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Prognostic significance of the degree of lymphatic vessel invasion in locally advanced, surgically resectable pancreatic head cancer

A single center experience

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Abstract

Little is known concerning the prognostic significance of the degree of lymphatic vessel invasion in pancreatic head cancer. To address this gap in knowledge, we retrospectively examined 60 patients with locally advanced, surgically resectable pancreatic head cancer who underwent pancreaticoduodenectomy and lymph node (LN) dissection.

All cases were histopathologically diagnosed as ductal adenocarcinoma, stage II (25 pT3N0 cases, 35 pT3N1 cases). The following variables were investigated: age; sex; neoadjuvant therapy; adjuvant therapy; tumor size; tumor grade; invasion into the serosa, retropancreatic tissue, duodenum, bile duct, portal venous system and perineural area; cut margins; LN metastasis; and the number of invaded lymphatic vessels (LVI-score).

Univariate analysis demonstrated that LN metastasis and an LVI-score ≥5 were significantly associated with poor disease-free survival. Multivariate Cox regression analysis confirmed that LN metastasis and an LVI-score ≥7 were significantly associated with poor disease-free survival. Additionally, LVI-scores ≥9 and ≥10 were comparable to or surpassed the significance of LN metastasis based on the hazard ratio. Univariate analysis demonstrated that tumor size >30 mm, duodenal invasion, LN metastasis and an LVI-score ≥2 were significantly associated with poor overall survival. Multivariate Cox regression analysis confirmed that LN metastasis and LVI-scores ≥9 and ≥10 were significantly associated with poor overall survival, and an LVI-score ≥10 was comparable to or surpassed the significance of LN metastasis based on the hazard ratio.

Our study strongly suggests that a high degree of lymphatic vessel invasion is associated with a poor prognosis in patients with locally advanced, surgically resectable pancreatic head cancer.

Abbreviations: LN = lymph node, LVI-score = the lymphatic vessel invasion score.

Keywords: D2-40, ductal adenocarcinoma, lymphatic metastasis, pancreas, pancreaticoduodenectomy, prognosis, survival analysis

1. Introduction

Pancreatic cancer remains a serious disease, with an estimated 330,400 deaths (173,800 men and 156,600 women) in 2012 worldwide.[1] Despite exhaustive efforts to detect early-stage pancreatic ductal adenocarcinoma, only 10% to 20% or fewer of cases are surgically resectable at the time of diagnosis, resulting in low survival rates.[2] One study reported that the 5-year overall survival rate for localized cases (T1/2 N0 M0) is 41.5% to 43.7%, the 5-year overall survival rate for regional cases (T3/4 N0 M0 or any T N+ M0) is 14.4% to 16.7%, and the 5-year overall survival rate for distant cases (any T any N M+) is 3.7% to 4.3% in the United States and Germany.[3]

Pancreatic ductal adenocarcinoma metastasizes to lymph nodes (LNs) in greater than one-half to three-quarters of surgically resectable cases, and the presence of LN metastases is one of the most powerful predictors of postoperative survival.[4–7] Moreover, several studies have demonstrated that the metastatic LN ratio and the number of positive nodes are significant factors associated with overall survival in patients with resectable pancreatic ductal adenocarcinoma.[8–10] However, the number of retrieved and evaluated regional LNs is influenced by the extent of LN dissection

and the accuracy of pathological examination. Warschkow et al revealed that a higher number of retrieved regional LNs in pancreatic cancer decreases the rate of stage migration and is associated with an improved oncological outcome in node-negative and node-positive pancreatic cancer.[11] Thus, further elucidation of lymphatic pathology may help stratify pancreatic cancer patients to provide therapeutic strategies.

Given that lymphatic vessel invasion is the first step toward establishing lymphogenous metastasis of cancer, it is important to determine the pathological status of lymphatic vessel invasion in the primary lesion.[12] However, only a few studies have focused on this topic. Several studies reported that the presence of lymphatic vessel invasion was associated with survival in patients with resectable pancreatic cancer.[13,14] However, these studies did not possess robust, reproducible methodology for the assessment of lymphatic vessel invasion. Thus, our central question is the following: to what extent is lymphatic vessel invasion actually involved in the prognosis when lymphatic vessel invasion is assessed by a constant measurement criterion?

The aim of this study was to elucidate the prognostic significance of the degree of lymphatic vessel invasion based on a constant measurement criterion and sequential analysis using immunohistochemistry and a continuous natural number method in patients with locally advanced, surgically resectable pancreatic head cancer.

2. Materials and methods

2.1. Case collection and clinical evaluation

This retrospective study was approved by our institutional review board (No. A16-052). The inclusion criteria consisted of pancreaticoduodenectomy for pancreatic head cancer at Jichi Medical University Hospital. The exclusion criteria included stage I, III, and IV disease and a lack of available pathological samples and follow-up information. The medical records of potentially eligible patients were retrospectively and consecutively retrieved from our computerized database between January 2002 and December 2015. Board-certified surgeons reviewed the patients' medical charts and evaluated information regarding patient age, clinical history, surgical procedures, and administration of chemotherapy for pancreatic cancer.

2.2. Pathological evaluation

Materials obtained from pancreaticoduodenectomy and LN dissection were fixed in 15% formalin. Pancreatic and duodenal tissues were sliced at a nearly horizontal sectional view at approximately 5 mm intervals. Paraffin-embedded tissue blocks were generated from the slices by total segmentation. Routine histological examination was performed by board-certified anatomic pathologists using hematoxylin and eosin staining of these tissue samples. Diagnosis was based on the UICC TNM classification system[15] and the World Health Organization classification of tumors of the digestive system.[2]

Immunohistochemistry was performed on whole mount tissue sections that completely covered the maximum division surface of the tumor in each case using an avidin-biotin-peroxidase complex method, an automated staining system (Ventana Benchmark ULTRA; Roche Diagnostics; Tokyo, Japan), and an antibody against podoplanin (D2-40; Nichirei Bioscience; Tokyo, Japan) along with positive and negative controls according to the manufacturer's instructions. In addition, a serial section slide

stained with hematoxylin and eosin was made corresponding to each D2-40 immunohistochemical slide. Cancer cells surrounded by endothelium that were strongly and continuously positive for D2-40 were considered to represent lymphatic vessel invasion, whereas cancer cells adjacent to stromal cells that were weakly or intermittently positive for D2-40 and histologically interpreted to be fibrous components were not regarded as lymphatic vessel invasion. In addition, cancer cells with squamous differentiation that reacted with D2-40 were not considered to represent lymphatic vessel invasion. The degree of lymphatic vessel invasion was assessed using the lymphatic vessel invasion score (LVI-score), which was defined as the total number of invaded lymphatic vessels observed in the whole mounted section slides that covered the maximum division surface of the tumor. Lymphatic vessel invasion was evaluated twice, with an interval longer than one month, using these slides in each case, by a board-certified pathologist who was blinded to the prognostic data, and the final LVI-score was confirmed by reviewing these measurement results.

We also analyzed the number of invaded lymphatic vessels according to the following four anatomical locations: right, left, anterior, and posterior regions (Fig. 1).

