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Intramural Component of Venous, Lymphatic, and Perineural Invasion in Colon Cancer: A Threat or an Illusion?

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Background: Extramural venous invasion is an independent predictor of poor outcome in colorectal cancer, whereas the significance of the intramural component of venous and lymphatic and perineural invasion is unclear.

Aims: To evaluate the prognostic impact of intramural components for venous, lymphatic, and perineural invasions and the relation of these invasion patterns with clinicopathological features in patients with colon cancer.

Study Design: A retrospective cross-sectional study.

Methods: The analysis included 626 patients with colon cancer in stages II and III. All patients were divided into four categories (no invasion, intramural invasion only, extramural invasion only, or both intramural and extramural invasions) for vascular invasion, lymphatic invasion and perineural invasion. The primary outcomes were 5-year disease-free and overall survival.

Results: Right-sided (for vascular invasion, 24.7% vs. 33.9%, p = 0.007; for perineural invasion, 34.5% vs. 41.5%, $p = 0.034$) and dMMR tumors (for vascular invasion, 13.5% vs. 33.5, $p < 0.001$; for perineural invasion, 25% vs. 41.4%, $p = 0.004$) exhibited less venous and perineural invasion. Compared with no invasion, presence of intramural invasion only, did not exert any effect on disease-free or overall survival for vascular invasion, lymphatic invasion, and perineural invasion. Multivariate analyses revealed that the presence of both intramural and extramural invasion was independently associated with poor disease-free and overall survival for venous (hazard ratios: 2.39, $p = 0.001$; hazard ratios: 2.46, $p = 0.001$), lymphatic (hazard ratios: 2.456, $p < 0.001$; hazard ratios: 2.13, $p = 0.02$) and perineural invasion (hazard ratios: 2.99, $p < 0.001$; hazard ratios: 2.68, $p < 0.001$), respectively.

Conclusion: Our data strongly advocates the importance of reporting intramural and extramural components of invasion since the presence of intramural invasion alone may not be considered as a high-risk factor for systemic recurrence.

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INTRODUCTION

Colorectal cancer remains one of the most common malignancies in both genders and the third leading cause of mortality due to cancer.[1] Recurrence and survival rates depend mainly on basal staging and histopathological characteristics of the tumor. Staging criteria for colon cancer include the extension of the tumor in the colonic wall, the presence of nodal metastases, and/or distant disease spread as precised in TNM.[2] Adjuvant chemotherapy is recommended for stage III disease and stage II proficient mismatch repair (pMMR)/microsatellite stable (MSS) colon cancer with high-risk factors, such as T4 tumor, presence of tumor obstruction or perforation, poor differentiation, insufficient number of sampled lymph nodes, presence of lymphovascular invasion, and/or perineural invasion (PNI).[3]

Extramural venous invasion (EMVI) is a denominator of the adverse prognosis in colorectal cancer. However, the implication of the intramural component for venous, lymphatic, and even PNI is usually underrated. Depending on the depth and spreading route within the colonic wall, the invasion patterns are mainly classified as no invasion, intramural only, extramural only, and invasion of both compartments. Despite the efforts to discriminate these invasion models, both the College of American Pathologists (CAP) and Association of Directors of Anatomic and Surgical Pathology underscore only constant reporting of EMVI during routine specimen assessment.[4,5] The Royal College of Pathologists also advocates reporting the deepest level of invasion for venous spread, that is, extra or intramural (submucosal or muscular) but not for lymphatic invasion (LI) or PNI.[6] Since the standards for reporting of invasion level for VI, LI, and PNI differ among centers, the interpretation of these variables while making clinical decisions for adjuvant systemic therapies is challenging.

The aim of the current study is to evaluate the prognostic impact of intramural components for venous, lymphatic, and PNIs and the relation of these invasion patterns with clinicopathological features in patients with colon cancer.

MATERIALS AND METHODS

Retrospective analysis was performed on patients whose histopathological specimens were examined at the Acibadem Healthcare Group after radical surgery between December 2014 and December 2020. Patients with synchronous tumors and those younger than 18 years old were excluded. Rectal cancer, stage I metastatic colon cancer, and metastatic colon cancer patients who had undergone surgery for primary tumor were excluded because of discrepancies in tumor biology, treatment modalities, and prognosis. After institutional ethical board approval, data of patients were assembled from documentation of the pathology department, patient visit notes, and the social security index.

The study exclusively comprised patients with stage II and stage III colon adenocarcinoma. Patient demographics, all tumor characteristics described in the original pathology report, postoperative therapy, disease progression, and survival parameters were included. The patients were grouped into four categories based on the depth of invasion patterns for VI, LI, and PNI: no invasion, intramural invasion only, extramural invasion only, or both (intramural and extramural). The standardized pathological examination was accomplished by the members of the gastrointestinal pathology team. The disease stage was determined using the eighth edition of the TNM staging system by the American Joint Committee on Cancer (AJCC) (2). Tumor grading was performed depending on the classification of the World Health Organization.[7] Left-sided tumors included the sigmoid colon, descending colon, and splenic flexure, whereas right-sided tumors comprised the cecum, ascending colon, hepatic flexure, and transverse colon.

