Clinical implications of perineural invasion in patients with colorectal cancer

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Abstract

Perineural invasion (PNI) is a prominent characteristic of multiple solid tumors and indicates poor prognosis. Previous data concerning the impact of PNI on prognosis of patients with colorectal cancer (CRC) are conflicting, and little is known about risk factors of PNI. The aim of our study was to reveal the clinical implication of PNI on survival outcome and identify risk factors for the poor prognosis in patients with CRC.

We retrospectively reviewed 627 patients who were diagnosed with CRC and underwent curative surgical resection. The differences in several clinicopathologic characteristics were compared between PNI positive and PNI negative groups. Multivariate logistic regression analysis was performed to identify predictors of CRC with PNI. Five-year overall survival (OS) and disease-free survival (DFS) were calculated using the Kaplan–Meier method, and the difference in survival rate was assessed by the log-rank test. The variables that had prognostic potential, as indicated by univariate analyses, were subjected to multivariate analyses with the Cox proportional hazards regression model.

PNIIs were identified in 79 patients (12.6%). Age, T classification, N classification, M classification, UICC classification, and lymphovascular invasion were significantly associated with PNI. Multivariate logistic regression analysis demonstrated that only lymphovascular invasion was a predictor of PNI. Pathologic evidence of PNI was not associated with survival outcome (the 5-year OS \( P = .560 \) and DFS \( P = .083 \)). Cox proportional hazards regression model revealed that age and N2/3 classification were independent prognostic factors for poorer OS and DFS. M1 stage (95% confidence interval [CI] = 0.228–0.585, \( P = .000 \)), III/IV stage (95% CI = 0.335–0.920, \( P = .022 \)), and number of sampled lymph nodes (95% CI = 0.951–0.987, \( P = .001 \)) were independently prognostic for poorer OS, while history of other malignancy (95% CI = 1.133–2.813, \( P = .012 \)) was identified as an independent prognostic factor for poorer DFS.

Our study indicates that PNI is not an independent poor prognostic factor in patients with CRC and those patients with PNI may not benefit from postoperative adjuvant chemotherapy.

Abbreviations: BMI = body mass index, CRC = colorectal cancer, DFS = disease-free survival, OS = overall survival, PNI = perineural invasion, UICC = International Union Against Cancer.

Keywords: colorectal cancer, perineural invasion, prognosis

1. Introduction

Perineural invasion (PNI) is a prominent characteristic of multiple solid tumors (including pancreatic ductal adenocarcinoma, cutaneous squamous cell carcinoma of the head and neck, and prostate cancer) and indicates poor prognosis.[1–3] In the majority of cases, PNI is not associated with neurologic symptoms, however, local or referred pain is a warning sign of malignancy. The invasion of the surrounding nerves by cancer cells not only provides route for metastasis but also contributes to neural remodeling and changes in the neuronal milieu that can profoundly influence the microenvironment of tumor.[4]

The vast majority of colorectal cancer (CRC) cases are readily curable with complete surgical resection with and without adjuvant chemotherapy unless high-risk features are present. Recently, as a new biologic feature, PNI has attracted more and more attention in CRC. Previous data concerning the impact of PNI on prognosis of patients with CRC are conflicting, and little is known about risk factors of PNI.[5,6] To overcome limitations of previous studies on the relationship between PNI and CRC, which arise from small cohorts of patients and short follow-up periods, we collected detailed histopathologic and clinical data from our prospective follow-up database. This study was aimed to reveal the clinical implication of PNI on outcome and identify risk factors for the poor prognosis in patients with CRC.

2. Materials and methods

2.1. Patients

This study retrospectively reviewed 1429 patients who were diagnosed with CRC and underwent curative surgical resection at
First Affiliated Hospital of Anhui Medical University from January 11, 2012, through February 15, 2019. The patients were enrolled according to the following criteria: pathologically diagnosed with primary CRC; available test results for PNI status; available and complete clinical, pathologic and follow-up data. A total of 627 patients met these criteria and were included in this study. Age, gender, body mass index (BMI), diabetes mellitus, medical history of other malignancy, neoadjuvant chemotherapy, primary tumor site, histology, T classification, N classification, M classification, UICC classification, lymphovascular invasion, PNI, and number of sampled lymph nodes were extracted for statistical analysis. This study protocol was approved by the ethics committee of our college. All patients signed an informed consent regarding their understanding of the procedure and its potential complications as well as their approval of participation in the research.

