Original Paper

Is Perineural Invasion (PN) a Determinant of Disease Free Survival in Early Stage Colorectal Cancer?

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ABSTRACT

Background/Aims: The prognostic importance of perineural invasion (PN) in colorectal cancer (CRC) is unclear. The aim of this study to find out whether the PN was an independent stratification factor of postoperative relapse in curatively resected high-risk stage II & III CRC patients who were treated with adjuvant therapy. **Methodology:** Data of patients with high risk stage II & all stage III CRCs treated with adjuvant chemotherapy were retrospectively analyzed. Pathological features of final surgical specimen were noted. Disease-free survival was determined by Kaplan-Meier estimator, with differences determined by multivariate analysis using the Cox multiple hazards model. Results were compared using the log-rank test. **Results:** PN was found to be positive in 26% in the files of 593 eligible patients. In 21% of the reports PN status was not reported. Presence of PN in the resected primary tumors did not have independent effect on DFS. Further analyses for importance of PN on DFS of colon or rectal cancers did not show any effect. **Conclusions:** This study had failed to demonstrate any prognostic effect of PN for DFS in surgically resected stage II and III CRC patients who received adjuvant treatments.

Key Words: Perineural invasion, Colorectal cancer, Adjuvant, Survival, Prognosis.

INTRODUCTION

Perineural invasion (PN), which is a pathologic process characterized by tumor invasion of nervous structures and spread along nerve sheaths, has long been defined in cancer (1). Presence of PN in a cancer specimen reported to be associated with aggressive biological behavior. In head & neck, prostate, and pancreatic carcinomas PN was reported to be a marker of early recurrence (2-4). Albeit, College of American Pathologists (CAP) stated the PN as an insufficiently studied tumor characteristic as a prognostic factor for colorectal cancers (5). Few reports studied the prognostic significance of PNI in colorectal cancer (CRC) have been published (6-9). However, there has been an inconsistency between the results of these reports, placing the PN into a gray zone and thus made its prognostic significance debatable for CRC (10,11). To note, heterogeneous study characteristics were one of the reasons that may be responsible for this inconsistency.

In colorectal cancer, disease-free survival (DFS) was told to be a surrogate marker of overall survival. Patients who are candidates of adjuvant therapy have different DFS expectancies and currently the pathologic stage at diagnosis remains the best indicator for DFS and OS. However in the era of molecular diagnostics the prognostic role of easily accessible PN which can be determined by simpler methods in CRC has not been fully answered.

Therefore in this study, we aimed to find out whether the PN was an independent stratification factor of postoperative relapse in curatively resected UICC (international union against cancer) high-risk stage II & III CRC patients who were treated with adjuvant therapy.

METHODOLOGY

Patient population

Files of curatively resected (without gross or microscopic evidence of residual disease) stage II & III colorectal adenocarcinoma patients from two centers (Cerrahpasa Faculty of Medicine, Dept. of Medical Oncology & Marmara Faculty of Medicine, Dept. of Medical Oncology) were reviewed. There had to be no evidence of any distant metastasis as determined by the surgeon at operation, or on a preoperative computed tomography (CT) scan of the thorax, or on a CT/MRI (magnetic resonance imaging) of the abdomen and pelvis. Patients with recurrent disease at presentation or with preoperative chemoradiation for locally advanced rectal cancer were excluded from the study cohort. Full evaluation of the colon and rectum by colonoscopy was noted to ex-

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TABLE 1. Baseline characteristics of the study population.

