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PREOPERATIVE CA19.9 LEVEL PREDICTS LYMPH NODE METASTASIS IN RESECTABLE ADENOCARCINOMA OF THE HEAD OF THE PANCREAS: A FURTHER PLEA FOR BIOLOGICAL RESECTABILITY CRITERIA

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Editorial Office

International Journal of Surgery

Rome, May 17, 2022

Invited Article: "Preoperative CA 19.9 level predicts lymph node metastasis in resectable adenocarcinoma of the head of the pancreas: a further plea for biological resectability criteria"

HIGHLIGHTS

- **This is a retrospective multicenter study including a total of 2,034 patients underwent upfront pancreatoduodenectomy for pancreatic ductal adenocarcinoma.**
- **Primary endpoint was to define the relationships between preoperative CA 19.9 levels and the presence of lymph node metastasis at final histology; secondary endpoint of this study was to define a cut-off value of CA 19.9 that accurately predicts the presence of lymph node metastasis.**
- **The present study showed that the standard laboratory cut-off value of CA 19.9 (i.e., 37 U/mL) played a role in the prediction of nodal involvement, elevations of CA 19.9 levels above the standard laboratory cut-off are significantly associated with a high rate of nodal involvement (> 80%).**

- **At multivariable analysis, CA 19.9 was confirmed to be an independent risk factor for nodal involvement; more in detail every logarithmic increase of CA 19.9 resulted in doubling the odds of nodal positivity.**
- **In light of our findings, we can postulate that the presence of R-PDAC with CA 19.9 > 37 U/mL preoperative staging may suggest a shift from a "radiologically-related" upfront resectable status to a "biologically-related" borderline resectable status.**
- **A sub-analysis was performed in different classes of bilirubin, showing no impact of the different cholestasis severity strata in terms of lymph node positivity discrimination.**

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DATA STATEMENT

Data are available upon reasonable request made to the corresponding author.

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ABSTRACT

Introduction: Lymph-nodal involvement (N+) represents an adverse prognostic factor after pancreatoduodenectomy (PD) for pancreatic adenocarcinoma (PDAC). Preoperative diagnostic and staging modalities lack sensitivity for identifying N+. This study aimed to investigate preoperative CA19.9 in predicting the N+ stage in resectable-PDAC (R-PDAC).

Methods: Patients included in a multi-institutional retrospective database of PDs performed for R-PDAC from January 2000 to June 2021 were analyzed. A preoperative laboratory value of CA19.9 > 37 U/L was used in univariate and multivariate logistic regression analysis to determine a possible association with N+. Additionally, different cut-offs of CA19.9 related to the preoperative clinical T (cT) stage was assessed to evaluate the risk of N+.

Results: A total of 2034 PDs from thirteen centers were included in the study. CA19.9> 37 U/L was significantly associated with higher N+ at univariate and multivariate analysis ($p<0.001$). CA19.9 levels > 37 U/L were associated with N+ in 75.9%, 81.3%, and 85.7% of patients, respectively, in cT1, cT2, and cT3 tumors and with higher cut-off values for all cT stages.

Conclusion: Lymph nodal involvement is strongly related to preoperative CA19.9 levels. Specially in patients staged as cT3 the CA 19.9 could represent a valid and easy tool to suspect nodal involvement. Due to these findings, R-PDAC patients with elevated CA19.9 values should be considered in a more biologically advanced stage.

KEYWORDS

Pancreatic adenocarcinoma, pancreatoduodenectomy, CA 19.9, resectable pancreatic adenocarcinoma, lymph-node involvement, pancreatic cancer markers.

INTRODUCTION

Pancreatic cancer (PDAC) remains the fourth leading cause of death among all malignancies, with increasing incidence in both women and men. (1) Surgical resection is considered the gold standard curative option for PDAC. Unfortunately, up to 80% of patients are not eligible for surgery at the time of diagnosis due to locally advanced disease or systemic spread. (2,3)

Preoperative assessment of biological resectability is gaining momentum instead of still widely used anatomical resectability criteria. (4) A consensus meeting of the International Association of Pancreatology (IAP) introduced several biological factors, including carbohydrate antigen 19.9 (CA 19.9) level, in the definition of borderline resectable PDAC independent from anatomical characteristics. (5)

Moreover, loco-regional lymphadenopathy detected at staging is considered a worrisome feature. (6) Lymph node involvement significantly impacts the survival of patients with upfront resected PDAC. (7) It is suggested that anatomically resectable PDAC (R-PDAC) with suspicious biological features should be considered and treated as borderline resectable PDAC (BR-PDAC).

Unfortunately, preoperative confirmation of metastatic lymph nodes is suboptimal with standard imaging investigations. Several reports highlighted on using MRI, CT, or radiomics to increase the preoperative diagnostic rates of lymph node metastases; however, these radiological tools are nowadays related only to dimensional characteristics. (8–10)

The role of CA 19.9 at different cut-offs in predicting lymph node involvement, or margin status after surgery, is primarily debated in the literature with conflicting results. (11,12)

We hypothesised that R-PDAC patients with radiologically negative nodes and higher preoperative CA 19.9 values who undergo radical surgery would be found to have higher rates of positive nodes at pathology.

