

RESEARCH ARTICLE

Perineural Invasion Independent Prognostic Factors in Patients with Gastric Cancer Undergoing Curative Resection

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Abstract

Objective: The prognostic significance of perineural invasion (PNI) in gastric cancer has been previously investigated but not clearly clarified. The objective of our study was to investigate the role of PNI as prognostic factor in patients undergoing curative surgical resection and without distant metastasis in comparison with other clinicopathological factors. **Methods:** Between 2001 and 2010, 287 cases of gastric adenocarcinoma underwent radical gastrectomy recorded in hospital based registries. PNI was assessed as positive when cancer cells were seen in the perineurium or neural fascicles intramurally. Categorical and continuous variables were summarized using descriptive statistics and compared using chi-square and Mann-Whitney U tests, respectively. Cancer related survival rates were estimated by the Kaplan-Meier method. **Results:** PNI was positive in 211 of 287 cancers (73%), with a positive relation to lymph node metastases and advanced stage ($p=0.0001$, $p=0.0001$, respectively), mural invasion, and lymphatic and blood vessel invasion ($p=0.0001$, $p=0.0001$, respectively). The median survival of the PNI positive patients was significantly shorter than that of their PNI negative counterparts (24.1 versus 38.2 months, $p=0.008$). In the multivariate analysis, we detected PNI was an independent prognostic factor ($p=0.025$, HR=1.21, 95% CL 1.08-2.3) along with classical clinicopathological variables such as lymph node involvement ($p=0.001$), pT stage ($p=0.03$), and LVI ($p=0.017$), but not age, gender, tumour localization, stage, histologic type, and surgery procedure. **Conclusions:** PNI positivity in gastric cancers was related mural invasion, lymph node involvement, advanced stage and lymphatic and venous blood vessels. The presence of PNI appeared as an independent prognostic factor on survival on multivariate analysis, not influenced by tumor stage, lymph node metastases and other classical factors.

Keywords: Perineural invasion - gastric cancer - prognostic factors

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Introduction

Currently, gastric cancer is the second leading cause of cancer related death in both men and women (Ferlay et al., 2010; Ferlay, 2010). Incidence rates for gastric cancer have declined worldwide (Parkin, 1993; 2004; Bertuccio et al., 2009; Jemal et al., 2009). However, gastric cancer is the fourth most commonly diagnosed cancer (Ferlay et al., 2010). Gastric cancer prognosis has not improved much, and the five-year survival rates of all patients with gastric cancer have changed only slight over the past decades but remain under 20 percent (Starzyńska, 2007).

Total and subtotal gastrectomy with lymph node dissection is the cornerstone treatment of gastric cancer (Yokota et al., 2003). However, after curative resection if determined high risk for poor prognosis, these patients get benefits from additional adjuvant treatment (Macdonald et al., 2001).

Perineural invasion (PNI) is the infiltration of perineurium or neural fascicles around a tumor by cancer cells. It also has been called neurotropic carcinomatous spread and perineural spread. PNI is a distinct pathologic entity that can be observed in the absence of lymphatic

or vascular invasion. PNI is often seen in carcinomas head and neck, prostate, pancreas and biliary tract but is relatively rare in rectal carcinomas and it is reported to be crucial route for the local spread of tumour associated with poor prognosis in head and neck, prostate, pancreas and biliary tract cancers (Carter et al., 1983; Ueno, 2001). However, the prognostic importance of PNI in gastric cancer has been evaluated in a few studies. Prior some trials, PNI have not been provided any additional information to the classical prognostic factors (Tanaka et al., 1994; Duraker, 2003). But recently two studies, Bilici et al. and Tianhang et al. demonstrated that incidence of PNI in gastric cancer was high and PNI independent prognostic factor worse effect on survival (Tianhang et al., 2008; Bilici et al., 2010).

The objective of our study was to investigate the role of PNI as prognostic factor in patients who underwent curative surgical resection and without distant metastasis at gastric cancer and determined association of PNI with the other clinicopathological factors. A better understanding of PNI in gastric cancer may lend insight at tumor recurrence and metastasis and may be improved staging strategies and new treatment modality.