Characteristics	n (%)
Median age at dx (yrs) [range]	60 [18-82]
Gender	
Male	313 (52.8)
Female	280 (47.2)
Localization	
Rectum	298 (50.3)
Sigmoid	151 (25.5)
Descending	36 (6.1)
Transverse	15 (2.5)
Caecum & ascending	93 (15.7)
TNM stage	
II	242 (40.9)
III	351 (59.1)
Median tumor diameter (cm)	5 (1.5-16.5)
Depth of invasion	
T2	34 (5.7)
T3	469 (79)
T4	86 (14.5)
Unknown	4 (0.06)
Nodal involvement	
pN0	242 (40.8)
pN1	238 (40.1)
pN2	113 (19.1)
Median number of metastatic LNs	1 (0-31)
Median number of resected LNs	12 (0-99)
Histological grade	
Well	429 (72.3)
Poor	139 (23.4)
unknown	25 (4.1)
Mucine (+)	131 (22.1)
Mucinous adenocarcinoma	55 (9.3)
L (+)	301 (50.8)
V (+)	179 (30.2)
PN (+)	157 (26.5)
PN status unknown	125 (21.1)

clude other synchronous, unresected primary cancers.

Data of 593 eligible patients, who had been treated consecutively in our institutions between January 2000-December 2009 after curative resection with a final diagnosis of high risk stage II or stage III CRC, were retrospectively analyzed. All tumors were staged according to the Sixth UICC TNM staging system.

Clinical information about the age and gender of the patients, type of surgery, tumor location, histopathologic subtype(s), tumor size, histological grade, the depth of tumor invasion (T stage), lymph node involvement (N stage), lymphatic, vascular and perineural invasions (L, V and PN, respectively), resection margins, type of adjuvant chemotherapy and presence radiation therapy, disease recurrence and survival were obtained from patients charts. All of the H&E slides were examined by 2 pathologists (S.E., C.A.C.) with a special interest in colorectal cancer who were blinded to the patients' clinical information. PN was defined as tumor in the perineural space that surrounded at least one-third of the nerve without invading through the layer of epineurium or tumor cells within any of the 3 layers of the nerve sheath (1).

All patients, who had evidence of adequate organ functions as measured by the Eastern Cooperative Oncology Group (ECOG) performance status of 0-2, complete blood count and serum biochemical tests, received adjuvant treatments. Presence of risk factors like; poor differentiation, tumor perforation or obstruction, inadequate lymph node dissection (<12), or presence of lymphatic/vascular invasion were considered as high-risk criteria requiring adjuvant treatment for stage II colon cancer. Adjuvant treatments for colon cancers were either 5-FU based (Mayo regimen) or oxaliplatin based (FOLFOX-4/6 or FLOX). Adjuvant treatment of choice for rectal cancer was XRT (radiotherapy) plus concurrent 5-FU infusion/capecitabine, Patients who were given even one cycle of chemotherapy were included in the analysis. Adjuvant treatments were given within 3 months after definitive surgery.

Patients were followed-up every 3 months for the first 2 years and every 6 months thereafter as the guideline proposes. Complete blood counts (CBC), routine biochemical tests and serum CEA levels were measured at every visit and also in situations when clinically indicated. Imaging follow-up with CT and MRI was done according to contemporary guidelines. Determination of recurrence was made by clinical and radiological examinations or by histological confirmation whenever possible. In cases where relapse was controversial a final decision regarding the disease status of the patient was made by a council consisting of medical oncologists, general surgeons, radiologists, pathologists and specialists of nuclear medicine.

Aims

The primary aim of this study was to evaluate the reliability of PN as a potential determinant of DFS in patients with early-stage colorectal cancer given adjuvant treatments. Recurrences, disease-free periods, and deaths were tracked by investigators from the recorded cancer registries of individual institutions. A *relapse* is defined as reoccurrence of colorectal cancer either in the surgical bed or at a distant site or occurrence of a second primary colon/rectum cancer. *DFS* was defined as the time between the date of the operation and the first relapse, the occurrence of a second primary colon/rectum cancer, death from any cause with no recorded evidence of relapse, or the last date at which the patient was known to be free of disease.

Secondary aim of the study was to investigate the possible relationships of factors like age, sex, tumor size, TNM stage, T stage, N stage, grade, L, V, tumor localization, and mucine presence with PN.

TABLE 2. Descriptive properties of adjuvant treatments for patients with rectum and colon localization.

