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DNA ploidy combined with tumor stroma as a biomarker for predicting the prognosis of stage II colorectal cancer patients and identifying candidates for chemotherapy

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Abstract

Purpose The efficacy of postoperative adjuvant chemotherapy in patients with stage II colorectal cancer has been a subject of debate. This study aimed to evaluate the prognostic and predictive significance of DNA ploidy and stroma ratio in patients diagnosed with stage II colorectal cancer (CRC).

Methods Clinical data and tumor tissues from 179 patients with stage II CRC were collected retrospectively. DNA ploidy (P) and stroma (S) were assessed using automatic image analysis tools powered by machine learning.

Results Patients were categorized into three risk groups: PS-low (diploid and low stroma, PS-L), PS-intermediate (non-diploid or high stroma, PS-M), and PS-high (non-diploid and high-stroma, PS-H). According to the univariable model, the PS-H group exhibited significantly poorer 5-year overall survival rates at 73.0% compared to 87.8%, with a hazard ratio (HR) of 2.281 (95% CI: 0.946-5.502, $P=0.066$), as well as lower 5-year disease-free survival rates at 69.4% versus 86.6%, HR = 2.323 (95% CI: 1.016-5.308, $P=0.046$) among stage II colorectal cancer patients. Notably, chemotherapy was associated with improved overall survival [HR = 83.460 (95% CI: 0.179-38925.833), $P=0.003$] and disease-free survival [HR = 8.628 (95% CI: 1.059-70.265), $P=0$ .044] in individuals within the PS-high group.

Conclusion While ploidy and stroma alone do not possess predictive power regarding survival outcomes for stage II colorectal cancer patients, those receiving chemotherapy within the PS-H group demonstrated enhanced survival rates. Therefore, combining assessments of ploidy and stroma may serve as an adjunctive tool in clinical decision-making processes to guide chemotherapy treatment strategies for patients diagnosed with stage II colorectal cancer.

Keywords Stage II colorectal cancer, DNA ploidy, Stroma ratio, Adjuvant chemotherapy, Prognosis

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Introduction

Colorectal cancer (CRC) is the most prevalent malignant gastrointestinal tumor in China. In contrast, the incidence of colorectal cancer has gradually declined in the United States and other developed countries. However, both its incidence and mortality rates have risen significantly in China [1, 2]. For patients diagnosed with early-stage colorectal cancer, surgical resection remains the primary treatment modality. The decision regarding postoperative adjuvant chemotherapy primarily hinges on TNM staging and microsatellite instability status. Patients with stage I CRC generally exhibit a favorable prognosis post-surgery; thus, adjuvant chemotherapy is not typically recommended for this group. Conversely, individuals with stage III colorectal cancer can derive substantial benefits from adjuvant chemotherapy.

The prognosis for stage II patients presents considerable variability due to high population heterogeneity; consequently, the advantages and risks associated with postoperative adjuvant chemotherapy remain contentious [3, 4]. Numerous studies indicate that the efficacy rate of adjuvant chemotherapy for stage II colorectal cancer is less than 5% [5]. Decisions concerning adjuvant therapy for these patients are informed by various clinicopathological factors such as microsatellite stable status, T4 staging, fewer than 12 lymph nodes sampled during surgery, poorly differentiated tumors, vascular or perineural invasion presence, as well as intestinal obstruction or perforation [6]. It has been established that stage II CRC patients harboring MSI-H tumors tend to have a favorable prognosis and do not benefit from monotherapy using 5-FU as an adjunctive treatment option. Nevertheless, it is noteworthy that only 15-20% of sporadic cases of stage II colorectal cancer exhibit MSI-H characteristics [7, 8]. Despite this understanding, survival benefits following adjuvant therapy remain limited for high-risk patients. In certain studies, no significant differences were observed in overall survival (OS) or disease-free survival (DFS) among patients with clinicopathological high-risk tumors. Furthermore, those who received adjuvant chemotherapy may be at an increased risk of experiencing toxic side effects [5, 9]. Consequently, clinical methodologies are still being investigated to evaluate the prognostic risks and benefits of chemotherapy for patients diagnosed with stage II colorectal cancer [10].

In this study, DNA ploidy and tumor stroma were identified as predictive biomarkers for stage II colorectal cancer (CRC). Aneuploidy is a characteristic feature of cancer that occurs in nearly 90% of all tumors [11]. Patients with epithelial cancers exhibiting aneuploid tumors typically have a poor prognosis, as reviewed by Havard et al. [12]. Additionally, several studies have highlighted the stage-specific prognostic significance of aneuploidy in CRC [13]. Conversely, CRC is classified as an epithelial tumor; it's tissues comprise malignant epithelium and tumor stroma. The tumor stroma plays a critical role in supporting tumor survival, growth, and metastatic potential [14]. The integration of ploidy status and stromal composition has been shown to predict outcomes for European stage II CRC patients [15] as well as Chinese cohorts [16, 17]. A previous investigation demonstrated that the combination of DNA ploidy analysis alongside chromosome context and stromal assessment could effectively predict the efficacy of adjuvant therapy [17]. In this study, we aimed to explore the prognostic implications and predictive value associated with both ploidy levels and stromal ratios in patients diagnosed with stage II colorectal cancer.