This study aimed to compare the rates of pathologically positive nodes in two groups of R-PDAC patients with preoperative high or low CA 19.9 values in an international patient cohort.

MATERIALS AND METHODS

Study Design

In this retrospective international multicentre study, participating centres entered data on upfront pancreatoduodenectomy (PD) for R-PDAC performed between January 1, 2000, and June 30, 2021 into the "International Validation of CA19.9 Serum Level in the Prediction of Lymph-Nodes Status in Resectable Pancreatic Ductal Adenocarcinoma in Normo-Albumin Serum Levels Patient (ICALYRA)" project database.

The study protocol was approved by the Ethical Committee of the coordinator centre (PAR 119/21 (OSS)), and the Local Ethics Boards of all the involved centres. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed to create the study. The study has been reported in line with the Strengthening The Reporting Of Cohort Studies in Surgery (STROCSS) criteria, Supplemental Digital Content 1, http://links.lww.com/JS9/B80. (13)

Population

Patients aged ≥ 18 years with R-PDAC undergoing upfront PD for PDAC, with no preoperative radiological suspicion of lymph node metastases.

Endpoints

The primary endpoint of this study was to define the relationships between preoperative CA 19.9 levels and the presence of lymph node metastasis at final histology.

The secondary endpoint of this study was to define a cut-off value of CA 19.9 that accurately predicts the presence of lymph node metastasis.

Data Collection

Data were retrospectively collected from prospective institutional databases of each participating centre. The guarantor of the data quality was the data manager of the study group (AC). Data errors and missingness were identified across the database and solved, when possible, with specific queries.

Definitions

The cut-off value of CA 19.9 to differentiate between high- and low-value patients corresponded to the internationally recognised normal value of 37 U/mL. (14)

The CA 19.9 and bilirubin values reported in the database were obtained during the same preoperative diagnostic phase. In other words, the CA 19.9 value corresponds to the bilirubin value recorded for each patient at that specific moment.

R-PDACs were defined as pancreatic head tumours with a) no preoperative radiological evidence of portal vein and superior mesentery vein involvement, b) clear fat planes around the celiac trunk, hepatic artery, and superior mesentery artery, and c) no distant metastases. (4, 6–12)

BR-PDAC were defined as pancreatic PDAC with venous involvement of the superior mesenteric vein or portal vein with distortion or narrowing of the vein or occlusion of the vein with suitable vessel proximal and distal, allowing for safe resection and replacement. No tumor contact/invasion of superior mesenteric artery, celiac trunk or common hepatic artery.(4,5)

Pathology reporting followed the Union for International Cancer Control (UICC) staging system, 8th edition. (15)

To be included, patients should have undergone at least a standard lymphadenectomy for pancreatoduodenectomy as described by the International Study Group on Pancreatic Surgery (ISGPS). (16)

Positive margins (R1) were defined as tumours located ≤ 1 mm from the resection margin.

Patients diagnosed with distant metastases during the preoperative workup or surgical exploration were not included in the database.

Statistical analysis

Continuous variables were reported as medians and 1st–3rd quartiles (Q1–Q3). Categorical variables were described as numbers and percentages. Comparisons between groups were made using Fisher's exact test or the chi-square test for categorical variables, as appropriate. The Mann-Whitney test was used for continuous variables. Missing data involved less than 10% of patients (SM-Table 1, Supplemental Digital Content 2, http://links.lww.com/JS9/B81). Missing data were

handled with a single imputation method using a median of nearby points imputation. The median instead of the mean was adopted for skewed distribution of the managed variables.

Multivariable logistic regression analysis was run to identify the risk factors for nodal positivity after surgery. The investigated variables were selected using a 'full model' approach. A backward Wald method was adopted for constructing the final model. Odds ratios (OR) and 95% confidence intervals (95%CI) were reported for significant variables.

Only preoperatively available variables were included in this multivariable analysis model with the intent to avoid potential immortal bias phenomena in the construction of the model.

The predictive ability of CA 19.9 level for diagnosing nodal positivity was performed. The area under the curve (AUC) and 95%CI were reported. The diagnostic odds ratio (DOR) value was also evaluated for different CA 19.9 cut-off values, corresponding to previous values reported in the literature. (14)

Variables with a $p < 0.05$ were considered statistically significant. Statistical analyses and plots were run using the SPSS statistical package version 27.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 2,034 patients were included in the study. Patient characteristics are summarised in Table 1. The population was divided into two groups according to the preoperative CA 19.9 value: the low-value group (LVG; n = 596, 29.3%), and the high-value group (HVG; n = 1,438, 70.7%) (Table 1).

