Original Article

Clinicopathological outcomes of microsatellite instability in colorectal cancer

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ABSTRACT

Aims: This study aims to evaluate the histopathological features and prognostic parameters of tumors with microsatellite instability (MSI) compared with those without MSI in patients who underwent surgery for colorectal cancer (CRC).

Setting and Design: Follow-up for CRC at Istanbul Sultan 2. Abdulhamid Han Training and Research Hospital was retrospectively evaluated between March 2017 and March 2021.

Methods and Material: The patients were divided into two groups: those with and without MSI. Groups were compared in survival parameters. As a secondary result, groups were compared in pathological parameters such as stage, tumor diameter, degree of differentiation, and lymphovascular, and perineural invasion.

Statistical Analysis Used: Survival calculations were performed using the Kaplan–Meier analysis method. The effects of various prognostic factors related to tumor and patient characteristics on disease-free and overall survival (OS) were investigated by log-rank test.

Results: Two hundred fourteen patients were analyzed. The median age of the patients was 66 (30–89), and 59.3% ($n = 127$) were male. There were 25 patients in the MSI group and 189 patients in the non-MSI group. We found that MSI tumors had a significantly higher differentiation degree than non-MSI tumors and larger tumor diameters. MSI tumors frequently settled in the proximal colon, and more lymph nodes were removed in the resection material. MSI tumors had longer disease-free survival, cancer-specific survival, and overall survival.

Conclusions: By diagnosing microsatellite instability, CRCs can be divided into two groups. The histopathological features of the tumor and the prognosis of the disease differ between these groups. MSI can be a predictive marker in the patient's follow-up and treatment.

KEY WORDS: Colorectal cancer, colorectal carcinoma, microsatellite instability, prognosis, prognostic factor, recurrence, TNM staging, survival

INTRODUCTION

According to Global Cancer Statistics, more than 1.9 million new cases of colorectal cancer (CRC) and 935,000 deaths are estimated to occur in 2020, representing approximately one in ten cancer cases and deaths. Overall, it ranks third in colorectal incidence but second in mortality.[1]

In CRC evolution, the acquisition of genomic instability is a critical point, and there are at least two different pathways in the pathogenesis of CRC: the chromosomal instability pathway (85%) and the microsatellite instability (MSI) pathway (15%).[2]

MSI is a phenotype that occurs due to a malfunction in the DNA repair mechanism and is seen in approximately 15% of CRCs. CRCs with MSI have different clinical features, such as a tendency to settle in the proximal colon, poor differentiation, and more lymphocytic infiltration in the tumor. It has been shown that CRC with MSI has a better prognosis and responds differently to chemotherapy than CRC with microsatellite stability (MSS).[3]

We aimed to evaluate the different histopathological features of tumors with MSI compared with MSS in patients who underwent surgery for CRC. We also planned to determine how MSI affects prognostic

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parameters such as mortality rate, recurrence, disease-free survival, cancer-specific survival, and overall survival.

SUBJECTS AND METHODS

Study design and study population

This study was approved by the University of Health Sciences, Sancaktepe Şehit Prof.Dr. İlhan Varank Training and Research Hospital Scientific Research Ethics Committee (Number: 07.04.2021-2021/137) and registered with ClinicalTrials.gov (NCT05162248).

In this study, 231 patients were followed up after undergoing colorectal surgery at Istanbul Sultan 2. Abdulhamid Han Training and Research Hospital Oncology Clinic between March 2017 and March 2021 were examined. After excluding those whose MSI status was not studied in the pathology material in the postoperative period and those for whom adequate follow-up data could not be obtained, 214 patients were analyzed.

Data collection

The following parameters were analyzed from the follow-up files of the patients: sex, whether they received adjuvant or neoadjuvant treatment, dates of diagnosis, dates of surgery, last follow-up dates, relapse cases, date of relapse, and date of death. MSI status, stage, tumor diameter, degree of differentiation, and lymphovascular and perineural invasion status of the tumor were registered by examining the pathology reports.

