Prognostic value of desmoplastic stromal reaction, tumor budding and tumor-stroma ratio in stage II colorectal cancer

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Background: Existing high-risk factors are insufficient to accurately predict the postoperative recurrence risk of stage II colorectal cancer (CRC). The discovery of additional prognostic markers may be the key to improving the current status of stage II CRC treatment. The present study aimed to evaluate the relationship among desmoplastic reaction (DR), tumor budding (TBd), the tumor-stroma ratio (TSR) and their prognostic value for relapse-free survival (RFS).

Methods: In this study, 207 patients with histologically confirmed stage II CRC from January 2012 to August 2018 were retrospectively reviewed from a single center; the cohort was divided into subgroups based on low or high TSR, and low, intermediate or high DR and TBd. Kaplan–Meier curve analysis and log-rank test were applied to examine RFS among subgroups. Univariate and multivariate Cox proportional hazards analyses were used to identify independent factors associated with RFS, and a nomogram was subsequently developed.

Results: Abnormal CA242, CEA, T4 stage, presence of hypertension, internal obstruction or perforation (IOP), lymphovascular or/and perineural invasion (PNI), number of nodes examined less than 12, low-frequency microsatellite instability (MSI-L), higher Ki-67 and immature DR were associated with a lower RFS. In multivariable analysis, DR (HR =2.111; 95% CI: 1.184–3.766; P=0.011), LVI (HR =1.919; 95% CI: 1.004–3.669; P=0.049) and PNI (HR =2.724; 95% CI: 1.362–5.448; P=0.005) were prognostic factors for RFS. On this basis, a nomogram that integrated DR and clinicopathologic predictors for predicting RFS passed the calibration and had an area under the curve of 0.826.

Conclusions: The prognostic significance of DR outperformed TBd and TSR, therefore, we recommend adding DR as a biomarker in routine pathological reports. The novel nomogram combining these factors may be used as a reliable and effective tool for the prediction of RFS in stage II CRC, thus helping optimize therapeutic regimens under cooperation of oncologists and surgeons. Further multicentric studies are required for validation of this novel, simple and cost-effective prognostic model.

Introduction

Colorectal cancer (CRC) is the third most prevalent cancer worldwide with a high mortality rate (1); in China, the incidence of CRC increased to the second most common in 2020 (2). Approximately 25% of CRC patients are in stage II. Surgical resection for curative purposes is the mainstay of treatment for patients with stage II CRC. However, 20% to 25% of these patients still experience tumor recurrence after surgery (3), and treatment strategies after surgical resection are controversial (4,5). Recently, the tumor microenvironment has been recognized to play an important role in patient prognosis. The discovery of biomarkers that can predict the tumor microenvironment may be the key to overcoming the current treatment status.

The tumor microenvironment is a bidirectional, dynamic and intricate interaction network between stromal cells and tumor cells that is closely related to tumor proliferation, spread, metastasis, recurrence and chemotherapy resistance (6,7). The tumor-stroma ratio (TSR) is an estimate of the proportion of epithelial and stromal cells. Numerous studies have considered the TSR as an independent prognostic biomarker in CRC, with a high amount of stroma (low TSR) being predictive of an adverse prognosis (8-12). Desmoplastic reaction (DR) is the proliferation of myofibroblasts in the stroma of invasive cancer (13,14). Similar to TSR, DR is an important part of the tumor microenvironment and has been identified as an independent factor for poor prognosis in stage II CRC (15). Furthermore, tumor budding (TBd) is an acknowledged prognostic factor in CRC and other carcinomas. The presence of TBd has been associated with worse prognoses in CRCs (16,17). The International Tumor Budding Consensus Conference (ITBCC) (17) has proposed to include the presence of TBd in CRC staging systems and recommended using a three-tier system based on the quantification of tumor buds.

Recent studies revealed that immature DR, high TBd and low TSR were significantly associated with several high-risk clinicopathological factors and poor overall survival (OS) in gall bladder carcinoma (18), but the prognostic role of these parameters in CRC, especially in the Chinese population, remains controversial (19,20). In addition, due to the differences in biological behavior in stages I to IV CRC, our study design included only stage II patients of the Chinese population to perform a meaningful relapse-free survival (RFS) analysis. Hence, this study sought to investigate the prognostic value of DR, TBd and TSR, and combined them to build a prognostic model for the prediction of RFS in stage II CRC patients. We present the following article in accordance with the TRIPOD reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-758/rc).

Methods

Patients

This single-center, retrospective study was approved by the Ethics Committee of Tianjin Medical University Cancer Institute & Hospital (TMUCIH) (No. EK2022175), and a waiver of patient consent was granted. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

A total of 688 stage II CRC patients who underwent surgical resection at TMUCIH between January 2012 and August 2018 were enrolled in this study. The exclusion criteria were as follows: (I) synchronous multiple tumors (n=55); (II) received preoperative neoadjuvant therapy (n=78); (III) insufficient clinicopathology data (n=212) and (IV) lost to follow-up (n=136). In total, 207 patients with stage II CRC were included. Data on clinicopathological factors including age, gender, hypertension history, diabetes history, smoking history, family history of cancer, preoperative CEA, CA724, CA199, and CA242 levels, histological type, differentiation grade, T stage, presence or absence of lymphovascular invasion (LVI), perineural invasion (PNI), internal obstruction or perforation (IOP) status, number of lymph nodes examined (≥12 *vs.* <12), mismatch repair status, Ki-67 expression level and history of postoperative adjunctive chemotherapy (present *vs.* absent) were obtained from medical records and pathology reports.

All patients were followed-up for at least 3 years from the date of surgical resection. The RFS as the primary endpoint was defined as the date from surgery to confirmed clinical recurrence, death or last available contact.

Assessment of DR categorization, TBd and TSR

Hematoxylin and eosin-stained slides of the resection specimens were re-reviewed by two pathologists. They were blinded to the clinical information while confirming the findings of TBd, TSR, and DR. If the two pathologists disagreed with the diagnosis, they would convene to discuss and decide the final diagnosis.

DR

DR categorization was performed according to the Ueno method (21-23), which classified DR into three types: immature, intermediate and mature. Immature DR was defined as the presence of myxoid stroma at the tumor invasive front involving at least one high microscopic field (×40); cases without myxoid stroma larger than the ×40 objective field and with the presence of keloid-like collagens at the tumor invasive front were classified as intermediate DR; and those without keloid-like collagen or myxoid stroma were classified as mature DR (*Figure 1A-1C*).

