Perineural invasion as a predictive factor for survival outcome in gastric cancer patients: a systematic review and meta-analysis

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ABSTRACT

Aims The prognostic significance of perineural invasion (PNI) for gastric cancer (GC) patients was under debate. This study aimed to review relevant studies and evaluate the impact of PNI on the survival outcome of GC patients.

Methods Systematic literature search was performed using PubMed and Embase databases. The relevant data were extracted, and the association between PNI and clinicopathological characteristics or survival outcome in GC patients were evaluated using a fixed-effect model or random-effect model.

Results A total 13 studies involving 7004 GC patients were included in this meta-analysis. The positive rate of PNI was 35.9% (2512/7004) in GC patients, ranging from 6.9% to 75.6%. There were significant relationships between PNI and a series of unfavourable clinicopathological factors including undifferentiated histology type (OR: 1.78, 95% CI 1.37 to 2.33, p<0.001; I^2=75.3%), diffuse type (OR: 1.96, 95% CI 1.07 to 3.60, p=0.029; I^2=79.5%), lymphatic invasion (OR: 7.00, 95% CI 3.76 to 13.03, p<0.001; I^2=83.6%), vascular invasion (OR: 5.79, 95% CI 1.59 to 21.13, p=0.008; I^2=95.8%), deeper tumour invasion (OR: 4.79, 95% CI 3.65 to 6.28, p<0.001; I^2=65.0%) and lymph node metastasis (OR: 3.60, 95% CI 2.37 to 5.47, p<0.001; I^2=89.6%). In addition, PNI was significantly associated with worse survival outcome in GC patients (HR: 1.69, 95% CI 1.38 to 2.06, p<0.001; I^2=71.0%).

Conclusion PNI was frequently detected in surgically resected specimens of GC patients, and it was a predictive factor for survival outcomes in these patients.

INTRODUCTION

Although the overall incidence of GC has declined in the past few years, it remains the common cause of cancer-related deaths in the world according to Global Cancer Statistics 2018.[1] The optimal treatment option for resectable GC patients remains curative resection with negative margins and adequate lymphadenectomy.[2] However, a large proportion of GC patients experienced tumour recurrence and had a poor survival outcome even after curative resection. The depth of tumour invasion (T stage) and lymph node metastasis (N stage) were important prognostic factors for GC patients.[3 4] To better select patients and determine appropriate therapeutic strategies, identifying as many biological or pathological indicators for overall survival (OS) and recurrence as possible still be necessary.

Perineural invasion (PNI), which also has been called neurotropic carcinomatous spread or perineural spread, was a pathological feature characterised by the infiltration of tumour cells along the perineurium or the neural fascicles. This is an important pathway for the local spread of cancer cells, which represented an aggressive biological behaviour of tumours.[5] According to the previous reports, the prevalence of PNI in GC patients varied from 31.7% to 65.0%.[6–9] Positive PNI was not infrequent in surgically resected specimens of GC patients with the improvement of detection technology and pathologists' experience. However, we still lack sufficient understanding of PNI pathogenesis for cancer patients up to now. Besides, the definition of PNI was under debate. Some researchers hold that it should be defined as the presence of tumour cells in any three layers of the nerve sheath (epineurium, perineurium and endoneurium) or in foci outside the nerve sheath with the involvement of 33% the nerve circumference.[10]

It has been reported that PNI was significantly associated with recurrence and poor survival outcome in pancreatic cancer, prostate cancer and colorectal cancer.[11–14] Furthermore, there have been several studies evaluating the prognostic significance of PNI for GC patients, but the results were conflicting. Some studies regarded PNI as a useful prognostic factor for GC patients who underwent curative resection,[7 8 15] but others reported that it could not provide more additional prognostic information than well-known clinicopathological factors such as tumour, node, metastases (TNM) stage and differentiation type.[9 16] In view of no consensus on this topic, more research evidence need to be provided to help us better understand the prognostic significance of PNI and guide individual treatment for GC patients. In the present study, we reviewed relevant studies and performed a meta-analysis to determine the relationship between PNI and clinicopathological characteristics and evaluate the impact of PNI on survival outcome in GC patients.

METHODS

Literature search strategy

The systematic search for relevant studies was independently conducted by two investigators using PubMed, Embase and Cochrane Library databases