2.3. Clinicopathological parameters and endpoints

The following variables were investigated: age, sex, neoadjuvant therapy, adjuvant therapy, tumor size as measured histopathologically by the greatest dimension, histological tumor grade, serosal invasion, retropancreatic tissue invasion, duodenal invasion, bile duct invasion, portal venous system invasion, intrapancreatic perineural invasion, pancreatic cut margin, dissected peripancreatic tissue margin, LN metastasis, and the LVI-score. Disease-free survival and overall survival were chosen as endpoints. Disease-free survival was defined as the time from pancreaticoduodenectomy to the following events: locoregional recurrence, distant metastasis, second primary same or other cancer, death from same or other cancer, non-cancer-related death, or treatment-related death.[16] Overall survival was defined as the time from pancreaticoduodenectomy to death, irrespective of the cause.[16]

2.4. Statistical analysis

Fisher's exact test was used to investigate the relationship between lymphatic vessel invasion and clinicopathological findings. Pearson's correlation coefficient was used to examine correlations between the tumor size as measured histopathologically by the greatest dimension and the LVI-score. Friedman's test was used to investigate differences in the frequency of lymphatic vessel invasion according to the anatomical location. A receiver operating characteristic curve was used to analyze the diagnostic performance of the LVI-score to determine the cutoff point that yielded the highest combined sensitivity and specificity with respect to distinguishing patients with events described above from those without such events. The Kaplan–Meier method was used to estimate survival rates. All explanatory variables were dichotomized for survival analysis and compared using the log-rank test. The Cox proportional hazard model was used to identify independent risk factors for survival. A value of $P < .05$ was considered statistically significant (two-tailed test). These statistical analyses were performed with IBM SPSS Statistics 21 (IBM Japan; Tokyo, Japan) or EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical
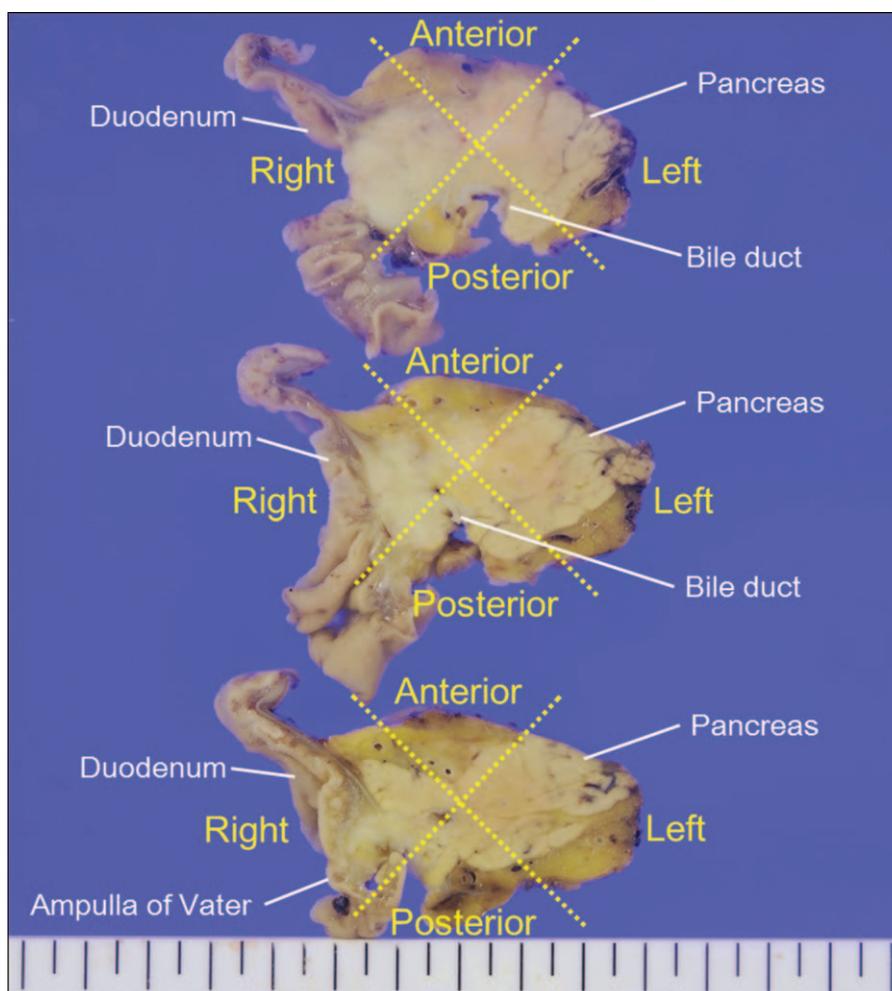
Figure 1. Anatomical classification of the pancreatic head portion (horizontal view).

user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).[17]

3. Results

3.1. Patient characteristics

A total of 63 patients who underwent pancreaticoduodenectomy and LN dissection for pancreatic head cancer were identified from our database during the study period, among which three cases were stage I and were thereby eliminated based on the exclusion criteria. The remaining 60 cases were all stage II and eligible for the study. The median age was 70 years (range 36–83 years), and the mean patient age was 67.8 years (S.E. = 1.3). The study included 29 males and 31 females. Nine patients underwent neoadjuvant therapy, including 7 patients who underwent chemoradiotherapy with gemcitabine or tegafur and 2 patients who underwent gemcitabine-based chemotherapy. Forty-seven patients underwent adjuvant chemotherapy with gemcitabine or tegafur or a combination of these drugs. The 60 cases were further classified as stage IIA: pT3N0M0 (25 cases) and stage IIB: pT3N1M0 (35 cases). All 60 cases were histopathologically diagnosed as ductal adenocarcinoma, including two cases of adenosquamous carcinoma and one case of undifferentiated carcinoma. The median tumor size was 30 mm (range 12–64 mm), and the mean tumor size was 30.7 mm (S.E. = 1.4). Three cases were classified as histological tumor grade 1, 26 cases were classified as grade 2, and 31 cases were classified as grade 3. The median number of dissected LNs was 16 (range 2–39), and the median number of metastatic LNs was 1 (range 0–12). Only one patient exhibited cancer invasion into the extrapancreatic nerve plexus, and only 1 patient was positive for the bile duct cut-end margin.

3.2. Characteristics of lymphatic vessel invasion

Forty-four patients exhibited lymphatic vessel invasions, as identified by the D2–40 immunohistochemical glass slides that completely covered the maximum division surface of the tumor (Fig. 2). The median LVI-score was 2 (range 0–112). The median number of D2-40 immunohistochemical glass slides used for evaluating lymphatic vessel invasion was 2 (range 1–4).

Lymphatic vessel invasion was frequently observed in patients with LN metastasis (Fisher's exact test, $P < .001$) and in patients with bile duct invasion ($P = .003$) but was much less frequently observed in patients who received neoadjuvant therapy ($P < .001$) (Table 1). Pearson's correlation coefficient for the relationship between the tumor size and LVI-score was 0.220 (Pearson's
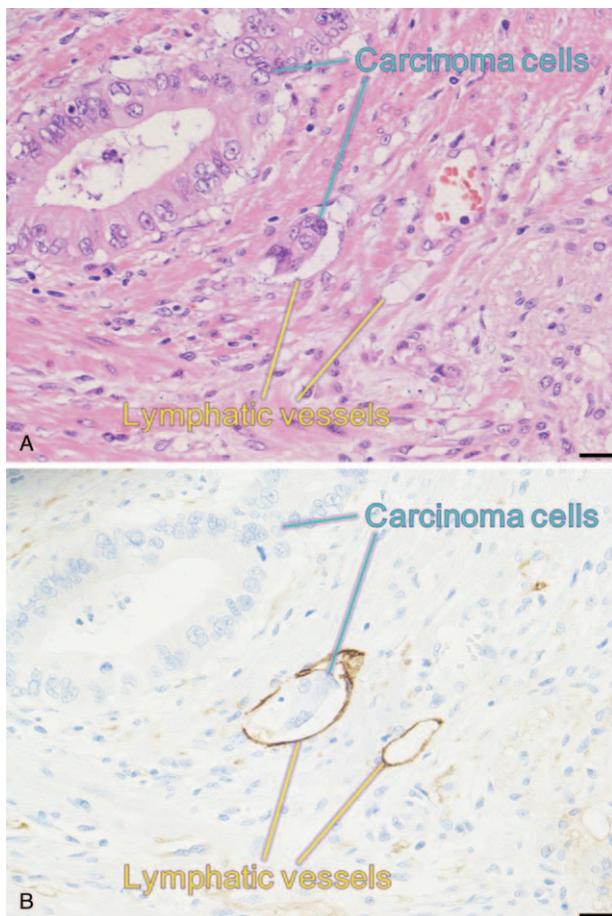
Figure 2. Representative histopathological findings of pancreatic head cancer. (A) Barely visible lymphatic vessel invasion of ductal adenocarcinoma cells shown by hematoxylin and eosin staining (the bar indicates 20 μm). (B) Clearly visible lymphatic vessel invasion of ductal adenocarcinoma cells shown by D2-40 immunohistochemistry (serial section of A, the bar indicates 20 μm).

