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Low lymphocyte monocyte ratio after neoadjuvant therapy predicts poor survival after pancreatectomy in patients with borderline resectable pancreatic cancer

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ABSTRACT

Background: The impact of systemic immune inflammatory markers to predict survival in patients receiving neoadjuvant therapy for borderline resectable pancreatic cancer has not been sufficiently investigated. This study aims to evaluate whether systemic immune inflammatory markers after neoadjuvant therapy followed by pancreatectomy become preoperative prognostic factors to predict survival in patients with borderline resectable pancreatic cancer.

Methods: We retrospectively reviewed 67 borderline resectable pancreatic cancer patients receiving neoadjuvant therapy and 58 borderline resectable pancreatic cancer patients undergoing upfront surgery between 2010 and 2016. The association between survival and systemic immune inflammatory markers was evaluated by univariate and multivariate analysis. The neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, lymphocyte-to-monocyte ratio, and prognostic nutrition index were assessed as systemic immune inflammatory markers.

Results: In univariate analysis, the postneoadjuvant neutrophil-to-lymphocyte ratio, lymphocyte-to-monocyte ratio, and prognostic nutrition index are significantly associated with survival as systemic immune inflammatory markers. The optimal cutoff value of the postneoadjuvant neutrophil-to-lymphocyte ratio, lymphocyte-to-monocyte ratio, and prognostic nutrition index were 2.5, 3.0, and 45, respectively. Patients with a lymphocyte-to-monocyte ratio <3.0 had significantly poor survival compared with those who had a lymphocyte-to-monocyte ratio ≥3.0 (14.9 months vs 31.7 months, $P = .006$). The multivariate analysis identified the following as predictors of poor prognosis: postneoadjuvant lymphocyte-to-monocyte ratio <3.0 (HR 2.59; 95% CI 1.02–6.58; $P = .045$), T4 (HR 1.82; 95% CI 1.11–3.01; $P = .029$), lymph node status (HR 2.62; 95% CI 1.24–5.51; $P = .012$), and no completion of adjuvant therapy (HR 7.09; 95% CI 3.08–16.4; $P < .001$).

Conclusion: A low lymphocyte-to-monocyte ratio after neoadjuvant therapy is useful prognostic information for patients with borderline resectable pancreatic cancer. This result might indicate a potential role of lymphocyte-to-monocyte ratios in stratification of treatment strategy in borderline resectable pancreatic cancer patients.

Introduction

According to the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines, pancreatic cancer (PC) can be classified as resectable, unresectable, or borderline resectable, using multidetector computed tomography (CT).[1] In the treatment

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of PC, surgical resection followed by adjuvant therapy is the only procedure that may lead to longer survival.[2,3] Borderline resectable PC (BRPC) has a particularly high risk of margin-positive resection and postoperative recurrence, although BRPC may technically be resectable. Neoadjuvant therapy is therefore recommended for BRPC patients in the NCCN Guidelines.[1] Neoadjuvant therapy followed by surgery might increase R0 rates compared with upfront surgery, according to several studies, and it could provide clinical benefits for BRPC.[4–6] However, in BRPC, survival remains poor, with a high risk of early recurrence after pancreatectomy regardless of neoadjuvant therapy followed by pancreatectomy.[7,8] Preoperative prognostic assessment is therefore crucial for evaluation of whether surgery is adopted in multimodal therapy for patients with BRPC. Preoperative prognostic factors after neoadjuvant therapy in BRPC remain unclear.

Several studies have reported that various systemic immune inflammatory markers, such as the neutrophil-to-lymphocyte ratio (NLR),[9,10] platelet-to-lymphocyte ratio (PLR),[11,12] lymphocyte-to-monocyte ratio (LMR),[13,14] and prognostic nutrition index (PNI)[15,16] have important roles in the prediction of PC survival. However, few studies have investigated the impact of systemic immune inflammatory markers to predict survival in patients receiving neoadjuvant therapy for BRPC. Although neoadjuvant therapy may cause hematologic adverse effects, such as neutropenia or lymphopenia because of its toxicities, little data are available on the association between neoadjuvant therapy and systemic immune inflammatory markers. Evaluation on how systemic immune inflammatory markers are affected by neoadjuvant therapy affects is necessary.

This study aims to evaluate whether systemic immune inflammatory markers after neoadjuvant therapy followed by pancreatectomy become preoperative prognostic factors to predict survival in patients with BRPC.

Patients and Methods

Patient characteristics

The prospective database of this study was retrospectively reviewed for 67 BRPC patients who received completion of neoadjuvant therapy followed by pancreatectomy and 58 BRPC patients with upfront surgery between January 2010 and December 2016 at Wakayama Medical University Hospital ([WMUH] Japan). Of 125 BRPC patients, 2 patients who were dead because of operation were excluded from the analysis of overall survival to evaluate whether systemic immune inflammatory markers were oncologically associated with survival. Since March 2010, neoadjuvant therapy for BRPC was begun at our institute with patients who have radiologic artery involvement (BRPC-A). Our indication for neoadjuvant therapy until December 2013 was only BRPC-A patients, and it included BRPC with portal and/or superior mesenteric vein (PV/SMV) involvement (BRPC-V) patients since then. BRPC was decided on based on the NCCN Clinical Practice Guidelines.[1] This study was approved by the WMUH Institutional Review Board (No. 2419).

Neoadjuvant therapy and postoperative adjuvant therapy

The regimes of neoadjuvant therapy have changed during the course of our study period.[4,17–19] The regimes of neoadjuvant therapy used in this study were as follows:

- Radiation with concurrent S-1 (oral 5-fluorouracil [5-FU] prodrug tegafur, with oteracil and gimeracil) as chemoradiotherapy ($n = 24$),[4,17]
- Gemcitabine plus S-1 therapy (n = 14),[4,17]
- Modified FOLFIRINOX[18] (without bolus 5-FU and leucovorin, also decreased the dose of irinotecan) ($n = 6$), or
- Nab-paclitaxel plus gemcitabine therapy[19] ($n = 22$)

Chemoradiotherapy regimens consisted of external-beam radiation with 50 Gy in 25 fractions with concurrent S-1 (oral 5-FU prodrug tegafur, with oteracil and gimeracil) at 80 mg/m^2 per day administered on alternate days for 6 weeks.[4,17] Gemcitabine plus S-1 therapy involves concurrent S-1 at 80 mg/m^2 per day with alternate-day administration for 9 weeks and gemcitabine at 800 mg/m^2 on days 1, 8, 22, 29, 43, and 50.[4,17] FIRINOX treatment was the modified FOLFIRINOX treatment (without bolus 5-FU and leucovorin and a decreased dose of irinotecan), which was given as follows: 2-hour intravenous (IV) infusion of oxaliplatin at 85 mg/m^2 (during which irinotecan was also intravenously infused during 90 minutes at 150 mg/m^2), followed by a continuous IV infusion of 5-FU during 46 hours at 2,400 mg/m^2.[18] This regimen was repeated every 2 weeks and was done in 4 cycles for 8 weeks or 8 cycles for 16 weeks. Nab-paclitaxel plus gemcitabine therapy consisted of a 30-minute IV infusion of nab-paclitaxel at a dose of 125 mg/m^2, followed by a 30-minute intravenous infusion of gemcitabine at a dose of 1,000 mg/m^2, on days 1, 8, and 15 during a 4-week period as 1 cycle of regimen.[19] Patients received 2 cycles of this regimen for 8 weeks.