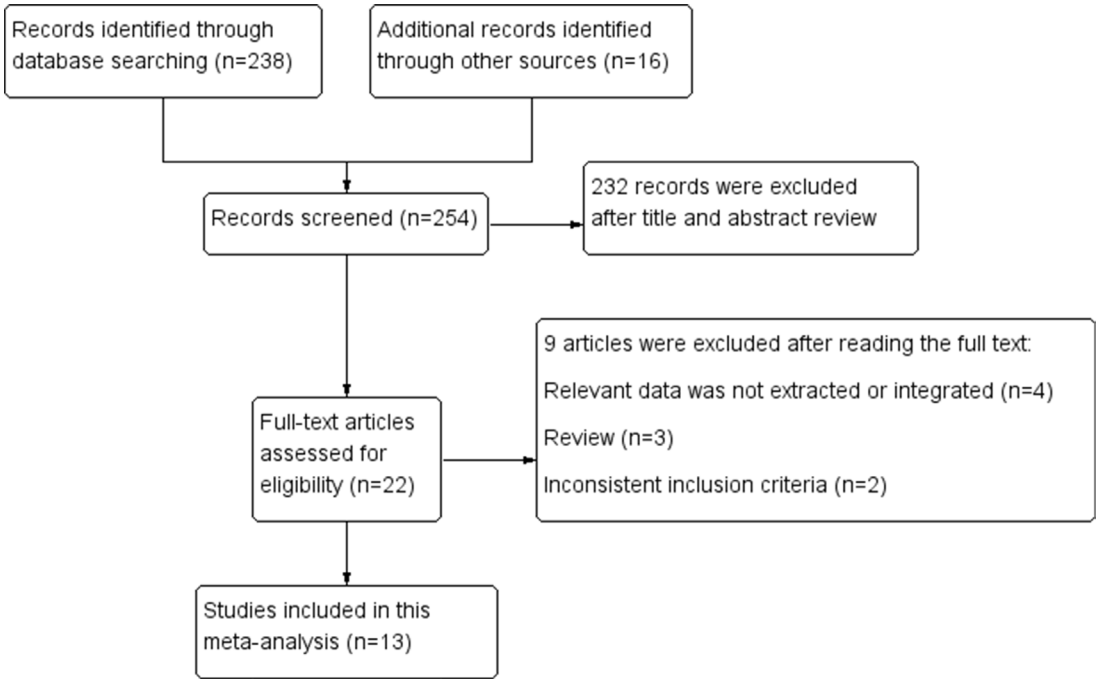

Figure 1 The flow diagram of study selection.

up to April 2019. The following keywords and search terms were used for this search strategy: "gastric cancer" or "gastric adenocarcinoma" and "perineural invasion". Through these combined keywords, the primary collection of studies was defined. Besides, the reference lists of relevant articles were manually searched to identify additional relevant studies. The search was limited to published full articles in English and Chinese language. The titles and abstracts of each retrieved studies were scanned by two investigators for evaluating the topic relevance. The full text of potentially relevant studies was obtained and further assessed.

Eligibility criteria

Studies included in this systematic review and meta-analysis were required to meet the following criteria: (1) positive PNI was defined as the presence of tumour cells along the perineurium or the neural fascicles in surgically resected specimens. All GC patients were divided into two groups based on the PNI status (positive PNI and negative PNI group). (2) Included studies investigated the relationship between PNI and clinicopathological characteristics or evaluated the prognostic significance of PNI for GC patients. (3) The survival outcome was presented as OS. HR with 95% CIs should be reported, and these data could be extracted directly from multivariate Cox regression analysis or calculated indirectly from original studies. (4) In case of the same study cohorts or the same populations, only the most informative or highest quality or the latest study was included in this meta-analysis. Studies were excluded if they were letters, comments, conference abstracts, review articles or case reports.

Data extraction and quality assessment

The data from included studies were independently extracted by two investigators after reviewing the abstracts and full text. Any discrepancies were resolved by discussion among all investigators until consensus was reached. The following information was collected from included studies: first author, publication year, country of study population, study design, study period and sample size. In addition, the following clinicopathological characteristics were extracted: age (≥60 vs <60 years), gender (male vs female), tumour size (≥3 cm vs <3 cm or ≥5 cm vs <5 cm), tumour location (upper and middle 1/3 vs low 1/3), differentiation type (undifferentiated vs differentiated type), Lauren classification (diffuse type and intestinal type), Borrmann type (III–IV vs I–II), T stage (T3-T4 vs T1-T2), lymph node metastasis (yes vs no), lymphatic invasion (yes vs no), vascular invasion (yes vs no), pathological TNM stage (III–IV vs I–II) and HR with 95% CIs for OS. All predefined outcomes were summarised in a 2*2 contingency table for further analysis.

The methodological quality of individual retrospective studies was independently evaluated by two investigators using the Newcastle-Ottawa Quality Assessment Scale.[17] All included studies were scored according to the scoring items. Studies with ≥7 points were recognised as high-quality in methodology for observational cohort studies. Our study was designed, performed and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.[18]

Statistical analysis

ORs and 95% CIs were used as measures to assess the relationship between PNI and clinicopathological characteristics. The HR and its corresponding 95% CIs for OS were pooled and calculated to evaluate the prognostic significance of PNI for GC patients. The pooled HR greater than one indicated a poorer survival outcome in the research group (positive PNI) as opposed to the control group (negative PNI). The degrees of heterogeneity between different studies were quantified and evaluated using the Cochran Q test and I^2 statistic. The results were expressed as I^2 value and Q test p value, where $I^2>50\%$ and/or $p<0.01$ was considered as a high heterogeneity. If a significant statistical heterogeneity was observed, a random effect model (inverse variance method) should be adopted; otherwise, a fixed-effect model should be used.

