Can Lymphovascular and Perineural Invasion be Additional Staging Criteria in Colorectal Cancer?

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ABSTRACT

Objective: To identify additional staging information, venous, lymphatic, and neural invasion as potential prognostic factors in colorectal cancer (CRC).

Study Design: A descriptive study.

Place and Duration of the Study: University of Health Sciences, Tepecik Training and Research Hospital, Izmir, Turkey; from May 2007 to June 2019.

Methodology: Retrospective analyses were performed on 855 CRC patients, who were treated with surgery. Patient and treatment characteristics, lymphovascular (LVI), and perineural (PNI) invasion were documented. The impact of LVI and PNI was determined using Cox proportional hazards model.

Results: The cohort examined had 346 (40.5%) LVI and 150 (17.5%) PNI positive patients. After surgery, mortality was 18.4% for LVI and 8% for PNI patients. Although increased ASA score (for ASA 2 hazard ratio [HR]=0.555, p=0.001 and ASA 3-4 HR=0.723, p=0.014), adjuvant chemotherapy (HR=2.5, p<0.001), LVI (HR=1.961, p<0.001) and PNI (HR=1.625, p<0.001) involvement increased the risk of death based on univariate analysis, multivariate Cox analysis showed a risk of death increase with increased ASA score (for ASA 2 HR=0.53, p<0.001 and ASA 3-4 HR=0.703, p=0.008), adjuvant chemotherapy (HR=2.114, p<0.001) and LVI involvement (HR=1.640, p<0.001).

Conclusion: LVI and PNI may be useful in identifying CRC patients who might benefit the most from adjuvant systemic therapy. On the other hand, the presence of LVI and PNI reflects a shorter patient survival.

Key Words: *Lymphovascular invasion, Perineural invasion, Colorectal cancer, Cancer staging.*

INTRODUCTION

Colorectal cancer (CRC) is a disease that is usually encountered in the elderly population and is accompanied by unavoidably high morbidity and mortality. The incidence of CRC has been growing fast and is expected to increase by 60% by 2030.[1] Worldwide, CRC accounts for 9.4% of all incident cancer in men and 10.1% in women with adenocarcinoma being the most common type of CRC observed.[2,3] Besides, CRC is the second most common cause of cancer-related death in both men and women.[2,4]

Although recent advances in CRC treatment have improved patient prognosis and nearly 65% of patients attain 5-year overall survival in high-income countries, this parameter has remained below 50% in low-income countries.[5]

Many recent studies have reported that the stage at diagnosis was the most important prognostic factor in CRC patients. Brenner *et al.* reported that the 5-year relative survival of patients in the USA diagnosed with localised CRC was 90.1%, with regional spread CRC was 69.2%, and with distant tumor spread was 11.7%.[5] The localised spread of the tumor is mediated by the invasion of tumor cells; this is an important feature of solid tumors and is indicative of a poor prognosis.[6] Lymphovascular invasion (LVI) and perineural invasion (PNI) caused by localized tumor spread are considered poor prognostic criteria and affect treatment plans. However, LVI and PNI are not included in classifications.

In recent years, PNI has increasingly been considered to be relevant to the prognosis of CRC. The infiltration of cancer cells into nerve tissue not only provides a new pathway for metastasis but can biologically alter tumor behavior by providing a new environment for tumor cells.[7] Recent data concerning the prognostic significance of PNI on patients with CRC are conflicting, and the risk associated with PNI is currently unclear.[6-8]

The aim of this study was to investigate the relationship between LVI and PNI and overall survival in CRC, to examine

the clinical implications of LVI and PNI on outcomes, and to identify risk factors for poor prognosis in CRC patients.