After completion of neoadjuvant therapies, blood examination, preoperative restaging CT scans, and staging laparoscopy were performed to exclude disease progression and to assess resectability. In this study, restaging CT scans were performed after neoadjuvant therapy was completed, and radiologic tumor response was reviewed in accordance with response evaluation criteria in solid tumors (RECIST) v 1.1.[20] Patients with no evidence of progressive disease were planned to undergo pancreatectomy within 2–8 weeks after the last dose of chemotherapy. After completion of neoadjuvant therapies, blood chemical tests, preoperative restaging CT scans, and staging laparoscopy were performed to exclude disease progression and to assess resectability.

Postoperative adjuvant therapy using gemcitabine or S-1-based chemotherapy depending on the physicians' choice was provided to patients, unless contraindicated by a patient's condition. All resected pancreatic tumors were pathologically confirmed to be invasive ductal carcinoma. Cases with invasive carcinoma derived from intraductal papillary mucinous neoplasms (IPMN) were not registered in this study. The tumor, node, metastasis (TNM) classification for pancreatic tumors by the 8th Union for International Cancer Control (UICC) was applied.[21] The tumor characteristics, including T and N status, tumor size, histologic type, surgical margin status, and peritoneal cytology were microscopically examined. The pathologic response to neoadjuvant therapy was evaluated according to the grading system reported by Evans et al.[22]

Relative dose intensity

Dose intensity is the total amount of drug given by the standard schedule of each drug. Relative dose intensity (RDI) was identified as the dose intensity achieved relative to the standard schedule of each drug and can be expressed as a percentage.

Data collection for systemic immune inflammatory markers

NLR, PLR, LMR, and PNI were evaluated as systemic immune inflammatory markers. NLR and PLR were calculated as the absolute count of neutrophils and platelets, respectively, divided by the absolute lymphocyte count. Similarly, LMR was calculated as the ratio of the lymphocyte count to the absolute count of monocytes. PNI was calculated according to 10 × albumin (g/dl) + 0.005 × total

Table I
Characteristics of 125 patients with BRPC

	Neoadjuvant ($n = 67$)	Upfront surgery (n=58)
Age (years)	68 (46–80)	70 (48–84)
Sex (male/female)	40/27	34/24
Neoadjuvant therapy *n* (%)		
radiation with concurrent S-1	25 (35)	
gemcitabine plus S-1	14 (22)	
modified FOLFIRINOX	6 (9)	
nab-paclitaxel plus gemcitabine	22 (34)	
Resectability (BR-PV/BR-A) *n* (%)	17 (25)/50 (75)	24 (41)/34 (59)
Tumor location (Head/body and tail) *n* (%)	39 (58)/28 (42)	46 (79)/12 (21)
RECIST 1.1, (PR/SD/PD) *n* (%)	10(15)/56(84)/1(1)	
Operative procedure *n* (%)		
pylorus resecting pancreaticoduodenectomy	39 (58)	42 (72)
distal pancreatectomy	1 (2)	6 (10)
distal pancreatectomy with celiac axis	25 (37)	9 (16)
total pancreatectomy	2 (3)	1 (2)
Vessel resection *n* (%)	44 (66)	35 (60)
Transfusion *n* (%)	8 (13)	13 (22)
Postoperative complication > grade III n (%)	17 (25)	14 (24)
UICC stage (IIa/IIb/III)	20(30)/41(61)/6(9)	7(12)/48(83)/3(5)
T stage (T3/T4)	61 (91)/ 6 (9)	55 (95)/ 3 (5)
N stage (N0/N1)	20 (30)/47 (70)	7 (12)/51 (88)
Margin status (R0/R1)	54 (81)/13 (19)	48 (83)/10 (17)
Pathologic response to neoadjuvant therapy I/IIA/IIB/III	21(32)/32(48)/13(19)/1(1)	
Implementation of adjuvant therapy n (%)	49 (73)	49 (85)
Completion of adjuvant therapy n (%)	30 (45)	20 (35)

Note: Pathologic response to neoadjuvant therapy is based on Evans' classification.
BR-PV, borderline resectable pancreatic cancer with portal vein involvement; *BR-A*, borderline resectable pancreatic cancer with artery involvement; *RECIST*, response evaluation criteria in solid tumors; *PR*, partial response; *SD*, stable disease; *PD*, progress disease; *UICC*, International Union Against Cancer; *R*, resectability.

lymphocyte count (per mm^3).[23] Data from complete blood counts (CBCs) and serum albumin were collected before and after neoadjuvant therapy. The most recent CBC from within 2 weeks before neoadjuvant therapy was used for calculating preneoadjuvant systemic immune inflammatory markers before neoadjuvant therapy. To calculate postneoadjuvant systemic immune inflammatory markers after neoadjuvant therapy, the most recent CBC from within 2 weeks before surgery after completion of neoadjuvant therapy was used.

The changes in NLR or LMR before and after neoadjuvant therapy were classified into three categories as follows: On evaluation of LMR, "increase" indicated that patients who received neoadjuvant therapy had more than a 20% increase in LMR compared with the value before neoadjuvant therapy, and "decrease" was defined as more than a 20% decrease in LMR. Others were defined as "stable." Similarly, on evaluation of NLR, "increase" was defined as more than a 20% increase in postneoadjuvant NLR compared with the value before neoadjuvant therapy, and "decrease" was defined as more than a 20% decrease in postneoadjuvant NLR. Others were defined as "stable."

Postoperative complications

Postoperative complication was redefined as more than grade II based on the Clavien classification.[24] Severe complications were defined as conditions that were grade III or more based on the Clavien classification. Clinically relevant pancreatic fistula was defined based on International Study Group on Pancreatic Fistula (ISGPF) guidelines.[25] Delayed gastric emptying (DGE) was defined by the International Study Group of Pancreatic Surgery (ISGPS) clinical criteria based on the clinical course and postoperative management.[26] Mortality was defined as death within 90 days of surgery.

Statistical analysis

Continuous variables were expressed as the median values ± standard error. Overall survival was calculated from the date of surgery to either the date of death or last follow-up. Overall survival was estimated using the Kaplan-Meier method, and the differences were tested using the log-rank test. Patients who were still alive at the time of the last follow-up were censored. Patients with BRPC were dichotomized into two groups based on the cutoff value of each systemic immune inflammatory marker as follows: NLR, PLR, LMR, and PNI. Then, the Kaplan-Meier method and a Cox proportional hazards model were used to evaluate the association between preneoadjuvant and postneoadjuvant systemic immune inflammatory markers as cutoff values and overall survival. Optimal cutoff values for systemic immune inflammatory markers were determined by the receiver operating characteristics (ROC) curve and the area under the curve (AUC). On the ROC curve, the point with both maximum sensitivity and specificity was selected for the best cutoff values. Univariate and multivariate analyses were performed using a Cox proportional hazard model to evaluate significant prognostic predictors. A value of $P < .05$ was considered to be statistically significant. All statistical analyses were performed using SPSS software, v 20 (SPSS, Chicago, IL, USA).

