Journal of Gastrointestinal Cancer (2024) 55:227–236
https://doi.org/10.1007/s12029-023-00953-0

RESEARCH

The Prognostic Role of Mismatch Repair Status and CDX-2 Expression with Inflammatory Markers and Pathological Risk Factors in Stage II and III Colon Cancer: Multicenter Real-Life Data

Sabin Goktas Aydin[1] · Omer Fatih Olmez[1] · Oguzhan Selvi[2] · Caglayan Geredeli[2] · Ferhat Ozden[3] · Ahmet Bilici[1] · Ozgur Acikgoz[1] · Ebru Karci[1] · Yasin Kutlu[1] · Jamshid Hamdard[1] · Ahmet Aydin[4]

Accepted: 9 June 2023 / Published online: 22 June 2023

Abstract

Objective Colorectal cancer is common worldwide, and adjuvant treatment's benefit is still controversial. We designed this study to determine the role of MSI and CDX-2 status determined by immunohistochemistry (IHC) combined with the inflammatory markers and pathological parameters in predicting disease recurrence in stage II and III colon cancer.

Methods A total of 226 stage II/III colon cancer patients with a median age of 59 years who underwent initial surgery were included in this retrospective study. The pathologic assessment of MSI and CDX-2 was performed twice by immunohistochemistry (IHC) and two different pathologists. No staining/weak staining below 10% of the tumor was accepted as CDX-2 negative, and any MSI clones with weak staining below 10% were accepted as MSI-H. The laboratory parameters were noted at the initial diagnosis.

Results One hundred twenty-one and 105 patients were diagnosed with stage III and II colon cancer. 58.0% of patients were male, 46 (20.4%) of tumor tissue were MSS, and 17 (7.5%) were CDX-2 negative. One hundred twenty-nine tumors were localized in the right colon. Disease recurrence was significantly correlated with tumor localization, CDX-2 status, stage at diagnosis, and preoperatively median CRP and CEA levels. DFS rates for MSS patients with CDX-2 negative and positive were 36.7% and 98.1%, respectively [$p < 0.001$]. There was no significant correlation between MSI status and CDX-2 status. MSI status, the presence of adjuvant treatment, and systemic inflammatory markers were not significant prognostic factors for DFS. CDX-2 status [HR:0.08, CI 95% 0.03–0.17, $p < 0.001$ HR: 1.7, CI 95% 1.1–3.0, $p = 0.03$], disease stage [HR:2.6, CI 95% 1.43–4.74], and preoperatively CEA levels [HR:4.1 CI 95% 2.18–785, $p < 0.001$ were independent significant prognostic factors for DFS.

Conclusion CDX-2 loss was an independent prognostic factor for DFS and disease recurrence in early-stage colon cancer. MSS patients with CDX-2 loss had significantly worse survival outcomes, and this might be the reason for deciding on adjuvant chemotherapy.

Keywords Colon cancer · CDX-2 · MSI · Inflammatory marker · Disease-free survival

Introduction

Colorectal cancer is commonly diagnosed worldwide, and the incidence and mortality vary between countries. The only curative treatment is surgical resection for colon cancer [1]. The adjuvant therapy, which's absolute benefit is demonstrated in stage III disease, is used to eradicate micrometastases [2]. The shared decision-making of adjuvant treatment in stage II disease is recommended [4]. Clinicopathologic and molecular features are defined to assess the risk of disease recurrence as microsatellite instability [MSI] for deficient mismatch repair [MMR] proteins, the number of lymph nodes [fewer than 12 nodes in the surgical specimen], T4 or perforated/obstructed tumor, poorly differentiated histology, and lymph vascular or perineural invasion, especially in stage II disease [4, 5].

Despite this, these factors fail to predict recurrence accurately. To date, 12-gene recurrence score and other microarray-based tests have been created to estimate the recurrence risk and to use as prognostic tools. However, these tests are not commonly used due to the cost and heterogeneity of colorectal cancer [6].

Extended author information available on the last page of the article

Defective MMR status or MSI-H status is defined as a germline mutation in one or more MRR genes, double somatic MMR gene inactivation, and somatic hypomethylation of the MLH-1 gene promoter. High MSI status [MSI-H] is a well-known positive prognostic factor for early-stage colon cancer, with an incidence of up to 22% in early cancer. dMMR causes resistance to 5-Fluorouracil; therefore, stage II colon cancer patients with dMMR are not eligible for adjuvant treatment [7, 8]. On the other hand, in an advanced setting, MSI-H is a poor prognostic factor. Nevertheless, dMMR predicts response to anti-PD-1 immunotherapy treatment [9].

Caudal-type homeobox transcription factor 2 [CDX-2], which regulates the proliferation and differentiation of intestinal epithelial cells, is a diagnostic and prognostic marker in colon cancer. The lack of CDX2 expression is associated with a high risk of relapse and poor survival. In addition, patients with stage II colon cancer who have a loss of CDX-2 expression benefit more from adjuvant treatment [10–12].

The significance of inflammation in cancer development and prognosis has been determined in many studies. Neutrophil/lymphocyte ratio [NLR], platelet/lymphocyte ratio [PLR], and systemic immune-inflammation index [SII] as inflammatory parameters have been associated with colon cancer prognosis and recurrence of the disease. In addition, the correlation between C-reactive, another inflammatory marker protein [CRP], and progression is well-known in many cancers [13–16].

The primary purpose of this study is to evaluate the role of MSI and CDX-2 status determined by immunohistochemistry (IHC) combined with the inflammatory markers and pathological parameters in predicting disease recurrence in early-stage colon cancer.

Materials and Methods

Between 2014 and 2021, 226 patients with stage II/III colon cancer who underwent initial surgical resection at Istanbul Medipol University Hospital and Okmeydani Training and Research Hospital were included in this study. The radiological staging was evaluated by AJCC/UICC 8th edition (American Joint Committee on Cancer/Union for International Cancer Control).