HVG patients had a significantly higher median level of total bilirubin (1.6 vs. 1.0 mg/dL; $p < 0.001$), a larger median tumour diameter at radiology (28 vs. 25 mm; $p < 0.001$), and a higher median value of CA 19.9 (255.6 vs. 12.4 U/mL; $p < 0.001$). Preoperative median albumin levels were significantly higher in the LVG patients (3.8 vs. 3.6 mg/dL; $p < 0.001$). No differences were observed between the two groups regarding age, sex, BMI, or diabetes.

The pathological reports confirmed the data showed by the preoperative radiological staging, with a larger median tumour size in the HVG patients (32 vs. 30 mm; $p < 0.001$). The pathological T stage

showed a significant difference, with the T1-T2 stage reported in 82.5 vs. 77.4% in LVG vs. HVG patients, respectively ($p < 0.001$).

The number of harvested lymph nodes was similar in the two groups, with a median number of 27 (Q1–Q3 = 18–38) in the LVG and 26 (Q1–Q3 = 18–37) in the HVG ($p = 0.16$). The N stage was statistically different between the two groups ($p < 0.001$), with an N0 reported in 32.4% vs. 18.4% in LVG vs. HVG patients, respectively. Two-hundred nine (35.5%) LVG patients and 546 (38.0%) HVG patients were classified as N1. Lastly, the N2 stage was present in 194 (32.6%) LVG patients and 627 (43.6%) HVG patients.

One-hundred ninety-nine (33.4%) LVG patients and 634 (44.1%) HVG patients had R1 ($p < 0.001$). In addition, three (0.5%) LVG patients and four (0.3%) HVG patients were classified as R2.

High and low tumour grades were reported in 370 (25.7%) and 201 (14.0%) HGV patients vs. 142 (23.8%) and 54 (9.1%) LVG patients ($p < 0.001$).

Risk factors for positive lymph nodes at pathology

At multivariable logistic regression analysis, log10 CA 19.9 (OR = 1.41, 95%CI = 1.26–1.59; $p < 0.001$) and log10 total bilirubin (OR = 1.29, 95%CI = 1.03–1.61; $p = 0.03$) correlated with positive pathological N status (Table 2). Patient age (OR = 0.98, 95%C I= 0.97–0.98; $p < 0.001$) and albumin levels (OR = 0.74, 95%CI = 0.61–0.99; $p = 0.002$) correlated with statistically significant decreased odds. The radiological tumour dimension did not show any statistical relevance. A separate analysis of risk factors for pathological N2 status showed a positive correlation with log10 CA 19.9 (OR = 1.36, 95%CI = 1.23–1.50; $p < 0.001$) and radiological tumour size (OR = 1.01, 95%CI = 1.01–1.02; $p < 0.001$). Patient age (OR = 0.99, 95%CI = 0.98–0.99; $p = 0.001$) and serum albumin levels (OR = 0.74, 95%CI = 0.64–0.87; $p < 0.001$) also confirmed a potential protective role in the setting of pathological N2 positivity.

Lymph nodes positivity at pathology, preoperative CA 19.9 levels, and radiological T status

Results of a sub-analysis of five patient cohorts stratified according to the CA 19.9 values and the number of harvested nodes, number of positive nodes, the ratio of positive nodes, and N1 and N2 stages, are shown in Table 3.

In all the sub-classes with CA 19.9 > 37 U/mL, the percentage of N+ patients increased from 80.1% to 83.4%. The percentage of N2 patients also increased progressively with the increase of CA 19.9 values, with 52.8% of cases observed when CA 19.9 values were > 1,000 U/mL. Interestingly, the number of harvested nodes was not statistically different in the different sub-classes.

A similar analysis was also performed according to the different radiological T stages (Table 3). This percentage increased with increasing CA 19.9 levels and tumour diameter, with 87.8% being N+ and 59.2% being N2 observed in cT3 patients with CA 19.9 values > 1,000 U/mL.

Prediction of lymph nodes positivity

CA 19.9 showed a sufficient ability to predict the risk of positive lymph nodes (AUC = 0.60, 95%CI = 0.57–0.63; $p < 0.001$) (Table 4). Testing different cut-offs, a value of 37 U/mL showed a sensitivity of 74.4% and a specificity of 42.1%, with the best DOR among the different threshold values tested (DOR = 2.1).

Similar prediction abilities of CA 19.9 were observed for the N2 status (AUC = 0.59, 95%CI = 0.56–0.61; $p < 0.001$) (Table 4). A cut-off of 37 U/mL had a sensitivity and specificity of 76.7% and 33.1%, respectively (DOR = 1.6).

A sub-analysis was performed, in which the prediction ability of CA 19.9 was estimated according to the initial radiological T stage (Table 5). In cT1 and cT2 patients, the diagnostic ability of CA 19.9 for an N+ status was poor-to-sufficient, with an AUC ranging from 0.57 to 0.60. In cT3 patients, the diagnostic ability increased (AUC = 0.71, 95%CI = 0.63–0.79; $p < 0.001$). Using a cut-off of 37 U/mL, sensitivity and specificity were 80.0% and 60.3%, respectively (DOR = 6.1).

