Inclusion Of Perineural Invasion With AJCC-TNM Staging: Outcomes From A South East Asian Cohort Of Curatively Treated Gastric Cancer Patients

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Abstract

Background: The incidence of perineural invasion (PNI) in patients with gastric cancer (GC) is high, and patients with PNI positive disease have a poor prognosis compared to PNI negative disease. The present study aims to study the incidence and evaluate the impact of PNI on the survival outcome of a cohort of Eastern Asian GC patients.

Material and Methods: All consecutive patients undergoing curative gastrectomy were included in the study. The incidence of PNI and correlation with different clinicopathological features and overall survival was performed.

Results: 59.54% had PNI positive disease and the median OS of PNI+ve patients was 29.3 months, while it was not reached in PNI-ve patients. The PNI positivity was a significant prognostic factor for overall survival both on univariate and multivariate analysis. On TNM-PNI staging, those with TNM stage I / II patients with PNI+ve disease had similar OS to all stage III patients ($p=0.835$) and was worse than that of PNI-ve patients ($p<0.05$).

Conclusion: The incidence of PNI in gastric cancer is high. The inclusion of PNI with AJCC-TNM staging may better stratify prognostic staging in curatively treated gastric cancer patients.

Introduction

Worldwide, Gastric cancer (GC) is the second most common neoplasm after colorectal cancer and is the leading cause of death due to digestive tract cancer.[1] Curative surgery with D2 lymphadenectomy is the standard approach in most treatment guidelines. Neoadjuvant/adjuvant treatment is administered to reduce recurrence and improve survival. It is essential to identify prognostic factors to select the patients better to tailor the treatments available and direct experimental approaches. [2, 3] Perineural invasion (PNI) is a process of cancer cells invading the nerves, which is also called the neurotropic carcinomatous spread. Vascular and lymphatic routes are well-documented as hallmarks of malignancy long before. However, tumor spread along the nerve has been described in the mid-1800s. PNI has recently emerged as an important and independent morphological prognostic factor in several malignancies such as pancreas, colon and rectum, prostate, head and neck, biliary tract, and stomach. Sometimes, PNI may be the only route of distant metastasis without lymphatic and vascular spread. The morphology and clinical significance of PNI are well-characterized, but its molecular mechanism is highly complex and not well elucidated.

This study aims to evaluate the impact of PNI in the long-term survival of GC patients undergoing curative surgery and discuss the impact of PNI on AJCC prognostic staging.

Material & Methods

All patients who had undergone curative radical gastrectomy from 1st May 2016 to 30th December 2020 were included in the study. The data were retrieved from the prospectively maintained standalone surgical oncology database (SQL Server Express 2012/power builder 9.0). All procedures were followed according to the Institutional ethical standards and the Helsinki Declaration of 1975, as revised in 1983.

Data Collection:

The database was queried for the following data from the database (demography, location of the tumor, neoadjuvant chemotherapy, type of surgery, curative/palliative surgery, stage, histopathology, differentiation, perineural invasion, lymphovascular invasion, adjuvant therapy details, last follow-up date, any recurrence, and mortality details). The 8th edition AJCC TNM system was used for staging purposes.

Inclusion and Exclusion Criteria:

All consecutive patients who underwent curative radical gastrectomy were included in the analysis. The patients who had undergone surgery with palliative intent and those with incomplete data were excluded from the analysis.

Perineural Invasion:

The resected gastrectomy specimens received in the histopathology lab were grossed and reported as per College of American Pathologists (CAP) protocol. Hematoxylin and eosin-stained slides were prepared using a standard protocol of tissue processing. PNI status was evaluated using the criteria proposed by Batsakis JG [4]. The presence of PNI was considered when the invasion was in, around, and through the nerves. [Figure 1] Though IHCs (S100 and NSE) help pick up microscopic foci of PNI, they were not used in this study.

Statistical Analysis:

Overall survival was calculated as the time difference between the date of diagnosis and the date of death or last contact with the patient. STATA software Version 11 (Stata Corp LP, TX) was used for data analysis. Categorical data were expressed as proportion or percentage. Numerical data were expressed as mean or median (+/-SD). The difference between categorical data was compared using the Chi-squared test / Fisher's exact test. The difference between numerical data was compared using the student's t-test / ANOVA test. Survival was calculated using the Kaplan-Meier curve, and the difference in survival was compared using the log-rank test/cox proportional hazards. A p-value of <0.05 was considered significant.

