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ORIGINAL ARTICLE – GASTROINTESTINAL ONCOLOGY

Prognostic Significance of Perineural Invasion in Patients with Gastric Cancer Who Underwent Curative Resection

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ABSTRACT

Background. The prognostic significance of perineural invasion (PNI) in gastric cancer has been previously investigated in a few studies, but had not reached a consensus. The aim of this study was to determine the prognostic value of PNI in patients with gastric cancer who underwent curative resection.

Materials and Methods. We retrospectively analyzed 238 patients who had undergone curative gastrectomy. Paraffin sections of surgical specimens from all patients were stained with hematoxylin and eosin. PNI was defined when carcinoma cells infiltrated into the perineurium or neural fascicles. PNI and the other prognostic factors were evaluated by univariate and multivariate analysis.

Results. PNI was detected as positive in 180 of the 238 patients (75.6%). pT stage, tumor size, lymph node metastasis, clinical stage, tumor differentiation, Borrmann classification, histological type, lymphatic vessel invasion, and blood vessel invasion were closely associated with the presence of PNI. The PNI-positive tumors had significantly larger size and more lymph node metastasis than the PNI-negative tumors ($P = .001$ and $P < .001$, respectively). The median survival of the PNI-positive patients was significantly worse than that of the PNI-negative patients (28.1 vs. 64.9 months, $P = .001$). Multivariate analysis indicated that the positivity of PNI was an independent prognostic factor ($P = .02$, hazard ratio [HR]: 2.75; 95% confidence interval [95% CI]:1.12–3.13) as were classical clinicopathological features.

Conclusion. Our results showed that the frequency of PNI was high in patients with gastric cancer who underwent curative gastrectomy and the proportion of PNI positivity increased with progression and clinical stage of disease. PNI may be useful in detecting patients who had poor prognosis after curative resection in gastric cancer.

The incidence and mortality of gastric cancer have decreased dramatically over the past several decades; nonetheless, the disease remains a major public health issue as the fourth most common cancer and second leading cause of cancer death worldwide.[1,2] Although the incidence rate has declined, its prognosis has not improved much, and the cumulative 5-year survival rates of all patients with gastric cancer have changed only slightly over the past 4 decades but remain under 20%.[3]

Radical surgery in combination with systemic lymph node dissection is the current treatment of choice for gastric cancer.[4] A correct definition of poor prognostic factors may help to guide more aggressive adjuvant treatments protocols, postoperatively.[5,6] So it is important to determine new biological or pathological indicators related to survival in addition to well-known prognostic factors such as TNM staging classification and clinical stage.

Perineural invasion (PNI) is the process of neoplastic invasion of nerves, and it also has been called neurotropic carcinomatous spread and perineural spread. PNI is the infiltration of the perineurium or neural fascicles around a

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tumor by cancer cells. It is commonly detected in carcinomas of the pancreas and biliary tract, but is relatively rare in rectal cancer.[7–13] It is reported to be a crucial route for the local spread of tumor associated with poor prognosis in pancreas and biliary tract cancers. However, the prognostic importance of PNI in gastric cancer has been evaluated in a few studies, but in these trials PNI did not provide any additional information to the classical prognostic factors.[14–16] Recently, Tianhang et al. indicated that the incidence of PNI in gastric cancer was high, and it corresponded to the progression of disease. Finally, the authors concluded that PNI may provide additional information for identifying patients who are at high risk for poor prognosis.[17] In the present study, we investigated the value of PNI as a prognostic factor in patients with gastric carcinoma who underwent curative resection and without distant metastasis. Furthermore, the association of PNI with the other clinicopathological factors and the effect of PNI on survival were also analyzed.