To explore potential source of heterogeneity, subgroup analysis was performed according to the population geography (eastern vs western), tumour stage (I–III vs I–IV stage), sample

Table 1 Clinicopathological characteristics of included studies

Study	Country	Inclusion period	Sample size (PNI+/PNI−)	PNI (%)	Follow-up median (range)	Resection	TNM stage	Survival data (PNI+ vs PNI−)	Quality
De Franco *et al*[20]	Italy	1995–2012	455 (162/293)	33.9	80.3 months (IQR: 38–122)	R0/R+	I–IV	5 years-DSS: 20.6% vs 65.7%; HR: 1.99 (1.24–3.19)	7
Zhou *et al*[9]	China	2001–2006	160 (104/56)	65.0	43 months (3–120)	R0	I–III	5 years-OS: 35.6% vs 62.5%; HR: 1.469 (0.896–2.409)	7
Selçukbiricik *et al*[19]	Turkey	2001–2010	287 (211/76)	73.0	N/A	R0	I–III	Median OS: 24.1 months vs 38.2 months; HR: 1.21 (1.08–2.3)	6
Aurello *et al*[8]	Italy	2004–2014	103 (47/56)	45.6	26 months (2–138)	R0	I–III	5 years-OS: 19% vs 59%; HR: 2.44 (1.34–4.45) 5 years-DFS: 22% vs 69%; HR: 2.35 (1.18–4.67)	7
Jiang *et al*[7]	China	2002–2006	518 (186/332)	35.9	50 months (3–98)	R0	I–III	5 years-OS: 22.6% vs 56.0%; HR: 1.901 (1.495–2.418)	7
Bilici *et al*[15]	Turkey	2003–2009	238 (180/58)	75.6	29.5 months (7.5–73)	R0	I–III	Median OS: 28.1 vs 64.9 months; HR: 2.75 (1.12–3.13)	7
Tanaka *et al*[21]	Japan	1982–1989	283 (139/144)	49.1	N/A	R0	I–III	5 years-OS: 23.7% vs 71.3%; T2: 24.6% vs 76.2%; T3-T4: 22.0% vs 48.8%	6
Tianhang *et al*[6]	China	2000–2005	1632 (518/1114)	31.7	47.4 months (25–84)	R0/R+	I–IV	Median OS: 21.0 vs 57.9 months; HR: 3.23 (2.6–8.11)	7
Scartozzi *et al*[22]	Italy	N/A	739 (51/688)	6.9	N/A	R0	I–IV	OS, HR: 1.41 (1.065–2.174)	6
Duraker *et al*[16]	Turkey	1993–2000	354 (211/143)	59.6	N/A	R0/R+	I–IV	OS, HR: 1.10 (0.89–1.37); T3, HR: 1.16 (0.91–1.48)	6
Setälä *et al*[23]	Finland	1976–1988	256 (116/140)	45.0	N/A	R0/R+	I–IV	5-OS: 15% vs 40%	6
Chen *et al*[25]	China	1999–2015	1801 (509/1292)	28.3	37.3 months (1–99)	R0/R+	I–IV	5-OS: 49.1% vs 60.7%; HR: 1.272 (1.066–1.516)	7
Xie *et al*[24]	China	2004–2008	178 (78/100)	43.8	N/A	R0	I–IV	Median OS: 28.6 vs 44.3 months; HR: 2.257 (1.268–4.17)	7

DFS, disease-free survival; DSS, disease-special survival; OS, overall survival; PNI, perineural invasion; TNM, tumour, node, metastases.

size (≥350 vs <350), publication year (after 2010 vs before 2010), resection type (R0 vs R^+), the incidence of positive PNI (≥40% vs <40%) and the quality of included studies (≥7 vs <7). Also, the funnel plot was constructed and potential publication bias was detected through visual inspection of its symmetry. All statistical analyses for evaluating the association between PNI and clinicopathological characteristics or its prognostic value were performed by the Stata V.13.0 software. The p value <0.05 was considered to be of statistical significance.

RESULTS

Search results and study characteristics

The flow diagram of study selection was shown in figure 1. From an electronic database search, a total of 254 potentially relevant studies were initially identified with the predefined search strategy. After scanning the titles and abstracts, 232 irrelevant studies were further excluded. Full text of the remaining 22 studies were obtained for review and assessment. Among these studies, three were review articles, four studies did not report related outcomes or data for pooled analysis and two studies did not meet the inclusion criteria. These studies were not included in this systematic review and meta-analysis. Ultimately, we identified 13 relevant studies for evaluating the clinicopathological characteristics and prognostic significance of PNI in GC patients.[6–9 15 16 19–25]

The baseline characteristics of 13 included studies were summarised in table 1. No randomised controlled trial was eligible for inclusion. All studies were retrospective in nature and were published between 1994 and 2018, and the research period ranged from 1976 to 2014. Five studies were from China, three studies were from Italy, three studies were from Turkey, one study was from Japan and one study was from Finland.