2.2. Determination of PNI status

About 4-μm thick 10% formalin-fixed, paraffin-embedded tissue sections of CRC were analyzed for PNI and other pathologic parameters were carried out by 1 institutional gastrointestinal pathologist. Questionable cases were reexamined by 2 pathologists. PNI was defined as tumor cells found within the perineural space or the infiltration of cancer cells into the endoneurium. The pathologic staging was grouped according to the American Joint Committee on Cancer Staging Manual, 8th edition.

2.3. Statistical analysis

All measurement data were represented as (x ± s) and the count data were expressed in percentage. Patient characteristics were compared using t tests for continuous variables and Chi-squared or Fisher exact tests for categorical variables. To select final predictors of PNI, all candidate predictors with a P < .2 in univariate analysis were included in a multivariate logistic regression model. Variates with P < .05 in the multivariate analysis were deemed independent predictors. Five-year overall survival (OS) and disease-free survival (DFS) were calculated using the Kaplan–Meier method, and the difference in survival rate was assessed by the log-rank test. OS was measured from the date of diagnosis to the date of death or last follow-up. DFS was defined as the time from diagnosis to the first occurrence of any of the following events: recurrence of ACC at any site or death from any cause without documentation of a cancer-related event. The variables that had prognostic potential, as indicated by univariate analyses, were subjected to multivariate analyses with the Cox proportional hazards regression model. All analyses were performed on statistical package SPSS version 23.0. All P values were 2-sided, and a value of < .05 indicated statistical significance. Variables used to stratify survival included age, gender, BMI, diabetes mellitus, medical history of other malignancy, neoadjuvant chemotherapy, primary tumor site, histology, T classification, N classification, M classification, UICC classification, number of sampled lymph nodes, and lymphovascular invasion.

3. Results

3.1. Patient and tumor characteristics

Total of 627 patients with CRC met inclusive criteria and were enrolled our study. The clinical and pathologic characteristics of the patients are shown in Table 1. All patients underwent surgical resection of the primary tumor and the diagnosis of adenocarcinoma was made pathologically. Of the 627 CRCs, 79 (12.6%) and 548 (87.4%) tumors were classified as PNI positive (PNI+) and PNI negative (PNI−), respectively. Significant differences between CRC with and without PNI were observed with respect to age (P = .038), T classification (P = .015), N classification (P = .000), M classification (P = .000), UICC classification (P = .000), and lymphovascular invasion (P = .000); whereas, there were no significant differences with respect to gender (P = .984), BMI (P = .770), type 2 diabetes mellitus (T2DM; P = .578), history of other malignancy (P = .322), neoadjuvant chemotherapy (P = .765), primary tumor site (P = .746), histology (P = .180), and number of sampled lymph nodes (P = .507). A total of 151 (24.1%) patients had T2DM and 77 (12.3%) patients had history of other malignancy. Neoadjuvant chemotherapy such as FOLFOX (infusional fluorouracil, leucovorin plus oxaliplatin) or CAPOX (capecitabine plus oxaliplatin) was observed in 27 (4.3%) patients. According to logistic regression analysis (Table 2), only lymphovascular invasion was an independent factor in CRC with PNI.

![Figure 1](https://example.com/figure1.png)

Figure 1. (A) Colorectal cancer (CRC) with perineural invasion and (B) CRC without perineural invasion.
with outcome (the 5-year OS [P = .560] and DFS [P = .083]) (Fig. 2). Age and N2/3 classification were independent prognostic factors for poorer OS and DFS. M1 stage (95% confidence interval [CI] = 0.228–0.585, P = .000), III/IV stage (95% CI = 0.335–0.920, P = .022), number of sampled lymph nodes (95% CI = 0.951–0.987, P = .001) were independently prognostic for poorer OS, while history of other malignancy was identified as an independent prognostic factor for poorer DFS.