All patients with CRC were categorized according to the Tumor-Node-Metastasis classification of the American Joint Committee on Cancer.[4,5] For patients with CRC, postoperative

Table 1: Clinicopathologic characteristics according to MSI status in patients with colorectal cancer

Characteristics	Overall population (*n*=214) *n* (%)	MSS (*n*=189) *n* (%)	MSI (*n*=25) *n* (%)	*Z*/χ^2	*P*
Age (years, median-range)	66 (30-89)	66 (30-89)	62 (33-88)	−0.772[a]	0.440
Age group				1.326[b]	0.250
≤65	101 (47.2)	86 (45.5)	15 (60.0)		
>65	113 (52.8)	103 (54.5)	10 (40.0)		
Sex				0.335[b]	0.563
Female	87 (40.7)	75 (39.7)	12 (48.0)		
Male	127 (59.3)	114 (60.3)	13 (52.0)		
Depth of invasion				0.192[c]	0.908
T1/2	22 (10.3)	20 (10.6)	2 (8.0)		
T3	156 (72.9)	137 (72.5)	19 (76.0)		
T4	36 (16.8)	32 (16.9)	4 (16.0)		
Lymph node metastasis				0.681[c]	0.711
N0	131 (61.2)	114 (60.3)	17 (68.0)		
N1	57 (26.6)	52 (27.5)	5 (20.0)		
N2	26 (12.1)	23 (12.2)	3 (12.0)		
TNM Stage				2.773[c]	0.250
Stage I-II	105 (49.1)	90 (47.6)	15 (60.0)		
Stage III	94 (43.9)	84 (44.4)	10 (40.0)		
Stage IV	15 (7.0)	15 (7.9)	0 (0.0)		
Harvested lymph node (LN) (median-range)	18 (4-78)	17 (4-78)	26 (12-70)	−4.019[a]	<0.001*
Metastatic lymph node (*n*=83) (median-range)	2 (1-31)	2 (1-31)	2.5 (1-10)	−0.508[a]	0.611
Tumor size (millimeter, median-range)	49 (10-120)	45 (10-120)	65 (25-120)	−2.941[a]	0.003*
Differentiation				8.702[c]	0.013*
Grade I	67 (31.3)	57 (30.2)	10 (40.0)		
Grade II	131 (61.2)	121 (64.0)	10 (40.0)		
Grade III	16 (7.5)	11 (5.8)	5 (20.0)		
Lymphatic invasion				0.493[b]	0.482
Yes	104 (48.6)	94 (49.7)	10 (40.0)		
No	110 (51.4)	95 (50.3)	15 (60.0)		
Vascular invasion				0.000[b]	1.000
Yes	95 (44.4)	84 (44.4)	11 (44.0)		
No	119 (55.6)	105 (55.6)	14 (56.0)		
Perineural invasion				2.780[b]	0.095
Yes	75 (35.0)	62 (32.8)	13 (52.0)		
No	139 (65.0)	127 (67.2)	12 (48.0)		
Extracapsular invasion (*n*=83)					0.041*
Yes	16 (19.3)	12 (16.0)	4 (50.0)		
No	67 (80.7)	63 (84.0)	4 (50.0)		
Tumor location				33.066[c]	<0.001*
Right colon	55 (25.7)	41 (21.7)	14 (56.0)		
Left colon	34 (15.9)	31 (16.4)	3 (12.0)		
Rectum	49 (22.9)	49 (25.9)	0 (0.0)		
Sigmoid colon	66 (30.8)	61 (32.3)	5 (20.0)		
Transverse colon	2 (0.9)	0 (0.0)	2 (8.0)		
Cecum	8 (3.7)	7 (3.7)	1 (4.0)		

*P<0,05; P>0,05; a (Z): Mann-Whitney U test; χ^2: Chi-square test (b: continuity correction, c: Pearson Chi-square test, d: Fisher's exact test)

adjuvant treatment and follow-up were administered by National Comprehensive Cancer Network (NCCN) guidelines.[6,7]

Disease-free survival (DFS), cancer-specific survival, and overall survival (OS) were calculated using the recorded dates. The patients were divided into two groups: MSS and MSI. One hundred eighty-nine patients in the MSI group and 25 in the MSS group were studied.