In this meta-analysis, a total of 7004 GC patients underwent surgical treatment, including 2512 patients with positive PNI and 4492 patients without PNI, with a range of sample size from 103 to 1801. According to the included studies, the positive rate of PNI was 35.9% in GC patients, ranging from 6.9% to 75.6%.

Relationship between PNI and clinicopathological characteristics

The results of meta-analysis indicated that positive PNI was significantly associated with undifferentiated histology type (OR: 1.78, 95% CI 1.37 to 2.33, p<0.001; I^2=75.3%), diffuse type (OR: 1.96, 95% CI 1.07 to 3.60, p=0.029; I^2=79.5%), upper or middle 1/3 tumour (OR: 1.26, 95% CI 1.12 to 1.42, p<0.001; I^2=40.5%), deeper tumour invasion (T3-T4 vs T1-T2: OR: 4.79, 95% CI 3.65 to 6.28, p<0.001; I^2=65.0%), lymph node metastasis (OR: 3.60, 95% CI 2.37 to 5.47, p<0.001; I^2=89.6%) and more advanced tumour stage (stage III–IV vs stage I–II: OR: 4.43, 95% CI 3.40 to 5.75, p<0.001; I^2=61.5%) (figures 2–4). On the other hand, lymphatic invasion (OR: 7.00, 95% CI 3.76 to 13.03, p<0.001; I^2=83.6%) and vascular invasion (OR: 5.79, 95% CI 1.59 to 21.13, p=0.008; I^2=95.8%) were more frequent in GC patients with PNI (table 2 and online supplementary figure 1). The cases with PNI had a larger tumour size than those without PNI (≥3 cm vs <3 cm: OR: 2.92, 95% CI 1.98 to 4.28, p<0.001; I^2=0%; ≥5 cm vs <5 cm: OR: 1.83, 95% CI 1.59 to 2.11, p<0.001; I^2=0%) (table 2 and online supplementary figure 2). However, there was no significant difference between positive PNI and negative PNI in terms of patients age, gender and Borrmann type (table 2 and online supplementary figure 3).

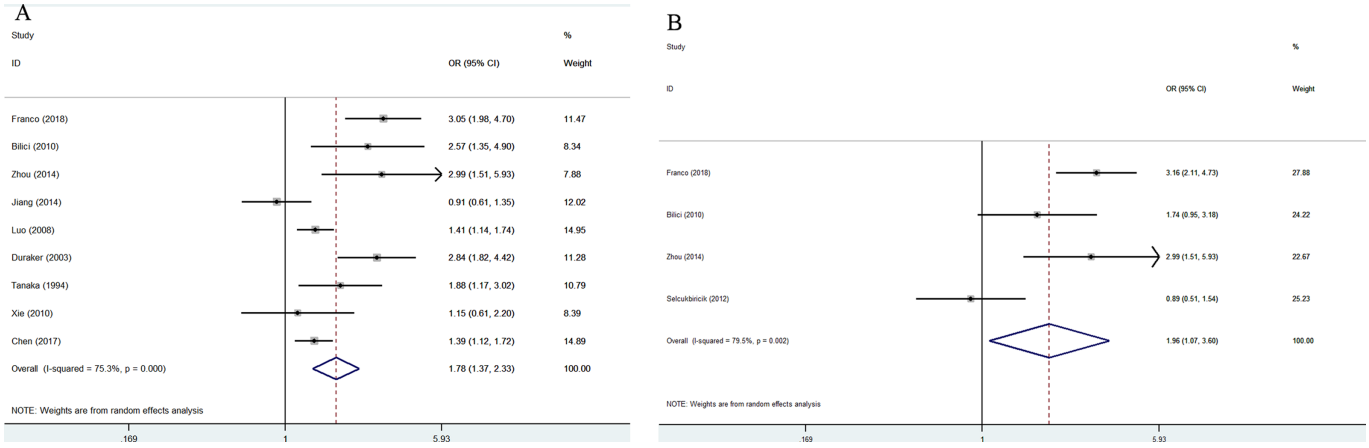

Figure 2 Forest plots evaluating the relationship between perineural invasion and clinicopathological factors for gastric cancer patients. (A) Differentiation type (undifferentiated vs differentiated type); (B) Lauren classification (diffuse type vs intestinal type).

Prognostic significance of PNI in GC patients

A total of 11 studies involving 5906 GC patients evaluated the impact of positive PNI on survival outcome, and nine of these studies reported a significant result. The meta-analysis of 11 studies revealed a pooled HR of 1.69 (95% CI 1.38 to 2.06, p<0.001), with a significant heterogeneity (I^2=71.0%, p<0.001) (figure 5). This result indicated that positive PNI was a predictive factor for survival outcome in GC patients.