	Adjuvant Chemotherapy, n (%)		Adjuvant Radiotherapy, n(%)	
	5-FU based regimens	Oxaliplatin-based regimens	Not received or unknown	Received
Rectum cancer patients (n=298)	282 (94.6)	16 (5.4)	17 (5.6)	281 (94.2)
Colon cancer patients (n=295)	220 (74.6)	75 (25.4)	-	-

Statistical analysis

Statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL) software. The relationship between PN positivity and the other clinicopathological factors were analyzed by the chi-square test. Survival analysis and curves were established according to the Kaplan–Meier method and compared by the log-rank test. Multivariate analysis to assess the role of PN and the other clinicopathological features, which had a *p* value <0.1 on univariate analyses, as determinants for DFS was performed by the Cox regression analysis. Multivariate *p* values were used to characterize the independence of these factors. The 95% confidence interval (95% CI) was used to quantify the relationship between DFS and each independent factor. A *p* value less than .05 were considered to be statistically significant.

RESULTS

Study population

The clinicopathological features of the study population are shown in **Table 1**. The median follow-up of 593 eligible patients was 32.8 months (range: 1.8-213). Median patient age at the time of resection was 60 years (range: 19-82). Forty-seven percent of the patients were women. The majority of the tumors occurred either in the rectum (50%), the sigmoid (25%), or the caecum and the right colon (15%). All patients had undergone R0 resections (negative gross and microscopic margins) and received appropiate adjuvant treatment including chemotherapy and/or radiotherapy. All patients with colon cancer received adjuvant chemotherapy with either 5-FU or oxaliplatin-based regimens. Ninety-four percent of the patients with rectal cancer (RC) received adjuvant radiation with chemotherapy. Only 5.4% of the RC patients received adjuvant oxaliplatin-based regimens (Table 2). Most common types for surgery were as follows: low-anterior resection (LAR) 39.8%, abdominoperineal resection (APR) 15.8%, left hemicolectomy 22.1%, and right hemicolectomy 15%.

PNI and other clinicopathological features

Twenty-six percent of patients in our study population (157 of 593 patients) had PN-positive tumors. About 34% percent of rectal cancers, 37% of sigmoid cancers, and 29% of the remaining colon cancers were PN positive (*p>0.05*) (**Figure 1**). PN status was not reported in 125 patients (21.1%). Percentages of underreporting for PN were equally distributed among rectal cancers, sigmoid cancers and rest of the colon cancers (22%, 21%, and 17%, respectively, *p>0.05*).

TABLE 3. Frequency of PN positivity according to grade, lymphatic and vascular invasion.

	PN (+)
Grade	
Low-grade	30.8%
High-grade	42.5%
Lymphatic invasion	
(-)	12.1%
(+)	46.1%
Vascular invasion	
(-)	22.8%
(+)	52%

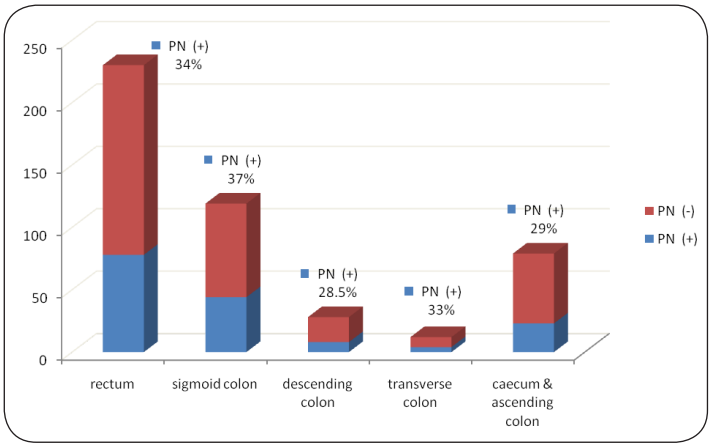

FIGURE 1. About 34% percent of rectal cancers, 37% of sigmoid cancers, and 29% of the remaining colon cancers were PN positive (*p>0.05*).

