 2 shows the sensitivity, specificity, PPV and NPV of the two prognostic markers. For the CA 19.9 marker, the upper reference value (37 U/mL) of the normal range was used as the cut-off point. For the NLR, the cut-off point used was the value corresponding to the Youden index obtained from our sample, which was 2.20.

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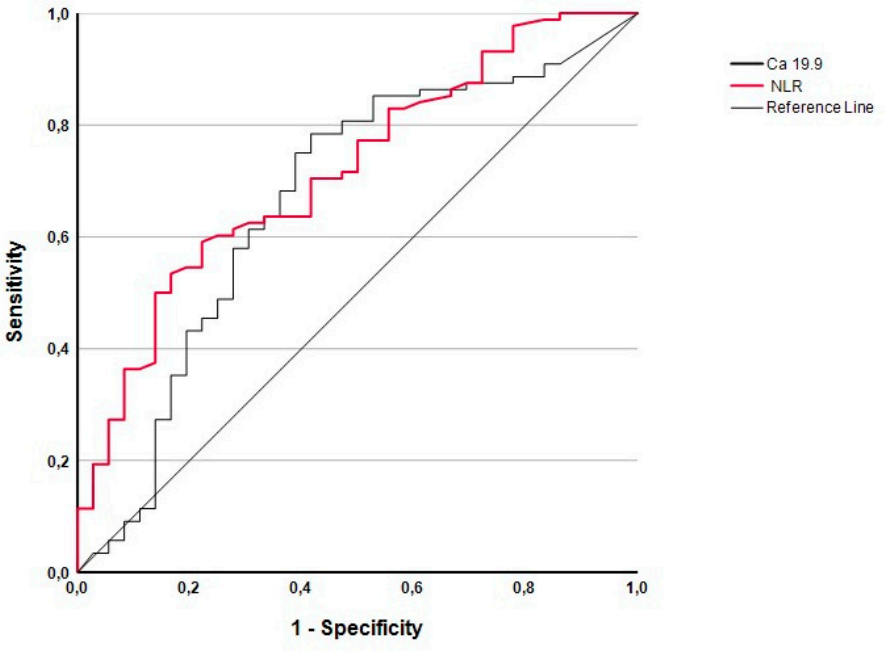

Area Under the Curve				
Variables	Area	SE	p	95% CI
Ca 19.9	0,67	0,06	0,004	0,56 - 0,78
NLR	0,72	0,05	0,000	0,62 - 0,81

Figure 4. - ROC curves and Areas under the Curve of CA 19.9 and NLR.

Table 2. - Diagnostic accuracy of CA 19.9 and NLR with respect to perineural infiltration. NLR: Neutrophil-to-Lymphocite Ratio. PPV: Positive Predictive Value. NPV: Negative Predictive Value.

	Sensitivity	Specificity	PPV	PPN
CA 19.9	65.9%	62.9%	81.7%	42.3%
NLR	62.1%	73.2%	84.3%	45.5%

3.8. Multivariate Analysis

The variables sex, baseline NLR and CA 19.9 values were introduced in a logistic regression model. CA 19.9 values were found positively skewed, and therefore, a logarithmic transformation was performed to convert the variable into approximately normal. Both, baseline NLR levels (p=0.012; OR: 1.95; 95%CI: 1.16-3.29) as well as CA 19.9 levels (p=0.026; OR: 1.67; 95%CI: 1.06-2.64) remained independent prognostic factors for perineural infiltration.

4. Discussion

There is a growing interest in identifying accessible, easy-to-evaluate, and cost-effective prognostic markers in the management of certain tumors, such as periampullary and pancreatic cancer. In these cases, early detection of risk factors for poor outcome is considered essential in order to optimally plan the sequence of application of the different available therapeutic modalities and the radicality of surgery.

This does not usually cause problems in the case of borderline or locally advanced tumors, which are easily detectable with preoperative radiological studies. But it may present difficulties in early-stage tumors. Perineural infiltration, an ominous sign of poor evolution and which may already be

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present in early stages[13], cannot be diagnosed until the resection specimen is studied histologically after surgery.

