Perineural Invasion Is an Independent Predictor of Outcome in Colorectal Cancer

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ABSTRACT

Purpose

Perineural invasion (PNI) is associated with decreased survival in several malignancies, but its significance in colorectal cancer (CRC) remains to be clearly defined. We evaluated PNI as a potential prognostic indicator in CRC, focusing on its significance in node-negative patients.

Patients and Methods

We identified 269 consecutive patients who had CRC resected at our institution. Tumors were rereviewed for PNI by a pathologist blinded to the patients' outcomes. Overall and disease-free survivals were determined using the Kaplan-Meier method, with differences determined by multivariate analysis using the Cox multiple hazards model. Results were compared using the log-rank test.

Results

PNI was identified in less than 0.5% of the initial pathology reports. On rereview, 22% of tumors in our series were found to be PNI positive. The 5-year disease-free survival rate was four-fold greater for patients with PNI-negative tumors versus those with PNI-positive tumors (65% v 16%, respectively; $P < .0001$). The 5-year overall survival rate was 72% for PNI-negative tumors versus 25% for PNI-positive tumors. On multivariate analysis, PNI was an independent prognostic factor for both cancer-specific overall and disease-free survival. In a subset analysis comparing patients with node-negative disease with patients with stage III disease, the 5-year disease-free survival rate was 56% for stage III patients versus 29% for patients with node-negative, PNI-positive tumors ($P = .0002$). Similar results were seen for overall survival.

Conclusion

PNI is grossly underreported in CRC and could serve as an independent prognostic factor of outcomes in these patients. PNI should be considered when stratifying CRC patients for adjuvant treatment.

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INTRODUCTION

Colorectal cancer (CRC) affects approximately 148,810 people and accounts for 49,960 deaths annually, making it the second leading cause of cancer death in the United States.¹ Pathologic and clinical staging variables are currently the main prognostic determinants and serve as the basis for patient selection for adjuvant therapy. Adjuvant chemotherapy is considered standard of care for node-positive (stage III) patients. Because of an overall better prognosis, node-negative patients (stages I and II) are not routinely offered chemotherapy. Local and/or distant recurrences are the main causes of death for patients with CRC. Identifying pathologic factors associated with disease recurrence and poor outcome may lead to more accurate clinical staging and may assist in identifying patients with node-

negative disease who could potentially benefit from adjuvant therapy.

It is becoming increasingly evident that tumor-stromal interactions play a critical role in tumor growth and progression. Angiogenesis, inflammation, and matrix remodeling are examples of the dynamic stromal processes at play in the tumor microenvironment essential for tumor development. Peripheral nerves have long been overlooked as inert bystanders in solid malignancies, yet they are gaining recognition as potentially important components of the tumor microenvironment. Perineural invasion (PNI) is a pathologic process characterized by tumor invasion of nervous structures and spread along nerve sheaths.² The pathogenesis of PNI likely involves complex signaling between tumor cells, stromal cells, and the nerves, but this area of research is still largely in its infancy.³⁻⁸ PNI is known to be a

marker for a more aggressive tumor phenotype and poor prognosis in several malignancies, most notably head and neck and prostate cancers.⁹⁻¹⁴ In this analysis, we aim to determine the prognostic significance of PNI in CRC, particularly in patients with node-negative disease who currently receive no adjuvant therapy.

PATIENTS AND METHODS

Patients and Database

The records of 269 consecutive patients with colorectal adenocarcinomas who underwent surgical resection of their tumors at the Michael E. DeBakey Veterans Affairs Medical Center between January 1995 and June 2000 were reviewed (Table 1). Twenty patients were excluded because specimens and/or slides were no longer available for analysis. Data pertaining to demographics, staging, pathology, and outcomes were reviewed for each patient and entered into a comprehensive database. The observation time in this unselected cohort was the interval between surgical resection date and the last contact (death or last follow-up). The mean duration of follow-up was 6.8 years (range, 4.6 to 10.4 years). Recurrences, deaths, and disease-free periods were tracked by trained cancer registrars and recorded in the institution's cancer registry. The determination of cancer-related versus cancer-unrelated death was made by the tumor registrar tracking that particular patient. In cases where the cancer registrar was unable to make a determination regarding disease status or cause of death, the patient's electronic medical record, which included clinic visit notes, imaging studies, carcinoembryonic antigen determinations, and surveillance endoscopy reports, was reviewed by a surgical oncologist for determination of disease status or cause of death. In cases where patients were lost to VA follow-up, patients or their next of kin were contacted. All patient information and pathology material was collected under a protocol approved by the Institutional Review Board of the Baylor College of Medicine.