Microsatellite instability analysis

Immunohistochemistry (IHC) assays and polymerase chain reaction (PCR) were used to detect of MSI. IHC uses antibodies against MLH1, MSH2, MSH6, and PMS2 to assess tissue samples' mismatch repair (MMR) conditions.[8] MSI testing with polymerase chain reaction (PCR) was used if the result of IHC was uncertain.[9]

Sample size

Guided by other studies whose d value, which is the effect size index, is reported $d = 0.20$ (X2:0.10–0.30 small effect size), $\alpha = 0.05$ (error margin), $1-\beta = 0.80$ (power), and it was calculated as a minimum of 197 cases with the help of the G*Power 3 (version 3.1 for Windows) package program, accompanied by the specified criteria.[10,11]

Statistical analysis

While evaluating the findings obtained in the study, the SPSS version 25.0 (IBM Corp., Armonk, NY, USA) program was used for statistical analysis. Descriptive statistical methods (number, percentage, median, etc.) were used while evaluating the study data. Survival calculations were performed using the Kaplan–Meier analysis method. The effects of various prognostic factors related to tumor and patient characteristics on disease-free and overall survival were investigated by log-rank test. In addition, the impact of multiple prognostic factors on disease-free and OS was analyzed using the multivariate Cox regression test. Proportional differences between groups were calculated with Chi-square tests (Pearson Chi-square, continuity correction, Fisher's exact test), and quantitative differences were calculated with the Mann–Whitney U test. The results were evaluated at the 95% confidence interval and the significance level of $P < 0.05$.

Study outcomes

The primary outcome of this study was to ascertain whether there was a difference between MSS and MSI in terms of clinical indicators such as recurrence, mortality, DFS, cancer-specific survival, DFS, and OS. As a secondary outcome, the groups were compared in terms of pathological parameters such as stage, T stage, N stage, tumor diameter, degree of differentiation, and lymphovascular and perineural invasion status.

RESULTS

Baseline characteristics

After exclusion criteria, 214 patients were analyzed [Figure 1]. The microsatellite status of patients was 88.3% ($n = 189$) MSS and 11.7% ($n = 25$) MSI. The median age of the patients was