Patients' data were retrospectively obtained from patients' charts with respect to age, gender, tumor location, number of lymph nodes dissected, the presence of adjuvant chemotherapy, tumor size, pathologic poor risk factors, and laboratory data, which were collected preoperatively.

The major inclusion criteria were the commencement of adjuvant chemotherapy after surgery within 8 weeks. Additionally, patients with enough surgical tissue which was eligible to re-evaluate were included in this study. MSI and CDX status were assessed twice by different pathologists. Patients with ECOG PS 3/4 who were not candidates for adjuvant chemotherapy and patients with loss of follow-up were excluded from data analysis. The presence of synchronous or metachronous tumor was another exclusion criteria.

Surgery was performed in different centers and by different surgical teams, but surgeons followed similar protocols. To detail, laparoscopy-assisted colectomy was preferred in 176 patients. The remaining surgical technique was open colectomy. Restoration of bowel continuity using a primary anastomosis was accomplished in 192 patients. The remaining patients had temporary proximal diverting colostomy/ileostomy.

The primary end-point of this study was to determine the prognostic role of MSI and CDX-2 status for DFS in stage II and III colon cancer. The secondary endpoints were to assess possible prognostic markers, including systemic inflammatory markers on survival, and to assess the predictive role of CDX-2 and MSI status.

Inflammatory Markers

NLR is calculated as absolute neutrophil count [neutrophil count/mL]/absolute lymphocyte count [lymphocyte count/mL]; PLR is calculated as absolute platelet count [platelet count/mL]/absolute lymphocyte count. Therefore, the SII was defined as follows: SII = Platelets × neutrophil/lymphocytes [13–16].

Immunohistochemical Analysis

Four μm thick sections of the paraffin-embedded blocks were obtained. Automated IHC for MMR and CDX2 expression was performed for all cases on a BenchMark ULTRA staining instrument [Ventana Medical Systems, Tucson, AZ]. The antibody clones used were all Ventana ready-to-use monoclonal antibodies applied with the OptiView DAB IHC Detection Kit and OptiView Amplification Kit following the manufacturer's instructions. Antibody clones were MSH-2 [clone G219-1129, mouse monoclonal], MSH-6 [clone 44, mouse monoclonal, MSH-6 [cloneSP93, rabbit monoclonal], MLH-1 [clone M1, mouse monoclonal], PMS-2 [clone A16-4, mouse monoclonal], and CDX2 [clone ERP2764Y]. Positive external controls [colon adenocarcinoma tissue] were included in all the slides.

Heterogeneous staining was defined with MMR and CDX2 in some cases [strongly positive tumor areas mixed with weakly positive or negative tumor areas]. Thus, the arbitrary cut-off value of approximately 10% of the tumor was used. No staining and only weak staining below 10% of the tumor accepted as CDX-2 negative /low, and > 10% of the tumor as CDX-2 positive. Figure 1 shows the microscopic IHC images for CDX-2 staining. Same as CDX-2, if

any MMR clone with weak staining or staining below 10% was accepted as MSI-H, the others were accepted as MSS.

Statistical Analysis

SPSS 22.0 [SPSS Inc., Chicago, IL, USA] software was used for all statistical analyses. Descriptive parameters were quoted as the median. Because the distribution of study parameters was non-normal, nonparametric tests were used. The chi-squared test and Fisher's exact test compared the relationship between clinicopathological factors and disease recurrence. Inflammatory markers were dichotomized at the median as a cut-off value. The survival analysis and curves were established using the Kaplan-Meier method and compared with the log-rank test. Disease-free survival was defined as the time between the operation and recurrence dates. The 95% confidence [CI] was used to quantify the relationship between survival time and each independent factor. All p values were two-sided in tests, and p values less than or equal to 0.05 were considered statistically significant.

Results

One hundred thirty-one [58.0%] patients were male, and 95 patients [42.0%] were female, with a median age of 59 years [range:21–89]. T and N status were as follows: 3, 11, 153, and 59 tumors were T1, T2, T3, and T4, respectively. 46.5% of patients were diagnosed pathologically with stage II colon cancer, and 53.5% were stage III colon cancer. Forty-six tumor [20.4%] specimens were MSI-H, and 180 [79.6%] were MSS. CDX-2 positivity was detected in 209 patients [92.5%] and negativity in 17 [7.5%]. 57.1% and 42.9% of tumors were localized in the right and left colon, respectively. Pathological risk assessment; LVI was detected in 123 [53.9%] patients, obstruction, or perforation in 9 [3.9%] patients, poorly differentiation in 16 [7%] patients, close or positive margins in 2 patients, and inadequate lymph node dissection in 5 [2.2%] patients. One hundred ninety-one patients received adjuvant treatment.

Patients were categorized, and the characteristics were compared according to MSI status. There was no significant difference between gender, size, and nodal status of the primary tumor, stage, histopathologic type, clinicopathologic risk factors, and the number of patients who received adjuvant treatment between the MSS and MSI-H groups. As expected, the rate of MSI-H patients was significantly higher in right-sided tumors [$p = 0.03$]. The clinicopathologic features of patients according to MSI status are depicted in Table 1.

At a median follow of 34.3 months (range 3.5–96.6 months), the 36-month DFS and OS rates were 68.0% and 89.0%, respectively. Disease recurrence occurred in 65 patients. Disease-related death occurred in 34 patients.

There was a significant correlation between the distal or local disease recurrence with CDX-2 status [$p = 0.001$], stage at diagnosis [< 0.001], and the localization of the tumor [0.01]. Additionally, disease recurrence rates were significantly higher in patients with CEA > 4.5 ng/ml [$p < 0.001$] and CRP > 8.2 mg/l [$p = 0.03$]. There was no significant correlation between the recurrence of the disease and MSI status. Moreover, the association between disease recurrence and inflammatory markers was not observed [Tables 2].