Assessment of Tumor Stage and Resection Level

Tumor stage was established postoperatively by either a medical or a surgical oncologist who was participating in our institution's multidisciplinary tumor board at the time of the patient's treatment. Stages 0 to IV were defined according to the 2002 sixth edition of the American Joint Commission on Cancer (AJCC) TNM staging system.¹⁵ Completeness of resection was determined for each patient based on the operative and pathology reports and classified as follows: R0 (negative gross and pathologic margins), R1 (negative gross with positive microscopic margins), and R2 (positive gross margins).

Histopathologic Analysis

For each patient included in the study, original hematoxylin and eosin-stained slides from the tumor resection were collected from the pathology department. All slides containing tumor were rereviewed for PNI by a single GI pathologist with expertise in PNI who was blinded to any patient data including stage of disease and outcome. PNI was defined as tumor cells within any layer of the nerve sheath or tumor in the perineural space that involved at least one third of the nerve circumference.

Statistical Analysis

Overall survival time was censored at the time of last follow-up for alive patients or at the time of death. Disease-free survival was censored at the time of last follow-up if the patient had remained disease free or at the time of non-cancer-related death. The influence of PNI on overall and disease-free survival was estimated using the Kaplan-Meier method. The association of PNI with various clinical characteristics was assessed using the Fisher's exact test. The effect of PNI on recurrence and cancer-specific survival was analyzed using the Cox regression model, controlling for age, number of lymph nodes resected, AJCC stage, and whether or not the patient received adjuvant therapy. The association between time to recurrence or death and PNI status was tested using the log-rank test. Differences between means were compared using the *t* test and analysis of variance. Analyses were performed with Prism, version 4 (GraphPad, San Diego, CA). Results were considered significant at $P < .05$.

Table 1. Disease-Free and Overall Survival Among 249 Colorectal Cancer Patients Who Underwent Primary Resection

	Patients		Disease-Free Survival		Overall Survival	
	No.	%	5 year (%)	<i>P</i>	5 year (%)	<i>P</i>
Age, years (mean, 67)				.02		.008
< 67	112	45.0	49		55	
≥ 67	137	55.0	34		40	
Sex				.7		.8
Male	246	98.8	55		61	
Female	3	1.0	66		67	
Location				.2		.4
Right	65	26.1	40		42.4	
Transverse	11	4.4	45		44.6	
Left	18	7.2	39		37.4	
Rectosigmoid	89	35.7	49		57	
Rectum	66	26.5	31		40	
Tumor stage				< .0001		< .0001
pTis	23	9.6	74		85	
pT1	33	13.8	94		97	
pT2	23	9.6	74		78	
pT3	136	56.9	43		50	
pT4	24	10	33		37	
Nodal status				< .0001		< .0001
Negative	146	58.6	68		73	
Positive	90	36.1	32		40	
Nx	13	5.2	67		67	
Distant metastasis				< .0001		< .0001
M0	168	67.5	67		74	
M1	39	15.7	0		6	
Mx	42	16.9	62		62	
AJCC stage				< .0001		< .0001
0	23	9.2	57		64	
1	46	18.5	67		67	
2	75	30.1	46		53	
3	59	23.7	40		50	
4	46	18.5	0		3.9	
Resection level completeness				< .0001		< .0001
R0	198	79.5	50.6		56.7	
R1	13	5.2	14.9		22.6	
R2	38	15.3	0		2.8	
Differentiation				.0002		< .0001
Poorly	54	21.7	35		37	
Moderately	120	48.2	51		60	
Well	35	14.1	64		71	
Mucinous	4	1.6	34		33	
N/A	41	16.5	83		88	
No. of lymph nodes analyzed				.5		.5
< 8	125	50.6	58		64	
≥ 8	122	49.3	53		59	
Perineural invasion				< .0001		< .0001
Yes	55	22.1	16		25	
No	194	77.9	65		72	

Abbreviation: AJCC, American Joint Committee on Cancer.

