Original Article

Perineural Invasion is a Prognostic Factor in Patients with Colorectal Cancer

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Key Words
Perineural invasion;
Colorectal cancer;
Prognostic factor

Purpose. Identification of prognostic factors in colorectal cancer has taken center stage in recent research, and to improve the survival rate of colorectal cancer in Taiwan and elsewhere, additional studies are required to investigate the prognostic value of perineural invasion in colorectal cancer patients.

Methods. From a prospective database in a single medical center, patients with colorectal cancer who had undergone surgical resection between January 2010 and December 2014 were identified. For all subjects, patient characteristics, cancer-specific survival, and overall survival were evaluated. Data from complete pathological reports, including perineural invasion (PNI), round cell infiltration, and infiltrative invasive pattern of cancer tissue, were analyzed.

Results. In total, 2582 patients met the eligibility criteria, of which 236 were excluded due to missing clinical or pathological data, and 72 were lost to follow-up. Thus, 2274 patients were included in the analysis. Patients were predominantly male (60.3%) and ≥ 65 years of age (55.1%). Perineural invasion was significantly associated with poor cancer-specific survival ($p < 0.001$) and overall survival ($p < 0.001$). There were more patients with perineural invasion in the groups that underwent emergent surgery and with advanced TNM staging ($p < 0.001$). Pathologic analysis showed that PNI was associated with lymphovascular invasion, isolated cancer nodule at mesentery, and inflammatory change around the cancer ($p < 0.001$).

Conclusion. In CRC patients, PNI was significantly associated with poor cancer-specific survival and overall survival rate. PNI was demonstrated to be an independent prognostic factor in stage II CRC patients. Further molecular studies and treatment strategies are warranted.

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The incidence of cancer in Taiwan has steadily been rising since 1982, and in recent years, colorectal cancer (CRC) has been ranked the most common cancer in males and the second most common cancer in females.[1] In 2016, CRC was the third leading cause of death from cancer, with 5,722 deaths associated with this disease.[1] The most important predictors of prognosis in patients with CRC are the depth of tumor invasion (T), lymph node (N) and distant metastasis (M), but the search continues for prognostic factors in addition to pathological TNM stage for CRC.[2,3] Perineural invasion (PNI) is a key pathologic predictor for several cancers, including that of the pancreas, prostate, biliary tract, and stomach.[2]

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However, progress in understanding whether PNI is an optimal predictor of CRC has been considerably slower than that in other solid tumors. Therefore, the aim of the present study was to evaluate PNI as a predictor of CRC and examine its association with clinicopathological parameters in patients with CRC.

Materials and Methods

Previously, patient evaluation and pathological data collection were prospectively performed at Taipei Veterans General Hospital (Taipei, Taiwan). In the current study, the prognostic value of PNI in a series of patients who underwent surgery for CRC in our institution between January 2010 and December 2014 were retrospectively investigated.

Patient data collected included age, gender, tumor location, type of surgery, and pathological TNM staging. Based on the pathologic report, the 7th American Joint Committee on Cancer (AJCC) staging manual was used for cancer staging and included 1) blood vascular invasion, 2) lymphatic vessel invasion (LVI), 3) PNI, 4) isolated cancer nodule at mesentery, 5) inflammatory change around cancer, 6) infiltrative invasive pattern of cancer tissue, and 7) signet ring cell component. As recommended by the College of American Pathologists, the pathologic status complied with protocol for the examination of specimens from patients with adenocarcinoma of the colon and rectum.

For histopathologic analysis, the PNI status of each specimen was examined by specialty pathologists in our institution. PNI was defined according to the presence of tumor cell invasion to the perineurium, and which including two types: intramural and extramural. Intramural was mean tumor invasion to nerves in Meissner plexus and Auerbach's plexus. The extramural is mean the tumor cell invasion to nerve in subserosal soft tissue. Hematoxylin and eosin (H&E) staining was used to identify PNI positivity; and no other special stains were used.