Presence of vascular invasion in the submucosal or muscular layers of the colon was defined as intramural vascular invasion (IMVI). Detection of tumor cells within a space lined by endothelium, containing erythrocytes, or is surrounded by a muscle rim, was defined as extramural vascular invasion (EMVI).[8] When necessary, in addition to hematoxylin and eosin stain, additional methods, such as immunohistochemistry (IHC) or elastin stains, were used to detect EMVI. A histopathological picture of intramural and EMVI is shown in Figures 1 and 2.

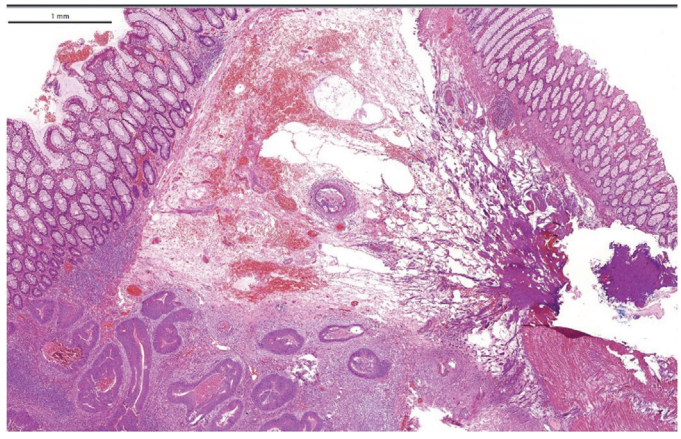

FIG. 1. Intramural venous invasion H&E x 3.1

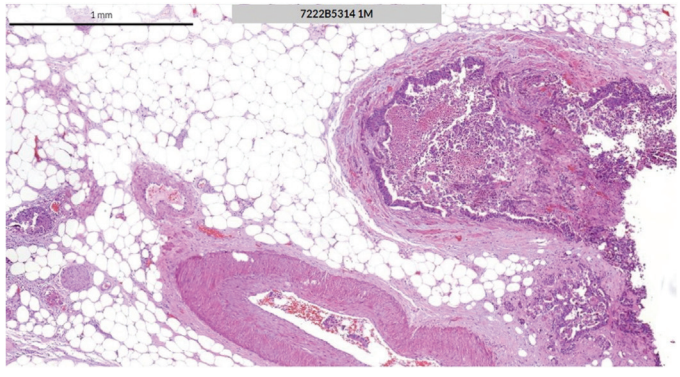

FIG. 2. Extramural venous invasion H&E x 26

LI was considered as positive only when cancer cells are detected within an endothelial-lined lymphatic channel. For discriminating LI from retraction artifact, D2-40 IHC was preferred to recognize explicitly the lymphatic vessel endothelial layer.[9] Detection of LI beyond the muscularis propria has been reported as an extramural invasion. If the invasion was limited to the submucosal and/or muscular layer, it was reported as an intramural invasion. A histopathological picture of intramural LI is shown in Figure 3.

PNI was defined as the presence of cancer cells within the perineurium of any nerve structure, such as the Meissner plexus or Auerbach plexus.[10,11] The detection of cancer cell spread along the Auerbach plexus zone was defined as intramural PNI, whereas invasion or spreading of tumor cells along nerve fascicles beyond the muscularis propria was defined as extramural PNI.[12] Histopathological pictures of intramural and extramural PNI are shown in Figures 4 and 5.