METHODOLOGY

Retrospective analyses were performed on 855 CRC patients, surgically treated between May 2007 and June 2019. Demographic features of the patient population, carcinoembryonic antigen (CEA) levels, tumor features, administered treatment and surgical procedures as well as tumor classifications according to survival status, were evaluated. Patients' diagnosis, tumor subtype, histologic differentiation, and presence of lymphovascular invasion (LVI) or PNI were evaluated by histopathology after surgery. The histologic differentiation subtypes (none, cancer-*in-situ*, well-, moderate-, and poorly-differentiated) and American Joint Committee on Cancer (AJCC) staging system were evaluated according to survival status, PNI, and LVI of the patients.

The findings were analysed with patients categorised according to survival status, histologic differentiation, and AJCC staging system. The endpoint of this study was overall survival (OS), which was defined as the time from surgery to the date of last follow-up or death from any cause.

Software package SPSS for Windows version 25.0 (SPSS, Chicago, IL, USA) was used for statistical analyses. Descriptive statistics included mean and standard deviation. Crosstables were reported as percent ratio. Variables with continuous data were first ascertained for normality of distribution by the shape of the distribution pattern in the Kolmogorov-Smirnov test. The data were then statistically compared using independent sample t test for normally distributed samples. Variables with categorical data were compared using Chi-square test. Survival rates were assessed using the Kaplan-Meier method and compared with the log-rank test. The Cox proportional hazard model used to calculate the hazard ratio (HR) and 95% confidence interval (CI). Variables with $p < 0.1$ in the univariate analysis were entered into a multivariate Cox analysis with stepwise backward selection. Two-sided p value below 0.05 was considered to be statistically significant.

RESULTS

Table 1 summarises demographic and clinic pathologic characteristics of the patients (518 males, 337 females; mean age 62 ± 12 years, range 18 to 96 years) due to survival status. Overall mortality rate was 34.5% (n=295). The ratio of patients with both LVI positivity and mortality among all patients was 18.4%, while the ratio of LVI negative survivors among all patients was 43.4% ($p < 0.001$). Similarly, the ratio of patients with both PNI positivity and mortality among all patients was 8.0%, while the ratio of LVI negative survivors among all patients was 55.9% (p=0.002). The mortality rate was seen to increase statistically significantly with worsening histological differentiation and in IIIB, IIIC, and IVA stages of the AJCC staging system.

Analyses of demographic, clinicopathologic, and tumor classification characteristics of the patients according to LVI and PNI

are shown in Table II. Age, gender, and the ASA score did not show any statistically significant difference based on LVI and PNI. In patients undergoing adjuvant chemotherapy (n=576), LVI positivity was 47.7% and PNI positivity was 21.2%, which was statistically significant ($p < 0.05$). In patients undergoing neoadjuvant chemoradiotherapy (n=139), LVI positivity was 20.9% and negativity was 79.1%; while PNI positivity was 16.5%, and negativity was 83.5%. This difference was statistically significant for LVI ($p < 0.001$); but not for PNI (p=0.736). LVI positivity was detected in 53.2% of patients with mortality ($p < 0.001$), and PNI positivity was 23.1% for patients with mortality (p=0.002).

The univariate and multivariate Cox regression analyses for overall survival are presented in Table III. Univariate and multivariate analyses and Kaplan-Meier survival plots showed no significant difference between patients who were grouped as younger and older than 65 years. A higher ASA score was associated with a higher risk of death and statistically significant differences in OS were found between ASA subgroups. Patients who underwent AC had a greater risk of death and showed a significantly lower OS. The univariate analysis also showed that LVI and PNI were statistically significant risk factors. The LVI positive group showed a significantly lower OS, like PNI positive group. In the multivariate analysis, the variables associated with poor OS were increased ASA score, presence of LVI and undergoing AC. However, age and PNI did not show statistical significance in the multivariate analysis (Figure 1).