Univariate analysis for DFS revealed that CDX-2 status [$p > 0.01$], stage of disease [$p < 0.001$], localization of tumor [$p = 0.01$], gender [$p = 0.02$], lymphovascular invasion [$p = 0.01$], and preoperatively CEA levels [$p < 0.001$] were found to be significant prognostic indicators. On the other hand, MSI status, presence of adjuvant treatment, CRP levels, SII, NLR, and PLR, were not found to be significant prognostic factors for DFS. The statistical DFS contribution ofadjuvant

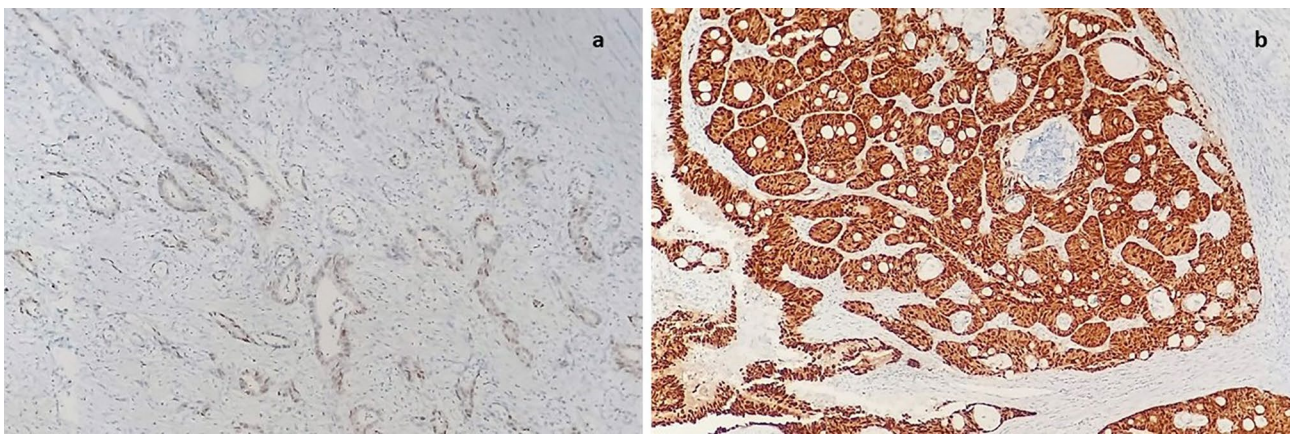

Fig. 1 IHC images for CDX-2 staining negative (**a**) positive (**b**)

Table 1 Clinicopathologic features of patients according to MSI status

Characteristics	Total patients	MSI status		
	226 (%)	MSS	MSI-H	*p*
		N (%)	N (%)	
Age, years	59	59	57	0.7
Median, range	(21–89)	(21–89)	(26–82)	
Gender				
Male	131 (58.0)	107 (81.7)	24 (18.3)	0.4
Female	95 (42.0)	73 (76.8)	22 (23.2)	
T status				
T1	3 (1.3)	3 (100)	0	
T2	11 (4.9)	9 (81.8)	2 (18.2)	0.1
T3	153 (67.7)	116 (75.8)	37 (24.2)	
T4	59 (26.1)	52 (88.1)	7 (11.9)	
N status				
N0	105 (46.5)	81 (77.1)	24 (22.9))	0.3
N1	75 (33.2)	64 (85.3)	11 (14.7))	
N2	46 (20.4)	35 (76.1)	11 (23.9)	
Pathological stage				
Stage II	105 (46.5)	81 (77.1)	24 (22.9)	0.2
Stage III	121 (53.5)	99 (81.8)	22 (18.2)	
Histologic type				
Adenocarcinoma	193 (85.4)	156 (80.8)	37 (19.2)	0.3
Mucinous carcinoma	33 (14.6)	24 (72.7)	9 (27.3)	
CDX-2 status				
Low/absent	17 (7.5)	16 (94.1)	1 (5.9)	0.1
Present	209 (92.5)	164 (78.5)	45 (21.5)	
Tumor localization				
Right	129 (57.1)	96 (74.4)	33 (25.6)	**0.03**
Left	97 (42.9)	84 (86.6)	13 (13.4)	
Clinicopathologic risk factors				
Obstruction/perforation	9 (3.9)	8 (4.4)	1 (2.1)	0.4
LVI	123 (35.9)	80 (44.2)	25 (53.2)	0.2
Poorly differentiated	16 (7)	12 (6.6)	4 (8.5)	0.8
Close/positive margins	2 (0.9)	2 (1.1)	0	0.4
Inadequate LND	5 (2.2)	5 (2.8)	0	0.2
Adjuvant treatment				
Absent	35 (15.5)	24 (68.6)	11 (31.4)	0.06
Present	191 (84.5)	156 (81.7)	35 (18.3)	

**TNM* tumor, node, metastasis, *LVI* lymph vascular invasion, *MSI* microsatellite instability, *MSS* microsatellite stable, *MSI-H* microsatellite instability-high, *CDX-2* caudal type homeobox 2, *LND* lymph node dissection

therapy in stage II and III disease could not be demonstrated [p = 0.07, p = 0.6, respectively] in the subgroup analysis. The patients with CDX-2 loss/absence died from disease progression before 30 months. Thus 3-year-DFS rate was not applicable in this group. Table 3 summarizes the univariateanalysis with 3-years-DFS rates and multivariate analysis.

COX-regression analysis indicated that CDX-2 status [HR:0.08, CI 95% 0.03–0.17, $p < 0.001$], localization of tumor [HR: 1.7, CI 95% 1.1–3.0, $p = 0.03$], disease stage [HR:2.6, CI 95% 1.43–4.74], and preoperatively CEA levels [HR:4.1 CI 95% 2.18–785, $p < 0.001$] were significant independent prognostic factors for DFS. However, in multivariate analysis, MSI status was not a prognostic factor for DFS in stage II and III diseases.

