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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

A novel histologic grading system based on lymphovascular invasion, perineural invasion, and tumor budding in colorectal cancer

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Abstract

Purpose This study aimed to evaluate the prognostic significance of lymphovascular (LVI), perineural invasion (PNI), and tumor budding positivity in patients with colorectal cancer.

Methods From January 2008 to December 2011, 3707 consecutive patients who underwent curative surgery for stage I–III colorectal cancer were assessed. These patients were then categorized into four groups based on LVI, PNI, and tumor budding (risk grouping): all negative ($n=1495$), 1 + only ($n=1063$), 2 + only ($n=861$), and all positive ($n=288$).

Results With a median follow-up period of 52 months, the 5-year disease-free survival rates of the risk groups were significantly different in terms of cancer staging (stage I, Stage II, and Stage III: $P=0.006$, $P<0.001$, and $P<0.001$, respectively). In the multivariate analysis, risk grouping was an independent prognostic factor of disease-free survival. Preoperative carcinoembryonic antigen level, tumor size, T category, and N category were independent predictors of LVI, PNI, and tumor budding positivity.

Conclusion Risk grouping based on LVI, PNI, and tumor budding positivity is a strong predictor of disease-free survival in patients with colorectal cancer.

Keywords Prognosis · Lymphovascular invasion · Perineural invasion · Tumor budding · Colorectal cancer

Introduction

Presence of lymphovascular invasion (LVI) or perineural invasion (PNI) has been an important prognostic factor of colorectal cancer (Huh et al. 2010a; b; Knijn et al. 2016; Liebig et al. 2009; Lim et al. 2010; Shinto et al. 2006; Yuan et al. 2017). Detection of cancer cells around the vessels or neural tissues indicates progression of metastasis; therefore, it is considered an indicator of poor prognosis. Tumor budding is a pathologic feature characterized by the detachment of tumor cells from the invasive margin of a tumor and migration into the peritumoral stroma. More recently, tumor budding has also been considered as a potentially significant prognostic factor of colorectal cancer (Koelzer et al. 2016; Oh et al. 2018; Shinto et al. 2006; Ueno et al. 2002; van Wyk et al. 2015; Wang et al. 2009). However, only a few studies have reported the prognostic role of LVI, PNI, and tumor budding simultaneously in colorectal cancer. Moreover, the combined impact of LVI, PNI, and tumor budding positivity on survival from and recurrence of colorectal cancer is unknown. Thus, this study evaluated the potential prognostic significance of a novel grading system based on of LVI, PNI, and/or tumor budding positivity in patients with colorectal cancer who underwent curative resection.

Methods

Consecutive patients who underwent curative surgery for colorectal cancer at our institution between January 2008 and December 2011 were retrospectively assessed. Patients with recurrent disease, stage IV cancer, familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer; those who underwent palliative surgery and local resection; and those without records of LVI, PNI, and tumor budding, or sufficient follow-up data were excluded from the study

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cohort. Patients who underwent a preoperative chemoradiotherapy for rectal cancer were also not included. A total of 3707 patients were eligible. This study was reviewed and approved by the appropriate institutional review board (2018-11-089).

All patients underwent curative resection of tumors, which were staged according to the 7th American Joint Committee on Cancer (AJCC) TNM classification. Resected specimens were evaluated for the depth of tumor invasion, lymph node involvement, histologic grade, LVI, PNI, and tumor budding as previously described (Huh et al. 2013; Oh et al. 2018). LVI was defined by the presence of tumor cells within the endothelium-lined space or the destruction of a lymphovascular wall by tumor cells (Lim et al. 2010). PNI was defined as the presence of extraneural cancer cells (Huh et al. 2010b). Tumor budding was defined as the presence of single tumor cells or small clusters of fewer than 5 cells in the stroma at the invasive tumor margin (Ueno et al. 2002). In 3707 patients, a clinical risk scoring based on LVI, PNI, and tumor budding was performed according to the following criteria: no-risk group, 0 factors; low-risk group, 1 factor; intermediate-risk group, 2 factors; and high-risk group, 3 factors.

5-fluorouracil-based adjuvant chemotherapy was preferentially considered for patients with stage II and III diseases based on pathological examination. However, it was based on the patient's health status and compliance and physician preference. The patients were followed-up every 3 months for the first 2 years after surgery, then every 6 months for up to 5 years, and annually thereafter. On a semiannual basis or when recurrence was suspected, follow-up examinations were performed, which included assessment of clinical history, physical examination, serum carcinoembryonic antigen (CEA) assay, chest radiography, chest and abdominopelvic computed tomography or magnetic resonance imaging, and positron emission tomography scan, if available. Colonoscopy was performed after the first year and then biennially.