Among the classic preoperative prognostic factors, CA 19.9 is the main biomarker predicting survival and resectability in biliopancreatic neoplasms. Patients who have elevated CA 19-9 levels at diagnosis are biologically borderline resectable regardless of anatomic resectability, and neoadjuvant systemic therapy is recommended[5].

In our study, in an attempt to detect perineural invasion early, we demonstrate that both the tumor marker CA 19-9, and especially the inflammatory marker LNR, are good predictors of the presence of perineural invasion, and may be useful as an indirect sign of poor prognosis.

Multivariate analysis showed that an NLR above level 2.20 was an independent prognostic factor for perineural involvement in the surgical specimen. A patient with a NLR > 2.20 was 95% more likely to have perineural infiltration. Something similar happened with CA 19.9. An elevation of this marker indicated a 67% likelihood of perineural infiltration.

However, up to 10-15% of patients (Lewis negative phenotype) may fail to produce CA 19.9[32], and its use can be limited by false negatives and false positives, especially in the presence of obstructive jaundice[33].

Moreover, we found a group of 24 patients (19.5% of the total sample) with normal CA 19.9 levels and high NLR levels. Of these, 75% had perineural infiltration. Therefore, it is in these situations when the NLR would become more relevant. If our results are confirmed in other studies, patients diagnosed with pancreatic cancer with high levels of NLR and a normal CA 19.9 before surgery, should be referred to the oncologist for neoadjuvant therapy.

Inflammation has been long recognized as a key aspect in the development and prognosis of different neoplasms[34]. Neutrophils are promoters of angiogenesis and may play a role in tumor cell proliferation and the development of metastases[35]. Signals emanating from cancer cells educate neutrophils to facilitate support networks that lead to its expansive spread. These functions can occur locally in or around the tumor microenvironment, as well as systemically in distant organs[36]. In this sense, NLR should be considered a good systemic inflammatory marker.

In fact, a close relationship between preoperative NLR and long-term survival in pancreatic cancer has been demonstrated[21–25]. Only few authors have not found this association in their series[37,38]. Other inflammatory prognostic markers such as C-reactive protein, alone or in combination with albumin[39,40] or certain scores such as the Systemic Immune-inflammatory index[41] have also been used. This aspect was not analyzed in our study.

The role of perineural invasion in cancer biology has been underestimated, often considered a passive process with less significance compared to lymphatic and hematogenous dissemination. However, new evidence shows neural regulation in cancer and cancer-induced axonogenesis. It is being emphasized that nerves are emerging regulators of cancer initiation, progression, and metastasis[42]. Some authors[12] even suggest that curative pancreatic tumor resection with long-term survival could only be achieved in perineural invasion negative patients.

Perineural infiltration is a complex process that depends on a combination of biological, molecular, and tumor microenvironment factors[43].

The periampullary area and the head of the pancreas have extensive innervation from the autonomic nervous system through the celiac plexus and the superior mesenteric artery[44]. Due to the high neurotropism of tumor cells in pancreatic adenocarcinoma, these plexuses represent a pathway for dissemination and a possible cause of local recurrence and lymphatic metastases[45]. Thus, perineural infiltration is detected in almost 75% of early-stage pancreatic adenocarcinomas, suggesting that it represents an early sign of tumor progression[31,46].

In our series, we have included neuroendocrine and some very poorly differentiated tumors. One third of our patients with neuroendocrine tumors had perineural infiltration. We know that perineural infiltration may be present to varying degrees in neuroendocrine tumors[15], and that it also behaves as an important predictor of survival[47].

In this context, we believe that the location of the tumor may be even more relevant than the histological type of neoplasia in predicting perineural invasion. The complex neural network in the

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periampullary region provides tumors with proximity to nerves, thereby increasing the risk of neural invasion. It has been observed that there is a significant difference in the rate of neural invasion between intrahepatic and extrahepatic anatomical locations, despite both belonging to the category of cholangiocarcinomas.

Two meta-analyses have been performed[10,48] to evaluate the **influence of neural invasion on survival** and tumor recurrence in pancreatic ductal adenocarcinomas. They concluded that PNI appears to be an independent prognostic factor for overall survival, disease-free survival and progression-free survival in these neoplasms. The authors emphasized that PNI should be increasingly considered in patient stratification and in the development of novel therapeutic algorithms.