Methods

Patients

A continuous cohort of Stage II colorectal patients who underwent radical resection at the PLA General Hospital from January 1, 2011, to December 31, 2014, were enrolled retrospectively. Patients who received neoadjuvant therapy and without formalin-fixed paraffin-embedded pathological tissues were excluded. All patients were followed up until death or March 7, 2019. This study was approved by the Clinical Research Ethics Committee at PLA General Hospital.

Refinement of sample preparation and imaging

One or two 50 μm formalin-fixed paraffin-embedded (FFPE) sections were obtained from each FFPE tumor tissue block. The nuclei were isolated using a modified Hedley method [18]. In brief, the FFPE sections underwent deparaffinization, rehydration, and enzymatic digestion to facilitate nuclear isolation. The resulting nuclear suspension was filtered through a 60 μm mesh nylon filter and subsequently centrifuged onto a slide. Following this process, the nuclei were stained with Feulgen dye and imaged using a digital scanner (MBMbio Intelligence-400, MBMbio, China) equipped with a 546 nm green filter.

Measurement of DNA ploidy

The DNA Ploidy Working Station (Room 4, Kent, UK) was employed for the analysis of DNA ploidy as previously described [19]. This platform automates the classification and analysis of scanned cell nuclei utilizing machine learning algorithms. It categorizes cell nuclei into epithelial nuclei, stromal nuclei, lymphocyte nuclei (reference cells), as well as those deemed unsuitable for analysis due to fragmentation or overlap; such unsuitable nuclei must be excluded from further evaluation.

The integrated optical density (IOD) of both epithelial and reference nuclei was generated based on ploidy histograms. Tumor nucleus DNA ploidy was classified into three categories: diploid, aneuploid, and tetraploid; in this study, aneuploid and tetraploid classifications were collectively categorized as nondiploid.

Tumor-stroma ratio

Whole-slide images of H&E-stained tissue sections were scanned using a digital scanner (MBM Intelligence, China). Initially, tumor regions within these images were annotated by a pathologist; thereafter, the stroma ratio within the tumor tissue was automatically reported via the Stroma Analyzer tool (Room 4, Kent, UK), consistent with previous reports [15]. Stromal ratios equal to or less than 50% were designated as low stroma while those exceeding 50% were classified as high stroma [20].

Statistical analysis

The primary endpoints assessed in this study included overall survival (OS) and disease-free survival (DFS). OS was defined as the interval from the date of the first surgery to either the date of death for any reason or the date of the last follow-up. DFS was defined as the number of days from the date of the first operation to either death from any cause or to the occurrence of first local recurrence or metastasis. Statistical analyses were conducted using SPSS version 23.0. Kaplan–Meier survival curves were generated, and log-rank tests were employed to compare OS and DFS. Both univariate and multivariate analyses were performed utilizing Cox proportional hazards regression models. The threshold for statistical significance was established at 0.05.

Results

Patient demographics

In this study, a total of 182 patients pathologically diagnosed with stage II colorectal cancer (CRC) were collected. However, upon detection, the FFPE samples of three patients were found to not meet the requirements, and ultimately, 179 patients were included in the analysis. The median age of these patients was 61 years (range: 29-80), with a male predominance observed (69.83% versus 30.17%). Most participants had colon cancer, accounting for 71.51% of cases. Additionally, 61.45% received adjuvant chemotherapy (capecitabine combined with oxaliplatin for a duration of six to eight cycles) following surgical intervention. At the conclusion of follow-up, median OS and DFS were recorded at 60 months (25th-75th percentiles: 47-77 months) and 59 months (25th-75th percentiles: 47-77 months), respectively. A significant majority exhibited nondiploid characteristics (69.27%) and presented with a low stroma ratio (79.89%). Additional patient characteristics and their distributions are detailed in Table 1.

1. Univariate Prognostic Factors for Overall Survival (OS) and Disease-Free Survival (DFS) in Patients with Colorectal Cancer

The results of the univariate analyses regarding the 5-year OS and DFS rates among stage II colorectal cancer (CRC) patients are summarized in Table 2.