Table 1

Association between lymphatic vessel invasion and clinico-pathological factors in 60 patients with stage II pancreatic head cancer.

Clinicopathological factors	Lymphatic vessel invasion		P-value of Fisher's exact test
	Absent	Present	
Age (years)			
≤70	5	26	.081
>70	26	18	
Sex			
Male	9	20	.563
Female	7	24	
Neoadjuvant therapy			
Absent	8	43	<.001*
Present	8	1	
Adjuvant therapy			
Absent	3	10	1.000
Present	13	34	
Tumor size (mm)†			
≤30	13	24	.076
>30	3	20	
Histological grade			
Grade 1-2	10	19	.247
Grade 3	6	25	
Serosal invasion			
Absent	7	23	.771
Present	9	21	
Retropancreatic tissue invasion			
Absent	3	14	.359
Present	13	30	
Duodenal invasion			
Absent	8	13	.220
Present	8	31	
Bile duct invasion			
Absent	8	5	.003*
Present	5	39	
Portal venous system invasion			
Absent	12	39	.230
Present	4	5	
Intrapancreatic perineural invasion			
Absent	3	2	.112
Present	13	42	
Pancreatic cut end margin			
Negative	14	38	1.000
Positive	2	6	
Dissected peripancreatic tissue margin			
Negative	12	34	1.000
Positive	4	10	
Lymph node metastasis			
Absent (Stage IIA)	13	12	<.001*
Present (Stage IIB)	3	32	

* P < .05.
† Tumor size denotes the greatest histopathological dimension.

correlation coefficient, $P = .091$). Lymphatic vessel invasion was more frequently observed in the right and posterior regions of the pancreaticoduodenectomy material than in the left and anterior regions (Friedman's test, $P < .001$) (Table 2).

3.3. Disease-free survival analysis

The median disease-free survival time was 12.1 months (95% confidence interval 9.0–15.1 months), and the 5-year disease-free survival rate was 20% in our study cohort of 60 patients. The area under the receiver operating characteristic curve used to analyze the diagnostic performance of the LVI-score for disease-free survival was 0.673 (95% confidence interval 0.530–0.817), and the LVI-score that yielded the maximal sensitivity and specificity was 3; at this threshold, the sensitivity was 0.533, and the specificity was 0.733 (Supplemental Digital Content 1, http://links.lww.com/MD/C682). Kaplan–Meier curves and the log-rank test demonstrated that LN metastasis as well as LVI-scores of ≥5, ≥6, ≥7, ≥8, ≥9 and ≥10 were significantly associated with poor disease-free survival (Supplemental Digital Content 2, http://links.lww.com/MD/C682, Fig. 3). Multivariate Cox regression analysis confirmed that LN metastasis and LVI-scores of ≥7, ≥8, ≥9 or ≥10 were significantly associated with poor disease-free survival (Table 3). Most notably, LVI-scores of ≥9

and ≥10 were as or more significant than LN metastasis based on hazard ratio analysis.

In the subgroup analysis, an LVI-score ≥10 was significantly associated with poor disease-free survival in 25 cases of stage IIA disease (pT3N0) (Table 4, Fig. 4), whereas LVI-scores of ≥7, ≥9 and ≥10 were significantly associated with poor disease-free survival in 35 cases of stage IIB disease (pT3N1) (Table 5, Fig. 5). In this subgroup, the area under the receiver operating characteristic curve used to analyze the diagnostic performance of the LVI-score for disease-free survival was 0.66 (95% confidence interval 0.46–0.85), and the LVI-score that yielded the maximal sensitivity and specificity was 2; at this threshold, the sensitivity was 0.375, and the specificity was 0.889 (Supplemental Digital Content 3, http://links.lww.com/MD/C682).

3.4. Overall survival analysis

The median overall survival time was 50.1 months (95% confidence interval 28.9–71.3 months), and the 5-year overall survival rate was 34.7% in our study cohort consisting of 60

Table 2

Regional differences of the number of invaded lymphatic vessels observed in 45 patients with stage II pancreatic head cancer.

Region	Mean rank	Median number of invaded lymphatic vessels (range)	Right	Left	Anterior	Posterior
Right	2.92	1 (0–112)	n/a	0.004[*]	0.029[*]	1.000
Left	1.99	0 (0–14)	0.004[*]	n/a	1.000	0.003[*]
Anterior	2.16	0 (0–14)	0.029[*]	1.000	n/a	0.026[*]
Posterior	2.93	1 (0–14)	1.000	0.003[*]	0.026[*]	n/a