Lastly, a similar sub-analysis was performed in which the diagnostic ability of CA 19.9 was estimated according to the post-operative pathological T stage (Table 6). The highest diagnostic

ability was found in pT3 patients (AUC = 0.67, 95%CI = 0.60–0.75; $p < 0.001$). Using a cut-off of 37 U/mL, sensitivity and specificity were 83.1% and 54.9%, respectively (DOR = 6.0).

Analysis of potential confounding factors

Different cut-offs of CA 19.9 were additionally analysed at different bilirubin levels for the prediction of lymph node involvement. As shown in SM-Table 2, Supplemental Digital Content 3, http://links.lww.com/JS9/B82, the percentages of nodal involvement remain substantially similar in the different CA 19.9 strata no matter if the bilirubin levels were normal (≤ 1.50 mg/dL), borderline (1.51–3.00 mg/dL), or high (≥ 3.00 mg/dL).

An additional analysis was performed in CA 19.9 *non-secretors*, namely in the sub-cohort of patients with a CA 19.9 level ≤ 37 U/mL (n = 596). Dividing this sub-cohort into four groups (CA 19.9 0.1–2.0 U/mL, 2.1–9.9 U/mL, 10.0–19.9 U/mL, and 20.0–37.0 U/mL), no differences were found among the strata in terms of nodal involvement (SM-Table 3, Supplemental Digital Content 4, http://links.lww.com/JS9/B83).

DISCUSSION

The present study showed that the standard laboratory cut-off value of CA 19.9 (i.e., 37 U/mL) played a role in the prediction of nodal involvement. Elevations of CA 19.9 levels above the standard laboratory cut-off are significantly associated with a high rate of nodal involvement (> 80%).

The investigation of the standard laboratory cut-off of CA 19.9 represents one of the main strengths and innovative aspects of the study, consenting to adopt a user-friendly and cheap tool during the patient staging workup. Other strengths of the study were the large sample size of the investigated cohort, the established pancreatic surgery experience of all the involved centres, and the reliable statistical method.

At multivariable analysis, CA 19.9 was confirmed to be an independent risk factor for nodal involvement. Interestingly, radiological morphology aspects failed to be independent predictors of nodal involvement, confirming the relevance of biological aspects in the discrimination of patients

at high risk for worrisome features. As previously reported, pathological tumour information was not included in the construction of the model, with the intent to avoid the risk of immortal biases. Our intent was, in fact, to construct a model based only on preoperative features able to investigate the real value of CA 19.9 in the setting of the therapeutic decision-making process.

Every logarithmic increase of CA 19.9 resulted in doubling the odds of nodal positivity. Furthermore, in cT3 patients, the odds increased up to 6 times, allowing us to consider this subclass of patients as the one who could more significantly benefit from the ability of CA 19.9 to predict lymph node involvement.

Another result observed in the present study was that different CA 19.9 values showed similar abilities to predict lymph node positivity in the different cT and pT sub-classes of patients. This result suggests that the reliable clinical role of the T staging should be further improved in correctly predicting lymph node staging with the integration of CA 19.9.

The negative role of lymph node involvement is well-known in upfront resected R-PDAC. (17) The Heidelberg group demonstrated that a 5-year overall survival (OS) was related to the presence of positive lymph nodes and that CA 19.9 levels impacted OS only in early follow-up. Moreover, the authors reported that CA 19.9 < 37 U/mL and the number of positive lymph nodes were the only predictors of 5-year disease-free survival. (7)

In line with this evidence, the IAP consensus meeting stated that patients with anatomically R-PDAC with CA 19.9 levels higher than 500 U/mL, or regional lymph node metastases diagnosed by biopsy or suspicious imaging, should be staged as BR-PDAC beyond the classical anatomical criteria. (4)

Similarly, the National Comprehensive Cancer Network (NCCN) guidelines list CA 19.9 levels, large primary tumours, large regional lymph nodes, excessive weight loss, and extreme pain as high-risk features. R-PDAC with these features should be staged as biological BR-PDAC. (6)

Notably, no unique cut-off value of CA 19.9 was mentioned, and only radiological dimensional criteria were considered suspicious of lymph node involvement.

The results of the present study confirm these aspects, showing how preoperative CA 19.9 levels should be increasingly central in lymph node status evaluation and, therefore, in R-PDAC management using the standard laboratory cut-off.

Preoperative diagnosis of regional lymph node metastases is essential for optimal patient management, as the staging will shift from upfront resectable to BR-PDAC. Despite recent technological advancements, pancreatic cancer preoperative radiological nodal staging still lacks sensitivity. (18)

Although the impact of the lymph node stage and CA 19.9 levels on survival are well-reported in literature, results on the ability of CA 19.9 to predict lymph node involvement are inconsistent.