Statistical analyses were performed using the SPSS software for Windows (version 18.0; SPSS Inc., Chicago, IL, the USA). Categorical variables were analyzed using the Chi square test, and continuous variables were analyzed using the student's *t* test. One-way analysis of variance with least significant difference multiple comparisons was used for analysis of quantitative differences between the groups. The Cox proportional hazards regression model was used to identify the variables that could independently influence disease-free survival and risk grouping. Variables with a @@*P* value < 0.05 by univariate analysis were entered into multivariate analysis using a Cox proportional model. Survival curves were calculated using the Kaplan–Meier method, and differences between the curves were evaluated using the log-rank test. A *P* value ≤ 0.05 was considered statistically significant.

Results

The clinicopathologic characteristics of the 3707 patients are summarized in Table 1. The participants consisted of 2227 men (60.1%), with a median age of 60 (range: 16–90) years. Of these patients, 2692 (72.6%) and 1015 (27.4%) had colon and rectal cancer, respectively. Using the 7th AJCC TNM staging system, 863, 1194, and 1650 patients had pathologic stage I, II, and III cancers, respectively. LVI was observed in 1472 (39.7%) patients, PNI was noted in 506 (13.6%) patients, and tumor budding was identified in 1671 (45.1%) patients. Using the risk grouping criteria, 1495 (40.3%), 1063 (28.7%), 861 (23.2%), and 288 patients (7.8%) were classified under the no-, low-, intermediate-, and high-risk groups, respectively.

Comparisons of the patients according to the risk grouping are shown in Table 2. Preoperative serum CEA levels, type of surgery, maximal diameter of the tumor, differentiation, number of resected lymph nodes, circumferential

Table 1 Characteristics of the patient ($n = 3707$)

	No. (%)
Median age, years (range)	60 (16–90)
Sex	
Male	2227 (60.1)
Female	1480 (39.9)
Tumor location	
Colon	2692 (72.6)
Rectum	1015 (27.4)
Median maximal tumor size, cm (range)	4.2 (0.1–21.0)
Median preoperative CEA, ng/mL (range)	2.6 (0.1–840.0)
Type of surgery	
Open	1197 (32.3)
Laparoscopic	2510 (67.7)
Median no. of resected lymph nodes (range)	18 (1–138)
Postoperative chemotherapy	1777/2548 (69.7)
Pathologic TNM stage	
Pathologic TNM stage	
I	863 (23.3)
II	1194 (32.2)
III	1650 (44.5)
Lymphovascular invasion	1472 (39.7)
Perineural invasion	506 (13.6)
Tumor budding	1671 (45.1)
Risk grouping	
No	1495 (40.3)
Low	1063 (28.7)
Intermediate	861 (23.2)
High	288 (7.8)