Results

Patient Characteristics:

A total of 212 consecutive patients underwent surgery for gastric malignancy during the study period, and 131 patients met the above inclusion criteria [eFigure 1 in Supplement]. The mean age was 57.17 (+/- 11.12) years, and the male to female ratio was 2.2:1.

The incidence of PNI was 78 (59.54%) patients in this cohort. On univariate analysis, the incidence of PNI was significantly associated with location of tumour (74.42% in proximal, gastroesophageal junction & diffuse tumours vs 52.27% in distal & body tumours, $p=0.015$); surgery type (distal gastrectomy 50% vs total gastrectomy 82.05%, $p=0.003$), differentiation (well differentiated 34.62% vs moderately differentiated 53.33% vs poorly differentiated 76.79%, $p=0.002$), clinical stage (early 45.10% vs locally advanced 68.75%, $p=0.007$), AJCC "T" stage (T1 7.69% vs T2 26.32% vs T3 59.57% vs T4 84.62%, $p=0.001$), and AJCC "N" stage (N0 43.75%, vs N+ 64.65%, $p=0.036$). On multivariate analysis, incidence of PNI was significantly high as the AJCC "T" stage increases ($p=0.001$) [Table 1].

Table 1
Demography and Patient Characteristics (n=131)

Variable	Sub-Groups	Incidence		Univariate Analysis	Multivariate Analysis
		PNI -	PNI +	p-value	p-value
Age	≤ 50 Years	31 (39.24%)	48 (60.76%)	0.726	0.340
	> 50 Years	22 (42.31%)	30 (57.69%)		
Gender	Female	16 (39.02%)	25 (60.98%)	0.821	0.516
	Male	37 (41.11%)	53 (58.89%)		
Tumour Location	GE Junction, Proximal and Diffuse	11 (25.58%)	32 (74.42%)	0.015	0.299
	Body, Distal	42 (47.73%)	46 (52.27%)		
Surgery Type	Total Gastrectomy	7 (17.95%)	32 (82.05%)	0.003	0.797
	Distal Gastrectomy	40 (50%)	40 (50%)		
	Others	6 (50%)	6 (50%)		
Morphology	Proliferative	12 (60%)	8 (40%)	0.053	0.088
	Non-Proliferative	41 (40%)	70 (60%)		
Differentiation	Well Differentiated	17 (65.38%)	9 (34.62%)	0.002	0.301
	Moderately Differentiated	14 (46.67%)	16 (53.33%)		
	Poorly Differentiated	13 (23.21%)	43 (76.79%)		
	Unknown	9 (47.37%)	10 (52.63%)		
Clinical Stage	Early	28 (54.90%)	23 (45.10%)	0.007	0.415
	Locally Advanced	25 (31.25%)	55 (68.75%)		

Variable	Sub-Groups	Incidence		Univariate Analysis	Multivariate Analysis
		PNI -	PNI +	p-value	p-value
Lymphovascular Invasion	Yes	31 (35.23%)	57 (64.77%)	0.081	0.094
	No	22 (51.16%)	21 (48.84%)		
AJCC "T" Stage	T1	12 (92.31%)	1 (7.69%)	0.001	0.002
	T2				
	T3	14 (73.68%)	5 (26.32%)		
	T4	19 (40.43%)	28 (59.57%)		
		8 (15.38%)	44 (84.62%)		
AJCC "N" Stage	N0	18 (56.25%)	14 (43.75%)	0.036	0.104
	N+	35 (35.35%)	64 (64.65%)		
Adjuvant Therapy	Chemotherapy	35 (37.23%)	59 (62.77%)	0.231	0.649
	Chemoradiation	0	4 (100%)	0.094	–
Recurrence	Yes	6 (26.09%)	17 (73.91%)		
Mortality	Yes	10 (21.74%)	36 (78.26%)		
Median Overall Survival (months)		Not Reached	29.3 months		

Recurrence, Mortality and Overall Survival:

After a median follow-up of 17.7 months, 23 (17.56%) patients had a recurrence, and 46 (35.11%) patients died. The median overall survival (OS) of the cohort of 131 patients was 35.87 months. The median OS for patients with PNI+ve was 29.3 months, while the median survival was not reached for PNI-ve patients. The PNI was an independent prognostic factor influencing OS with the 3-year OS of 65.14% (95% CI 42.06-80.89) for PNI -ve vs. 32.50% (95%CI 17.90-47.99) for PNI+ve patients (p=0.009) [Figure 2]. On the log-rank test of different subgroups, PNI positivity was significantly associated (p-value<0.05) with poor survival in patients with age>50 years, male gender, tumors in the body & antro-pyloric region of the stomach, and non-proliferative gross morphology, any clinical-stage, any lymphovascular invasion, pathological T4, pathological node-positive, and N3 disease [Figure 3], [eTable 1 in Supplement]. On

multivariate analysis with Cox regression, PNI positivity was an independent prognostic factor (HR 2.73, 95% CI 1.194-6.244, $p=0.01$).