TBd

TBd was scored using the proposed three-tier approach by the ITBCC 2016 international criteria (17), which relied on the quantification of the buds at the hotspot (in a field measuring 0.785 mm^2) and was divided into three categories: less than 5 (BD1), 5 to 9 (BD2) and greater than or equal to 10 (BD3) (*Figure 1D-1F*).

TSR

TSR, also known as the tumor stroma percentage, was defined as the proportion of stromal area at the most invasive region in a microscopic field with a ×10 objective magnification. Patients were divided into TSR high (less than or equal to 50% stroma) and TSR low (greater than 50% stroma) groups using a cutoff value of 50% (24) (*Figure 1G,1H*).

Nomogram development and performance evaluation

Kaplan–Meier curve analysis and the log-rank test were applied to examine RFS in subgroups. The Cox proportional hazards model was used for univariate and multivariate analyses. To provide an individual predictive graphical presentation, we subsequently constructed a nomogram on the basis of multivariate Cox analysis in the cohort.

To evaluate the performance of the prediction model, a calibration curve with bootstrapping was constructed by measuring the agreement between the predicted and actual 3-year RFS. Furthermore, a receiver operating characteristic (ROC) curve was produced to further verify the prediction performance of the model by calculating the area under the curve (AUC) as well as accuracy, sensitivity and specificity.

Other statistical analyses

All statistical calculations were performed with SPSS statistical software (version 20.0) or R software (version 6.1). The data were presented as numbers (%) for categorical variables. Normally distributed continuous variables were expressed as the mean [standard deviation (SD)], while non-normally distributed continuous variables were expressed as the median and range. The chi-square test or Fisher's exact test was used for association analysis between categorical variables where appropriate. R packages of "rms," "survival," "calibrate," "pROC," and "OptimalCutpoints" were used to construct the prognostic nomogram, plot calibration curves, ROC curves and cutoff value. The optimal cutoff point was estimated by Youden's J index (25). A two-sided P value less than 0.05 in the statistical test was statistically significant. All confidence intervals (CIs) were stated at the 95% confidence level.

Results

Patient characteristics

The baseline characteristics of the 207 patients are summarized in *Table 1*. Briefly, there were 126 males and 81 females of whom 51 patients older than 65 years. The mean age at the time of surgery was 58.31±10.43 years. Fifty (24.15%) patients of the entire population had disease recurrence, and all of them experienced recurrence within 3 years. A total of 17 patients were confirmed by surgery and histopathology, and the other patients were diagnosed by radiologic features. The mean RFS was 1,039.33±475.09 days (range, 33–1,802 days).

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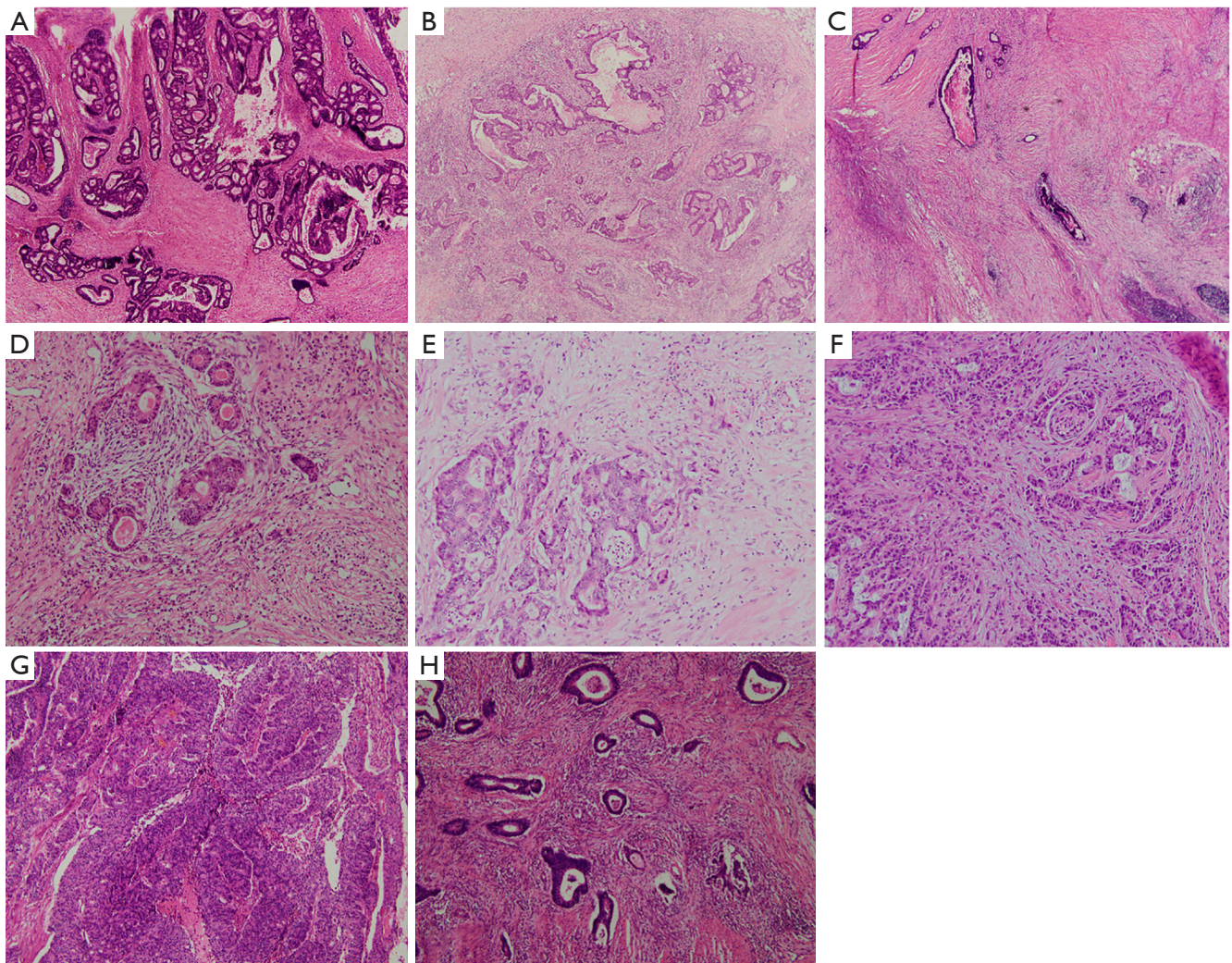

Figure 1 Examples of a DR, TBd, and TSR on hematoxylin and eosin stained slides. (A) Mature; (B) intermediate; (C) immature; (D) BD1; (E) BD2; (F) BD3; (G) TSR high (≤50% stroma); (H) TSR low (>50% stroma). A-C with magnification ×40; D-F with magnification ×200; G,H with magnification ×100. DR, desmoplastic reaction; TBd, tumor budding; TSR, tumor-stroma ratio.