Table 1 Demographic and clinical characteristics of the patients

Variables	N (%)
Age	
≧65	63 (35.20%)
< 65	116 (64.80%)
Gender	
Male	125 (69.83%)
Female	54 (30.17%)
Tumor site	
Colon	128 (71.51%)
Rectal	51 (28.49%)
Lymph nodes sampling	
≥ 12	132 (73.74%)
< 12	45 (25.14%)
NA[a]	2 (1.12%)
pT stage	
pT3	89 (49.72%)
pT4	90 (50.28%)
Adjuvant therapy	
YES	110 (61.45%)
No	68 (37.99%)
NA[a]	1 (0.56%)
Mismatch repair status	
dMMR	61 (34.08%)
pMMR	24 (13.41%)
NA[a]	94 (52.51%)
Intestinal obstruction	
No	156 (87.15%)
Yes	23 (12.85%)
Vascular invasion	
No	170 (94.97%)
Yes	9 (5.03%)
Ploidy	
Diploid	55 (30.73%)
Nondiploid	124 (69.27%)
Stroma	
Low-stroma ratio	143 (79.89%)
High-stroma ratio	36 (20.11%)
Total	179

NA[a]: data not available

Table 2 Univariate analysis of the prognostic factors for 5-year overall survival and 5-year disease-free survival in stage II colorectal cancer patients

	5 year OS				5 year DFS			
Variables	**N (events)**	**OS (%)**	**HR (95% CI)**	***P* value**	**N (events)**	**DFS (%)**	**HR (95% CI)**	***P* value**
Lymph nodes sampling				0.656				0.592
≥ 12	132 (17)	85.51	1		132 (19	84.15	1	
< 12	45 (7)	84.39	1.222 (0.507–2.946)		45 (8)	81.96	1.253 (0.549–2.863)	
pT stage				0.144				0.257
pT3	89 (9)	88.87	1		89 (11)	86.76	1	
pT4	90 (15)	82.46	1.854 (0.810–4.243)		90 (16)	81.34	1.561 (0.723–3.368)	
Adjuvant therapy				< 0.001				0.002
YES	110 (6)	94.26	1		110 (9)	91.67	1	
No	68 (18)	70.86	5.404 (2.144–13.621)		68 (18)	70.86	3.467 (1.557–7.721)	
Clinical risk group				0.196				0.239
Low risk	56 (5)	89.38	1		56 (6)	87.76	1	
High-risk	123 (19)	83.95	1.917 (0.715–5.137)		123 (21)	82.27	1.726 (0.696–4.278)	
Ploidy				0.504				0.565
Diploid	55 (6)	88.69	1		55 (7)	87.27	1	
Nondiploid	124 (18)	83.91	1.370 (0.544–3.452)		124 (20)	82.27	1.287 (0.544–3.045)	
Stroma				0.075				0.062
Low-stroma fraction	143 (16)	87.93	1		143 (18)	86.66	1	
High-stroma fraction	36 (8)	75.4	2.163 (0.926–5.056)		36 (9)	72.5	2.142 (0.962–4.771)	
Ploidy and Stroma				0.066				0.046
PS-L&M	150 (17)	87.8	1		150 (19)	86.6	1	
PS-H	29 (7)	73.0	2.281 (0.946–5.502)		29 (8)	69.4	2.323 (1.016–5.308)	

Based on the findings related to ploidy (P) and stroma ratio (S), nondiploidy or a high stroma ratio was identified as a significant risk factor for these patients. Consequently, the patients were categorized into three groups: the low-risk group based on ploidy and stroma (PS-L; diploid and low stroma), the intermediate-risk group (PS-M; nondiploid or high stroma), and the high-risk group (PS-H; nondiploid and high stroma). Given that survival curves for both PS-L and PS-M groups were sufficiently close to overlap concerning OS and DFS, these two groups were combined for subsequent analysis. Among stage II CRC patients, those classified within the PS high-risk group exhibited a significantly elevated risk of disease recurrence [5-year DFS: 69.4%, hazard ratio (HR): 2.323 (95% CI: 1.016-5.308), $P=0.046$]. Conversely, factors such as T stage, number of lymph nodes sampled, along with other clinical indicators deemed high-risk demonstrated lesser prognostic significance in this patient cohort.

2. Univariate Prognostic Factors for Overall Survival (OS) and Disease-Free Survival (DFS) in Patients with Colon Cancer

In stage II colon cancer patients, both stromal characteristics alone or their combination with ploidy presented notable prognostic value. Specifically, individuals exhibiting a high tumor-stroma ratio (TSR) experienced poorer outcomes regarding their 5-year DFS [69.4% vs 86.9%, HR: 2.476 (1.011-6.060), $P=0.047$] compared to those with lower TSR values as detailed in Table 3. Furthermore, patients belonging to the PS high-risk category showed diminished outcomes across both metrics—5-year OS [69.2% vs 88.8%, HR: 2.792 (1.032-7.551), $P=0.043$] as well as 5-year DFS [64.4% vs 86.8%, HR: 2.800(1.116-7.021), $P=0.028$] when contrasted against individuals from low-and intermediate-risk PS cohorts—as illustrated in Table 3 and Fig. 1.The univariate analysis results are comprehensively outlined in Table 3.