PN-positivity correlated with established risk factors for poor outcome such as increasing T and N stages, L and V (**Table 3, Figure 2**). Twenty percent of high-risk stage II and 43% of stage III patients were PN positive. PN positivity also correlated with worsening tumor grade. Thirty-one percent of low-grade tumors were PN positive compared with 42.5% high-grade tumors. These findings all suggest that PN may correlate with disease relapse in CRC.

TABLE 4. Results of analysis of disease-free survival at 3 years by individual factors.

Factors	Disease-free survival at 3 years		
	Univariate Analysis (p value)	*Multivariate analysis (p value)*	*Relative risk (95% CI)*
Age	NS	-	
Gender	NS	-	
Family history	NS	-	
Localization (colon vs rectum)	0.005	0.003	1.88(1.24-2.87)
Maximum tumor diameter	NS	-	
TNM stage	< 0.001	-	
T category (T2+T3 vs T4)	0.001	0.023	1.89(1.14-3.14)
N stage	< 0.001	0.003	2.03(1.24-3.33)
L	< 0.001	NS	
V	0.003	NS	
PN	< 0.001	0.094	
Mucine presence	0.035	NS	
Grade (low vs high)	0.057	NS	
Total number of LN dissected	NS	-	
Type of adjuvant treatment (5-FU vs oxaliplatin based)	NS	-	

L: lymphatic invasion, V: vascular invasion, PN: perineural invasion, LN: lymph nodes, NS: non significant.

Role of PN as Prognostic Factor of DFS

The prognostic significance of PN as well as other clinical and pathologic variables were investigated by univariate analyses. Localization of the tumor (rectum vs. colon), pT stage, pN stage, AJCC stage, L, V, and PN status, grade, and presence of mucine significantly influenced the disease-free survival (**Table 4**). Age, gender, family history of colon cancer, maximal tumor diameter, number of total lymph nodes harvested, and type of adjuvant therapy did not significantly affect the outcome. The 3-year disease-free survival rate was 71.9% for patients with PN negative tumors versus 47.9% for patients with PN-positive tumors (*p<0.001*; **Figure 3**).

A Cox multivariate analysis was used to assess the influence of all significant covariates on DFS. Multivariate analysis showed that tumor location, pT stage and pN stage were significantly and independently associated with a worse DFS (**Table 4**). However presence of PN in the resected primary tumors did not have independent effect on postoperative relapse.

Further analyses to detect the possible effect of PN on colon and rectum cancers were done separately. But no independent effect of PN on DFS of patients with either colon cancer or rectum cancer was seen (Data not shown).

DISCUSSION

CRC remains the second leading cause of cancer related death (12). Discovery of novel factors or defining the exact role of old ones for CRC that are predictive or prognostic may affect the treatment outcomes of a huge burden of patients. PN is one of these factors, with its utility in predicting postoperative relapse in patients with curatively resected CRC and received adjuvant therapies has not been studied thoroughly although the prognostic value of PN has been propagated previously in a number of CRC studies with different population characteristics (6-9). So, in this study we sought out to determine whether PN is an independent determinant of postoperative relapse in curatively resected CRC patients who were given adjuvant treatments.

By definition, treatment effects are neglected while testing the prognostic importance of a given parameter, however it is obvious that the endpoint of overall survival is much more prone to be affected by the types of treatment and the numbers of lines of chemotherapies given. Moreover time to first relapse is reported to be a surrogate for overall survival as an outcome measure in CRC. It possibly excludes the treatment related bias in this cohort in which treatment choices of particular groups of patients –for those with relapsed CRC - have varied over time due to change in valid scientific evidence. Therefore we intentionally set the primary endpoint as "disease-free survival" by which, to us, made our cohort more homogenous in terms of treatment effects.

Several papers have pointed out the under reporting of PN as an issue in pathology reports (13). Five to fifteen percent of PN positivity in first reports increased to around 20-30% on revision of the slides by experienced GI pathologists (9,14-17). Although PN status was not reported in about 21% of our patients, PNI positivity was 26.5% in our cohort. This ratio was comparable with the frequencies after revisions that were published in the literature. We thought that it is mostly, but not completely, related with our experienced GI pathologists who had carefully analyzed and dictated all CRC cases and our pathology revision policy for patients who had a CRC diagnosis elsewhere.