Association among the investigated parameters

Tables 2-4 show the correlations between DR, TBd, and TSR and the clinicopathological parameters of patients.

DR was significantly associated with CEA level (P=0.035), PNI (P=0.024), IOP status (P=0.030), TBd (P<0.001), TSR (P<0.001) and adjuvant therapy (P=0.015). TBd was significantly associated with histological grade (P=0.046), hypertension (P=0.004), DR (P<0.001), TSR (P<0.001) and adjuvant therapy (P<0.001); and TSR was significantly associated with age (P=0.048), PNI (P=0.015), DR (P<0.001) and TBd (P<0.001).

Patients with immature DR had a significantly higher

proportion of BD3 and low TSR; in contrast, participants with mature and intermediate DR had higher proportions of BD1 and high TSR, respectively. Patients with a low TSR more commonly presented with BD3, whereas patients with a TSR >50% more commonly presented with BD1.

Survival analysis

Overall, our results showed abnormal CA242, CEA, T4 stage, presence of hypertension, LVI, PNI, IOP, number of nodes examined less than 12, MSI-L, higher Ki-67 and immature DR to be associated with a lower RFS

Table 1 Baseline clinicopathological characteristics and the distribution of the patients

Characteristics	All patients (N=207)
Gender, n (%)	
Male	126 (60.9)
Female	81 (39.1)
Age, n (%)	
>65 years	51 (24.6)
≤65 years	156 (75.4)
Histologic grade, n (%)	
Well	30 (14.5)
Moderate	116 (56.0)
Poor	61 (29.5)
Location, n (%)	
Ascending colon	23 (11.1)
Transverse colon	4 (1.9)
Descending colon	8 (3.9)
Sigmoid colon	36 (17.4)
Rectum	136 (75.7)
Smoking, n (%)	
No	164 (79.2)
Yes	43 (20.8)
Hypertension, n (%)	
No	152 (73.4)
Yes	55 (26.6)
Family history of cancer, n (%)	
No	178 (86.0)
Yes	29 (14.0)
Diabetes, n (%)	
No	187 (90.3)
Yes	20 (9.7)
CEA level, n (%)	
Normal	115 (55.6)
Abnormal	92 (44.4)
CA242, n (%)	
Normal	164 (79.2)
Abnormal	43 (20.8)

Table 1 (continued)

Table 1 (continued)

Characteristics	All patients (N=207)
CA724, n (%)	
Normal	164 (76.9)
Abnormal	43 (23.1)
CA199, n (%)	
Normal	176 (85.0)
Abnormal	31 (15.0)
Ki-67 level, n (%)	
Low	9 (4.3)
High	198 (95.7)
T stage, n (%)	
T3	132 (63.8)
T4	75 (36.2)
Lymphovascular invasion, n (%)	
Absent	160 (77.3)
Present	47 (22.7)
Perineural invasion, n (%)	
Absent	189 (91.3)
Present	18 (8.7)
IOP status, n (%)	
No	188 (90.8)
Yes	19 (9.2)
Number of nodes examined, n (%)	
12 or more	175 (86.3)
Less than 12	32 (13.7)
Mismatch repair status, n (%)	
MSI-L	160 (77.3)
MSI-H	47 (22.7)
DR, n (%)	
Mature	101 (48.8)
Intermediate	62 (30.0)
Immature	44 (21.2)
TBd, n (%)	
<5 (BD1)	108 (52.2)
5–9 (BD2)	45 (21.7)
≥10 (BD3)	54 (26.1)

Table 1 (continued)

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Table 1 (*continued*)

Characteristics	All patients (N=207)
TSR, n (%)	
Low (stroma-high)	93 (44.9)
High (stroma-low)	114 (55.1)
Adjuvant therapy, n (%)	
No	72 (34.8)
Yes	135 (65.2)

MSI, microsatellite instability; CEA, carcinoembryonic antigen; CA242, carbohydrate antigen 242; CA724, carbohydrate antigen 724; CA199, carbohydrate antigen199; IOP status, internal obstruction or perforation; DR, desmoplastic reaction; TBd, tumour budding; TSR, tumor-stroma ratio.

(Table S1). Kaplan–Meier curves with the log-rank test for RFS according to the presence of DR, TSR and TBd are presented in *Figure 2*. Patients with higher TB (BD3) and low TSR (stroma-high tumors) tended to have a worse RFS; however, the difference was not statistically significant (P=0.13 and 0.15, respectively). In addition, it is worth noting that the findings from Kaplan–Meier curves showed similar survival curves for the DR-intermediate and DR-mature groups, but only the DR-immature group had a significantly worse prognosis compared to the other two groups. According to this, in the univariate and multivariate analyses that followed, DR was categorized into 2 groups: immature DR and other DR types, as previously reported (15,26).

The univariate and multivariate Cox proportional hazards regression analyses for RFS in CRC are detailed in *Table 5*. In the univariate analyses of RFS, pT stage (P=0.040), LVI (P<0.001), PNI (P<0.001), IOP status (P=0.001), number of nodes examined (P<0.001), mismatch repair status (P=0.019), CEA (P=0.001), CA242 (P=0.024), DR (P<0.001) and adjuvant chemotherapy (P=0.001) were associated with RFS. When subjected to multivariate analysis, DR remained prognostic for RFS (HR =2.111; 95% CI: 1.184–3.766; P=0.011). LVI (HR 1.919; 95% CI 1.004-3.669; P=0.049) and PNI (HR =2.724; 95% CI: 1.362–5.448; P=0.005) were the only other parameters significantly associated with RFS.

Construction and validation of a nomogram

A nomogram was developed based on the above prediction model (*Figure 3A*), providing the predicted 3-year disease recurrence probability for each patient. The calibration curve of the nomogram for the probability of disease recurrence showed a good agreement between the predicted and actual survival (*Figure 3B*). Moreover, an ROC curve was plotted for the evaluation of the model performance (*Figure 4*). An optimal cut-off value that maximized the sum of sensitivity and specificity in the ROC curve was identified. The best cut-off point was 0.235. The prediction model yielded an AUC of 0.826 (95% CI: 0.758–0.895; accuracy 0.749; sensitivity 0.780; specificity 0.739) in our cohort.