In summary, adjuvant therapy has been shown to enhance both the overall survival rate at five years as well as disease-free survival rates among patients diagnosed with either colorectal or colon cancer (Tables 2 and 3). The 5-year overall survival (OS) rate for colon cancer patients who received adjuvant chemotherapy was 95.9%, in contrast to a rate of 70.9% for those who did not receive such treatment [HR 6.920, 95%

Table 3 Univariate analysis of the prognostic factors for 5-year overall survival and 5-year disease-free survival in stage II colon cancer patients

	5 year OS				5 year DFS			
Variable	**N (events)**	**OS (%)**	**HR (95% CI)**	***P* value**	**N (events)**	**DFS (%)**	**HR (95% CI)**	***P* value**
Lymph nodes sampling				0.654				0.540
≥ 12	101 (13)	85.4	1		101 (15)	83.4	1	
< 12	25 (4)	84.0	1.292 (0.421–3.963)		25 (5)	79.3	1.373 (0.499–3.778)	
pT stage				0.137				0.264
pT3	58 (5)	90.2	1		58 (7)	86.6	1	
pT4	70 (12)	81.5	2.211 (0.777–6.285)		70 (13)	80.1	1.690 (0.673–4.242)	
Adjuvant therapy				0.002				0.013
YES	73 (3)	95.9	1		73 (6)	91.5	1	
No	54 (14)	70.9	6.920 (1.987–24.093)		54 (14)	70.9	3.353 (1.288–8.729)	
Clinical risk group				0.186				0.224
Low risk	40 (3)	90.6	1		40 (4)	88.0	1	
High-risk	88 (14)	83.2	2.323 (0.667–8.089)		88 (16)	80.7	1.973 (0.659–5.907)	
Ploidy				0.355				0.401
Diploid	43 (4)	90.7	1		43 (5)	88.4	1	
Nondiploid	85 (13)	82.6	1.697 (0.553–5.204)		85 (15)	80.1	1.542 (0.561–4.244)	
Stroma				0.055				0.047
Low-stroma ratio	99 (10)	89.1	1		99 (12)	86.9	1	
High-stroma ratio	29 (7)	73.0	2.574 (0.979–6.763)		29 (8)	69.4	2.476 (1.011–6.060)	
Ploidy and Stroma				0.043				0.028
PS-L&M	106 (11)	88.8	1		106 (13)	86.8	1	
PS-H	22 (6)	69.2	2.792 (1.032–7.551)		22 (7)	64.4	2.800 (1.116–7.021)	

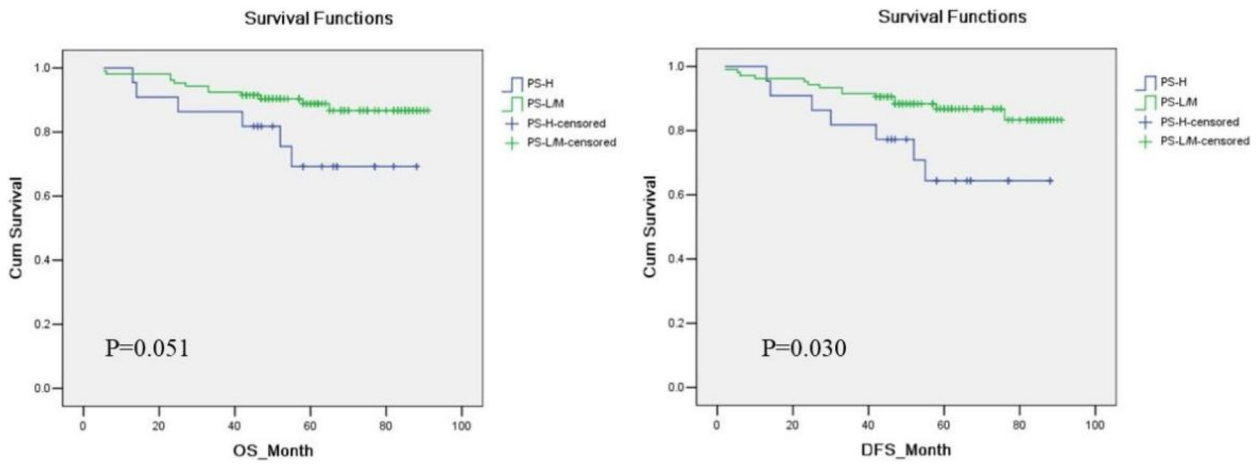

Fig. 1 Kaplan–Meier plots illustrating overall survival (OS, **A**) and disease-free survival (DFS, **B**) in stage II colon cancer patients when stratified by ploidy and stroma. PS-LM: Ploidy and stroma low risk (diploidy and low stroma) and middle risk (aneuploidy or high stroma); PS-H: Ploidy and high-risk stroma (aneuploidy and high stroma)

CI (1.987-24.093), $P=0.002$] (Table 3). Notably, other high-risk clinical factors in colon cancer patients did not demonstrate significant prognostic value for either OS or disease-free survival (DFS) (Table 3).