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Materials and Methods

Patients

Gastric cancer patients treated at Istanbul University Cerrahpasa Medical School between 2001-2010 were retrospectively evaluated. Cancer was coded according to the International Classification of Disease for Oncology, 3rd edition (ICD-O-3) (A- Fritz et al., 2000). We included only gastric adenocarcinoma in this study. Patients were included when adenocarcinoma of stomach underwent either partial gastrectomy or total gastrectomy with regional lymph node dissection as curative intent and survival data were available. Chemotherapy concurrent with postoperative radiotherapy (RT) was protracted 5-fluorouracil (5FU) infusion. Five cycles of adjuvant bolus 5FU (425 mg/m²/day) and leucovorin (20 mg/m²/day) as Mayo regimen on days 1-5 every 28 days were administered after surgery, as indicated. Bolus 5FU and leucovorin was employed on days 1-4 every 28 days concurrent with postoperative RT in the 2nd and 3th cycles of planned 5 cycles of adjuvant chemotherapy (Macdonald et al., 2001). Patients were not included in the study if they had metastatic disease, positive surgical margins, poor performance status (Eastern Cooperative Oncology Group (ECOG) >2), inadequate renal and hepatic function, other second primary cancers. Those not undergoing surgery for various reasons were also excluded.

Clinic information about age, gender, operation type, tumor location, histopathology, tumor stage, lymph node involvement, mural invasion, perineural invasion, lymphatic and blood vessel invasion, resection margin, treatment and outcome were obtain from patients chart. Gastric adenocarcinoma stage was determined according to seventh edition of the International Union Against Cancer guidelines (Wittekind, 2010). We divided cases two sub group based of anatomic localization of stomach. Group 1; proximal stomach (cardia) and group 2: noncardia (gastric antrum, pylorus, lesser curvature and greater curvature of stomach) (Devesa, 1998).

Histopathological evaluation

Surgical specimens were fixed in 10% formalin and embedded in paraffin. Paraffin-embedded blocks were cut into 5- μ m-thick sections, and stained with haematoxylin and eosin. PNI was assessed as positive when cancer cells were seen in the perineurium or neural fascicles intramurally. Cases were divided based on histopathological subtype into: group 1, intestinal gastric cancer; group 2, diffuse gastric cancer; and group 3; mixed adenocarcinoma (Laurén, 1965).

Follow-up

Patients were followed every 3 months for 2 years and every 6 months between 2-5 years and annually thereafter. Evaluation included clinical examination, complete blood count, serum chemistries, thorax and abdomen CT and endoscopy as indicated. Recurrence was diagnosed on the basis of imaging findings. Pathologic confirmation was obtained in selected cases. Follow-up data of patients were collected by telephoning the patients and their relatives or outpatients service.

Statistical Analysis

Categorical and continuous variables were summarized using descriptive statistics (e.g., median, range, frequency, and percentage) and compared with chi-square and Mann-Whitney U tests, respectively. Disease free survival (DFS) was defined as the time from the diagnosis to the detection of any local or distant recurrence or date of death respectively. Overall survival (OS) was defined from the time of diagnosis to death of any cause.

Survival rates were estimated by the Kaplan-Meier method and compared by the long-rank test. We evaluated effect of perineural invasion and other clinicopathological features as prognostic factors on survival by Cox regression analyses. The Cox proportional-hazards model was used to calculate hazard ratio and 95 percent confidence intervals. All analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA) software. The statistical level of significance was defined as $p < 0.05$.

Results

Between 2001 and 2010, there were 287 cases of gastric adenocarcinoma that had undergone radical gastrectomy recorded in hospital based registries. The median age at diagnosis was 59 years (range=23-89) and 75(26%) female, 212(74%) male; lymph node metastases (68%), pT3 (52%) and stage III (58%).

Patients PNI was positive in 211 of 287 patients (73%). There was no association between PNI positivity and gender, age, stomach localization, histologic type and surgery procedure type. Tumors with PNI positive had more lymph node metastases and advanced stage ($p=0.0001$, $p=0.0001$, respectively). The positivity of PNI was also significantly increased in mural invasion, lymphatic and blood vessel invasion ($p=0.0001$, $p=0.0001$, respectively). The details of between PNI and clinicopathological factors are shown in Table 1.

Overall survival analyses were performed in 287 patients. The median survival of the PNI positive patients was significantly shorter than that of the PNI negative patients (24.1 versus 38.2 months, $p=0.008$, Figure 1). The positivity of PNI was closely associated with worse OS of patients who undergone curative resection in gastric adenocarcinoma in the univariate analysis (long-rank, $p=0.008$). Therefore, we performed multivariate analysis with Cox proportional hazard model in order to further evaluate the prognostic significance of PNI and other clinicopathological factors.

