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Investigation of the effect of perineural invasion on survival rates in rectal tumors

Rektum tümörlerinde perinöral invazyonun sağkalım oranlarına etkisinin araştırılması

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Abstract

Purpose: Colorectal cancers compose the third most common cancer group leading to death, with increasing rates in developed countries after lung and breast cancers. Rectal cancers make up about 30% of all colorectal cancers. Although many factors affecting recurrence and survival in rectal cancer have been identified, the stage of the tumor is the most important prognostic factor. However, many additional parameters that can be associated with survival and affect the adjuvant treatment plan have been identified besides the stage. Perineural invasion (PNI) is one of the parameters that can be associated with survival and affect the adjuvant treatment plan in colorectal cancers. The aim of this study is to investigate the effects of various histopathological prognostic factors, including PNI on disease-free survival, overall survival, and recurrence rates in the study group.

Materials and methods: The clinical records of patients diagnosed with rectal cancer and operated on at the Department of General Surgery, Faculty of Medicine, Pamukkale University between 2008-2014 were reviewed. To investigate the factors affecting disease-free survival, recurrence rates, and overall survival, the presence of PNI, serum CEA levels, cTNM stage, pT stage, pN stage, number of metastatic LNs, tumor budding status, histopathological grades, lymphocyte infiltration, and presence of desmoplasia were evaluated in patients.

Results: A total of 124 patients were included in the study. 42 patients (33.9%) had PNI while 82 patients (66.1%) did not. Of the 45 patients with recurrence, 26 had PNI while 19 did not have PNI. Of the 79 patients without recurrence, 16 had PNI while 63 did not exhibit any evidence of PNI. Similarly, PNI was seen more frequently in deaths related to the disease. The 3-year average survival of patients with and without PNI was 55% and 89%, respectively (p<0.05). The 5-year survival was 38% and 83% respectively (p<0.05). Similarly, there was a significant relationship between the presence of PNI and 3-year disease-free survival (46.8% and 84.1% respectively, p<0.05) and 5-year disease-free survival (22.3% and 80.4% respectively, p<0.05). In the multivariate analysis in which other prognostic factors were also evaluated, the presence of PNI was found to be significantly associated with both overall survival (HR:3.4, 95% CI:1.7-7.2) and disease-free survival (HR:3.0, 95% CI:1.6-5.6).

Conclusion: PNI presence is a strong and independent prognostic indicator for survival. Large-scale, randomized, prospective studies are needed to determine whether PNI presence should be taken into account in the staging of colorectal cancer or play a role in choosing adjuvant therapy.

Key words: Rectal cancer, perineural invasion, survival.

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Öz

Amaç: Kolorektal kanserler insidansı gelişmiş ülkelerde giderek artan oranlarda akciğer ve meme kanserlerinden sonra en sık ölüme yol açan üçüncü kanser grubunu oluşturmaktadır. Tüm kolorektal kanserlerin %30 kadarını ise rektum kanserleri oluşturmaktadır. Rektum kanserlerinde nüks gelişimi ve sağkalım üzerine etkisi olabilecek birçok faktör tanımlanmış olmakla birlikte en önemli prognostik faktör tümörün evresidir. Ancak evre ile birlikte sağkalımla ilişkili olabilecek ve adjuvan tedavi planını etkileyebilecek birçok ek parametre tanımlanmıştır. Perinöral invazyon (PNİ) da kolorektal kanserlerde sağkalımla ilişkili olabilecek ve adjuvan tedavi planını etkileyebilecek parametrelerden birisidir. Bu çalışmanın amacı rektum kanseri nedeniyle opere edilen hastaların başta PNİ olmak üzere çeşitli histopatolojik prognostik faktörlerin hastalıksız sağkalıma, genel sağkalıma, rekürrens oranlarına etkilerinin araştırılmasıdır.

Gereç ve yöntem: Pamukkale Üniversitesi Tıp Fakültesi Hastanesi'nde 2008-2014 yılları arasında rektum kanseri tanısı koyularak Genel Cerrahi Anabilim Dalı tarafından opere edilen hastaların klinik kayıtları incelendi. Olgularda hastalıksız sağkalıma, nüks oranlarına ve genel sağkalıma etki eden faktörleri araştırmak amacıyla başta PNİ olmak üzere serum CEA düzeyleri, tümör evresi (cTNM, pT, ve pN evresi), metastatik LN sayıları, tümör budding durumu, tümör gradeleri, lenfosit infiltrasyonu ve desmoplazi varlığı değerlendirilmiştir.