Results

Patient characteristics

Table I presents the characteristics of the 67 BRPC patients who received completion of neoadjuvant therapy followed by pancreatectomy and 58 BRPC patients with upfront surgery. Of 67 BRPC patients receiving neoadjuvant therapy, 17 (25%) patients were classified as borderline resectable based on venous involvement and 50 (75%) patients were classified as borderline resectable based on arterial involvement. On the other hand, of the 58 BRPC patients with upfront surgery, 24 patients (41%) were classified as borderline resectable based on venous involvement and 34 patients (59%) were classified as borderline resectable based on arterial involvement. A total of 42 (63%) patients received chemotherapy and a

Table II
Postoperative complications of 125 patients with BRPC

	Neoadjuvant ($n = 67$)	Upfront surgery ($n = 58$)
Severe complications*	14 (21%)	14 (24%)
Clinically relevant pancreatic fistula†	6 (9.0%)	7 (12%)
grade B	2 (3.0%)	5 (8.6%)
grade C	4 (6.0%)	2 (3.4%)
Intra-abdominal abscess	8 (11.9%)	8 (14%)
Intra-abdominal bleeding	4 (6.0%)	2 (3.4%)
Delayed gastric emptying	5 (7.5%)	2 (3.4%)
Portal vein thrombosis	1 (1.5%)	1 (1.5%)
Necrosis of the stomach	1 (1.5%)	0 (0%)
Mortality	2 (3.0%)	0 (0%)

* Severe complications were considered to be those classified as III or more according to Clavien-Dindo classification.
† Pancreatic fistula was defined by the International Study Group on Pancreatic Fistula (ISGPF).

Table III
Association between the types of neoadjuvant therapy and systemic immune inflammatory markers

	S − 1 + radiation ($n = 23$)	Gemcitabine + S − 1 ($n = 14$)	mFOLFIRINOX ($n = 6$)	nab-paclitaxel + gemcitabine ($n = 22$)	*P*
Preadjuvant therapy					
NLR, number (range)	2.7 (1.6–5.2)	3.5 (2.0–4.4)	3.5 (2.0–5.1)	2.7 (1.4–4.7)	.101
PLR, number (range)	157 (94–309)	181 (141–240)	205 (138–219)	199 (138–316)	.103
LMR, number (range)	4.0 (1.0–17.0)	3.5 (1.4–8.0)	3.5 (2.5–5.8)	3.9 (1.6–10.0)	.715
PNI, number (range)	47 (35–56)	49 (37–59)	49 (32–55)	48 (39–58)	.447
Postadjuvant therapy					
NLR, number (range)	4.0 (1.1–23.4)	2.9 (1.2–17.6)	2.8 (0.6–8.3)	2.7 (0.7–6.2)	.132
PLR, number (range)	186 (67–128)	213 (66–502)	164 (106–717)	205 (64–417)	.596
LMR, number (range)	1.7 (0.1–4.3)	2.9 (0.7–8.5)	2.7 (0.6–6.2)	3.5 (1.2–16.3)	.005
PNI, number (range)	42 (33–52)	44 (37–57)	40 (35–49)	42 (34–54)	.190

LMR, lymphocyte monocyte ratio; *NLR*, neutrophil lymphocyte ratio; *PLR*, platelet lymphocyte ratio; *PNI*, prognostic nutrition index.

total of 25 (37%) patients received chemoradiotherapy. The R0 rate was 80.6% ($n = 54$) in patients with neoadjuvant therapy and 83% ($n = 48$) in patients with upfront surgery. Implementation rate of adjuvant therapy with gemcitabine-based chemotherapy or S-1-based chemotherapy was 73% ($n = 49$) in patients with neoadjuvant therapy and 85% ($n = 49$) in patients with upfront surgery. The completion rate of adjuvant therapy was 45% ($n = 30$) in patients with neoadjuvant therapy and 35% ($n = 20$) in patients with upfront surgery.

A detailed summary of postoperative complications after pancreatectomy is presented in Table II. Severe complications, classified as III and IV according to Clavien classification, occurred in 14 patients (20.9%) with neoadjuvant therapy and 14 patients (24%) with upfront surgery. Pancreatic fistula grade C based on the ISGPF[25] occurred in 4 patients (6.0%) with neoadjuvant therapy and 2 patients (3.4%) with upfront surgery. The mortality rate was 3.0% (2 of the initial 67 patients with neoadjuvant therapy). One patient died of uncontrollable arterial hemorrhage without pancreatic fistula on postoperative day 28. Another patient died of multiple organ failure from clinically relevant pancreatic fistula on postoperative day 50. The mortality rate was 0% in patients with upfront surgery.

Systemic immune inflammatory markers before and after neoadjuvant therapy

The duration of neoadjuvant therapy depends on applied regimens. The median time from initiation of neoadjuvant therapy to completion of neoadjuvant therapy was 55 days (range, 32–140 days). The median time from completion of neoadjuvant therapy to surgery was 21 days (range, 11–42 days). All patients ($n = 67$) in this study underwent pancreatectomy within 8 weeks after the last dose of chemotherapy. The duration of neoadjuvant therapy (HR 1.01, 95% CI 0.99–1.01; $P = .722$) and time from completion of neoadjuvant therapy to surgery (HR 1.03, 95% CI 0.99–1.07; $P = .191$) were not associated with survival.

In Table III, the association between the types of neoadjuvant therapy and systemic immune inflammatory markers are summarized. Postadjuvant LMR was associated with the types of neoadjuvant therapy (S − 1 + radiation 1.7 versus gemcitabine + S − 1 2.9 versus mFOLFIRINOX 2.7 versus nab-paclitaxel + gemcitabine 3.5, $P = .005$), although other systemic immune inflammatory markers were not associated with the types of neoadjuvant therapy (Table III). The heterogeneity in the neoadjuvant therapy regimens of this study affected LMR after neoadjuvant therapy.

The ROC curve found the cutoff values of the preneoadjuvant and postneoadjuvant NLR, PLR, LMR, and PNI (Table IV). Each cutoff value of NLR, PLR, LMR, and PNI was found to have the highest log-rank statistic of any cut point. The optimal cutoff values for preneoadjuvant NLR, PNR, LMR, and PNI were 2.9, 183, 4.0, and 48.5, respectively, and the AUC for NLR, PNR, LMR, and PNI were 0.537, 0.501, 0.608, and 0.609, respectively. On the other hand, the optimal cutoff values for postneoadjuvant NLR, PNR, LMR, and PNI were 2.5, 160, 3.0, and 45, respectively, and the AUC for the NLR, PNR, LMR, and PNI were 0.756, 0.568, 0.847, and 0.729, respectively.