[*] $P < .05$.
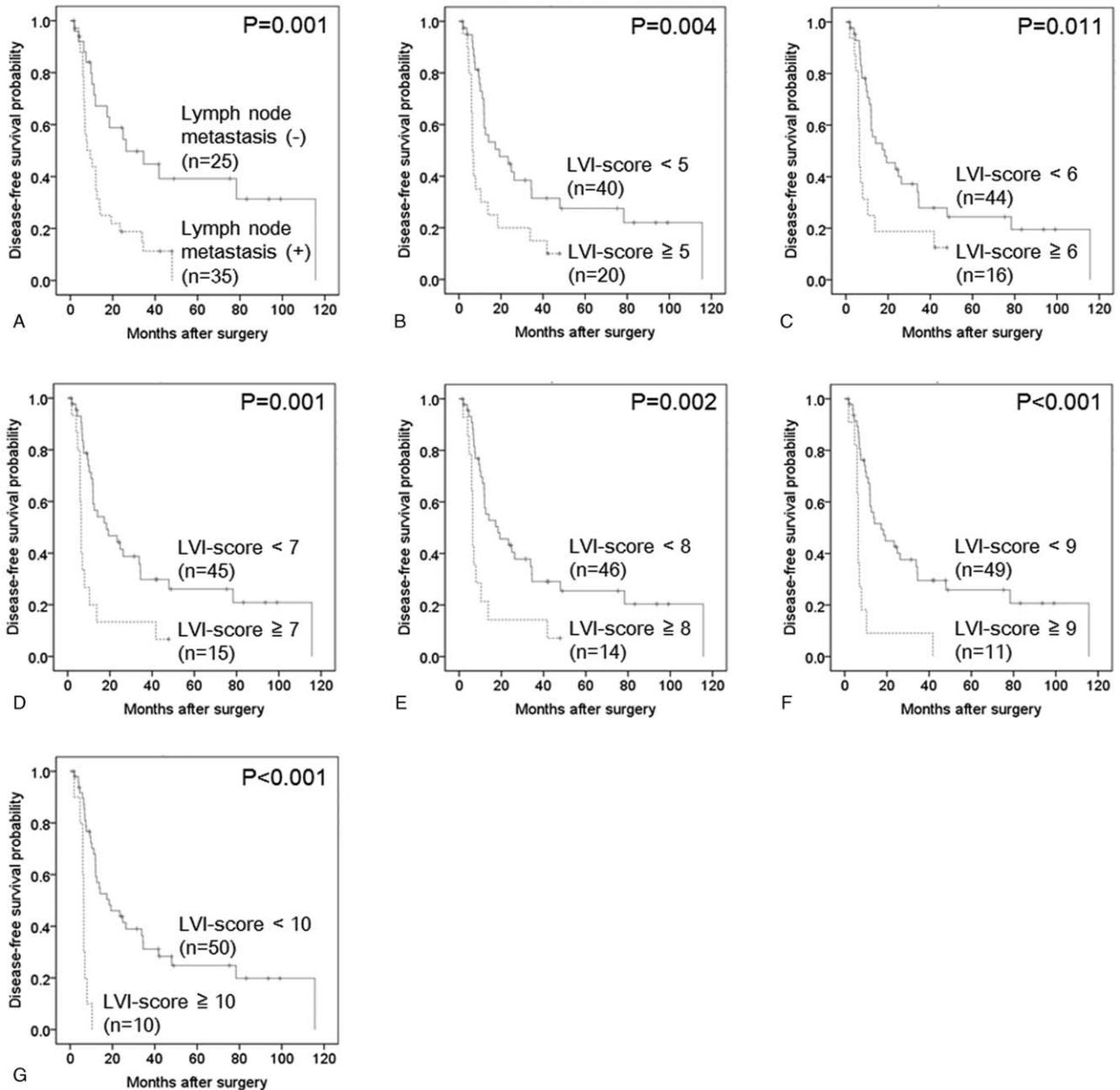
Figure 3. Kaplan–Meier curves showing the disease-free survival of 60 patients with pancreatic head cancer. (A) Lymph node metastasis (−) versus lymph node metastasis (+) ($P = .001$). (B) Lymphatic vessel invasion (LVI)-score <5 versus LVI-score ≥5 ($P = .004$). (C) LVI-score <6 versus LVI-score ≥6 ($P = .011$). (D) LVI-score <7 versus LVI-score ≥7 ($P = .001$). (E) LVI-score <8 versus LVI-score ≥8 ($P = .002$). (F) LVI-score <9 versus LVI-score ≥9 ($P < .001$). (G) LVI-score <10 versus LVI-score ≥10 ($P < .001$). LVI = lymphatic vessel invasion.

Table 3

Multivariate Cox regression analysis of disease-free survival in 60 patients with stage II pancreatic head cancer.

| | Clinicopatholgical factors | Multivariate Cox regression | | |
		Hazard ratio	95% CI	P-value
Pair 1	Lymph node metastasis	2.349	1.185–4.654	.014[*]
	LVI-score ≥ 7	2.257	1.141–4.464	.019[*]
Pair 2	Lymph node metastasis	2.417	1.224–4.775	.011[*]
	LVI-score ≥ 8	2.045	1.025–4.080	.042[*]
Pair 3	Lymph node metastasis	2.707	1.385–5.292	.004[*]
	LVI-score ≥ 9	3.575	1.694–7.544	.001[*]
Pair 4	Lymph node metastasis	2.187	1.084–4.414	.029[*]
	LVI-score ≥ 10	5.065	2.084–12.308	<.001[*]

CI = confidence interval, LVI = lymphatic vessel invasion.

[*] P < .05.

patients. The area under the receiver operating characteristic curve used to analyze the diagnostic performance of the LVI-score for predicting overall survival was 0.710 (95% confidence interval 0.580–0.842), and the LVI-score that yielded the maximal sensitivity and specificity was 3; at this threshold, the sensitivity was 0.656, and the specificity was 0.750 (Supplemental Digital Content 4, http://links.lww.com/MD/C682). Kaplan–Meier curves and the log-rank test demonstrated that tumor size >30 mm; duodenal invasion; LN metastasis; and LVI-scores of ≥2, ≥3, ≥4, ≥5, ≥6, ≥7, ≥8, ≥9 and ≥10 were significantly associated with poor overall survival (Supplemental Digital Content 4, http://links.lww.com/MD/C682, Fig. 6). Multivariate Cox regression analysis confirmed that LN metastasis and LVI-scores ≥9 or ≥10 were significantly associated with poor overall survival (Table 6). Most notably, an LVI-score ≥10 was as or more significant than LN metastasis based on hazard ratio analysis.

In the subgroup analysis, no variable was significantly associated with overall survival in 25 cases of stage IIA disease (pT3N0) (Table 7), whereas portal venous system invasion and

Table 4

Disease-free survival analysis of 25 patients with stage IIA (pT3N0) pancreatic head cancer.

| Clinicopatholgical factors | | Number of case | Number of event | Disease-free survival month | | Log rank test |
				Median	95% CI	P-value
Age (years)	≤70	13	9	34.5	5.9–63.1	.907
	>70	12	7	26.3	5.7–46.8	
Sex	Male	12	8	18.4	5.8–31.1	.348
	Female	13	8	41.8	16.5–67.1	
Neoadjuvant therapy	Absent	17	11	34.5	6.8–62.3	.680
	Present	8	5	24.9	9.4–40.3	
Tumor size (mm)[†]	≤30	23	16	n/e	n/e	.153
	>30	2	0	n/e	n/e	
Histological grade	Grade 1–2	11	7	78.4	0.0–164.3	.248
	Grade 3	14	9	24.9	3.2–46.5	
Serosal invasion	Absent	15	12	18.5	0.0–37.5	.127
	Present	10	4	78.3	0.0–179.3	
Retropancreatic tissue invasion	Absent	7	5	24.9	5.5–44.2	.978
	Present	18	11	34.5	7.4–61.7	
Duodenal invasion	Absent	16	13	24.9	13.7–36.0	.096
	Present	9	3	n/e	n/e	
Bile duct invasion	Absent	9	5	24.9	0.0–58.3	.647
	Present	16	11	34.5	5.3–63.7	
Portal venous system invasion	Absent	21	14	34.5	11.0–58.0	.760
	Present	4	2	10.2	n/e	
Intrapancreatic perineural invasion	Absent	4	3	17.3	3.9–30.7	.713
	Present	21	13	34.5	7.8–61.2	
Pancreatic cut end margin	Negative	24	16	n/e	n/e	.460
	Positive	1	0	n/e	n/e	
Dissected peripancreatic tissue margin	Negative	21	15	26.2	4.0–48.4	.244
	Positive	4	1	n/e	n/e	
LVI-score ≥ 1	Absent	13	7	24.9	4.7–45.0	.810
	Present	12	9	34.5	0.0–75.8	
LVI-score ≥ 2	Absent	18	10	26.3	0.0–86.2	.642
	Present	7	6	34.5	0.0–75.8	
LVI-score ≥ 3	Absent	21	12	26.3	7.8–44.7	.762
	Present	4	4	18.5	0.0–57.6	
LVI-score ≥ 4	Absent	21	12	26.3	7.8–44.7	.762
	Present	4	4	18.5	0.0–57.6	
LVI-score ≥ 5	Absent	22	13	34.5	0.0–92.8	.177
	Present	3	3	18.5	0.0–45.0	
LVI-score ≥ 6	Absent	23	14	26.2	4.2–48.3	.257
	Present	2	2	1.9	n/e	
LVI-score ≥ 7	Absent	23	14	26.2	4.2–48.3	.257
	Present	2	2	1.9	n/e	
LVI-score ≥ 8	Absent	23	14	26.2	4.2–48.3	.257
	Present	2	2	1.9	n/e	
LVI-score ≥ 9	Absent	23	14	26.2	4.2–48.3	.257
	Present	2	2	1.9	n/e	
LVI-score ≥ 10	Absent	24	15	34.5	11.9–57.1	<.001[*]
	Present	1	1	1.7	n/e	

CI = confidence interval, LVI = lymphatic vessel invasion, n/e = not estimated.