In stage II disease, the median DFS was 13.6 months in the CDX2 negative group and 88.1 months in the CDX2 positive group. In stage III disease, the median DFS was 12.5 and 58.3 months in CDX2 negative and positive groups,

Table 2 Distant or local recurrence rates according to pathologic and laboratory findings

Characteristics	Distant or local recurrence of disease		
	Absent *n* (%)	Present *n* (%)	*p*
Gender			
Male	99 (61.5)	32 (49.2)	**0.06**
Female	62 (38.5)	33 (50.8)	
MSI status			
MSS	124 (77.0)	56 (86.2)	0.08
MSI-H	37 (23.0)	9 (13.8)	
CDX-2 status			
Absent	6 (3.7)	11 (16.9)	**0.001**
Present	155 (96.3)	54 (83.1)	
Tumour Localization			
Right	100 (62.1)	29 (44.6)	**0.01**
Left	61 (37.9)	36 (55.4)	
Stage			
II	87 (54.0)	18 (27.7)	**< 0.001**
III	74 (46.0)	47 (72.3)	
CEA (ng/ml)			
≤ 4.5	103 (64.0)	12 (18.5)	**< 0.001**
> 4.5	58 (36.0)	53 (81.5)	
CRP (mg/l)			
≤ 8.2	88 (54.7)	26 (40.0)	**0.03**
> 8.2	73 (45.3)	39 (60.0)	
BMI			
≤ 26.5	80 (49.7)	33 (50.8)	0.5
> 26.5	81 (50.3)	32 (49.2)	
NLR			
≤ 2.6	78 (48.4)	33 (50.8)	0.4
> 2.6	83 (51.6)	32 (49.2)	
PLR			
≤ 156.2	81 (50.3)	32 (49.2)	0.5
> 156.2	80 (49.7)	33 (50.8)	
SII			
≤ 806	82 (50.9)	31 (47.7)	0.3
> 806	79 (49.1)	34 (52.3)	

MSI microsatellite instability, *MSS* Microsatellite Stable, *MSI-H* microsatellite instability-high, *CDX-2* caudal type homeobox 2, *CEA* carcinoembryonic antigen, *NLR* neutrophil/lymphocyte ratio, *PLR* platelet/lymphocyte ratio, *LMR* lymphocyte/monocyte ratio, *SII* systemic immune-inflammation index, *BMI* body mass index, *CRP* C-reactive protein

Table 3 Univariate analysis with 3-year DFS rates and Multivariate analysis for DFS

Factor	Disease-free survival				
	Univariate analysis		Multivariate analysis		
	p	3-years-DFS rates %	*p*	HR	95% CI
Gender	**0.02**		0.5	1.1	0.70–1.97
Female		79.2			
Male		73			
CDX 2 status				0.08	0.03–0.17
Low/absent	**< 0.001**	NA	**< 0.001**		
Present		92.9			
MSI status	0.1				
MSS		63.1	-		
MSI-H		84.5			
Stage of disease	**< 0.001**		**0.002**	2.6	1.43–4.74
Stage II		81.8			
Stage III		56.6			
Histology	0.1	-			
Localization of tumor	**0.01**		**0.02**	1.7	1.1–3.0
Right colon		74			
Left colon		60.1			
Lymph vascular invasion	**0.01**		0.8	1	0.6–1.75
Absent		73			
Present		60.4			
Inadequate LND	0.8	-			
Obstruction/ perforation	0.6	-			
CEA level	**< 0.001**		**< 0.001**	4.1	2.18–7.85
CEA ≤ 4.5		87.3			
CEA > 4.5		50.5			
CRP	0.1	-			
SII	0.4	-			
NLR	0.7	-			
PLR	0.8	-			
BMI	0.6	-			

LVI lymph vascular invasion, *MSI* microsatellite instability, *MSS* Microsatellite Stable, *MSI-H* microsatellite instability-high, *CDX-2* caudal type homeobox 2, *LND* lymph node dissection, *NLR* Neutrophil/lymphocyte ratio, *PLR* platelet/lymphocyte ratio, *SII* Systemic Immune-inflammation Index, *BMI* body mass index, *CRP* C-reactive protein, *CEA* carcinoembryonic antigen

respectively [Fig. 2]. The difference was statistically significant [$p < 001$]. When evaluated according to localization, the MSI status of left colon tumors had significant prognostic importance on DFS. The median DFS of patients with left colon tumor and MSS was 51.6 months and 76.8 months in patients with MSI-H [$p = 0.009$]. In contrast, a significant relationship was not found between MSI status and right-sided tumors. MSI status had an impact on DFS concerning stage. Three-year DFS rates of MSS tumors were 76.7% and 52.9% in stages II and III, respectively [Fig. 3]. There was no significant correlation between CDX-2 and MSI status [$p = 0.66$]. Since only one patient in the MSI-H group was CDX-2 negative, DFS analysis was performed according to CDX status in the MSS group. Twelve-month DFS rates for MSS patients with CDX-2 negative and positive were 36.7% and 98.1%, respectively [$p < 0.001$].