DISCUSSION

The aim of the current study was to investigate the prognostic significance of PNI in a population of CRC patients who underwent curative surgical resection, to evaluate the significance of PNI on clinical outcomes, and to identify risk factors leading to poor prognosis. The study results revealed that high ASA score, presence of LVI, and undergoing AC were the significant risk factors for mortality; moreover, these factors had a significant effect on OS. Although univariate analysis indicated the presence of PNI as a risk factor and showed a lower OS rate in a Cox hazard model, multivariate analysis did not support this data.

Early diagnosis, good tumor staging, as well as successful surgical and medical interventions are the cornerstones for improved outcomes in CRC patients.[1] Primary tumor staging is one of the most important steps in determining treatment strategy and predicting the prognosis of the disease in CRC patients.[9] The classification of the AJCC, according to the TNM stage, provides valuable prognostic information and guides therapy decisions.[5,10] Its success in predicting prognosis has been found to be effective in early and late stage patients; however, success in prognosis prediction is reported to be lower for intermediate-stage CRC patients.[2,3] Due to the inherent complications in intermediate-stage CRC, among the parameters of the currently used TNM staging system of AJCC, the depth of local invasion and anatomic extent of CRC at diagnosis have become the most important factors for predicting survival.[10]

Table I: Demographic and clinicopathologic characteristics of the patients and analyses for survival status (n=855).

Demographic and clinicopathologic characteristics		Survival status		
		Exitus	Alive	p
Age, n (%)	<65	182 (21.3)	323 (37.8)	0.256
	>65	113 (13.2)	237 (27.7)	
Gender, n (%)	Female	118 (13.8)	219 (25.6)	0.800
	Male	177 (20.7)	341 (39.9)	
Adjuvant chemotherapy, n (%)	Early mortality	33 (3.9)	0(0%)	<0.001
	Performed	203 (23.7)	373 (43.6)	
	Not Performed	59 (6.9)	187 (21.9)	
Neoadjuvant Chemoradiotherapy, n (%) Performed		39 (4.6)	100 (11.7)	0.081
American Society of Anesthesiologists Score, n (%)	1	46 (5.4)	121 (14.2)	0.003
	2	157 (18.4)	319 (37.3)	
	3-4	92 (10.8)	120 (14)	
Carcinoembryonic Antigen groups, n (%)	<5	152 (18.8)	378 (46.7)	<0.001
	5-10	40 (4.9)	83 (10.2)	
	>10	79 (9.8)	78 (9.6)	
Lymphovascular invasion, n (%)	Positive	157 (18.4)	189 (22.1)	<0.001
	Negative	138 (16.1)	371 (43.4)	
Perineural invasion, n (%)	Positive	68 (8.0)	82 (9.6)	0.002
	Negative	227 (26.5)	478 (55.9)	
Histologic differentiation, n (%)	None	11 (1.3)	31 (3.6)	0.013
	Cancer-in-situ	1 (0.1)	13 (1.5)	
	Well-differentiated	20 (2.3)	66 (7.7)	
	Moderately-differentiated	245 (28.7)	422 (49.4)	
	Poorly-differentiated	18 (2.1)	28 (3.3)	
The American Joint Committee on Cancer staging classification, n (%)	0	8 (1.0)	26 (3.0)	<0.001
	I	23 (2.7)	84 (9.8)	
	IIA	56 (6.5)	179 (20.9)	
	IIB	28 (3.3)	49 (5.7)	
	IIC	7 (0.8)	16 (1.9)	
	IIIA	4 (0.5)	23 (2.7)	
	IIIB	83 (9.7)	132 (15.4)	
	IIIC	40 (4.7)	30 (3.5)	
	IVA	39 (4.6)	17 (2)	
	IVB	1 (0.1)	1 (0.1)	
	IVC	6 (0.7)	3 (0.4)	