4. Discussion

Recently, as a new biologic feature, PNI has attracted more and more attention in CRC. At present, to explore the prognostic role of PNI in CRC, the main objectives are as follows: to improve the clinical staging standards and to evaluate the necessity of postoperative adjuvant chemotherapy.

The PNI was found in 12.6% of our patients, which is similar to the results of previous studies. The reported incidence of PNI in patients with CRC ranges between 9% and 30%, but its prognostic significance is still controversial.[5,6,9] Quah and colleagues claimed that PNI status can be used to facilitate the selection of patients with stage II CRC for adjuvant chemotherapy.[10] Fujita and coworkers also reported that PNI significantly reduced the survival rates in patients with stages II and III CRC and might be useful for characterizing patients who might benefit from adjuvant system therapy.[10] On the contrary, Burdy et al suggested that PNI was a significant prognostic factor in univariate analysis but was not significant in multivariate analysis in patients undergoing surgery for T3/4N0 colon cancer.[9] Moreover, in Di Fabio and coworkers’ study, the study group showed that only T4 stage and age over 70 were independent factors associated with significantly poor cancer-related survival in patients with stage II CRC.[11] We concur with this and confirmed PNI is not a prognostic factor for the survival of patients with CRC.

Zhou et al suggested that PNI status could be used as a complementary factor for TNM staging.[12] Their results[5,13] indicated that PNI significantly influenced the survival outcomes of TNM stages II and III patients, which was consistent with the findings of some studies. By combining TNM staging and PNI status, patient prognosis could be stratified into 3 levels: stage II PNI negative; stage II PNI positive/stage III PNI negative; and stage III PNI positive. Patients at stages II and III could be further divided according to their PNI status, thereby providing a basis for individualized auxiliary treatment.[13,14] Liebig et al reported that stage II PNI-positive patients have poorer prognosis than stage III patients.[13] Some study indicated that the value of cancer

### Table 1
Clinicopathologic characteristics of the patients.

| Features                        | PNI (+) | PNI (-) | P value |
|---------------------------------|---------|---------|---------|
| Total                           | 79 (12.6%) | 548 (87.4%) | .038   |
| Age, yr                         | 65.19±14.56 | 66.43±12.56 | .988   |
| Gender, n (%)                   | Male 42 (53.2%) | 292 (53.3%) |   |
| - Female 37 (46.8%)             | 256 (46.7%) |   |
| BMI, kg/m²                      | 25.75±4.66 | 24.89±4.48 | .770   |
| Type 2 diabetes mellitus, n (%) | Yes 21 (26.6%) | 130 (23.7%) | .578   |
| - No 56 (73.4%)                 | 418 (76.3%) |   |
| Other malignancy, n (%)         | Yes 7 (8.9%) | 70 (12.8%) | .322   |
| - No 72 (91.1%)                 | 478 (87.2%) |   |
| Neoadjuvant chemotherapy, n (%) | Yes 4 (5.1%) | 23 (4.2%) | .765   |
| - No 75 (94.9%)                 | 525 (95.8%) |   |
| Location, n (%)                 | Right† 23 (29.1) | 150 (27.4) | .746   |
| - Left‡ 56 (70.9)               | 398 (72.6) |   |
| Histology (por:wel/mod)         | Yes 57 (72.2) | 432 (78.8) | .180   |
| - No 22 (27.8)                  | 116 (21.2) |   |
| T classification, n (%)         | T1/2 8 (10.1) | 120 (21.9) | .015   |
| - T3/4 71 (89.9)                | 428 (78.1) |   |
| N classification, n (%)         | N0/1 51 (64.6) | 458 (83.6) | .000   |
| - N2/3 28 (35.4)                | 90 (16.4) |   |
| M classification, n (%)         | M0 56 (70.9) | 482 (88.0) | .000   |
| - M1 23 (29.1)                  | 66 (12.0) |   |
| UICC classification, n (%)      | U1 27 (34.2) | 319 (58.2) | .000   |
| - U3 52 (65.8)                  | 229 (41.8) |   |
| Lymphovascular invasion, n (%)  | Yes 60 (75.9) | 172 (31.4) | .000   |
| - No 19 (24.1)                  | 376 (68.6) |   |
| Number of sampled lymph nodes, n (%) | 21.92±15.51 | 21.49±12.00 | .507   |