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Bulgular: Bu çalışma toplam 124 hasta analiz edilmiştir. Hastaların 42 tanesinde (%33,9) PNİ saptanırken kalan 82 hastada (%66,1) PNİ saptanmadı. Nüks saptanan 45 hastanın 26 tanesinde PNİ saptanırken 19 tanesinde saptanmamıştır. Nüks saptanmayan 79 hastanın 16 tanesinde PNİ varken 63 tanesinde PNİ saptanmamıştır. Benzer şekilde hastalığa bağlı ölümlerde PNİ anlamlı olarak daha sık görülmektedir. PNİ olan ve olmayan hastaların 3 yıllık ortalama sağkalımları sırası ile %55 ve %89 olarak saptandı (p<0,05). 5 yıllık sağkalımları ise sırası ile %38 ve %83 olarak saptandı (p<0,05). Benzer şekilde PNİ varlığı ile 3 yıllık hastalıksız sağkalım (sırası ile %46,8 ve %84,1, p<0,05) ve 5 yıllık hastalıksız sağkalımları (sırası ile %22,3 ve %80,4, p<0,05) arasında anlamlı ilişki bulundu. Diğer prognostik faktörlerin de incelendiği multivariate analizde PNİ varlığı hem genel sağkalım için (HR:3,4; 1,7-6,7; %95CI, p<0,001) hem de hastalıksız sağkalım için (HR:2,5; 1,2-4,9; %95 CI, p<0,001) bağımsız prognostik faktör olarak saptanmıştır.
Sonuç: PNİ varlığının sağkalım için güçlü ve bağımsız bir prognostik belirteç olduğu saptanmıştır. PNİ varlığının kolorektal kanser evrelemesinde dikkate alınması ya da adjuvan tedavi seçiminde rol oynayabilmesi için geniş kapsamlı randomize prospektif olarak dizayn edilmiş çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Rektal kanser, perinöral invazyon, sağkalım.

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Introduction

Colorectal cancers are the third most common cancers leading to death in developed countries following lung and breast cancers [1]. Approximately 800,000 new colorectal cancer patients are diagnosed worldwide each year, which is about 10% of all cancers with an increasing incidence. Among all, rectal cancers forms over one-third of colorectal cancers [2]. Rectal cancer is a complex disease, primarily due to its location with relatively high recurrence rates generally ranging from 20% to 50% [3], with higher rates in patients with a more advanced initial tumor stage. Although many factors affecting recurrence and survival rates in rectal cancer have been identified, the stage of the tumor is the most important prognostic factor [3]. The five-year survival rates for patients vary according to the stages in the TNM system [3]. However, many additional parameters that can be associated with survival and the adjuvant treatment decision have been identified besides stage.

Perineural invasion (PNI) is one of the parameters that can be associated with survival and affect the adjuvant treatment plan in colorectal cancers [4]. The invasion of tumor cells into the lymphatic or blood vessels is an important indicator in the metastatic process. Perineural invasion (PNI) is not a subcategory of lymphovascular space invasion (LVSI), and there are no lymphatic or vascular vessels in the perineural space. PNI is directly related to survival in colorectal cancers [4]. In a study by Liebig et al. [5], the five-year disease-free survival rates were reported as 16% and 65% for patients with and without PNI, respectively, while the general survival rates were 25% and 72% for the same patients.

The aim of our study is to evaluate the effect of perineural invasion (PNI) along with serum Carcinoembryonic antigen (CEA) levels and the other histopathological prognostic factors (The TNM Classification of Malignant Tumours (TNM) stage, primary tumor invasion (pT stage), regional lymph nodal metastasis (pN stage) pT stage, metastatic LN count, tumor budding condition, grade, lymphocyte infiltration and presence of desmoplasia) on disease-free survival, overall survival, and recurrence rates in patients who underwent surgery for rectal tumor.

Material and method

The clinical records of patients diagnosed with rectal cancer who underwent surgery at a tertiary referral center between 2008 and 2014 were obtained from the patient database. The study design was approved by the University Ethics Committee (07.03.2017/04). All patients had biopsy-confirmed rectal adenocarcinoma. Demographic information, forms of neoadjuvant therapy, surgical methods, clinicopathological data, and survival times were collected through direct patient interviews. Data collection was terminated at August 2017. Cases who have died or dropped out were not considered as “at risk”. Patients were labeled as “censored” if they were lost before the cencoring time. The available time-to-event was censored as the date patient’s death or dropout. Otherwise,

all outcome parameters were recruited for all "non-censored" subjects at the date of study termination.

The assessment included PNI and serum CEA levels, cTNM stage, pT stage, pN stage, metastatic LN count, tumor budding, histopathological grades, lymphocyte infiltration, and the presence of desmoplasia. Patients with distal organ metastasis, synchronous malignancy in another system, previously operated rectal cancer, or Adenomatous polyposis coli syndrome (APC) were excluded, leaving 124 patients in the study.

Tumor localization was classified as lower rectum (tumors up to 5 cm from the anal canal), middle rectum (tumors between 5 to 10 cm from the anal canal), and upper rectum (tumors farther than 10 cm from the anal canal). Standard surgery was performed based on the principles of total mesorectal excision for middle and lower rectal tumors. The TNM staging system defined by the Union for International Cancer Control (UICC), American Joint Committee on Cancer, and Union Internationale Contre Le Cancer workgroups was used for staging [6].

Decision on neoadjuvant chemotherapy, radiotherapy, or chemoradiotherapy was discussed in a multidisciplinary team. Stage 2-3 diseases were treated with first-step chemotherapy. Patient condition and acceptance of therapy were considered during the decision-making process. The disease-free survival period was defined as the time between the first operation and the date of clinical or histopathological recurrence. The overall survival period was defined as the time between the first operation and the date of last contact, either in person or by phone, or the date of death.

Patients were invited for follow-up visits every three months for the first two years after surgery, every six months for the next three years, and once a year thereafter. Patients who missed scheduled visits were contacted by phone for clinical information. In addition to symptoms, patients underwent physical examination, serum CEA level testing, chest radiography, and computerized and positron emission tomography (PET) imaging in case of doubt. Recurrence was diagnosed based on clinical findings, imaging, and histopathological results, if applicable. Recurrence was classified into three categories: local recurrence, distal recurrence, and both local and distal recurrence.