Despite advances in molecular and genetic markers that support staging systems, the presence of lymphovascular invasion (LVI) or perineural invasion (PNI) is not addressed in the T staging of TNM and is also not regarded as a prognostic factor that may affect the care of the patient.[1,10] Many studies have indicated that the presence of LVI or PNI has significant negative effects on prognosis in CRC.[6,7] By definition, detection of LVI, which refers to the invasion of tumor cells to the lymphatic or blood vessel wall by crossing the full fold, or PNI which refers to the growth of tumor in, around, and through nerves and nerve sheaths, suggests that the metastatic process has begun. As the tumor is no longer localised, LVI and PNI are likely to have a direct relationship with survival and recurrence.[1,2,10,11] In the decision for chemotherapy, radiotherapy, and surgical options, it is highly important to identify low risk patients who will do well with minimal intervention and those patients who have poor prognosis that need radical surgery and adjuvant treatments.[2] The literature highlights the importance of LVI and PNI in the evaluation of survival in CRC; however, there is currently no consensus on their unification with staging and decisions regarding treatment options.[2,11]

The most important characteristic of malignant cells is their ability to separate from the primary tumor and disseminate into the systemic circulation leading to metastasis. Metastasis is the primary factor that causes tumor recurrence and is associated with a poor prognosis.[4,12] LVI has been ascertained in many studies as a poor prognostic factor with suggestions to add the assessment of LVI to the current TNM staging system.[4,13,14] In the present study, light microscopy assessment indicated that LVI and PNI were found in CRC patients with post-treatment mortality, which was comparable with previously reported findings.[4] Additionally, the present study showed a significant correlation between increased serum levels of CEA, increased histological tumor grade, advanced tumor stage, and the presence of LVI and PNI. Both LVI and PNI are considered as indicators of the depth of invasion and spread to regional lymph nodes, and the presence of either condition may be considered as evidence of a more aggressive tumor.[5,7,15]

Similar to LVI, PNI is associated with metastatic progress, and is considered a poor prognostic factor in the literature.[2,16-19] In a review article, the process of PNI occurs by reciprocal signalling between tumor cells and nerves in which neurotrophic growth factors that are recognised as neurotrophins, play a major role.[7] Neurotrophins are considered as a therapeutic target because of the increased expression of neurotrophins by tumor cells is likely to be an escape mechanism from dependence on paracrine expression by stromal elements.[7,18]

Table II. Demographic, clinicopathologic, and tumor classification characteristics of the patients according to lymphovascular invasion and perineural invasion (n=855).