More recently, other series have been published with a large number of patients with the aim of evaluating the impact of perineural invasion in patients who had undergone pancreatic cancer surgery.

Felsenstein et al[12] found that, out of 571 patients, 93% exhibited tumors with perineural invasion. R0 resection rate was 62.9%., and PNI was observed in the majority of R0 resected patients (91.4%). PNI was associated with worse disease-free and long-term survival. The potential impact of different variables on perineural invasion was also assessed. This variable correlated with advanced tumor stage and lymphatic dissemination in univariate analysis, but in multivariate analysis, only lymph node metastasis was a significant predictor of PNI.

Crippa et al[9] also published the results of a multicenter study assessing the clinical impact of perineural invasion in pancreatic cancer. PNI was found in 87% of 778 patients included in the study. R0 resection was achieved in 62.9%, and perineural invasion was detected in the majority of R0 tumors (91%). PNI was identified in multivariate analysis as a relevant prognostic factor for both tumor recurrence and survival after pancreatoduodenectomy.

In our serie, PNI was detected in 70% of the patients. R0 resection rate was 69.1%, and perineural invasion was observed in only 57.9% of R0 tumors. These differences could be explained by the type of patients included in our series: the majority were initially considered resectable. More complex patients were probably included in the two previously mentioned studies.

Schouten et al[13], in a nationwide observational cohort study of 1630 Dutch patients, confirmed the previous finding that perineural invasion seems to be an important prognostic factor in patients with radically resected (R0) and node-negative (pN0) pancreatic cancer. They concluded that PNI is strongly associated with worse survival, particularly in resected patients with relatively favorable pathological features.

It is challenging to identify perineural invasion before surgery. In our series, perineural infiltration was statistically significantly associated with NLR, CA 19.9, CEA, histological type, tumor stage and lymph node involvement.

We have not found in the literature consulted any reference relating LNR to the presence of perineural invasion. Regarding CA 19.9, our results are comparable with the reported rates of Wang et al[16]. However, CA 19.9 was not related to PNI in the series of Felsenstein et al[12], Crippa et al[9], and Zou et al[49]. We note that the distribution of the CA 19.9 variable was highly skewed and that a logarithmic transformation had to be performed to analyze its significance.

This marker is not applicable to detect PNI in neuroendocrine tumors. In our series, NLR values were also not associated with the presence of PNI. In these cases, higher tumor grades seem to be more associated with the existence of PNI[50].

We confirm, as did other authors, that perineural infiltration was most frequently observed in ductal adenocarcinomas[14], followed by biliary adenocarcinomas, undifferentiated carcinomas, neuroendocrine tumors, and intestinal adenocarcinomas. These differences were statistically significant. Also, a greater number of patients with PNI were detected in the more advanced stages of the disease.

In an attempt to improve the preoperative diagnosis of PNI, some alternatives have been proposed. Zhang et al[51] suggest that PNI could be diagnosed preoperatively by evaluating abdominal enhanced MSCT images with high accuracy, combined with serum tumor marker levels.

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In this study, classification of fat tissue around celiac trunk incorporated with tumor size, CA19-9, CEA, age and sex, showed the highest AUC as 0.939, with specificity of 95.0% and sensitivity of 90.4%. Zou et al[49] developed a nomogram using preoperative total bilirubin, preoperative blood glucose, and preoperative CA19-9. It achieved good AUC values of 0.753 and 0.737 in predicting PNI in training and validation cohorts, respectively. Other authors[18] argue that certain CT findings provides sufficient diagnostic information to detect PNI in patients with resectable tumors of the pancreatic head before surgery.

Regarding the predictive capability of the preoperative markers under consideration, the area under the ROC curve obtained was better for the NLR (0.72) than for the CA 19.9 marker (0.67). In this latter case, AUROC was lower than expected. Nevertheless, with both markers, a PPV greater than 80% was achieved. This implies that 80% of patients with NLR levels >2.2 and/or CA 19.9 levels >37 will have perineural infiltration.