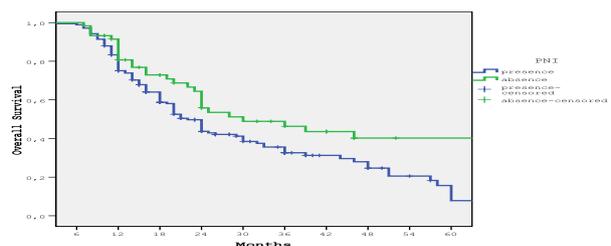


Figure 1. OS Curves (Kaplan-Meier) of the PNI-Positive Patients (Median=24.1 months) was Significantly Worse Than Patients of the PNI-Negative Patients (Median 38.2, $p=0.008$, Long-Rank Test).

In the multivariate analysis, we detected PNI was an independent prognostic factor ($p=0.025$ HR=1.21, 95% CL 1.08-2.3) as were classical clinicopathological variable such as lymph node involvement ($p=0.001$), pT stage ($p=0.03$), LVI ($p=0.017$). Other hand, age, gender, tumour localization, stage, histologic type, surgery procedure, analyses we was not determined effect on survival in multivariate analysis.

Discussion

PNI was demonstrated as a crucial route of spread for pancreatic and biliary tract and colorectal cancer. PNI has been recognized in many series as prevalent pathologic features of colorectal cancer and is reported in up to 33% of these tumors at the time resection (Knudsen et al., 1983; Ueno, 2001). In otherwise PNI rate were much higher in pancreatic cancer (70-100%) and 75-85% in biliary tract cancer (Nagakawa et al., 1993; Seki et al., 1993). It is reported to be crucial route for the local spread of tumour associated with poor prognosis in head and neck, prostate, pancreas and biliary tract cancers (Carter et al., 1983; Ueno, 2001).

In our studies, we investigated the effect of PNI on survival in patients with gastric carcinoma who underwent

Table 1. C Association between PNI and Clinicopathological Features 287 Patients who Underwent Radical Gastrectomy and Lymph Node Dissection

Variable	PNI ve- n (%)	PNI ve+ n (%)	P value
Gender			0.5
Female	20 (27)	58 (27)	
Male	55 (73)	154 (73)	
Time to diagnosis age			0.43
<59 years old	43 (29)	103 (71)	
≥59 years old	35 (25)	106 (75)	
Stomach localization			0.4
Cardia (Proximal stomach)	8 (24)	25 (76)	
None-cardia (distal stomach)	70 (28)	184 (72)	
Histologic type			0.6
Diffuse adenocarcinoma	27 (29)	67 (71)	
Intestinal adenocarcinoma	51 (26)	142 (74)	
Surgery procedure type			0.39
Subtotal gastrectomy	37 (30)	89 (70)	
Total gastrectomy	41 (26)	120 (74)	
pT* stage			0.0001
pT1	11 (85)	2 (15)	
pT2	21 (40)	31 (60)	
pT3	38 (20)	128 (80)	
pT4	9 (19)	37 (81)	
Lymph node metastasis			0.0001
Absence	44 (54)	39 (46)	
Presence	34 (17)	160 (83)	
Lymphatic and blood vessel invasion			0.0001
Absence	45 (79)	12 (21)	
Presence	33 (14)	197 (86)	
Time to diagnosis stage			0.0001
Stage I	18 (56)	14 (44)	
Stage II	14 (32)	30 (68)	
Stage III	46 (22)	165 (78)	

*Pathologic T stage

Table 2. Multivariate Analysis of the Prognostic Factors in Overall Survival Patients.

Variable	HR	95% CI	P
PNI			0.025
Absent	1		
Present	1-21	(1.08-2.3)	
LVI			0.017
Absent	1		
Present	1-31	(1.12-2.43)	
pT Stage			0.03
pT1	1		
pT2	1-2	(1.08-1.71)	
pT3	1-72	(1.31-2.33)	
pT4	1-3	(1.09-1.49)	
Lymph node metastases			0.001
Absent	1		
Present	8-9	(2.7-14.1)	

*HR: Hazard ratio, CI: Confidence interval

curative resection. In this study, we stained specimen with haematoxylin and eosin to determine the positivity of PNI in patients with gastric adenocarcinoma and we found that 211 of the 287 patients (73%) were PNI. We found that there was a statistical different with respect to Overall survival between PNI positive and negative patients. The median OS for patients with PNI positive and negative patients was 24.1 and 38.2 months ($p=0.008$, long-rank) respectively. At multivariate analysis, the presence of PNI appeared as an independent prognostic factor on survival. It can be an independent prognostic factor which is not influenced by tumor stage, lymph node metastases and other classical factors.