Demographic, clinicopathologic and tumor classification characteristics		Total	Lymphovascular invasion			Perineural invasion		
			Positive	Negative	p	Positive	Negative	p
Age groups, n (%)	<65	505 (59.1)	215 (25.1)	290 (33.9)	0.132	91 (10.6)	414 (48.4)	0.660
	>65	350 (40.9)	131 (15.3)	219 (25.6)		59 (6.9)	291 (34)	
Gender, n (%)	Female	337 (39.4)	128 (15)	209 (24.4)	0.232	54 (6.3)	283 (33.1)	0.346
	Male	518 (60.6)	218 (25.5)	300 (35.1)		96 (11.2)	422 (49.4)	
Adjuvant chemotherapy, n (%)	Early mortality	33 (3.9)	18 (2.1)	15 (1.8)	<0.001	9 (1.1)	24 (2.8)	<0.001
	Performed	576 (67.4)	275 (32.2)	301 (35.2)		122 (14.3)	454 (53.1)	
	Not Performed	246 (28.8)	53 (6.2)	193 (22.6)		19 (2.2)	227 (26.5)	
Neoadjuvant chemoradiotherapy, n (%)		139 (16.3)	29 (3.4)	110 (12.9)	<0.001	23 (2.7)	116 (13.6)	0.736
American Society of Anesthesiologists Score, n (%)	1	167 (19.5)	70 (8.2)	97 (11.3)	0.453	26 (3)	141 (16.5)	0.755
	2	476 (55.7)	198 (23.2)	278 (32.5)		86 (10.1)	390 (45.6)	
	3-4	212 (24.8)	78 (9.1)	134 (15.7)		38 (4.4)	174 (20.4)	
Carcinoembryonic antigen (ng/mL), Mean±SD		14.14±45.60	21.5±65.71	9.21±22.94	<0.001	23.57±54.74	12.16±43.22	0.007
Mortality, n (%)		295 (34.5)	157 (18.4)	138 (16.1)	<0.001	68 (8.0)	227 (26.5)	0.002
Lymphovascular invasion, n (%)		346 (40.5)	-	-	-	107 (12.5)	239 (28.0)	<0.001
Perineural invasion, n (%)		150 (17.5)	107 (12.5)	43 (5.0)	<0.001	-	-	-
Histologic differentiation, n (%)	None	42 (4.9)	4 (0.5)	38 (4.4)	<0.001	4 (0.5)	38 (4.4)	0.006
	Cancer-in-situ	14 (1.6)	0(0)	14 (1.6)		0(0)	14 (1.6)	
	Well	86 (10.1)	31 (3.6)	55 (6.4)		10 (1.2)	76 (8.9)	
	Moderately	667 (78.0)	283 (33.1)	384 (44.9)		121 (14.2)	546 (63.9)	
	Poorly	46 (5.4)	28 (3.3)	18 (2.1)		15 (1.8)	31 (3.6)	
The American Joint Committee on Cancer Staging System, n (%)	0	34 (4.0)	4 (0.4)	30 (3.6)	<0.001	3 (0.3)	31 (3.7)	<0.001
	I	107 (12.5)	10 (1.2)	97 (11.3)		5 (0.6)	102 (11.9)	
	IIA	235 (27.5)	69 (8.1)	166 (19.4)		26 (3)	209 (24.4)	
	IIB	77 (9.0)	20 (2.3)	57 (6.7)		12 (1.4)	65 (7.6)	
	IIC	23 (2.7)	10 (1.2)	13 (1.5)		3 (0.4)	20 (2.3)	
	IIIA	27 (3.2)	12 (1.4)	15 (1.8)		2 (0.2)	25 (2.9)	
	IIIB	215 (25.1)	120 (14)	95 (11.1)		51 (6.0)	164 (19.1)	
	IIIC	70 (8.2)	58 (6.8)	12 (1.4)		29 (3.4)	41 (4.8)	
	IVA	56 (6.5)	35 (4.1)	21(2.5)		16 (1.9)	40 (4.7)	
	IVB	2 (0.2)	2 (0.2)	0(0)		1 (0.1)	1 (0.1)	
	IVC	9 (1.1)	6 (0.7)	3 (0.4)		2 (0.2)	7 (0.8)	

Table III: Univariate and multivariate Cox regression analyses for overall survival.

|		Univariate Cox analyses			Multivariate Cox analysis		
		p	Hazard Ratio	95% Confidence Interval	p	Hazard Ratio	95% Confidence Interval
Age	65 and over	1 (Reference)			1 (Reference)		
	Under 65	0.145	0.84	0.664- 1.062	0.137	0.836	0.659- 1.059
American Society of Anesthesiologists Score	1	1 (Reference)			1 (Reference)		
	2	0.001	0.555	0.389- 0.791	<0.001	0.530	0.371-0.756
	3-4	0.014	0.723	0.559- 0.936	0.008	0.703	0.542-0.910
Lymphovascular invasion	Negative	1 (Reference)			1 (Reference)		
	Positive	<0.001	1.961	1.56- 2.466	<0.001	1.640	1.289-2.088
Perineural invasion	Negative	1 (Reference)			1 (Reference)		
	Positive	<0.001	1.625	1.238- 2.131	0.185	1.210	0.913-1.604
Adjuvant chemotherapy	Negative	1 (Reference)			1 (Reference)		
	Positive	<0.001	2.500	1.879- 3.328	<0.001	2.114	1.588- 2.814