RESULTS

Patient Characteristics and Pathologic Variables

Patient characteristics and pathologic variables of our cohort are listed in Table 1. Mean patient age at the time of resection was 67 years (range, 39 to 95 years). Because our patient population was selected from a Veterans Affairs medical center, almost all of the patients were men. The majority of the tumors occurred either in the rectum (27%), the rectosigmoid (36%), or the right colon (26%), which included the hepatic flexure and appendix. Eighty percent of tumors (198 of 254 tumors) were classified as R0 resections (negative gross and microscopic margins). The remaining tumors were classified as either R1 (negative gross with positive microscopic margins) or R2 resections (gross disease left behind after resection; 5% and 15%, respectively). Seventy-seven percent of patients (49 of 63 patients) with stage III or IV disease who underwent resection with curative intent also underwent adjuvant and/or neoadjuvant chemotherapy. Seventeen percent of patients (18 of 105 patients) with node-negative disease who underwent R0 resections also received adjuvant chemotherapy and/or radiation therapy. Of these, 39% (seven of 18 patients) were patients with rectal or rectosigmoid lesions.

Incidence of PNI in CRC Patients

Twenty-two percent of patients in our series (55 of 249 patients) had PNI-positive tumors. Only 16% of these patients (nine of 55 patients) were identified as PNI positive in the original pathology report, demonstrating the historically gross under-reporting of this pathologic finding. Thirty percent of rectal cancers were PNI positive compared with 19% of colon cancers ($P = .3$). Of colon masses, tumors in the ascending and descending colon had the greatest PNI-positive rates (25% and 22%, respectively; $P = .3$) compared with tumors located in the transverse and sigmoid colon (Fig 1).

Fig 1. Incidence of perineural invasion (PNI) varies by tumor location. Tumors in the rectum had the highest PNI-positive rate (30%), followed by tumors in the right colon (25%) and left colon (22%). Tumors in the transverse colon and rectosigmoid showed the lowest frequency of PNI positivity (18% and 15%, respectively). These results did not reach statistical significance.

Relation Between PNI and Known Prognostic Factors in CRC

PNI-positive rates correlated with established risk factors for poor outcome in CRC. None of the stage 0 (carcinoma in situ) or stage I tumors exhibited PNI, whereas 20% of stage II, 24% of stage III, and 57% of stage IV tumors were PNI positive (Fig 2A). Patients with stage IV disease were three times more likely to have

Fig 2. Associations between perineural invasion (PNI) and established risk factors for poor outcome in colorectal cancer. PNI-positive rates increase with (A) advanced stage and (B) worsening tumor grade. (C) Patients with PNI-positive tumors are significantly more likely to have metastatic disease at diagnosis (five-fold increase compared with patients with PNI-negative tumors).

PNI-positive tumors than patients with either stage II or III disease (odds ratio = 4.8; relative risk = 3.0; $P < .0001$; Fig 2A). PNI-positive rates also correlated with worsening tumor grade. Just 14% of well-differentiated tumors were PNI positive compared with 23% and 41% of moderately and poorly differentiated tumors, respectively ($P < .01$; Fig 2B). Patients with PNI-positive tumors were almost five times more likely to have metastatic disease at the time of diagnosis compared with patients with PNI-negative tumors (47% v 10%, respectively; relative risk = 4.6; odds ratio = 7.8; $P < .0001$; Fig 2C). These findings all suggest that PNI may correlate with disease progression in CRC.