In a series published by Kim et al., CA 19.9 could not predict nodal involvement in R-PDAC at cut-offs of 93, 500, and 1000 U/mL. (19)

Bergquist et al. analysed a large series of patients from the National Cancer Data Base (NCDB) and demonstrated that CA 19.9 > 37 U/mL harmed OS of early-stage PDAC and that the CA 19.9 elevation was related to significantly higher rates of positive nodes. (20)

These results are in line with the findings of the present study. However, some differences should be noted. First, the main aim of the NCDB study was the OS. Secondly, the study included PDAC regardless of pancreatic tumour location. Thirdly, Bergquist et al. did not perform a matched analysis between CA 19.9 levels and clinical T stages. Lastly, due to the NCDB format, all values of CA 19.9 > 98 U/mL were considered together and did not allow further analysis with different CA 19.9 levels. (20) Regarding this last aspect, the present study showed that the percentages of lymph node involvement remain similar when using different CA 19.9 cut-off levels higher than 37 U/mL. This result demonstrated that CA 19.9 > 37 U/mL is enough to have a very high risk, around 80%, of lymph node involvement also in radiologically R-PDAC.

In light of our findings, we can postulate that the presence of R-PDAC with CA 19.9 > 37 U/mL preoperative staging may suggest a shift from a “radiologically-related” upfront resectable status to a “biologically-related” borderline resectable status. This shift is not without clinical implications,

considering the potential clinical value of neo-adjuvant therapies in this setting. In R-PDAC patients, it has been proven that the purpose of neo-adjuvant therapies is to treat nodal and distant micro-metastatic disease and to assess the biological chemosensitivity of the tumours. (21)

The Dutch Pancreatic Cancer Group demonstrated in the PREOPANC Trial that neo-adjuvant therapies could also affect the number of positive lymph nodes as a secondary endpoint. (22)

The potential role of neo-adjuvant therapies should be considered mainly in the sub-group of patients with cT3 status and CA 19.9 > 37 U/mL, corresponding to the non-negligible percentage of 7.4% in the present cohort. Obviously, prospectively designed trials are required for confirming the relevance of this approach in this sub-class of patients.

Interestingly, the present study also reported a non-negligible rate of patients with normal CA 19.9 (i.e., CA 19.9 *non-secretors*) and lymph node involvement. When we investigated the CA 19.9 *non-secretors*, we were not able to define a specific cut-off of low risk discriminating patients with laboratory normal CA 19.9 values. This group of patients deserves further investigation with the intent to explore other biological markers able to discriminate among non-secretive tumours.

The study presents some limitations. First, the study is retrospective in nature. Second, the re-staging was performed on medical records, and not by a centralised direct imaging review. The 10% of the population that did not express CA 19.9 could play a role in influencing the results. Furthermore, methods used in the preoperative, surgical, and pathology work-up could have changed over the study period, especially regarding the margin status analysis.

A relevant aspect to consider is that CA 19.9 can be influenced by other conditions like nutritional status and cholestasis. (11,20,23) As for the albumin levels, an independent predictive ability of both albumin and CA 19.9 was observed in the multivariable model, excluding a potential collinearity phenomenon and, therefore, excluding a specific correlation between these two variables. As for the bilirubin levels, several studies observed that cholestasis may influence CA 19.9 levels. (20,23) However, Anger et al., logarithmised CA 19.9 and bilirubin to create a model

for the calculation of an individually corrected CA 19.9 level in jaundice patients without changing the CA 19.9 staging ability. (23)

In the present study, we observed that both CA 19.9 and bilirubin were independent predictors for N+ status, excluding potential collinearity phenomena. Moreover, a sub-analysis was performed in different classes of bilirubin, showing no impact of the different cholestasis severity strata in terms of lymph node positivity discrimination.

Another limit was the inevitable differences in surgical techniques among the different participating centres. However, we noted that the median number of harvested lymph nodes was similar in the different centres, indicating a similar quality among the different centres involved (i.e., adequate lymph node sampling).

Lastly, the findings of this study are applied to R-PDAC located in the head of the pancreas and cannot be extended to other patient categories.

CONCLUSIONS

The standard laboratory normal cut-off value for CA 19.9 can predict lymph node metastases in radiological R-PDAC. This result can support surgeons and oncologists in the decision-making process in identifying patients with biologically advanced pancreatic cancer that should move from an upfront resectable stage to a borderline resectable stage.

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Table 1. Characteristics of the investigated population and in the two groups of patients with high and low CA 19.9.