### Table 2
Multivariate analysis of factors predicting colorectal cancer with PNI.

| Features                        | Odds ratio | 95% CI   | P value |
|---------------------------------|------------|----------|---------|
| Age                             | 1.008      | 0.988–1.028 | .442   |
| Histology (por:wel/mod)         | 0.820      | 0.465–1.445 | .493   |
| T classification (T1/2:T3/4)    | 1.620      | 0.712–3.684 | .250   |
| N classification (N0/1:N2/3)    | 1.234      | 0.648–2.352 | .522   |
| M classification (M0:M1)        | 1.691      | 0.882–3.242 | .114   |
| UICC classification (III/IV)    | 0.889      | 0.446–1.773 | .738   |
| Lymphovascular invasion (yes:no)| 0.169      | 0.093–0.307 | .000   |

CI = confidence interval, mod = moderately differentiated adenocarcinoma, PNI = perineural invasion, por = poorly differentiated adenocarcinoma, wel = well-differentiated adenocarcinoma.

BMI = body mass index, mod = moderately differentiated adenocarcinoma, PNI = perineural invasion, por = poorly differentiated adenocarcinoma, wel = well-differentiated adenocarcinoma.

† Right = cecum, ascending colon, and transverse colon.
‡ Left = descending colon, sigmoid colon, rectosigmoid colon, and rectum.
staging could be enhanced by PNI assessment using a grading system based on PNI location within the bowel.[16,17] Previous reports indicated that PNI can be detected in 10% to 35% of the resected tumor samples of CRC, and it increases with higher tumor grade and stage.[18,19] However, the current cancer evaluation system of PNI lacks uniform standards and concrete guidelines.

Several studies have shown that the number of lymph nodes dissected during surgery is an important prognostic factor in patients with stage II CRC.[6,20] Harvesting more lymph nodes is associated with a better OS in patients with stage II CRC. Huh et al suggested that PNI did not impact OS at the completion of their analysis.[13] Therefore, further large-scale studies with long-term follow-up periods are necessary to determine the prognostic role of PNI for OS in patients with stage II CRC. This study’s limitations deserve commentary. First, this was a nonrandomized retrospective analysis from a single center, and as such, there were potential biases for comparison. Second, we did not evaluate the circumferential resection margin using the resection specimen and the effect of adjuvant chemotherapy on patients with 6 or fewer examined lymph nodes have poorer outcomes than those with a higher number examined due to under staging.[20] They also observed that recovering more lymph nodes is associated with a better OS in patients with stage II CRC. Caplin and coworkers suggested that stage II

### Table 3

| Variable                                      | Comparator vs reference | Univariate analysis | Multivariate analysis |
|-----------------------------------------------|-------------------------|---------------------|----------------------|
|                                               |                         | HR (95% CI)         | P value              | HR (95% CI)         | P value |
| Age                                           |                         | 1.012–1.043         | .000                 | 1.018–1.050         | .000    |
| Gender                                        | Male vs female          | 0.726–1.448         | .886                 | 0.641–1.229         | .612    |
| BMI                                           | 0.940–1.014             | .210                | 0.940–1.020          | .308                |
| Type 2 diabetes mellitus                      | Yes vs no               | 0.745–1.620         | .636                 | 0.665–1.498         | .994    |
| Other malignancy                              | Yes vs no               | 0.795–2.062         | .310                 | 0.596–1.618         | .943    |
| Neoadjuvant chemotherapy                      | Yes vs no               | 1.767–6.187         | .000                 | 0.849–3.624         | .129    |
| Location                                      | Right vs left†          | 0.873–1.831         | .215                 | 0.948–2.065         | .091    |
| Histology                                     | Por vs wel/mod          | 1.025–2.214         | .037                 | 0.828–1.806         | .312    |
| T classification                              | T1/2 vs T3/4            | 0.220–0.760         | .005                 | 0.366–1.360         | .297    |
| N classification                              | N0/1 vs N2/3            | 0.218–0.443         | .000                 | 0.372–0.953         | .031    |
| M classification                              | M0 vs M1               | 0.192–0.402         | .000                 | 0.228–0.585         | .000    |
| TNM classification                            | I/II vs III/IV          | 0.226–0.472         | .000                 | 0.335–0.920         | .022    |
| Lymphovascular invasion                       | Yes vs no               | 1.312–2.633         | .000                 | 0.755–1.774         | .502    |
| Perineural invasion                           | Yes vs no               | 1.184–2.880         | .007                 | 0.706–1.900         | .560    |
| Number of sampled lymph nodes                 | 0.962–0.994             | .009                | 0.951–0.987          | .001                |