[†] Tumor size denotes the greatest histopathological dimension.

[*] P < .05.
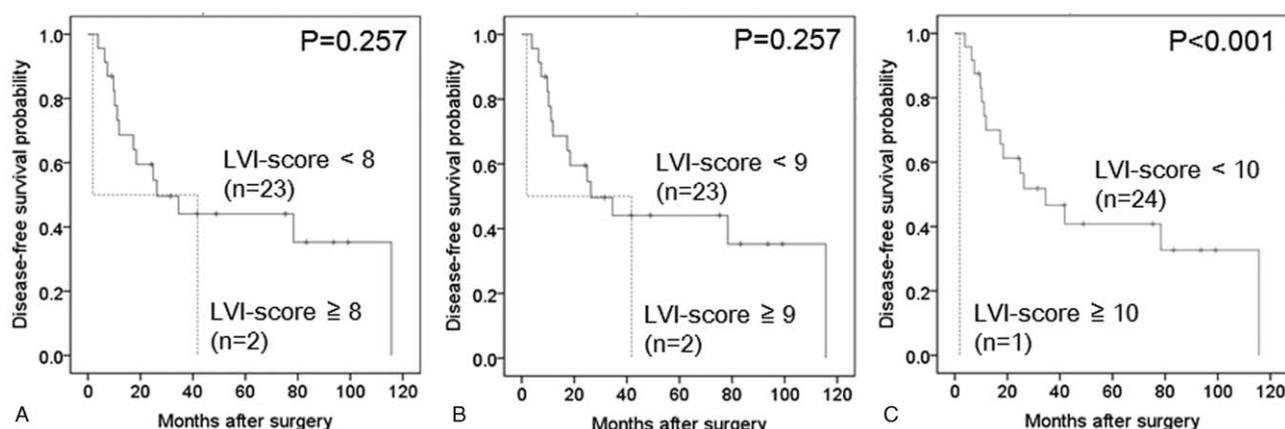
Figure 4. Kaplan–Meier curves depicting the disease-free survival of 25 patients with stage IIA pancreatic head cancer. (A) Lymphatic vessel invasion (LVI)-score <8 versus LVI-score ≥8 (*P* = .257). (B) LVI-score <9 versus LVI-score ≥9 (*P* = .257). (C) LVI-score <10 versus LVI-score ≥10 (*P* < .001). LVI = lymphatic vessel invasion.

Table 5

Disease-free survival analysis of 35 patients with stage IIB (pT3pN1) pancreatic head cancer.

Clinicopatholgical factors		Number of case	Number of event	Disease-free survival month Median	95% CI	Log rank test P-value
Age (years)	≤70	18	15	6.9	0.0–14.2	.359
	>70	17	14	7.9	4.6–11.2	
Sex	Male	17	15	12.6	3.6–21.6	.407
	Female	18	14	6.8	4.8–8.8	
Neoadjuvant therapy	Absent	34	28	7.9	3.4–12.5	.957
	Present	1	1	12.0	n/e	
Tumor size (mm)[†]	≤30	14	12	12.0	8.3–15.7	.597
	>30	21	17	6.9	5.5–8.4	
Histological grade	Grade 1–2	18	15	12.0	4.0–20.0	.593
	Grade 3	17	14	7.6	4.5–10.8	
Serosal invasion	Absent	15	12	10.3	2.5–18.2	.249
	Present	20	17	7.9	5.2–10.6	
Retropancreatic tissue invasion	Absent	10	8	6.8	5.7–8.0	.238
	Present	25	21	12.0	5.6–18.4	
Duodenal invasion	Absent	5	3	11.9	0.0–37.1	.107
	Present	30	26	7.6	6.2–9.1	
Bile duct invasion	Absent	4	3	12.0	7.9–16.1	.953
	Present	31	26	7.6	5.9–9.4	
Portal venous system invasion	Absent	30	25	11.9	6.3–17.6	.106
	Present	5	4	6.6	6.0–7.2	
Intrapancreatic perineural invasion	Absent	1	1	9.4	n/e	.750
	Present	34	28	7.9	1.2–14.7	
Pancreatic cut end margin	Negative	28	23	7.9	2.4–13.5	.671
	Positive	7	6	9.4	3.2–15.6	
Dissected peripancreatic tissue margin	Negative	25	20	7.9	5.1–10.8	.702
	Positive	10	9	10.3	2.3–18.4	
LVI-score ≥ 1	Absent	3	3	12.0	3.4–20.6	.724
	Present	32	26	7.9	3.7–12.2	
LVI-score ≥ 2	Absent	8	7	9.4	3.5–15.4	.756
	Present	27	22	7.9	3.7–12.1	
LVI-score ≥ 3	Absent	11	9	12.0	7.9–15.9	.912
	Present	24	20	7.0	5.3–8.7	
LVI-score ≥ 4	Absent	15	12	12.0	8.0–15.1	.446
	Present	20	17	6.8	6.2–7.4	
LVI-score ≥ 5	Absent	18	14	12.0	11.2–12.9	.170
	Present	17	15	6.4	5.8–7.1	
LVI-score ≥ 6	Absent	21	17	12.0	11.8–12.2	.240
	Present	14	12	6.4	6.2–6.6	
LVI-score ≥ 7	Absent	22	17	12.0	11.1–13.0	.028[*]
	Present	13	12	6.4	5.9–7.0	
LVI-score ≥ 8	Absent	23	18	12.0	11.1–13.0	.067
	Present	12	11	6.4	6.3–6.6	
LVI-score ≥ 9	Absent	26	20	12.0	11.0–13.1	.001[*]
	Present	9	9	6.4	6.3–6.6	
LVI-score ≥ 10	Absent	26	20	12.0	11.0–13.1	.001[*]
	Present	9	9	6.4	6.3–6.6	

CI = confidence interval, LVI = lymphatic vessel invasion, n/e = not estimated.

[†] Tumor size denotes the greatest histopathological dimension.

[*] *P* < .05.
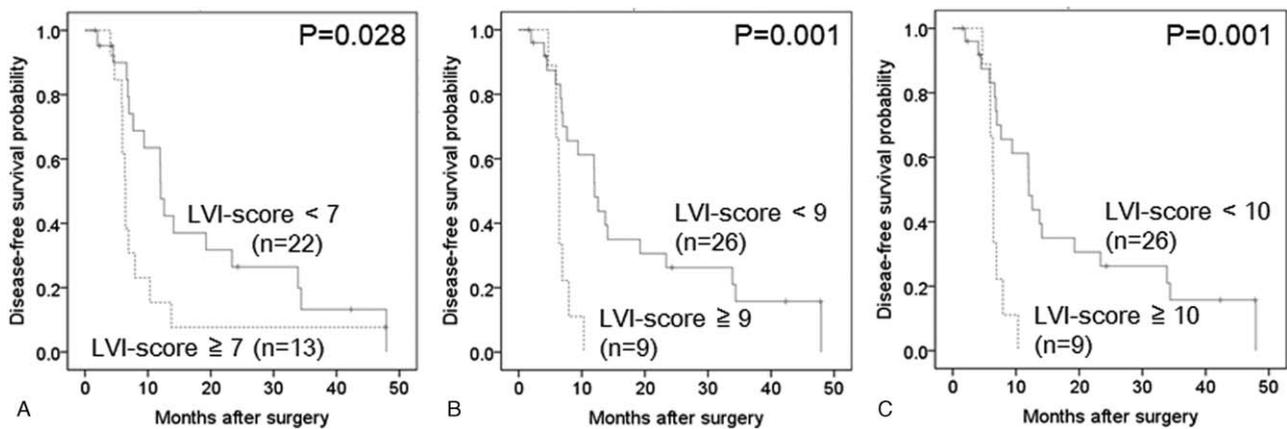
Figure 5. Kaplan–Meier curves depicting the disease-free survival of 35 patients with stage IIB pancreatic head cancer. (A) Lymphatic vessel invasion (LVI)-score <7 versus LVI-score ≥7 (P = .028). (B) LVI-score <9 versus LVI-score ≥9 (P = .001). (C) LVI-score <10 versus LVI-score ≥10 (P = .001). LVI = lymphatic vessel invasion.