Variables	Entire Cohort (n=2,034)	Low-value CA 19.9 (n=596, 29.3%)	High-value CA 19.9 (n=1,438, 70.7%)	p-value
		Median (1st-3rd Q) or n (%)		
Age, years	70 (63-76)	70 (63-76)	70 (62-76)	0.72
Male sex	1,057 (51.9)	312 (52.3)	745 (51.8)	0.85
BMI ≥30	179 (8.8)	46 (7.7)	133 (9.2)	0.30
T2DM	566 (27.8)	150 (25.2)	416 (28.9)	0.09
ASA 3-4	1,029 (50.5)	294 (49.3)	735 (51.1)	0.47
Pre-operative CA 19.9, U/L	116.1 (30.0-501.0)	12.4 (4.3-24.0)	255.6 (98.4-860.5)	<0.001
Pre-operative albumin, mg/dL	3.6 (3.3-4.1)	3.8 (3.3-4.1)	3.6 (3.2-4.0)	<0.001
Pre-operative total bilirubin, mg/dL	1.4 (0.7-4.0)	1.0 (0.6-2.7)	1.6 (0.7-4.9)	<0.001
Radiological tumor size, mm	27 (20-35)	25 (20-35)	28 (22-35)	<0.001
Radiological T (UICC 8 ed)				
T1	540 (26.6)	208 (34.9)	332 (23.1)	
T2	1,276 (62.7)	321 (53.9)	955 (66.4)	<0.001
T3	218 (10.7)	67 (11.2)	151 (10.5)	
Pathological tumor size, mm	30 (25-40)	30 (22-37)	32 (25-40)	<0.001
Pathological T (UICC 8 ed)				
T1	306 (15.1)	130 (21.8)	176 (12.2)	
T2	1,300 (63.9)	362 (60.7)	938 (65.2)	<0.001
T3	396 (19.4)	94 (15.8)	302 (21.0)	
T4	32 (1.6)	10 (1.7)	22 (1.5)	
Pathological N (UICC 8 ed)				
N0	458 (22.6)	193 (32.4)	265 (18.4)	
N1	755 (37.2)	209 (35.1)	546 (38.0)	<0.001
N2	821 (40.3)	194 (32.6)	627 (43.6)	

Harvested nodes, n	26 (18-37)	27 (18-38)	26 (18-37)	0.16
Positive nodes, n	3 (1-6)	2 (0-5)	3 (1-6)	<0.001
Ratio of nodes positive/harvested, %	10 (0-20)	10 (0-20)	10 (0-30)	<0.001
Margin Status				
R1	833 (40.9)	199 (33.4)	634 (44.1)	<0.001
R2	7 (0.3)	3 (0.5)	4 (0.3)	
Tumor grading				
G1	181 (8.9)	78 (13.1)	103 (7.2)	
G2	1,066 (52.4)	318 (53.4)	748 (52.0)	
G3	512 (25.2)	142 (23.8)	370 (25.7)	<0.001
G4	255 (12.5)	54 (9.1)	201 (14.0)	
No grading evaluable	20 (1.0)	4 (0.7)	16 (1.1)	

Abbreviations: n, number; Q, quartile; BMI, body mass index; T2DM, type-2 diabetes mellitus; ASA, American Society of Anesthesiologists; UICC, Union for International Cancer Control.

Table 2. Multivariable logistic regression analyses for the risk factors of positive nodes at pathology. Backward Wald method.

Variables	Beta	SE	Wald	OR	95.0%CI	p
Any pN status (*)						
Log10 CA 19.9, per 0.1 U/L	0.35	0.06	33.85	1.41	1.26-1.59	<0.001
Patient age, years	-0.02	0.006	11.74	0.98	0.97-0.99	<0.001
Albumin, per 0.1 g/dl	-0.30	0.10	9.26	0.74	0.61-0.90	0.002
Log10 total bilirubin, per 0.1 mg/dL	0.25	0.11	4.84	1.29	1.03-1.61	0.03
Constant	2.90	0.58	25.37	18.24	-	<0.001
pN2 status (**)						
Log10 CA 19.9, per 0.1 U/L	0.31	0.05	36.37	1.36	1.23-1.50	<0.001
Albumin, per 0.1 g/dl	-0.30	0.08	14.63	0.74	0.64-0.87	<0.001
Tumor size, per 1 mm	0.01	0.004	11.03	1.01	1.01-1.02	<0.001
Patient age, years	-0.02	0.005	10.51	0.99	0.98-0.99	0.001
Constant	0.66	0.47	1.97	1.94	-	0.16

Hosmer-Lemeshow test: 0.18 (*); 0.91 (**)

Variables initially introduced into the mathematical models: patient age, patient BMI, T2DM, ASA 3-4, log10 CA 19.9, log10 total bilirubin, albumin, tumor size.

Abbreviations: SE, standard error; OR, odds ratio; CI, confidence intervals; BMI, body mass index; T2DM, type-2 diabetes mellitus; ASA, American Society of Anesthesiologists.

Table 3. Pathologic lymph nodes positivity results according to clustered preoperative CA 19.9 levels in the entire population and in the subclasses of patients according to the radiological T status.