Resection specimens were analyzed for invasion depth, LN retention and count, histological type and grade, LVSI, PNI, lymphocyte infiltration, and presence of desmoplastic reaction. LVSI was considered positive when there were tumoral cells in endothelium-filled gaps or invasion of the lymphovascular wall by tumor cells. PNI was indicated by the presence of tumor cells around neural tissues that surrounded at least 1/3 of the neural tissue but did not invade the epineurium or nerve sheath. Tumor budding was defined as the presence of individual tumor cells or clusters of less than five tumor cells on the resection margin. Paraffin blocks were stained with standard hematoxylin and eosin stain and examined under a light microscope.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA). Descriptive statistics were presented as mean, and standard deviation. The normal distribution was tested using the Kolmogorov-Smirnov test. The student t-test was used to compare the mean of the variables that match the normal distribution, and the Mann–Whitney U test was used for variables that do not match the normal distribution. The chi-square test was used to compare the qualitative data. Survival analyses were performed with Kaplan-Meier curves. The log-rank test was used to compare the survival curves. First, univariate analysis was conducted to examine which variables had an impact on survival. Cox regression was then used in the multivariate analysis of the variables, which were statistically significant ($p<0.05$) in the univariate analysis. The results were assessed at a confidence interval of 95 percent. $P<0.05$ was considered statistically significant.

Results

One hundred twenty-four patients were operated with the diagnosis of rectal cancer. The mean age of the patients was 62 years (26 to 93 years), 42 patients were female (33.9%) and 82 patients were male (66.1%). Neoadjuvant therapy was administered to 87 patients

(70.2%). 99 patients (79.8%) received adjuvant chemotherapy, and only seven patients (5.6%) received adjuvant radiotherapy. Recurrence was observed in 45 patients (36.3%) in total. Recurrence arise as local in three patients, as distal metastasis in 31 patients, and as both local and distal recurrence in 11 of the patients. In total 47 patients (37.9%) died for a reason attributable to the disease. The localization of the tumor was recorded as the lower rectum for 31 patients (25%), the mid-rectum for 35 patients (28.2%), and the upper rectum for 58 patients (46.8%). CEA readings were available for 123 patients, and nine of them had a high CEA. When prognostic factors of the histopathological specimens were considered, 42 patients (33.9%) had PNI while 82 patients (66.1%) did not have PNI. Other clinical and histopathological features of the patients are given in Table 1.

Table 1. Demographic and clinicopathological features of the patients

	N	%
Sex	124	
Female	42	33.9
Male	82	66.1
Age (average=62)		
<62	65	52.4
≥62	59	47.6
Neoadjuvant therapy		
Administered	87	70.2
Not administered	37	29.8
Adjuvant therapy		
Chemotherapy	99	79.8
Radiotherapy	7	5.6
Recurrence		
Present	45	36.3
Not present	79	63.7
Localization of recurrence	(N=45)	
Local recurrence	3	6.7
Distal metastasis	31	68.9
Local + distal recurrence	11	24.4
Disease-related death		
Yes	47	37.9
No	77	62.1
Tumor localization		
Lower rectum	31	25.0
Middle rectum	35	28.2
Upper rectum	58	46.8
Elevated CEA levels	(N=123)	
Present	9	7.3
Not present	114	92.7
cTNM stage		
0	11	8.9
1	0	0
2	1	0.8
3	97	78.2
4	15	12.1
pT stage		
0	38	30.6
1	8	6.5
2	21	16.9
3	46	37.1
4	11	8.9

Table 1. Demographic and clinicopathological features of the patients (continued)

	N	%
pN stage		
0	81	65.3
1	25	20.2
2	18	14.5
Histopathology		
No residual tumor	7	5.6
Adenocarcinoma	24	19.4
Well-differentiated adenocarcinoma	5	4.0
Moderately differentiated adenocarcinoma	60	48.4
Poorly differentiated adenocarcinoma	12	9.7
Signet ring cell	1	0.8
Mucinous adenocarcinoma	15	12.1
Tumor budding		
Present	39	31.5
Not present	85	68.5
LVSI		
Present	47	37.9
Not present	77	62.1
Lymphocyte infiltration		
Present	56	45.2
Not present	68	54.8
Desmoplasia		
Present	55	44.4
Not present	69	55.6
PNI		
Present	42	33.9
Not present	82	66.1

CEA: carcinoembryonic antigen, LVSI: lymphovascular space invasion, PNI: perineural invasion

Classification of the clinicopathological features of the patients by the presence of PNI is summarized in Table 2. PNI was detected in 26, and not detected in 19, out of 45 patients with recurrence. Similarly, PNI is observed significantly more often in disease-related deaths. The pN stage tumor budding, LVSI and the number of metastatic lymph nodes are significantly higher in the patients with PNI. Lymphocyte infiltration and desmoplasia however, were significantly lower in patients with PNI. Additionally, there was no significant difference between the patients with PNI and without PNI in terms of sex, age, neoadjuvant treatment, recurrence patterns, localization of the primary tumor, CEA elevation, cTNM stage, pT stage, histopathological subtypes and the average number of lymph nodes extracted (Table 2).

