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Influence of perineural invasion in predicting overall survival and disease-free survival in patients With locally advanced gastric cancer

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Abstract

BACKGROUND: The aim of the present study was to evaluate the prognostic significance of perineural invasion (PNI) in locally advanced gastric cancer patients who underwent D2 gastrectomy and adjuvant chemotherapy.

METHODS: The records of a series of 103 patients undergoing D2 gastrectomy with curative intent combined with adjuvant chemotherapy from January 2004 to December 2014 were retrospectively reviewed.

RESULTS: PNI was positive in 47 (45.6%) specimens. The 1-, 3-, and 5-year overall survival rates were 81%, 55%, and 42%, respectively. The 1-, 3-, and 5-year disease-free survival (DFS) rates were 76%, 57%, and 49%, respectively. A multivariate analysis showed that age number of positive lymph nodes, T stage, and PNI were independently associated with overall survival. Regarding DFS, the multivariate analysis showed that only PNI was independently associated with DFS.

CONCLUSIONS: PNI and T stage and positive lymph nodes are independent markers of poor prognosis in patients with gastric cancer. PNI should be incorporated in the postoperative staging system for planning follow-up after surgery and in our opinion to propose more aggressive postoperative therapies in PNI-positive patients.

Gastric cancer is the fourth most common malignancy and the second most common cause of cancer-related death worldwide.[1] Tumor stage, tumor size, grade of differentiation, and lymph node metastasis are well-established prognostic factors and lymphovascular invasion (LVI), which is also known to be an independent factor for lymph node metastasis and for survival.[2] The prognostic value of LVI, in fact, has been investigated by many authors, confirming the importance of this histopathologic parameter on patient's outcome.[2,3]

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Perineural invasion (PNI), also named neurotropic carcinomatous spread or perineural spread, is the process through which cancer cells invade perinerium and neural fascicles. This is an important pathway for local spread of cancers and is also related to cancerous pain, cancer recurrence, and poor prognosis.

Up to now, the understanding of PNI pathogenesis is still in its infancy. However, this is recognized to be associated with a more aggressive tumor and with a poorer prognosis in several malignancies, such as head and neck tumors and prostate cancer.[4–8] Regarding gastric cancer, PNI's prognostic value has not reached any general consensus, and the role of its positivity on predicting outcomes after curative gastric resection for cancer is still under debate. Bilici et al[9] found that the median survival of PNI-positive patients was worse than those having PNI-negative cancers and demonstrated that this is a useful prognostic factor for curative gastric cancer surgery. However, in the study presented by Duraker et al[10] in 2003, although the positivity of PNI was 59.6% among patients, this did not provide any additional prognostic information to the well-established oncologic parameters. Other studies furthermore analyzed the usefulness of PNI in predicting outcomes of patients with gastric cancer, describing no association with survival.[11–14]

Because of the lack of consensus, in this article, we evaluated the prognostic significance of PNI in locally advanced gastric cancer patients who underwent curative D2 gastrectomy and adjuvant chemotherapy.

Patients and Methods

The records of a series of 126 consecutive patients who underwent resection for gastric cancer from January 2004 to December 2014, at S. Andrea Hospital, La Sapienza, University of Rome, were retrospectively reviewed. All patients had undergone gastrectomy and modified D2 lymphadenectomy with curative intent according to tumor location and extent of disease. Only patients who underwent curative-intent surgery were included.

Combined resection of other organs, such as the spleen, pancreas, and colon, was performed in case of direct invasion. Patients with residual disease either microscopically (R1) or macroscopically (R2) were excluded from the study. None of the patients included in the study had liver or distant metastases at time of surgery.

No patients underwent neoadjuvant chemotherapy, and all the patients were treated with adjuvant chemotherapy (oxaliplatin and capecitabine) after surgery as proposed in the "Associazione Italiana di Oncologia Medica" guidelines (www.aiom.it). Standard clinicopathologic data were collected, including age, sex, operation type, tumor size, tumor location, type of procedure, histologic type and grade of the tumor, depth of invasion, number of lymph nodes harvested, number of metastatic lymph nodes, lymph node ratio (LNR), LVI and PNI, and final American Joint Committee on Cancer pathologic stage of disease. T stage and nodal status were determined using the American Joint committee on *Cancer Staging Manual*, Seventh Edition.[15] The LNR was calculated based on the relationship between positive nodes and total nodes of the specimen.[16]

For histologic parameters, multiple samples were obtained from every surgical specimen, 4-μm-thick sections of each formalin-fixed paraffin-embedded tissue block were cut and hematoxylin and eosin stained. Each slide was carefully and separately reviewed by 2 of the authors (G.L.R.V. and F.S.) in blind. Doubtful cases were further discussed together to reach concordance.