Table V presents the association between preneoadjuvant and postneoadjuvant NLR, PNR, LMR, and PNI and survival by univariate analysis when patients with BRPC with neoadjuvant therapy were dichotomized into two groups based on each cutoff value. Postneoadjuvant NLR, LMR, and PNI were significantly associated with survival. On the other hand, Preneoadjuvant NLR, PLR, LMR, PNI, and postneoadjuvant PLR were not associated with survival. Next, median survival time (MST) of postadjuvant NLR, LMR, and PNI divided based on each cutoff value were compared with that of the upfront surgery group. MST among postadjuvant NLR $\geq$ 2.5 group versus postadjuvant NLR < 2.5 group versus upfront surgery group was as follows: postadjuvant NLR $\geq$ 2.5 group, 31.7 months; postadjuvant NLR < 2.5 group, 15.6 months; and upfront surgery group, 13.7 months (Fig 1, *A*). Survival in patients with postadjuvant NLR $\geq$ 2.5 was significantly better than that in patients with postadjuvant NLR < 2.5 group ($P = .041$) and in patients with upfront surgery ($P = .074$). However, there was no significant difference in survival

Table IV
The cutoff values of preneoadjuvant and postneoadjuvant systemic immune inflammatory markers

Markers	Cutoff	AUC	Sensitivity (%)	Specificity (%)	95% CI
Preneoadjuvant					
NLR	3.0	0.537	51.0	57.1	0.371–0.704
PLR	183	0.501	53.0	64.3	0.352–0.651
LMR	4.0	0.608	61.2	61.1	0.445–0.0771
PNI	48.5	0.609	57.1	57.1	0.459–0.760
Postneoadjuvant					
NLR	2.5	0.756	68.6	71.4	0.619–0.892
PLR	160	0.568	78.4	50.0	0.378–0.757
LMR	3.0	0.847	72.5%	85.7%	0.725–0.970
PNI	45.0	0.729	76.5%	57.1%	0.580–0.878

CI, confidence interval.

between patients with postadjuvant NLR < 2.5 and those with upfront surgery ($P = .680$). MST among LMR ≥ 3.0 group versus LMR < 3.0 group versus upfront surgery group was as follows: postadjuvant LMR ≥ 3.0 group, 31.7 months; postadjuvant LMR < 3.0 group, 14.9 months; and upfront surgery group, 13.7 months (Fig 1, *B*). Survival in patients with postadjuvant LMR ≥ 3.0 was significantly better than that in patients with postadjuvant LMR < 3.0 group ($P = .003$) and in patients with upfront surgery ($P = .014$). However, there was no significant difference in survival between patients with postadjuvant LMR < 3.0 and those with upfront surgery ($P = .315$). On the other hand, MST among postadjuvant PNI ≥ 45 group versus postadjuvant PNI < 45 group versus upfront surgery group was as follows: postadjuvant PNI ≥ 45 group, 29.9 months; postadjuvant PNI < 45 group, 14.9 months; and upfront surgery group, 13.7 months (Fig 1, *C*). Overall survival in patients with postadjuvant PNI ≥ 45 was significantly better than that in patients with postadjuvant PNI < 45 group ($P = .036$) and in patients with upfront surgery ($P = .044$). However, overall survival of patients with postadjuvant PNI < 45 group was equivalent with that of those with upfront surgery ($P = .614$).

The association between postadjuvant LMR and clinicopathologic characteristics

The association between postadjuvant LMR and clinicopathologic characteristics is summarized (Table VI). Postadjuvant LMR ≥ 3.0 was closely associated with higher pathologic response to neoadjuvant therapy (IIB or III) (35% versus 13%, $P = .036$). Moreover, high postadjuvant LMR was also closely associated with low postadjuvant NLR (65%) and high postadjuvant PNI (87%). However, high postadjuvant LMR was not associated with other oncologic parameters such as T stage, positive lymph nodes, RECIST response, or CA19-9 levels.

Association between the change of LMR and survival

Much higher postadjuvant LMR did not correlate with overall survival (R value = 0.177, P = .160), although postadjuvant LMR ≥ 3.0 was associated with better survival.

Figures 2, *A* and *B* present the association between the change of NLR or LMR and survival. Of 65 patients, 28 were assigned to "increase" NLR cohort, 24 were assigned to "decrease" NLR cohort, and 13 were assigned to "stable" NLR cohort. Regarding the change in NLR before and after neoadjuvant therapy, survival in patients with increased NLR was as similar to those with stable NLR (MST 15.6 months versus MST 16.1 months; $P = .777$). However, patients with decreased NLR had significantly better survival compared with those with increased NLR (MST 31.7 months versus MST 15.6 months; $P = .021$). On the other hand, regarding the association between the change of LMR and survival, of 65 patients, 12 were assigned to "increase" LMR cohort, 37 were assigned to "decrease" LMR cohort, and 16 were assigned to "stable" LMR cohort. Regarding the change in LMR before and after neoadjuvant therapy, survival in patients with increased LMR was as similar to those with stable LMR (MST 25.7 months versus MST 21.6 months; $P = .776$). However, patients with increased LMR had significantly better survival compared with those with decreased LMR (MST 25.7 months versus MST 14.9 months; $P = .030$). Increased LMR correlated negatively with decreased NLR (R value = −0.369, $P = .003$).

Table V
Association between overall survival and systemic immune inflammatory markers

Markers	Categories	*n*	MST (month)	Hazard ratio	95% CI	*P* value
Preneoadjuvant						
NLR	≥ 3.0	35	16.7	1.01	0.570–1.77	.987
	< 3.0	30	18.7			
PLR	≥ 183	34	17.5	1.07	0.604–1.88	.825
	< 183	31	18.1			
LMR	≥ 4.0	28	26.2			
	< 4.0	37	16.0	1.56	0.859–2.82	.142
PNI	≥ 48.5	35	18.7			
	< 48.5	29	15.6	1.03	0.583–1.82	.920
Postneoadjuvant						
NLR	≥ 2.5	26	15.6	1.85	1.02–3.36	.044
	< 2.5	39	31.7			
PLR	≥ 160	23	15.9	1.42	0.776–2.59	.253
	< 160	42	31.7			
LMR	≥ 3.0	26	31.7			
	< 3.0	39	14.9	2.49	1.34–4.62	.003
PNI	≥ 45.0	20	29.9			
	< 45.0	45	14.9	1.96	1.03-3.71	.039

CI, confidence interval; *MST*, median survival time.

Prognostic factors in BRPC patients with neoadjuvant therapy followed by pancreatectomy

Table VII presents the results of the univariate and multivariate analyses of the demographic characteristics, tumor characteristics, and postoperative complications in patients with neoadjuvant therapy followed by pancreatectomy.

The types of neoadjuvant therapy was not associated with survival (S − 1 + radiation 14.9 months versus gemcitabine + S − 1 18.1 months versus mFOLFIRINOX 18.4 months versus nab-paclitaxel + gemcitabine 24.9 months, $P = .211$) (Table VII). Similarly, completion rate of adjuvant therapy were equivalent across various neoadjuvant therapy regimens (S − 1 + radiation 43.5% versus gemcitabine + S − 1 57.1% versus mFOLFIRINOX 66.7% versus nab-paclitaxel + gemcitabine 36.4%, $P = .456$). The heterogeneity in the neoadjuvant therapy regimens of this study did not affect completion of adjuvant therapy and survival in patients with BRPC. The restaging CT scan after neoadjuvant therapy showed 10 patients (15.4%) with partial response, 54 patients (83.0%) with stable disease, and 1 patient (1.5%) with progressive disease attributable to progressive local disease. Overall survival in patients with partial response was similar with overall survival in those with stable disease (partial response; MST 20.0 months versus stable disease; MST 16.7 months; $P = .244$). Preneoadjuvant CA19-9 (HR 1.85, 95% CI 0.25–1.16; P = .115) and postneoadjuvant CA19-9 (HR 1.37, 95% CI 0.41–1.29; P = .284) were also not associated with survival.

