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ORIGINAL SCIENTIFIC REPORT

Prognostic Impact of Perineural Invasion in Rectal Cancer After Neoadjuvant Chemoradiotherapy

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Abstract

Background Perineural invasion (PNI) has emerged as an important factor related to colorectal cancer spread; however, the impact of neoadjuvant chemoradiotherapy (nCRT) on PNI remains unclear. Herein, we investigated the prognostic value of PNI, along with lymphovascular invasion (LVI), in rectal cancer patients treated with nCRT.

Methods This single-center observational study of pathologic variables, including PNI and LVI, analyzed 1411 invasive rectal cancer patients (965 and 446 patients treated with primary resection and nCRT, respectively).

Results The overall detection rates of LVI and PNI were 16.7 and 28.8%, respectively. The incidence of LVI was significantly lower in patients treated with nCRT (8.1 vs. 20.6%, $P < .001$); this was confirmed by multivariate analysis. However, PNI was not affected by nCRT (with nCRT 28.3% vs. without nCRT 29.1%, $P = .786$). In the 446 patients with nCRT, multivariate analysis revealed that PNI was an independent prognostic factor for both disease-free survival (DFS) and overall survival (OS). For the prediction of both 5-year DFS and OS, the C-index for the combinations of T-stage with the PNI (TPNI) system showed favorable result, especially in patients with a total number of harvested lymph nodes <8.

Conclusion PNI is a meaningful prognostic factor for rectal cancer patients treated with nCRT, especially when <8 lymph nodes are harvested. The lack of influence of nCRT on the PNI incidence suggests that residual tumor cells with PNI are more radioresistant or biologically aggressive than those without.

Introduction

The extent of colorectal cancer (CRC) at diagnosis is the most important factor for predicting survival. Despite advances in incorporating molecular and genetic markers to staging systems, the currently used TNM staging system of the American Joint Committee on Cancer/the International Union Against Cancer concerns only the anatomic extent of disease [1, 2]. In this system, pathologic staging for rectal cancer overlaps with colon cancer in many important aspects. However, since the introduction of neoadjuvant chemoradiotherapy (nCRT) [3–5], the role of pathologic TNM staging following nCRT has not been only fully addressed also related to some limitation.

Particularly, while the TNM stage provides relatively accurate prognostic stratification for patients with early-stage and advanced-stage CRC, for patients with intermediate-stage disease (i.e., stage II), it is less informative [6]. Therefore, it is essential to ensure adequate lymph node dissection and thoroughness of the lymph node surgical specimens in these patients. However, the findings must be

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interpreted with caution when few lymph nodes are retrieved in rectal cancer patients treated with nCRT. For colon cancer, higher numbers of lymph nodes have been shown to be associated with better oncologic outcomes [7]. However, the number of lymph nodes retrieved in rectal cancer patients post-nCRT is lower than that in cases treated without nCRT, and some studies have shown an inverse correlation between the number of lymph nodes and survival [8–10].

In addition to accurate assignment of each disease stage, supplemental pathologic features have been suggested for further risk stratification of stage II CRC. For example, perineural invasion (PNI), lymphovascular invasion (LVI), surgical resection margin, and high tumor grade have been validated and used as adverse prognostic factors after additional chemotherapy. Similar to the pathologic TNM stage, these factors are currently not considered for the clinical decision-making process for adjuvant chemotherapy in rectal cancer following nCRT. However, recent studies have shown significant effects of PNI and LVI in locally advanced rectal cancer patients treated with nCRT, raising questions about the roles of these additional prognostic factors [11–15]. In particular, there is a growing interest in PNI as a key pathologic feature of a potential fourth route of cancer spread, in addition to the three well-known routes of direct invasion, lymphatic spread, and hematogenous spread.

Herein, we examined the clinical utility of PNI and LVI in predicting survival in a large prospective cohort of rectal cancer patients treated with nCRT. A quantitative comparison of the impacts of nodal metastatic status, PNI, and LVI on the patients' prognoses was made. We also examined the influence of nCRT on the incidences of PNI and LVI to provide additional insight into the biology of rectal cancer.

Methods

Patients

Prospectively collected data from 1767 patients with invasive rectal adenocarcinoma who underwent potentially curative surgery were reviewed retrospectively. Of them, patients with metastatic disease (n = 153), who underwent transanal mass excision (n = 137), and/or who achieved a pathologic complete response (n = 66) were excluded. The study was approved by the Institutional Review Board of Chonnam National University Hwasun Hospital. Written informed consent was obtained from all patients before the study.

The initial clinical assessment included digital rectal examination, rigid sigmoidoscopy, abdominopelvic and chest computed tomography, rectal magnetic resonance imaging, serum carcinoembryonic antigen (CEA) level assessment, and positron emission tomography. The distance from the anal verge to the lower edge of the tumor was measured using rigid sigmoidoscopy. nCRT consisted of 4500–5040 cGy in 25–28 fractions to the pelvis using the four-field box technique. During the first and fifth weeks of radiotherapy, 5-fluorouracil (425 mg/m^2/day) and leucovorin (20 mg/m^2/day) were intravenously administered. All procedures were required to meet the principles of total mesorectal excision or partial mesorectal excision when the tumor was located in the upper one-third of the rectum [16].