Discussion

In this retrospective study, two important clinical findings were discovered. First, TBd, TSR, and DR were significantly associated with conventional clinicopathological prognostic factors in stage II CRC. Second, DR was an independent prognostic factor in addition to LVI and PNI in the multivariate analysis of RFS. Furthermore, we developed an easy-to-use nomogram incorporating the above variables for the preoperative individualized prediction of disease recurrence in patients with stage II CRC.

Adjuvant chemotherapy (fluorouracil plus oxaliplatin regimen) was recommended only for stage II CRC patients with high risk factors for recurrence, defined by the NCCN guidelines (27), including T4 tumor stage, poor histologic grade, obstruction or perforation, LVI, PNI, a small number (<12) of lymph nodes, and positive margins. However, the methodology of identifying patients with high recurrence risk remains controversial (28). Several studies have found that according to these clinicopathological factors with a high recurrence risk, many stage II CRC patients defined as having a high recurrence risk have no recurrence and have long-term survival, while some patients without high recurrence risk factors experience recurrence (29,30). Hence, the high recurrence risk factors, as judged only by "macroscopic" and "submicroscopic" indicators such as anatomy and histopathology alone, cannot always correctly predict the prognosis of patients. Recently, an increasing number of people have realized that in addition to the heterogeneity of the tumor itself, the heterogeneity of the tumor microenvironment is also an important factor.

According to our study, we showed an association between TBd and TSR. Consistent with our study results, van Wyk (31) reported that TBd was positively correlated with tumor stroma. However, the current study showed that

Table 2 The association between DR and clinicopathological characteristics

Characteristics	DR			P value
	Immature (N=44)	Intermediate (N=62)	Mature (N=101)	
Gender, n (%)				0.526
Male	30 (68.2)	36 (58.1)	60 (59.4)	
Female	14 (31.8)	26 (41.9)	41 (40.6)	
Age, n (%)				0.714
>65 years	9 (20.5)	17 (27.4)	25 (24.8)	
≤65 years	35 (79.5)	45 (72.6)	76 (75.2)	
Histologic grade, n (%)				0.158
Well	5 (11.4)	5 (8.1)	20 (19.8)	
Moderate	22 (50.0)	39 (62.9)	55 (54.5)	
Poor	17 (38.6)	18 (29.0)	26 (25.7)	
Location, n (%)				0.815
Ascending colon	4 (9.1)	10 (16.2)	9 (8.9)	
Transverse colon	1 (2.3)	0 (0.0)	3 (3.0)	
Descending colon	3 (6.8)	2 (3.2)	3 (3.0)	
Sigmoid colon	7 (15.9)	9 (14.5)	20 (19.8)	
Rectum	29 (65.9)	41 (66.1)	66 (65.3)	
Smoking, n (%)				0.712
No	36 (81.8)	47 (75.8)	81 (80.2)	
Yes	8 (18.2)	15 (24.2)	20 (19.8)	
Hypertension, n (%)				0.768
No	34 (77.3)	44 (71.0)	74 (73.4)	
Yes	10 (22.7)	18 (29.0)	27 (26.7)	
Family history of cancer, n (%)				0.318
No	38 (86.4)	50 (80.6)	90 (89.1)	
Yes	6 (13.6)	12 (19.4)	11 (10.9)	
Diabetes, n (%)				0.615
No	38 (86.4)	57 (92.0)	92 (91.1)	
Yes	6 (13.6)	5 (8.0)	9 (8.9)	
CEA level, n (%)				0.035*
Normal	17 (38.6)	36 (58.1)	62 (61.4)	
Abnormal	27 (61.4)	26 (41.9)	39 (38.6)	
CA242, n (%)				0.567
Normal	33 (75.0)	48 (77.4)	83 (82.2)	
Abnormal	11 (25.0)	14 (22.6)	18 (17.8)	

Table 2 (*continued*)

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Table 2 (*continued*)

Characteristics	DR			P value
	Immature (N=44)	Intermediate (N=62)	Mature (N=101)	
CA724, n (%)				0.743
Normal	35 (79.5)	51 (82.3)	78 (77.2)	
Abnormal	9 (20.5)	11 (17.7)	23 (22.8)	
CA199, n (%)				0.271
Normal	36 (81.8)	50 (80.6)	90 (89.1)	
Abnormal	8 (18.2)	12 (19.4)	11 (10.9)	
Ki-67 level, n (%)				0.190
Low	4 (9.1)	1 (1.6)	4 (4.0)	
High	40 (90.9)	61 (98.4)	97 (96.0)	
T stage, n (%)				0.156
T3	26 (59.1)	35 (56.5)	71 (70.3)	
T4	18 (40.9)	27 (43.5)	30 (29.7)	
Lymphovascular invasion, n (%)				0.420
Absent	32 (72.7)	46 (74.2)	82 (81.2)	
Present	12 (27.3)	16 (25.8)	19 (18.8)	
Perineural invasion, n (%)				0.024*
Absent	36 (81.8)	56 (90.3)	97 (96.0)	
Present	8 (18.2)	6 (9.7)	4 (4.0)	
IOP status, n (%)				0.030*
No	37 (84.1)	54 (87.1)	97 (96.0)	
Yes	7 (15.9)	8 (12.9)	4 (4.0)	
Nodes examined, n (%)				0.502
12 or more	35 (79.5)	52 (83.9)	88 (87.1)	
Less than 12	9 (20.5)	10 (16.1)	13 (12.9)	
Mismatch repair status, n (%)				0.909
MSI-L	35 (79.5)	48 (77.4)	77 (76.2)	
MSI-H	9 (20.5)	14 (22.6)	24 (23.8)	
TBd, n (%)				0.000*
<5 (BD1)	5 (11.4)	28 (45.2)	75 (74.3)	
5–9 (BD2)	13 (29.5)	21 (33.9)	11 (10.9)	
≥10 (BD3)	26 (59.1)	13 (20.9)	15 (14.8)	
TSR, n (%)				0.000*
Low (stroma-high)	36 (81.8)	30 (48.4)	27 (26.7)	
High (stroma-low)	8 (18.2)	32 (51.6)	74 (73.3)	

Table 2 (*continued*)

Table 2 (*continued*)

Characteristics	DR			P value
	Immature (N=44)	Intermediate (N=62)	Mature (N=101)	
Adjuvant therapy, n (%)				0.015*
No	12 (27.3)	15 (24.2)	45 (44.6)	
Yes	32 (72.7)	47 (75.8)	56 (55.4)	

*P<0.05. MSI, microsatellite instability; CEA, carcinoembryonic antigen; CA242, carbohydrate antigen 242; CA724, carbohydrate antigen 724; CA199, carbohydrate antigen199; IOP status, internal obstruction or perforation; DR, desmoplastic reaction; TBd, tumour budding; TSR, tumor-stroma ratio.