CA 19.9 U/mL	n	N+	N1	N2	Harvested	Number of N+	Ratio N+/harvested, %
Entire population							
≤37	596	403 (67.6)	209 (35.1)	194 (32.6)	27 (18-38)	2 (0-5)	10 (0-20)
38-249	709	568 (80.1)	295 (41.6)	273 (38.5)	25 (17-36)	3 (1-5)	10 (0-20)
250-499	220	181 (82.3)	89 (40.5)	92 (41.8)	26 (17-36)	3 (1-6)	10 (0-30)
500-999	193	161 (83.4)	66 (34.2)	95 (49.2)	26 (19-38)	3 (1-7)	10 (0-30)
≥1,000	316	263 (83.2)	96 (30.4)	167 (52.8)	26 (19-38)	4 (1-8)	10 (10-30)
p-value	-	<0.001	<0.001		0.34	<0.001	<0.001
cT1							
≤37	208	130 (62.5)	71 (34.1)	59 (28.4)	24 (17-34)	1 (0-4)	0 (0-20)
38-249	203	154 (75.9)	85 (41.9)	69 (34.0)	22 (16-33)	2 (1-5)	10 (0-20)
250-499	49	43 (87.8)	23 (46.9)	20 (40.8)	23 (17-33)	2 (1-6)	10 (0-35)
500-999	28	21 (75.0)	10 (35.7)	11 (39.3)	23 (16-33)	2 (0-7)	10 (0-28)
≥1,000	52	39 (75.0)	21 (40.4)	18 (34.6)	23 (16-39)	2 (0-5)	10 (0-20)
p-value	-	0.002	0.03		0.94	0.02	0.009
cT2							
≤37	321	241 (75.1)	129 (40.2)	112 (34.9)	28 (18-38)	2 (1-5)	10 (0-20)
38-249	443	360 (81.3)	186 (42.0)	174 (39.3)	26 (18-38)	3 (1-6)	10 (0-20)
250-499	149	120 (80.5)	56 (37.6)	64 (43.0)	26 (17-36)	3 (1-6)	10 (0-30)
500-999	148	127 (85.8)	51 (34.5)	76 (51.4)	27 (20-38)	4 (2-8)	20 (10-30)
≥1,000	215	181 (84.2)	61 (28.4)	120 (55.8)	26 (19-37)	4 (1-8)	20 (10-30)
p-value	-	0.03	<0.001		0.44	<0.001	<0.001
cT3							
≤37	67	32 (47.8)	9 (13.4)	23 (34.3)	29 (19-49)	0 (0-5)	0 (0-10)
38-249	63	54 (85.7)	24 (38.1)	30 (47.6)	25 (16-41)	3 (2-6)	10 (10-20)
250-499	22	48 (81.8)	10 (45.5)	8 (36.4)	37 (26-55)	3 (2-5)	10 (0-20)
500-999	17	13 (76.5)	5 (29.4)	8 (47.1)	28 (22-47)	3 (1-5)	10 (0-25)

≥1,000	49	43 (87.8)	14 (28.6)	29 (59.2)	29 (20-45)	5 (3-8)	20 (10-30)
p-value	-	<0.0001	<0.001		0.19	0.001	<0.001

Abbreviations: n, number; N+, nodal positivity at pathology; N1, nodal 1 stage; N2, nodal 2 stage; cT1, radiological T1 stage; cT2, radiological T2 stage; cT3, radiological T3 stage.

Table 4. Predictive ability of CA 19.9 for pN and pN2 positivity.

AUC (95.0% CI)	SE	p-value	CA 19.9 U/mL cut-off (centile)	Sensitivity	Specificity	DOR
pN positivity (pN1 plus pN2)						
0.60 (0.57-0.63)	0.02	<0.001	37 (29th)	74.4	42.1	2.1
			250 (64th)	38.4	72.9	1.7
			500 (75th)	26.9	81.4	1.6
			1,000 (85th)	16.8	88.4	1.5
			1,827 (90th)	11.4	95.0	2.6
pN2						
0.59 (0.56-0.61)	0.01	<0.001	37 (29th)	76.7	33.1	1.6
			250 (64th)	43.1	69.1	1.7
			500 (75th)	31.9	79.6	1.8
			1,000 (85th)	20.6	87.7	1.8
			2,578 (92th)	11.4	95.0	2.4

Abbreviations: AUC, area under the curve; SE, standard error; DOR, diagnostic odds ratio; pN1, pathological nodal 1 stage; pN2, pathological nodal 2 stage.

Table 5. Predictive ability of CA 19.9 for pN positivity. Population stratified for radiological T status.

AUC (95.0% CI)	SE	p-value	CA 19.9 U/mL cut-off (centile)	Sensitivity	Specificity	DOR
cT1						
0.60 (0.54-0.65)	0.03	0.001	37 (29th)	66.4	50.3	2.0
			250 (64th)	26.9	83.0	1.8
			500 (75th)	15.5	86.3	1.2
			1,000 (85th)	10.1	91.5	1.2
cT2						
0.57 (0.53-0.61)	0.02	0.001	37 (29th)	76.7	32.0	1.5
			250 (64th)	41.7	66.0	1.4
			500 (75th)	29.9	77.7	1.5
			1,000 (85th)	17.8	86.2	1.4
cT3						
0.71 (0.63-0.79)	0.04	<0.001	37 (29th)	80.0	60.3	6.1
			250 (64th)	46.3	75.9	2.7
			500 (75th)	35.6	82.8	2.7
			1,000 (85th)	27.5	89.7	3.3

Abbreviations: AUC, area under the curve; SE, standard error; DOR, diagnostic odds ratio; cT1, radiological T1 stage; cT2, radiological T2 stage; cT3, radiological T3 stage.