Our findings showed that PN was closely associated with aggressive disease characteristics such as higher T, N, and AJCC stages, L, V, and poor differentiation as consistent with the literature. However frequency of PN was similar in rectal, sigmoid, and rest of the colon cancers. This is a different finding from some of the previous reports. Liebig et al. reported that tumors in the rectum had the highest PN-positivity rate (30%), followed by tumors in the right colon (25%) and left colon (22%) (9). And added that this finding was related with the rich autonomic nerve plexuses that surround the rectum. We argue that both Liebeg and Poechl (18) data regarding the frequency of PN in different parts of the colorectum was confounded with advanced/metastatic disease since PN status was not reported specifically

for different parts of the colorectum with corresponding AJCC stage. On the other hand in parallel with our results, Huh et al. (16) found equal PN presence of 50% in rectum and colon cancers in curatively resected stage II CRC patients. Furthermore, we did not find differences in the frequency of PN among rectum, sigmoid and other colon cancers within either stage II or III disease. It is obvious that PN is associated with more advanced disease in CRC, but on the basis of available data it is insufficient for us to explain the relative aggressiveness of rectal cancer compared to colon cancer by PN as it was proposed by Liebig et al. (9).

Previous studies have suggested a significant correlation between PN in CRC and increased locoregional recurrence, lower 5-year survival (9-11,15-17,19-22). In our study, although it was significant in univariate analysis, we show that PN is not an independent determinant of postoperative relapse in curatively resected stage II&III CRC patients who were treated in the adjuvant setting. At this point we would like to underlie the difference of our study population. This is a relatively homogenous group of CRC patients which we would like to predict their disease-free survival period upfront. But other than well-known factors such as T and N stages, unfortunately so proposed "prognostic PN" did not give more information about postoperative relapse risk of this group of patients. This contradictory result is, however, not surprising; a prognostic factor for OS does not necessarily give information about prognosis for DFS. As in the case of BRAF mutation in CRC, current information about this mutation is it determines prognosis only after the metastases develops. We know that not all factors are responsible for the whole stages of development, progression and metastases of cancers. PN itself, or the mechanism(s) that result(s) in PN shall be responsible for poor prognosis only after CRC relapse occurs. At this point our findings are against for PN as being an independent factor of postoperative relapse.

According to the College of American Pathologists (CAP), PN status is currently not a required feature of the pathology report for colon and rectal tumors (5). It was classified as "Category III" which denotes those factors not yet sufficiently studied to determine their prognostic value. Although we could not find an independent effect on prognosis, we still routinely report PN status of CRC patients at our institutions, hoping that the data accumulation will provide valuable insight into this debatable topic.

Obviously, this study has inherent limitations of any retrospective study. We would have more conclusive results if we had revised the pathology specimens in terms of perineural invasion. Nevertheless PN was found in 26% of curatively resected CRC patients. Presence of PN was not different in regards to the tumor localization. And in conclusion, this study had failed to demonstrate any prognostic effect of PN for DFS that was reported in the final pathology reports of surgical resection in stage II and III CRC patients who received adjuvant treatments.