Three-year survival rate was 89% for patients without PNI and 55% for patients with PNI (p<0.05). Five-year survival rates were 83% and 38% respectively. The mean survival was 153.6 months (134.8-172.4±9.6) for those without PNI, and 59.2 months (42.6-75.9±8.5) for those with PNI (p<0.05). Median survival was 48 months for the patients with PNI, and was not applicable for the patients without PNI since fewer than 50 percent of them were lost. Figure 1 shows the Kaplan–Meier curves of overall survival rate of the patients based on the presence of PNI.

Considering the factors that contribute to overall survival; neoadjuvant treatment, tumor localization, CEA elevation, cTNM stage, pT stage, pN stage, tumor budding, presence of LVSI, presence of PNI, presence of lymphocyte infiltration, and presence of desmoplasia were analyzed in univariate analysis. Of those variables, pN stage, tumor budding, LVSI, PNI, presence of lymphocyte infiltration and desmoplasia were found to affect the overall survival (Table 3). The factors that affected overall survival in univariate analysis were then analyzed by multivariate analysis using cox regression. The presence of PNI (HR 3.4; 1.7-6.7; 95 percent CI), lymphocyte infiltration (HR 0.41; 0.21-0.80; 95 percent CI) and desmoplasia (HR: 0.47; 0.23-0.94; 95 percent CI) were identified as independent variables that affected overall survival (Table 4).

Table 2. Comparison of clinicopathological characteristics of patients by PNI status

	PNI present (N=42)	PNI not present (N=82)	*p*
Sex			
Female	15 (35.7)	27 (32.9)	>0.05
Male	27 (64.3)	55 (67.1)	
Age (average=62)	61	62	
<62			>0.05
≥62			
Neoadjuvant therapy			
Administered	30 (71.4)	57 (69.5)	>0.05
Not administered	12 (28.6)	25 (30.5)	
Recurrence (percentages relate to the column)			
Present	26 (61.9)	19 (23.2)	**<0.001**
Not present	16 (38.1)	63 (76.8)	
Localization of recurrence (N=45)			
Local recurrence	2 (7.7)	1 (5.3)	>0.05
Distal metastasis	15 (57.7)	16 (84.2)	
Local + distal recurrence	9 (34.6)	2.4 (10.5)	
Disease-related death			
Yes	30 (71.4)	17 (20.7)	**<0.001**
No	12 (28.6)	65 (79.3)	
Tumor localization			
Lower rectum	10 (23.8)	21 (25.6)	>0.05
Middle rectum	17 (40.5)	18 (22.0)	
Upper rectum	15 (35.7)	43 (52.4)	
Elevated CEA			
Present	4 (9.5)	5 (6.1)	>0.05
Not present	38 (90.5)	76 (92.7)	
cTNM stage			
0	0	11 (13.4)	
1	0	0	>0.05
2	0	1 (1.2)	
3	36 (85.7)	61 (74.4)	
4	6 (14.3)	9 (11)	
pT stage			
0	12 (28.6)	26 (31.7)	
1	1 (2.4)	7 (8.5)	>0.05
2	4 (9.5)	17 (20.7)	
3	18 (42.9)	28 (34.1)	
4	7 (16.7)	4 (4.9)	
pN stage			
0	15 (35.7)	66 (80.5)	**<0.001**
1	10 (23.8)	15 (18.3)	
2	17 (40.5)	1 (1.2)	
Histopathology			
No residual tumor	0	7 (8.5)	
Adenocarcinoma	5 (11.9)	19 (23.2)	
Well-differentiated adenocarcinoma	1 (2.4)	4 (4.9)	>0.05
Moderately differentiated adenocarcinoma	22 (52.4)	38 (46.3)	
Poorly differentiated adenocarcinoma	5 (11.9)	7 (8.5)	
Signet ring cell	1 (2.4)	0	
Mucinous adenocarcinoma	8 (19)	7 (8.5)	
Tumor budding			
Present	26 (61.9)	13 (15.9)	**<0.001**
Not present	16 (38.1)	69 (84.1)	

Table 2. Comparison of clinicopathological characteristics of patients by PNI status (continued)

	PNI present (N=42)	PNI not present (N=82)	*p*
LVSI			
Present	29 (69.0)	18 (22.0)	**<0.001**
Not present	13 (31.0)	64 (78.0)	
Lymphocyte infiltration			
Present	13 (31.0)	43 (47.6)	**<0.05**
Not present	29 (69.0)	39 (52.4)	
Desmoplasia			
Present	13 (31.0)	42 (51.2)	**<0.05**
Not present	29 (69.0)	40 (48.8)	
Average number of LN extracted	10.3	7.9	>0.05
Metastatic LN count	3.4	0.5	**<0.001**

CEA: carcinoembryonic antigen, LVSI: lymphovascular space invasion, PNI: perineural invasion, $p<0.05$ revealed statisticaly significant