CEA carcinoembryonic antigen

Table 2 Comparison of patients according to the risk grouping

	No risk ($n=1495$)	Low risk ($n=1063$)	Intermediate risk ($n=861$)	High risk ($n=288$)	P value
Age, years					0.202
< 60	676 (45.2)	511 (48.1)	403 (46.8)	148 (51.4)	
≥ 60	819 (54.8)	552 (51.9)	458 (53.2)	140 (48.6)	
Sex					0.496
Male	908 (60.7)	626 (58.9)	528 (61.3)	165 (57.3)	
Female	587 (39.3)	437 (41.1)	333 (38.7)	123 (42.7)	
Preoperative CEA level, ng/mL					< 0.001
< 5	1298 (86.8)	848 (79.8)	639 (74.2)	193 (67.0)	
≥ 5	168 (11.2)	192 (18.1)	203 (23.6)	88 (30.6)	
Unknown	29 (1.9)	23 (2.2)	19 (2.2)	7 (2.4)	
Location of the tumor					0.343
Colon	1086 (72.6)	789 (74.2)	607 (70.5)	210 (72.9)	
Rectum	409 (27.4)	274 (25.8)	254 (29.5)	78 (27.1)	
Type of surgery					< 0.001
Open	421 (28.2)	359 (33.8)	308 (35.8)	109 (37.8)	
Laparoscopic	1074 (71.8)	704 (66.2)	553 (61.2)	179 (62.2)	
Maximal diameter of the tumor, cm					< 0.001
< 4.2	842 (56.3)	500 (47.0)	391 (45.4)	133 (46.2)	
≥ 4.2	653 (43.7)	563 (53.0)	470 (54.6)	155 (53.8)	
Differentiation					< 0.001
Well + moderate	1422 (95.1)	977 (91.9)	745 (86.5)	250 (86.8)	
Poor + mucinous	73 (4.9)	86 (8.1)	116 (13.5)	38 (13.2)	
No. of resected lymph nodes					0.004
< 12	221 (14.8)	116 (10.9)	125 (14.5)	27 (9.4)	
≥ 12	1274 (85.2)	947 (89.1)	736 (85.5)	261 (90.6)	
Circumferential resection margin, mm					< 0.001
< 1	9 (0.6)	20 (1.9)	24 (2.8)	22 (7.6)	
≥ 1	1486 (99.4)	1043 (98.1)	837 (97.2)	266 (92.4)	
Postoperative chemotherapy					< 0.001
No	504 (33.7)	174 (16.4)	75 (8.7)	18 (6.3)	
Yes	478 (32.0)	577 (54.3)	548 (63.6)	174 (60.4)	
Unknown	513 (34.3)	312 (29.4)	238 (27.6)	96 (33.3)	
Pathologic T category					< 0.001
T0–T2	696 (46.6)	262 (24.6)	104 (12.1)	3 (1.0)	
T3–T4	799 (53.4)	801 (75.4)	757 (87.9)	285 (99.0)	
Pathologic N category					< 0.001
N−	1293 (86.5)	532 (50.0)	197 (22.9)	35 (12.2)	
N+	202 (13.5)	531 (50.0)	664 (77.1)	253 (87.8)	
Pathologic TNM category					< 0.001
I	641 (42.9)	176 (16.5)	46 (5.3)	0	
II	652 (43.6)	356 (33.5)	151 (17.5)	35 (12.2)	
III	202 (13.5)	531 (50.0)	664 (77.2)	253 (87.8)	
Postoperative morbidity					0.131
No	1185 (79.3)	866 (81.5)	683 (79.3)	217 (75.3)	
Yes	310 (20.7)	197 (18.5)	178 (20.7)	71 (24.7)	

CEA carcinoembryonic antigen

resection margin, postoperative chemotherapy, pathologic T category, pathologic N category, and pathologic TNM category significantly differed among the four groups.

With a median follow-up period of 51.7 (range 0.1–87.9) months, the 5-year disease-free survival rate of this cohort was 84.3%. When the risk groups were divided according to pathologic TNM stage (stage I, II, and III diseases), their 5-year disease-free survival rates were significantly different ($P = 0.006$, $P < 0.001$, and $P < 0.001$, respectively; Fig. 1a, b, c).

The univariate analysis indicated that the factors associated with disease-free survival were preoperative serum CEA levels, location of the tumor, type of surgery, maximal diameter of the tumor, differentiation, circumferential resection margin, postoperative chemotherapy, pathologic T category, pathologic N category, and risk grouping (Table 3). The multivariate analysis revealed that preoperative serum CEA levels, location of the tumor, type of surgery, differentiation, postoperative chemotherapy, pathologic T category, pathologic N category, and risk grouping were independent prognostic factors of disease-free survival (Table 3).

Given the importance of LVI, PNI, and tumor budding positivity on survival, we performed a multivariate analysis to identify the factors independently associated with the high-risk group (Table 4). Preoperative serum CEA level ($P = 0.014$), maximal diameter of the tumor ($P = 0.001$), pathologic T category ($P < 0.001$), and pathologic N category ($P < 0.001$) were independent predictors of all positivity for LVI, PNI, and tumor budding.

Discussion

We investigated the prognostic impact of a novel grading system based on LVI, PNI, and/or tumor budding in patients with colorectal cancer. The long-term survival rates of the risk groups obtained using the novel grading system were significantly different according to pathologic TNM stage. Moreover, the risk grouping was an independent prognostic factor of survival.

Lymphatic spread of cancer cells via the lymphatic channels or venules may be a critical early step for the metastasis of tumors in the lymph node (Stacker et al. 2002). LVI is a widely recognized unfavorable prognostic factor of survival in patients with colorectal cancer (Blumberg et al. 1998; Lim et al. 2010; Minsky et al. 1988). LVI was found in 39.7% of our patients, and its incidence rate ranged from 5.2 to 89.5% (Lim et al. 2010; Sternberg et al. 2002; Yuan et al. 2017). This wide variation in the positivity rates of LVI may be due to several factors, including differences in patient cohorts and tumor characteristics, different criteria for LVI positivity, and variations in the use of special stains.