Table 3 showed the results of subgroup analysis for OS in these patients. We found that geographic area, publication year, sample size, tumour stage, resection type, the prevalence of PNI and the quality of included studies had no significant impact on the pooled results for OS, which further supported a strong consistent association between PNI and poor survival outcome in GC patients. Besides, the sensitivity analysis suggested that the pooled results remain significant when those studies with the largest weight were in turn removed.

Three studies provided available data on the number of recurrence cases. The pooled result showed that the patients with positive PNI had a higher risk of recurrence than those with PNI negative despite a significant heterogeneity (OR: 3.60, 95% CI 1.67 to 7.74, p<0.001; I^2=76.5%).

Publication bias

Based on the visual assessment of funnel plots, the asymmetric distribution of main studies was not observed in the pooled analysis of HR for OS (figure 6).

DISCUSSION

In the present study, we performed a systematic review and meta-analysis to determine the relationship between PNI and other clinicopathological characteristics and evaluate the prognostic significance of PNI for GC patients. The results demonstrated that PNI was significantly associated with a series of unfavourable clinicopathological factors including undifferentiated histology type, diffuse type, tumour size, lymphatic invasion, vascular invasion, lymph node metastasis and tumour stage. The patients with positive PNI were more likely to have more aggressive oncological features than those without PNI.

Previous studies reported a strong association between lymphovascular invasion and PNI.[7 15 26] The abundant lymphatic network around the nerves and direct infiltration of the vasa nervorum by cancer cells may partly explain why PNI and lymphovascular invasion were more likely to be simultaneously detected in resected specimens.[15] Hwang *et al* retrospectively

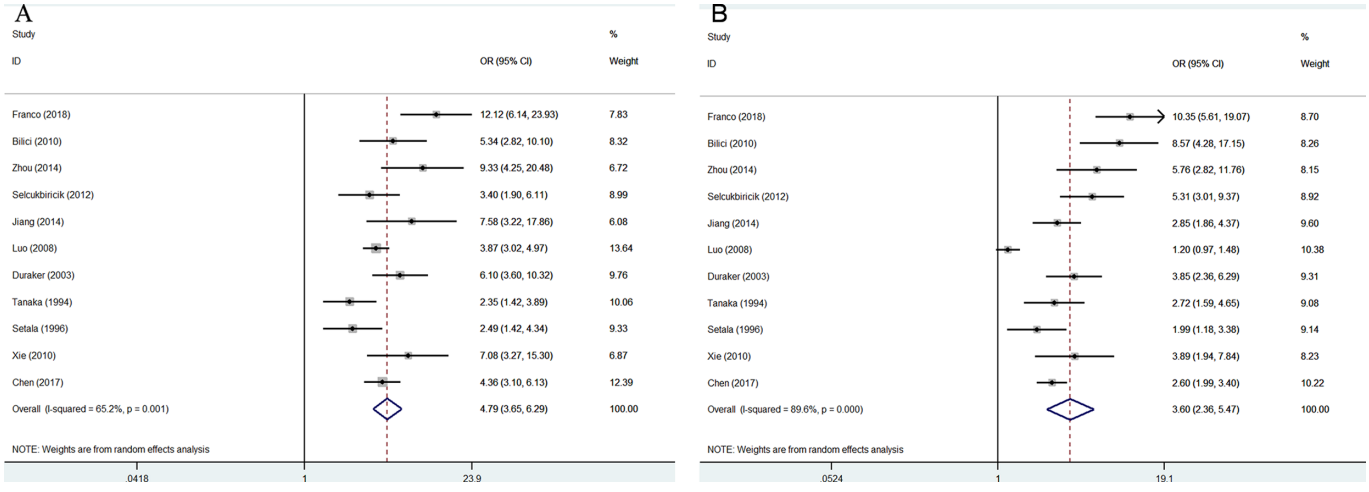

Figure 3 Forest plots evaluating the relationship between perineural invasion and clinicopathological factors for gastric cancer patients. (A) T stage (T3-T4 vs T1-T2); (B) lymph node metastasis (yes vs no).