PATIENTS AND METHODS

This study included a total of 238 patients with gastric cancer who had undergone curative gastrectomy at Dr. Lutfi Kirdar Kartal Education and Research Hospital, between May 2003 and January 2009. The clinicopathologic findings were determined according to the Japanese Classification of Gastric Carcinoma (JCGC).[18] All patients underwent either distal partial gastrectomy, proximal partial gastrectomy, or total gastrectomy with regional lymph nodes dissection as curative intent. The eligibility criteria consisted of histologically confirmed R_0 gastric resection, which was defined as no macroscopic or microscopic residual tumor. Postoperative survival expectancy of patients was longer than 3 months. The patients with distant metastasis at diagnosis were excluded from the study.

Clinical information about age, gender, resection type, tumor location, histopathology, tumor stage, tumor size, histologic grade, lymph node involvement, the depth of tumor invasion, lymphatic vessel invasion and blood vessel invasion, resection margins, Borrmann classification, histological type, adjuvant chemotherapy and radiation therapy type, responses to treatment, and survival were obtained from patients charts.

Histopathological Evaluation

Surgical specimens were fixed in 10% formalin and embedded in the paraffin. Paraffin-embedded blocks were cut into 5-μm thick sections and stained with hematoxylin and eosin; the endoneurium of nerve fibers and perineurium around the nerve fasciculi were strongly stained. PNI was assessed as positive when cancer cells infiltrated into the perineurium or neural fasciculus intramurally (Fig. 1).

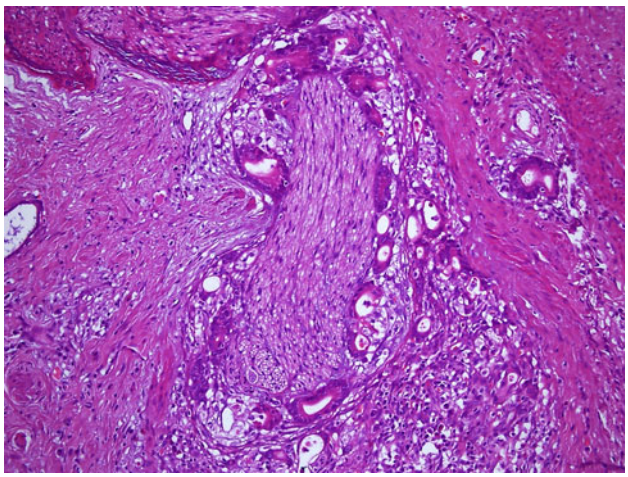

FIG. 1 Tumor cells infiltrating into the perineurium or neural fasciculus are seen (hematoxylin-eosin staining). ×200

The depth of tumor invasion, lymph node involvement (N_0, no metastasis; $N_{1,\ 1–6}$ metastatic lymph nodes; N_2, 7–15 metastatic lymph nodes; N_3, >15 metastatic lymph nodes) and distant metastasis, stage grouping, and tumor grade were classified according to the 1997 UICC TNM staging classification for gastric cancer.[19] In addition, the histologic type of gastric carcinoma was grouped with respect to the histological classification for gastric carcinoma by the World Health Organization (WHO).[20] Early gastric cancer was defined as a gastric carcinoma confined to the mucosa and/or submucosal and designated as pT1, regardless of the lymph node metastatic status. Locally advanced gastric cancer was also defined as an invasive gastric cancer with no distant metastasis, regardless of lymph node metastatic status (pT2–T4, N0–3, M0).[18,19]

Statistical Analysis

Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL) software. The relationship between PNI positivity and the other clinicopathological factors were analyzed by the chi-square test and Fisher exact test. Survival analysis and curves were established according to the Kaplan–Meier method and compared by the log-rank test. Disease-free survival (DFS) was defined as the time from curative surgery to disease progression or recurrence or to the date of death or lost in follow-up. Overall survival (OS) was described as the time from diagnosis to the date of the patient's death or lost in follow-up. Multivariate analysis to assess the role of PNI and the other clinicopathological features as prognostic factors were performed by the Cox regression analysis. Multivariate *P* values were used to characterize the independence of

these factors. The 95% confidence (95% CI) was used to quantify the relationship between survival time and each independent factor. All P values were two-sided in tests, and P values less than .05 were considered to be statistically significant.