Role of PNI As Prognostic Factor of Outcome in CRC Patients

The prognostic significance of PNI as well as other clinical and pathologic variables was investigated by univariate analyses. PNI status, AJCC stage, TNM stage, grade, and resection level significantly influenced both disease-free and overall survival (Table 1). Age, sex, and tumor location did not significantly affect outcome. The 5-year disease-free survival rate was four-fold greater for patients with PNI-negative tumors versus patients with PNI-positive tumors (65% v 16%, respectively; $P < .0001$; Fig 3A). Similar results were found for

overall survival. Patients with PNI-negative tumors exhibited a three-fold increase in 5-year overall survival compared with patients with PNI-positive tumors (72% v 25%, respectively; $P < .0001$; Fig 3B).

Among patients who underwent R0 resection of their CRC (198 of 249 patients, 80%), PNI remained a significant predictor of poor outcome. The 5-year disease-free survival rate was more than two-fold greater among patients with PNI-negative tumors versus those with PNI-positive tumors (72% v 34%, respectively; $P = .0004$; Fig 3C). Overall survival at 5 years was 78% in patients with PNI-negative tumors compared with 52% in patients with PNI-positive tumors ($P = .0007$; Fig 3D).

PNI Is an Independent Prognostic Factor of Outcome in CRC Patients

A Cox multiple regression model was used to assess the influence of all significant covariates on survival in the subgroup of patients who underwent R0 resections. After controlling for age, grade, number of lymph nodes resected, AJCC stage, and adjuvant therapy, multivariate analysis confirmed that the presence of PNI was significantly and independently associated with a worse prognosis (Table 2). The presence of PNI in the resected primary tumors doubled the likelihood of developing a CRC recurrence (hazard ratio = 2.17; 95% CI, 1.16 to

Fig 3. Kaplan-Meier curves depicting (A) disease-free and (B) overall survival based on perineural invasion (PNI) status among 249 patients who underwent primary resection of their colorectal cancer, and Kaplan-Meier curves depicting (C) disease-free and (D) overall survival based on PNI status among 198 patients who underwent R0 resection of their colorectal cancer.

Table 2. Cox Multivariate Regression Models Were Used to Assess the Influence of PNI on Cancer-Specific Overall and Disease-Free Survival

	Cancer-Specific Overall Survival		
	Hazard Ratio	P	95% CI
Cancer-specific overall survival			
PNI	2.12	.03	1.009 to 4.14
Age	1.01	.65	0.98 to 1.03
Grade (baseline, well differentiated)			
Poorly differentiated	2.55	.11	0.82 to 7.97
Moderately differentiated	1.57	.41	0.54 to 4.60
Other	1.36	.69	0.30 to 6.24
Total lymph nodes $\geq$ 8	0.63	.18	0.32 to 1.23
AJCC stage (baseline, 0)			
1	0.79	.78	0.16 to 3.86
2	1.70	.53	0.33 to 8.84
3	3.12	.21	0.52 to 18.53
4	18.18	.00	2.61 to 126.74
Adjuvant therapy	0.65	.23	0.32 to 1.31
Cancer-specific disease-free survival			
PNI	2.17	.02	1.16 to 4.04
Age	0.99	.56	0.97 to 1.02
Grade (baseline, well differentiated)			
Poorly differentiated	1.28	.59	0.52 to 3.15
Moderately differentiated	0.93	.85	0.42 to 2.06
Other	0.74	.66	0.20 to 2.78
Total lymph nodes $\geq$ 8	0.66	.17	0.37 to 1.20
AJCC stage (baseline, 0)			
1	0.56	.40	0.14 to 2.20
2	1.11	.89	0.26 to 4.74
3	2.11	.35	0.44 to 10.12
4	12.01	.00	2.17 to 66.30
Adjuvant therapy	0.69	.24	0.37 to 1.28

Abbreviations: PNI, perineural invasion; AJCC, American Joint Committee on Cancer.

4.04; $P = .02$) and of dying of CRC (hazard ratio = 2.12; 95% CI, 1.09 to 4.14; $P = .03$) in our patient cohort.