The cut-off point for NLR is highly debated. Forget et al[52], identified that the normal NLR values in an adult, non-geriatric, population in good health are between 0.78 and 3.53. Here we used 2.2 as cut-off point, which was the value that maximized the sum of sensitivity and specificity in our series, according to the Youden index. Interestingly, it was the same NLR cutoff point used by Hasegawa et al[53] in their study. Inoue et al[54] used a NLR cutoff point of 2. However, in most of the series included in the systematic review by Stevens et al[39], cut-off points around 5 were used.

Our study has some limitations. First, it is a single-center, retrospective study with a limited number of patients. Additionally, other potential confounding factors such as nutritional status or symptom duration have not been analyzed. Therefore, these results should be confirmed in larger cohorts in prospective multicenter studies. Nevertheless, these findings emphasize and demonstrate the value of an easily accessible inflammatory marker for the clinician, the NLR, in patients with periampullary and pancreatic cancer, especially in patients with normal CA19.9.

5. Conclusions

In conclusion, the results of this study demonstrate that the Neutrophil Lymphocyte Ratio (NLR) at the time of diagnosis is a simple, easily available, and cost-effective indicator that can be used to suspect the presence of perineural invasion. It would help select those patients who are candidates for neoadjuvant treatment and/or more radical surgeries, especially in patients with normal CA 19.9. Furthermore, this prognostic factor should be useful as stratification parameter for future trials.

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References

1. He J, Ahuja N, Makary MA, Cameron JL, Eckhauser FE, Choti MA, et al. 2564 resected periampullary adenocarcinomas at a single institution: Trends over three decades. HPB. 2014;16(1):83–90.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209–49.
3. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res. 2014 Jun;74(11):2913–21.
4. Gillen S, Schuster T, Meyer Zum Büschenfelde C, Friess H, Kleeff J. Preoperative/neoadjuvant therapy in pancreatic cancer: a systematic review and meta-analysis of response and resection percentages. PLoS Med. 2010 Apr;7(4):e1000267.