LVI-scores ≥9 and ≥10 were significantly associated with poor overall survival in 35 cases of stage IIB disease (pT3N1) (Table 8, Fig. 7). In this subgroup, the area under the receiver operating characteristic curve used to analyze the diagnostic performance of the LVI-score for overall survival was 0.526 (95% confidence interval 0.26–0.80), and the LVI-score that yielded the maximal sensitivity and specificity was 9; at this threshold, the sensitivity was 0.167, and the specificity was 0.947 (Supplemental Digital Content 6, http://links.lww.com/MD/C682).

4. Discussion

The main finding of this study was that a high degree of lymphatic vessel invasion is an independent prognostic factor for both disease-free and overall survival in patients with locally advanced, surgically resectable pancreatic head cancer. In addition, subgroup analysis based on the presence or absence of LN metastasis demonstrated that a high degree of lymphatic vessel invasion was significantly associated with shorter disease-free survival in patients without LN metastasis (LVI-score ≥10) and those with LN metastasis (LVI-score ≥7, ≥9 and ≥10), whereas a high degree of lymphatic vessel invasion was significantly associated with shorter overall survival exclusively in patients with LN metastasis (LVI-score ≥9 and ≥10).

In routine diagnostic practice, histopathological grading for lymphatic vessel invasion is recommended using a four-tier code described by the Japan Pancreatic Society: ly0 (no evidence of invasion), ly1 (slight invasion), ly2 (moderate invasion), and ly3 (marked invasion).[18] However, this definition is vague and can lead to inter-observer variability. Our approach to assess the degree of lymphatic vessel invasion is simple, objective and inexpensive, requiring the preparation of only a few immunohistochemical slides. Furthermore, the findings are predictive of the prognosis by counting at most 10 invaded lymphatic vessels in each case.

Given that the lymphatic system is a continuum including lymphatic capillaries, collecting lymphatic vessels, intervening LNs and the thoracic duct, LN metastasis itself represents only a part of the lymphogenous spread of cancer.[19,20] The intralymphatic cancer cells observed in the primary lesion can be interpreted as not only a predictor of LN metastasis but also as a

result of downstream lymphostasis by lymphogenous spread of cancer outside the primary lesion.[19,20] Therefore, it is reasonable that a high degree of lymphatic vessel invasion is associated with a poor prognosis of pancreatic cancer.

It is interesting to note that lymphatic vessel invasion was more frequently observed in the right and posterior regions of the pancreaticoduodenectomy material than in the left and anterior regions. This finding may be due to the inherent anatomical distribution of the lymphatics. The right region contains duodenal lymphatics, which are well developed through the wall and drain into the downstream lymphatics around the upper para-aortic region.[21,22] The lymphatics in the posterior region are also well developed with a number of collecting lymphatics that drain into the downstream lymphatics around the upper para-aortic region.[21–23]

In the present study, decreased lymphatic vessel invasion was associated with neoadjuvant therapy. Seven of the 9 patients receiving neoadjuvant therapy exhibited no lymphatic vessel invasion. Of these, 4 patients underwent radiotherapy combined with gemcitabine chemotherapy, and 3 patients underwent radiotherapy combined with tegafur. Although the mechanism remains to be elucidated, we hypothesize that chemoradiotherapy plays a key role in damaging both cancer-associated, small-sized lymphatic vessels and intralymphatic cancer cells.[24–27]

The present study has several limitations. First, only the lymphatic vessels that were strongly and continuously positive for D2-40 by immunohistochemistry were assessed. However, D2-40 expression could be weakened in certain pathological conditions or in a large-sized collecting lymphatic vessel, and therefore we could have potentially overlooked some invaded lymphatic vessels. To ensure accuracy, use of other lymphatic endothelial markers, such as prox1, may be useful.[28] Second, only a single pathologist assessed the lymphatic vessel invasion, which could be a source of bias. However, the pathologist who assessed lymphatic vessel invasion was board-certified with 10 years of experience in tumor pathology; therefore, we believe that the degree of measurement error due to the rater is within acceptable levels. Third, this study was performed using a relatively small number of cases of stage II pancreatic head cancer in a single institution; therefore, it might be difficult to generalize our results. For example, we found that the difference in
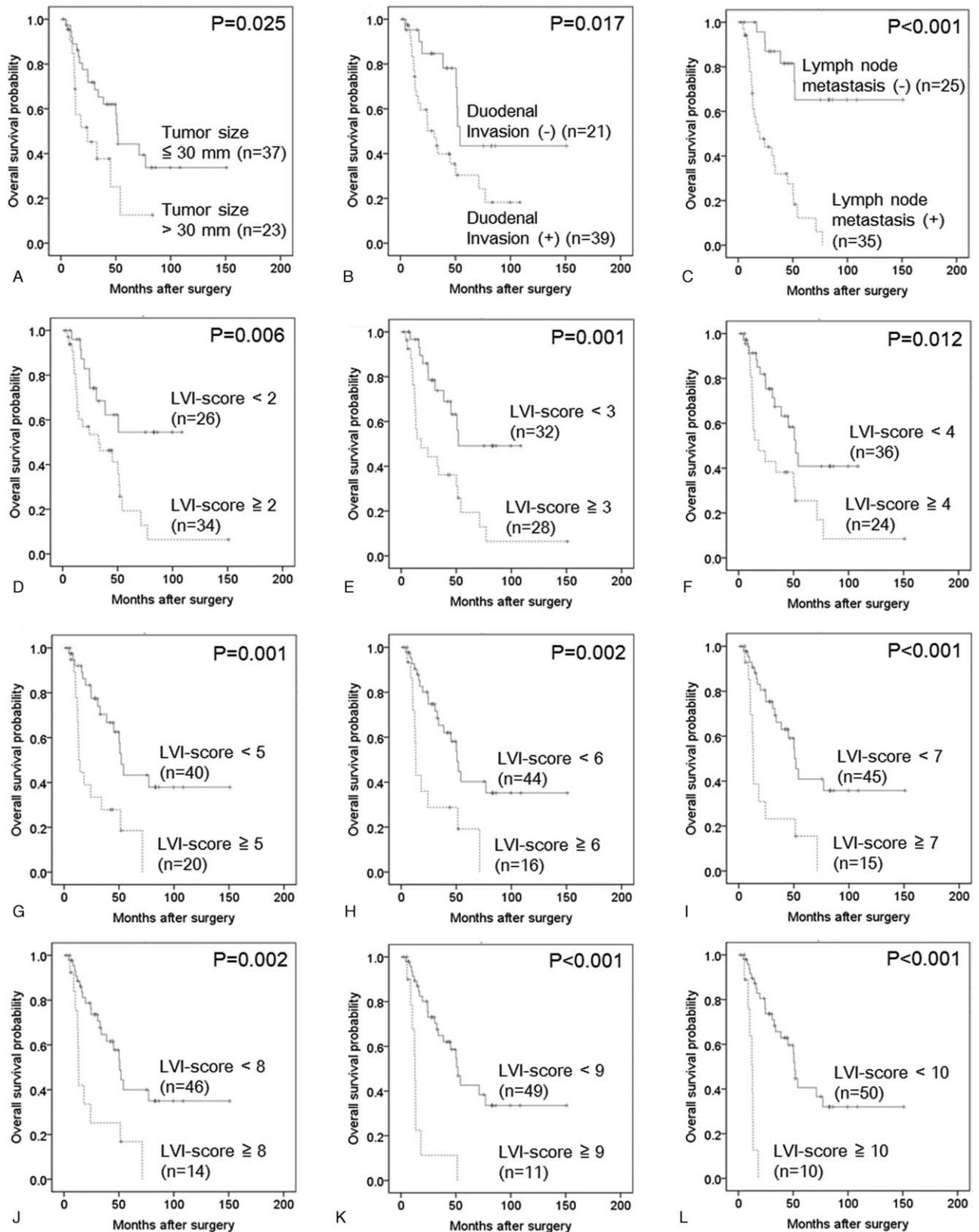
Figure 6. Kaplan–Meier curves depicting the overall survival of 60 patients with pancreatic head cancer. (A) Tumor size ≤30 mm versus tumor size >30 mm (*P* = .025). (B) Duodenal invasion (−) versus duodenal invasion (+) (*P* = .017). (C) Lymph node metastasis (−) versus lymph node metastasis (+) (*P* < .001). (D) Lymphatic vessel invasion (LVI)-score <2 versus LVI-score ≥2 (*P* = .006). (E) LVI-score <3 versus LVI-score ≥3 (*P* = .001). (F) LVI-score <4 versus LVI-score ≥4 (*P* = .012). (G) LVI-score <5 versus LVI-score ≥5 (*P* = .001). (H) LVI-score <6 versus LVI-score ≥6 (*P* = .002). (I) LVI-score <7 versus LVI-score ≥7 (*P* < .001). (J) LVI-score <8 versus LVI-score ≥8 (*P* = .002). (K) LVI-score <9 versus LVI-score ≥9 (*P* < .001). (L) LVI-score <10 versus LVI-score ≥10 (*P* < .001). LVI = lymphatic vessel invasion.