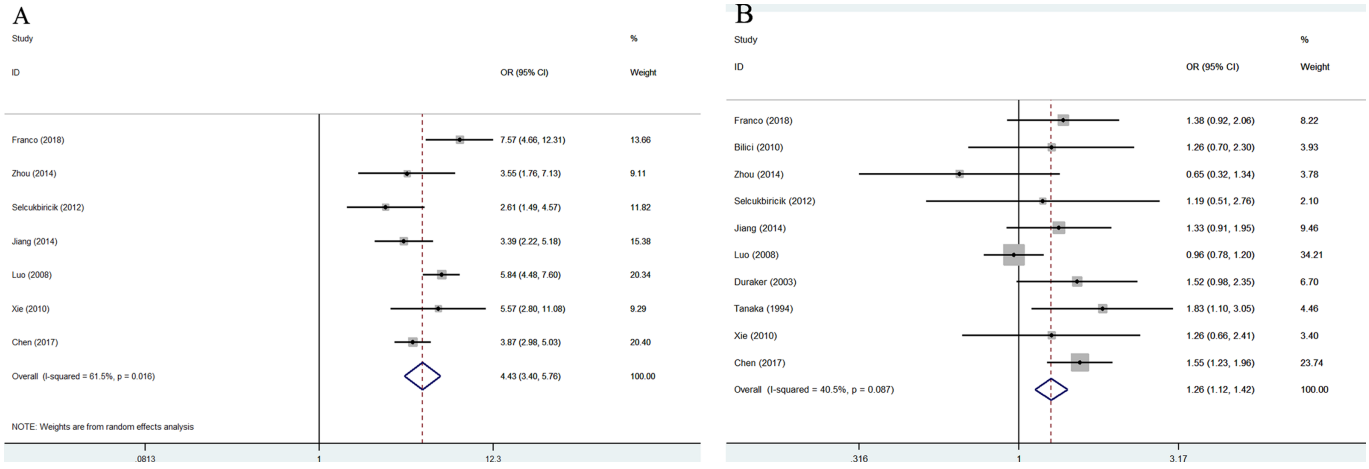

Figure 4 Forest plots evaluating the relationship between perineural invasion and clinicopathological factors for gastric cancer patients. (A) Pathological tumour, node, metastases stage (stage III–IV vs stage I–II); (B) tumour location (upper and middle 1/3 vs low 1/3).

reviewed histopathological information of 206 consecutive stage II–III patients who underwent curative resection for GC, the results demonstrated that the proportion of concomitant existence of PNI and lymphovascular invasion accounted for 42.7% of all patients.[26] In terms of survival outcome, the median disease-free survival (DFS) and OS time for the patients with both positive PNI and positive lymphovascular invasion were the poorest among all GC patients. These data suggested a high propensity between PNI and lymphovascular invasion. In addition, positive PNI seem to be more frequent in the upper and middle 1/3 tumours. The distribution of large autonomic nerves was relatively broader in the upper and middle 1/3 tumours, so cancer cells could easily spread through the gap between the nerves and tissue.[7] Also, tumour size was significantly larger, tumour invasion was significantly deeper and tumour stage was more advanced in patients with positive PNI than those with negative PNI. These findings seem to further support a fact that the patients with positive PNI had a poorer survival outcome than those without PNI. According to the study of Aurello *et al*,[8] the 5-year DFS rate of positive PNI and negative PNI patients was 22% and 69%, respectively. Similarly, Bilici *et al* reported that the median OS of positive PNI patients was 28.1 months, which was significantly worse than that of negative PNI patients (64.9 months, p=0.001).[15]

Although PNI was significantly associated with poorer survival outcome in GC patients, some studies reported that it could not provide more additional prognostic information than other well-known clinicopathological factors.[16 20] Zhou *et al* found that PNI was not an independent prognostic factor after classical and well-established clinicopathological variables were adjusted in Cox multivariate regression analysis.[9] Similar results were reported by De Franco *et al* in the multivariate analysis. However, further subgroup analysis based on the Lauren histology type indicated that PNI was a valuable prognostic factor for GC patients with intestinal type (HR: 1.99, 95% CI 1.24 to 3.19, p=0.005), but not for those with diffuse type.[20] In view of current conflicting results, we extracted adjusted HR and its corresponding 95% CIs for OS and performed a pooled analysis to determine the prognostic significance of PNI. The results indicated that PNI was a predictive factor for survival outcome in GC patients. It has been proved that the depth of tumour invasion (T category) and the number of positive lymph nodes (N category) were two important prognostic factors for GC patients.[3 4] The TNM classification system based on these prognostic parameters has been widely used to predict survival outcome of GC patients and guide postoperative treatment strategy. Recently, Jiang *et al* incorporated PNI into the 7th edition of TNM staging system for GC, and the results indicated that the novel staging system

Table 2 Meta-analysis results for the relationship between perineural invasion and clinicopathological factors in gastric cancer patients