3. Multivariate Analysis of Prognostic Factors for OS and DFS in Patients with Colon Cancer

In the multivariate analysis, the pathological T stage, number of lymph nodes sampled, adjuvant therapy, and ploidy-stroma were included as covariates. The results indicated that the main contributing factor to the 5-year OS among stage II colon cancer patients was the administration of adjuvant chemotherapy [HR 5.230 (95% CI: 1.711-15.986), $P=0.004$]. Furthermore,

adjuvant chemotherapy also significantly influenced the 5-year DFS [HR 2.923; 95% CI: 1.171-7.296, $P=0.022$]. A summary of the multivariate analyses across individual datasets is presented in Table 4.

4. Univariate Analysis of Adjuvant Therapy for Overall Survival (OS) and Disease-Free Survival (DFS) in Stage II Colorectal Cancer Patients Stratified by Independent Variables

In patients with colorectal cancer, the 5-year overall survival (OS) rate for those who received adjuvant chemotherapy was 94.26%, compared to 70.86% for those who did not receive such treatment [HR 5.404, 95% CI (2.144-13.621), $P<0.001$] (Table 2). Similarly, the 5-year disease-free survival (DFS) rate was observed to be 91.67% in patients treated with adjuvant chemotherapy, while it was only 70.86% in those without this intervention [HR 3.467, 95% CI (1.557-7.721), $P=0.002$] (Table 2). These findings indicate that adjuvant therapy significantly enhances both OS and DFS among colorectal cancer patients in this study. However, previous research has suggested that stage II colorectal cancer patients may derive benefits from limited amounts of chemotherapy; conversely, some individuals might experience adverse toxic side effects as a result of treatment. To identify which patients would benefit most from chemotherapy, we stratified them based on clinical risk factors, ploidy status, and stromal characteristics. As illustrated in Table 5, patients with nondiploid tumors exhibited significant improvements in OS [HR 4.781; 95% CI: (1.735-13.17), $P=0.002$] and DFS [HR 2.921; (1.237-6.9), $P=0.015$] following chemotherapy administration. In contrast, there were no statistically significant differences observed regarding OS ($P=0.088$) or DFS ($P=0.12$) between diploid tumor patients who did or did not receive chemotherapy. Furthermore, the benefits associated with adjuvant chemotherapy concerning OS and DFS were not found to be significant within the PS-low-risk group [($P=0.283$) or ($P=0.369$), respectively]. However, an improvement in OS [HR3.462; (95%CI:1.155-10.373), $P=0.027$] due to chemotherapy was noted within the PS-intermediate group. Additionally, substantial enhancements were recorded for both OS [HR 83.460; (95%CI:0.179-38,925.833), $P=0.003$] and DFS [HR 8.628;(95%CI:1.059-70.265), $P=0.044$] among patients classified within the PS-high group (Table 5, Fig. 2).

For the clinical factors, patients with pathological T4 [OS, HR 9.376; 95% CI: (2.112-41.63), $P=0.003$; DFS, HR 6.22; 95% CI: (1.769-21.86), $P=0.004$] and those classified as clinical high-risk [OS, HR 4.172; 95% CI: (1.599-10.89), $P=0.004$; DFS, HR 3.031; 95% CI: (1.269-7.243), $P=0.013$] demonstrated a significant benefit from adjuvant chemotherapy treatment. Conversely, patients with pathological T4 and clinical high-risk characteristics who did not receive chemotherapy exhibited poorer overall survival (OS) and disease-free survival (DFS) (Table 5).

Discussion

Stage II accounts for approximately 20-30% of colorectal cancer patients, and the use of adjuvant chemotherapy has long been a subject of debate [4, 5, 9]. The decision-making process regarding chemotherapy is primarily based on traditional histopathological features as outlined in the NCCN or ESMO guidelines. However, according to the QUASAR study [5], the absolute improvement in the five-year overall survival (OS) rate for patients receiving adjuvant chemotherapy was merely 3.6%. Therefore, there is an urgent need for novel biomarkers that can accurately identify populations most likely to benefit from adjuvant chemotherapy.

