Revised article for consideration for publication in *HPB*

Prognostic value of positive histological margins in patients with pancreatic head ductal adenocarcinoma and lymph node involvement: an international multicentric study

Gaëtan-Romain Joliat[1,2], Pierre Allemann[1], Ismail Labgaa[1], Jesse Sulzer[3], Dionisios Vrochides[3], Alessandro Zerbi[4,5], Gennaro Nappo[4], Julie Perinel[6], Mustapha Adham[6], Michael F. Nentwich[7], Jakob R. Izbicki[7], Nicolas Demartines[1], Markus Schäfer[1].

[1] Department of Visceral Surgery, Lausanne University Hospital CHUV, Lausanne, Switzerland.

[2] Graduate School for Health Sciences, University of Bern, Switzerland.

[3] Division of Hepatobiliary and Pancreatic Surgery, Carolinas Medical Center, Charlotte, USA.

[4] Humanitas Clinical and Research Center, Milan, Italy.

[5] Department of Biomedical Sciences, Humanitas University, Milan, Italy.

[6] Department of Digestive Surgery, Edouard Herriot Hospital, Lyon, France.

[7] Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

This article was presented in parts at the Annual Congress of the Swiss Surgical Society in Bern, Switzerland (May 15-17, 2019) and at the European Pancreatic Club meeting in Bergen, Norway (June 26-29, 2019).

Financial support: none declared

Conflict of interests: none declared

Article type: original article

Manuscript word count: 2618

Keywords: pancreatectomy; cancer; resection status; lymphatic involvement.

Short running head: R1 resection after pancreatoduodenectomy

Correspondence to:

Nicolas Demartines, MD, FACS, FRCS

Prof. and Chairman

Department of Visceral Surgery

Lausanne University Hospital CHUV

Rue du Bugnon 46

1011 Lausanne, Switzerland

Phone: +41 21 314 24 00

Fax: +41 21 314 23 11

Email: demartines@chuv.ch

Requests for reprints should be addressed to the corresponding author.

Abstract (word count: 202)

Background

Resection margin status and lymph node (LN) involvement are known prognostic factors for patients who undergo pancreatoduodenectomy for pancreatic ductal adenocarcinoma (PDAC). This study aimed to compare overall survival (OS) and disease-free survival (DFS) by resection margin status in patients with PDAC and LN involvement.

Methods

A retrospective international multicentric study was performed including four Western tertiary centers. Multivariable Cox analysis was performed to identify prognostic factors of OS and DFS. Median OS and DFS were calculated using Kaplan-Meier curves and compared using log-rank tests.

Results

A cohort of 814 patients with pancreatoduodenectomy for PDAC were analyzed. A total of 651 patients had LN involvement (80%). On multivariable analysis R1 resection was not an independent factor of worse OS and DFS in patients with LN involvement (HR 1.1, p=0.565; HR 1.2, p=0.174). Only tumor size, grade, and adjuvant chemotherapy were associated with OS and DFS. Median OS and DFS were similar between patients with R0 and R1 resections (23 *vs.* 20 months, p=0.196; 15 *vs*. 14 months, p=0.080).

Conclusion

Resection status was not identified as predictor of OS or DFS in PDAC patients with LN involvement. Extensive surgery to achieve R0 resection in such patients might not influence the disease course.

Introduction

For patients undergoing pancreatoduodenectomy for pancreatic ductal adenocarcinoma (PDAC), there are several known prognostic factors.[1-3] Complete surgical resection (R0 resection) has been shown to be associated with a better prognosis in terms of recurrence and survival compared to patients with positive margin resections (R1 or R2 resections).[4–6]

Lymph node (LN) involvement appeared as a major prognostic factor in patients with PDAC.[7,8] LN involvement increases the risk of recurrence and negatively impacts survival.[7]

To date, the impact of resection margin status (R status) in PDAC patients with proven LN involvement remains to be elucidated. The aim of the present study was to assess whether the R status (R0 or R1) had an impact on overall survival (OS) and disease recurrence in patients who underwent upfront pancreatoduodenectomy for PDAC and were shown to have LN involvement.