RESULTS

A total of 238 patients who had undergone radical gastrectomy for gastric cancer were retrospectively analyzed; 80 patients were women and 158 were men. The median age was 59 years, ranging from 29 to 85 years. There were 126 patients who were younger than 60 years (52.9%). Based on the number of lymph node metastasis, 50 (21%) patients were classified as pN0, 80 (33.6%) as pN1, 63 (26.5%) as pN2, and 45 (18.9%) as pN3. The majority of patients were pT3 (126 patients, 52.9%) and clinical stage III (102 patients, 42.9%).

PNI was detected as positive in 180 of the 238 patients (75.6%). The depth of invasion (pT stage), tumor size, lymph node metastasis, clinical stage, tumor differentiation, Borrmann classification, histological type, lymphatic vessel invasion, and blood vessel invasion were closely associated with the PNI positivity. On the other hand, the relationship between PNI positivity and gender, age, tumor site, surgery type was not detected. The associations between PNI and clinicopathological factors are shown in Table 1.

Tumors with PNI positive were larger in size and had more lymph node metastases than those in the PNI-negative patients ($P = .001$ and $<.001$, respectively). The positivity of PNI was also significantly increased in tumors with undifferentiated histology ($P = .009$), mural invasion ($P < .001$), in advanced stage ($P < .001$), lymphatic vessel invasion ($P < .001$), blood vessel invasion ($P < .001$). Recurrence occurred in 42.8% of patients (102 of 238), and PNI-positivity was detected in 86 of 102 patients (84.3%) with recurrent disease in our study. The recurrence was also related to the PNI positivity ($P = .009$).

At the median follow-up of 29.5 months (range, 7.5–73 months), 3-year OS and the median OS time were 73.9% and 64.9 months (SE 21.7, 95% CI 22.2–77.3), respectively, for patients who had no PNI positivity, whereas they were 40.8% and 28.1 months (SE 4.3, 95% CI 19.6–36.6), respectively, for the PNI-positive patients. The median survival of the PNI-positive patients was significantly worse than that of the PNI-negative patients (28.1 vs. 64.9 months, $P = .001$, Fig. 2).

The positivity of PNI was closely associated with OS of patients with radically resected gastric cancer in the univariate analysis ($P = .001$). Therefore, we performed multivariate analysis with Cox regression method in order to further evaluate the prognostic significance of PNI and the other clinicopathological factors. Multivariate analysis indicated that the positivity of PNI was an independent prognostic factor ($P = .02$, HR 2.75, 95% CI 1.12–3.13) as were classical clinicopathological features such as pN stage ($P = .001$), tumor differentiation ($P = .01$), pT stage ($P = .04$), age ($P = .01$), surgery type ($P = .02$), tumor location ($P = .03$), and Borrmann classification ($P = .03$). Furthermore, as previously known, the absence of recurrence was an independent prognostic indicator by multivariate analysis. Table 2 shows the results of multivariate analysis in all patients.

In subgroup analysis, PNI was only associated with surgery type and tumor site ($P = .001$ and .001, respectively) in patients with early-stage gastric carcinoma. Survival analysis could not be performed for PNI because of small sample size in this group. However, the significant correlation between PNI positivity and tumor differentiation, Borrmann classification, pT stage, pN stage, clinical stage, lymphatic vessel invasion, and blood vessel invasion was detected in patients with locally advanced gastric cancer (pT2–T4,N0–3,M0). The relationship between PNI positivity and gender, age, tumor site, and surgery type was not detected. The associations between PNI and clinicopathological factors are listed for locally advanced gastric cancer patients in Table 3.

Tumors with PNI positive had more lymph nodes metastasis than those in the PNI-negative patients ($P < .001$). The positivity of PNI was also significantly increased in tumors with undifferentiated histology ($P = .03$), mural invasion ($P = .001$), in advanced stage ($P < .001$), lymphatic vessel invasion ($P < .001$), and blood vessel invasion ($P < .001$) in patients with locally advanced gastric cancer. The median survival and 3-years OS interval of the PNI-positive patients was significantly lower than that of the PNI-negative patients in locally advanced stage subgroup (27.9 months, 38.5% vs. 60.3 months, 69.8%, respectively, $P = .025$, Fig. 3). In the multivariate analysis, the presence of PNI ($P = .03$, HR 1.75, 95% CI 0.52–3.10) was found to be an independent prognostic factor, as were pN stage ($P = .02$) and age ($P = .01$) for OS in patients with locally advanced gastric carcinoma (Table 4).