LVI was considered positive when either single tumor cells or cell clusters were clearly visible within an endothelium-lined vessel-like structure. PNI was defined as the presence of cancer cells along nerves and/or within the epineurial, perineurial, and endoneurial spaces of the neuronal sheath including cases in which the cell circumscribed at least 33% of the nerves.[17]

Data of last follow-up or death, recurrence-related information, such as site of recurrence and treatment, and vital status were also collected. Patients who died within 30 days of surgery and also those who lost to follow-up were excluded. Ultimately, 103 patients were eligible. The last follow-up was dated on June 2015. Clinicopathologic characteristics of the 103 patients are listed in Table 1.

Statistical analysis

Overall survival (OS) was defined as the interval between the date of operation and the date of death for any cause or last patient visit. Disease-free survival (DFS) was defined as the time from operation until tumor relapse either local or distant. Comparison of categorical variables was performed using the chi-square test. Kaplan–Meier curves were generated for OS and DFS, and differences in survival rates between groups were compared by the log-rank test. A backward stepwise Cox regression model was used to identify variables influencing OS and DFS. Multivariate analysis was performed using those variables that have significant independent relationship with OS and DFS. Significance was defined as a P value $<.05$. All statistical analyses were performed using the SPSS for Mac, version 17.0 (SPSS, Inc., Chicago, IL).

Results

Thirty-three (32%) patients presented with gastrointestinal bleeding, 15 (14.5%) with obstruction, and 55 (53.3%) complaining of pain and discomfort. Lymph node invasion was suspected in 63 (61.1%) patients at radiologic evaluation before surgery.

A total gastrectomy was performed in 57 (55.3%) patients, 46 (44.6%) underwent a subtotal distal gastrectomy, whereas 16 (15.5%) received a combined procedure because of direct tumor invasion; more in detail,

Table 1 Patient's perioperative characteristics

Variables	N = 103
Sex (F/M)	48/55
Age > 70 y	57 (55%)
Mean	70.2 ± 11
ASA score (I/II/III)	22/31/50
Tumor site	
Antrum/pyloric	47 (46%)
Proximal/upper third	24 (23%)
Body/middle third	30 (29%)
Gastric stump*	2 (2%)
Type of operation	
Total gastrectomy	57 (55%)
Distal subtotal gastrectomy	46 (45%)
Combined procedures	16/103 (15%)
Tumor size (mm)	15 ± 18
No. of lymph nodes retrieved	28.7 ± 14
No. of metastatic lymph nodes	7.2 ± 11
Lymph node ratio	.2 ± .2
Lauren type	
Diffuse type	29 (28%)
Intestinal type	68 (66%)
Mixed type	6 (6%)
Depth of invasion	
T1	19 (18%)
T2	34 (33%)
T3	29 (28%)
T4	21 (20%)
Node status	
N0	38 (37%)
N1	24 (23%)
N2	13 (13%)
N3	28 (27%)
Stage Ib/II/III	22/25/56
Grading (G1/G2/G3/G4)	8/20/69/8
LVI	78 (78%)
PNI	47 (46%)

ASA = American Society of Anesthesiology; F = female; LVI = lymphovascular invasion; M = male; PNI = perineural invasion.
*After previous subtotal gastrectomy.

11 (10.6%) patients required a combined splenectomy, 3 (2.9%) underwent a right hemicolectomy, 1 (.9%) a jejunal loop resection, and 1 (.9%) patient underwent a pancreaticoduodenectomy because of direct tumor invasion. After pathologic examination, mean tumor diameter was 15 ± 18.4 mm; all resections were R0 on all margins. PNI was positive in 47 (45.6%) specimens, whereas LVI was present in 78 (75.7%).

The median duration of follow-up was 26 months (2 to 138 months). Of 103 patients, 57 patients (55.3%) had died and 46 (44.6%) patients were alive at the end of follow-up. Forty-two patients (73.6%) of the study died from cancer-related causes. The 1-, 3-, and 5-year OS rates were 81%, 55%, and 42%, respectively. After R0 resection, 45 patients (44%) developed a recurrence that was locoregional in most of the cases (56%). The 1-, 3-, and 5-year DFS rates were 76%, 57%, and 49%, respectively. Survival curves are shown in Fig. 1A,B. At a subgroup analysis, patients with positive PNI had a significantly worse 5-year actuarial OS (59% vs 19%, $P = .0001$; Fig. 2A) and DFS compared with patients without PNI (69% vs 22%, $P = .0001$; Fig. 2B).