Methods

Patients

The study period extended from January 1, 2000 to December 31, 2017. All consecutive patients who underwent pancreatoduodenectomy for PDAC were prospectively collected and retrospectively analyzed. PDAC diagnosis was based on histopathology of the surgical specimen. Patients with ampullary tumors or distal cholangiocarcinomas were excluded. Patients treated with neoadjuvant treatment were also excluded. Eligible patients were ≥18 years old and did not refuse to have their data used for research. Patients with R2 resection (macroscopic tumoral invasion of the surgical margins) or presence of distant metastases were excluded. Patients from four international tertiary centers were included: department of Visceral Surgery, Lausanne University Hospital CHUV (Lausanne, Switzerland), division of Hepatobiliary and Pancreatic Surgery, Carolinas Medical Center (Charlotte, USA), Pancreatic Surgery Section, Humanitas Cancer Center (Milan, Italy), and department of Digestive Surgery, Edouard Herriot Hospital (Lyon, France). Data were extracted from prospectively maintained databases of the respective institutions. Patients were followed using regular CT scans and tumor marker (CA19-9) measurements.

This study was approved by the local Ethics committee. It was conducted in accordance with the Declaration of Helsinki and followed the rules of Good Clinical Practice.

Pathological definitions

Tumors were classified using the TNM staging system according to the 8th edition of the AJCC. All centers used during the study period and presently use the "1 mm rule" for the resection margin status as defined by the British Royal College of Pathologists (R0 defined as absence of cancer cells within 1 mm of all resection margins).[9] As these recommendations were first published in 2009, all cases where the above definition was not used were

retrospectively reviewed and regraded if necessary. Axial slicing of the surgical specimen was used in all centers. All resection margins were inked and analyzed to assess medial, lateral, anterior, and posterior tumoral involvement. All institutions performed standard lymphadenectomy during pancreatoduodenectomy as defined by the International Study Group for Pancreatic Surgery.[10] All LN present in the surgical specimens were individually evaluated on histology for the presence of tumoral cells. The entirety of each LN was assessed. After all individual LN were sampled, the connective tissue and peripancreatic fat was assessed to be sure that no LN was missed. Standardization of the methodology to assess the LN was based on the recommendation of the British Royal College of Pathologists (last edition October 2019). Of note, direct invasion of the LN by the primary tumor was classified in this study as N1 as recommended by the British Royal College of Pathologists. The use of the above protocol and definitions permitted to have homogeneity and conformity between the participating centers.

End points and postoperative outcomes

Primary end points were OS rates in the R0 and R1 groups in patients with LN involvement. Secondary end points were disease-free survival (DFS); overall, loco-regional, and distant recurrence rates, respectively.

Severity of postoperative complications occurring during hospital stay or within 30 postoperative days were graded according to Clavien classification.[11] Grade V was defined as perioperative mortality.[11] The Comprehensive Complication Index (CCI) was calculated as well.[12] Date of death was established based on institutional, national, or follow-up data specific to each center.

OS was calculated from operation date to last follow-up date or date of patient death from any cause. DFS was calculated from operation date to first cancer-related event, last

follow-up date or date of patient death from any cause. In patients without event at time of analysis, data were censored on the date of last follow-up. Patients who died perioperatively were not taken into account in the survival analyses.

Recurrences were defined as appearance of recurrent tumoral disease based on radiological or pathological evidences. Recurrences were separated into local (loco-regional) and distant (metastatic disease). Local recurrence was defined as tumor recurrence in the pancreas bed, remnant pancreas or along the pancreatic vascular axis (superior mesenteric artery/vein, portal vein/hepatic artery, celiac trunk). Follow-up was performed every 6 months with measure of CA19-9 and CT-scan during the first 2 years. Then, it was adapted based on the oncologic evolution of the disease. Adjuvant treatment was considered at the beginning of the study period for patients with R1 resections or lymph node involvement on pathology and became a standard after all resection in the past few years.

Statistical analysis

Qualitative variables were compared using chi-square test and quantitative variables using Mann-Whitney *U* test or Student *t*-test depending on the normality of the variables. Survival analysis was performed using Kaplan-Meier curves. Comparisons between survivals (OS and DFS) were made using a log-rank test. For OS and DFS, multivariable analyses using a Cox proportional-hazards model were performed to assess prognostic factors (hazard ratio, HR, with 95% confidence interval, CI). Items were included in the multivariable analysis if p-value on univariable analysis was <0.1. P-values <0.05 were considered statistically significant and all tests were two-sided. All statistical analyses were performed using SPSS Statistics for Mac, version 25 (IBM Corp., Armonk, NY, USA).