DISCUSSION

In this study, we stained specimens with hematoxylin and eosin to determine the positivity of PNI in patients with gastric cancer and found that 180 of the 238 patients (75.6%) were PNI positive. The tumor size, pT stage, lymph node metastasis, clinical stage, tumor differentiation, Borrmann classification, histological type, lymphatic

TABLE 1 The relationship between PNI and clinicopathological factors in patients with gastric cancer who underwent curative resection

Factors	PNI positive *n* (%)	PNI negative *n* (%)	*P* value
All	180 (75.6)	58 (24.4)	–
Gender			
Male	120 (66.6)	38 (65.5)	.87
Female	60 (33.4)	20 (34.5)	
Age (year)			
≤60	92 (51.1)	34 (58.6)	.36
>60	88 (48.9)	24 (41.4)	
Tumor site			
Upper	36 (20)	9 (15.5)	.22
Middle	56 (31)	19 (32.7)	
Lower	78 (43)	30 (51.8)	
Diffuse	10 (6)	–	
Surgery type			
Proximal	33 (18.3)	6 (10.3)	.24
Distal	66 (36.6)	27 (46.5)	
Total	81 (45.1)	25 (43.2)	
Tumor size			
≤3 cm	26 (14.4)	22 (37.9)	.001
≤6 cm	77 (42.8)	17 (29.3)	
>6 cm	77 (42.8)	19 (32.8)	
Tumor differentiation			
Well differentiated	4 (2.2)	4 (6.8)	.009
Moderately differentiated	87 (48.3)	38 (65.5)	
Poorly differentiated	89 (49.5)	16 (27.7)	
Borrmann classification			
Type I	2 (1.1)	7 (12)	.016
Type II	102 (56.6)	24 (41.3)	
Type III	55 (30.5)	27 (46.7)	
Type IV	21 (11.8)	–	
Histological type			
Intestinal	84 (46.7)	35 (60.3)	.039
Diffuse	96 (53.3)	23 (39.7)	
pT stage			
T1	1 (0.5)	14 (24)	<.001
T2	49 (27.2)	25 (43)	
T3	109 (60.5)	17 (29.5)	
T4	21 (11.8)	2 (3.5)	
pN stage			
N0	20 (11.1)	30 (52)	<.001
N1	60 (33.3)	20 (34.4)	
N2	59 (32.7)	4 (6.8)	
N3	41 (22.9)	4 (6.8)	
Clinical stage			
Stage I	9 (5)	25 (43.1)	<.001
Stage II	23 (12.7)	18 (31)	
Stage III	91 (50.5)	11 (18.9)	
Stage IV	57 (31.6)	4 (7)	
Lymphatic vessel invasion			
Absence	22 (12.2)	38 (65.5)	<.001
Presence	158 (87.8)	20 (34.5)	
Blood vessel invasion			
Absence	30 (16.6)	40 (68.9)	<.001
Presence	150 (83.4)	18 (31.1)	
Recurrence			
Absence	94 (52.2)	42 (72.4)	.009
Presence	86 (47.8)	16 (27.6)	