Statistical analysis was carried out using the SPSS/PC v.20 for Windows statistical package. The association of clinicopathological parameters with PNI was examined using the Chi-square test ($\chi 2$ test). Overall survival rate and cancer-specific survival rate was analyzed by the Kaplan-Meier method. Multivariate analysis of overall survival rate was used by Cox regression.

Results

Patients

In total, 2582 patients met the eligibility criteria, of which 236 were excluded due to missing clinical or pathological data, and 72 were lost to follow-up. Thus, 2274 patients were included in the analysis. The clinicopathological characteristics are summarized in Table 1. Patients were predominantly male (60.3%) and ≥ 65 years of age (55.1%). Median age was 67 years (range, 22-99). Of the total, 112 patients (4.9%) received emergent surgery. Only 25.4% of the tumors were located in the right colon. Of the various stages, 293 patients (12.9%) were pT2, 1311 (57.7%) were pT3, and 261 (11.4%) were pT4. PNI was observed in 268 patients (11.8%), particularly in patients who underwent emergent surgery and in those with elder age, advanced TNM stage, lymphovascular invasion, and isolated cancer nodule at mesentery (Table 2).

Surgical outcomes

Patients with PNI exhibited poor overall survival rate ($p < 0.001$) and cancer-specific survival rate ($p < 0.001$). Stage II CRC patients also exhibited poor overall survival rate ($p < 0.001$) and cancer-specific survival rate ($p = 0.009$), but this was not the case in patients with stages I, III, and IV CRC (Fig. 1). The same result is also appeared in groups of tumors in colon and rectum respectively (Fig. 2). For stage II patients with PNI, chemotherapy had trend of benefit (Fig. 3). In the univariate analysis of prognostic factors for patients of all stages, elder age, emergent surgery, advanced TNM stage, lymphovascular invasion, PNI, and isolated cancer nodule at mesentery, showed poor overall survival rates and cancer-specific survival rates (Fig. 4). In stage II patients, age, emergent surgery, and PNI had poor overall and cancer-specific

Table 1. Association between clinical parameters and perineurai invasion (PNI)

Parameters	Patients (%)	Patients with PNI (%)	*p*-value
Age (range: 22~99, average: 66.87 years)			0.072
< 65	1020 (44.9%)	134 (13.1%)	
≥ 65	1254 (55.1%)	134 (10.7%)	
Gender			0.944
Male	1371 (60.3%)	161 (11.7%)	
Female	903 (39.7%)	107 (11.8%)	
Surgery			< 0.001
Emergency	112 (4.9%)	26 (23.2%)	
Elective	2162 (95.1%)	242 (11.2%)	
Location			0.121
Right	578 (25.4%)	71 (12.1%)	
Left	804 (35.4%)	107 (13.3%)	
Rectum	847 (37.2%)	85 (10.0%)	
Multiple	45 (2.0%)	5 (10.9%)	
TNM stage			< 0.001
Stage 0	124 (5.5%)	0 (0%)	
Stage I	298 (13.1%)	3 (1.0%)	
Stage II	875 (38.5%)	58 (6.6%)	
Stage III	606 (26.6%)	109 (18.0%)	
Stage IV	371 (16.3%)	98 (26.1%)	
T stage			< 0.001
T0	124 (5.5%)	0 (0%)	
T1	285 (12.5%)	1 (0.4%)	
T2	293 (12.9%)	12 (4.1%)	
T3	1311 (57.7%)	183 (14.0%)	
T4	261 (11.4%)	72 (27.3%)	
N stage			< 0.001
N0	1374 (60.4%)	73 (5.3%)	
N1	530 (23.3%)	92 (17.4%)	
N2	370 (16.3%)	103 (27.8%)	
PNI			
PNI (+)	268 (11.8%)	N/A	N/A
PNI (-)	2006 (88.2%)		

Table 2. Association between pathological parameters and perineural invasion (PNI)