Results

Overall cohort

There were 869 patients from four tertiary centers who underwent pancreatoduodenectomy for PDAC during the study period (**Fig. 1**).

Postoperative complications (Clavien grades I-IV) occurred in 57% (464/814) of the patients and median CCI was 0 (IQR 0-27.6, mean CCI 16.1 ± 27.1). Mortality rate (Clavien grade V) was 28/814 (3%). At a median follow-up of 51 months, median OS was 25 months (95% CI: 22-28) and median DFS was 14 months (95% CI: 13-15). Patients with LN involvement had worse median OS and DFS compared to patients without LN involvement (21 [95% CI: 19-23] *vs.* 57 [95% CI: 38-76] months, p<0.001 and 13 [95% CI: 12-14] *vs.* 38 [95% CI: 25-51] months, p<0.001, respectively, **supplementary eFig. 1**). Patients with R0 resection had better median OS and DFS compared to patients with R1 resection (29 [95% CI: 25-34] *vs.* 22 [95% CI: 19-25] months, p=0.001 and 17 [95% CI: 15-19] *vs.* 13 [95% CI: 12-14] months, p<0.001, respectively, **supplementary eFig. 2**). Among the 163 patients with absence of LN involvement, patients with R0 resection had better median OS and DFS compared to patients with R1 resection (58 [95% CI: 38-82] *vs*. 33 [95% CI: 25-41] months, p=0.045 and 45 [95% CI: 33-57] *vs*. 15 [95% CI: 1-34] months, p=0.048, respectively, **supplementary eFig. 3**). On multivariable analysis, R status was an independent predictive factor of OS (HR: 1.7, 95% CI: 1.1-3.2, p=0.042) in patients with absence of LN involvement.

Patients with lymph node involvement

Table 1 shows the preoperative characteristics, demographics, pathological and surgical details, and adjuvant treatments of patients with LN involvement (n=651, 80%) divided according to R status. In the 299 patients with R1 resections, 182 (61%) had microscopic

invasion of the vascular margin (superior mesenteric vein or artery margin), 85 (28%) of the anterior or posterior margin, 27 (9%) of the pancreatic neck, 4 (1%) of the duodenum/stomach, and 1 (1%) of the common bile duct. Moreover, in the group with vascular resection (n=124), median OS were similar between R0 and R1 resections (21 [95% CI: 19-23] *vs*. 23 [95% CI: 18-28] months, p=0.332). Of note, in the group with vascular and R1 resections (n=75), 39 (52%) patients had microscopic invasion of the vascular margin.

At a median follow-up of 58 months, recurrence rates were 209/352 (59%) in the R0 group and 204/299 (68%) in the R1 group. Loco-regional recurrences appeared in 39 (11%) and 42 (14%) patients, distant recurrences (metastases) in 107 (30%) and 98 (33%) patients, and combined loco-regional and distant recurrences in 63 (18%) and 64 (21%) patients in the R0 and R1 groups, respectively. At last follow-up, 427 patients (66%) had died and 68 (10%) were lost to follow-up.

On multivariable Cox regression, tumor size, tumor grade, and adjuvant chemotherapy were predictors of OS. R status was not an independent predictive factor of OS (HR: 1.1, 95% CI: 0.8-1.4, p=0.565, **Table 2**). Median OS were similar between R0 and R1 groups (23 [95% CI: 20-26] *vs*. 20 [95% CI: 17-23] months, p=0.196, **Fig. 2a**). The OS rates at 1, 2, and 3 years were 69%, 37%, and 22% in the R0 group and 69%, 34%, and 13% in the R1 group, respectively.

Median DFS were similar between R0 and R1 groups (15 [95% CI: 13-17] *vs*. 14 [95% CI: 13-15] months, p=0.080, **Fig. 2b**). The DFS rates at 1, 2, and 3 years were 52%, 22%, and 12% in the R0 group and 48%, 19%, and 7% in the R1 group, respectively. Among patients with LN involvement, multivariable analysis showed that R status was not independently associated with DFS (HR: 1.2, 95% CI: 0.9-1.5, p=0.174). Only tumor size,

tumor differentiation, and adjuvant chemotherapy were found to be predictive of DFS (**Table 3**).