Duraker et al. demonstrated that PNI was positive 59.6% patients in gastric adenocarcinoma by stained specimen with haematoxylin and they show progression free survival significant worse PNI positive than without PNI patients. but PNI that do not effect on survival in multivariate analysis (Duraker, 2003). Tanaka et al examined PNI in gastric cancer by staining laminin using an immunohistochemical method and they demonstrated that PNI was 49.1% of patients gastric cancer and they found PNI for diffuse gastric cancer 80% and they found PNI positive patients had poor prognosis (Tanaka et al., 1994: 1997). Tianhang et al. PNI positive found 31.7% in gastric cancer (Tianhang et al., 2008) and Bilici et al. indicated that PNI positive 75.6% in patients who resected curative intent (Bilici et al., 2010). Last two studies demonstrated PNI independent prognostic factor on survival (Tianhang et al., 2008; Bilici et al., 2010).

Prior few studies demonstrated PNI positivity was from 31.7 to 75.6% (Duraker, 2003; Bilici et al., 2010). This situation may be each study had different patients population based of time diagnosis stage, clinicopathological features and demonstrated different technical stained PNI.

Prior studies detected PNI related with the rate of large tumors and lymph node metastases, advanced stage, lymph and blood vessel invasion (22-26). In current study, PNI positivity, we found 15%, 60%, 80%, and 81% in pT1, pT2, pT3, pT4 respectively. It is increased when mural invasion increased ($p=0.0001$). In addition, we found as

same priors studies, lymph node involvement, advanced stage, lymph and vessel blood invasion were significant higher in the PNI positive than negative patients.

The pathogenesis of PNI has not yet been sufficiently clarified. Nagakawa et al reported that the high incidence of PNI in pancreas and biliary tract cancer was related to rich autonomic innervation of these organs. They think that cancer cell infiltrated the perineural space directly through the perineurium from the cancer nest. It is thought that cancer cells could invade into the perineurium and infiltrate the perineural space and interstitium with little resistance but they also indicated that the pathogenesis of entry of cancer cells into the nerves remains unelucidated (Nagakawa et al., 1993). Seki et al found that PNI which is frequently determined in bile duct cancer. It was not dependent only anatomical specificity, but also special ability of the cancer cell to recognize neural tissue easily by secreting the neural cell adhesion molecule (NCAM). They also demonstrated a significant correlation between PNI and lymphatic and venous blood vessels in bile duct cancer and they suggested that PNI of cancer cells occurred not only as result of direct infiltration, but also by invading the lymphatic vessels and veins around the nerves (Seki et al., 1993). However, the perineural space is now usually accepted as an independent route of cancer spread because it is anatomical different the lymphatic canals (Takahashi et al., 1997). In current study, we indicated that the PNI positivity was closely related to lymphatic and vessel bloods invasion. But, PNI positivity independent variable which is not influenced lymphatic and venous bloods vessels effect on survival.

In conclusion, our study demonstrated PNI positivity high percent with patients gastric cancer who underwent curative resection. PNI positivity was related mural invasion, lymph node involvement, advanced stage and lymphatic and venous blood vessels. The presence of PNI appeared as an independent prognostic factor on survival at multivariate analysis. It can be an independent prognostic factor which is not influenced by tumor stage, lymph node metastases and other classical factors. A better understanding of PNI in gastric cancer may lend insight at tumor recurrence and metastasis and may be improved staging strategies and new treatment modality.