Parameters	Patients	PNI (%)	*p*-value
Vascular invasion			< 0.001
Yes (+)	346 (15.3%)	122 (35.3%)	
No (-)	1928 (84.7%)	146 (7.5%)	
Lymphatic invasion			< 0.001
Yes (+)	577 (25.4%)	185 (32.0%)	
No (-)	1697 (74.6%)	83 (4.9%)	
Isolated cancer nodule at mesentery			< 0.001
Yes (+)	82 (3.6%)	44 (53.7%)	
No (-)	2192 (96.4%)	224 (10.2%)	
Inflammatory change around cancer			< 0.001
Yes (+)	456 (20.0%)	80 (17.5%)	
No (-)	1818 (80.0%)	188 (10.3%)	
Infiltrative invasion pattern of cancer tissue			0.064
Yes (+)	1337 (58.8%)	172 (12.9%)	
No (-)	937 (41.2%)	96 (10.2%)	

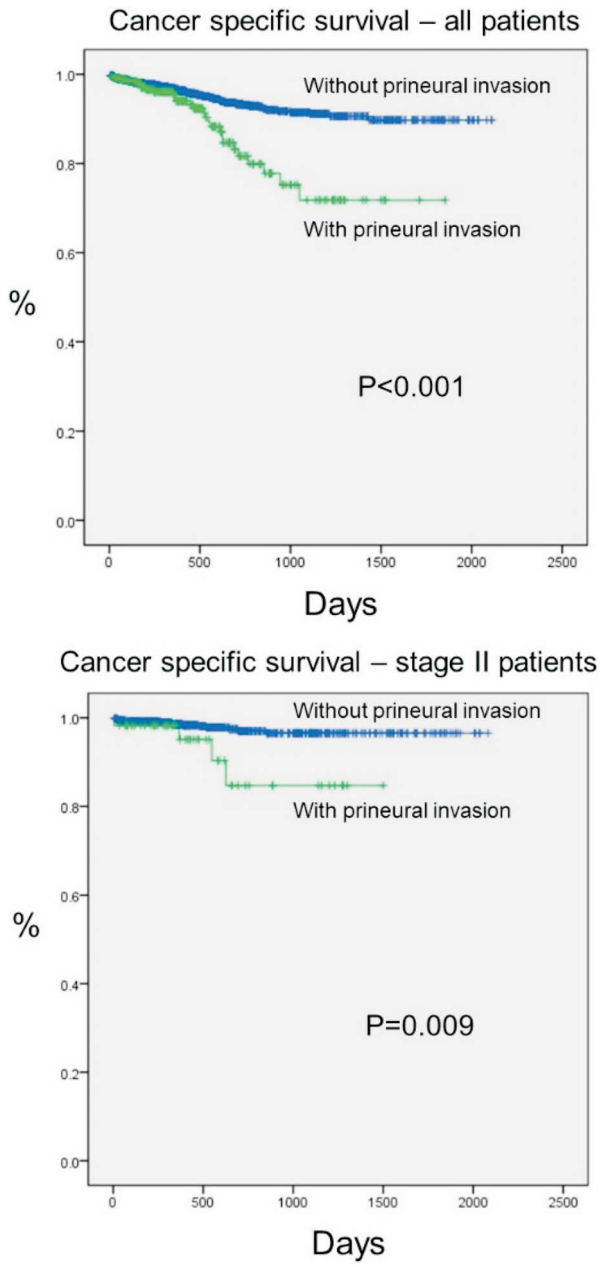

Fig. 1. Cancer-specific survival rates in all-stage patients and stage II patients.