Impact of PNI on Survival in Node-Negative CRC Patients

As shown in Table 2, disease-free and overall survival rates were significantly affected by PNI status independent of tumor stage. Among node-negative patients, the 5-year disease-free survival for PNI-negative patients was almost three-fold greater than for PNI-positive patients (82% v 29%, respectively; $P = .0005$; Fig 4A). In fact, node-negative patients with PNI had a significantly worse disease-free survival rate than node-positive patients (29% v 56%, respectively; $P = .0002$; Fig 4A). Similar results were seen for overall survival, where the 5-year overall survival for node-negative patients with PNI-positive tumors was 43% compared with 87% for patients with PNI-negative tumors. Similarly, node-negative but PNI-positive patients had a significantly lower 5-year overall survival rate compared with node-positive patients (43% v 67%, respectively; $P = .002$; Fig 4B).

DISCUSSION

PNI has become an increasingly relevant yet understudied aspect of tumor biology in cancers including CRC. PNI was first reported in

head and neck cancers describing their spread along nerves toward the intracranial fossa.^{16,17} PNI continues to be investigated in head and neck and prostate cancers.¹⁸⁻²¹ The role of PNI and its utility to clinicians continue to be debated. Because CRC remains the second leading cause of cancer death, we sought out to determine the impact of PNI in CRC in our patient population. Furthermore, our particular interest is in determining the potential role of PNI in therapy stratification in node-negative CRC patients currently not receiving adjuvant chemotherapy who may benefit from existing regimens currently limited to patients with node-positive disease.

Less than 20% of PNI-positive tumors in our series were identified as such on pathologic analysis at the time of resection. The increased detection rate seen on our rereview of slides is most likely because our pathologists did not routinely report PNI until recently. Furthermore, the rereview was performed by a pathologist with expertise in PNI who was blinded to outcomes in this study. Our findings are consistent with a previous study in head and neck cancers in which PNI had been a standard part of the original pathology report and had been initially reported as negative but revealed a 100% increase in the detection rate from 30% to 60% PNI positivity.²² According to the College of American Pathologists, PNI status is currently not a required feature of the pathology report for colon and rectal tumors.^{23,24} As a result of this study, it is now routinely reported at our institution. We believe that PNI status should become standard in pathology reports for colorectal tumors.

In this study, rectal cancers showed a greater incidence of PNI than colon cancers. This finding is not altogether surprising given the rich autonomic nerve plexuses that surround the rectum in the pelvis. Because we found that PNI is associated with more aggressive disease, we hypothesize that the observed higher PNI-positive rates in rectal cancers may partially explain why rectal cancers have worse outcomes stage-for-stage than colon cancers. Interestingly, colon cancers in retroperitonealized segments of the colon (ascending and descending colon cancers) showed higher incidences of PNI than the colon segments not attached to the retroperitoneum (transverse and sigmoid colon), suggesting that the physical proximity of these tumors to the autonomic nerve-rich retroperitoneum may increase the likelihood of PNI in these tumors.

We have concluded that PNI is associated with more advanced disease in CRC based on its correlation with poor tumor differentiation and higher stage. Studies in other malignancies have led to similar conclusions. For instance, in pancreatic cancer, the presence of extrapancreatic neural plexus invasion correlates with the amount of intrapancreatic PNI, suggesting that PNI outside of the tumor is the result of significant nerve invasion inside the pancreas.^{25,26} Several series have shown that PNI on prostate needle biopsy is predictive of higher T stage and extracapsular invasion at resection, suggesting a correlation with more advanced disease.^{10,27} We have also demonstrated in our series that PNI is associated with a five-fold increase in metastatic disease at the time of diagnosis. This further supports a role for PNI not just in disease progression, but also in tumor metastasis. Fifty-seven percent of patients with metastatic disease were PNI positive compared with a PNI-positive rate of 22% for all colorectal tumors.