In the subgroups of N1 (<4 positive LN, n=330) and N2 (≥4 positive LN, n=301) patients, R0 and R1 patients had similar median OS (N1 subgroup: R0: 27 months, 95% CI: 20-34 *vs*. R1: 23 months, 95% CI: 16-30, p=0.085; N2 subgroup: R0: 18 months, 95% CI: 15-22 *vs*. R1: 17 months, 95% CI: 14-20, p=0.999, **supplementary eFig. 4**). Similar findings were found for median DFS in the N1 subgroup (R0: 18 months, 95% CI: 14-22 *vs*. R1: 16 months, 95% CI: 12-20, p=0.107) and in the N2 subgroup (R0: 13 months, 95% CI: 12-14 *vs*. R1: 13 months, 95% CI: 11-15, p=0.667).

Adjuvant chemotherapy was found as a prognostic factor of OS and DFS (**Tables 2** and **3**). In patients with LN involvement who received adjuvant chemotherapy, no difference in median OS and DFS was found between the R0 and R1 groups (29 [95% CI: 26-32] *vs*. 24 [95% CI: 21-27] months, p=0.112 and 15 [95% CI: 11-19] *vs.* 12 [95% CI: 10-14] months, p=0.062). In the 481 patients who received adjuvant chemotherapy (n=336) or radiochemotherapy (n=145), 74% (354/481) received gemcitabine alone, 16% (75/481) gemcitabine combined with other substances (paclitaxel n=25, capecitabine n=20, cisplatin/epirubicin/capecitabine n=16, oxaliplatine n=9, fluorouracil n=2, erlotinib n=2, cisplatin n=1), 4% (19/481) oxaliplatine combined with other substances (fluorouracil/irinotecan n=9, fluorouracil n=6, capecitabine n=4), and 1% (6/481) various substances (capecitabine n=3, fluorouracil n=2, fluorouracil/irinotecan n=1). In 27 patients (5%), the used regimen was unknown. The median time of adjuvant chemotherapy was 6 months (IQR 5-6).

Discussion

The main findings of this multicentric study were that R0 and R1 resections defined by the "1 mm rule" had similar OS and DFS in patients with LN involvement. Moreover, resection status was not an independent prognostic factor of OS or DFS in these patients.

The results of this study highlight the important prognostic role of LN involvement in patients with PDAC. In the present cohort of 869 patients, LN involvement had worse OS and DFS compared to patients without LN involvement. Moreover, resection status had no influence on recurrence and survival in patients with LN involvement, whereas it had a prognostic role in patients without LN involvement. A recent paper by Tummers *et al.* evaluated the impact on recurrence and survival of the resection margin status after pancreas surgery.[13] The authors found that patients with R1 resection defined by the "1 mm rule" and LN involvement had shorter time to loco-regional recurrence compared to R0 resection and LN involvement.[13] Moreover, they also found that median OS was similar in patients with R0 and R1 resections in case of LN involvement (17 *vs*. 14 months, p=0.068).[13] These results corroborate the findings of the present study regarding OS, but not regarding recurrence. It is important to mention that the study by Tummers *et al.* included all types of pancreatectomy, and even a few patients with metastasis.[13] In a recent bicentric study by Honselmann *et al*., R1 resection defined according to the "1 mm rule" of the British Royal College of Pathologists was independently associated with an increased risk of local recurrence irrespective of LN involvement (285 patients had LN involvement) contrarily to the results of the present study.[7] In their study, PDAC of all pancreatic regions were considered and a higher incidence of tumors were well or moderately differentiated (G1-G2: 69%) compared to the present study (52%). In the present study, LN involvement played a more preponderant prognostic role compared to resection margin status as R0 and R1 resections had similar long-term outcomes (OS and DFS) in patients with LN involvement. LN involvement reflects

tumor aggressiveness and local dissemination. This finding reinforces the importance of multimodal treatment for PDAC and assumes that extended resections to obtain R0 resection might be less critical in case of LN involvement. Preoperatively, the aim of the surgical resection should nevertheless remain obtaining R0 resection.