BMI = body mass index, CI = confidence interval, HR = hazard ratio, mod = moderately differentiated adenocarcinoma, por = poorly differentiated adenocarcinoma, wel = well-differentiated adenocarcinoma.

† Right: cecum, ascending colon, and transverse colon.

### Table 4

| Variable                                      | Comparator vs reference | Univariate analysis | Multivariate analysis |
|-----------------------------------------------|-------------------------|---------------------|----------------------|
|                                               |                         | HR (95% CI)         | P value              | HR (95% CI)         | P value |
| Age                                           |                         | 0.972–0.998         | .029                 | 0.966–0.994         | .005    |
| Gender                                        | Male vs female          | 0.914–1.795         | .150                 | 0.987–1.975         | .059    |
| BMI                                           | 0.978–1.053             | .447                | 0.974–1.050          | .561                |
| Type 2 diabetes mellitus                      | Yes vs no               | 0.662–1.436         | .897                 | 0.612–1.359         | .651    |
| Other malignancy                              | Yes vs no               | 1.046–2.523         | .031                 | 1.133–2.813         | .012    |
| Neoadjuvant chemotherapy                      | Yes vs no               | 1.000–3.624         | .050                 | 0.811–3.681         | .156    |
| Location                                      | Right vs left†          | 0.675–1.434         | .932                 | 0.736–1.591         | .688    |
| Histology                                     | Por vs wel/mod          | 0.854–1.837         | .249                 | 0.758–1.652         | .571    |
| T classification                              | T1/2 vs T3/4            | 0.223–0.729         | .003                 | 0.298–1.050         | .071    |
| N classification                              | N0/1 vs N2/3            | 0.290–0.583         | .000                 | 0.448–1.113         | .134    |
| M classification                              | M0 vs M1               | 0.266–0.559         | .000                 | 0.333–0.952         | .030    |
| TNM classification                            | I/II vs III/IV          | 0.207–0.612         | .000                 | 0.520–1.368         | .491    |
| Lymphovascular invasion                       | Yes vs no               | 1.267–2.469         | .001                 | 0.771–1.687         | .511    |
| Perineural invasion                           | Yes vs no               | 1.294–2.934         | .001                 | 0.948–2.375         | .083    |
| Number of sampled lymph nodes                 | 0.990–1.016             | .651                | 0.981–1.009          | .489                |

BMI = body mass index, CI = confidence interval, HR = hazard ratio, mod = moderately differentiated adenocarcinoma, por = poorly differentiated adenocarcinoma, wel = well-differentiated adenocarcinoma.

† Right: cecum, ascending colon, and transverse colon.

1 Left: descending colon, sigmoid colon, rectosigmoid colon, and rectum.
outcomes. However, our data reflect that PNI is not an independent poor prognostic factor in patients with CRC and could not guide therapeutic regimen. M1 stage, III/IV stage, and the number of sampled lymph nodes were independently prognostic for poorer OS, while history of other malignancy could not guide therapeutic regimen. M1 stage, III/IV stage, and independent poor prognostic factor in patients with CRC and DFS. Both age and N2/3 classification were significantly correlated with poorer OS and DFS. The results of the present analysis will hopefully lead to a prospective randomized study with the ultimate goal of a centralized national program for prognostic value of PNI in CRC.

Author contributions
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Figure 2. Five-year (A) overall survival and (B) disease-free survival rates calculated by the Kaplan–Meier method. PNI = perineural invasion.