Previous studies have suggested in univariate analysis a significant correlation between PNI in CRC and increased locoregional recurrence, lower 5-year survival, and increased likelihood of finding metastatic disease at the time of resection.²⁸⁻³³ In our study, we show

Fig 4. Disease-free and overall survival of patients with R0 resections according to TNM stage and perineural invasion (PNI) status. (A) The 5-year disease-free survival rate was 56% for patients with stage III disease compared with 29% for patients with node-negative, PNI-positive disease ($P = .0002$). (B) Five-year overall survival rate was 67% for patients with stage III disease compared with 43% for patients with node-negative, PNI-positive disease ($P = .002$).

that PNI is associated with decreased survival on multivariate analysis and, therefore, establish that PNI is an independent predictor of outcome in CRC patients. In fact, multivariate analysis revealed that patients with PNI-positive tumors were approximately twice as likely to die from their CRC within 5 years of diagnosis when compared with their stage-matched, PNI-negative counterparts. Our results reinforce findings of other multivariate analyses of PNI in CRC,³⁴⁻³⁸ despite their inclusion of only node-negative patients,^{34,36,37} obstructed patients,³⁵ or older staging criteria.^{35,36} Furthermore, we not only clearly define PNI, but we also rereviewed all available slides from each specimen—revealing initial under-reporting. Our incidence of PNI based on pathology reports (3.5%) correlates with that reported by others,³⁸ whereas the 22% incidence found after our rereview was significantly higher. Burdy et al³⁹ did not find PNI to be an independent prognostic factor on multivariate analysis; however, only 108 patients over a 20-year period were included in this study.

Of particular importance are patients with node-negative CRC. Although these patients do not currently receive adjuvant therapy available to node-positive patients, it is well known that a subset of these patients will die of aggressive recurrent and/or metastatic CRC. Attempts to prospectively identify this subset of node-negative CRC patients who could potentially benefit from available adjuvant therapy have proven unsuccessful. In our study, node-negative patients with PNI-positive tumors had significantly worse outcomes compared with node-positive patients. This finding highlights the robust prognostic significance of PNI in CRC and underscores the need for taking PNI status into account when stratifying patients for adjuvant therapy. Underscoring this concept, node-negative patients in our series who received adjuvant chemotherapy had survival rates similar to node-negative, PNI-negative patients who did not receive adjuvant treatment and had far better survival outcomes than stage III patients. Although the number of these node-negative patients in our series who received adjuvant chemotherapy was too low to draw a statically significant difference, this trend supports our hypothesis that node-negative, PNI-positive patients should be managed similarly to stage III patients and receive currently available adjuvant therapy.

Mechanisms of PNI remain poorly understood. The clear association between PNI and metastases in several cancers strongly suggests a role for PNI in tumor dissemination. The most common metastatic sites of CRC are the liver and the retroperitoneum, both richly innervated by autonomic nerve fibers. The sympathetic fibers innervating the liver share a preganglionic origin with the sympathetic nerves that innervate the colon and rectum.⁴⁰ Although this hypothetical route for CRC metastases has not been investigated, a histologic study by Pour et al⁴¹ involving analysis of serial nerve sections from three pancreas cancer specimens revealed that cancer cells could be followed within the nerves from the body of the tumor, along the superior mesenteric artery to the celiac ganglia, without invasion of the surrounding tissues. Another potential explanation for the observed association between PNI and CRC metastases may be that metastatic CRC cells exhibit neurotropism, and on reaching, by either hematogenous or lymphatic spread, the nerve-rich retroperitoneum and/or liver, they would establish a paracrine interaction with these nerve fibers that would facilitate metastatic growth. The liver's intraparenchymal nerve fibers could contribute to the development of metastasis by providing an immunoprivileged environment capable of sheltering tumor cells from immune elements. In support of this hypothesis, recent studies have shown that the autonomic nerves of the liver directly regulate apoptotic pathways of their target hepatocytes.^{42,43} Furthermore, studies in prostate cancer have shown decreased apoptosis of prostate cancer cells in the perineural environment, both in vitro and in human prostate cancer specimens.^{44,45} Tumor cells having acquired an advantageous PNI phenotype could perhaps benefit from this antiapoptotic activity in the immediate perineural environment.

In conclusion, PNI is an underreported phenomenon in CRC. Our data strongly suggest that PNI could function as an independent prognostic factor of outcomes in CRC and support consideration of PNI status in primary CRC specimens for therapy stratification. In particular, we advocate consideration of node-negative patients who are PNI positive for treatment with currently available effective adjuvant therapies. Further investigations into the molecular basis of PNI could help develop therapeutic strategies targeted toward this aggressive tumor phenotype.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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