Prognostic factors of OS and DFS in the multivariable analysis were tumor size, differentiation grade, and adjuvant chemotherapy. Several studies found that tumor size, grade, and chemotherapy were independent predictors of recurrence and survival.[14–16] Groot *et al.* assessed potential predictors of early recurrence (<12 months) in resected patients with PDAC.[14] Several factors were found in particular tumor size >3 cm on CT-scan and poor tumor differentiation similarly as in the present cohort. Tumor grading is therefore a key prognostic marker in PDAC. In the present study, patients with differentiation grade 4 had drastically shorter OS and DFS compared to patients with grade 1 (for R0: from 34 to 12 months and from 21 to 4 months, respectively, data not shown). This emphasizes the importance of having precise histological analysis for a more specific prediction of the oncologic prognosis. Preoperative LN biopsy guided by endoscopic ultrasound or intraoperative LN samplings in case of doubts of LN involvement on preoperative imaging could be performed to further tailor the oncologic approach (e.g., neoadjuvant treatment) and operative strategy (e.g., vascular resection).[17–19]

The results found in this study raise different important points. First, if LN involvement can be preoperatively diagnosed with high accuracy, extensive surgery with vascular or multi-organ resection might not contribute to long-term survival. Hence, it might be of interest to develop a precise management algorithm when LN involvement is found intraoperatively to individually tailor the operative resection. Secondly, if LN involvement is preoperatively proven, all these patients might be candidates for neoadjuvant treatments as positive LN involvement is the initial step to develop systemic disease.[20,21] The results of this

study reinforce the hypothesis that LN status is among the strongest prognostic factors.[6] This study suggests the preponderant prognostic role of LN status and that when LN involvement is present the resection status loses its prognostic value.

The present study has some limitations that need to be acknowledged. First of all, this is a retrospective study, which has inherent biases linked to potential mistakes during data collection, missing data, or follow-up difficulties. Another limitation lies in the diversity of postoperative treatments that were performed in the different centers during the study period.

In conclusion, this multicentric international cohort of patients with PDAC who underwent upfront pancreatoduodenectomy revealed that resection margins (R0 or R1 resection) did not influence OS and DFS in case of LN involvement. In these patients, the quest of R0 resection at any price needs to be redefined.

Acknowledgment: none.

Specific author contributions:

Gaëtan-Romain Joliat: conception, design, acquisition of data, analysis and interpretation, drafting of the article, final approval, and agreement to be accountable for all aspects of the work.

Pierre Allemann: conception, design, analysis and interpretation, critical revision of the article, final approval, and agreement to be accountable for all aspects of the work.

Ismail Labgaa: acquisition of data, analysis and interpretation, drafting of the article, critical revision of the article, final approval, and agreement to be accountable for all aspects of the work.

Jesse Sulzer: acquisition of data, critical revision of the article, final approval, and agreement to be accountable for all aspects of the work.

Dionisios Vrochides: acquisition of data, critical revision of the article, final approval, and agreement to be accountable for all aspects of the work.

Alessandro Zerbi: acquisition of data, critical revision of the article, final approval, and agreement to be accountable for all aspects of the work.

Gennaro Nappo: acquisition of data, critical revision of the article, final approval, and agreement to be accountable for all aspects of the work.

Julie Perinel: acquisition of data, critical revision of the article, final approval, and agreement to be accountable for all aspects of the work.

Mustapha Adham: acquisition of data, critical revision of the article, final approval, and agreement to be accountable for all aspects of the work.

Michael F. Nentwich: acquisition of data, critical revision of the article, final approval, and agreement to be accountable for all aspects of the work.

Jakob R. Izbicki: acquisition of data, critical revision of the article, final approval, and agreement to be accountable for all aspects of the work.

Nicolas Demartines: analysis and interpretation, critical revision of the article, final approval, and agreement to be accountable for all aspects of the work.

Markus Schäfer: conception, design, analysis and interpretation, drafting of the article, critical revision of the article, final approval, and agreement to be accountable for all aspects of the work.

References

1. Wolfgang CL, Herman JM, Laheru DA, et al. Recent progress in pancreatic cancer. CA Cancer J Clin. 2013;63:318–48.

2. Conroy T, Hammel P, Hebbar M, et al. FOLFIRINOX or Gemcitabine as Adjuvant Therapy for Pancreatic Cancer. N Engl J Med. 2018;379:2395–406.

3. Cameron JL, Riall TS, Coleman J, Belcher KA. One thousand consecutive pancreaticoduodenectomies. Ann Surg. 2006;244:10–5.

4. Demir IE, Jäger C, Schlitter AM, et al. R0 Versus R1 Resection Matters after Pancreaticoduodenectomy, and Less after Distal or Total Pancreatectomy for Pancreatic Cancer. Ann Surg. 2018;268:1058–68.