The univariate analysis showed that age ($P = .003$), number of positive lymph nodes ($P = .0001$), LNR ($P = .0001$), tumor stage ($P = .005$), LVI ($P = .007$), and PNI ($P = .0001$) were associated with OS. A multivariate analysis of these significant variables showed that age ($P = .001$), number of positive lymph nodes ($P = .03$), T stage ($P = .04$), and PNI ($P = .004$) were independently associated with OS (Table 2).

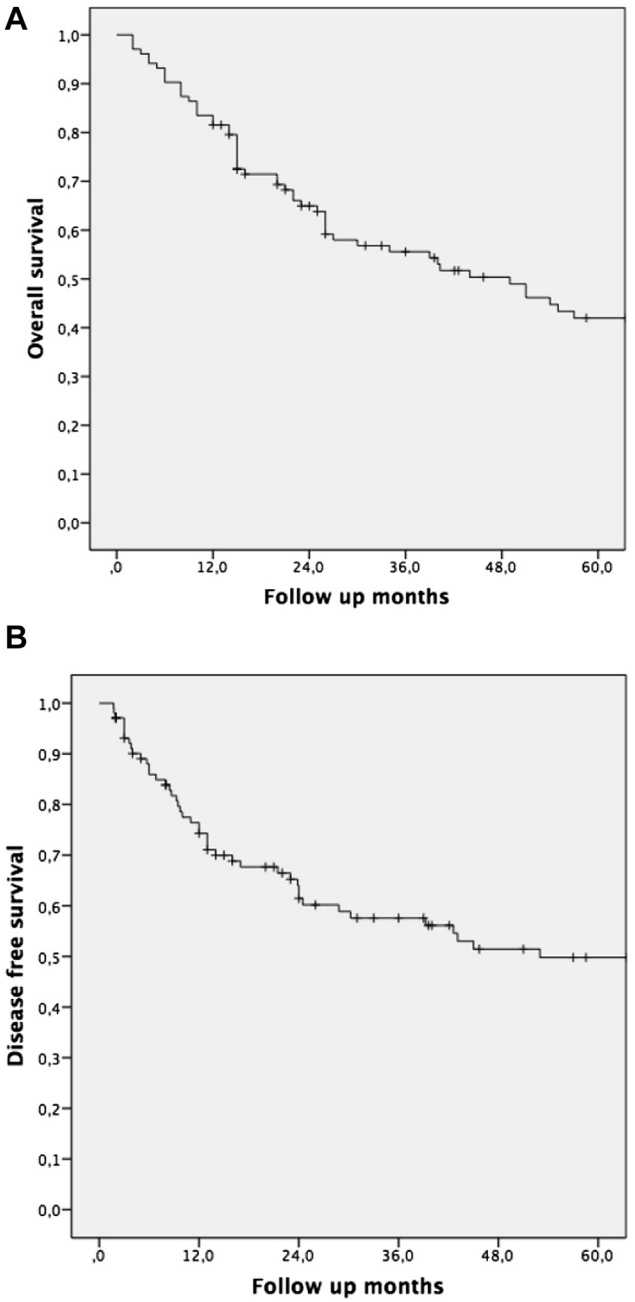

Figure 1 (A) Patient's OS curve. (B) Patient's DFS curve.