5. Bergquist JR, Puig CA, Shubert CR, Groeschl RT, Habermann EB, Kendrick ML, et al. Carbohydrate antigen 19-9 elevation in anatomically resectable, early stage pancreatic cancer is independently associated with decreased overall survival and an indication for neoadjuvant therapy: A national cancer database study. J Am Coll Surg. 2016 Jul 1;223(1):52–65.
6. Kamisawa T, Wood LD, Itoi T, Takaori K. Pancreatic cancer. Lancet. 2016 Jul;388(10039):73–85.
7. Strobel O, Lorenz P, Hinz U, Gaida M, König AK, Hank T, et al. Actual Five-year Survival After Upfront Resection for Pancreatic Ductal Adenocarcinoma: Who Beats the Odds? Ann Surg. 2022 May;275(5):962–71.
8. Bilici A. Prognostic factors related with survival in patients with pancreatic adenocarcinoma. World J Gastroenterol. 2014 Aug;20(31):10802–12.
9. Crippa S, Pergolini I, Javed AA, Honselmann KC, Weiss MJ, Di Salvo F, et al. Implications of Perineural Invasion on Disease Recurrence and Survival After Pancreatectomy for Pancreatic Head Ductal Adenocarcinoma. Ann Surg. 2022 Aug;276(2):378–85.
10. Schorn S, Demir IE, Haller B, Scheufele F, Reyes CM, Tieftrunk E, 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 Mar;26(1):105–15.
11. Wang Y, Liu Z, Tian Y, Zhao H, Fu X. Periampullary cancer and neurological interactions: current understanding and future research directions. Front Oncol. 2024 Mar 19;14:1370111.
12. Felsenstein M, Lindhammer F, Feist M, Hillebrandt KH, Timmermann L, Benzing C, et al. Perineural Invasion in Pancreatic Ductal Adenocarcinoma (PDAC): A Saboteur of Curative Intended Therapies? J Clin Med. 2022 Apr;11(9).
13. Schouten TJ, Kroon VJ, Besselink MG, Bosscha K, Busch OR, Crobach ASLP, et al. Perineural Invasion is an Important Prognostic Factor in Patients With Radically Resected (R0) and Node-negative (pN0) Pancreatic Cancer. Ann Surg [Internet]. 2024 May 6 [cited 2024 May 20]; Available from: https://pubmed.ncbi.nlm.nih.gov/38708885/
14. Liebl F, Demir IE, Mayer K, Schuster T, D'Haese JG, Becker K, et al. The impact of neural invasion severity in gastrointestinal malignancies: a clinicopathological study. Ann Surg. 2014 Nov;260(5):900–7; discussion 907–8.
15. Izumo W, Higuchi R, Furukawa T, Yazawa T, Uemura S, Matsunaga Y, et al. Evaluation of the Significance of Lymphatic, Microvascular and Perineural Invasion in Patients With Pancreatic Neuroendocrine Neoplasms. Cancer Diagnosis & Prognosis [Internet]. 2022 Mar 3 [cited 2024 May 20];2(2):150. Available from: /pmc/articles/PMC8962817/
16. Wang PH, Song N, Shi L Bin, Zhang QH, Chen ZY. The relationship between multiple clinicopathological features and nerve invasion in pancreatic cancer. Hepatobiliary & Pancreatic Diseases International. 2013 Oct 15;12(5):546–51.
17. Noda Y, Pisuchpen N, Parakh A, Srinivas-Rao S, Kinowaki Y, Mino-Kenudson M, et al. Does CT overestimate extra-pancreatic perineural invasion in patients with pancreatic ductal adenocarcinoma following neoadjuvant chemoradiation therapy? Br J Radiol [Internet]. 2024 Feb 28 [cited 2024 May 16];97(1155):607–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/38305574
18. Khristenko E, Shrainer I, Setdikova G, Palkina O, Sinitsyn V, Lyadov V. Preoperative CT-based detection of extrapancreatic perineural invasion in pancreatic cancer. Sci Rep [Internet]. 2021 Jan 19 [cited 2024 May 16];11(1):1800. Available from: http://www.ncbi.nlm.nih.gov/pubmed/33469112
19. Chang ST, Jeffrey RB, Patel BN, DiMaio MA, Rosenberg J, Willmann JK, et al. Preoperative Multidetector CT Diagnosis of Extrapancreatic Perineural or Duodenal Invasion Is Associated with Reduced Postoperative Survival after Pancreaticoduodenectomy for Pancreatic Adenocarcinoma: Preliminary Experience and Implications for Patient Care. Radiology. 2016 Dec;281(3):816–25.
20. Roxburgh CSD, McMillan DC. Role of systemic inflammatory response in predicting survival in patients with primary operable cancer. Future Oncol. 2010 Jan;6(1):149–63.
21. Mei Z, Shi L, Wang B, Yang J, Xiao Z, Du P, et al. Prognostic role of pretreatment blood neutrophil-to-lymphocyte ratio in advanced cancer survivors: A systematic review and meta-analysis of 66 cohort studies. Cancer Treat Rev. 2017 Jul;58.
22. Cheng H, Long F, Jaiswar M, Yang L, Wang C, Zhou Z. Prognostic role of the neutrophil-to-lymphocyte ratio in pancreatic cancer: a meta-analysis. Sci Rep. 2015 Jul;5:11026.
23. Yang JJ, Hu ZG, Shi WX, Deng T, He SQ, Yuan SG. Prognostic significance of neutrophil to lymphocyte ratio in pancreatic cancer: A meta-analysis. World J Gastroenterol [Internet]. 2015 Mar 7 [cited 2024 May 22];21(9):2807–15. Available from: https://www.wjgnet.com/1007-9327/full/v21/i9/2807.htm
24. Mowbray NG, Griffith D, Hammoda M, Shingler G, Kambal A, Al-Sarireh B. A meta-analysis of the utility of the neutrophil-to-lymphocyte ratio in predicting survival after pancreatic cancer resection. HPB [Internet]. 2018 May 1 [cited 2024 May 22];20(5):379–84. Available from: http://www.hpbonline.org/article/S1365182X17311966/fulltext