5. Nitta T, Nakamura T, Mitsuhashi T, et al. The impact of margin status determined by the one-millimeter rule on tumor recurrence and survival following pancreaticoduodenectomy for pancreatic ductal adenocarcinoma. Surg Today. 2017;47:490–7.

6. Delpero JR, Jeune F, Bachellier P, et al. Prognostic Value of Resection Margin Involvement After Pancreaticoduodenectomy for Ductal Adenocarcinoma Updates From a French Prospective Multicenter Study. Ann Surg. 2017;266:787–96.

7. Honselmann KC, Pergolini I, Castillo CF-D, et al. Timing But Not Patterns of Recurrence Is Different Between Node-negative and Node-positive Resected Pancreatic Cancer. Ann Surg. (2019). doi: 10.1097/SLA.0000000000003123.

8. Lim JE, Chien MW, Earle CC. Prognostic factors following curative resection for pancreatic adenocarcinoma: a population-based, linked database analysis of 396 patients. Ann Surg. 2003;237:74–85.

9. Campbell F, Smith RA, Whelan P, et al. Classification of R1 resections for pancreatic cancer: the prognostic relevance of tumour involvement within 1 mm of a resection margin. Histopathology. 2009;55:277–83.

10. Tol JAMG, Gouma DJ, Bassi C, et al. Definition of a standard lymphadenectomy in surgery for pancreatic ductal adenocarcinoma: a consensus statement by the International Study Group on Pancreatic Surgery (ISGPS). Surgery. 2014;156:591–600.

11. Dindo D, Demartines N, Clavien P-A. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004;240:205–13.

12. Slankamenac K, Graf R, Barkun J, Puhan MA, Clavien P-A. The comprehensive complication index: a novel continuous scale to measure surgical morbidity. Ann Surg. 2013;258:1–7.

13. Tummers WS, Groen JV, Sibinga Mulder BG, et al. Impact of resection margin status on recurrence and survival in pancreatic cancer surgery. Br J Surg. 2019;106:1055-65.

14. Groot VP, Gemenetzis G, Blair AB, et al. Defining and Predicting Early Recurrence in 957 Patients With Resected Pancreatic Ductal Adenocarcinoma. Ann Surg. 2019;269:1154-62.

15. Hartwig W, Hackert T, Hinz U, et al. Pancreatic cancer surgery in the new millennium: better prediction of outcome. Ann Surg. 2011;254:311–9.

16. Winter JM, Cameron JL, Campbell KA, et al. 1423 pancreaticoduodenectomies for pancreatic cancer: A single-institution experience. J Gastrointest Surg. 2006;10:1199–210.

17. Hewitt MJ, McPhail MJW, Possamai L, Dhar A, Vlavianos P, Monahan KJ. EUS-guided FNA for diagnosis of solid pancreatic neoplasms: a meta-analysis. Gastrointest Endosc. 2012;75:319–31.

18. Lewis JJ, Kowalski TE. Endoscopic Ultrasound and Fine Needle Aspiration in Pancreatic Cancer. Cancer J. 2012;18:523–9.

19. van Rijssen LB, Narwade P, van Huijgevoort NCM, et al. Prognostic value of lymph node metastases detected during surgical exploration for pancreatic or periampullary cancer: a systematic review and meta-analysis. HPB. 2016;18:559–66.

20. Conroy T, Bachet J-B, Ayav A, et al. Current standards and new innovative approaches for treatment of pancreatic cancer. European Journal of Cancer. 2016;57:10–22.

21. Roland C, D Yang A, Katz M, et al. Neoadjuvant Therapy is Associated with a Reduced Lymph Node Ratio in Patients with Potentially Resectable Pancreatic Cancer. Ann Surg Oncol. 2014;22:1168-75.

Figure legends.

Figure 1. Flowchart of the study patients.