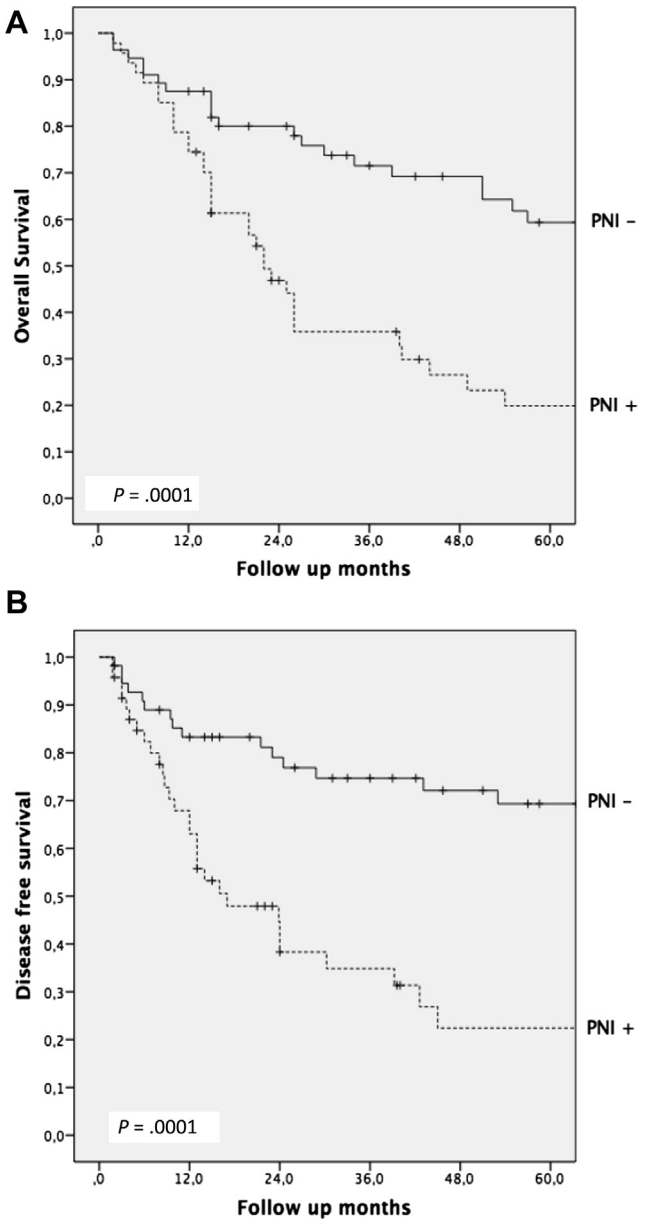

Figure 2 (A) Patient's OS according to perineural invasion. (B) Patient's DFS according to perineural invasion.

Regarding DFS, the univariate analysis showed that number of positive lymph nodes ($P = .0001$), LNR ($P = .0001$), tumor stage ($P = .02$), PNI ($P = .0001$), and LVI ($P = .002$) were significant. A multivariate analysis of these significant variables showed that only PNI ($P = .01$) was independently associated with DFS (Table 3).

Comments

The incidence of PNI has been already documented in previous studies, ranging from 6.8% to 75.6% in oncologic patients and approximately 20% in colonic and rectal carcinoma.[18] This incidence is described as much higher in pancreatic carcinoma (50% to 80%) and in carcinoma of the biliary tract (85% to 88%).[19–23] No clear association between PNI and metastases has been evidenced in all these studies concerning different type of cancers.

The exact molecular mechanism mediating the interaction between cancer and neural cells during PNI is poorly understood. Xia et al[24] using an in vitro tumor–neural cell culture system demonstrated that gastric cancer cells not only promote neural progenitor cells' proliferation but also can enhance neurite elongation and branching of postmitotic neural cells. Such morphologic changes contribute to the observation that neural cells can facilitate migration and invasion of cancer cells toward neuronal axons. Furthermore, PNI is described as being related to the close anatomical association between the tumor and the neural plexus and on the special ability of cancer cells to easily recognize neural tissue by secreting neural cell adhesion molecule.[25,26]

Deng et al[27] in their systematic review and meta-analysis demonstrated the prognostic role of PNI in gastric cancer patients, showing that this is independent from lymph nodes status, tumor size, and tumor grade and a range of other biologic variables on multivariate analysis. Despite this, only few of the included studies reported outcomes specifically concerning PNI, analyzing patients with positive or negative PNI by subgroups. Furthermore, heterogeneity between studies was described for both OS and DFS analysis when pooling results and only 4 articles could be included in the analysis concerning DFS.

Jiang et al[28] in a group of patients who underwent radical resection for gastric carcinoma found that the presence on PNI had a significant correlation with size of tumor ($\geq$5 cm), lymphatic venous invasion, deeper tumor invasion (T4), lymph node metastases (N3), and deeper TNM stage (III), which were proved to be associated with worse survival outcomes. In the multivariate analysis, presence of PNI and classical oncologic variables maintained significance and was recognized as an independent prognostic factor in gastric carcinoma patients with poor survival.

On the other hand, Duraker et al found that although PNI was positive in 211 of the 354 patients (59.6%) with gastric carcinoma and the incidence of PNI increased with disease stage, this variable did not provide any additional information to the classical and well-established oncologic prognostic parameters; this was subsequently confirmed in other studies worldwide.[10–14]

In the present article, we investigated incidence and prognostic value of PNI in a group of patients who underwent potentially curative resection for gastric carcinoma. The incidence of PNI detected by histopathology analysis of surgical specimens was 45.6%.