Tumor staging was determined using the seventh edition TNM system by 2 experienced gastrointestinal pathologists. Tumor regression induced by nCRT was defined by the ratio of fibrosis to residual viable tumor cells. The tumor regression grades (TRGs) were defined as TRG1 (<25% fibrosis), TRG2 (25–50% fibrosis), TRG3 (50–75% fibrosis), and TRG4 (>75% fibrosis). Pathologic complete response (pCR) was defined as the absence of viable tumor cells, with no lymph node involvement. PNI-positivity was defined as viable tumor cells within any layer of the nerve sheath or in the perineural space that involved > 33% of the nerve's circumference [14, 17]. LVI-positivity was assessed as tumor cells in an endothelial-lined channel [18]. No distinction between lymphatic/venous vessel invasion and mural/extramural invasion has been described [19].

Statistical analysis

The Chi-square test was used to analyze differences in categorical variables. Multivariate logistic regression was used to identify independent factors influencing the detection rates of PNI-positivity and LVI-positivity. The Kaplan–Meier method and log-rank test were used to evaluate the effects of each variable on survival. Statistical measure of survival discrimination was computed using the concordance index (C-index), a generalization of the area under the receiver operating characteristic curve, in which values close to 1.0 and 0.5 indicate perfect and no predictive discrimination, respectively [20]. Multivariate survival analyses were performed using Cox regression models with calculation of the hazard ratios (HRs) and 95% confidence intervals (CIs). P values <.05 were considered significant. Statistical analysis was performed using R statistical software, version 3.1.1 (R Project for Statistical Computing, Vienna, Austria).

Results

The overall detection rates of LVI and PNI were 16.7 and 28.8%, respectively. Both LVI and PNI were associated with more aggressive clinical and pathologic tumor

Table 1 Clinicopathologic characteristics depending on the absence or presence of perineural invasion and lymphovascular invasion

	Perineural invasion			Lymphovascular invasion		
	Negative	Positive	*P*	Negative	Positive	*P*
Age (years)			.190			1
<65	490 (69.5)	215 (30.5)		588 (83.4)	117 (16.6)	
≥65	514 (72.8)	192 (27.2)		588 (83.3)	118 (16.7)	
Gender			.463			.369
Male	671 (71.8)	263 (28.2)		772 (82.7)	162 (17.3)	
Female	333 (69.8)	144 (30.2)		404 (84.7)	73 (15.3)	
Tumor location			.361			.072
Low	310 (72.9)	115 (27.1)		370 (87.1)	55 (12.9)	
Middle	469 (70.1)	200 (29.9)		548 (81.9)	121 (18.1)	
Upper	179 (71.9)	70 (28.1)		206 (82.7)	43 (17.3)	
Tumor size			**<.001**			.130
<4	552 (76.6)	169 (23.4)		612 (84.9)	109 (15.1)	
≥4	452 (65.5)	238 (34.5)		564 (81.7)	126 (18.2)	
Tumor stage			**<.001**			**<.001**
T0–Tis	8	0		8	0	
T1	172 (96.6)	6 (3.4)		161 (90.4)	17 (9.6)	
T2	279 (90.9)	28 (9.1)		270 (87.9)	37 (12.1)	
T3	516 (60.9)	331 (39.1)		693 (81.8)	154 (18.2)	
T4	29 (40.8)	42 (59.2)		44 (62.0)	27 (38.0)	
Nodal involvement			**<.001**			**<.001**
N0	727 (81.0)	171 (19.0)		808 (90.0)	90 (10.0)	
N1	215 (62.9)	127 (37.1)		278 (81.3)	64 (18.7)	
N2	62 (36.3)	109 (63.7)		90 (52.6)	81 (47.4)	
AJCC stage			**<.001**			**<.001**
1	388 (93.7)	26 (6.3)		375 (90.6)	39 (9.4)	
2	341 (70.2)	145 (29.8)		435 (89.5)	51 (10.5)	
3	275 (53.8)	236 (46.2)		366 (71.6)	145 (28.4)	
Differentiation			**<.001**			**<.001**
Well	441 (78.6)	120 (21.4)		498 (88.8)	63 (11.2)	
Moderately	499 (65.9)	258 (34.1)		607 (80.2)	150 (19.8)	
Poorly/mucinous	48 (62.3)	29 (37.7)		55 (71.4)	22 (28.6)	
Circumferential resection margin			**<.001**			**<.001**
Involved	55 (47.8)	60 (52.1)		82 (71.3)	33 (28.3)	
Not involved	949 (67.3)	347 (24.6)		1094 (84.4)	202 (15.6)	
No. of lymph nodes analyzed			.172			.686
<12	211 (74.6)	72 (25.4)		233 (82.3)	50 (17.7)	
≥12	790 (70.2)	335 (29.8)		940 (83.6)	185 (16.4)	
Operation type			**.037**			**<.001**
LAR	774 (71.6)	307 (28.4)		888 (82.1)	193 (17.9)	
ISR	167 (74.6)	57 (25.4)		204 (91.1)	20 (8.9)	
APR	56 (59.6)	38 (40.4)		73 (77.7)	21 (22.3)	
Hartmann's op.	7 (58.3)	5 (41.7)		11 (91.7)	1 (8.3)	
Neoadjuvant CRT			.786			**<.001**
No	684 (70.9)	281 (29.1)		766 (79.4)	199 (20.6)	
Yes	320 (71.7)	126 (28.3)		410 (91.9)	36 (8.1)	