In the univariate analysis, postneoadjuvant NLR, postneoadjuvant LMR, postneoadjuvant PNI, T factor, lymph node status (N1), postoperative adjuvant therapy, and completion of adjuvant therapy were identified as prognostic factors. In the multivariate Cox proportional hazard analysis of the prognostic factors, postneoadjuvant LMR < 3.0 in systemic immune inflammatory markers

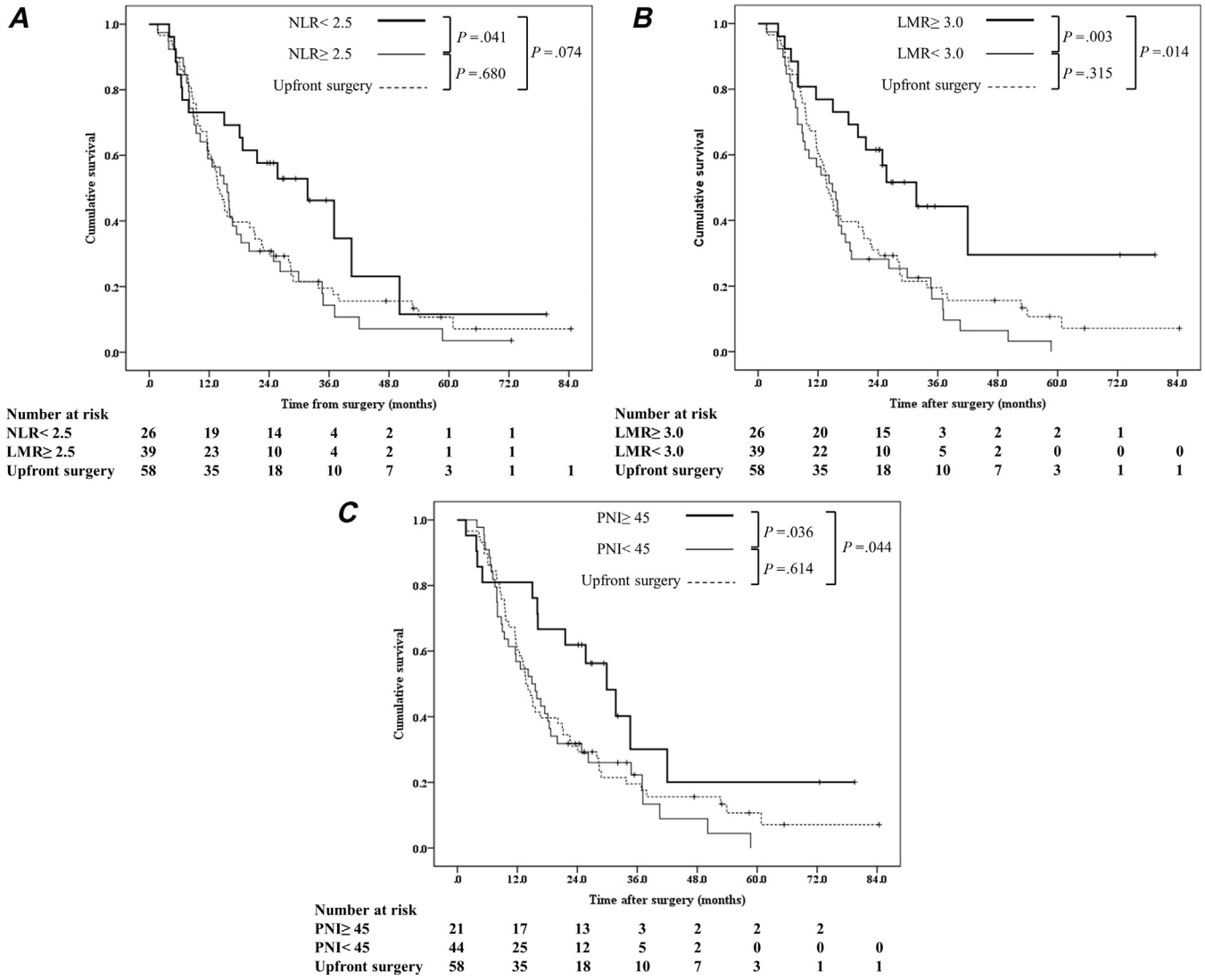

Fig 1. (*A*) Comparison of overall survival among postadjuvant NLR ≥ 2.5 versus postadjuvant NLR < 2.5 versus upfront surgery. (*B*) Comparison of overall survival among postadjuvant LMR ≥ 3.0 versus postadjuvant LMR < 3.0 versus upfront surgery. (*C*) Comparison of overall survival among postadjuvant PNI ≥ 45 versus postadjuvant PNI < 45 versus upfront surgery.

was detected as an independent prognostic factor (HR 2.59; 95% CI 1.02–6.58; $P = .045$). Regarding demographics and tumor characteristics, the multivariate analysis also identified T4 (HR 1.82; 95% CI 1.11–3.01; $P = .029$), lymph node status (N1) (HR 2.62; 95% CI 1.24–5.51; $P = .012$) and no completion of adjuvant therapy (HR 7.09; 95% CI 3.08–16.4; $P < .001$) as predictors of poor prognosis.

Discussion

This is the first study to report that low LMR after neoadjuvant therapy is a predictor of poor survival in BRPC patients with neoadjuvant therapy followed by pancreatectomy. Preoperative LMR is an important prognostic biomarker for pancreatic cancer, according to an earlier meta-analysis.[27] However, few studies have investigated how LMR after neoadjuvant therapy predicts survival after pancreatectomy in patients with BRPC. The important point is that postneoadjuvant LMR, but not preneoadjuvant LMR, is an independent prognostic factor in BRPC patients who received neoadjuvant therapy. There may be a reason that neoadjuvant therapy causes hematologic toxicity because of its myelosuppressive effect.[17,18] Preneoadjuvant LMR might not therefore precisely reflect survival because lymphocyte counts or monocyte counts were affected by neoadjuvant therapy.

The mechanism by which low LMR predicts poor survival in pancreatic cancer has not been fully defined. However, lymphocytes, especially tumor-infiltrating lymphocytes, such as CD4+ and CD8+ T lymphocytes, have crucial roles in enhancement of antitumor immune response.[28–30] CD4+ T lymphocytes have been shown to be critical in initiation and maintenance of immune responses against cancer cells by secreting cytokines, such as IL-2, or by activating antigen-presenting cells, including dendritic cells.[28–30] CD8+ T lymphocytes, which recognize tumor-associated antigens in the context of MHC class I molecules, directly target tumor cells and inhibit tumor cell proliferation. Several studies reported that lymphopenia is associated with poor prognosis in pancreatic cancer.[28–30] On the other hand, monocytes have roles in promoting tumor invasion and angiogenesis. Tumor-associated macrophages, which develop from circulating monocytes, directly suppress the T-cell mediated antitumor immune response and secrete potent proangiogenic factors, such as vascular endothelial growth factor A or tumor necrosis factor α (TNF-α).[31–33] Lower LMR may therefore represent inadequate antitumor immunity because