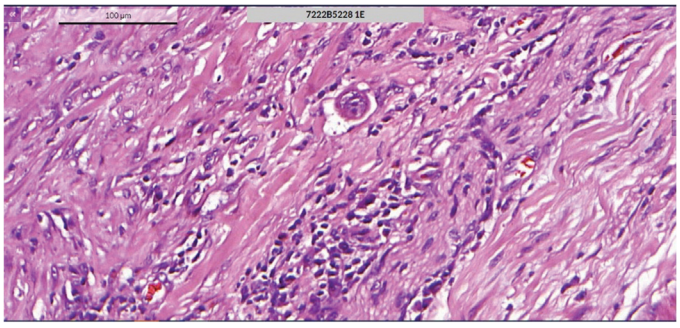

FIG. 3. Intramural lymphatic invasion . H&E x 43

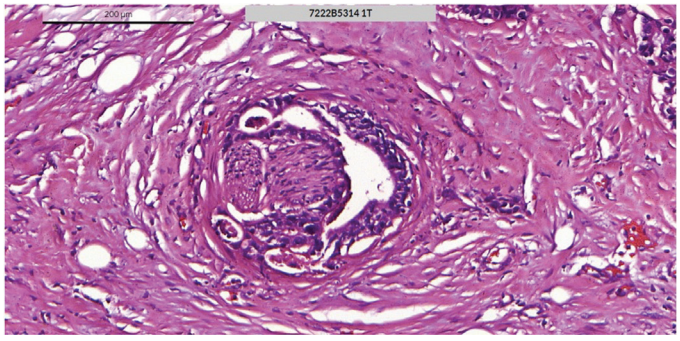

FIG. 4. Intramural perineural invasion H&E x 15

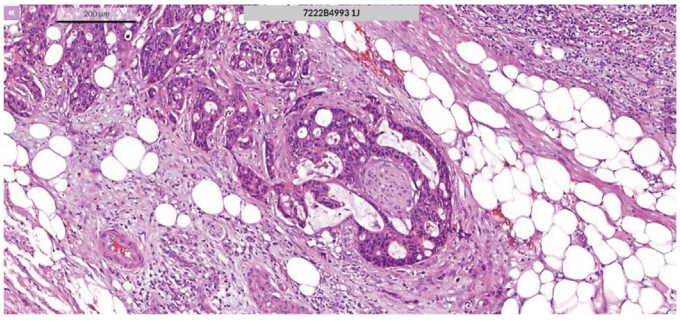

FIG. 5. Extramural perineural invasion H&Ex26

The primary outcomes of the study were 5-year disease-free survival (DFS) and overall survival (OS). Disease progression was defined as locoregional recurrence and/or newly emerged distant metastases. OS was the time interval from the date of surgical intervention to death for any reason. Data on long-term follow-up and survival were obtained from the TR Social Security Index. After evaluation of the final pathology report, the attending medical oncologist decided whether adjuvant chemotherapy was appropriate based on National Comprehensive Cancer Network (NCCN) guidelines and patient-related factors, such as performance status, comorbid diseases, and patient's consent to therapy.[13]

Estimation of the impact of VI, LI, and PNI on OS and DFS was made by the Kaplan-Meier method using a log-rank test. The chi-square (X^2) test was used to evaluate the association of these parameters with various categorical clinical and pathological variables. Based on the primary end points of the study, multivariate analyses were performed, including no invasion, IM invasion only, EM invasion only, and IM + EM invasion for VI, LI, and PNI, to identify independent factors associated with DFS and OS. Hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) were estimated.

The risk of lymph node metastasis increases around 10% with a gradual increase in T level, which refers to the invasion of the main tumor. Considering these data, we presume that the presence of any risk factor we evaluated will increase from 0% to 10%. The sample size, n = 73, in each group was required on the basis of α error of 0.05 and a β of 80%. $p < 0.05$ was assumed to be statistically significant and was derived from two-tailed tests.

RESULTS

Baseline Characteristics

Six hundred twenty-six patients (359 men, 57.3%) were included. The mean age of the patients was 62.58 ± 12.72. The cohort included 295 (47.1%) patients in stage II and 331 (52.9%) patients in stage III; 91.8% of patients in stage III and 37.3% in stage II received adjuvant chemotherapy. The histological and clinicopathological characteristics of the participants are outlined in Table 1.

VI was observed in 189 (30.1%) patients (43 [6.9%] patients with intramural only, 75 [11.9%] with extramural only, and 71 [11.3%] with both intra/extramural invasion). LI was detected in 349 (55.7%) patients (161 [25.7%] patients with intramural only, 18 [2.9%] with extramural only, and 170 [27.2%] with both intra/extramural invasion). PNI was detected in 242 (38.6%) patients (68 [10.9%] patients with intramural only, 68 [10.9%] with extramural only, and 106 [16.9%] with both intra/extramural invasion).

Correlation of Invasion Patterns with Clinicopathologic Factors

There was no statistically significant association between gender and VI, LI, or PNI. The depth and rate of LI were not related with tumor-sidedness. However, less VI and PNI was observed among right-sided tumors compared with left-sided tumors (for VI, 24.7% vs. 33.9%, $p = 0.007$; for PNI, 34.5% vs. 41.5%, $p = 0.034$) (Table 2).

Stage III patients exhibited consistently higher rates of PNI, LI, and VI compared with stage II patients (for PNI, 51.4% vs. 24.4%, $p < 0.001$; for LI, 69.8% vs. 39.7%. $p < 0.001$; for VI, 36.9% vs. 22.7%, $p < 0.001$). The rate of VI and PNI was significantly decreased for patients with dMMR tumors (for VI, 13.5% vs. 33.5, $p < 0.001$; for PNI, 25% vs. 41.4%, $p = 0.004$), but LI rates were similar compared with pMMR tumors.