Figure 2. Kaplan-Meier curves of overall survival (OS) and disease-free survival (DFS) of patients with lymph node involvement according to resection status.

a) median OS 23 months (R0), 95% CI 20-26 *vs*. 20 months (R1), 95% CI 17-23, p=0.196

b) median DFS 15 months (R0), 95% CI 13-17 *vs*. 14 months (R1), 95% CI 13-15, p=0.080

Supplementary eFigure 1. Kaplan-Meier curves of overall survival (OS) and disease-free survival (DFS) of all patients (n=786, the 28 patients with perioperative death were excluded) according to lymph node involvement.

a) median OS 21 months (N+), 95% CI 19-23 *vs.* 57 months (N0), 95% CI 38-76, p<0.001

b) median DFS 13 months (N+), 95% CI 12-14 *vs.* 38 months (N0), 95% CI 25-51, p<0.001

Supplementary eFigure 2. Kaplan-Meier curves of overall survival (OS) and disease-free survival (DFS) of all patients (n=786, the 28 patients with perioperative death were excluded) according to resection status.

a) median OS 29 months (R0), 95% CI 25-34 *vs.* 22 months (R1), 95% CI 19-25, p=0.001

b) median DFS 17 months (R0), 95% CI 15-19 *vs.* 13 months (R1), 95% CI 12-14, p<0.001

Supplementary eFigure 3. Kaplan-Meier curves of overall survival (OS) and disease-free survival (DFS) of patients without lymph node involvement (n=155, the 8 patients with perioperative death were excluded) according to resection status.

a) median OS 58 months (R0), 95% CI 38-82 *vs*. 33 months (R1), 95% CI 25-41, p=0.045

b) median DFS 45 months (R0), 95% CI 33-57 *vs*. 15 months (R1), 95% CI 1-34, p=0.048

Supplementary eFigure 4. Kaplan-Meier curves of disease-free survival (DFS) of patients with lymph node involvement (n=631) according to resection status and stratified in N1 (<4 positive lymph nodes) and N2 (≥4 positive lymph nodes) subgroups.

a) N1 patients (n=330): median DFS 18 months (R0), 95% CI 14-22 months *vs*. 16 months (R1), 95% CI 12-20 months, p=0.107

b) N2 patients (n=301): median DFS 13 months (R0), 95% CI 12-14 months *vs*. 13 months (R1), 95% CI 11-15 months, p=0.667

Table 1. Preoperative characteristics, demographics, pathological and surgical details, and adjuvant treatment of patients with lymph node involvement (n=651) divided according to resection margin status (R0 and R1).

	R0 resection N=352	R1 resection N=299	P-value
Age, *years**	68 (60-75)	68 (60-75)	0.430
Sex (women)	169 (48%)	141 (47%)	0.860
Body-mass index, *kg/m²**	24 (22-27)	25 (22-27)	0.505
Active smoker	75 (21%)	55 (18%)	0.700
Pre-existing diabetes	72 (20%)	72 (24%)	0.257
Jaundice	257 (73%)	194 (65%)	**0.025**
Preoperative biliary stenting	215 (61%)	175 (59%)	0.459
ASA score I-II	160 (46%)	161 (54%)	0.221
Highest CA 19-9, *U/ml**	133 (27-582)	200 (40-717)	0.082
Tumor size on CT, *mm**	25 (20-30)	25 (20-31)	0.794
Tumor size on pathology, *mm**	30 (25-38)	32 (25-40)	**0.015**
T stage 1-2	50 (14%)	33 (11%)	0.227
Collected lymph nodes*	19 (13-25)	22 (16-28)	**<0.001**
N1/N2	194 (55%)/158 (45%)	142 (47%)/157 (53%)	0.052
Vascular invasion (V1)[a]	228 (65%)	227 (76%)	**0.003**
Tumor grade G1-G2	199 (57%)	141 (47%)	**0.008**
Classic Whipple[b]	122 (35%)	78 (26%)	**0.018**
PJ anastomosis	291 (83%)	256 (86%)	0.345
Portal vein resection	47 (13%)	72 (24%)	**0.018**
Arterial resection	2 (1%)	3 (1%)	0.526
Operation time, *min**.	353 (300-450)	423 (325-497)	**<0.001**
Intraoperative blood loss, *ml**	400 (250-600)	475 (300-800)	0.052
Intraoperative blood transfusion	84 (24%)	93 (31%)	0.364

Adjuvant treatment			
Radiochemotherapy	65 (18%)	80 (27%)	**0.012**
Chemotherapy only	191 (54%)	145 (49%)	0.142
Radiotherapy only	5 (1%)	9 (3%)	0.164

Data appear as number and percentage.

* Median and interquartile range.

Significant values appear in bold.

ASA: American Society of Anesthesiologists, CA: carbohydrate antigen, CT: computed tomography, PJ : pancreaticojejunal.

a) Defined as microvascular invasion on pathology (V1 according to TNM staging).

b) The rest of the patients underwent pylorus-preserving Whipple.