Table VI
Association between postneoadjuvant LMR and clinicopathologic factors

	LMR < 3.0 (n = 39)	LMR ≥ 3.0 (n = 26)	P value
Age (years)	68±9	67±9	.760
Sex (male/female)	25/14	13/13	.258
RECIST 1.1, (PR/SD/PD) n (%)	5(13)/33(85)/1(2)	5(19)/21(81)/0(0)	.574
UICC stage (IIa / IIb / III) n (%)	11(28)/23(59)/5(13)	9(35)/16(62)/1(4)	.452
T stage (T3/T4) n (%)	34 (87)/5 (13)	25 (96)/1 (4)	.221
N stage (N0/N1) n (%)	11 (28)/28 (72)	9 (35)/17 (65)	.583
Margin status (R0/R1) n (%)	29 (74)/10 (26)	23 (89)/3 (11)	.164
Pathological response to neoadjuvant therapy n (%)			
I, IIA / IIB, III	34 (87)/5 (13)	17 (65)/9 (35)	.036
Preadjuvant CA19-9 (U/ml) n (%)			
< 37/≥ 37	5 (13)/33 (87)	6 (23)/20 (77)	.280
Postadjuvant CA19-9 (U/ml) n (%)			
< 37/≥ 37	13 (33)/26 (77)	12(46)/14 (54)	.298
Postadjuvant NLR n (%)			
< 2.5/≥ 2.5	9 (23)/30 (77)	17 (65)/9 (35)	.001
Postadjuvant PLR n (%)			
< 160/≥ 160	11 (28)/28(72)	12 (87)/14(13)	.138
Postadjuvant PNI n (%)			
< 45/≥ 45	32 (82)/7 (18)	14(13) /12 (87)	.003
Implementation of adjuvant therapy n (%)	29 (74)	20 (77)	.814
Completion of adjuvant therapy n (%)	15 (39)	15 (59)	.128

Note: Pathologic response to neoadjuvant therapy is based on Evans' classification.
BR-PV, borderline resectable pancreatic cancer with portal vein involvement; *BR-A*, borderline resectable pancreatic cancer with artery involvement; *RECIST*: response evaluation criteria in solid tumors; *PR*, partial response; *SD*, stable disease; *PD*, progress disease; *UICC*, International Union Against Cancer; *R*, resectability.

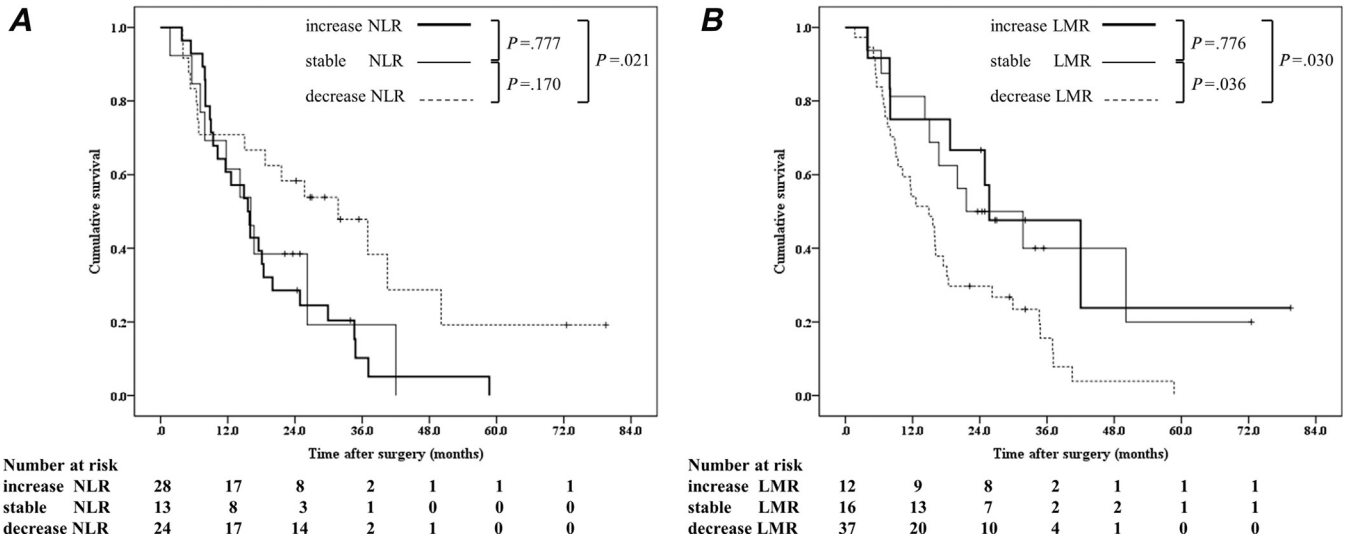

Fig 2. (*A*) Comparison of overall survival in the change of NLR before and after neoadjuvant therapy. (*B*) Comparison of overall survival in the change of LMR before and after neoadjuvant therapy.

of decreased lymphocyte counts and promotion of a tumor inflammatory microenvironment because of increased monocyte counts. However, it would be more important to compare the association between immunologic response within the tumor and the change of lymphocyte in peripheral blood in BRPC patients with pancreatectomy after neoadjuvant therapy. Unfortunately, resected tumor specimens for immunohistochemistry had not been obtained among the patients in this study. Regarding the tumor microenvironment of pancreatic cancer, one study reported that high levels of tumor-infiltrating CD4+ and CD8+ T lymphocytes in the tumor microenvironment were significantly associated with longer survival.[30] Moreover, another study reported that neoadjuvant therapy may induce the accumulation of tumor-infiltrating CD4+ and CD8+ T lymphocytes in the tumor microenvironment of pancreatic cancer.[34]

In this study, in addition to postneoadjuvant LMR, postneoadjuvant PNI tended to be another useful prognostic factor other than preneoadjuvant PNI in BRPC patients with neoadjuvant therapy followed by pancreatectomy. Some studies report that PNI, which was calculated based on albumin levels and lymphocyte counts, not only predicts survival, but also is widely used as a parameter for nutritional assessment and predicts survival in patients with pancreatic cancer.[15,16] Neoadjuvant therapy has been reported to have the potential to deteriorate a patient's nutritional status because of the adverse effects.[35,36] Both lymphocyte counts and albumin levels are integral parts of PNI and these factors could reflect nutrition status. Therefore, postneoadjuvant PNI may more accurately predict survival and nutrition status of patients with BRPC receiving neoadjuvant therapy than preneoadjuvant PNI.