Table 3 The association between TBd and clinicopathological characteristics

Characteristics	TBd			P value
	BD1 (N=108)	BD2 (N=45)	BD3 (N=54)	
Gender, n (%)				0.852
Male	65 (60.2)	29 (64.4)	32 (59.3)	
Female	43 (39.8)	16 (35.6)	22 (40.7)	
Age, n (%)				0.217
>65 years	24 (22.2)	9 (20.0)	18 (33.3)	
≤65 years	84 (77.8)	36 (80.0)	36 (66.7)	
Histologic grade, n (%)				0.046*
Well	20 (18.5)	6 (13.3)	4 (7.4)	
Moderate	65 (60.2)	24 (53.3)	27 (50.0)	
Poor	23 (21.3)	15 (33.4)	23 (42.6)	
Location, n (%)				0.382
Ascending colon	16 (14.8)	2 (4.4)	5 (9.3)	
Transverse colon	2 (1.9)	1 (2.2)	1 (1.9)	
Descending colon	4 (3.7)	2 (4.4)	2 (3.7)	
Sigmoid colon	16 (14.8)	8 (17.8)	12 (22.2)	
Rectum	70 (64.8)	32 (71.1)	34 (62.9)	
Smoking, n (%)				0.886
No	85 (78.7)	35 (77.8)	44 (81.5)	
Yes	23 (21.3)	10 (22.2)	10 (18.5)	
Hypertension, n (%)				0.004*
No	70 (64.8)	34 (75.6)	48 (88.9)	
Yes	38 (35.2)	11 (24.4)	6 (11.1)	
Family history of cancer, n (%)				0.504
No	91 (84.3)	38 (84.4)	49 (90.7)	
Yes	17 (15.7)	7 (15.6)	5 (9.3)	

Table 3 (*continued*)

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Table 3 (*continued*)

Characteristics	TBd			P value
	BD1 (N=108)	BD2 (N=45)	BD3 (N=54)	
Diabetes, n (%)				0.106
No	102 (94.4)	39 (86.7)	46 (85.2)	
Yes	6 (5.6)	6 (13.3)	8 (14.8)	
CEA level, n (%)				0.396
Normal	63 (58.3)	21 (46.7)	31 (57.4)	
Abnormal	45 (41.7)	24 (53.3)	23 (42.6)	
CA242, n (%)				0.431
Normal	89 (82.4)	33 (73.3)	42 (77.8)	
Abnormal	19 (17.6)	12 (26.7)	12 (22.2)	
CA724, n (%)				0.852
Normal	84 (77.8)	36 (80.0)	44 (81.5)	
Abnormal	24 (22.2)	9 (20.0)	10 (18.5)	
CA199, n (%)				0.114
Normal	97 (89.9)	35 (77.8)	44 (81.5)	
Abnormal	11 (10.1)	10 (22.2)	10 (18.5)	
Ki-67 level, n (%)				0.962
Low	5 (4.6)	2 (4.4)	2 (3.7)	
High	103 (95.4)	43 (95.6)	52 (96.3)	
T stage, n (%)				0.203
T3	74 (68.5)	24 (53.3)	34 (63.0)	
T4	34 (31.5)	21 (46.7)	20 (37.0)	
Lymphovascular invasion, n (%)				0.503
Absent	87 (80.6)	33 (73.3)	40 (74.1)	
Present	21 (19.4)	12 (26.7)	14 (25.9)	
Perineural invasion, n (%)				0.738
Absent	100 (92.6)	41 (91.1)	48 (88.9)	
Present	8 (7.4)	4 (8.9)	6 (11.1)	
IOP status, n (%)				0.164
No	102 (94.4)	39 (86.7)	47 (87.0)	
Yes	6 (5.6)	6 (13.3)	7 (13.0)	
Nodes examined, n (%)				0.470
12 or more	94 (87.0)	38 (84.4)	43 (79.6)	
Less than 12	14 (13.0)	7 (15.6)	11 (20.4)	

Table 3 (*continued*)

Table 3 (*continued*)

Characteristics	TBd			P value
	BD1 (N=108)	BD2 (N=45)	BD3 (N=54)	
Mismatch repair status, n (%)				0.712
MSI-L	81 (75.0)	36 (80.0)	43 (79.6)	
MSI-H	27 (25.0)	9 (20.0)	11 (20.4)	
DR, n (%)				0.000*
Mature	75 (69.4)	11 (24.4)	16 (29.6)	
Intermediate	28 (25.9)	21 (46.7)	13 (24.1)	
Immature	5 (4.7)	13 (28.9)	25 (46.3)	
TSR, n (%)				0.000*
Low (stroma-high)	28 (25.9)	23 (51.1)	42 (77.8)	
High (stroma-low)	80 (74.1)	22 (48.9)	12 (22.2)	
Adjuvant therapy, n (%)				0.000*
No	47 (43.5)	11 (24.4)	14 (25.9)	
Yes	61 (56.5)	34 (75.6)	40 (74.1)	

*$P<0.05$. MSI, microsatellite instability; CEA, carcinoembryonic antigen; CA242, carbohydrate antigen 242; CA724, carbohydrate antigen 724; CA199, carbohydrate antigen199; IOP status, internal obstruction or perforation; DR, desmoplastic reaction; TBd, tumour budding; TSR, tumor-stroma ratio.