The rate of PNI and LI was decreased for low-grade tumors compared with high-grade tumors (for PNI, 37.1% vs. 48.3%, $p = 0.031$; for LI, 53.2% vs. 71.3%, $p = 0.004$), while VI rates were similar for both groups.

TABLE 1. Demographic and Histopathological Characteristics of Patients

		n (%)
Gender	Male	359 (57.3)
Tumor location	Right-sided	255 (40.7)
	Left-sided	371 (59.3)
Stage	2	295 (47.1)
	3	331 (52.9)
T stage	T0/1/2	26 (4.2)
	T3/4	600 (95.8)
N stage	N0	295 (47.1)
	N1	236 (37.8)
	N2	95 (15.1)
Grade	Low	539 (86.1)
	High	87 (13.9)
Lymphatic invasion	Absent	277 (44.2)
	IMLI only	161 (25.7)
	EMLI only	18 (2.9)
	IMLI+EMLI	170 (27.2)
Vascular invasion	Absent	437 (69.8)
	IMVI only	43 (6.9)
	EMVI only	75 (12.0)
	IMVI+EMVI	71 (11.3)
Perineural invasion	Absent	384 (61.3)
	IMPNI only	68 (10.9)
	EMPNI only	68 (10.9)
	IMPNI + EMPNI	106 (16.9)
Mismatch repair	pMMR	522 (83.4)
	dMMR	104 (16.6)
Recurrence	Absent	530 (84.7)
	Present	96 (15.3)
	Locoregional	24 (3.8)
	Distant	72 (11.5)
Survival	Alive	537 (85.8)
	Dead	89 (14.2)

IMLI: intramural lymphatic invasion; EMLI: extramural lymphatic invasion; IMVI: intramural vascular invasion; EMVI: extramural vascular invasion; IMPNI: intramural perineural invasion; EMPNI: extramural perineural invasion; pMMR: proficient mismatch repair; dMMR: deficient mismatch repair

Survival Outcomes

Median follow-up time was 44.57 ± 19.62 (2-87) months. Ninety-six patients (15%) had disease recurrence, and 89 (14.2%) died during the follow-up. Twenty-four (3.8%) patients had locoregional recurrence, and 72 (11.5%) had distant metastases. Among the stage II and III patients who had received adjuvant chemotherapy, the rates of local and distal recurrences were 20 (4.8%) and 58 (14.1%), respectively.

The presence of venous, lymphatic, and PNI was strongly associated with poor DFS and OS (Table 3). The presence of "only" intramural VI, LI, and PNI and "no invasion" did not display statistically significant differences regarding estimated 5-year DFS and OS rates (for VI 87.2 vs. 88.4%, $p = 0.84$; 88.3 vs. 90.7%, $p = 0.90$; for LI 89.5 vs. 85.1%, $p = 0.13$; 89.5 vs. 89.4%, $p = 0.9$; for PNI 89.1 vs. 80.9%, $p = 0.26$; 90.6 vs. 84.8%, $p = 0.12$, respectively). However, the presence of both intramural and extramural compartment invasion was associated with worst DFS and OS compared to no invasion (for VI 87.2 vs. 73.2%, $p < 0.001$; 88.3 vs. 74.6%, $p < 0.001$; for LI 89.5 vs. 77.1%, $p < 0.001$; 89.5 vs. 81.8% $p = 0.001$; for PNI 89.1 vs. 70.8%, $p < 0.001$; 90.6 vs. 75.5%, $p < 0.001$, respectively).

Multivariate analyses revealed that the presence of both intramural and extramural invasion was independently associated with poor DFS and OS for venous (HR: 2.39; 95% CI, 1.42-4.03; $p = 0.001$; HR: 2.46; 95% CI, 2.46-4.14; $p = 0.001$), lymphatic (HR: 2.45; 95% CI, 1.51-3.97; $p < 0.001$; HR: 2.13; 95% CI, 1.32-3.43; $p = 0.02$) and PNI (HR: 2.99; 95% CI, 1.88-4.76; $p < 0.001$; HR: 2.68; 95% CI, 1.65-4.37; $p < 0.001$), respectively (Table 4). Detection of merely extramural venous and perineural compartment invasion without an intramural component demonstrated poor DFS but not OS (for VI, HR: 1.79; 95% CI, 1.02-3.12; $p = 0.04$; for PNI, HR: 1.86; 95% CI, 1.001-3.473; $p = 0.049$).