Table VII
Univariate and multivariate Cox hazard regression analysis for overall survival of BRPC with neoadjuvant therapy followed by pancreatectomy

Factors	*n*	MST (months)	Univariate analysis			Multivariate analysis		
			HR	95% CI	*P* value	HR	95% CI	*P* value
Sex								
Male	38	16.1	1.18	0.64–1.13	.252			
Female	27	24.9						
Age (years)								
< 70	36	18.1						
≥ 70	29	16.7	1.01	0.57–1.78	.978			
Preoperative biliary drainage								
Yes	7	16.7	1.23	0.29–2.28	.695			
No	58	29.9						
Neoadjuvant therapy								
Chemotherapy	42	20.0						
chemoradiotherapy	23	14.9	1.72	0.33–1.01	.055			
Neoadjuvant therapy								
S − 1 + radiation	23	14.9			.211			
Gemcitabine + S − 1	14	18.1	0.102	0.27–1.13				
mFOLFIRINOX	6	18.4	0.772	0.33–2.29				
nab-paclitaxel + gemcitabine	22	24.9	0.095	0.26–1.11				
RECIST classification								
PR	10	20.0						
SD/PD	55	16.7	1.65	0.70–3.88	.298			
Preneoadjuvant CA19-9								
< 37 IU/L	11	37.1						
≥ 37 IU/L	54	15.6	1.85	0.25–1.16	.115			
Postneoadjuvant CA19-9								
< 37 IU/L	25	20.0						
≥ 37 IU/L	40	14.9	1.37	0.41–1.29	.284			
Postneoadjuvant NLR								
< 2.5	26	31.7						
≥ 2.5	39	15.6	1.85	1.02–3.36	.044	1.09	0.52–2.29	.826
Postneoadjuvant LMR								
< 3.0	26	14.9	2.49	1.34–4.62	.003	2.59	1.02–6.58	.045
≥ 3.0	39	31.7						
Postneoadjuvant PNI								
< 45	45	14.9	1.96	1.03–3.71	0.036	1.10	0.44–2.72	.838
≥ 45	20	29.9						
Tumor location								
Head	41	17.5						
Body and tail	24	16.7	1.12	0.85–1.48	.430			
T stage								
T3	59	18.4						
T4	6	7.5	1.59	1.03–2.44	.037	1.82	1.11–3.01	.018
N stage								
N0	20	37.0						
N1	45	15.0	2.47	1.26–4.82	.008	2.62	1.24–5.51	.012
Surgical margins								
R0	52	18.7						
R1	13	9.4	1.24	0.88–1.73	.215			
Severe complication								
Yes	14	15.6	1.19	0.88–1.61	.267			
No	51	18.7						
Pathologic response to neoadjuvant								
I, IIA	51	16.1	1.49	0.69–3.19	.301			
IIB, III	14	17.5						
Adjuvant therapy								
Yes	49	20.0						
No	16	7.5	2.38	1.20–4.76	.012	1.05	0.40–2.28	.917
Completion of adjuvant therapy								
Yes	30	34.6						
No	35	8.8	5.56	2.86–11.1	< .001	7.09	3.08–16.4	< .001

Note: Pathologic response to neoadjuvant therapy is based on Evans' classification.
CI, confidence interval; *HR RECIST*, response evaluation criteria in solid tumors; *MST*, median survival time; *PR*, partial response; *SD*, stable disease; *PD*, progress disease; *UICC*, International Union Against Cancer; *R*, resectability.

Some studies have reported that neoadjuvant therapy for pancreatic cancer reduces circulating lymphocyte counts during the therapy itself and may affect the adaptive immune system because of chemotherapy toxicity.[37,38] On the other hand, several studies have reported that neoadjuvant therapy improved survival for BRPC patients compared with upfront surgery.[4–6] One of the advantages of neoadjuvant therapy is to deliver high-dose intensity without the influence of postoperative outcomes. In contrast, higher dose intensity may introduce a risk to induce more severe myelosuppression. In this study, RDI negatively correlated with postadjuvant LMR (R value = −0.281, $P = .023$). Perioperative management should be required to reconcile the discrepancy

between the advantages and disadvantages of neoadjuvant therapy. Lower LMR might be induced not only by myelosuppression because of chemotherapy toxicity, but also by malnutrition attributable to chemotherapy toxicity. This study could not evaluate whether lower postadjuvant LMR was improved by a longer waiting time before surgery. However, it is an urgent issue to take measures against myelosuppression or malnutrition attributable to chemotherapy toxicity. One study reported that enteral nutrition support during neoadjuvant chemotherapy reduced the incidence of chemotherapy-related hematological toxicities such as neutropenia or lymphopenia in patients with esophageal cancers.[39] Therefore, the implementation of adequate nutritional support during neoadjuvant therapy may lead to better survival by improving postneoadjuvant LMR in patients with BRPC. At the same time, more effective neoadjuvant therapy should be added for BRPC patients with low LMR because survival of BRPC patients with low LMR after neoadjuvant therapy was as poor as that of BRPC patients with upfront surgery.

Some studies have reported that a decline in CA19-9 after neoadjuvant therapy was associated with better survival.[40,41] However, in this study, normalization of postneoadjuvant CA19-9 was not a prognostic factor in patients with BRPC. A reason for this may be that 11 of 65 patients (16.9%) in this study had CA19-9 nonproducing pancreatic cancer attributable to the lack of the Lewis gene. CA19-9 nonproducing pancreatic cancer could potentially confound the analysis regarding the association between CA19-9 and survival.

This study has several limitations because it is a retrospective review. First, there was selection bias regarding the indication for, regimens of, and administration intervals of neoadjuvant therapy. The regimes of neoadjuvant therapy have changed during the course of our study period. However, the heterogeneity in the neoadjuvant therapy regimens of this study did not affect survival in patients with BRPC. Second, lymphocyte subpopulations or the functional status of lymphocytes against pancreatic cancer was not assessed to clarify the association between lower LMR after neoadjuvant therapy and poor survival in BRPC. The study relatively consisted of a small sample size in a single institution. Therefore, further validation study in a large population across multiple centers is required to confirm this result.

In conclusion, low LMR after neoadjuvant therapy shows useful prognostic information for BRPC patients with pancreatectomy after neoadjuvant therapy as one of systemic immune inflammatory markers. This result might indicate the potential role of LMR to stratify a treatment strategy in BRPC patients. The implementation of adequate nutritional support during neoadjuvant therapy may improve postneoadjuvant LMR. Furthermore, the study of the management during neoadjuvant therapy to prevent low postneoadjuvant LMR will be required to improve survival in patients with BRPC.