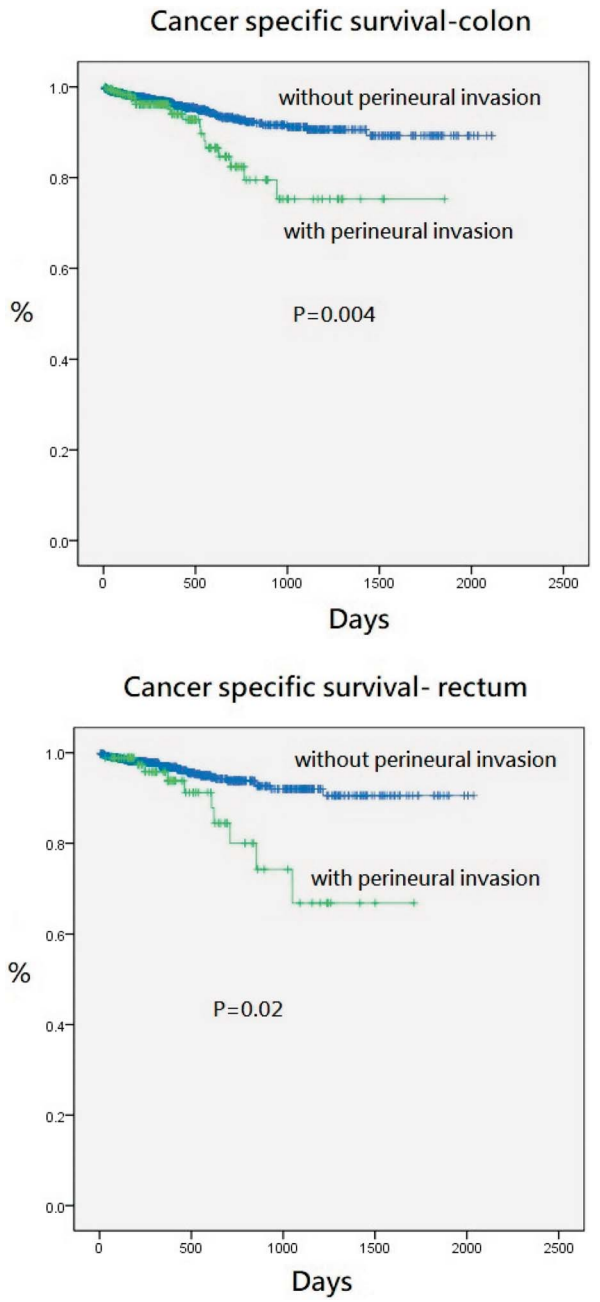

Fig. 2. Cancer-specific survival rates in all-stage patients for tumor s in colon and rectum.

survival rates. In multivariate analysis of stage II patients, age, emergent surgery, and PNI were the risk factors for poor overall survival rates. PNI also may be an independent prognostic factor (HR, 3.65; 95% CI, 1.363-9.779; $p = 0.010$) (Table 3). The relationship between the routes of regional metastasis and the depth of tumor invasion are summarized in Table 4. The rations of PNI, LVI, and lymph node metastasis (LNM), are more expression in patients with advanced T stage ($p < 0.001$). Moreover, the ration of PNI is less than LVI and LNM.