Table 4 The association between TSR and clinicopathological characteristics

Characteristics	TSR		P value
	Low (stroma-high) (N=93)	High (stroma-low) (N=114)	
Gender, n (%)			0.123
Male	62 (66.7)	64 (56.1)	
Female	31 (33.3)	50 (43.9)	
Age, n (%)			0.048*
>65 years	29 (31.2)	22 (19.3)	
≤65 years	64 (68.9)	92 (80.7)	
Histologic grade, n (%)			0.221
Well	13 (14.0)	17 (14.9)	
Moderate	47 (50.5)	69 (60.5)	
Poor	33 (35.5)	28 (24.6)	
Location, n (%)			0.300
Ascending colon	7 (7.5)	16 (14.0)	
Transverse colon	2 (2.2)	2 (1.8)	

Table 4 (*continued*)

Table 4 (*continued*)

Characteristics	TSR		P value
	Low (stroma-high) (N=93)	High (stroma-low) (N=114)	
Descending colon	4 (4.3)	4 (3.5)	
Sigmoid colon	21 (22.6)	15 (13.2)	
Rectum	59 (63.4)	77 (67.5)	
Smoking, n (%)			0.356
No	71 (76.3)	93 (81.6)	
Yes	22 (23.7)	21 (18.4)	
Hypertension, n (%)			0.241
No	72 (77.4)	80 (70.2)	
Yes	21 (22.6)	34 (29.8)	
Family history of cancer, n (%)			0.696
No	79 (84.9)	99 (86.8)	
Yes	14 (15.1)	15 (13.2)	

Table 4 (*continued*)

Table 4 (*continued*)

Characteristics	TSR		P value
	Low (stroma-high) (N=93)	High (stroma-low) (N=114)	
Diabetes, n (%)			0.995
No	84 (90.3)	103 (90.4)	
Yes	9 (9.7)	11 (9.6)	
CEA level, n (%)			0.925
Normal	52 (55.9)	63 (55.3)	
Abnormal	41 (44.1)	51 (44.7)	
CA242, n (%)			0.356
Normal	71 (76.3)	93 (81.6)	
Abnormal	22 (23.7)	21 (18.4)	
CA724, n (%)			0.815
Normal	73 (78.5)	91 (79.8)	
Abnormal	20 (21.5)	23 (20.2)	
CA199, n (%)			0.111
Normal	75 (80.6)	101 (88.6)	
Abnormal	18 (19.4)	13 (11.4)	
Ki-67 level, n (%)			0.318
Low	6 (6.5)	3 (2.6)	
High	87 (93.5)	111 (97.4)	
T stage, n(%)			0.930
T3	59 (63.4)	73 (64.0)	
T4	34 (36.6)	41 (36.0)	
Lymphovascular invasion, n (%)			0.710
Absent	73 (78.5)	87 (76.3)	
Present	20 (21.5)	27 (23.7)	
Perineural invasion, n (%)			0.015*
Absent	80 (86.0)	109 (95.6)	
Present	13 (14.0)	5 (4.4)	
IOP status, n (%)			0.822
No	84 (90.3)	104 (91.2)	
Yes	9 (9.7)	10 (8.8)	
Nodes examined, n (%)			0.530
12 or more	77 (82.8)	98 (86.0)	
Less than 12	16 (17.2)	16 (14.0)	

Table 4 (*continued*)

Table 4 (*continued*)

Characteristics	TSR		P value
	Low (stroma-high) (N=93)	High (stroma-low) (N=114)	
Mismatch repair status, n (%)			0.05
MSI-L	66 (71.0)	94 (82.5)	
MSI-H	27 (29.0)	20 (17.5)	
DR, n (%)			0.000*
Mature	27 (29.0)	74 (64.9)	
Intermediate	30 (32.3)	32 (28.1)	
Immature	36 (38.7)	8 (7.0)	
TBd, n (%)			0.000*
<5 (BD1)	28 (30.1)	80 (70.2)	
5–9 (BD2)	23 (24.7)	22 (19.3)	
≥10 (BD3)	42 (45.2)	12 (10.5)	
Adjuvant therapy, n (%)			0.117
No	27 (29.0)	45 (39.5)	
Yes	66 (71.0)	69 (60.5)	

*P<0.05. MSI, microsatellite instability; CEA, carcinoembryonic antigen; CA242, carbohydrate antigen 242; CA724, carbohydrate antigen 724; CA199, carbohydrate antigen199; IOP status, internal obstruction or perforation; DR, desmoplastic reaction; TBd, tumour budding; TSR, tumor-stroma ratio.

both TBd and TSR failed to predict RFS outcomes. To the best of our knowledge, the prognostic value of TSR and TBd in CRC has been extensively studied, but the results remain controversial; for example, Mesker *et al.* (32) was the first study to show that a high TSR associated with both longer DFS and OS is an independent prognostic variable for colon cancer. Other reports have reached the opposite conclusion (8,9,20,33). Similarly, a wealth of studies has identified the independent prognostic value of TBd for the prediction of patient clinical outcomes in CRC (34-37), but this is contradictory to other studies that have not found any significant correlation between TBd and OS or RFS (19,38). We speculated that these findings may be attributable to the differences in the study populations. In our cohort, we found that only 55.1% of tumors had a high TSR, which is lower than that reported by others (70.8% [9], 70.51% [19], and 73.00% [32]). Additionally, our study included patients with stage II CRC, of which rectal cancer constituted the majority (65.7%), whereas the study by Koelzer *et al.* (39)
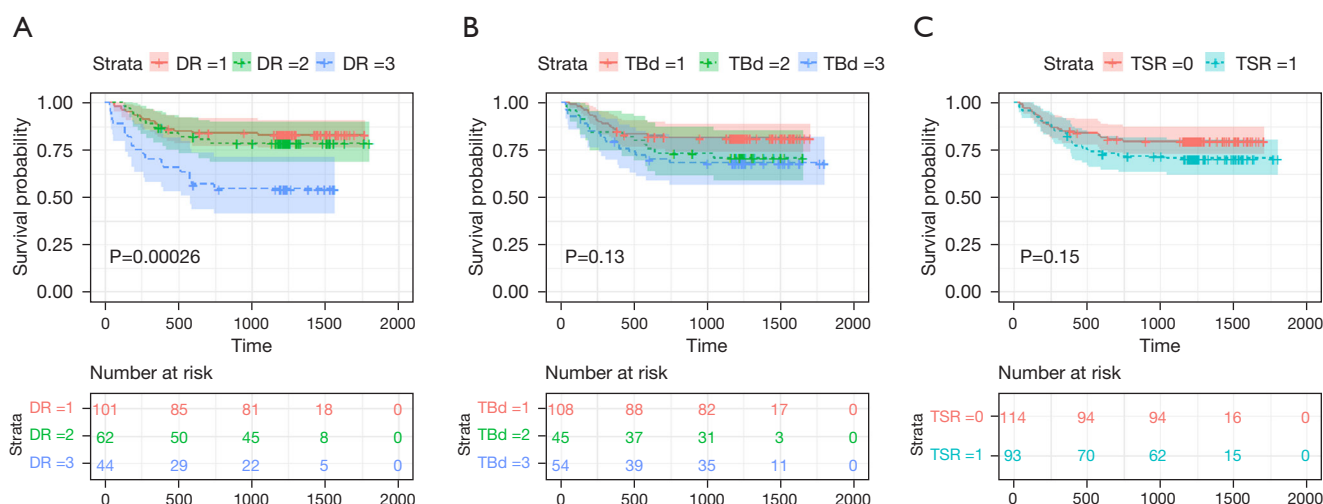
Figure 2 Kaplan-Meier survival curves for RFS (time unit: days). (A) DR; (B) TBd; (C) TSR. DR1, DR-mature; DR2, DR-intermediate; DR3, DR-immature; TBd1, BD1; TBd2, BD2; TBd3, BD3; TSR0, high TSR (stroma-low); TSR1, low TSR (stroma-high). RFS, relapse-free survival; DR, desmoplastic reaction; TBd, tumor budding; TSR, tumor-stroma ratio.