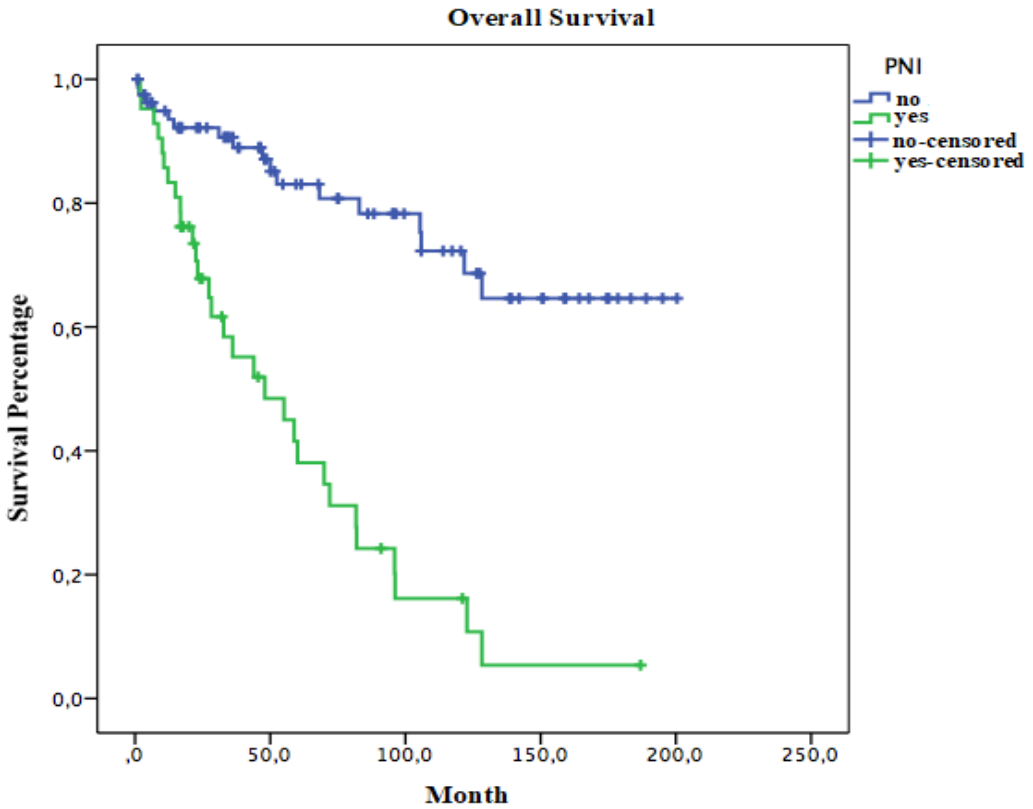

Figure 1. The Kaplan–Meier curve that shows the overall survival rates of patients by PNI status (Log rank test $p<0.05$)

Table 3. Univariate analysis of the factors that affect overall survival

Factor	*P*
Neoadjuvant therapy	>0.05
Tumor localization	>0.05
Elevated CEA	>0.05
cTNM stage	>0.05
pT stage	>0.05
pN stage	**<0.01**
Tumor budding	**<0.01**
LVSI	**<0.01**
PNI	**<0.01**
Lymphocyte infiltration	**<0.05**
Presence of desmoplasia	**<0.05**

CEA: carcinoembryonic antigen, LVSI: lymphovascular space invasion, PNI: perineural invasion
p<0.05 revealed statisticaly significant

Table 4. Multivariate analysis of the factors that affect overall survival

Factor	*P*	HR	95% CI
pN stage	>0.05		
Tumor budding	>0.05		
LVSI	>0.05		
PNI	**<0.001**	3.4	1.7-6.7
Lymphocyte infiltration	**<0.05**	0.4	0.2-0.8
Presence of desmoplasia	**<0.05**	0.5	0.2-0.9

LVSI: lymphovascular space invasion, PNI: perineural invasion, *p*<0.05 revealed statisticaly significant
HR:Hazard ratio, CI:confidence

The mean disease-free survival for the patients without PNI was 146.9 months (127.1-166.7±10) and the mean disease-free survival for the patients with PNI was 56.2 months (33.0-79.3±11.8) (*p*<0.05). Median disease-free survival was 32 months for the patients with PNI, and was not applicable to the patients without PNI since fewer than 50 percent of them had recurrence. Figure 2 shows the Kaplan–Meier curves of disease-free survival of the patients based on the presence of PNI.

While LN metastasis was not detected in 81 patients, it was present in 43 patients. Association of positive PNI with an increased risk of LN metastasis was shown above. The effect of the presence of PNI on the patients with and without LN metastasis was also investigated. Mean three-year survival rate was 87%, and mean survival was 148 months (127-170±10 months) for the patients without LN metastasis and PNI. Since less than half of the patients in this group died, the median survival value was not available. Mean three-year survival rate was 76%, and mean survival was 58 months in the presence of PNI for the patients without LN metastasis. Overall survival decreases significantly even in the absence of LN metastasis when PNI is present (*p*<0.01). Figure 3 shows the correlation between the presence of PNI and overall survival for the patients without LN metastasis.

Mean three-year survival rate was 92.3%, and average survival was 163 months (130-195±16 months) for the patients with LN metastasis without PNI. Since less than half of the patients in this group died, the median survival value was not available. Average three-year survival rate was 48%, and mean survival was 36 months in the presence of PNI for the patients with LN metastasis. Presence of PNI significantly reduces overall survival for the patients with LN metastasis as well (*p*<0.01). Figure 4 shows the correlation between the presence of PNI and overall survival for the patients with LN metastasis.

As presented in the figures above, three-year and five-year survival rates of the patients with PNI and without LN metastasis (76% and 46%, respectively) are lower than the three-year and five-year survival rates of the patients with LN metastasis and without PNI (92% and 82% respectively). In this respect, the presence of

PNI appears to be a more significant prognostic factor than LN metastasis for the survival of patients. It is seen that similar outcomes also apply to the disease-free survival periods.