Table 1 continued

	Perineural invasion			Lymphovascular invasion		
	Negative	Positive	*P*	Negative	Positive	*P*
Preoperative CEA			**<.001**			**.007**
<5	736 (75.5)	239 (24.5)		828 (84.9)	147 (15.1)	
≥5	244 (61.0)	158 (39.3)		317 (78.9)	85 (21.1)	

Bold font indicates significance

APR abdominoperineal resection, *CEA* carcinoembryonic antigen, *CRT* chemoradiotherapy, *LAR* low anterior resection, *ISR* intersphincteric resection

features (higher T- and N-stages, poor differentiation, more frequent circumferential resection margin involvement, and elevated preoperative serum CEA levels). However, the detection rate of LVI was significantly lower in patients treated with versus without nCRT (8.1 vs. 20.6%, $P < .001$), whereas no difference was observed for PNI (28.3 vs. 29.1%, $P = .786$) (Table 1). In the multivariate logistic regression analysis, the PNI-positivity rate did not correlate with nCRT, whereas the LVI-positivity rate was almost three times lower in tumors treated with versus without nCRT (odds ratio 2.804; 95% CI 1.922–4.191; $P < .001$; Table 2). This observation was more remarkable when the patients were stratified by pathologic stage. While no difference in PNI-positivity was observed between patients treated with or without nCRT in each pathologic stage, the LVI-positivity rate was significantly decreased in each stage ($P = .006$, .005, and <.001 for stages I–III, respectively; Table 3).

Overall, the median follow-up in the 446 patients treated with nCRT was 33.4 months (interquartile range 22.6–53.4). Forty-three (9.6%) and 125 (28.0%) patients experienced local recurrence and distant metastasis, respectively. The prognostic significance of PNI, LVI, and other clinicopathologic variables was investigated by univariate analyses (Table 4). PNI-positivity ($P < .001$) and LVI-positivity ($P < .001$), the T-stage ($P < .001$), N-stage ($P < .001$), and circumferential resection margin involvement ($P < .001$) were related to poorer 5-year disease-free survival (DFS). Similar results were observed for overall survival (OS). In addition to the factors associated with

Table 2 Multivariate logistic regression analyses for perineural invasion and for lymphovascular invasion

Predictive factor	Perineural invasion			Lymphovascular invasion		
	OR	95% CI	*P*	OR	95% CI	*P*
Tumor stage (T0–T2 vs. T3–T4)	6.651	4.597–9.899	**<.001**	–	–	–
Nodal stage (negative vs. positive)	2.324	1.798–3.007	**<.001**	3.154	2.346–4.259	**<.001**
Tumor differentiation (W vs. M/P)	1.510	1.156–1.978	**.002**	1.831	1.331–2.545	**<.001**
Neoadjuvant CRT (no vs. yes)	–	–	–	2.804	1.922–4.191	**<.001**

Bold font indicates significance

CRT chemoradiotherapy, *M* moderately, *P* poorly, *W* well

Table 3 Association between perineural invasion and lymphovascular invasion and neoadjuvant CRT according to TNM stage

	Perineural invasion					Lymphovascular invasion				
	Neoadjuvant CRT (−)		Neoadjuvant CRT (+)		*P*	Neoadjuvant CRT (−)		Neoadjuvant CRT (+)		*P*
	Negative	Positive	Negative	Positive		Negative	Positive	Negative	Positive	
Stage 1	269 (94.1)	17 (5.9)	119 (93.0)	9 (7.0)	.839	251 (87.8)	35 (12.2)	124 (96.9)	4 (3.1)	**.006**
Stage 2	221 (71.5)	88 (28.5)	120 (67.8)	57 (32.2)	.447	267 (86.4)	42 (13.6)	168 (94.9)	9 (5.1)	**.005**
Stage 3	194 (52.4)	176 (47.6)	81 (57.4)	60 (42.6)	.359	248 (67.0)	122 (33.0)	118 (83.7)	23 (16.3)	**<.001**

Bold font indicates significance

CRT chemoradiotherapy

Table 4 Disease-free and overall survival among 446 rectal cancer patients who underwent radical resection after neoadjuvant chemoradiotherapy

Factor	Patients No.	Disease-free survival 5 years (%)	*P*	Overall survival 5 years (%)	*P*
All	446	63.7			
Age (years)			.481		.181
≤65	219	57.6		82.1	
>65	227	57.9		64.2	
Sex			.328		.88
Male	318	58.3		80.4	
Female	128	58.5		83.7	
Distance from AV (cm)			.374		**.041**
≤4	140	62.8		68.8	
>4	306	55.7		82.8	
ypT category			**<.001**		**.007**
1–2	152	74.6		89.9	
3–4	294	49.2		75.4	
ypN category			**<.001**		**.008**
Negative	303	67.7		84.9	
Positive	143	38.4		72.5	
CRM			**<.001**		**<.001**
Negative	404	60.5		84.0	
Positive	42	34.6		51.3	
Differentiation			.836		.079
Well/moderate	412	59.7		81.6	
Poorly	33	50.0		74.7	
Lymphovascular invasion			**<.001**		**<.001**
No	410	61.7		83.2	
Yes	36	19.3		58.2	
Perineural invasion			**<.001**		**<.001**
No	320	68.6		86.9	
Yes	126	29.4		64.3	
Grouped TRG			.227		**.037**
2 + 3	290	57.4		78.0	
0 + 1	156	59.8		87.9	
Preoperative CEA (ng/ml)			.123		**.006**
<5	335	61.7		83.3	
≥5	108	48.2		74.4	
Harvested lymph nodes			.424		.098
<8	55	63.5		70.8	
≥8	391	58.0		84.2	