Discussion

CRC remains the third leading cause of cancer-

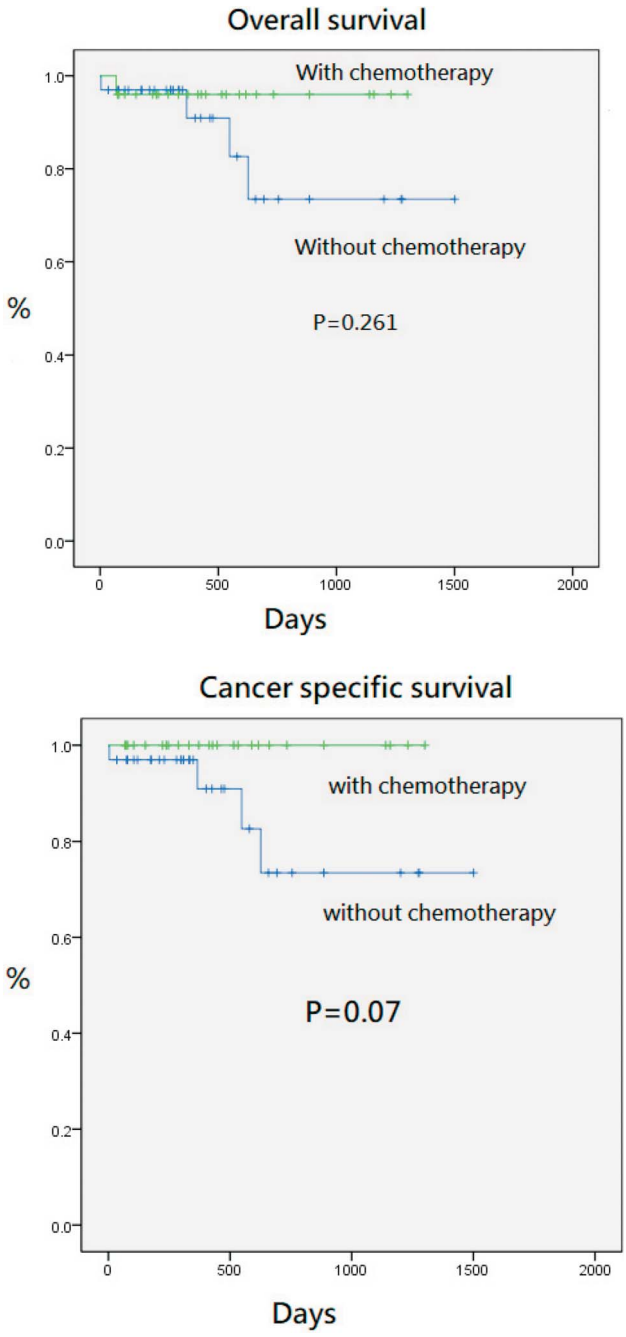

Fig. 3. The outcome of chemotherapy for survival rates in stage II patients with perineural invasion (PNI).

related deaths in Taiwan.[1] The identification and validation of novel prognostic or predictive factors for CRC may positively impact the treatment outcome. Several histologic factors revealed a poor outcome according to the National Comprehensive Cancer Network guidelines, including i) tumor invasion to serosal layer (T4), ii) closed/indeterminate/or positive margins, iii) poorly differentiated histology, iv) less than 12 lymph nodes examined in the resected specimen, and v) the presence of perineural and lymphatic/vascular invasion.[4] Though the prognostic significance of PNI in head and neck, prostate, and breast cancer is known,[5] literature surrounding PNI in colorectal cancer is scant. The reported incidence of PNI in patients with CRC ranges between 8 and 20%,[2] which is in concordance with our study, wherein PNI was found in 11.8% of patients in the present cohort. A study on 507 patients with stage I-II CRC under treatment identified PNI in 57 patients (11.2%).[6] Compared to CRC patients without PNI, 5-year disease-free survival was significantly poorer in CRC patients with PNI (73.5% vs. 88.6%; $p = 0.02$).[6] Further, a recent meta-analysis concluded that disease-free survival rate in stage II patients with PNI was similar to that found in patients with stage III disease (HR, 1.67; 95% CI, 0.53-5.25; $p = 0.038$).[6,7] Similar to our observation, a review of 38 studies suggested that PNI may be an independent prognostic factor in stage II CRC patients[2] and was consistently associated with poor differentiation, T stage, incidence of metastasis at time of diagnosis, and lymphovascular invasion.[2] In our study, PNI was associated with T stage, N stage, and emergent surgery (Table 1). Pathological patterns revealed that PNI was associated with lymphovascular invasion, isolated cancer nodule at mesentery, and inflammatory changes around the cancer (Table 2). Thus, even though PNI is not routinely used in prognosis of CRC in clinical practice, its association with poor outcomes in CRC patients must be considered for appropriate management of the disease.

In NCCN guideline, chemotherapy had benefit in high risk stage II CRC patients. Our study revealed chemotherapy in stage II CRC patient with PNI had some trend of benefit only. The discrepancy maybe due to less study sample. (Only 24 patients received chemotherapy in stage II patients with PNI).