Survival curves of our case series revealed a reasonable 5-year actuarial OS and DFS for gastric cancer patients, in accordance with the literature. Furthermore, the long-term follow-up although of a relatively limited case series improves the statistical value of the curves. Confirmation from future studies could strengthen our results. What is interesting is that at a subgroup analysis, patients with PNI

Table 2 Predictive factors for overall survival

	Univariate analysis		Multivariate analysis	
Variables	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Female sex	.84 (.50–1.42)	.52		
Age	1.04 (1.01–1.07)	.003	1.05 (1.01–1.08)	.001
Tumore size (mm)	1.00 (.99–1.01)	.40		
N lymph nodes retrieved	1.00 (.98–1.02)	.44		
N positive lymph nodes	1.03 (1.02–1.05)	.001	1.03 (1.00–1.07)	.03
LNR	5.64 (2.42–13.14)	.001	.61 (.11–3.35)	.57
T stage				
I	1	.005	1	.04
II	2.84 (1.06–7.61)	.03	2.19 (.68–7.02)	.18
III	5.55 (2.02–15.22)	.001	5.08 (1.44–17.91)	.01
IV	5.05 (1.73–14.72)	.003	3.74 (1.03–13.54)	.04
TNM stage III	2.02 (.63–4.16)	.34		
Diffuse histology	1.08 (.60–1.96)	.78		
Poor differentiation (G4)	1.41 (.81–2.60)	.21		
Lymphovascular invasion	2.70 (1.31–5.54)	.007	1.08 (.42–2.75)	.86
Perineural invasion	3.07 (1.77–5.30)	.0001	2.44 (1.34–4.45)	.004

CI = confidence interval; LNR = lymph node ratio; OR = odds ratio; TNM = tumor-node-metastasis.

had a significantly worse OS and DFS, suggesting the role of neural cancer cell invasion in survival. These data were furthermore confirmed in the Cox regression analysis in which PNI was recognized to be an independent factor predicting oncologic outcomes.

Other well-known oncologic variables, such as positive lymph nodes and T stage, resulted significantly associated with OS at multivariate analysis. These 2 important prognostic factors are currently used for stratifying patients to decide the best treatment strategy. Jiang et al[28] incorporated PNI into N3 and TNM stage III in patients with gastric carcinoma through the redefinition of stage III in III_{PNI} and N stage in N_{PNI}. Their under-categorization reached a lower −2 log-likelihood value and a higher hazard ratio and 95% confidence interval, which represented an optimum prognostic stratification, together with better homogeneity, and discriminatory ability. We believe that the results of our study could confirm the usefulness of this incorporation because of the link between PNI and survival.

Most gastric cancer patients, even after curative resection, experienced recurrence with a rate of 60% and median length of survival after recurrence of 7.4 months in case of distant metastases and 10.4 months in case of local recurrence.[29] In this context, considering the results of the present study in which PNI turned to be statistically and independently associated with DFS, this, in our opinion

Table 3 Predictive factors for disease-free survival

	Univariate analysis		Multivariate analysis	
Variables	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Female sex	.85 (.63–1.14)	.29		
Age	1.01 (.98–1.04)	.32		
Tumor size (mm)	1.00 (.99–1.02)	.45		
N lymph nodes retrieved	1.01 (.99–1.03)	.26		
N positive lymph nodes	1.04 (1.02–1.06)	.0001	1.03 (.98–1.07)	.15
LNR	9.32 (3.55–24.51)	.0001	1.11 (.14–8.58)	.91
T stage				
I	1	.02	1	.10
II	14.76 (1.97–110.13)	.009	6.70 (.82–54.81)	.07
III	20.17 (2.66–152.84)	.004	10.40 (1.20–89.77)	.03
IV	11.21 (1.37–91.83)	.02	5.26 (.57–48.05)	.14
TNM stage III	1.64 (.25–4.33)	.29		
Diffuse histology	1.13 (.68–1.89)	.62		
Poor differentiation (G4)	12.72 (.68–20.33)	.93		
Lymphovascular invasion	5.04 (1.79–14.14)	.002	1.44 (.45–4.65)	.53
Perineural invasion	3.86 (2.05–7.29)	.0001	2.35 (1.18–4.67)	.01

CI = confidence interval; LNR = lymph node ratio; OR = odds ratio; TNM = tumor-node-metastasis.

should be incorporated in gastric cancer postsurgery therapy stratification and weighed together with other known adverse factors to have more elements to consider an aggressive oncologic therapy in perineural positive patients.

This study has some limitation mostly consisting in its relatively small sample size, retrospective design, and single-center conduction. Therefore, a large-scale prospective validation study is needed to confirm these results.

In summary, PNI is an independent marker of poor prognosis in patients with gastric cancer. Identification of sensitive markers for patients who had undergone curative gastrectomy and who are at high risk of recurrence would provide useful information for planning follow-up after surgery or intensive adjuvant chemotherapy.

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