Bold font indicates significance

AV anal verge, *CEA* carcinoembryonic antigen, *CRM* circumferential resection margin, *TRG* tumor regression grade

DFS, the distance from the anal verge ($P = .041$), TRG ($P = .037$), and preoperative serum CEA level ($P = .006$) were also related to the 5-year OS. Cox multiple regression was used to identify independent prognostic factors of survival (Table 5). After controlling for the T-stage, N-stage, and circumferential resection margin involvement, both PNI-positivity (HR 1.968; 95% CI 1.207–2.773; $P < .001$) and LVI-positivity (HR 1.687; 95% CI 1.076–2.646; $P = .022$) were significantly and independently associated with worse DFS. PNI-positivity was associated with a twofold risk of death (HR 2.018; 95% CI 1.102–3.694; $P < .001$) in our cohort. However,

Table 5 Multivariate analysis of different covariables on 5-year disease-free survival and overall survival after neoadjuvant CRT

Variable (reference)	Disease-free survival			Overall survival		
	HR	95% CI	*P*	HR	95% CI	*P*
ypN category (N0 vs. N+)	1.755	1.253–2.457	**.001**	–	–	–
ypT category (T0–T2 vs. T3–T4)	1.452	0.950–2.219	.084	–	–	–
Perineural invasion (negative vs. positive)	1.968	1.207–2.773	**<.001**	2.018	1.102–3.694	**<.001**
Lymphovascular invasion (negative vs. positive)	1.687	1.076–2.646	**.022**	–	–	–
TRG (<50 vs. ≥50%)	–	–	–	0.448	0.259–0.776	**.004**
CRM (positive vs. negative)	0.590	0.377–0.925	**.021**	0.382	0.198–0.736	**.004**
Total lymph nodes (<8 vs. ≥8)				0.484	0.248–0.946	**.033**

Bold font indicates significance

CRT chemoradiotherapy, *TRG* tumor regression grade, *CRM* circumferential resection margin

the N-stage and LVI-positivity did not significantly predict OS.

Given the prognostic importance of these pathologic variables, novel staging systems were developed using combinations of the T-stage with the LVI (TLVI; 1: T0–T2 and LVI-negative, 2: T3–T4 and LVI-negative, and 3: any T and LVI-positive), PNI (TPNI; 1: T0–T2 and PNI-negative, 2: T3–T4 and PNI-negative, and 3: any T and PNI-positive), and nodal statuses (TNM stages). The associations of these systems with survival in stages I–III disease are shown in Fig. 1. All systems significantly predicted both the 5-year DFS and OS. To quantify which system best predicted the actual outcome, we calculated the C-index for each system using the 5-year DFS data (Table 6). The C-indices for the TPNI, TLVI, and TNM systems were 0.651, 0.611, and 0.627, respectively. Similar results were obtained for the 5-year OS, indicating a modest improvement in prognostic discrimination using TPNI. Finally, subgroup analyses were performed according to the total number of harvested lymph nodes (<8 and ≥8) (Figs. 2, 3). Regardless of the number of harvested lymph nodes, the TPNI system provided superior predictive value for the risk stratifications of both DFS and OS (Table 7). In particular, although neither the TNM nor TLVI system could predict 5-year DFS (P = .116 and .072, respectively) in cases with insufficient lymph nodes (<8), the TPNI system was not only significantly associated with the 5-year DFS (P = .018), but also provided the highest C-index (TNM 0.583; TPNI 0.634; TLVI 0.599).

Discussion

Pathologic TNM staging is a well-established independent prognostic predictor in locally advanced rectal cancer after nCRT [21–27]. A German study reported the prognostic significance of the pathologic T and N categories [22], and, recently, long-term data (median follow-up 132 months) have confirmed these findings [27]. The authors reported that the 10-year DFS rates were related to the pathologic tumor stage (89.5, 95.2, 77.9, 65.7, and 40.0% for ypT0, ypT1, ypT2, ypT3, and ypT4 tumors, respectively), as well as the presence of involved lymph nodes in the surgical specimen (83.5, 59.4, and 27.5% for ypN0, ypN1, and ypN2 disease, respectively) [27].

However, the prognostic value of the pathologic TNM stage after nCRT is also limited. As mentioned above, accurate determination of ypN status is pivotal for the staging of rectal cancer post-nCRT and is more challenging than in cases treated without nCRT. Generally, the greater the number of lymph nodes harvested, the lower the risk of nodal understaging [28, 29]. However, nCRT in rectal cancer patients is known to cause a decrease in the harvested lymph nodes. On histology, nCRT leads to lymphocyte depletion, atrophy, and stromal fibrosis, and eventually decreased normal lymph node size [30]. In a Dutch randomized controlled rectal cancer study of preoperative short-course nCRT, the number of lymph nodes harvested was significantly lower in the nCRT group (7.7 vs. 9.7; $P < .001$) compared with the surgery-alone group [31]. A recent meta-analysis reported mean decreases in the numbers of resected lymph nodes of 3.9 and 2.1 in the nCRT-groups and preoperative radiotherapy-only groups, respectively. Surprisingly, in a population-based study using Surveillance, Epidemiology, and End Results data, the number of lymph nodes harvested was not only significantly lower in patients treated with nCRT (7 vs. 10; $P < .001$) than in patients undergoing surgery alone, but ypNx was observed in 16% of these patients. Additionally, the College of American Pathologists recommend harvesting ≥12 nodes to confirm accurate staging in node-negative patients [28, 29]; however, 12 nodes were only assessed in 20% of patients treated with preoperative radiotherapy in one previous study [32]. Importantly, in addition to the concern of understaging, other impacts of decreased lymph nodes harvested post-nCRT have not been