The patients who underwent upfront surgery (those without neoadjuvant treatment, $n=91$) were tested whether PNI status had any influence on survival in different subgroups (TNM stage, T stage, and N stage). The patients who underwent neoadjuvant therapy were excluded from this analysis to exclude the influence of chemotherapy on PNI in the post-chemotherapy resected specimen. In this cohort of upfront surgery patients, the PNI was incorporated to TNM staging (TNM-PNI staging). The patients with TNM stage I / II patients with PNI+ve disease had worse overall survival than that of PNI-ve patients. While the patients with TNM stage I / II disease with PNI+ve disease had similar survival to TNM stage III patients ($p>0.05$) [Figure 2B]. The patients with stage III-PNI -ve disease had similar survival to stage I/II PNI-ve disease ($p>0.05$) [Table 2]. Similarly, the T stages (pT4 vs. pT1-3, the pT1/3 group were clubbed together due to fewer patients in T1/2), and N stages could be prognosticated into two subgroups by PNI status. The patients with pT1-3 with PNI +ve had similar survival to patients with pT4 disease (pT4 with PNI -ve and +ve together) [Figure 2C]. Similarly, it was observed that the patients with pN0 & PNI +ve disease had worse overall survival, which was similar to patients of pN+ disease (pN+ with PNI -ve and +ve together) ($p\text{-value}>0.05$) [Figure 2D][6].

Table 2
AJCC TNM Stage and TNM-PNI Stage Grouping vs Hazards of Death

AJCC TNM Staging			TNM-PNI Grouping			
TNM Stage	Hazard Ratio (95% CI)	p-value	TNM-PNI	No of Patients	Hazard Ratio (95% CI)	p-value
Stage I	1	–	Stage I-PNI Neg	17 (18.68%)	1	–
			Stage I-PNI Pos	3 (3.30%)	4.18 (0.26 - 66.97)	0.312
Stage II	5.94 (1.28 - 7.55)	0.023	Stage II-PNI Neg	9 (9.89%)	7.48 (0.67 - 83.01)	0.101
			Stage II-PNI Pos	14 (15.38%)	10.49 (1.29 - 85.44)	0.028
Stage III	6.03 (1.39 - 6.06)	0.016	Stage III-PNI Neg	14 (15.38%)	2.87 (0.26 - 31.89)	0.389
			Stage III-PNI Pos	34 (37.36%)	13.52 (1.79 - 102.10)	0.012

Discussion

In the present article, we studied the incidence and prognostic value of PNI in patients who underwent curative resection for gastric adenocarcinoma. The incidence of PNI detected by histopathology analysis after surgery was 59.54%. The PNI positivity was higher in proximal, gastroesophageal junction, diffuse tumors; who had undergone total gastrectomy; less differentiated tumors; advanced disease; higher T or N stage. However, on multivariate analysis, only the AJCC T stage was significantly associated with a higher incidence of PNI.. The median OS of the whole cohort was 35.87 months, while that of PNI+ve was 29.3 months PNI-ve patients did not reach median survival. On univariate and multivariate analysis, the PNI positivity was a significant prognostic factor. The hazard of death in PNI +ve disease was worse in all subgroups. However, it was significant in older than 50 years, males, tumors in the body & antro-pyloric region of the stomach, non-proliferative gross morphology, pathological T4, pathological node-positive, and N3 disease.

The incidence of PNI in gastric cancer has been reported by various series from 31.7 - 73% [Table 3]. Different authors have studied the factors associated with PNI positivity. (e.g., the site of the tumor, Borrmann classification, the depth of invasion (pT stage), tumor size, differentiation, lymph node metastasis (pN stage), clinical stage, histological type, lymphatic vessel invasion, and blood vessel invasion, pathological stage were closely associated with increased PNI positivity). But all studies consistently showed an increase in PNI positivity rate as the AJCC TNM stage, T stage, or N stage increases. (ref) The present study also showed a similar trend in the PNI positivity rate.