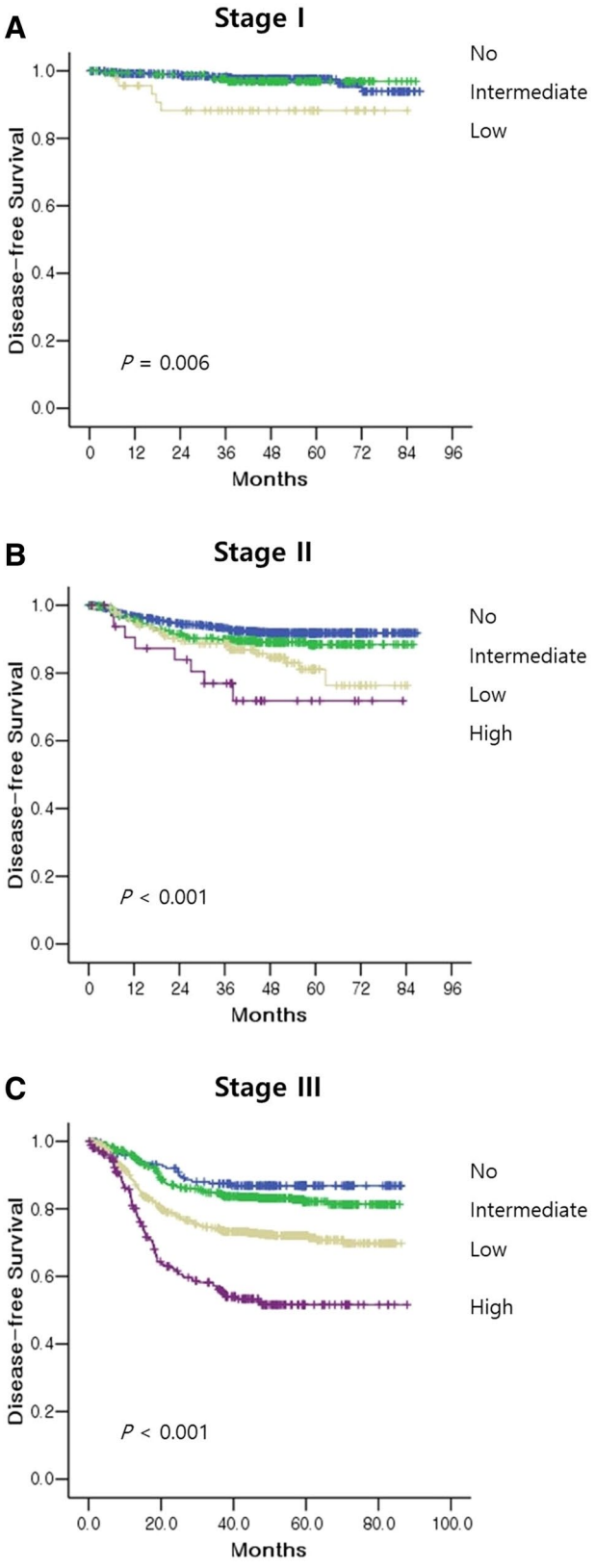

Fig. 1 Disease-free survival curves according to the risk grouping and TNM stage in patients with **a** stage I, **b** stage II, and **c** stage III

Table 3 Univariate and multivariate analyses of the factors associated with 5-year disease-free survival

Factor	Univariate analysis		Multivariate analysis		
	Survival (%)	*P* value	HR	95% CI	*P* value
Age, years		0.084			
< 60	85.2				
≥ 60	83.4				
Sex		0.999			
Male	84.2				
Female	84.4				
Preoperative CEA, ng/mL		< 0.001			
< 5	86.6		1		
≥ 5	74.8		1.325	1.087–1.616	0.005
Location of the tumor		< 0.001			
Colon	86.8		1		
Rectum	77.4		1.863	1.553–2.235	< 0.001
Type of surgery		< 0.001			
Open	78.7		1		
Laparoscopic	86.9		0.816	0.678–0.982	0.031
Maximal diameter of the tumor, cm		< 0.001			
< 4.2	87.0		1		
≥ 4.2	81.5		1.087	0.901–1.313	0.384
Differentiation		< 0.001			
Well + moderate	85.1		1		
Poor + mucinous	74.8		1.441	1.117–1.859	0.005
No. of resected lymph nodes		0.705			
< 12	84.4				
≥ 12	84.4				
Circumferential resection margin, mm		< 0.001			
< 1	66.5		1		
≥ 1	84.6		0.665	0.435–1.016	0.059
Postoperative chemotherapy		0.001			
No	88.9		1		
Yes	82.6		1.738	1.287–2.348	< 0.001
Pathologic T category		< 0.001			
T0–T2	95.2		1		
T3–T4	79.9		2.945	2.070–4.191	< 0.001
Pathologic N category		< 0.001			
N-	92.3		1		
N+	74.2		2.163	1.700–2.752	< 0.001
Risk grouping		< 0.001			
No	93.6		1		
Low	86.7		1.409	1.049–1.892	0.023
Intermediate	74.7		2.295	1.704–3.092	< 0.001
High	54.0		3.973	2.847–5.545	< 0.001