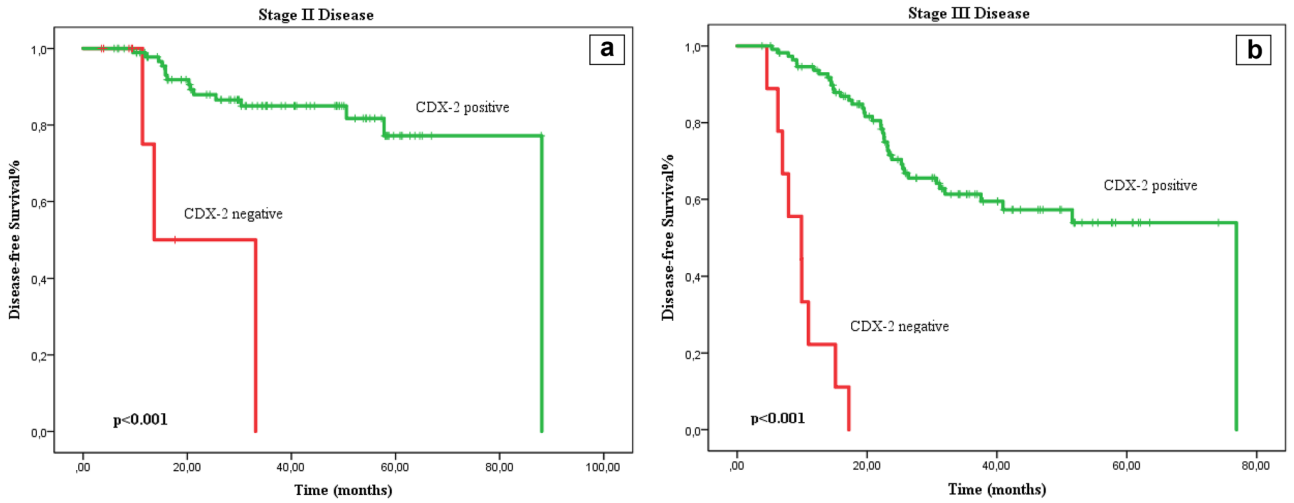

Fig. 2 Disease-free survival curve with respect to CDX-2 in stage II disease [**a**] and stage III disease [**b**]

Although none of the important prognostic factors for OS was detected in the entire cohort, when we classified it according to stage II, CDX-2 status, median CEA level, LVI, and gender were prognostic factors for OS [$p < 0.001$, $p = 0.04$, $p = 0.003$, respectively]. In addition, univariate analysis for OS in stage III disease revealed that CDX-2 status [$p = 0.02$ CI%95 59.6–106.9] and median CEA level [$p = 0.01$ CI%95 51.1–70.0] were prognostic factors.

Discussion

The primary management of early-stage colon cancer is curative resection. After surgery, the aim is to eradicate micro-metastases, thereby reducing the recurrence risk. In stage III disease, the benefits of adjuvant treatment have been demonstrated well; however, it is controversial in stage II [3–5]. Therefore, we aimed to determine the role of CDX2 expression, MSI status, and inflammatory markers in predicting the recurrence of early-stage colon cancer.

Clinicopathologic, molecular prognostic, and predictive factors are described for stage II disease as follows: MMR deficiency, presence of BRAF mutation, T4 primary, high-grade/poorly differentiated histology, LVI, PNI, bowel obstruction or perforation, close or positive margins; inadequately sampled lymph nodes [less than 13 in the surgical specimen], and high preoperatively CEA level [5, 13–21].

Takagawa et al. demonstrated that disease recurrence was significantly higher in stage II/III colon cancer patients with CEA levels higher than ten ng/ml than < 10 ng/

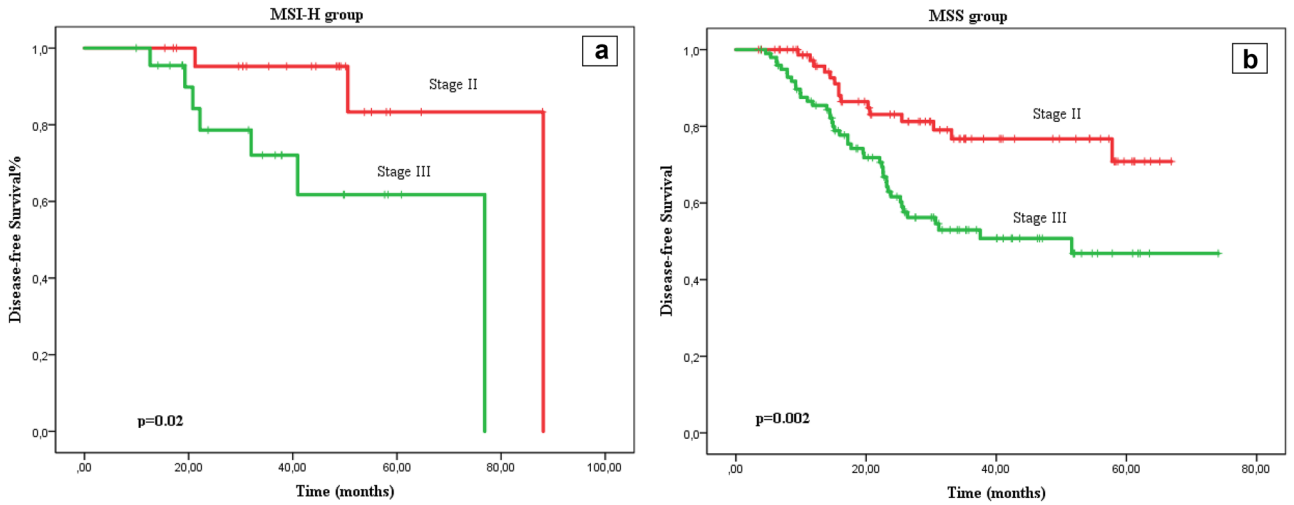

Fig. 3 Disease-free survival curve with respect to stage of disease in MSI-H group [**a**] and MSS group [**b**]

ml. Additionally, the 5-year DFS rate was 90.7% versus 77.2% [$p = 0.002$] in patients with CEA < 10 ng/ml and CEA > 10 ng/ml [22]. Betge et al. studied the efficacy of LVI on DFS and disease recurrence. They showed that 5-year cancer-specific survival rates were 35% and 64%; disease recurrence rates were 61% and 31% in patients with LVI versus lack of LVI [18]. Similarly, in our study, DFS was significantly associated with preoperatively CEA level and LVI status [$p < 0.001$ and $p = 0.001$, respectively]. The disease recurrence rate was statistically higher in the median preoperatively CEA > 4.5 patients [$p < 0.001$]. Our cohort was insufficient in the number of patients with inadequate lymph node dissection, bowel obstruction/perforation, and positive or close margin. Therefore, we could not demonstrate any statistical significance between DSF and the other clinicopathologic features.