DISCUSSION

This is the largest series evaluating the prognostic significance of the intramural constituent of VI, LI, and PNI simultaneously on clinical outcomes of stage II and III colon cancer patients. Our results revealed that, compared with no invasion, the presence of IMVI without an extramural component did not have any effect on DFS or OS. In our patient population, the rates of EMVI and IMVI were 23.3% and 6.8%, respectively, which was consistent with the study by Leijjsen et al.[14] A meta-analysis evaluating the role of intramural invasion in colorectal cancer has reported the overall incidence of EMVI and IMVI as 24.3% and 12.5%, respectively.[15] Four of six articles in this meta-analysis that directly compared IMVI to EMVI did not show any significant differences in their prognostic impact. However, all these studies have included both rectum and colon cancer patients; thus, the results may be misleading due to distinct biological and molecular features of these cancers.

Vascular involvement is one of the most frequent routes of tumor propagation, mainly through venous and lymphatic vessels.[16] Yet dissemination of tumor along nerves is a less accentuated mode.

TABLE 2. Relationship of Invasion Patterns with Clinicopathologic Factors

		Female	Right-sided	Stage II	Mucinous*	Low grade	dMMR
Lymphatic invasion	**No invasion (*n* = 277)**	114 (41.2%)	124 (44.8%)	178 (63.3%)	98 (35.4%)	252 (90.9%)	50 (18.1%)
	IM only (*n* = 161)	82 (50.9%)	58 (36.1%)	71 (44.1%)	47 (29.2%)	137 (85.1%)	24 (14.9%)
	EM only (*n* = 18)	6 (33.3%)	4 (22.2%)	5 (27.8%)	8 (44.5%)	15 (83.3%)	4 (22.2%)
	Both (*n* = 170)	65 (38.2%)	69 (40.6%)	41 (24.1%)	62 (36.4%)	135 (36.5%)	26 (15.3%)
	p-value	.080	.083	<.001	.378	.004	.759
Vascular invasion	**No invasion (*n* = 437)**	193 (44.2%)	192 (43.9%)	228 (52.2%)	168 (38.4%)	374 (85.6%)	90 (20.6%)
	IM only (*n* = 43)	20 (46.5%)	18 (41.9%)	22 (51.2%)	12 (27.9%)	38 (88.4%)	6 (13.9%)
	EM only (*n* = 75)	26 (34.7%)	17 (22.7%)	22 (29.3%)	17 (22.7%)	68 (90.7%)	6 (8%)
	Both (*n* = 71)	28 (39.4%)	28 (39.4%)	23 (32.4%)	18 (25.4%)	59 (83.1%)	2 (2.8%)
	p-value	.404	.007	<.001	.011	.419	.001
Perineural invasion	**No invasion (*n* = 384)**	169 (44.0%)	167 (43.5%)	223 (58.1%)	137 (35.7%)	339 (88.3%)	78 (20.3%)
	IM only (*n* = 68)	27 (39.7%)	30 (44.1%)	30 (44.1%)	19 (27.9%)	52 (76.5%)	12 (17.6%)
	EM only (*n* = 68)	27 (39.7%)	17 (25%)	15 (22.1%)	20 (29.4%)	59 (86.8%)	6 (8.8%)
	Both (*n* = 106)	44 (41.5%)	41 (38.7%)	27 (25.5%)	39 (36.8%)	89 (83.9%)	8 (7.5%)
	p-value	.843	.034	<.001	.466	.031	.004

IM: intramural; EM: extramural; dMMR: deficient mismatch repair.
*Tumor tissue with mucinous component.

However, there is substantial evidence regarding the unfavorable impact of PNI on survival outcomes of colorectal cancer.[12,17,18] Accordingly, PNI had been integrated as an additional prognostic tool in the 7th edition of the *AJCC Cancer Staging Manual*, and as a poor-risk factor in the NCCN guideline that should be considered for adjuvant chemotherapy decision.[13,19] There is scarce data about the clinical significance of reporting the intramural and extramural component of PNI.[20-22] The Japanese Society for Cancer of the Colon and Rectum had suggested a grading system for PNI based on the location of PNI within the bowel and categorized cases as Pn0 (no PNI), Pn1a (intramural PNI only), and Pn1b (extramural PNI) in a multi-institutional study involving 2,845 patients with colorectal cancer.[12] The 5-year DFS rates were 88%, 70%, and 48%, for the three different categories, respectively. In our study, outcomes were also poorer with increasing depth of invasion for PNI; the 5-year DFS was 89.1%, 85.3%, 80.9%, and 70.8% for no PNI, intramural PNI only, extramural PNI only, and both, respectively.