PNI perineural invasion

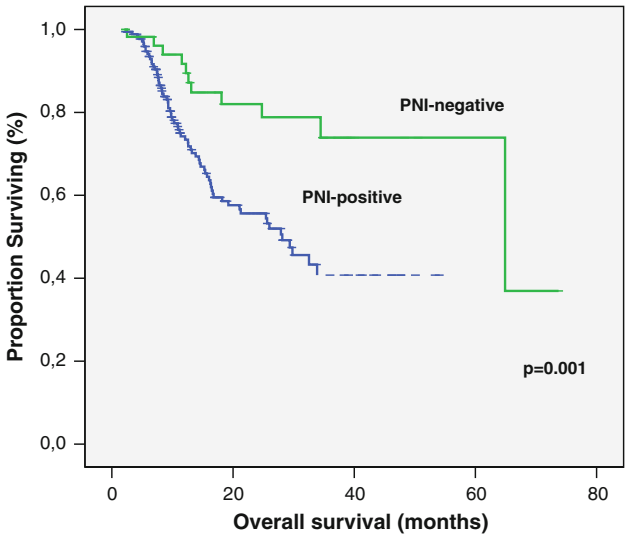

FIG. 2 Overall survival curves of the PNI-positive patients (median; 28.1 months) was significantly worse than that of the PNI-negative patients (median; 64.9 months)

vessel invasion, and blood vessel invasion were closely associated with the PNI positivity. The positivity rate of PNI increased when tumors were undifferentiated and the depth of invasion, the size, and clinical stage of tumor increased. By multivariate analysis we found that PNI is an independent prognostic factor for survival of patients with gastric cancer who underwent curative gastrectomy and lymph node dissection ($P = .02$), in addition to already known important clinicopathological prognostic indicators such as pN stage, tumor differentiation, pT stage, age, surgery type, tumor location, and Borrmann classification.

PNI was documented as a crucial route of spread for pancreatic and biliary tract cancers. The rate of positivity for PNI was approximately 20% in colonic and rectal cancers, but much higher in pancreatic cancer (45–100%) and 85–88% in biliary tract carcinomas.[7–11,21,22] The

TABLE 2 Multivariate analysis of the prognostic factors in patients with radically resected gastric cancer

Factors	HR	95% CI	*P*
Sex			.56
Male	1.18	0.66–2.09	
Female			
Age			.01
≤60 years	1.91	1.11–3.28	
>60 years			
Tumor location			.03
Upper, middle, lower, diffuse	1.47	1.02–2.12	
Tumor differentiation			.01
Well-differentiated	1.10	0.28–3.65	
Moderately differentiated	1.21	0.22–6.55	
Poorly differentiated	1.65	0.81–3.38	
Tumor size			.20
<3 cm	0.91	0.59–3.11	
<6 cm	0.87	0.30–2.52	
>6 cm	0.96	0.44–2.07	
pT stage			.04
T1	0.23	0.11–1.32	
T2	0.86	0.65–1.44	
T3	0.40	0.16–0.93	
T4	0.30	0.20–1.11	
Borrmann classification			.03
Type I	0.26	0.12–0.97	
Type II	0.44	0.40–1.14	
Type III	1.21	0.92–3.01	
Type IV	1.01	0.54–2.87	
Histological type			.24
Intestinal	0.72	0.41–1.25	
Diffuse	0.99	0.23–1.17	
Surgery type			.02
Proximal	1.11	0.84–1.32	
Distal	5.39	3.51–11.61	
Total	0.95	0.61–1.69	
pN stage			.001
N0	0.33	0.54–0.93	
N1	0.45	0.13–1.12	
N2	1.28	0.91–4.18	
N3	4.56	1.88–10.22	
Clinical stage			.25
Stage I	0.99	0.34–1.12	
Stage II	2.04	0.12–4.56	
Stage III	1.55	0.26–3.12	
Stage IV	1.83	0.47–1.99	
Lymphatic vessel invasion absence vs. presence	3.07	0.79–11.87	.10
Blood vessel invasion absence vs. presence	1.12	0.21–5.86	.88
Perineural invasion absence vs. presence	2.75	1.12–3.13	.02
Recurrence absence vs. presence	6.23	4.30–11.89	<.001