Table 6. Population stratified for pathological T status. Risk for pN positivity.

AUC (95.0%CI)	SE	p-value	CA 19.9 U/mL cut-off (centile)	Sensitivity	Specificity	DOR
pT1						
0.57 (0.50-0.63)	0.03	0.050	37 (29th)	63.5	50.4	1.8
			250 (64th)	23.8	83.2	1.5
			500 (75th)	14.4	88.0	1.2
			1,000 (85th)	7.7	93.6	1.2
pT2						
0.57 (0.53-0.61)	0.02	0.001	37 (29th)	73.9	34.1	1.5
			250 (64th)	38.0	69.0	1.4
			500 (75th)	26.8	79.5	1.4
			1,000 (85th)	16.1	87.2	1.3
pT3						
0.67 (0.60-0.75)	0.04	<0.001	37 (29th)	83.1	54.9	6.0
			250 (64th)	49.5	70.4	2.3
			500 (75th)	35.1	78.9	2.0
			1,000 (85th)	24.6	84.5	1.8
pT4						
0.42 (0.12-0.72)	0.16	0.61	37 (29th)	71.4	0.25	0.01
			250 (64th)	25.0	50.0	0.3
			500 (75th)	17.9	50.0	0.2
			1,000 (85th)	14.3	50.0	0.2

Abbreviations: AUC, area under the curve; SE, standard error; DOR, diagnostic odds ratio; pT1, radiological T1 stage; pT2, pathological T2 stage; pT3, pathological T3 stage; pT4, pathological T4 stage.

SM-Table 1. Missing data in the entire population (N=2,034).

Variables	Missing data (n)	(%)
Age	0	0.0
Sex	0	0.0
BMI≥30	0	0.0
T2DM	0	0.0
ASA Score 3-4	0	0.0
Preoperative CA 19.9	0	0.0
Preoperative albumin	40	2.0
Preoperative total bilirubin	52	2.6
Tumor size at imaging	0	0.0
Tumor size at pathology	0	0.0
Vascular resection	0	0.0
Vascular resection type	0	0.0
R1-R2	0	0.0
Tumor grading	0	0.0
Harvested nodes	0	0.0
Positive nodes	0	0.0

Abbreviations: BMI, body mass index; T2DM, type-2 diabetes mellitus; ASA, American Society of Anesthesiologists.

SM-Table 2. Pathologic lymph nodes positivity results according to clustered preoperative CA 19.9 levels in the subclasses of patients according to the total bilirubin value at the same time of CA 19.9 measurement.

CA 19.9 U/mL	n	N+	N2
Total bilirubin 0.10-1.50 mg/dL			
≤37	378	243 (64.3)	109 (28.8)
38-249	376	295 (78.5)	134 (35.6)
250-499	106	87 (82.1)	45 (42.5)
500-999	87	70 (80.5)	42 (48.3)
≥1,000	116	94 (81.0)	57 (49.1)
p-value		<0.001	<0.001
Total bilirubin 1.51-3.00 mg/dL			
≤37	79	58 (73.4)	36 (45.6)
38-249	128	105 (82.0)	51 (39.8)
250-499	28	25 (89.3)	12 (42.9)
500-999	33	29 (87.9)	16 (48.5)
≥1,000	57	49 (86.0)	27 (47.4)
p-value	-	0.18	0.82
Total bilirubin > 3.00 mg/dL			
≤37	139	102 (73.4)	49 (35.3)
38-249	205	168 (82.0)	88 (42.9)
250-499	86	69 (80.2)	35 (40.7)
500-999	73	62 (84.9)	37 (50.7)
≥1,000	143	120 (83.9)	83 (58.0)
p-value	-	0.15	0.002

Abbreviations: n, number; N+, nodal positivity at pathology; N2, nodal 2 stage.

SM-Table 3. Pathologic lymph nodes positivity results according to clustered preoperative CA 19.9 levels: only patients with CA19.9 ≤37 U/mL.

CA 19.9 U/mL	**n**	**N+**	**N2**
0.1-2.0	113	76 (67.3)	37 (32.7)
2.1-9.9	131	88 (67.2)	31 (31.3)
10.0-19.9	155	97 (62.6)	55 (35.5)
20.0-37.0	197	142 (72.1)	61 (31.0)
p-value	-	0.31	0.82

Abbreviations: n, number; N+, nodal positivity at pathology; N2, nodal 2 stage.