The clinical significance of detecting extramural invasion without an intramural component has not been previously discussed in detail. Various studies have subdivided the vascular invasion patterns into three classes: IMVI only, EMVI only, and both IMVI and EMVI.[23-25] The overall rate of "EMVI only," according to these studies, including heterogeneous patient populations, was 15.3%.[15] EMVI without IMVI may be considered as an unnoticed intramural component that could have been detected with a cautious re-examination of pathological slides, an increase in the number of tissue blocks being analyzed, or with the aid of supplementary stains. However, "EMVI only" merits special attention rather than a misinterpretation since survival rates for this group have differed compared with the invasion of both intramural and extramural compartments in other studies, including the current analysis.[14,25]

Current guidelines regarding adjuvant systemic chemotherapy for node-positive patients are clear. However, the decision of adjuvant chemotherapy for MSS/pMMR stage II colon cancer is mainly dependent on the presence of high-risk features, such as T4 tumors, insufficient number of sampled lymph nodes, bowel obstruction or perforation, poor differentiation, and lymphovascular invasion. As outlined in our study, to identify the worst prognostic group mainly within stage II colon cancer, more explicit prognosticators rather than a general term of "lymphovascular invasion" are necessary.[26,27] Currently, personalized approaches with genetic tests, such as circulating tumor DNA or prognostic classifiers like Oncotype Dx or Immunoscore, are incorporated in the decision-making process for the adjuvant treatment of stage II colon cancer. However, such tests are still unavailable worldwide and cost effective to be applicable in routine daily practice, unlike histopathological analyses.[28-31] CAP guidelines recommend disclosing lymphovascular invasion as a combined entity and only entail reporting intramural and an extramural compartment for venous invasion, despite the prerequisite for a more comprehensive reporting practice to detect poor prognostic factors.[4] In accordance, the Royal College of Pathologists advises reporting the deepest level of venous invasion as extra or intramural (submucosal or muscular) separately, but similar attention is invalid for LI or PNI.[6] Our study is the first to elucidate the prognostic significance of intramural and extramural components for LI and PNI, likewise VI, within the same cohort.

Similar to VI, disease-free and OS rates for patients with an only intramural component of PNI and LI did not exhibit any difference compared with no invasion. Furthermore, the presence of both (intra and extramural) components of VI, LI, and PNI was an independent indicator of poorer outcomes.

TABLE 3. Invasion Specific Survival Outcomes

		Disease-free survival			Overall survival		
		n/event	5 yr	*p*	*n*/event	5 yr	p
Lymphatic	Absent	29/277	89.5%	.002	32/277	89.5%	.003
	IMLI only	24/161	85.1%		17/161	89.4%	
	EMLI only	4/14	77.8%		4/14	77.8%	
	EMLI+IMLI	39/170	77.1%		36/170	81.8%	
	Absent	29/277	89.5%	.133	32/277	89.5%	.917
	IMLI only	24/161	85.1%		17/161	89.4%	
	Absent	29/277	89.5%	.081	32/277	89.5%	.118
	EMLI only	4/14	77.8%		4/14	77.8%	
	Absent	29/277	89.5%	<.001	32/277	89.5%	.001
	EMLI+IMLI	39/170	77.1%		36/170	81.8%	
	IMLI only	24/161	85.1%	.370	17/161	89.4%	.098
	EMLI only	4/14	77.8%		4/14	77.8%	
	IMLI only	24/161	85.1%	.061	17/161	89.4%	.005
	EMLI+IMLI	39/170	77.1%		36/170	81.8%	
	EMLI only	4/14	77.8%	.992	4/14	77.8%	.995
	EMLI+IMLI	39/170	77.1%		36/170	81.8%	
Vascular	Absent	56/437	87.2%	.003	56/437	88.3%	.005
	IMVI only	4/43	88.4%		5/43	90.7%	
	EMVI only	16/75	78.7%		9/75	89.3%	
	IMVI+EMVI	19/71	73.2%		19/71	74.6%	
	Absent	56/437	87.2%	.844	56/437	88.3%	.904
	IMVI only	4/43	88.4%		5/43	90.7%	
	Absent	56/437	87.2%	.036	56/437	88.3%	.788
	EMVI only	16/75	78.7%		9/75	89.3%	
	Absent	56/437	87.2%	.001	56/437	88.3%	<.001
	IMVI+EMVI	19/71	73.2%		19/71	74.6%	
	IMVI only	4/43	88.4%	.146	5/43	90.7%	.790
	EMVI only	16/75	78.7%		9/75	89.3%	
	IMVI only	4/43	88.4%	.034	5/43	90.7%	.045
	IMVI+EMVI	19/71	73.2%		19/71	74.6%	
	EMVI only	16/75	78.7%	.323	9/75	89.3%	.034
	IMVI+EMVI	19/71	73.2%		19/71	74.6%	
Perineural	Absent	42/384	89.1%	<.001	41/384	90.6%	.001
	IMPNI only	10/68	85.3%		11/68	86.8%	
	EMPNI only	13/68	80.9%		10/68	83.8%	
	IMPNI+EMPNI	31/106	70.8%		27/106	75.5%	
	Absent	42/384	89.1%	.262	41/384	90.6%	.123
	IMPNI only	10/68	85.3%		11/68	86.8%	
	Absent	42/384	89.1%	.044	41/384	90.6%	.215
	EMPNI only	13/68	80.9%		10/68	83.8%	
	Absent	42/384	89.1%	<.001	41/384	90.6%	<.001
	IMPNI+EMPNI	31/106	70.8%		27/106	75.5%	
	IMPNI only	10/68	85.3%	.566	11/68	86.8%	.823
	EMPNI only	13/68	80.9%		10/68	83.8%	
	IMPNI only	10/68	85.3%	.040	11/68	86.8%	.208
	IMPNI+EMPNI	31/106	70.8%		27/106	75.5%	
	EMPNI only	13/68	80.9%	.153	10/68	83.8%	.142
	IMPNI+EMPNI	31/106	70.8%		27/106	75.5%	