Table 5 Univariate and multivariate cox regression analysis for relapse-free survival in stage II CRC

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Gender			0.839			
Male		Referent				
Female	0.942	0.532–1.668				
Age			0.111			
≤65 years		Referent				
>65 years	1.023	0.995–1.052				
Histologic grade			0.328			
Well		Referent				
Moderate	1.408	0.542–3.657				
Poor	1.969	0.731–5.303				
Location			0.111			
Ascending colon		Referent				
Transverse colon	1.392	0.156–12.453				
Descending colon	2.582	0.578–11.544				
Sigmoid colon	2.598	0.855–7.896				
Rectum	1.166	0.409–3.324				

Table 5 (*continued*)

Table 5 (*continued*)

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Smoking			0.726			
No		Referent				
Yes	1.127	0.577–2.201				
Hypertension			0.054			
No		Referent				
Yes	0.475	0.223–1.013				
Family history of cancer			0.656			
No		Referent				
Yes	1.188	0.558–2.530				
Diabetes			0.823			
No		Referent				
Yes	1.111	0.441–2.799				
CEA			0.001*			0.190
Normal		Referent				
Abnormal	2.640	1.481–4.707		1.514	0.815–2.813	
CA724			0.109			
Normal		Referent				
Abnormal	1.657	0.894–3.074				
CA242			0.024*			0.091
Normal		Referent				
Abnormal	1.986	1.096–3.601		1.717	0.918–3.212	
CA199			0.075			
Normal		Referent				
Abnormal	1.837	0.940–3.589				
Ki-67 level			0.311			
Low		Referent				
High	1.01	0.990–1.031				
T stage			0.040*			0.269
T3		Referent				
T4	1.787	1.026–3.114		1.416	0.764–2.623	
Lymphovascular invasion			<0.001*			0.049*
Absent		Referent				
Present	3.100	1.767–5.439		1.919	1.004–3.669	

Table 5 (*continued*)

Table 5 (continued)

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Perineural invasion			<0.001*			0.005*
Absent		Referent				
Present	5.696	3.051–10.633		2.724	1.362–5.448	
IOP status			0.001*			0.240
Absent		Referent				
Present	2.976	1.522–5.817		1.571	0.740–3.334	
Number of nodes examined			<0.001*			0.085
12 or more		Referent				
Less than 12	3.301	1.834–5.940		1.777	0.925–3.417	
Mismatch repair status			0.019*			0.267
MSI-L		Referent				
MSI-H	0.331	0.132–0.835		0.574	0.215–1.530	
DR			<0.001*			
Mature + intermediate		Referent				
Immature	3.021	1.715–5.323		2.111	1.184–3.766	0.011*
TBd			0.135			
<5 (BD1)		Referent				
5–9 (BD2)	1.632	0.812–3.281				
≥10 (BD3)	1.872	0.981–3.574				
TSR			0.158			
High (stroma-low)		Referent				
Low (stroma-high)	1.493	0.856–2.604				
Adjuvant therapy			0.001*			0.400
No		Referent				
Yes	3.713	1.670–8.256		1.482	0.593–3.704	

*, P<0.05. MSI, microsatellite instability; CEA, carcinoembryonic antigen; CA242, carbohydrate antigen 242; CA724, carbohydrate antigen 724; CA199, carbohydrate antigen199; IOP status, internal obstruction or perforation; DR, desmoplastic reaction; TBd, tumour budding; TSR, tumor-stroma ratio.

contained only upper rectal cancers. Furthermore, the heterogeneity of stage II tumors is inevitable, and thus these might explain the divergence in the results obtained, although larger study sample sizes are needed before widespread implementation in clinical practice.