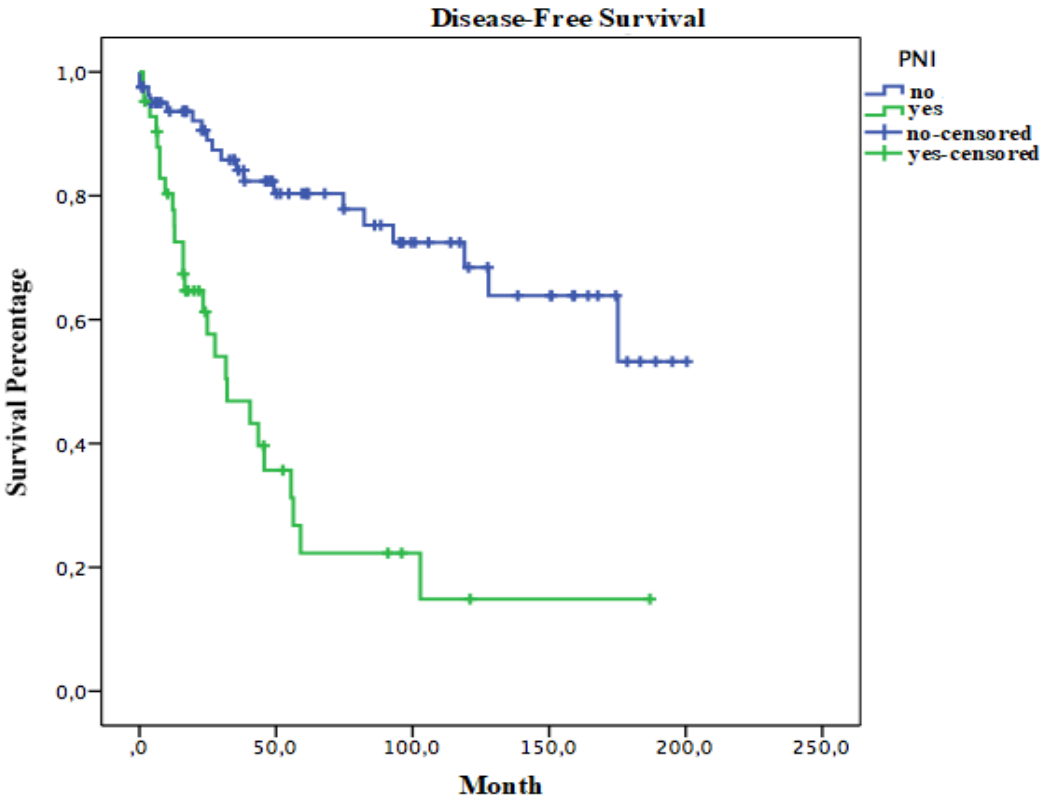

Figure 2. The Kaplan–Meier curve that shows the disease-free survival rates of patients by PNI status (Log rank test $p<0.05$)

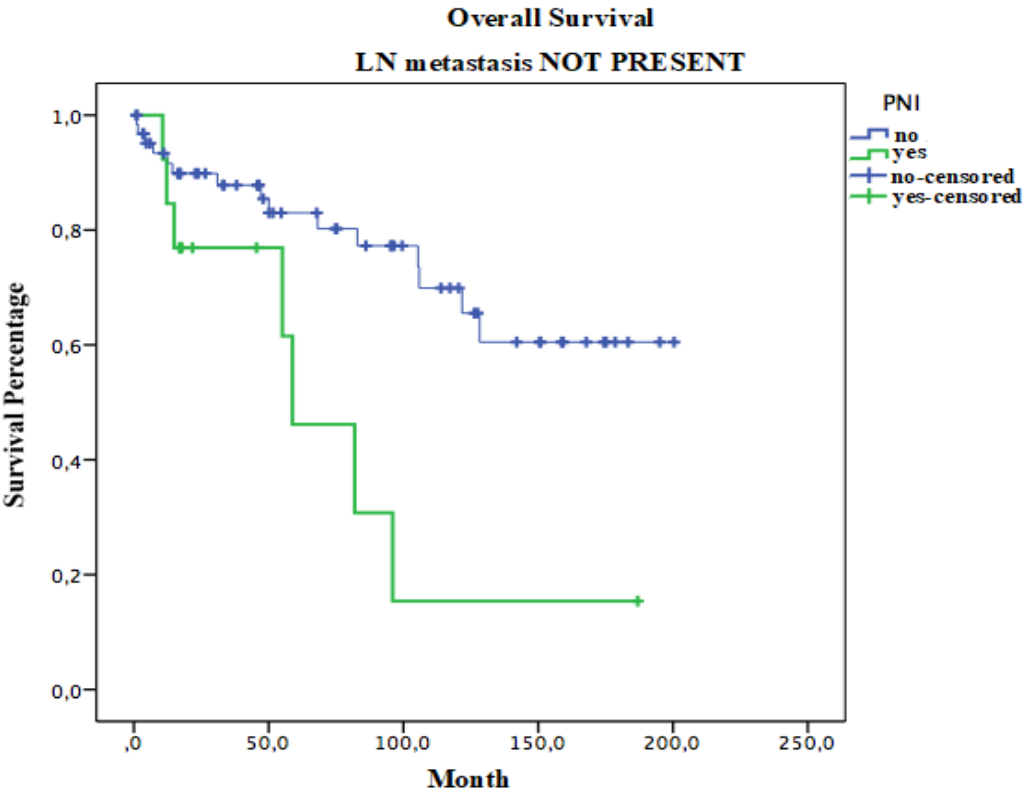

Figure 3. The Kaplan–Meier curve that shows the correlation between the presence of PNI and overall survival for the patients without LN metastasis (Log rank $p<0.01$)