IMLI: intramural lymphatic invasion; EMLI: extramural lymphatic invasion; IMVI: intramural vascular invasion; EMVI: extramural vascular invasion; IMPNI: intramural perineural invasion; EMPNI: extramural perineural invasion.

TABLE 4. Multivariate Analysis by Pathological Invasion Patterns

		Overall Survival		Disease-free Survival	
		HR (95% CI)	*p*	HR (95% CI)	*p*
Lymphatic	No invasion	Reference		Reference	
	IM invasion only	0.956 (0.531-1.722)	.880	1.513 (0.881-2.600)	.133
	EM invasion only	2.236 (0.790-6.328)	.129	2.464 (0.866-7.012)	.091
	IM+EM invasion	2.134 (1.324-3.438)	**.002**	2.456 (1.518-3.975)	**<.001**
Venous	No invasion	Reference		Reference	
	IM invasion only	0.936 (0.375-2.339)	.888	0.893 (0.358-2.230)	.809
	EM invasion only	1.096 (0.542-2.217)	.799	1.794 (1.028-3.129)	**.040**
	IM+EM invasion	2.461 (2.461-4.145)	**.001**	2.398 (1.424-4.038)	**.001**
Perineural	No invasion	Reference		Reference	
	IM invasion only	1.683 (0.865-3.276)	.126	1.467 (0.735-2.925)	.277
	EM invasion only	1.552 (0.777-3.099)	.213	1.864 (1.001-3.473)	**.049**
	IM+EM invasion	2.688 (1.652-4.372)	**<.001**	2.998 (1.884-4.769)	**<.001**

HR: hazard ratio; CI: confidence interval; IM: intramural; EM: extramural; OS: overall survival; DFS: disease-free survival.

There are several limitations to our study. Due to the relatively small number of patients and events for stage II disease, it was impossible to evaluate the prognostic effect and predictive value of the invasion patterns for adjuvant chemotherapy benefit specifically among this patient group. Detailed information regarding adjuvant chemotherapy was not available in some stage III patients.

The presence of intramural invasion only may not be considered as a high-risk factor for systemic recurrence. Our data strongly advocate the importance of consistently reporting intramural and extramural components of VI, LI, and PNI, which are not within the minimum essentials of current pathology guidelines.

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Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin.* 2022;72:7-33. [CrossRef]
2. Amin MB, Edge SB, Greene FL, et al, eds. AJCC Cancer Staging Manual. 8th ed. New York: Springer; 2017. [CrossRef]
3. Benson AB 3rd, Schrag D, Sommerfield MR, et al. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. *J Clin Oncol*. 2004;22:3408-3419. [CrossRef]
4. College of American Pathologists. Protocol for the Examination of Resection Specimens From Patients With Primary Carcinoma of the Colon and Rectum Version: 4.2.0.1. Protocol Posting Date: November 2021. [CrossRef]
5. Jass JR, O'Brien J, Riddell RH, Snover DC; Association of Directors of Anatomic and Surgical Pathology. Recommendations for the reporting of surgically resected specimens of colorectal carcinoma: Association of Directors of Anatomic and Surgical Pathology. *Am J Clin Pathol.* 2008;129:13-23. [CrossRef]
6. The Royal College of Pathologists. Standards and datasets for reporting cancers. Dataset for histopathological reporting of colorectal cancer. September 2018. [CrossRef]
7. Ahadi M, Sokolova A, Brown I, Chou A, Gill AJ. The 2019 World Health Organization Classification of appendiceal, colorectal and anal canal tumors: an update and critical assessment. *Pathology.* 2021;53:454-461. [CrossRef]
8. Talbot IC, Ritchie S, Leighton M, Hughes AO, Bussey HJ, Morson BC. Invasion of veins by carcinoma of the rectum: method of detection, histological features and significance. *Histopathology.* 1981;5:141-163. [CrossRef]
9. Ishii M, Ota M, Saito S, Kinugasa Y, Akamoto S, Ito I. Lymphatic vessel invasion detected by monoclonal antibody D2-40 as a predictor of lymph node metastasis in T1 colorectal cancer. *Int J Colorectal Dis.* 2009;24:1069-1074. [CrossRef]
10. Batsakis JG. Nerves and neurotropic carcinomas. *Ann Otol Rhinol Laryngol*. 1985;94:426-427. [CrossRef]
11. Betge J, Langner C. Vascular invasion, perineural invasion, and tumour budding: Predictors of outcome in colorectal cancer. *Acta Gastroenterol Belg*. 2011;74:516–529. [CrossRef]
12. Ueno H, Shirouzu K, Eishi Y, et al. Characterization of perineural invasion as a component of colorectal cancer staging. *Am J Surg Pathol.* 2013;37:1542–1549. [CrossRef]
13. National Comprehensive Cancer Network. Colon Cancer (Version 1.2022). [CrossRef]
14. Leijssen LGJ, Dinaux AM, Amri R, et al. Impact of intramural and extramural vascular invasion on stage II-III colon cancer outcomes. *J Surg Oncol*. 2019; 119:749–757. [CrossRef]
15. Knijn N, van Exsel UEM, de Noo ME, Nagtegaal ID. The value of intramural vascular invasion in colorectal cancer - a systematic review and meta-analysis. *Histopathology.* 2018;72:721-728. [CrossRef]
16. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. *Cell*. 2011;147:275–292. [CrossRef]
17. Liebig C, Ayala G, Wilks J, et al. Perineural invasion is an independent predictor of outcome in colorectal cancer. *J Clin Oncol.* 2009;27:5131–5137. [CrossRef]
18. Law WL, Chu KW. Anterior resection for rectal cancer with mesorectal excision : a prospective evaluation of 622 patients. *Ann Surg*. 2004;240:260-268. [CrossRef]