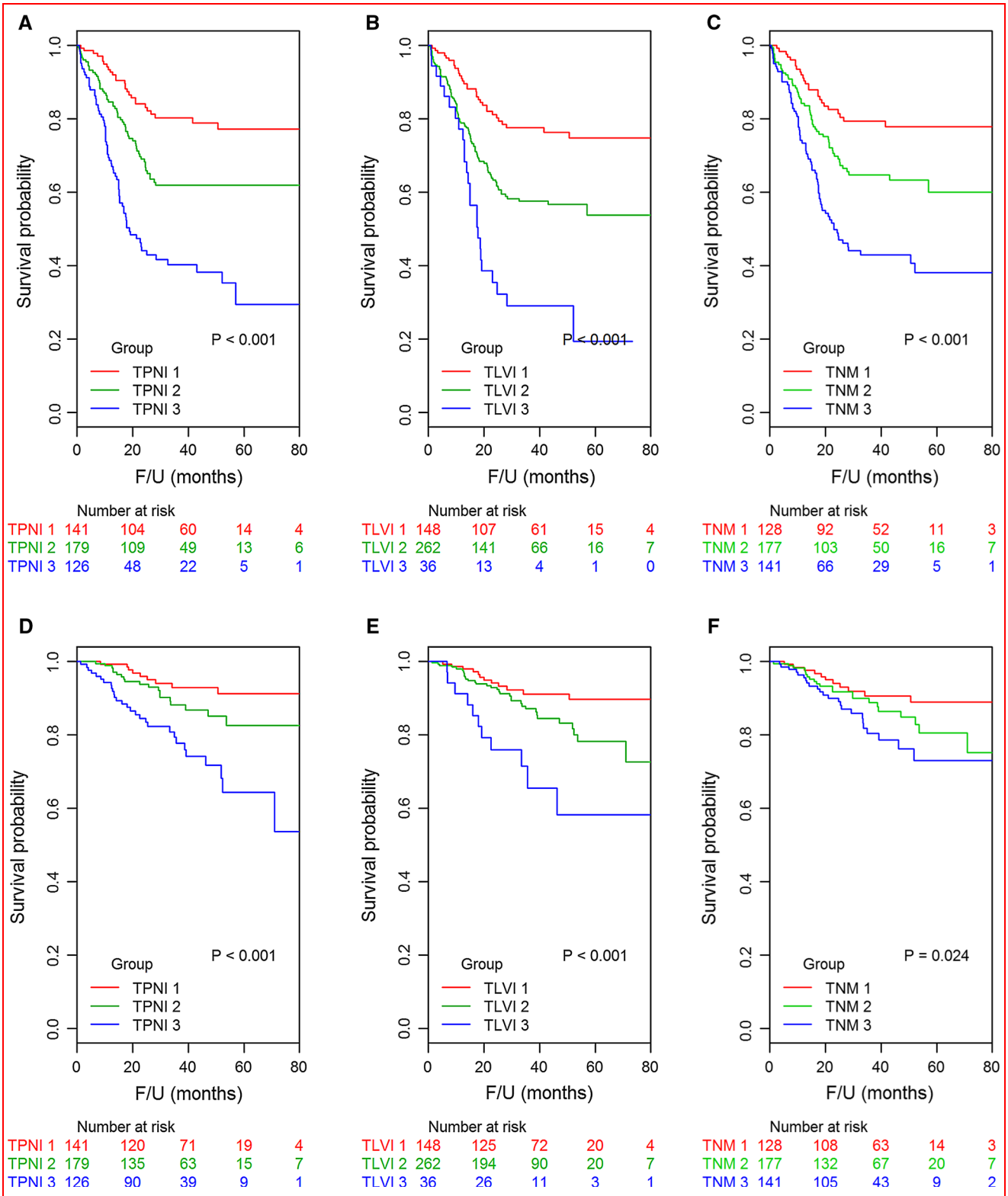

Fig. 1 Survival outcomes according to TPNI, TLVI, and TNM stages. Disease-free survival rate (**a**–**c**); overall survival rate (**d**–**f**)

Table 6 Comparison of discriminative ability using concordance index (C-index) among three different staging systems

	No.	5-Year DFS (95% CI)	*P*	C-index	5-Year OS (95% CI)	*P*	C-index
TNM stage			**<.001**	0.627 (0.583–0.671)		**.024**	0.583 (0.509–0.658)
1	128	77.8 (70.3–86.1)			89.0 (82.8–95.6)		
2	177	59.9 (50.8–70.8)			80.5 (72.3–89.7)		
3	141	38.0 (29.2–49.6)			73.0 (62.9–84.8)		
TPNI stage			**<.001**	0.651 (0.607–0.695)		**<.001**	0.656 (0.581–0.731)
1	141	77.3 (69.8–85.6)			91.3 (85.8–97.1)		
2	179	61.9 (54.6–70.2)			82.6 (74.7–91.2)		
3	126	29.4 (18.4–46.9)			64.3 (52.3–79.1)		
TLVI stage			**<.001**	0.611 (0.570–0.652)		**<.001**	0.606 (0.536–0.676)
1	148	74.8 (67.3–83.1)			89.6 (84.0–95.6)		
2	262	53.8 (46.1–62.9)			78.2 (70.7–86.5)		
3	36	19.3 (7.4–50.6)			58.2 (40.4–83.9)		

Bold font indicates significance

DFS disease-free survival, *OS* overall survival

evaluated. Contrary to non-irradiated CRC, in which an increased number of harvested lymph nodes are associated with improved survival [7], some studies have suggested that decreased or no harvested lymph nodes may reflect an increased response to nCRT. Habr-Gama et al. [33] reported that ypNx was found in 11% of patients, with 5-year DFS rates for patients with ypNx, ypN0, and ypN+ of 74, 59, and 30%, respectively.