References

- Ayala GE, Dai H, Ittmann M, et al (2004). Growth and survival mechanisms associated with perineural invasion in prostate cancer. *Cancer Res*, **64**, 6082-90.
- Bertuccio P, Chatenoud L, Levi F, et al (2009). Recent patterns in gastric cancer: a global overview. *Int J Cancer*, **125**, 666-73.
- Bilici A, Seker M, Ustaalioglu BB, et al (2010). Prognostic significance of perineural invasion in patients with gastric cancer who underwent curative resection. *Ann Surg Oncol*, **17**, 2037-44.
- Carter RL, Foster CS, Dinsdale EA, et al (1983). Perineural spread by squamous carcinomas of the head and neck: a morphological study using anti-axonal and anti-myelin monoclonal antibodies. *J Clin Pathol*, **36**, 269-75.
- Devesa SS, Blot WJ, Fraumeni JF Jr (1998). Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. *Cancer*, **83**, 2049-53.
- Duraker N, Sisman S, Can G (2003). The significance of perineural invasion as a prognostic factor in patients with gastric carcinoma. *Surg Today*, **33**, 95-100.
- Ferlay J, Shin HR, Bray F, et al (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, **127**, 2893-917.
- Jemal A, Siegel R, Ward E, et al (2009). Cancer statistics, 2009. *CA Cancer J Clin*, **59**, 225-49.
- Knudsen JB, Nilsson T, Sprechler M, et al (1983). N. Venous and nerve invasion as prognostic factors in postoperative survival of patients with resectable cancer of the rectum. *Dis Colon Rectum*, **26**, 613-7.
- Kurtz KA, Hoffman HT, Zimmerman MB, et al (2005). Perineural and vascular invasion in oral cavity squamous carcinoma: increased incidence on re-review of slides and by using immunohistochemical enhancement. *Arch Pathol Lab Med*, **129**, 354-9.
- Laurén P (1965). The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. *Acta Pathol Microbiol Scand*, **64**, 31.
- Macdonald JS, Smalley SR, Benedetti J, et al (2001). Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. *N Engl J Med*, **345**, 725-30.
- Maru N, Otori M, Kattan MW, et al (2001). Prognostic significance of the diameter of perineural invasion in radical prostatectomy specimens. *Hum Pathol*, **32**, 828-33.
- Nagakawa T, Mori K, Nakano T, et al (1993). Perineural invasion of carcinoma of the pancreas and biliary tract. *Br J Surg*, **80**, 619-21.
- Ozaki H, Hiraoka T, Mizumoto R, et al (1999). The prognostic significance of lymph node metastasis and intrapancreatic perineural invasion in pancreatic cancer after curative resection. *Surg Today*, **29**, 16-22.
- Parkin DM, Pisani P, Ferlay J (1993). Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int J Cancer*, **54**, 594-606.
- Parkin DM, Bray FI, Devesa SS (2001). Cancer burden in the year 2000. The global picture. *Eur J Cancer*, **37**, 4-66.
- Parkin DM (2004). International variation. *Oncogene*, **23**, 6329-40.
- Seki H, Tanaka J-I, Sato Y, et al (1993). Neural cell adhesion molecule (NCAM) and perineural invasion in bile duct cancer. *J Surg Oncol*, **53**, 78-83.
- Soo KC, Carter RL, O'Brien CJ, et al (1986). Prognostic implications of perineural spread in squamous carcinomas of the head and neck. *Laryngoscope*, **96**, 1145-8.
- Starzyńska T (2007). Dig Dis. *Molecular Epidemiol of Gastric Cancer*, **25**, 222-4.
- Takahashi T, Ishikura H, Motohara T, et al (1997). Perineural invasion by ductal adenocarcinoma of the pancreas. *J Surg Oncol*, **65**, 164-70.
- Tianhang L, Guoen F, Jianwei B, et al (2008). The effect of perineural invasion on overall survival in patients with gastric carcinoma. *J Gastrointest Surg*, **12**, 1263-7.
- Tanaka A, Yoshikawa H, Okuno K, et al (1997). The importance of neural invasion (NI) as a prognostic factor in diffuse invasive gastric cancer. *Surg Today*, **27**, 692-5.
- Tanaka A, Watanabe T, Okuno K, et al (1994). Perineural invasion as a predictor of recurrence of gastric cancer. *Cancer*, **73**, 550-5.
- Ueno H, Hase K, Mochizuki H (2001). Criteria for extramural perineural invasion as a prognostic factor in rectal cancer. *Br J Surg*, **88**, 994-1000.
- Wittekind C, Meyer H-J (2010). TNM Classification of Malignant Tumours (ed 7). Weinheim, Germany, Wiley.
- Yokota T, Ishiyama S, Saito T, et al (2003). Treatment strategy of limited surgery in the treatment guidelines for gastric cancer in Japan. *Lancet Oncol*, **4**, 423-8.