19. Edge SB, Byrd SR, Compton CC, Frıtz AG, Greene FL, Trottı A, editors. AJCC Cancer Staging Manual. 7th edition. Springer-Verlag; New York (NY): 2010:143-164. [CrossRef]
20. Cienfuegos JA, Martínez P, Baixauli J, et al. Perineural invasion is a major prognostic and predictive factor of response to adjuvant chemotherapy in stage I-II colon cancer. *Ann Surg Oncol.* 2017;24:1077–1084. [CrossRef]
21. Yang Y, Huang X, Sun J, et al. Prognostic value of perineural invasion in colorectal cancer: A meta-analysis. *J Gastrointest Surg.* 2015;19:1113–1122. [CrossRef]
22. Knijn N, Mogk SC, Teerenstra S, Simmer F, Nagtegaal ID. Perineural invasion is a strong prognostic factor in colorectal cancer: a systematic review. *Am J Surg Pathol.* 2016;40:103–112. [CrossRef]
23. Gibson KM, Chan C, Chapuis PH, Dent OF, Bokey L. Mural and extramural venous invasion and prognosis in colorectal cancer. *Dis Colon Rectum.* 2014;57;916-926. [CrossRef]
24. Minsky BD, Mies C, Recht A, Rich TA, Chaffey JT. Resectable adenocarcinoma of the rectosigmoid and rectum. II. The influence of blood vessel invasion. *Cancer.* 1988;61;1417-1424. [CrossRef]
25. Betge J, Pollheimer MJ, Lindtner RA, et al. Intramural and extramural vascular invasion in colorectal cancer: Prognostic significance and quality of pathology reporting. *Cancer.* 2012;118;628-638. [CrossRef]
26. Quasar Collaborative Group, Gray R, Barnwell J, et al. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. *Lancet.* 2007;370:2020–2029. [CrossRef]
27. Wilkinson NW, Yothers G, Lopa S, Costantino JP, Petrelli NJ, Wolmark N. Long-term survival results of surgery alone versus surgery plus 5-fluorouracil and leucovorin for stage II and stage III colon cancer: pooled analysis of NSABP C-01 through C-05. A baseline from which to compare modern adjuvant trials. *Ann Surg Oncol.* 2010;17:959–966. [CrossRef]
28. Anandappa G, Starling N, Begum R, et al. Minimal residual disease (MRD) detection with circulating tumor DNA (ctDNA) from personalized assays in stage II-III colorectal cancer patients in a U.K. multicenter prospective study (TRACC). *J Clin Oncol.* 2021;3. [CrossRef]
29. Tie J, Cohen JD, Lahouel K, et al. Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer. *N Eng J Med.* 2022;386:2261-2272. [CrossRef]
30. Srivastava G, Renfro LA, Behrens RJ, et al. Prospective multicenter study of the impact of oncotype DX colon cancer assay results on treatment recommendations in stage II colon cancer patients. *Oncologist.* 2014;19:492-497. [CrossRef]
31. Galon J, Hermitte F, Mlecnik B, et al. Immunoscore as a parameter predicting time to recurrence and disease-free survival in T4N0 stage II colon cancer patients. *J Clin Oncol.* 2020;38. [CrossRef]