HR hazard ratio, *CI* confidence interval

presence and prognostic importance of PNI have been investigated previously in patients with gastric cancer by only a few studies, but PNI was not found to be an independent prognostic factor for survival in these studies and a consensus was not reached.[14–16] Tanaka et al. in their two studies, examined PNI in gastric cancer by staining laminin using an immunohistochemical method. They found that PNI was positive in 49.1% of patients with gastric cancer.[16] Moreover, the other study performed by Tanaka et al.[15] detected PNI positivity for diffuse invasive gastric cancer in 60 of the 75 patients (80%). The authors found that advanced gastric cancer with the presence of PNI revealed poor prognosis in their two studies. However, Duraker et al.[14] indicated that PNI was positive in 211 of the 354 patients (59.6%) and the presence of PNI was high in gastric cancer and increased with the progression of disease, but PNI did not contribute any additional information to the classical prognostic parameters.

In our study, the impact of PNI on survival in patients with gastric carcinoma who underwent curative gastrectomy was investigated, and we found that there was a statistically significant difference with respect to OS between PNI-positive patients and patients without PNI. The median OS interval for patients with PNI-positive was 28.1 months, which was significantly worse than that of the patients with PNI-negative (64.9 months, $P = .001$). In a recently published study including large sample size carried out by Tianhang et al.[17] they researched the prognostic significance of PNI in gastric cancer patients and firstly found that the presence of PNI was an independent prognostic factor for OS. They concluded that their results identified well with what has been already shown by other studies hypothesizing that PNI may be a prognostic indicator in gastric carcinoma. In addition, the authors showed that it can be an independent prognostic factor that is not affected by classical clinicopathological factors such as tumor stage, tumor grade, and lymph node involvement.

In the multivariate analysis, we also detected that the positivity of PNI was an independent prognostic factor in gastric cancer patients who underwent radically resection as compatible with the study of Tianhang et al.[17] In addition, the rate of large tumors and lymph node metastasis were significantly higher in the PNI-positive patients than in the PNI-negative patients. The positivity of PNI was also detected as significantly related with tumor differentiation, the depth of gastric mural invasion, advanced stage, lymphatic vessel invasion, and blood vessel invasion. Duraker et al.[14] indicated that the ratio of undifferentiated tumors, tumors with vascular invasion, and lymph node metastasis was significantly higher in patients with PNI than in patients without PNI. Furthermore, they also found that the depth of mural invasion was associated with PNI-positivity similar to our findings. Although the authors confirmed that there was significant difference for OS in patient with PNI positive compared with PNI-negative patients by univariate analysis, an independent prognostic importance of PNI could not be proved in the multivariate analysis.

TABLE 3 Relationship between PNI and clinicopathological factors in patients with locally advanced gastric cancer

Factors	PNI positive *n* (%)	PNI negative *n* (%)	*P* value
Locally advanced-stage disease (pT_2–T_4,$N_{0–3}$,M_0)			
All	179 (80.2)	44 (19.8)	
Gender			.49
Male	119 (66.4)	30 (68.1)	
Female	60 (33.6)	14 (31.9)	
Age (year)			.61
≤60	92 (51.3)	25 (56.8)	
>60	87 (48.7)	19 (43.2)	
Surgery type			.66
Proximal	32 (17.8)	6 (13.8)	
Distal	66 (36.8)	15 (34)	
Total	81 (45.4)	23 (52.2)	
Tumor size			.35
≤3 cm	26 (14.6)	10(22.8)	
≤6 cm	76 (42.4)	15 (34.1)	
>6 cm	77 (43)	19 (43.1)	
Tumor differentiation			.03
Well differentiated	4 (2.3)	1 (3.2)	
Moderately differentiated	86 (48)	31 (70.4)	
Poorly differentiated	89 (49.7)	12(36.4)	
Borrmann classification			.009
Type I	2 (1.2)	7 (16)	
Type II	101 (56.4)	16 (36.3)	
Type III	55 (30.7)	21 (47.7)	
Type IV	21 (11.7)	–	
Histological type			
Intestinal	83 (46.4)	24 (54.5)	.20
Diffuse	96 (53.6)	20 (45.5)	
pT stage			.001
T2	96 (53.6)	20 (45.5)	
T3	49 (27.3)	25 (56.8)	
T4	109 (60.8)	17 (38.6)	
pN stage			<.001
N0	21 (11.9)	2 (4.6)	
N1	20 (11.3)	20 (45.4)	
N2	59 (32.9)	16 (36.4)	
N3	59 (32.9)	4 (9.1)	
Clinical stage			<.001
Stage IB	41 (22.9)	4 (9.1)	
Stage II	10 (5.8)	12 (27.2)	
Stage III	22 (12.2)	18 (40.9)	
Stage IV	90 (50.2)	10 (22.7)	
Lymphatic vessel invasion			<.001
Absence	57 (31.8)	4 (9.2)	
Presence	22 (12.3)	25 (56.8)	
Blood vessel invasion	157(87.7)	17 (43.2)	<.001
Absence	30 (16.8)	28(63.6)	
Presence	149 (83.2)	15 (36.4)	