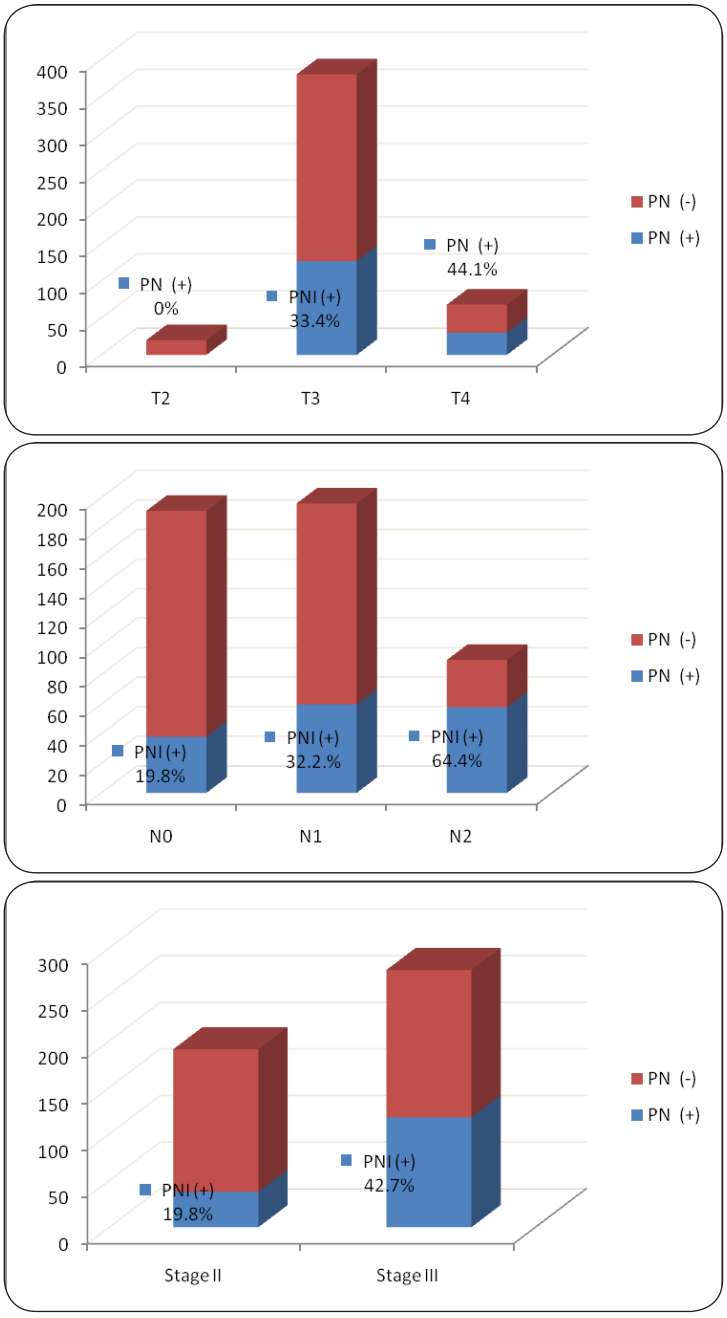

FIGURE 2. PN-positivity correlated with established risk factors for poor outcome such as increasing T and N stages, L and V.

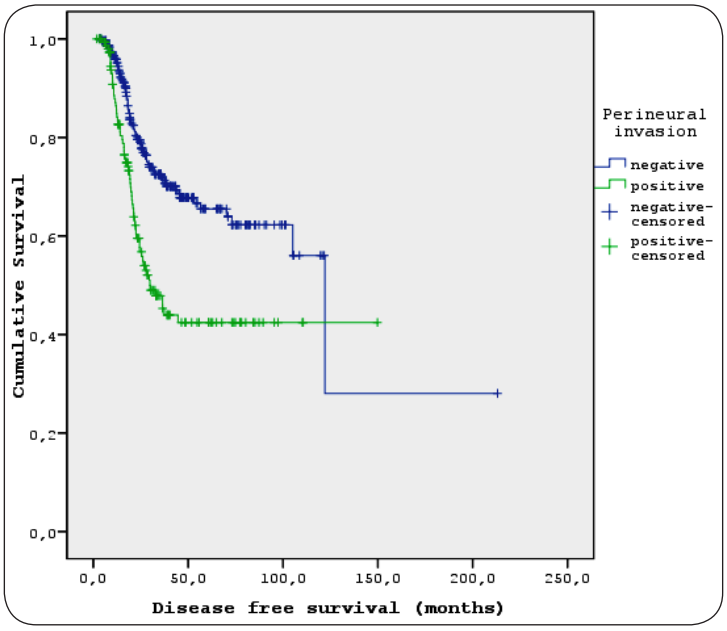

FIGURE 3. The 3-year disease-free survival rate was 71.9% for patients with PN negative tumors versus 47.9% for patients with PN-positive tumors (p<0.001).

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