CEA carcinoembryonic antigen, *HR* hazard ratio, *CI* confidence interval

PNI was found in 13.6% of our patients, which is similar to the results of previous studies (9–30%) (Fujita et al. 2007; Huh et al. 2010b; Liebig et al. 2009). Although the mechanisms associated with tumor cell dissemination via the nerves are still unclear, the association between PNI and metastases in several cancers strongly suggests that PNI play a role in cancer dissemination (Liebig et al. 2009). PNI is recognized as an independent predictor of survival in patients with colorectal cancer (Huh et al. 2010b; Knijn et al. 2016; Liebig et al. 2009). Moreover, PNI may be useful in identifying patients with

Table 4 Multivariate regression analysis of patients with colorectal cancer in the high-risk group

	Odds ratio (95% CI)	*P* value
Age (≥ 65 years)	1.222 (0.948–1.574)	0.121
Sex (male)	1.046 (0.810–1.352)	0.729
Preoperative serum CEA level (≥ 5 ng/mL)	1.428 (1.076–1.896)	0.014
Maximal diameter of the tumor (≥ 4.5 cm)	1.569 (1.210–2.036)	0.001
Location in the rectum	1.071 (0.804–1.426)	0.640
Differentiation (poor + mucinous)	1.222 (0.834–1.790)	0.304
Pathologic T category (T3 + T4)	26.290 (8.283–83.444)	< 0.001
Pathologic N category (positive)	6.731 (4.669–9.703)	< 0.001

High-risk group, all positive for lymphovascular invasion, perineural invasion, and tumor budding. *CEA* carcinoembryonic antigen, *CI* confidence interval

node-negative colorectal cancer who are more likely to benefit from adjuvant chemotherapy (Huh et al. 2010b).

Tumor budding has been gaining increased recognition as a predictor of lymph node metastasis and unfavorable survival in patients with colorectal cancer (Koelzer et al. 2016; Oh et al. 2018; van Wyk et al. 2015). Tumor budding is defined as a phenomenon in which cancer cells separate from the invading tumor and migrate into the stroma of the tumor (Rogers et al. 2014). Although the prognostic impact of tumor budding in colorectal cancer should be fully elucidate, tumor budding is an independent prognostic factor of colorectal cancer with an association with poor differentiation, LVI, and PNI based on several recent publications (Ohtsuki et al. 2008; Syk et al. 2011; Wang et al. 2009). Moreover, the grade of tumor budding was an independent prognostic factor of survival in patients with colon cancer (Oh et al. 2018).

We observed a significant association between the risk grouping and adverse histologic factors, such as elevated preoperative serum CEA level, poor differentiation, and advanced pathologic TNM stage. Preoperative serum CEA level, maximal diameter of the tumor, pathologic T category, and pathologic N category were independent predictors of all positivity of LVI, PNI, and tumor budding. In addition, the risk grouping was an independent prognostic factor of disease-free survival in the multivariate analysis. To the best of our knowledge, this study is the first to report on the prognostic significance of the novel histologic grading system based on LVI, PNI and/or tumor budding positivity in colorectal cancer. The evaluation of this risk grouping may be useful for stratifying and individualizing the patients who underwent colorectal cancer surgery. Due to the inherent limitations of a retrospective study and the relatively small sample size of this analysis, this result requires further validation via multicenter studies with larger sample sizes to obtain a firm conclusion.

In conclusion, a novel histologic grading of the risk grouping using LVI, PNI, and tumor budding is a strong predictor of disease-free survival in patients with colorectal cancer. The evaluation of the combination of these familial histologic factors may be used as a new clinically useful prognostic system individualizing prognosis and choosing an appropriate adjuvant therapy in patients with colorectal cancer.

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Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest.

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