PNI perineural invasion

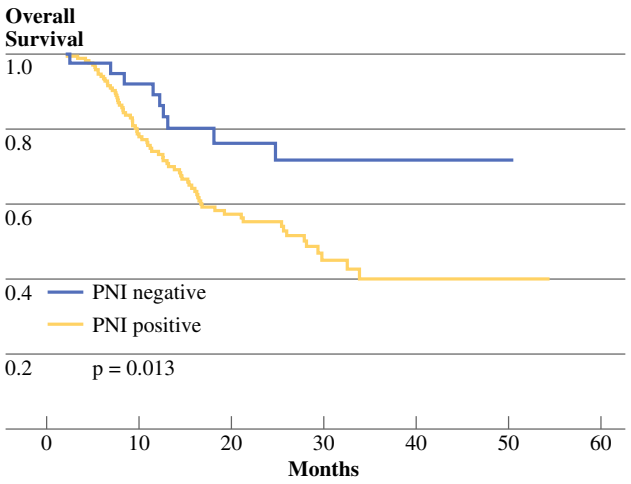

FIG. 3 Overall survival curves are shown in the PNI-positive or PNI-negative patients with locally advanced stage gastric cancer

The recurrence was also significantly related to the PNI positivity ($P = .009$). Tanaka et al.[16] indicated that recurrence correlated significantly with PNI, as were lymphatic vessel invasion and lymph node metastasis. Tianhang et al.[17] found that the occurrence of peritoneal metastasis but not hepatic metastasis was significantly associated with PNI positivity. In the present analysis, we did not investigate the association of peritoneal or hepatic metastasis with PNI positivity, because the patients with peritoneal and distant metastasis at diagnosis were excluded from the study, and only patients with R_0 gastric resection and without distant metastasis were included. Therefore, our study may be noteworthy for determining of the prognostic significance of PNI in patients with curative gastrectomy who have no metastasis. Although prospective studies are needed, our results contribute to the literature because of including only patients with early and locally advanced gastric cancer.

PNI positivity was detected in only 1 patient (6.6%) in early-stage gastric cancer subgroup. In addition, PNI was only associated with surgery type and tumor site in this subgroup. The relationship between survival and the PNI could not be investigated because of small sample size in patients who had pT1 tumor. However, PNI

TABLE 4 Multivariate analysis of the prognostic factors in patients with locally advanced gastric cancer

Factors	HR	95% CI	*P* value
Age			
≤60 years	0.42	0.23–0.77	.01
>60 years			
pT stage			
T2	0.20	0.10–0.64	.92
T3	0.29	0.12–0.71	
T4	0.99	0.54–1.23	
Borrmann classification			
Type I	0.98	0.47–1.21	.12
Type II	1.24	1.01–2.11	
Type III	1.74	0.96–2.11	
Type IV	2.72	1.23–3.44	
Tumor differentiation			
Well differentiated	0.67	0.13–0.78	.46
Moderately differentiated	1.04	0.20–3.26	
Poorly differentiated	1.86	1.03–3.28	
pN stage			
N0	0.27	0.12–1.09	.02
N1	0.31	0.20–1.45	
N2	0.78	0.54–1.55	
N3	1.67	1.04–3.45	
Clinical stage			
Stage IB	0.22	0.17–0.78	.44
Stage II	0.42	0.39–1.11	
Stage III	1.76	1.34–2.77	
Stage IV	1.89	1.54–2.66	
Lymphatic vessel invasion absence vs. presence	0.56	0.08–3.56	.55
Blood vessel invasion absence vs. presence	2.24	0.50–9.92	.28
Perineural invasion absence vs. presence	1.75	0.52–3.10	.03