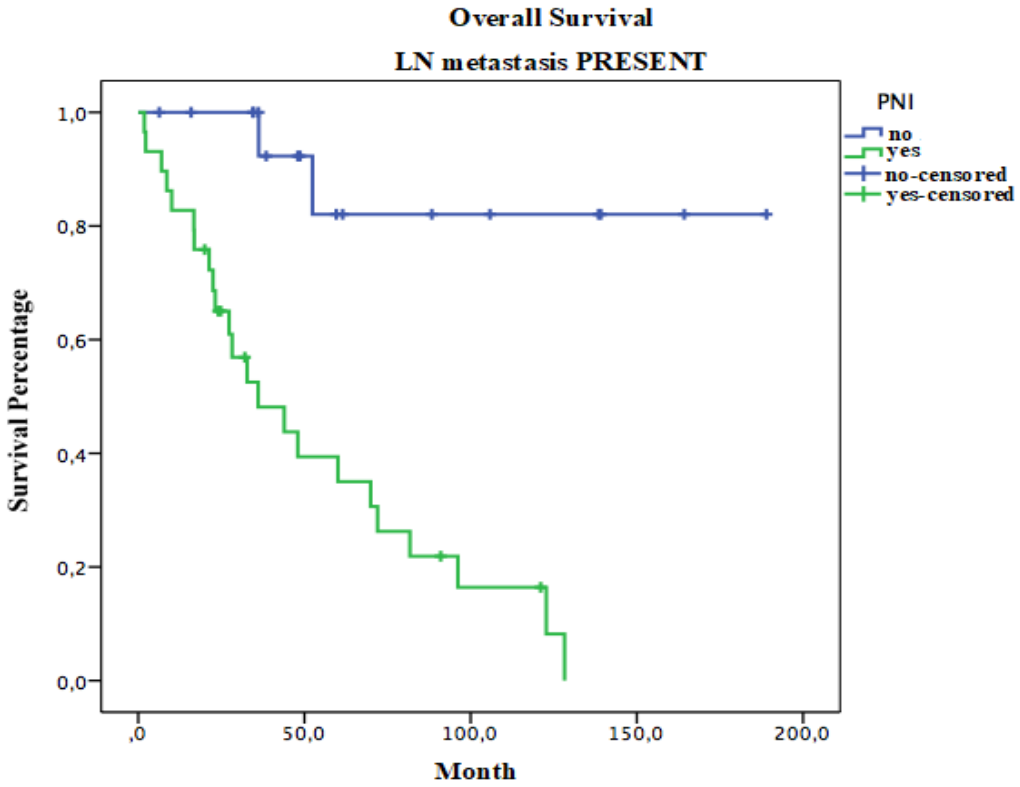

Figure 4. The Kaplan–Meier curve that shows the correlation between the presence of PNI and overall survival for the patients with LN metastasis (Log rank $p<0.01$)

Discussion

Our results documented that PNI was seen more frequently in deaths related to the disease. The average survival of patients with PNI was shorter than those without PNI. In the multivariate analysis in which other prognostic factors were also evaluated, the presence of PNI was found to be significantly associated with both overall survival and disease-free survival.

Rectal cancer is 1.5 times more prevalent in males than in females. Its incidence increases with age, and patients are diagnosed most often in the sixth and seventh decades [1, 2]. While female patients made up 33.9%, male patients accounted for 66.1% of our patient cohort, and the mean age of the patients was 62 years. Patient series in the literature revealed that the probability of a locally advanced stage tumor is higher, and vascular and neural invasion is more frequent on tumor margins in colorectal carcinoma cases diagnosed in patients aged under 60. It also revealed that young patients develop T4 and N2/3 tumors more often [7]. In contradiction to this data, the survival of young patients was significantly better than that of the older patients in our patient group.

PNI was defined for the first time as an indication of the spread of tumor to intracranial fossae in head and neck tumors [8]. There have been several recent studies on its prognostic importance in prostate cancer [9]. Indication of PNI in intrapancreatic tumors in pancreatic cancer indicates the spread of extrapancreatic tumor and invasion of extrapancreatic neural tissue [10]. Since colorectal cancers are the third most common cause of cancer-related deaths in developed countries, we aimed to conduct this study to investigate the effect of the presence of PNI on the prognosis of the disease. Rectal cancers are more likely to show PNI than colon cancers [5, 11]. A possible reason for this is that there are more rich nerve plexuses around the rectum than the colon. The higher rate of PNI associated with rectal cancers may explain why the prognosis of rectal cancers is worse than colon cancers at the same stage.

According to the AJCC cancer-staging manual (seventh edition), PNI is defined as a specific prognostic factor for colorectal cancer [12]. Nevertheless, some problems encountered in practical implementation of the definition of PNI await resolution. There is not a fully standardized criterion in the pathologic definition

of PNI in colorectal cancers in particular [13]. The level of aggrement among pathologists for the definition of PNI and reproducibility of the PNI diagnosis have not been studied yet.