In this situation, which can be interpreted as having either a high possibility of understaging or good tumor sensitivity ultimately leading to favorable oncologic outcome, supplemental prognostic factors are needed for patient stratification and therapeutic decision making. Herein, we investigated the prognostic significance of PNI and LVI as auxiliary pathologic factors for lymph node metastasis, especially in this paradoxical situation. We found that both PNI and LVI had significant prognostic roles, equal to lymph node metastasis, in patients treated with nCRT. Similarly, several studies have previously reported that PNI and/or LVI were associated with more aggressive disease features and poor outcomes in CRC [12–14, 17, 34–38]. To examine whether including PNI or LVI, as compared to lymph node metastasis, adds prognostic value, we introduced simple systems by combining the T-stage with PNI and LVI and calculated the C-index, which measures the discriminatory performance and predictive accuracy of each system. Consequently, we found that, overall, TPNI and TLVI were useful systems for risk discrimination, similar to the TNM stage. Of note, in patients with few harvested lymph nodes, the TPNI system showed better prediction than the TNM stage in predicting both 5-year DFS and OS.

In CRC, vascular spread and lymphatic spread are well-known pathways of distant metastasis. However, the route of dissemination along nerves is relatively poorly understood. Furthermore, in irradiated rectal specimens, it is more complicated to assess. In this regard, a surprising finding was that the LVI detection rate may be influenced by nCRT (Table 2). Accordingly, randomized controlled studies evaluating the detection rates of LVI and PNI in patients treated by nCRT followed by surgery versus surgery alone or postoperative CRT are warranted. Unfortunately, many previous trials did not report the detection rates of LVI and/or PNI, and only limited information on the impact of nCRT on them was provided. One Korean randomized phase 3 trial comparing nCRT and postoperative CRT with capecitabine showed that LVI was significantly less frequent in the nCRT arm (9 vs. 38%, $P < .001$). Contrary to our results, the PNI rate was also lower in the nCRT arm (20 vs. 11%, $P = .045$) [40]. However, when the cases with pCR ($n = 18$) were excluded, no difference in PNI was found (13 vs. 20%, $P = .212$), whereas the difference in LVI remained (10 vs 38%, $P < .001$). A single-center report from the MD Anderson Cancer Center showed similar results [35]; the authors found that the LVI rate in patients with a minor (≥50% residual cancer cells) pathologic response (47.3%) was significantly higher than in those with a major (>5 and <50% residual cancer cells) pathologic response (26.6%). However, the PNI rate did not differ according to the pathologic response (18.2 vs. 16%).

We suggest two possible explanations for why PNI was relatively less influenced by nCRT than LVI. First, the independent prognostic significance of PNI in rectal cancer patients treated with nCRT over LVI and lymph node metastasis suggests that the neural structures in the rectum may be more radioresistant than the lymphovascular structures. Ionized radiation works by directly damaging