Table 6

Multivariate Cox regression analysis of overall survival in 60 patients with stage II pancreatic head cancer.

Clinicopatholgical factors	Multivariate Cox regression		
	Hazard ratio	95% CI	*P*-value
Pair 1			
Tumor size[†] > 30 mm	0.753	0.307–1.846	.535
Duodenal invasion	1.083	0.419–2.800	.870
Lymph node metastasis	7.400	2.444–22.400	<.001[*]
LVI-score ≥ 9	6.765	2.470–18.525	<.001[*]
Pair 2			
Tumor size > 30 mm	0.687	0.265–1.777	.439
Duodenal invasion	0.961	0.374–2.468	.934
Lymph node metastasis	6.217	2.160–17.898	.001[*]
LVI-score ≥ 10	8.200	2.387–28.170	.001[*]

CI = confidence interval, LVI = lymphatic vessel invasion.
[†] Tumor size denotes the greatest histopathological dimension.

lymphatic vessel invasion frequency was significantly different between the neoadjuvant and non-neoadjuvant groups, but we could not demonstrate for a significant difference in the survival analysis between the groups, likely due to the small number of cases analyzed, which is inconsistent with the previously reported result.[29] Besides, in the Stage IIA (pT3N0) subgroup analyses using the area under the receiver operating characteristic curve, statistically significant associations could not been observed between the LVI-score and prognoses probably due to a low statistical power inherent to a small sample size, although the disease-free survival approached significance (the lower limit of 95% confidence interval was 0.46). However, to our knowledge, this study is the first to attempt to investigate the relationship between the degree of lymphatic vessel invasion and the prognosis of pancreatic cancer. Further comprehensive, large-scale surveys are needed to elucidate whether the LVI-score can be a robust prognostic predictor in pancreatic cancer patients without LN metastasis.

Table 7

Overall survival analysis of 25 patients with stage IIA (pT3N0) pancreatic head cancer.

Clinicopatholgical factors		Number of case	Number of event	Overall survival month		Log rank test
				Mean	95% CI	*P*-value
Age (years)	≤70	13	3	112.0	75.3–148–7	.922
	>70	12	3	78.4	58.0–98.7	
Sex	Male	13	4	75.2	61.5–88.9	.487
	Female	12	2	101.5	64.2–138.9	
Neoadjuvant therapy	Absent	17	4	113.3	82.5–144.1	.615
	Present	8	2	62.7	40.0–88.5	
Adjuvant therapy	Absent	4	1	63.2	32.1–94.3	.628
	Present	21	5	112.6	84.3–140.8	
Tumor size (mm)[*]	≤30	23	6	n/e	n/e	.437
	>30	2	0	n/e	n/e	
Histological grade	Grade 1–2	11	3	116.9	84.1–149.7	.874
	Grade 3	14	3	75.4	48.5–102.4	
Serosal invasion	Absent	15	5	100.9	67.0–134.8	.294
	Present	10	1	96.8	76.0–117.6	
Retropancreatic tissue invasion	Absent	7	1	122.8	75.2–170.3	.642
	Present	18	5	76.0	59.2–92.8	
Duodenal invasion	Absent	16	4	107.8	74.2–141.5	.913
	Present	9	2	89.8	67.0–112.6	
Bile duct invasion	Absent	9	1	74.5	59.4–89.6	.652
	Present	16	5	108.3	78.6–138.1	
Portal venous system invasion	Absent	21	5	112.0	83.6–140.4	.829
	Present	4	1	68.1	43.5–92.7	
Intrapancreatic perineural invasion	Absent	4	2	59.4	15.9–102.9	.143
	Present	21	4	117.8	89.7–145.8	
Pancreatic cut end margin	Negative	24	6	n/e	n/e	.705
	Positive	1	0	n/e	n/e	
Dissected peripancreatic tissue margin	Negative	21	6	n/e	n/e	.283
	Positive	6	0	n/e	n/e	
LVI-score ≥ 1	Absent	13	3	66.7	50.6–82.9	.816
	Present	12	3	109.3	71.8–146.8	
LVI-score ≥ 2	Absent	18	4	87.4	69.5–105.4	.797
	Present	7	2	84.7	31.8–137.6	
LVI-score ≥ 3	Absent	21	5	85.4	68.1–102.7	.916
	Present	4	1	101.0	32.1–170.0	
LVI-score ≥ 4	Absent	21	5	85.4	68.1–102.7	.916
	Present	4	1	101.0	32.1–170.0	
LVI-score ≥ 5	Absent	22	5	116.8	90.9–142.7	.453
	Present	3	1	51.3	51.3–51.3	
LVI-score ≥ 6	Absent	23	5	117.9	92.6–143.3	.184
	Present	2	1	51.3	51.3–51.3	
LVI-score ≥ 7	Absent	23	5	117.9	92.6–143.3	.184
	Present	2	1	51.3	51.3–51.3	
LVI-score ≥ 8	Absent	23	5	117.9	92.6–143.3	.184
	Present	2	1	51.3	51.3–51.3	
LVI-score ≥ 9	Absent	23	5	117.9	92.6–143.3	.184
	Present	2	1	51.3	51.3–51.3	
LVI-score ≥ 10	Absent	24	6	n/e	n/e	n/e
	Present	1	0	n/e	n/e	

Abbreviations: CI = confidence interval, LVI = lymphatic vessel invasion, n/e = not estimated.
[*] Tumor size denotes the greatest histopathological dimension.