According to the literature, the incidence rate of PNI in colorectal cancers is 10 to 35% [11]. In our series, it was 33.9% and it is coherent with the literature. The most significant reason for those different rates of incidence in the literature is attributable to the difference in the definition of PNI. While the presence of tumoral invasion around and within the neural tissue qualifies as PNI according to the definition made by Batsakis [14], Seefeld and Bargen require infiltration of the tumor into perineural and endoneural gaps before a diagnosis of PNI is reached [15]. According to recent studies, detection of extraneural tumoral invasion is also considered sufficient for the diagnosis of PNI [16]. Additionally, stricter definitions of PNI have also been made. Accordingly, it was suggested that diagnosis of PNI can be made only if tumoral cells are detected in the perineurium [17]. However, it was shown in the immunohistochemistry studies conducted with Glut1 that the perineurium cannot usually be seen by eosin staining, and when the tumors diagnosed with PNI using those criteria are re-examined, it was seen that tumoral invasion was actually outside the perineurium in a significant part of them [18]. Some researchers argue that PNI should be included in the staging system and reporting it in trichotomy is correlated with the prognosis. According to this view, it is emphasized that a correlation may be made with clinical prognosis when non-PNI cases are graded as Pn0, exclusively intramural PNI cases are graded as Pn1a, and extramural PNI cases are graded as Pn1b [19]. It is argued that this may also reduce the inter-observer variability in definition of PNI.

In our series, a significant difference in terms of prognosis was seen between patients with and without PNI, although there was not any significant difference between the cTNM stage and pT stage of the two groups. Although an association is expected between the presence of PNI and the size and aggressiveness of primary tumors, our series did not yield any statistical difference between the groups in this respect. The rate of LN metastasis was higher in PNI-positive cases. In addition, independently of LN mestastasis, detection of PNI in patients with and without LN metastasis was associated with poor prognosis. Moreover, both overall and disease-free survival rates of the patients who did not have PNI but had LN metastasis were better than those who had PNI but did not have LN metastasis. This indicates that presence of PNI is the most significant histopathological prognostic indicator of rectal cancer. Similarly, multivariate analyses in the literature showed that presence of PNI was a poor prognostic factor regardless of the stage of the disease [11].

Univariate analyses of previous studies established a correlation between the presence of PNI and an increase in the risk of locoregional recurrence risk, a decrease in five-year survival and an increase in the risk of metastatic disease [20-22]. Our study showed an increase in the risk of recurrence in the cases with PNI but recurrence patterns did not change based on the status of PNI.

As a result of our multivariate analysis, it was seen that the death risk of the PNI positive patients was 3.4 times higher than the PNI negative patients at the same stage. According to an 80-patient study conducted in Türkiye, in which factors that affected postoperative survival after curative surgery on rectal cancer were investigated, median survival was 26.12 months for the patients with PNI and 46.76 months for the patients without PNI [23].

As mentioned in the NCCN guidelines, presence of PNI should be determinant for the choice of adjuvant chemotherapy for stage 2 colorectal cancers in particular. An interesting outcome that we yielded in our study is that three-year survival rates of the patients without lymph node metastasis but with PNI was significantly lower than those who had lymph node metastasis but did not have PNI. This is an indication of a higher probability of recurrence and disease-related death for the patients with PNI who are at an early stage at the time of the operation. Therefore, one may think that patients who are in early stage but has PNI invasion may benefit from postoperative adjuvant treatment. However, no study has yet been conducted to clarify this. In this respect, Huh et al. [13] showed in their study that the presence of PNI in patients with early-stage colorectal carcinoma without lymph node metastasis indicated a poor

prognosis, which is in line with our findings.

A study conducted by the Japanese Society for Cancer of the Colon and Rectum (JSCCR) in recent years found a PNI rate of 17.7%, and established a correlation between PNI presence and the stages of T and N as well as tumor differentiation and LVSI [24]. It was shown in the multivariate analysis conducted under this polycentric study, where 2.485 patients were examined, that the presence of PNI is a poor prognostic factor for disease-free survival and overall survival. However, unlike in our study, immunohistochemical stains such as s100, synaptophysin and CD56 were used in the beginning to stain tumoral tissues in the aforementioned study.

In a study where patients with T1 and T2 colorectal cancer were examined, the presence of PNI in the early-stage disease was found to correlate with the lymph node metastasis [25]. Although the correlation of the presence of LVSI with lymph node metastasis in early-stage colorectal cancers is known, there are fewer studies in the literature on the importance of PNI in early-stage colorectal cancers.

In conclusion, according to the findings of our study, the presence of PNI is a prognostic factor independent of other poor prognostic factors, such as LVSI, LN metastasis or advanced stage of the disease, for both overall survival and disease-free survival.

Conflicts of interest: No conflict of interest was declared by the authors.

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Contributions of the authors to the article

U.S. and U.O. constructed and developed the theory, verified the analytical methods. U.O. and N.D. collected the patients' data. N.D. investigated the findings. U.O. performed the calculations, data analysis and created figures. U.S., N.D. and U.O. interpreted and discussed the results. U.O. provided critical feedback. U.O. wrote the manuscript with input from all authors. All authors discussed the results, reviewed and commented on the manuscript.