Conflict of interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

References

1. National Comprehensive Cancer Network. NCCN practice guidelines for pancreatic cancer, v2 Web site. https://www.nccn.org/professionals/physician_gls/PDF/pancreatic.pdf. Accessed July 10, 2018.
2. Uesaka K, Boku N, Fukutomi A, et al. Adjuvant chemotherapy of S-1 versus gemcitabine for resected pancreatic cancer: a phase 3, open-label, randomised, non-inferiority trial (JASPAC 01). *Lancet*. 2016;388:248–257.
3. Neoptolemos JP, Palmer DH, Ghaneh P, et al. Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patients with resected pancreatic cancer (ESPAC-4): a multicentre, open-label, randomised, phase 3 trial. *Lancet*. 2017;389:1011–1024.
4. Hirono S, Kawai M, Okada KI, et al. Treatment strategy for borderline resectable pancreatic cancer with radiographic artery involvement. *Pancreas*. 2016;45:1438–1446.
5. Jang JY, Han Y, Lee H, et al. oncological benefits of neoadjuvant chemoradiation with gemcitabine versus upfront surgery in patients with borderline resectable pancreatic cancer: a prospective, randomized, open-label, multicenter phase 2/3 trial. *Ann Surg*. 2018;268:215–222.
6. Blazer M, Wu C, Goldberg RM, et al. Neoadjuvant modified (m) FOLFIRINOX for locally advanced unresectable (LAPC) and borderline resectable (BRPC) adenocarcinoma of the pancreas. *Ann Surg Oncol*. 2015;22:1153–1159.
7. Groot VP, Gemenetzis G, Blair AB, et al. Defining and predicting early recurrence in 957 patients with resected pancreatic ductal adenocarcinoma. *Ann Surg*. 2018. https://doi.org/10.1097/SLA.0000000000002734.
8. Xia BT, Habib DA, Dhar VK, et al. Early recurrence and omission of adjuvant therapy after pancreaticoduodenectomy argue against a surgery-first approach. *Ann Surg Oncol*. 2016;23:4156–4164.
9. Okabayashi T, Shima Y, Sumiyoshi T, et al. a novel physiobiological parameter-based grading system for resectable pancreatic cancer. *Ann Surg Oncol*. 2018;25:1889–1895.
10. Stevens L, Pathak S, Nunes QM, et al. Prognostic significance of pre-operative C-reactive protein and the neutrophil-lymphocyte ratio in resectable pancreatic cancer: a systematic review. *HPB (Oxford)*. 2015;17:285–291.
11. Yu J, Ding Z, Yang Y, Liu S. Increased platelet-to-lymphocytes ratio is associated with poor long-term prognosis in patients with pancreatic cancer after surgery. *Medicine (Baltimore)*. 2018;97:e11002.
12. Shirai Y, Shiba H, Sakamoto T, et al. Preoperative platelet to lymphocyte ratio predicts outcome of patients with pancreatic ductal adenocarcinoma after pancreatic resection. *Surgery*. 2015;158:360–365.
13. Xue P, Hang J, Huang W, et al. Validation of lymphocyte-to-monocyte ratio as a prognostic factor in advanced pancreatic cancer: an East Asian cohort study of 2 countries. *Pancreas*. 2017;46:1011–1017.
14. Sierzega M, Lenart M, Rutkowska M, et al. preoperative neutrophil-lymphocyte and lymphocyte-monocyte ratios reflect immune cell population rearrangement in resectable pancreatic cancer. *Ann Surg Oncol*. 2017;24:808–815.
15. Geng Y, Qi Q, Sun M, Chen H, Wang P, Chen Z. Prognostic nutritional index predicts survival and correlates with systemic inflammatory response in advanced pancreatic cancer. *Eur J Surg Oncol*. 2015;41:1508–1514.
16. Lee SH, Chung MJ, Kim B, et al. the significance of the prognostic nutritional index for all stages of pancreatic cancer. *Nutr Cancer*. 2017;69:512–519.
17. Yamaue H, Satoi S, Kanbe T, et al. Phase II clinical study of alternate-day oral therapy with S-1 as first-line chemotherapy for locally-advanced and metastatic pancreatic cancer. *Cancer Chemother Pharmacol*. 2014;73:97–102.
18. Okada KI, Kawai M, Hirono S, et al. Impact of treatment duration of neoadjuvant FIRINOX in patients with borderline resectable pancreatic cancer: a pilot trial. *Cancer Chemother Pharmacol*. 2016;78:719–726.
19. Okada KI, Hirono S, Kawai M, et al. Phase I study of nab-paclitaxel plus gemcitabine as neoadjuvant therapy for borderline resectable pancreatic cancer. *Anticancer Res*. 2017;37:853–858.
20. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45:228–247.
21. Brierley JD, Gospodarowicz MK, Wittekind C. *TNM classification of malignant tumours*. 8th ed. Hoboken, NJ: Wiley-Blackwell; 2017.
22. Evans DB, Rich TA, Byrd DR, et al. Preoperative chemoradiation and pancreaticoduodenectomy for adenocarcinoma of the pancreas. *Arch Surg*. 1992;127:1335–1339.
23. Kanda M, Fujii T, Kodera Y, Nagai S, Takeda S, Nakao A. Nutritional predictors of postoperative outcome in pancreatic cancer. *Br J Surg*. 2011;98:268–274.
24. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg*. 2004;240:205–213.
25. Bassi C, Marchegiani G, Dervenis C, et al. The 2016 update of the International Study Group (ISGPS) definition and grading of postoperative pancreatic fistula: 11 years after. *Surgery*. 2017;161:584–591.
26. Wente MN, Bassi C, Dervenis C, et al. Delayed gastric emptying (DGE) after pancreatic surgery: a suggested definition by the International Study Group of Pancreatic Surgery (ISGPS). *Surgery*. 2007;142:761–768.
27. Li W, Tao L, Zhang L, Xiu D. Prognostic role of lymphocyte to monocyte ratio for patients with pancreatic cancer: a systematic review and meta-analysis. *Onco Targets Ther*. 2017;10:3391–3397.
28. Fukunaga A, Miyamoto M, Cho Y, et al. $CD8^+$ tumor-infiltrating lymphocytes together with $CD4^+$ tumor-infiltration lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. *Pancreas*. 2004;28:26–31.
29. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity*. 2004;21:137–148.
30. Ino Y, Yamazaki-Itoh R, Shimada K, et al. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. *Br J Cancer*. 2013;108:914–923.
31. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. 2008;454:436–444.

32. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell*. 2006;124:263–266.
33. Riabov V, Gudima A, Wang N, Mickley A, Orekhov A, Kzhyshkowska J. Role of tumor associated macrophages in tumor angiogenesis and lymphangiogenesis. *Front Physiology*. 2014;5:75.
34. Homma Y, Taniguchi K, Murakami T, et al. Immunological impact of neoadjuvant chemoradiotherapy in patients with borderline resectable pancreatic ductal adenocarcinoma. *Ann Surg Oncol*. 2014;21:670–676.
35. Motoori M, Yano M, Yasuda T, et al. Relationship between immunological parameters and the severity of neutropenia and effect of enteral nutrition on immune status during neoadjuvant chemotherapy on patients with advanced esophageal cancer. *Oncology*. 2012;83:91–100.
36. Reisinger KW, Bosmans JW, Uittenbogaart M, et al. Loss of skeletal muscle mass during neoadjuvant chemoradiotherapy predicts postoperative mortality in esophageal cancer surgery. *Ann Surg Oncol*. 2015;22:4445–4452.
37. Plate JM, Plate AE, Shott S, Bograd S, Harris JE. Effect of gemcitabine on immune cells in subjects with adenocarcinoma of the pancreas. *Cancer Immunol Immunother*. 2005;54:915–925.
38. Wild AT, Ye X, Ellsworth SG, et al. The association between chemoradiation-related lymphopenia and clinical outcomes in patients with locally advanced pancreatic adenocarcinoma. *Am J Clin Oncol*. 2015;38:259–265.
39. Miyata H, Yano M, Yasuda T, et al. Randomized study of clinical effect of enteral nutrition support during neoadjuvant chemotherapy on chemotherapy-related toxicity in patients with esophageal cancer. *Clin Nutr*. 2012;31:330–336.
40. Reni M, Zanon S, Balzano G, et al. Selecting patients for resection after primary chemotherapy for non-metastatic pancreatic adenocarcinoma. *Ann Oncol*. 2017;28:2786–2792.
41. Tsai S, George B, Wittmann D, et al. Importance of normalization of ca19-9 levels following neoadjuvant therapy in patients with localized pancreatic cancer. *Ann Surg*. 2018. https://doi.org/10.1097/SLA.0000000000003049.

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