DNA aneuploidy represents a significant contributor to chromosomal instability and tumorigenesis. Numerous studies have indicated a correlation between DNA aneuploidy and chromosome instability. Prognosis tends to be poorer for colorectal cancer patients with aneuploid tumors [21]. The tumor stroma consists of an extracellular matrix interspersed with fibroblasts, myofibroblasts, endothelial cells, as well as inflammatory and immune infiltrative cells; it plays a crucial role in tumor initiation, growth, invasion, and metastasis. Colorectal cancer

Table 4 Multivariate analysis of 5-year overall survival and 5-year disease-free survival prognostic factors in stage II colon cancer patients

	OS		DFS	
Variable	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**
Lymph nodes sampling (≥12 and <12)	1.667(0.520-5.343)	0.390	1.699(0.593-4.865)	0.324
pT stage (pT3 and pT4)	1.457(0.532-3.993)	0.464	1.246(0.500-3.102)	0.637
Adjuvant therapy (Yes and No)	5.230(1.711-15.986)	0.004	2.923(1.171-7.296)	0.022
Ploidy and Stroma (L&M and H)	2.390(0.842-6.784)	0.102	2.559(0.976-6.711)	0.056

Table 5 Univariate analysis of adjuvant therapy for OS and DFS in stage II CRC patients when stratified by independent variables

	OS		DFS	
Variables	**HR (95%CI)**	***P* value**	**HR (95%CI)**	***P* value**
Lymph nodes sampling				
≥ 12	3.906 (1.496–10.19)	0.005	2.839 (1.187–6.791)	0.019
< 12	7.591(1.463–39.38)	0.016	4.712 (1.121–19.8)	0.034
pT stage				
pT3	2.154 (0.6944–6.685)	0.184	1.587 (0.5501–4.576)	0.393
pT4	9.376 (2.112–41.63)	0.003	6.22 (1.769–21.86)	0.004
Clinical risk group				
Low risk	4.673 (0.9052–24.12)	0.066	3.059 (0.7301–12.82)	0.126
High risk	4.172 (1.599–10.89)	0.004	3.031 (1.269–7.243)	0.013
Ploidy				
Diploid	3.703 (0.8238–16.64)	0.088	3.277 (0.733–14.65)	0.12
Nondiploid	4.781 (1.735–13.17)	0.002	2.921 (1.237–6.9)	0.015
Stroma				
Low-stroma fraction	2.618 (1.05–6.527)	0.0389	2.032 (0.8596–4.802)	0.106
High-stroma fraction	129.805 (0.291-Inf)	0.118	13.74 (1.716–110.1)	0.0136
PS				
PS-L	2.403 (0.483–11.962)	0.283	2.082 (0.420–10.319)	0.369
PS-M	3.462 (1.155–10.373)	0.027	2.476 (0.917–6.686)	0.074
PS-H	83.460 (0.179–38,925.833)	0.003	8.628 (1.059–70.265)	0.044

The clinical risk group is referenced from the NCCN guidelines for stage II colorectal cancer high-risk factors

originates from epithelial tumor cells found within both malignant epithelium and tumor stroma. Substances secreted by highly invasive tumor cells into the stroma can even influence surrounding normal tissue cells and alter their metabolic processes. Consequently, the characteristics of the tumor stroma hold significant predictive value concerning both tumor behavior and treatment strategy selection [22].

In this study, we combined ploidy and stroma to predict the prognosis and chemotherapy benefits for patients with stage II colon and rectal cancer. Aneuploidy and high stromal content were identified as significant risk factors for recurrence. Patients exhibiting zero, one, or two high-risk factors were categorized into low-risk, intermediate-risk, and high-risk groups, respectively. Among patients with colon cancer, those presenting both aneuploidy and a high tumor-stroma ratio (TSR) demonstrated significantly poorer 5-year overall survival (OS) [HR 2.792 (95% CI: 1.032-7.551), $P=0.043$] and 5-year disease-free survival (DFS) [HR 2.800 (95% CI: 1.116-7.021), $P=0.028$], according to univariate analysis (Table 3). When incorporating pT stage, the number of lymph nodes sampled, and the administration of adjuvant chemotherapy as covariates in multivariate analysis, results indicated that chemotherapy could enhance OS ($P=0.004$) and DFS ($P=0.022$) in stage II colon cancer patients (Table 4). Furthermore, findings from the multivariate analysis suggested that patients in the high-risk group exhibited a trend towards poorer 5-year DFS outcomes ($P=0.056$; Table 4), although this difference did not reach statistical significance.