Factors	Studies	Patients	Pooled OR (95% CI)	P value	Heterogeneity I^2 (%)	Heterogeneity p value
Age (≥60 years)	5	2495	0.96 (0.81 to 1.14)	0.637	19.1	0.293
Gender (male)	10	5906	1.02 (0.91 to 1.15)	0.732	0	0.774
Tumour location (upper and middle 1/3)	10	5906	1.26 (1.12 to 1.42)	<0.001	40.5	0.087
Differentiation (poorly/undifferentiated)	9	5619	1.78 (1.37 to 2.33)	<0.001	75.3	<0.001
Lauren classification (Diffuse type)	4	1140	1.96 (1.07 to 3.60)	0.029	79.5	0.002
Lymphatic invasion (yes)	4	2777	7.00 (3.76 to 13.03)	<0.001	83.6	<0.001
Vessel invasion (yes)	4	1330	5.79 (1.59 to 21.13)	0.008	95.8	<0.001
Invasion depth (T3-T4)	11	6162	4.79 (3.65 to 6.28)	<0.001	65.0	<0.001
Lymph node metastasis (yes)	11	6162	3.60 (2.37 to 5.47)	<0.001	89.6	<0.001
Tumour size (≥3 cm)	3	752	2.92 (1.98 to 4.28)	<0.001	0	0.605
Tumour size (≥5 cm)	3	3951	1.83 (1.59 to 2.11)	<0.001	0	0.634
TNM stage (III–IV)	7	5031	4.43 (3.40 to 5.75)	<0.001	61.5	0.016
Borrmann type (III–IV)	4	2840	1.33 (0.86 to 2.06)	0.207	78.9	0.003

TNM, tumour, node, metastases.

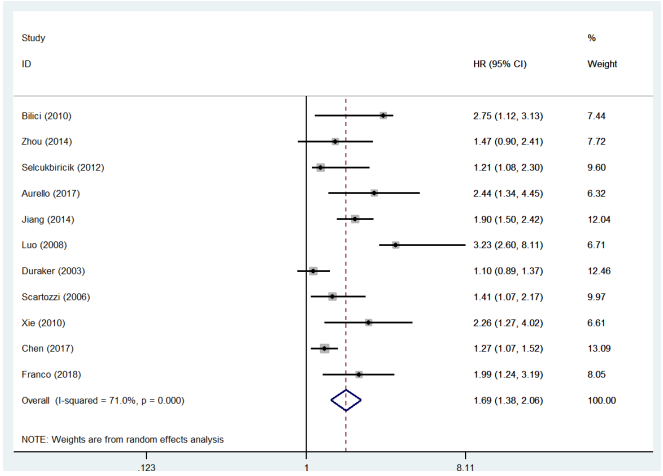

Figure 5 Forest plots evaluating the impact of perineural invasion on overall survival in gastric cancer patients.

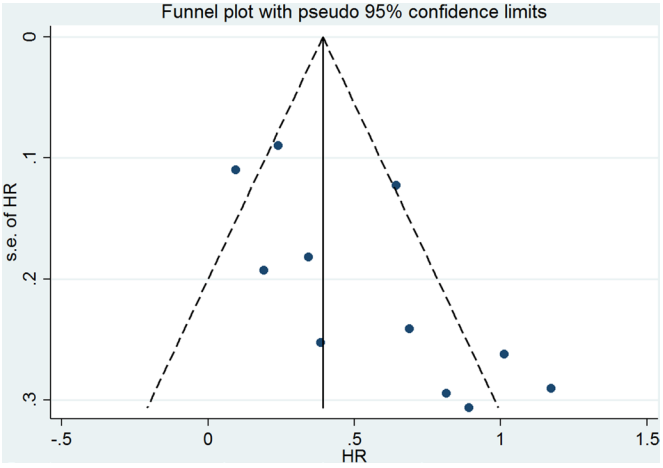

Figure 6 Funnel plot for the association between perineural invasion and survival outcome in gastric cancer patients.

could provide a better prognostic stratification for stage III patients than single TNM staging.[7] Given its prognostic significance in GC patients, a thorough follow-up plan and intensive adjuvant chemotherapy should be considered for those patients with positive PNI.

The exact pathological mechanism of PNI in a different type of cancers remains unclear. Some researchers attributed PNI pathogenesis to the close anatomical relationship between the tumour and neural plexus.[16] However, the incidence of PNI was not high in rectal cancer patients despite the proximity to the presacral autonomic nerve plexus.[27 28] Xia *et al* found that GC cells not only could promote the proliferation of neural progenitor cells but also enhance neurite elongation and branching of postmitotic neural cells in cancer-neural cell coculture condition in vitro.[29] A large amount of cell adhesion molecule and chemokines secreted from tumour cells may recruit and facilitate migration and invasion of cancer cells toward surrounding neuronal axons and neural tissue.[30] Lv *et al* reported that the expression level of CX3CL1 chemokines and its receptor CX3CR1 was significantly higher in GC patients with positive PNI than in those with negative PNI, showing a significant association between CX3CL1/CX3CR1 expression and PNI in gastric carcinoma patients.[31]

PNI was more frequent in pancreatic cancer and biliary tract carcinoma than other malignant tumours (approximately 80%–88% in pancreatic cancer and biliary tract carcinoma).[32 33] According to the previous reports, the incidence of PNI in surgically resected specimens of GC patients varied from 6.9% to 75.6%.[8 15 20 22] The significant variation among different studies for the incidence of PNI may be partially attributed to detection