HR hazard ratio, *CI* confidence interval

positivity was closely associated with tumor differentiation, Borrmann classification, pT stage, pN stage, clinical stage, lymphatic vessel invasion, and blood vessel invasion in patients with locally advanced gastric cancer (pT2–T4, N0–3, M0). The median survival and 3-year OS interval of the PNI-negative patients were significantly better than that of the PNI-positive patients in locally advanced stage subgroup (60.3 months, 69.8% vs. 27.9 months, 38.5%, respectively, $P = .025$). Moreover, multivariate analysis indicated that the presence of PNI was an independent prognostic factor, as were pN stage and age for OS in patients with locally advanced gastric carcinoma. Tianhang et al. found that there was significant relationship between clinical stage and PNI, and they indicated that both PNI and clinical stage were independent prognostic indicators. However, the subgroup analysis had not been carried out according to clinical stage in this study.[17]

The pathogenesis of PNI could not be sufficiently clarified, previously. Murakawa et al.[23] showed that the mechanism of neural invasion could be explained partly by the close anatomical association between the pancreas and celiac plexus. Nagakawa et al.[7] reported that the high incidence of PNI in pancreas and biliary tract cancers was related to rich autonomic innervation of these organs, but they also indicated that the pathogenesis of entry of cancer cells into the nerves remains unelucidated. However, in a study performed by Seki et al.[10] they stated that PNI which is frequently determined in bile duct cancer, was dependent not only on anatomical specificity, but also on the special ability of the cancer cells to recognize neural tissue easily by secreting the neural cell adhesion molecule (NCAM). Although the rectum is in close proximity to the presacral autonomic nerve plexus, the frequency of PNI in rectal cancer is low, ranging from 9.9 to 34.9%.[11–13] Kameda et al.[22] suggested that although the number of layers of the perineurium increased at the central nerve and tumor cells invaded the perineurium through that site causing PNI, the number of layers at the terminal of the nerves had decreased. As seen in both our and the other two series, the incidence of PNI was high in patients with gastric cancer in which the proximity of the stomach and celiac nerve plexus may influence the frequency of PNI and may play a role in this entity.[14,17]

Seki et al.[10] indicated that PNI was significantly correlated with lymphatic and venous invasion in bile duct cancer and demonstrated that PNI of cancer cells occurred not only as a result of direct infiltration, but also by invading the lymphatic vessels and veins around the nerves. On the other hand, the perineural space is now usually accepted as an independent route of cancer spread because it is anatomically and ultrastructurally different from the lymphatic canals.[8] In our study, we found that the positivity of PNI was closely related to lymphatic vessel invasion and blood vessel invasion. These associations may also play a role in the high presence of PNI in the present study.

The major limitations of this study are small sample size and low follow-up time. In addition, the retrospective nature of our study could be considered another significant limitation, and they may have influenced these results. However, although our results should be confirmed by prospective studies, we believe that our results contribute to the literature because of including only patients with early and locally advanced gastric cancer.

In conclusion, our study indicates that the frequency of PNI is high in patients with gastric cancer and without

distant metastasis who underwent R_0 curative gastrectomy, and the ratio of PNI positivity increases with progression and clinical stage of disease. In addition, PNI contributes important additional information as an independent prognostic indicator for survival to classical prognostic features such as pT stage, pN stage, lymphatic vessel invasion, and blood vessel invasion. It may be a useful and new prognostic factor in detecting patients who are at high risk for poor prognosis after curative resection in gastric cancer and may help to guide more aggressive adjuvant therapy.

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