DR refers to the presence of excess extracellular matrix at the tumor invasive front (40). Previous studies performed by Ueno *et al.* have shown that DR is associated with adverse clinicopathological features (advanced T stage and lymphatic or vascular invasion) in CRC (23,40). Meanwhile, DR is an independent factor for poor prognosis in stage II CRC (15). In addition, Ueno *et al.* (23) showed that compared with T stage, MSI and TBd, DR is the best prognostic factor for stage II colon cancer. In our study,
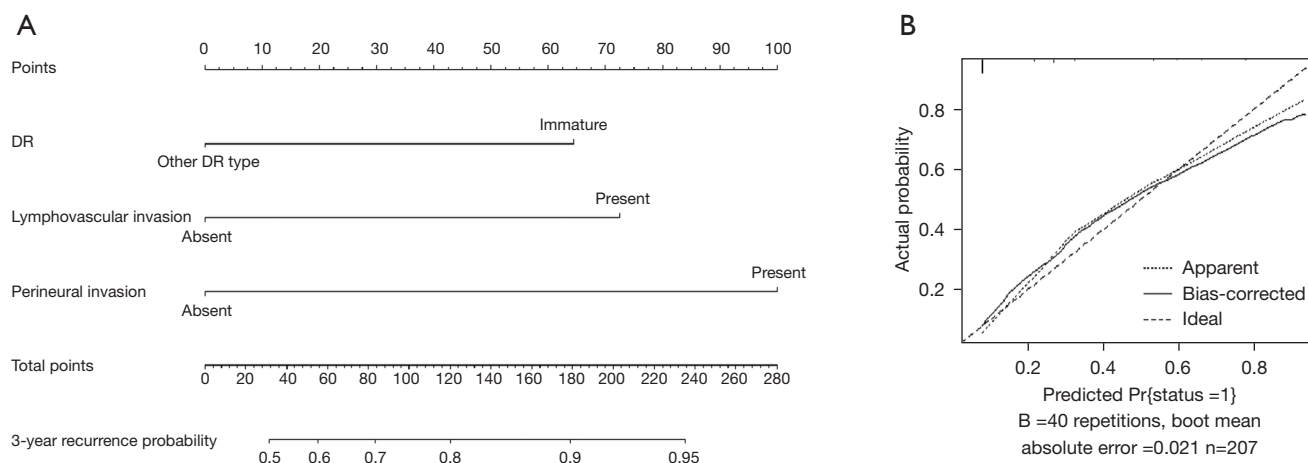
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Figure 3 Construction and validation of the nomogram. (A) A nomogram for differentiating and prediction of disease recurrence for stage II CRC patients. (B) Calibration curves of the prediction model. Instructions for use of the nomogram: First, assign the points of each characteristic of the patient by drawing a vertical line from that variable to the points scale. Then, sum all the points and draw a vertical line from the total points scale to disease recurrence axis to obtain the probability. DR, desmoplastic reaction; CRC, colorectal cancer.
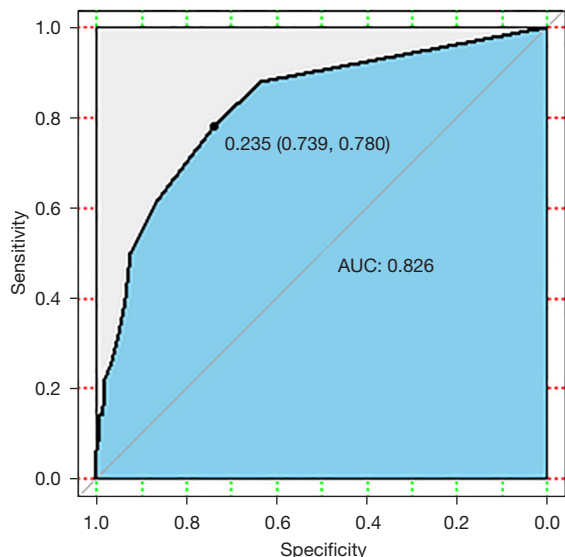
Figure 4 ROC curve analysis of the prediction model. ROC, receiver operating characteristic; AUC, area under the curve.

the DR categorization was the mature type in 101 patients (48.8%), the intermediate type in 62 patients (30.0%), and the immature type in 44 patients (21.2%). Moreover, this study found that DR was associated with CEA level, PNI, IOP, TSR and TBd. Additionally, our results revealed that DR categorization could stratify the risk of recurrence in stage II CRC patients. Compared with patients with mature stroma, patients with immature stroma had the

most unfavorable prognosis. Notably, compared to TSR and TBd, DR was the only independent factor for RFS, as demonstrated by multivariate analysis in the current study. Based on the current results, we showed that the prognostic significance of DR outperformed TBd and TSR and recommended adding DR as a biomarker in routine pathological reports.

Since many patients who are diagnosed with stage II CRC have concerns about their outcome after surgery, there is an urgent need to build a scoring system for reference. To date, several potential biomarkers, such as FOXP3⁺ tumor-infiltrating lymphocytes (TILs), microRNAs and radiomics signatures, have been included in nomograms to substratify these patients and guide individualized treatment decisions (41-43). However, these biomarkers still require further validation and are not included in the routine checklist for CRC.

In the present study, we constructed a predictive nomogram that can generate an individual probability of having disease recurrence by integrating both the significant preoperative clinicopathologic variables and DR, which is easily evaluated using hematoxylin-eosin-stained specimens. Therefore, both physicians and patients could use this novel, easy-to-use and cost-effective scoring system to make preoperative individualized predictions of the disease recurrence risk, in line with the current trends in personalized medicine (44).

Although this is a preliminary study, it still has some

limitations. First, as a retrospective study, there was an unavoidable selection bias. Second, this study included patients from 5 to 10 years ago, and the development of treatment for CRC during this period may have affected the prognosis. Finally, the data were based on a single cancer center and lacked external validation. For this reason, large multicenter studies are needed to further investigate the trends.

To conclude, our study highlighted the prognostic value of DR in stage II CRC. Moreover, the results of the study provided evidence that DR, as a novel histopathological predictor, could contribute to improve the prognostic performance for the prediction of RFS in stage II CRC after surgery. Therefore, DR should be considered in the routine pathological report. Furthermore, the significant correlation of DR with several conventional high-risk clinicopathological factors suggests that such a model with DR may have the potential to substratify the high-risk patients and predict patients who are more likely to benefit from postoperative adjuvant chemotherapy, although future multicentric and prospective studies are needed before widespread implementation in clinical practice.

Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://jgo. amegroups.com/article/view/10.21037/jgo-22-758/rc

Data Sharing Statement: Available at https://jgo.amegroups. com/article/view/10.21037/jgo-22-758/dss

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Conflicts of interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups. com/article/view/10.21037/jgo-22-758/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This retrospective study was approved by the Ethics Committee of Tianjin Medical University Cancer Institute & Hospital (No. EK2022175) and a waiver of patient consent was granted for the retrospective nature of the study.

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Table S1 Survival analysis for recurrence

Characteristics	P value	Method
Gender	0.839	Log rank
Age	0.059	Cox PH
Histologic grade	0.320	Log rank
Location	0.093	Log rank
Smoking	0.726	Log rank
Hypertension	0.049	Log rank
Family history of cancer	0.655	Log rank
Diabetes	0.823	Log rank
CEA level	0.001	Log rank
CA242	0.021	Log rank
CA724	0.105	Log rank
CA199	0.071	Log rank
Ki-67 level	0.038	Cox PH
T stage	0.037	Log rank
Lymphovascular invasion	<0.001	Log rank
Perineural invasion	<0.001	Log rank
IOP status	0.001	Log rank
Number of nodes examined	<0.001	Log rank
Mismatch repair status	0.014	Log rank
DR	<0.001	Log rank
TBd	0.127	Log rank
TSR	0.154	Log rank

MSI, microsatellite instability; CEA, carcinoembryonic antigen; CA242, carbohydrate antigen 242; CA724, carbohydrate antigen 724; CA199, carbohydrate antigen199; IOP status, internal obstruction or perforation; DR, desmoplastic reaction; TBd, tumour budding; TSR, tumor-stroma ratio.