Table 3 Subgroup analysis for the impact of PNI on survival outcome of gastric cancer patients

			Pooled results		Heterogeneity	
Subgroup analysis	**Studies**	**Patients**	**Effect size (95% CI)**	**P value**	**I^2 (%)**	**P value**
Region			HR			
Eastern	5	4289	1.61 (1.20 to 2.18)	<0.001	71.30	0.004
Western	6	2176	1.81 (1.33 to 2.47)	0.002	74.50	0.003
TNM stage			HR			
I–III	5	1306	1.81 (1.37 to 2.38)	<0.001	53.90	0.07
I–IV	6	5159	1.59 (1.22 to 2.08)	<0.001	73.20	0.002
Sample size			HR			
≥350	6	5499	1.59 (1.23 to 2.04)	<0.001	77.80	<0.001
<350	5	966	1.86 (1.33 to 2.61)	<0.001	55.70	0.06
Publication year			HR			
Before 2011	5	3141	1.89 (1.23 to 2.92)	0.004	82.00	<0.001
After 2011	6	3324	1.59 (1.27 to 1.98)	<0.001	59.20	0.032
Resection type			HR			
R0	7	2223	1.76 (1.42 to 2.19)	<0.001	45.00	0.091
R0 and R+	4	4242	1.57 (1.12 to 2.21)	0.009	80.40	0.002
PNI positive rate (%)			HR			
≥40	6	1320	1.67 (1.20 to 2.32)	<0.001	72.10	0.003
<40	5	5145	1.74 (1.32 to 2.30)	0.003	73.90	0.004
Study quality			HR			
≥7	8	5085	1.97 (1.53 to 2.53)	<0.001	68.80	0.002
<7	3	1380	1.18 (1.00 to 1.40)	0.048	0	0.501

PNI, perineural invasion; TNM, tumour, node, metastases.

technology and the experience of pathologists in addition to patient selection. In general, most of research institutions evaluated the presence of PNI using H&E staining. However, nerve fibres in histological sections were not always clearly identified by pathologists due to the invasion and severe damage by tumour cells.[9] Previous studies have demonstrated that the detection rate of PNI could be significantly improved by immunohistochemical staining using S-100 or laminin.[9 34] Zhou *et al* reported that the positive rate of PNI was 56.9% (91/160) in GC patients using S-100 stain, but only 38.1% of patients (61/160) were identified as positive PNI when the tissue sections were evaluated by HE staining.[9] Despite improved visual detection of nerves, the identification of PNI was still not an easy task for pathologists. It is very difficult to distinguish tumour cells from inflammatory cells even in S-100 stained slides, especially when these cells were small and distributed in a diffuse way. Recently, Zhou *et al* used double immunohistochemical staining to label nerve fibres and epithelial-derived tumour cells for evaluating the PNI status in GC patients. The results indicated that the positive rate of PNI was 65.0% (104/160) in the double staining group and 56.9% (91/160) in single S-100 staining group, respectively.[9] Compared with double staining, 19 false-negative and six false-positive cases were observed in single S-100 stained slides, especially for diffuse histology type.[9] In the future, detection methods for PNI need to be further improved for better diagnostic accuracy and efficiency.

In addition, the prognostic value of PNI may be partly depended on how it is evaluated by pathologists. Most of the current studies evaluated the PNI status in submucosa or muscularis propria (mural perineural invasion, mPNI), but few studies focus their attention on extramural perineural invasion (ePNI, invasion of the nerve plexus beyond muscularis propria). Recently, España-Ferrufino *et al* subdivided 73 pT3-T4a stage GC patients with positive PNI into mPNI and ePNI group, and they found that the patients with ePNI had a poorer disease-specific survival than those with mPNI.[35] However, these findings need to be confirmed by more studies.

The present study had some limitations. First, all included studies were retrospective in nature and selection bias should be considered. Second, different detection methods and diagnostic criteria for PNI as well as heterogeneous patient populations in included studies could result in a significant variation for the positive rate of PNI, which could further affect the prognostic evaluation.

In summary, the positive rate of PNI was high in surgically resected specimens of GC patients. Our results demonstrated that PNI was significantly associated with a series of unfavourable clinicopathological factors including undifferentiated histology type, diffuse type, larger tumour size, lymphovascular invasion, lymph node metastasis and advanced tumour stage. PNI was a predictive factor for survival outcome in GC patients, and it may provide additional prognostic information for these patients.

Take home messages

- The positive rate of perineural invasion (PNI) was very high in surgically resected specimens of gastric cancer (GC) patients.
- The patients with positive PNI were more likely to have more aggressive oncological features than those without PNI.
- PNI was a predictive factor for survival outcome in GC patients, and it may provide additional prognostic information for these patients.
- In view of its prognostic significance in gastric cancer patients, a thorough follow-up plan and intensive adjuvant chemotherapy should be considered for those patients with positive PNI.

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