25. Neumann CCM, Schneider F, Hilfenhaus G, Vecchione L, Felsenstein M, Ihlow J, et al. Inflammation-Based Prognostic Scores in Pancreatic Cancer Patients-A Single-Center Analysis of 1294 Patients within the Last Decade. Cancers (Basel). 2023 Apr;15(8).
26. Park SJ, Jang S, Han JK, Kim H, Kwon W, Jang JY, et al. Preoperative assessment of the resectability of pancreatic ductal adenocarcinoma on CT according to the NCCN Guidelines focusing on SMA/SMV branch invasion. Eur Radiol [Internet]. 2021 Sep 1 [cited 2024 May 27];31(9):6889–97. Available from: https://link.springer.com/article/10.1007/s00330-021-07847-4
27. Gómez-Mateo MDC, Sabater-Ortí L, Ferrández-Izquierdo A. Pathology handling of pancreatoduodenectomy specimens: Approaches and controversies. World J Gastrointest Oncol [Internet]. 2014 Sep 15 [cited 2024 May 16];6(9):351–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25232460
28. Youden W. Index for rating diagnostic tests. Cancer. 1950 Jan;3(1):32–5.
29. Clavien PA, Strasberg SM. Severity Grading of Surgical Complications. Ann Surg. 2009 Aug;250(2):197–8.
30. Allen PJ, Kuk D, Castillo CF Del, Basturk O, Wolfgang CL, Cameron JL, et al. Multi-Institutional validation study of the American Joint Commission on Cancer (8th edition) changes for T and N staging in patients with pancreatic adenocarcinoma. Ann Surg [Internet]. 2017 [cited 2024 May 23];265(1):185. Available from: /pmc/articles/PMC5611666/
31. Gasparini G, Pellegatta M, Crippa S, Lena MS, Belfiori G, Doglioni C, et al. Nerves and Pancreatic Cancer: New Insights into a Dangerous Relationship. Cancers (Basel). 2019 Jun;11(7).
32. Balaban DV, Marin FS, Manucu G, Zoican A, Ciochina M, Mina V, et al. Clinical characteristics and outcomes in carbohydrate antigen 19-9 negative pancreatic cancer. World J Clin Oncol [Internet]. 2022 Jul 24 [cited 2024 May 21];13(7):630–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/36157158
33. Ballehaninna UK, Chamberlain RS. The clinical utility of serum CA 19-9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: An evidence based appraisal. J Gastrointest Oncol. 2012 Jun;3(2):105–19.
34. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008 Jul 24;454(7203):436–44.
35. Liang W, Ferrara N. The Complex Role of Neutrophils in Tumor Angiogenesis and Metastasis. Cancer Immunol Res. 2016 Feb;4(2):83–91.
36. Coffelt SB, Wellenstein MD, De Visser KE. Neutrophils in cancer: neutral no more. Nat Rev Cancer [Internet]. 2016 Jun 24 [cited 2023 Jul 11];16(7):431–46. Available from: https://pubmed.ncbi.nlm.nih.gov/27282249/
37. Chawla A, Huang TL, Ibrahim AM, Hardacre JM, Siegel C, Ammori JB. Pretherapy neutrophil to lymphocyte ratio and platelet to lymphocyte ratio do not predict survival in resectable pancreatic cancer. HPB (Oxford). 2018 May;20(5):398–404.
38. Merlo I, Ardiles V, Sanchez-Clariá R, Fratantoni E, de Santibañes E, Pekolj J, et al. Prognostic Factors in Resected Pancreatic Ductal Adenocarcinoma: Is Neutrophil-Lymphocyte Ratio a Useful Marker? J Gastrointest Cancer. 2023 Jun;54(2):580–8.
39. Stevens L, Pathak S, Nunes QM, Pandanaboyana S, Macutkiewicz C, Smart N, et al. Prognostic significance of pre-operative C-reactive protein and the neutrophil-lymphocyte ratio in resectable pancreatic cancer: a systematic review. HPB (Oxford). 2015 Apr;17(4):285–91.
40. Hajibandeh S, Hajibandeh S, Romman S, Parente A, Laing RW, Satyadas T, et al. Preoperative C-Reactive Protein-to-Albumin Ratio and Its Ability to Predict Outcomes of Pancreatic Cancer Resection: A Systematic Review. Biomedicines [Internet]. 2023 Jul 1 [cited 2024 May 22];11(7). Available from: /pmc/articles/PMC10377035/
41. van 't Land FR, Aziz MH, Michiels N, Mieog JSD, Bonsing BA, Luelmo SAC, et al. Increasing Systemic Immune-inflammation Index During Treatment in Patients With Advanced Pancreatic Cancer is Associated With Poor Survival: A Retrospective, Multicenter, Cohort Study. Ann Surg. 2023 Dec 1;278(6):1018–23.
42. Faulkner S, Jobling P, March B, Jiang CC, Hondermarck H. Tumor Neurobiology and the War of Nerves in Cancer. Cancer Discov. 2019 Jun;9(6):702–10.
43. Li J, Kang R, Tang D. Cellular and molecular mechanisms of perineural invasion of pancreatic ductal adenocarcinoma. Cancer Commun [Internet]. 2021 Aug 1 [cited 2024 May 23];41(8):642. Available from: /pmc/articles/PMC8360640/
44. Yi SQ, Miwa K, Ohta T, Kayahara M, Kitagawa H, Tanaka A, et al. Innervation of the pancreas from the perspective of perineural invasion of pancreatic cancer. Pancreas. 2003 Oct;27(3):225–9.
45. Takahashi T, Ishikura H, Motohara T, Okushiba S, Dohke M, Katoh H. Perineural invasion by ductal adenocarcinoma of the pancreas. J Surg Oncol. 1997 Jul;65(3):164–70.
46. Luchini C, Veronese N, Nottegar A, Riva G, Pilati C, Mafficini A, et al. Perineural Invasion is a Strong Prognostic Moderator in Ampulla of Vater Carcinoma: A Meta-analysis. Pancreas. 2019 Jan;48(1):70–6.