High inflammation parameters significantly impact prognosis in many cancer types [13–16]. Systemic review and meta-analysis indicated that high levels of CRP, low albumin values, high NLR levels, and GPS score significantly correlated with poor survival rates [15]. We did not demonstrate any association between disease recurrence and SII, NLR, and PLR values except CRP level. However, we supported the previous studies by showing that disease recurrence occurs in patients with higher CRP values, as the median CRP value was > 8.2 in 61.2% of the patients with disease recurrence [$p = 0.04$]. But the correlation between higher CRP and poor survival was not observed in multivariate and univariate analysis in our cohort.

CDX-2 controls cell differentiation in the intestinal epithelium and is used as a marker in daily practice to verify or rule out an intestinal origin of a carcinoma especially colorectal carcinoma. Loss of CDX-2 has some prognostic impact on survival in several studies [10, 11, 23]. Dalerba et al. found that the 5-year disease-free survival was lower among CDX2 protein–negative patients than in CDX2 protein–positive colon cancer patients [hazard ratio, 2.42; 95% CI, 1.36 to 4.29; $p = 0.003$] [10]. Konukiewitz et al. showed worse survival with CDX2 protein low/absent group in the overall cohort Stage II/III [DFS: $p = 0.005$] and microsatellite stable and left-sided CRCs, respectively, but not in MSI-H or right-sided CRCs. The multivariate analysis could demonstrate no significant statistical difference [23]. CDX2 protein expression was evaluated by IHC and liquid chromatography in the study, which established the prognostic importance of CDX2 in DFS [12]. The association between CDX-2 loss and MSS and worse survival outcomes was also reported in the study by Toth et al. [24]. Similar to the literature, we analyzed 105 patients with stage II and 123 patients with stage III CRC and demonstrated significantly worse DFS with CDX-2 negative patients in the total cohort [HR:0.08, CI 95% 0.03–0.17, $p < 0.001$] and in stage II, stage III disease [$p < 0.001$, $p < 0.001$, respectively]. Moreover, loss of CDX2 was significantly correlated with disease recurrence [$p = 0.001$].

The localization of primary tumors has an impact on prognosis in CRC. Gene expression profiles differ between the right and left colon epithelium. CIMP-high, genome-wide hypermethylation causing epigenetic gene silencing, and MSI-high CRCs are more likely right-sided, and tumors with chromosomal instability are more likely left-sided [25]. High microsatellite instability [MSI-H] is a well-known positive prognostic factor for colorectal cancer, with an incidence of up to 22% in the early stage [26]. Retrospective studies showed a better prognosis in right-side tumors with stage II disease and a worse prognosis in stage III. It was likely associated with higher MSI-high tumors in right-sided location II cancers [27, 28]. Prospective studies showing inferior DFS in right-sided III disease who received adjuvant treatment supported the results of previous studies [HR, 0.70; 95% CI, 0.61–0.81] [29]. Real-world data from Germany showed significantly higher disease-free, relapse-free, and overall survival in favor of the MSI-H group compared with the MSS group. Also, in their large cohort, MSI-H status with an incidence of 23.7% was correlated with female gender, BRAF mutation, stage II disease and right-sided colon tumors [26].

In our study, the localization of the tumor was a significant independent prognostic factor for DFS [HR: 1.7, CI 95% 1.1–3.0, $p = 0.02$]. In our cohort, 129 tumors [57.1%] were localized in the right colon. Contrary, we did not demonstrate an impact of MSI status on DFS in stage II/III colon cancer. The small sample size and the detection of higher rates with MSI-H status in the right colon might affect the results. Also, 52.9% of left colon tumors were stage III disease [$p = 0.056$], and 77.8% were MSS [$p = 0.04$]. We supported the previous studies by demonstrating a significantly worse prognosis in left-sided tumors with MSS status [$p = 0.009$]. Contrary to the literature, in our study, 55.2% of patients with statistically significant recurrence were left colon tumors.

Hestetun et al. demonstrated that 45–81% of CDX-2 negative tumors had dMMR, 25–41% of dMMR tumors had CDX-2 negativity, and patients with MSS and negative CDX-2 had worse cancer-specific survival than the remaining patients with early-stage colon cancer [median 35.8 months vs 52.1–53.5 months, CI 45.6–58.6, $p = 0.001$] [11]. According to the latest ESMO guideline published in 2020, active follow-up is recommended for patients with stage II disease whose tumor is MSS or MSI-H and with no other risk factors [30]. Our study showed no significant correlation between CDX-2 and MSI status. Although it was not statistically significant due to the insufficient number of patients, CDX-2 loss was more common in MSS tumors, and CDX-2 loss was present in only one patient in the MSI-H group. Moreover, in the MSS group, CDX-2 status was a

significant prognostic factor for DFS and MSS tumors with CDX-2 loss had worse survival outcomes [$p < 0.001$] in our study. Despite ESMO guidelines, we believe CDX-2 loss in low-risk and MSS stage II disease may be candidates for adjuvant chemotherapy.

The major limitation of our study was to evaluate CDX2 and MSI status only by IHC. As mentioned in previous studies, evaluating CDX-2 status by liquid chromatography and PCR is a more sensitive and specific method. The data part did not include information about the comorbidities that may contribute to the morbidity rate. In our study, frequencies of MSI-H and loss of CDX2 expression were much higher than reported in previous studies [23, 24]. The reason might be the retrospective design which may lead to bias and evaluation of these factors only by the IHC technique. Additionally, the incidence of CDX-2 loss and MSI status in the Turkish population remains controversial [31]. Therefore, our study contributes to the literature by showing CDX and MSI status incidence.

Need for more data for the Turkish population-based investigations. To prevent inappropriate evaluation of MSI-H status/CDX2, two separate pathologists performed the IHC analysis. The other limitation was that the number of patients lacking CDX-2 was insufficient further to subgroup analysis and the comparison with MSI status.

The contribution of our study to the literature is as follows: we analyzed the prognostic effects of both clinicopathological factors and biochemical parameters on disease recurrence and DFS. IHC testing for CDX-2 and MSI status was a reliable method for the prognosis of early-stage colon cancer. Another interesting observation of our study is the dependence of CDX-2 status on tumor localization, as the presence of CDX-2 in the left and right colons was a significant prognostic marker that may lead to the treatment choice and the duration of treatment in the future as MSI status. Moreover, CDX-2 loss in Turkish people with early-stage colon cancer tended to be higher than in other populations. However, further studies are needed.