Rasheed et al. try to find the relationship between lymph node metastasis and depth of tumor invasion (T stage).[12] Our study revealed the PNI, LVI, and LNM are more expression in advanced T stage. However, the ratio of perineural invasion is less than LVI and

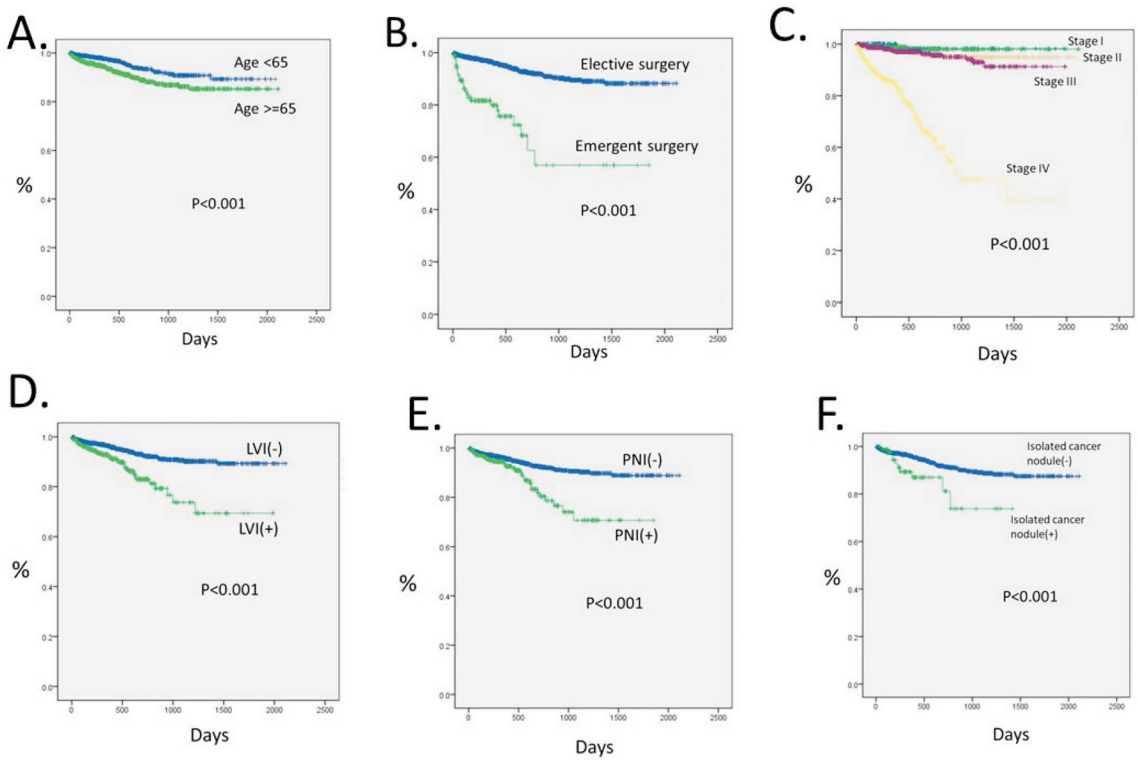

Fig. 4. Univariate analysis of prognostic factors for patients with colorectal cancer. Overall survival rate of: A. Age, B. Emergent surgery, C. Stage, D. Lymphovascular invasion, E. Perineural invasion, F. Isolated cancer nodule at mesentery.