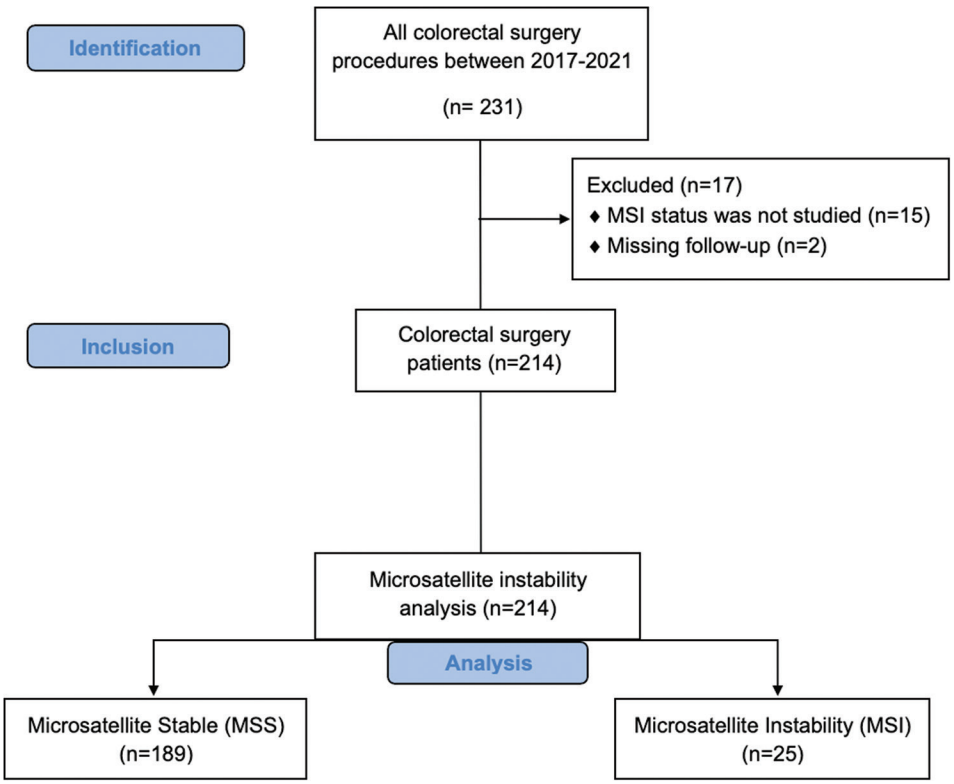

Figure 1: Flowchart of patient selection

66 (30–89), and 59.3% (n = 127) were male. It was determined that the demographic characteristics of the patients did not show any difference according to the MSI status ($P > 0.05$). The median number of lymph nodes harvested in 214 patients was 18 (4–78). The median lymph node count (MSI: 26 and MSS: 17; $P = <0.001$) was significantly higher in the MSI group. Grade III differentiation was higher in the MSI group (MSI grade III: 20% and MSS grade III: 5.8%; $P = 0.013$). While the presence of extracapsular invasion was detected in 19.3% (n = 16) of lymph node-positive tumors, the presence of extracapsular invasion (MSI: 50% and MSS: 16%; $P = 0.041$) was higher in the MSI group. When the groups were compared in terms of tumor localization, it was seen that the MSI group was more common in the right colon, while the MSS group was more located in the sigmoid colon and rectum. Details are shown in Table 1.

Death occurred in 33 of the 214 patients included in the study, and the last time of death was 80 months. The colon cancer-related mortality rate corresponding to this time was 50.2%, and its standard error was 0.152. The 5-year OS rate of the patients was 67%.

After excluding 15 clinically staged IV patients out of 214 patients included in the study, 30 of the remaining 199 patients had recurrence, and the last recurrence time was 64 months. The DFS rate corresponding to this time was 69.9%, and the standard error was 0.098. The 5-year DFS rate of the patients was calculated as 79.9%.

Assessment of survival according to MSI status

Table 2 shows the effect of MSI status on disease-free, cancer-specific and OS rates in all cases according to their stages. As a result of Kaplan–Meier survival analysis, although it was not statistically significant, the MSI group cases were disease-free (MSI: 82.7% and MSS: 68.1%; $P = 0.680$) [Figure 2], cancer-specific (MSI: 87.5% and MSS: 79%; $P = 0.840$ [Figure 3] and overall survival rates (MSI: 79.5% and MSS: 66.6%; $P = 0.824$) [Figure 4].

Although it was not statistically significant in stage III tumors, cancer-specific (MSI: 87.5% and MSS: 71.9%; $P = 0.660$) [Figure 5] and overall survival (MSI: 87.5% and MSS: 54.8%; $P = 0.743$) rates were higher in the MSI group [Figure 6].

Table 3 shows the DFS rates of the patients in the MSI and MSS groups according to their demographic and clinicopathological characteristics. In the MSS group, it was determined that patients with male sex status (58.4%), lymph node metastasis (65.5%), stage III (65.1%), and positive perineural invasion (62%) had a significantly lower DFS rate. ($P < 0.05$). In the MSI group, there was a statistically significant difference in disease-free survival only in the presence of metastatic lymph nodes (pN0: 91.7% and pN1-2: 55.6; $P = 0.046$).

Factors affecting long-term outcomes

Table 4 shows the distribution of independent factors associated with disease-free, cancer-specific, and OS of patients. According to the Cox regression analysis results, although it was statistically insignificant, it was determined that the MSS group than the MSI group, respectively; disease-free survival HR = 2.08 (0.53–8.26) times, cancer-specific survival HR = 1.83 (0.31–10.84) times, and overall survival HR = 2.17 (0.55–8.60) times more negatively affect.

Patients' disease-free, cancer-specific, and OS rates are given in Table 5 according to their demographic and clinicopathological characteristics. As a result of Kaplan–Meier survival analysis,