Table 8

Overall survival analysis of 35 patients with stage IIB (pT3pN1) pancreatic head cancer.

Clinicopatholgical factors		Number of case	Number of event	Overall survival month		Log rank test
				Median	95% CI	P-value
Age (years)	≤70	18	15	16.0	8.5–23.5	.684
	>70	17	11	30.8	3.8–57.7	
Sex	Male	17	13	14.5	5.6–23.3	.335
	Female	18	13	30.8	10.0–51.5	
Neoadjuvant therapy	Absent	34	25	19.5	0.0–39.2	.589
	Present	1	1	16.0	n/e	
Adjuvant therapy	Absent	9	6	10.3	9.0–11.6	.051
	Present	26	20	24.2	8.0–40.5	
Tumor size (mm)[†]	≤30	14	13	19.5	0.0–46.5	.415
	>30	21	13	18.1	5.1–31.1	
Histological grade	Grade 1–2	18	14	24.2	1.0–47.3	.378
	Grade 3	17	12	14.5	5.9–23.1	
Serosal invasion	Absent	15	11	33.9	1.5–66.2	.600
	Present	20	15	18.1	11.3–24.9	
Retropancreatic tissue invasion	Absent	10	7	13.1	10.4–15.9	.343
	Present	25	19	24.2	6.5–41.9	
Duodenal invasion	Absent	5	4	50.6	2.1–99.2	.576
	Present	30	22	16.0	3.3–28.7	
Bile duct invasion	Absent	4	3	19.5	13.9–25.1	.639
	Present	31	23	18.1	0.0–38.3	
Portal venous system invasion	Absent	30	23	24.2	4.6–43.8	.003[*]
	Present	5	3	10.3	1.5–19.0	
Intrapancreatic perineural invasion	Absent	1	1	19.5	n/e	.738
	Present	34	25	18.1	0.0–37.7	
Pancreatic cut end margin	Negative	28	23	16.0	3.0–29.1	.128
	Positive	7	3	77.0	n/e	
Dissected peripancreatic tissue margin	Negative	25	18	18.1	10.8–25.4	.971
	Positive	10	8	30.8	0.0–73.1	
LVI-score ≥ 1	Absent	3	2	16.0	n/e	.700
	Present	32	24	19.5	6.0–33.0	
LVI-score ≥ 2	Absent	8	5	30.8	16.7–44.8	.942
	Present	27	21	18.1	5.3–30.9	
LVI-score ≥ 3	Absent	11	6	30.8	9.3–52.2	.595
	Present	24	20	14.5	8.4–20.6	
LVI-score ≥ 4	Absent	15	10	30.8	14.1–47.4	.901
	Present	20	16	14.5	11.7–17.3	
LVI-score ≥ 5	Absent	18	12	32.8	11.3–54.3	.198
	Present	17	14	13.2	12.3–14.2	
LVI-score ≥ 6	Absent	21	15	32.8	16.8–48.7	.382
	Present	14	11	13.1	12.0–14.3	
LVI-score ≥ 7	Absent	22	15	32.8	27.3–38.2	.164
	Present	13	11	13.1	11.7–14.6	
LVI-score ≥ 8	Absent	23	16	32.8	15.7–49.8	.285
	Present	12	10	13.1	12.4–13.9	
LVI-score ≥ 9	Absent	26	18	32.8	19.9–45.6	<.001[*]
	Present	9	8	12.8	9.5–16.0	
LVI-score ≥ 10	Absent	26	18	32.8	19.9–45.6	<.001[*]
	Present	9	8	12.8	9.5–16.0	

CI = confidence interval, LVI = lymphatic vessel invasion, n/e = not estimated.

[*] P < .05.

[†] Tumor size denotes the greatest histopathological dimension.

It can be assumed that some tumors may be larger than others; thus, the number of invaded lymphatic vessels should be stated per unit area. However, measurement of the number of invaded lymphatic vessels per unit area of the tumor is not feasible due to the following reasons:

(1) demarcation of the tumor is often difficult in pancreatic cancer, which almost always contains a considerable degree of fibrous stromal elements, thereby leading to measurement uncertainty;

(2) lymphatic vessel invasion is not exclusively observed in the tumor;

lymphatic vessel invasion was actually often found in the duodenum or other extrapancreatic tissues apart from the tumor in the present case series, thereby leading to the loss of potentially important information about lymphatic vessel invasion as a whole. Furthermore, the correlation between the tumor size and the number of invaded lymphatic vessels was not significant in the present study, indicating that tumor sizes did not necessarily
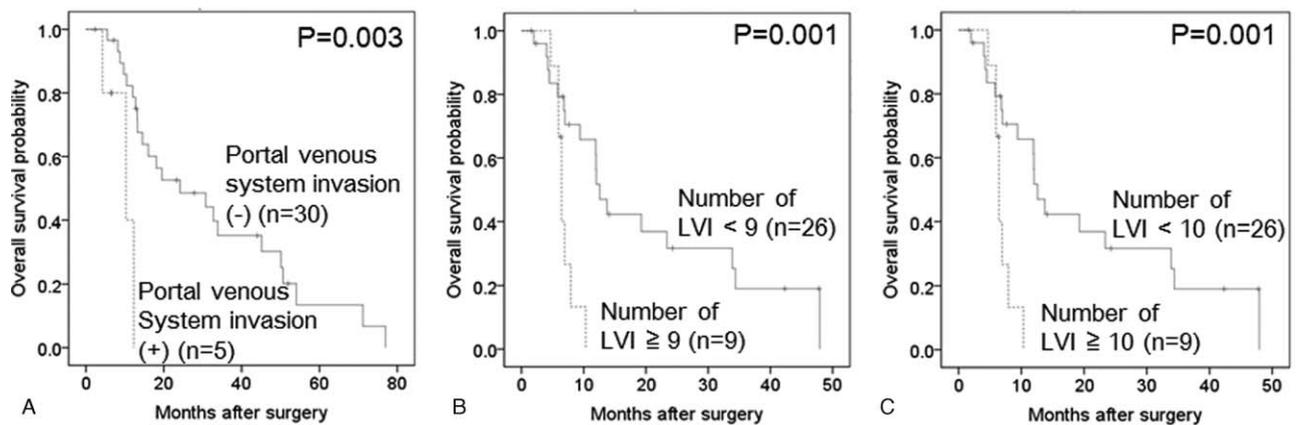
Figure 7. Kaplan–Meier curves depicting the overall survival of 35 patients with stage IIB pancreatic head cancer. (A) Portal venous system invasion (−) versus portal venous system invasion (+) (P = 0.003). (B) Lymphatic vessel invasion (LVI)-score <9 versus LVI-score ≥9 (P = .001). (C) LVI-score <10 versus LVI-score ≥10 (P = .001). LVI = lymphatic vessel invasion.

influence the number of invaded lymphatic vessels. We suggest that our methodology used in the present study can be applied for day-to-day clinical practice, and this approach may provide further insights into the development of lymphogenous metastasis of pancreatic cancer.

In conclusion, the present study suggests that a high degree of lymphatic vessel invasion is associated with a poor prognosis in patients with locally advanced, surgically resectable pancreatic head cancer. Further elucidation of the lymphatic pathology will help to stratify pancreatic cancer patients and establish future therapeutic strategies.

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Visualization: Kohei Morita and Hisashi Oshiro.
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Writing – review & editing: Kohei Morita and Hisashi Oshiro.

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