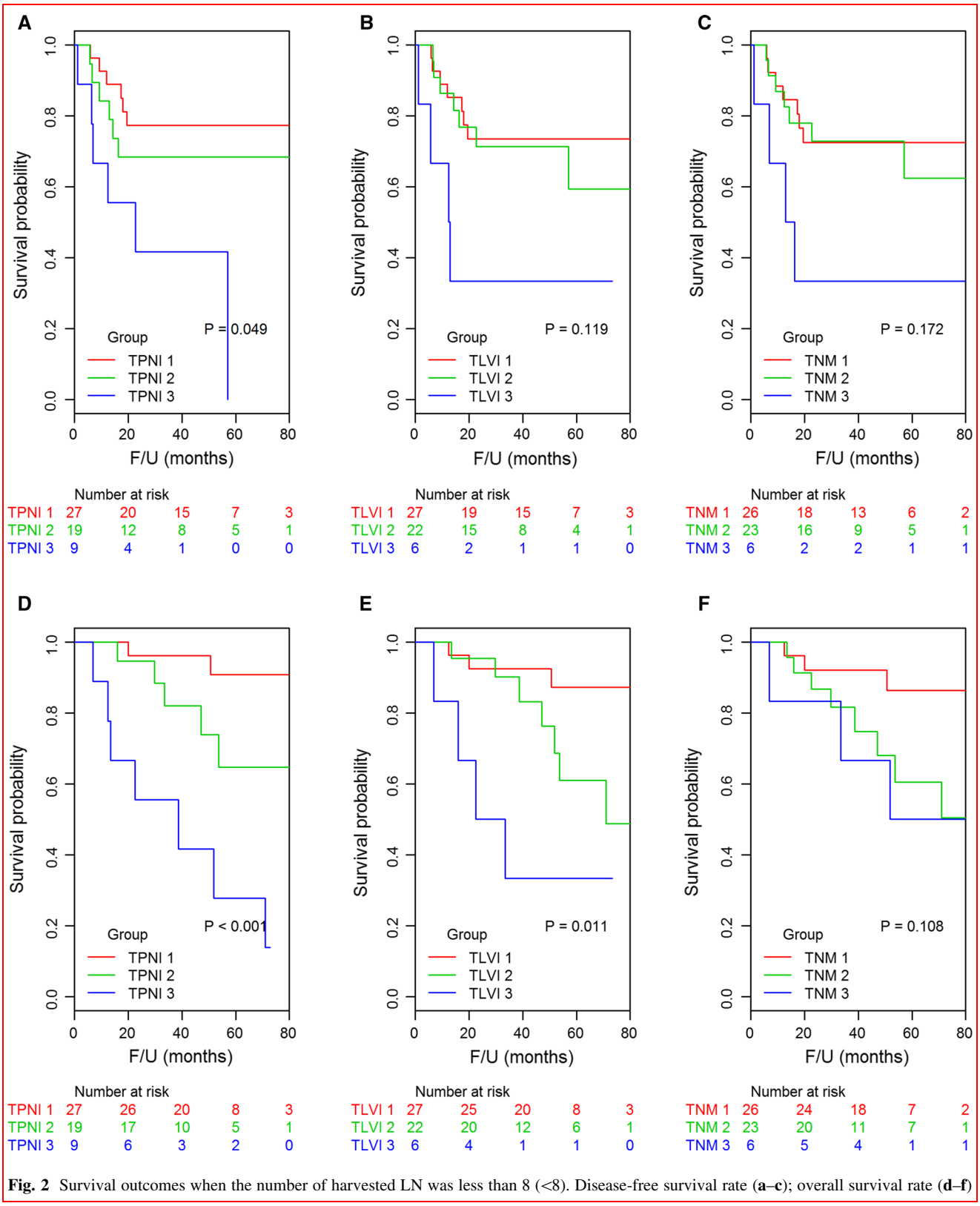

Fig. 2 Survival outcomes when the number of harvested LN was less than 8 (<8). Disease-free survival rate (**a**–**c**); overall survival rate (**d**–**f**)

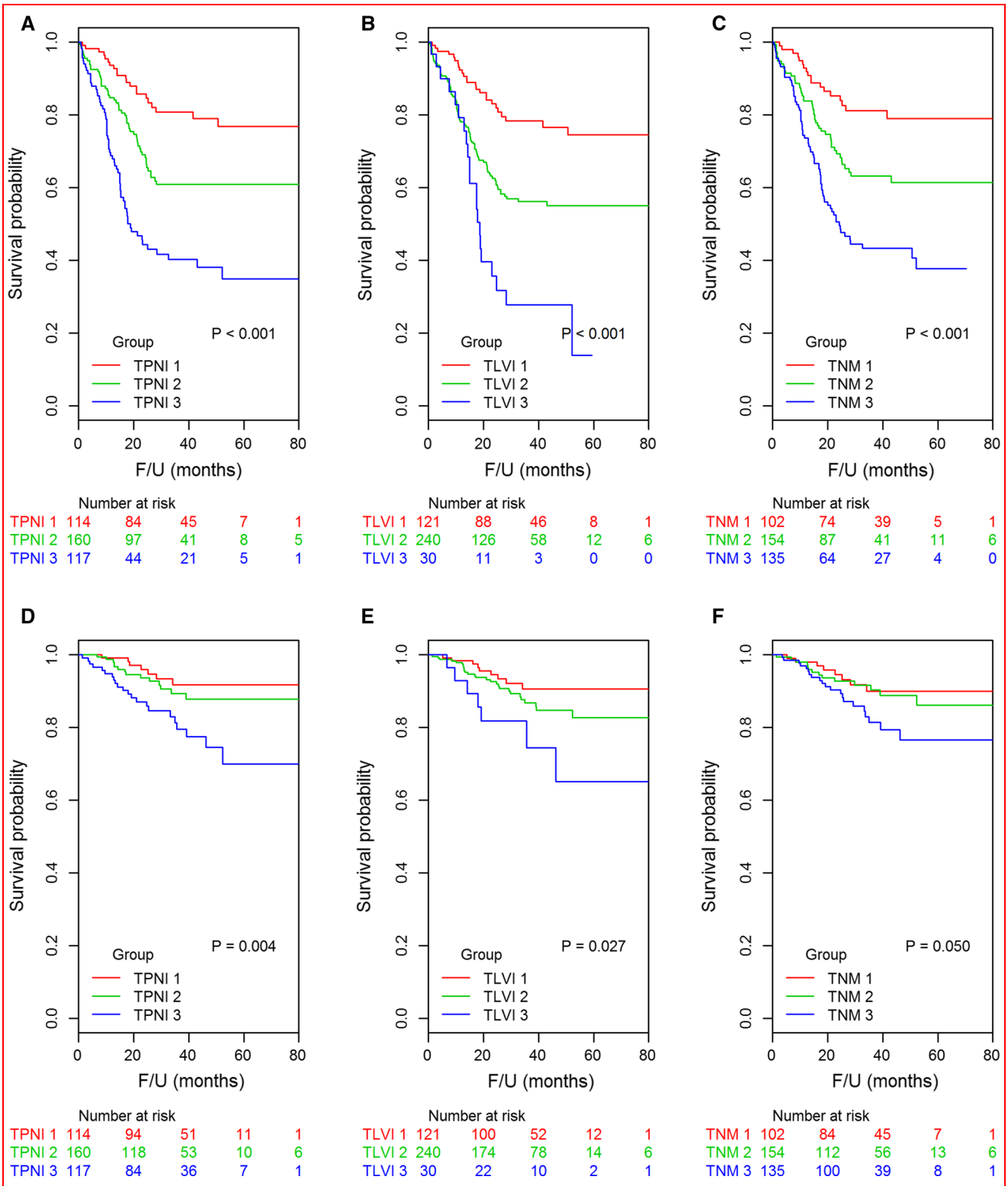

Fig. 3 Survival outcomes when the number of harvested LN was more than 8 (≥8). Disease-free survival rate (**a**–**c**); overall survival rate (**d**–**f**)