Table 2. Uni- and multivariable Cox regression analysis of potential pre- and intraoperative factors associated with overall survival in patients with lymph node involvement (n=631)[a].

	Univariable analysis		Multivariable analysis	
	HR, 95% CI	P-value	HR, 95% CI	P-value
Age, *years*	1.0 (1.0-1.0)	0.126		
Body-mass index, kg/m^2	1.0 (1.0-1.0)	0.835		
Pre-existing diabetes	1.2 (0.9-1.5)	0.163		
Jaundice	0.9 (0.8-1.1)	0.475		
Preoperative biliary stenting	1.2 (1.0-1.4)	0.106		
ASA score	1.2 (1.0-1.4)	**0.020**	1.2 (0.9-1.4)	0.216
CA 19-9, *U/ml*	1.0 (1.0-1.0)	**0.027**	1.0 (1.0-1.0)	0.225
Tumor size on pathology, *mm*	1.0 (1.0-1.0)	**<0.001**	1.0 (1.0-1.0)	**0.001**
T stage	1.1 (0.9-1.4)	0.239		
Vascular invasion (V1)[b]	1.1 (0.9-1.4)	0.360		
Grade (differentiation)	1.3 (1.1-1.5)	**0.002**	1.3 (1.1-1.6)	**0.013**
Resection status	1.2 (1.0-1.4)	0.086	1.1 (0.8-1.4)	0.565
Portal vein resection	1.1 (0.8-1.4)	0.480		
Intraoperative transfusion	1.6 (1.3-2.0)	**<0.001**	1.3 (1.0-1.7)	0.072
Adjuvant chemotherapy	2.4 (1.9-3.0)	**<0.001**	2.3 (1.7-3.1)	**<0.001**

Significant values appear in bold.

ASA: American Society of Anesthesiologists, CA: carbohydrate antigen, HR: hazard ratio, CI: confidence interval.

a) Patients with postoperative death (Clavien grade V) were excluded from the analysis.

b) Defined as microvascular invasion on pathology (V1 according to TNM staging).

Age, body-mass index, CA 19-9, and tumor size were treated as continuous variables.

Table 3. Uni- and multivariable Cox regression analysis of potential pre- and intraoperative factors associated with disease-free survival in patients with lymph node involvement (n=631)[a].

	Univariable analysis		Multivariable analysis	
	HR, 95% CI	P-value	HR, 95% CI	P-value
Age, *years*	1.0 (1.0-1.0)	0.211		
Body-mass index, kg/m^2	1.0 (1.0-1.0)	0.498		
Pre-existing diabetes	1.2 (1.0-1.5)	0.083	1.2 (0.9-1.5)	0.241
Jaundice	0.9 (0.7-1.0)	0.129		
Preoperative biliary stenting	1.1 (0.9-1.3)	0.312		
ASA score	1.0 (0.9-1.2)	0.895		
CA 19-9, *U/ml*	1.0 (1.0-1.0)	**0.005**	1.0 (1.0-1.0)	0.289
Tumor size on pathology, *mm*	1.0 (1.0-1.0)	**<0.001**	1.0 (1.0-1.0)	**<0.001**
T stage	1.0 (0.8-1.2)	0.880		
Vascular invasion (V1)[b]	1.2 (1.0-1.4)	0.107		
Grade (differentiation)	1.4 (1.2-1.6)	**<0.001**	1.3 (1.1-1.5)	**0.003**
Resection status	1.3 (1.1-1.5)	**0.010**	1.2 (0.9-1.5)	0.174
Portal vein resection	0.9 (0.7-1.1)	0.396		
Intraoperative transfusion	1.2 (1.0-1.5)	0.069	1.1 (0.9-1.4)	0.541
Adjuvant chemotherapy	1.9 (1.5-2.4)	**<0.001**	1.9 (1.4-2.5)	**<0.001**

Significant values appear in bold.

ASA: American Society of Anesthesiologists, CA: carbohydrate antigen, HR: hazard ratio, CI: confidence interval.

a) Patients with postoperative death (Clavien grade V) were excluded from the analysis.

b) Defined as microvascular invasion on pathology (V1 according to TNM staging).

Age, body-mass index, CA 19-9, and tumor size were treated as continuous variables.