Table 3
Published Studies on Perineural Invasion in Gastric Cancer and Survival

Author & Year	Resection	Stages	Incidence of PNI	Survival (PNI-ve vs. PNI+ve)
Tanaka et al. 1994 [5]	R0/R+	I/II/III	139/283 (49.1%)	5-year survival rate 71.3% vs.23.7%, p<0.001
Duraker et al. 2003 [6]	R0/R+	I/II/III/IV	211/354 (59.6%)	OS, HR: 1.10 (0.89–1.37); T3, HR: 1.16 (0.91–1.48)
Scartozzi et al. 2006 [2]	R0	I/II/III	211/287 (73%)	Median survival 38.2 months vs 24.1 months, p=0.008
Tianhang et al. 2008 [7]	R0/R+	I/II/III/IV	518/1,632(31.7%)	OS, HR =3.23, 95% CI=2.6–8.11, p<0.01
Bilici et al. 2010 [8]	R0	IB/II/III/IV	180/238 (75.6%)	Median OS, 64.9 vs.28.1 months, p=0.001
Selçukbiricik et al, 2012 [9]	R0	I/II/III	211/287 (73%)	Median OS, 38.2 vs. 24.1 months, p=0.008
Jiang et al. 2014 [10]	R0	I/II/III	186/518 (35.9%)	OS, HR =1.901, 95% CI = 1.495–2.418, P<0.001
Aurello et al. 2017 [3]	R0	Ib/II/III	47/103 (45.6%)	5-year actuarial OS (59% vs 19%, P=0 .0001) and DFS (69% vs 22%, P =0.0001)
De Franco et al. 2018 [11]	R0/R+	I/II/III/IV	162/455 (33.9%)	5-year cancer-related survival (CRS) was 65.7% vs. 20.6%, p < 0.001
Our series, 2021	R0	I/II/III	78/131 (59.54%)	3-year OS rate 65.14% vs. 32.50%, 0.009
eTable 1 in Supplement: Subgroup Analysis of 3-year Overall Survival (n=131)				

Different authors have studied the prognostic role of PNI in GC patients. [2, 3, 5, 6, 6–9, 11] The survival has consistently been reported to be significantly worse in PNI+ve patients than PNI -ve. [Table 3] In a study by Aurello et al. from Italy, PNI could effectively differentiate locally advanced gastric cancer into two statistically significant groups demonstrating different recurrence rates and survival. [3] In another study by Scartozzi et al., early gastric cancer can be effectively differentiated into biologically two prognostic groups.[2] Two meta-analyses published in 2008 and 2020 have shown a worse survival in PNI positive patients. [12, 13] The present study showed a worse survival in early gastric cancer (stage I/II) when PNI was positive. Thus, the presence of PNI can now be accepted as a poor prognostic factor in gastric cancer patients.

Several studies have demonstrated that incorporation of PNI in the TNM staging gives a more accurate prognostic group. [10, 14, 15] As per the data from previous studies and our analysis, PNI can effectively differentiate AJCC stage groups into PNI negative as a "good" and PNI positive as a "poor" prognostic

group. Attempts should be made to analyze large institutional / multi-institutional gastric cancer databases and validate the incorporation into the UICC AJCC staging system. Patients with PNI may be candidates for aggressive oncologic and experimental therapy. In addition, the role of PNI in conventional and experimental therapy may be established.

Limitations:

The limitations of the present study were the retrospective design and a relatively small sample size. Therefore, a large-scale prospective study is needed to confirm the results.

Conclusion

In summary, Perineural Invasion is an independent marker of poor prognosis in patients with gastric cancer. The presence of Perineural Invasion identifies poor-risk patients after curative gastrectomy who are at high risk of recurrence and worse survival. Those without Perineural Invasion have a better prognosis. Incorporating Perineural Invasion into TNM staging (TNM-PNI) may better prognosticate gastric cancer patients than the 8th edition of the AJCC staging system.

Declarations

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Figures

Figure 1

Histopathological Diagnosis of Perineural Invasion

Figure 1A. Tumor cells around the nerve bundle covering more than 2/3rd of the circumference, Figure 1B. Tumor cells in the perineural space (arrow), Figure 1C. Tumor cells invading the nerve bundle with acinar differentiation (yellow star), Figure 1D. Tumor cells and extracellular mucin invading the nerve bundle (green star), with surrounding inflammatory cells (arrow)

Figure 2

Kaplan-Meier Estimate of Overall Survival

Figure 2A: based on PNI (Perineural Invasion) Status, Figure 2B, 2C & 2D: Kaplan-Meier Survival Curve of patients who have undergone upfront surgery, Figure 2B: Overall Survival of Stage I/II with and without PNI vs Stage III, Figure 2C: Overall Survival of T1/2/3 with and without PNI vs T4, Figure 2D: Overall Survival of Node Negative Disease with and without PNI vs Node Positive Disease

Figure 3

Supplementary Files

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