Table 7 Comparison of discriminative ability using concordance index (C-index) among three different staging systems

	No.	5-Year DFS (95% CI)	*P*	C-index	5-Year OS (95% CI)	*P*	C-index
No. of harvested lymph nodes <8							
TNM stage			.116	0.583 (0.458–0.708)		.052	0.640 (0.492–78.8)
1	26	72.5 (57.1–92.2)			86.4 (72.9–100)		
2	23	62.4 (42.0–92.8)			60.5 (40.7–89.9)		
3	6	33.3 (10.8–100)			50.0 (22.5–100)		
TPNI stage			**.018**	0.634 (0.509–0.760)		**<.001**	0.770 (0.623–0.917)
1	27	77.3 (62.8–95.1)			90.8 (79.3–100)		
2	19	68.4 (50.4–92.9)			64.7 (43.2–96.7)		
3	9	0 (NA)			27.8 (8.9–86.9)		
TLVI stage			.072	0.599 (0.474–0.723)		**.003**	0.706 (0.560–0.852)
1	27	73.6 (58.5–92.5)			87.3 (74.7–100)		
2	22	59.4 (37.8–93.3)			61.0 (40.4–92.2)		
3	6	33.3 (10.8–100)			33.3 (10.8–100)		
No. of harvested lymph nodes ≥8							
TNM stage			**<.001**	0.630 (0.583–0.677)		**.018**	0.582 (0.497–0.666)
1	102	79.0 (70.5–88.5)			90.0 (83.5–97.0)		
2	154	61.4 (53.1–71.1)			86.1 (78.6–94.3)		
3	135	37.7 (28.4–50.1)			76.6 (67.2–87.2)		
TPNI stage			**<.001**	0.653 (0.606–0.700)		**.001**	0.634 (0.549–0.719)
1	114	76.8 (68.0–86.6)			91.8 (86.0–97.9)		
2	160	60.9 (53.0–69.9)			87.8 (81.6–94.4)		
3	117	34.9 (25.4–47.9)			69.9 (57.9–84.5)		
TLVI stage			**<.001**	0.611 (0.567–0.654)		**.008**	0.583 (0.505–0.661)
1	121	74.6 (66.0–84.3)			90.6 (84.8–96.8)		
2	240	55.1 (48.3–62.7)			82.7 (76.1–89.8)		
3	30	13.9 (3.1–63.1)			65.1 (45.1–94.0)		

Bold font indicates significance

DFS disease-free survival, *OS* overall survival

the DNA in malignant tumor cells. However, this non-specific DNA damage also affects normal rectal structures, with more prominent effects seen in rapidly dividing cells. In this regard, vascular endothelial cells are likely more vulnerable than enteric neural tissue. Indeed, vascular injury in normal tissues is a prominent feature after radiotherapy [39].

Obvious perivascular and intravascular fibrosis, intimal hyperplasia, and luminal hyperplasia can be found at rectal cancer surgery [39]. Besides structural changes and the associated microvascular atrophy or depletion, endothelial damage results in inflammation, coagulation, increased vascular permeability, and leukocyte migration. The resultant tissue hypoxia and oxidative damage can be attributed to reactive oxygen species, which also aggravate the fibrosis and/or necrosis in the tissues surrounding the blood vessels [40].

Huang et al. [41] reported that there was no difference in nerve density of the Meissner's plexus between patients who received abdominoperineal resection with versus without nCRT, and increased density of the nerve bundle in Auerbach's plexus was noted in patients undergoing nCRT [41]. Microvascular endothelial cell apoptosis is known to be associated with the tumor cell response to radiation, and recent data have suggested that the microenvironment in the space surrounding nerves can induce cancer invasion and survival [42, 43]. Furthermore, neuronal damage can trigger complex series of biochemical and genetic alterations that contribute to tumor growth or suppression of apoptosis [42, 44]. In this context, cancer cells invading neural tissue may be less influenced by any tumoricidal effects followed by microenvironmental changes after nCRT. Thus, it is plausible that cancer cells with PNI can tolerate or even proliferate in response to the factors participating in neuronal regeneration.

In conclusion, little is currently known about the radiation sensitivity of tumor cells invading or metastasizing to the lymph nodes, vessels, lymphatics, and peripheral neural

tissue, and standardized definitions are also lacking. To our knowledge, this is the first study comparing these pathologic factors quantitatively in rectal cancer patients treated with nCRT. Our data suggest that PNI and LVI predicted patient's poor survival outcomes who underwent nCRT, especially in ypStageII. The presence of PNI in rectal cancer specimens following nCRT is helpful to identify candidates for more intensive adjuvant treatment, especially among patients with few harvested low lymph nodes.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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