Conclusion

This study aimed to evaluate the role of MSI and CDX-2 status, along with inflammatory markers, in predicting disease recurrence in early-stage colon cancer. The findings showed that CDX-2 status, tumor localization, disease stage, and preoperative CEA levels were significant independent prognostic factors for DFS. MSI status had statistical importance on DFS between left- and right-sided tumors. However, MSI status did not prove to be a prognostic factor for DFS in the overall population. These data suggest that loss of CDX-2 in MSS patients could be treated with adjuvant chemotherapy and followed up carefully even if they have stage II disease and no known risk factors.

This study innovatively combines the assessment of MSI and CDX-2 status, along with inflammatory markers, to predict disease recurrence in early-stage colon cancer. The molecular mechanisms behind the enrichment of CDX2 loss and MSI status of tumors, as well as in right-sided CRCs, should be explored in further studies to address potential therapeutic implications.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12029-023-00953-0.

Author Contribution S. Goktas Aydin and O.F. Olmez designed the study. S. Goktas Aydin and O. F. Olmez wrote the manuscript. A. Bilici and S. Goktas Aydin conceived the statistical data analysis. Y. Kutlu, J. Hamdard, O. Selvi, C. Geredeli, O. Acikgoz, E. Karci, and A. Aydin contributed substantial input to the conception and acquisition of the work. F. Ozden was responsible for pathologic evaluation. All authors read and gave their stamp of approval for the submission of the final version of the manuscript.

Data Availability The data supporting this study's findings are not openly available. Further enquiries can be directed to the corresponding author.

Declarations

Ethics Approval The Local Ethics Committee of Istanbul Medipol University approved this multicenter study in December 2020 with the E-10840098-772.02-65178 decision number. Written informed consent had been obtained from patients.

Conflict of Interest The authors declare no competing interests.

References

1. https://www.uptodate.com/contents/colorectal-cancer-epidemiology-risk-factors-and-protective-factors/abstract/3Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. JAMA Oncol. 2017;3[3]:418.
2. Sargent D, Sobrero A, Grothey A, et al. Evidence for cure by adjuvant therapy in colon cancer: observations based on individual patient data from 20,898 patients on 18 randomized trials. J Clin Oncol. 2009;27:872.
3. Baxter NN, Kennedy EB, Bergsland E, et al. Adjuvant therapy for stage II colon cancer: ASCO guideline update. J Clin Oncol. 2022;40:892.
4. Quah HM, Chou JF, Gonen M, Shia J, Schrag D, Landmann RG, et al. Identification of patients with high-risk stage II colon cancer for adjuvant therapy. Dis Colon Rectum. 2008;51:503.
5. Roth AD, Delorenzi M, Tejpar S, et al. Integrated molecular and clinical prognostic factors analysis in stage II/III colon cancer. J Natl Cancer Inst. 2012;104:1635.
6. Roth AD, Delorenzi M, Tejpar S, Yan P, Klingbiel D, Fiocca R, et al. Relationship between tumor gene expression and recurrence in four independent studies of patients with stage II/III colon cancer treated with surgery alone or surgery plus adjuvant fluorouracil plus leucovorin. J Clin Oncol. 2010;28:3937.

7. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med. 2003;349:247.
8. Klingbiel D, Saridaki Z, Roth AD, Bosman FT, Delorenzi M, Tejpar S. Prognosis of stage II and III CC treated with adjuvant 5-fluorouracil or FOLFIRI in relation to microsatellite status: results of the PETACC-3 trial. Ann Oncol. 2015;26:126–32.
9. Ganesh K, Stadler ZK, Cercek A, Mendelsohn RB, Shia J, Segal NH, Diaz LA Jr. Immunotherapy in colorectal cancer: rationale, challenges, and potential. Nat Rev Gastroenterol Hepatol. 2019;16(6):361–75.
10. Dalerba P, Sahoo D, Paik S, Guo X, Yothers G, Song N, et al. CDX2 as a prognostic biomarker in stage II and stage III colon cancer. N Engl J Med. 2016;374(3):211–22.
11. Hestetun KE, Aasebø K, Rosenlund NB, Müller Y, Dahl O, Myklebust MP. Mismatch repair phenotype determines the implications of tumor grade and CDX2 expression in stage II-III colon cancer. Mod Pathol. 2021;34(1):161–70.
12. den Uil SH, de Wit M, Slebos RJC, Delis-van Diemen PM, Sanders J. Piersma SR et al Quantitative analysis of CDX2 protein expression improves its clinical utility as a prognostic biomarker in stage II and III colon cancer. Eur J Cancer. 2021;144:91–100.
13. Proctor MJ, McMillan DC, Morrison DS, Fletcher CD, Horgan PG, Clarke SJ. A derived neutrophil to lymphocyte ratio predicts survival in patients with cancer. Br J Canc. 2012;107[4]:695e699.
14. He YF, Luo HQ, Wang W, Chen J, Yao YW, Yan Y. et al. Preoperative NLR and PLR in the middle or lower ESCC patients with the radical operation. Eur J Canc Care. 2017;26[2].
15. Liao CK, Yu YL, Lin YC, Hsu YJ, Chern YJ, Chiang JM, You JF. Prognostic value of the C-reactive protein to albumin ratio in colorectal cancer: an updated systematic review and meta-analysis. World J Surg Oncol. 2021 May 1;19[1]:139. https://doi.org/10.1186/s12957-021-02253-y. PMID: 33933070; PMCID: PMC8088626.
16. Yamamoto T, Kawada K, Obama K. Inflammation-related biomarkers for the prediction of prognosis in colorectal cancer patients. Int J Mol Sci. 2021;22[15]:8002. https://doi.org/10.3390/ijms22158002. PMID: 34360768; PMCID: PMC8348168.
17. Amri R, Bordeianou LG, Berger DL. Effect of high-grade disease on outcomes of surgically treated colon cancer. Ann Surg Oncol. 2016;23:1157.
18. Betge J, Pollheimer MJ, Lindtner RA, Kornprat P, Schlemmer A, Rehak P. et al. Intramural and extramural vascular invasion in colorectal cancer: prognostic significance and quality of pathology reporting. Cancer. 2012;118:628.
19. Liebig C, Ayala G, Wilks J, Verstovsek G, Liu H, Agarwal N. et al. Perineural invasion is an independent predictor of outcome in colorectal cancer. J Clin Oncol. 2009;27:5131.
20. Chen HS, Sheen-Chen SM. Obstruction, and perforation in colorectal adenocarcinoma: an analysis of prognosis and current trends. Surgery. 2000;127:370.
21. Chang GJ, Rodriguez-Bigas MA, Skibber JM, Moyer VA. Lymph node evaluation and survival after curative resection of colon cancer: systematic review. J Natl Cancer Inst. 2007;99:433.
22. Takagawa R, Fujii S, Ohta M, Nagano Y, Kunisaki C, Yamagishi S. et al. Preoperative serum carcinoembryonic antigen level as a predictive factor of recurrence after curative resection of colorectal cancer. Ann Surg Oncol. 2008;15:3433.
23. Konukiewitz B, Schmitt M, Silva M, Pohl J, Lang C, Steiger K, Halfter K, et al. Loss of CDX2 in colorectal cancer is associated with histopathologic subtypes and microsatellite instability but is prognostically inferior to hematoxylin-eosin-based morphologic parameters from the WHO classification. Br J Cancer. 2021;125(12):1632–46.
24. Tóth C, Sükösd F, Valicsek E, Herpel E, Schirmacher P, Tiszlavicz L. Loss of CDX2 gene expression is associated with DNA repair proteins and is a crucial member of the Wnt signaling pathway in liver metastasis of colorectal cancer. Oncol Lett. 2018;15[3]:3586–93.
25. Lee MS, Menter DG, Kopetz S. Right versus left colon cancer biology: integrating the consensus molecular subtypes. J Natl Compr Canc Netw. 2017;15(3):411–9. https://doi.org/10.6004/jnccn.2017.0038. PMID: 28275039.
26. Noepel-Duennebacke S, Juette H, Feder IS, Kluxen L, Basara N, Hiller W, et al. High microsatellite instability [MSI-H] is associated with distinct clinical and molecular characteristics and an improved survival in early Colon cancer [CC]; real world data from the AIO molecular registry Colopredict Plus. Z Gastroenterol. 2020;58(6):533–41.
27. Meguid RA, Slidell MB, Wolfgang CL, Chang DC, Ahuja N. Is there a difference in survival between right- versus left-sided colon cancers? Ann Surg Oncol. 2008;15:2388–94.
28. Weiss JM, Pfau PR, O'Connor ES, King J, LoConte N, Kennedy G, et al. Mortality by stage for right- versus left-sided colon cancer: analysis of surveillance, epidemiology, and end results Medicare data. J Clin Oncol. 2011;29:4401–9.
29. Sinicrope FA, Mahoney MR, Smyrk TC, Thibodeau SN, Warren RS, Bertagnolli MM, et al. Prognostic impact of deficient DNA mismatch repair in patients with stage III colon cancer from a randomized trial of FOLFOX-based adjuvant chemotherapy. J Clin Oncol. 2013;31:3664–72.
30. Argilés G, Tabernero J, Labianca R, Hochhauser D, Salazar R, Iveson T, Laurent-Puig P, Quirke P, Yoshino T, Taieb J, Martinelli E, Arnold D; ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2020;31(10):1291–305.
31. Beypinar I, Demir H, Sakin A, Taskoylu BY, Sakalar T, Ergun Y, Korkmaz M, Ates O, Eren T, Turhal S, Artac M. The real-life data of BRAF mutation on the treatment of colorectal cancer: a TOG study. J Gastrointest Cancer. 2021;52(3):932–9.

Authors and Affiliations

Sabin Goktas Aydin[1] · Omer Fatih Olmez[1] · Oguzhan Selvi[2] · Caglayan Geredeli[2] · Ferhat Ozden[3] · Ahmet Bilici[1] · Ozgur Acikgoz[1] · Ebru Karci[1] · Yasin Kutlu[1] · Jamshid Hamdard[1] · Ahmet Aydin[4]

✉ Sabin Goktas Aydin
drsabingoktas@gmail.com

Omer Fatih Olmez
olmezof@gmail.com

Oguzhan Selvi
selvioguz@gmail.com

Caglayan Geredeli
caglayange@gmail.com

Ferhat Ozden
Ferhat.ozden@medipol.com.tr

Ahmet Bilici
ahmetknower@yahoo.com

Ozgur Acikgoz
ozgur.acikgoz@medipol.com.tr

Ebru Karci
ebru.karci@medipol.com.tr

Yasin Kutlu
yasin.kutlu@medipol.com.tr

Jamshid Hamdard
jamshid.hamdard@medipol.com.tr

Ahmet Aydin
bayrampasadahiliye@gmail.com

[1] Department of Medical Oncology, Medical Faculty, Istanbul Medipol University, TEM Avrupa Otoyolu Goztepe, Cikisi, No:1, Bagcilar 34214 Istanbul, Turkey

[2] Department of Medical Oncology, Okmeydani Training and Research Hospital, Kaptan Paşa Mahallesi, Darülaceze Cad. No:25, 34384 Okmeydani, Istanbul, Turkey

[3] Department of Pathology, Medical Faculty, Istanbul Medipol University, TEM Avrupa Otoyolu Goztepe, Cikisi, No:1, Bagcilar 34214 Istanbul, Turkey

[4] Department of Internal Medicine, Medical Faculty, Istanbul Medipol University, TEM Avrupa Otoyolu Goztepe, Cikisi, No:1, Bagcilar 34214, Istanbul, Turkey