The combination of ploidy and stroma has been shown to predict the prognosis of stage II colon cancer patients, as previously established. Danielsen et al. [15] conducted an analysis on the 5-year cancer-specific survival (CSS) rates of 1,029 patients from the QUASAR2, Gloucester, and Oslo University Hospital-Aker cohorts. They reported that the five-year CSS rates for the low-, intermediate-, and high-risk groups were 90%, 83%, and 73%, respectively ($P<0.001$). Furthermore, the prognostic efficacy of ploidy and stroma has recently been validated in both a Chinese clinical high-risk colon cohort [16] and a colorectal cohort [17]. In this study, ploidy and stroma were found to be predictive of a 5-year disease-free survival (DFS) [HR 2.323 (95% CI: 1.016-5.308), $P=0.046$; Table 2] in colorectal cancer patients, as well as predicting a 5-year overall survival (OS) [HR 2.792 (95% CI: 1.032-7.551), $P=0.043$; Table 3] and DFS [HR 2.800 (95% CI: 1.116-7.021), $P=0.028$; Table 3] specifically in colon cancer patients during univariate analysis. However, in multivariate analysis, while PS-high risk indicated a trend towards poorer outcomes for five-year DFS ($P=0.056$; Table 4) among Stage II colon cancer patients, this difference did not reach statistical significance due to the