Table 3. Multivariate analysis of overall survival rate in stage II patients

	p-value	HR	95.0% CI	
			Lower limit	Upper limit
Age ≥ 65 y	0.002	6.422	1.927	21.400
Emergent surgery	0.039	3.608	1.066	12.216
PNI	0.010	3.650	1.363	9.779

Table 4. Association between perineural invasion (PNI), lymphatic invasion (LVI), and positive of lymphadenopathy (N stage) regard to tumor invasion depth (T stage)

T stage	Patients (%)	PNI (%)	LVI (%)	N1 + N2 (%)
T1	285 (12.5%)	1 (0.4%)	22 (7.7%)	26 (9.1%)
T2	293 (12.9%)	12 (4.1%)	42 (14.3%)	48 (16.4%)
T3	1311 (57.7%)	183 (14.0%)	406 (31.0%)	648 (49.4%)
T4	261 (11.4%)	72 (27.3%)	136 (52.2%)	178 (68.2%)

lymphadenopathy. I hypothesize the perineural invasion is the one of varied method of microscopic tumor spread and the LMN was the macroscopic result. Besides, the ratio of LVI is more than PNI. It maybe caused by that fluidity of lymphovascular duct is better than neural structure.

Batsakis et al., first defined PNI and described it as tumor cell invasion in, around, and through nerves.[2,8] Later, Fujita et al. defined perineural invasion in cancer cells within the perineurium in the Auerbach's (myenteric) plexus adjacent to the tumor.[9] Liebig et al. defined the presence of tumor cells in the nerve sheath or tumor foci outside the nerve with involvement of more than 33% of the nerve's circumference in the perineural space.[10] Ueno et al. defined intramural PNI as cancer spread along Auerbach's (myenteric) plexus and extramural PNI as tumor cells spreading along nerves external to the muscularis propria.[2,11] Pragmatically, PNI must be found around the perineural space, and not just tumor cells in the layer of Auerbach's (myenteric) plexus. However, the incidence of PNI was not proportional to tumor size and depth of tumor invasion (T stage).

In the current study, we defined PNI as tumor cells

within or in contact with the perineurium, and included two types — intramural and extramural. The former indicated tumor cell invasion to nerves in Meissner plexus and Auerbach's (myenteric) plexus whereas the latter indicated tumor cell invasion to nerve in subserosal soft tissue. Since Auerbach's (myenteric) plexus was more easily identifiable, PNI was more evident in this structure. Nevertheless, the AJCC Cancer Staging Manual in its definition of PNI does not specify either the bowel layer or where PNI should be recorded.

Our study was not without limitations. First, this was a retrospective study from a single medical center with a small sample size, which subjected our results to selection bias. Second, while most other studies showed disease-free survival rates, retrieval of data on recurrence from follow-up studies was not performed in this study due to difficulties in studying and collecting data, including evidence from computed tomography (CT) or magnetic resonance imaging (MRI), elevated tumor markers, and pathological confirmation.

Conclusion

In CRC patients, PNI was significantly associated with poor cancer-specific survival and overall survival rate. Multivariate analysis of our study demonstrated PNI to be an independent prognostic factor in stage II CRC patients. While the results of the present study raise the possibility of improved treatment modalities for CRC patients in Taiwan, there is an urgent need for RCTs to confirm our findings.

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原　著

腫瘤細胞的神經侵犯是大腸直腸癌病患術後的預後因子

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目前許多的研究都在探討大腸直腸癌的各項預後因子。本研究旨在評估神經侵犯對於大腸直腸癌患者的臨床預後價值。本篇回顧性研究收錄了 2010 年至 2014 年於台北榮民總醫院的大腸直腸癌病患。我們分析了病患的基本資料、整體存活率、及腫瘤特定存活率。另外還收錄了完整的病理資料，包含是否有神經侵犯，圓細胞浸潤，腫瘤組織是否有整體的浸潤侵犯等。我們總共收錄了 2274 位病患。其中包含 1371 位男性及 903 位女性。結果顯示，不論是腫瘤特定存活率及整體存活率，神經侵犯都可以成為大腸直腸癌患者的良好預後因子。尤其是在第二期大腸直腸癌患者。另外，神經侵犯明顯和急診手術以、晚期癌症、圓細胞浸潤、以及整體腫瘤浸潤侵犯等惡性因子有相關性。

關鍵詞　神經侵犯、結腸直腸癌、預後因子。