 doi:10.20944/preprints202410.2483.v1

47. Andreasi V, Ricci C, Partelli S, Guarneri G, Ingaldi C, Muffatti F, et al. Predictors of disease recurrence after curative surgery for nonfunctioning pancreatic neuroendocrine neoplasms (NF-PanNENs): a systematic review and meta-analysis. J Endocrinol Invest [Internet]. 2022 Apr 1 [cited 2024 May 20];45(4):705–18. Available from: https://link.springer.com/article/10.1007/s40618-021-01705-2
48. Zhang JF, Hua R, Sun YW, Liu W, Huo YM, Liu DJ, et al. Influence of perineural invasion on survival and recurrence in patients with resected pancreatic cancer. Asian Pac J Cancer Prev. 2013;14(9):5133–9.
49. Zou W, Wu D, Wu Y, Zhou K, Lian Y, Chang G, et al. Nomogram predicts risk of perineural invasion based on serum biomarkers for pancreatic cancer. BMC Gastroenterol [Internet]. 2023 Dec 1 [cited 2024 May 23];23(1). Available from: /pmc/articles/PMC10508025/
50. Shyr BS, Shyr BU, Chen SC, Shyr YM, Wang SE. Impact of tumor grade on pancreatic neuroendocrine tumors. Asian J Surg. 2022 Dec 1;45(12):2659–63.
51. Zhang C, Wang L, Zheng Z, Yao J, He L, Li J. Preoperative diagnosis of perineural invasion in patients with periampullary carcinoma by MSCT imaging: preliminary observations and clinical implications. Abdom Radiol (NY). 2023 Feb;48(2):601–7.
52. Forget P, Khalifa C, Defour JP, Latinne D, Van Pel MC, De Kock M. What is the normal value of the neutrophil-to-lymphocyte ratio? BMC Res Notes. 2017 Dec 3;10(1):12.
53. Hasegawa S, Eguchi H, Tomokuni A, Tomimaru Y, Asaoka T, Wada H, et al. Pre-treatment neutrophil to lymphocyte ratio as a predictive marker for pathological response to preoperative chemoradiotherapy in pancreatic cancer. Oncol Lett. 2016 Feb;11(2):1560–6.
54. Inoue D, Ozaka M, Matsuyama M, Yamada I, Takano K, Saiura A, et al. Prognostic value of neutrophil-lymphocyte ratio and level of C-reactive protein in a large cohort of pancreatic cancer patients: a retrospective study in a single institute in Japan. Jpn J Clin Oncol. 2015 Jan;45(1):61–6.