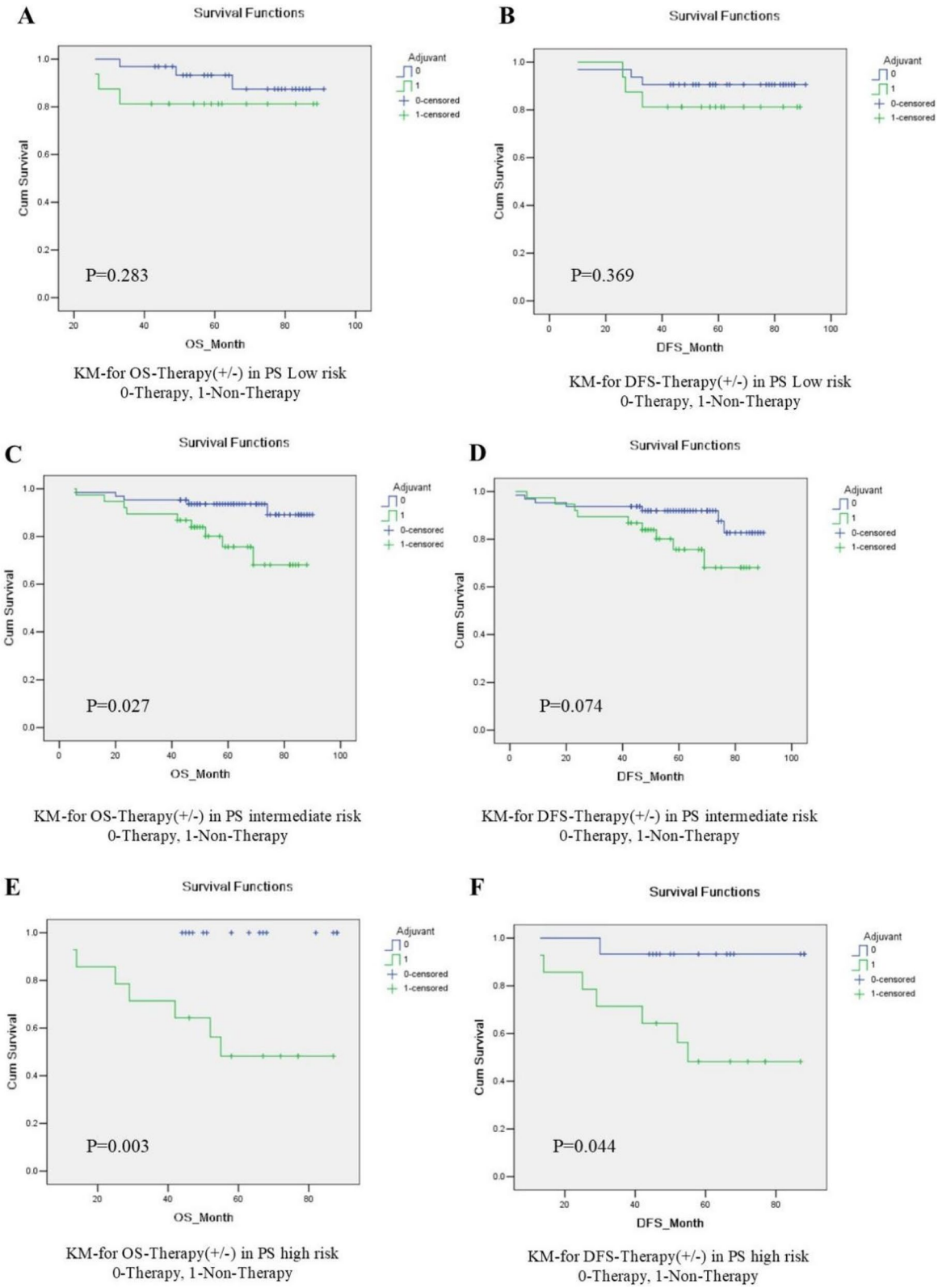

Fig. 2 Kaplan–Meier plots illustrating overall survival (OS) and disease-free survival (DFS) in stage II colorectal cancer patients after adjuvant therapy. **A** (OS) and **B** (DFS) of stage II colorectal cancer patients in the PS-low-risk groups. **C** (OS) and **D** (DFS) of stage II colorectal cancer patients in the PS-intermediate-risk groups. **E** (OS) and **F** (DFS) of stage II colorectal cancer patients in the PS- high-risk groups

limited number of patients within the PS-high group (colon $n=22$; colorectal $n=29$) coupled with relatively short follow-up duration compared to other Stage II colon cancer patient cohorts with favorable prognoses.

Given the ongoing debate surrounding adjuvant chemotherapy in early-stage colorectal cancer patients, we conducted an analysis of overall survival (OS) and disease-free survival (DFS) among patients who received either chemotherapy or observation. Our findings indicate that patients treated with chemotherapy exhibited a superior survival rate. We further stratified these patients based on pathological factors, ploidy, and stroma to identify those who might derive benefit from chemotherapy. Notably, adjuvant chemotherapy significantly improved the survival outcomes for patients presenting with T4 stage disease and high clinical risk factors. This suggests that ploidy and stroma may serve as complementary criteria for selecting candidates likely to benefit from chemotherapy.

According to the National Comprehensive Cancer Network (NCCN) and ESMO guidelines, microsatellite instability-high (MSI-H) or mismatch repair-deficient (dMMR) status serves as a favorable prognostic indicator for stage II colorectal cancer (CRC). Notably, patients with MSI-H/dMMR do not derive benefits from adjuvant chemotherapy; however, only 15% of sporadic CRC cases exhibit dMMR characteristics (70 out of 457) [23, 24]. An increasing array of biomarkers has been developed to stratify patients who may benefit from adjuvant chemotherapy. The Oncotype DX12 gene expression profile categorizes recurrence risk into three levels: low, intermediate, and high. This classification can be utilized for predicting recurrence risk [25, 26]. Nevertheless, the DX12 gene associated with CRC is limited in its predictive capacity; it can only forecast the risk of recurrence in early- and mid-stage CRC patients but does not assess the potential benefits of adjuvant chemotherapy. The immunoscore represents a novel tool that evaluates recurrence risk by analyzing formalin-fixed paraffin-embedded (FFPE) slides. Patients exhibiting high immunoscores are correlated with a reduced risk of recurrence and do not gain advantages from adjuvant chemotherapy [27]. In recent years, considerable research has concentrated on minimal residual disease (MRD), which involves capturing tumor-related DNA fragments present in the bloodstream. MRD-positive patients demonstrate significantly elevated rates of recurrence and metastasis post-surgery compared to their MRD-negative counterparts; those who are MRD-negative show survival rates comparable to those receiving chemotherapy for durations ranging from three to six months [28]. The emergence of these new biomarkers underscores the necessity for heightened attention towards early-stage colorectal cancer patients. Morphometric analyses focusing on ploidy and stroma have yielded a technically straightforward prognostic stratifier specifically for stage II CRC.

In conclusion, based on the findings of this study, neither ploidy nor stroma serves as a reliable predictor for the survival of patients with stage II colorectal cancer. However, patients in the PS-H group who receive chemotherapy demonstrate improved survival outcomes. The integration of ploidy and stroma may serve as an adjunct in clinical decision-making to inform chemotherapy strategies for treating stage II colorectal cancer patients. Additionally, we hope that more clinical evidence could be generated to support the clinical application of these new biomarkers.

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Authors' contributions
Xiaohui Du designed and directed this study, Yunshan Zhao and Xudong Zhao was responsible for data collection, Yunshan Zhao and Shaoyou Xia wrote the first draft of the article, Zufeng He and Zhigang Song carried out sample collection and sample detection, Fei Wang and Lijun Mao was in charge of statistical analysis. All authors reviewed the article. YunShan Zhao, ShaoYou Xia and Xudong Zhao contributed equally to this study.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was conducted in compliance with legal requirements following approval from the Ethics Committee of the Chinese PLA General Hospital. Informed consent was obtained from all individual participants involved in this research. The study adhered to the principles outlined in the Declaration of Helsinki.

Consent for publication
No identifying information pertaining to any participants included in this manuscript has been disclosed.

Competing interests
The authors declare no competing interests.

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