Furuta *et al.* reported that signalling through the GDNF receptor *via* increased integrin expression can affect the adhesion of cancer cells to the extracellular matrix (ECM) proteins and invasion in CRC.[18] It is also reported that tumor cells express matrix metalloproteinases for migration through the ECM.[4,7]

As further studies illuminate the mechanisms for the metastatic processes in LVI and PNI, future therapeutic strategies targeting these processes may inhibit the metastatic spread of cancer and prolong the survival of patients with CRC.[2,4,7,20]

Firstly, the current study has a retrospective design and consists of patients' data from a single centre. Secondly, several or potentially relevant variables and outcome measures (*e.g.*, local and systemic recurrence), were not available or were incompletely reported in the patient database and, therefore, could not be considered in the analysis. Those patients whose surgical treatment was performed in the clinic where the study was conducted but other cancer treatments were performed at other centres, or those patients with missing information, were excluded from the study. The authors did not have access to data on baseline functional status, which strongly influences the treatment decision-making process and outcomes for cancer

patients. There was no access to information on specific peri-operative complications, chemotherapy regimen, toxicity profile, and the number of treatment cycles received by the subjects. Despite the noted limitations, this study contributes towards understanding the short- and long-term prognostic implications of PNI and LVI in patients with CRC.
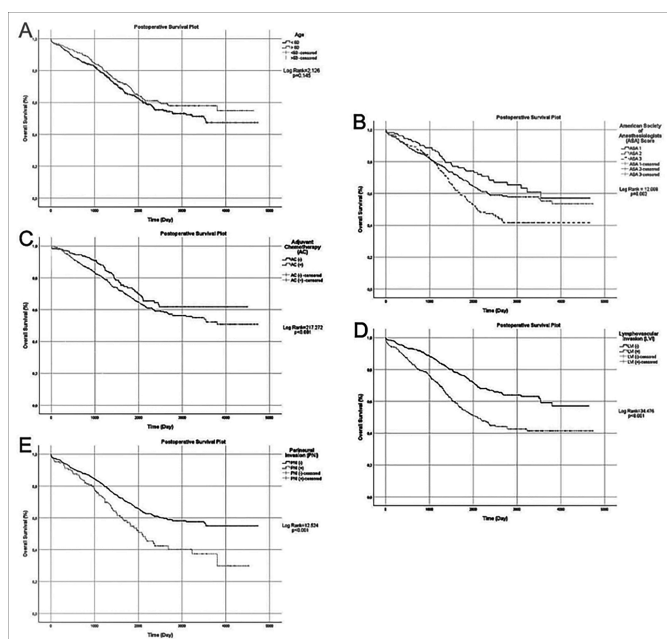
Figure 1. Cox regression analyses of overall survival after primer tumor resection of the colorectal carcinoma based on age (A), ASA score (B), adjuvant chemotherapy (C), histological lymphovascular invasion (LVI) (D) and perineural invasion (PNI) (E).

CONCLUSION

The results of this study support recent data on the prognostic significance of LVI and PNI that may provide a personalised estimate of prognosis as a guide for managing the treatment. Moreover, these findings will make a positive contribution and better clarify the role of LVI and PNI on the prognostication of CRC patients. Consequently, in the future, LVI and PNI may be added as criteria for staging CRC.

ETHICAL APPROVAL:
The study protocol was approved by the Ethics Committee of Tepecik Training and Research Hospital, Izmir, Turkey (Decision No. 2020/ 8-8).

PATIENTS' CONSENT:
Informed consents were obtained from all patients to publish the data concerning this study.

CONFLICT OF INTEREST:
The authors declared no conflict of interest.

AUTHORS′ CONTRIBUTION:
TK, AD: Conception and design, data collection, analysis and interpretation of data, literature search, drafting of the manuscript, writing and critical review, and final approval. Agreed to be responsible for all aspects of the study in

ensuring that questions regarding the accuracy or completeness of any part of the study are properly investigated and resolved.

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