Table 2: Disease-free, cancer-specific, and overall survival rates of patients

Characteristics	Disease-free survival			Cancer-specific survival			Overall survival		
	%	HR (95%CI)	*P*	%	HR (95%CI)	*P*	%	HR (95%CI)	*P*
MSI-All Stages									
MSS	68.1	1.3 (0.4-4.2)	0.680	79.0	1.2 (0.3-5.1)	0.840	66.6	1.1 (0.3-3.8)	0.824
MSI	82.7	1 (Ref)		87.5	1 (Ref)		79.5	1 (Ref)	
MSI-Stage I-II									
MSS	74.8	1.1 (0.1-9.6)	0.919	93.7	0.57 (0.1-5.5)	0.627	58.0	1.5 (0.3-7.4)	0.600
MSI	90.9	1 (Ref)		90.0	1 (Ref)		80.0	1 (Ref)	
MSI-Stage III									
MSS	65.1	1.1 (0.2-4.5)	0.951	71.9	1.6 (0.2-12.8)	0.66	54.8	1.4 (0.2-10.6)	0.743
MSI	65.6	1 (Ref)		87.5	1 (Ref)		87.5	1 (Ref)	
Stage I-II with Adjuvant Chemotherapy									
MSS	90.8	0.74 (0.1-7.1)	0.791	89.7	0.48 (0.04-5.3)	0.537	78.9	1.1 (0.1-9.6)	0.922
MSI	85.7	1 (Ref)		83.3	1 (Ref)		83.3	1 (Ref)	
Stage I-II without Adjuvant Chemotherapy									
MSS	71.9	24.7 (NA)	0.591	97.6	24.6 (NA)	0.705	63.2	3.4 (0.338.5)	0.291
MSI	100.0	1 (Ref)		100.0	1 (Ref)		75.0	1 (Ref)	
Stage III with Adjuvant Chemotherapy									
MSS	64.0	1.1 (0.3-4.9)	0.858	75.4	1.7 (0.2-15.6)	0.560	67.0	1.1 (0.1-8.2)	0.957
MSI	64.3	1 (Ref)		85.7	1 (Ref)		85.7	1 (Ref)	
Stage III without Adjuvant Chemotherapy									
MSS	73.3	25.5 (NA)	0.683	55.6	24.1 (NA)	0.630	52.4	24.2 (NA)	0.621
MSI	100.0	1 (Ref)		100.0	1 (Ref)		100.0	1 (Ref)	

P>0,05; Kaplan-Meier survival analysis, log rank test, HR: Hazard ratio, CI: Confidence interval, MSI: microsatellite instability, MSS: microsatellite stability, NA: Not available

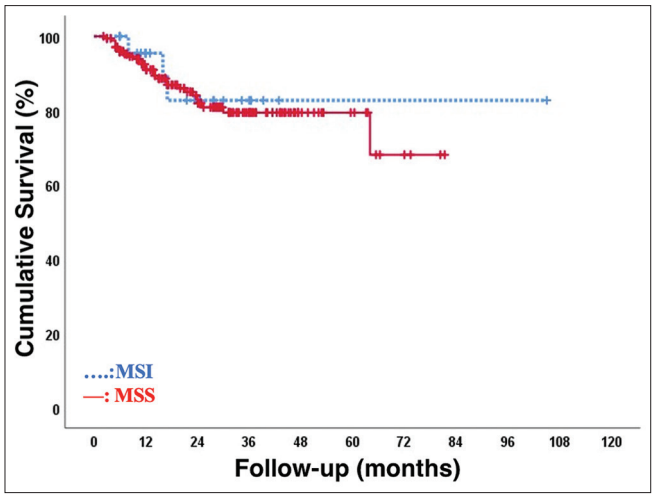

Figure 2: DFS curve of the patients according to MSI status

Table 3: Disease-free survival rates according to MSI status of patients

Characteristics	MSS (*n*=174)		MSI (*n*=25)	
	%	*P*	%	*P*
Age group				
≤65	78.8	0.608	88.9	0.323
>65	64.5		74.1	
Sex				
Female	88.8	0.041*	80.2	0.626
Male	58.4		85.7	
Depth of invasion				
T 1-2	93.8	0.194	100	0.658
T 3-4	64.8		81.6	
Lymph node metastasis				
N0	87.7	0.023*	91.7	0.046*
N1-2	65.5		55.6	
TNM Stage				
Stage I-II	93.5	0.001*	90.9	0.134
Stage III	65.1		65.6	
Tumor size (millimeter)				
<50 mm	71.8	0.108	85.7	0.761
≥50 mm	66.2		81.3	
Differentiation				
Grade I	86.2	0.220	100	0.187
Grade II-III	61.8		83.1	
Lymphatic invasion				
Yes	75.7	0.790	85.7	0.723
No	82.3		80.8	
Vascular invasion				
Yes	74.4	0.312	75.0	0.352
No	83.2		88.9	
Perineural invasion				
Yes	62.0	<0.001*	90.9	0.702
No	86.3		77.8	
Adjuvant Chemotherapy				
Yes	73.4	0.052	76.4	0.268
No	92.1		100	

*P<0,05; Cox regression analysis (method=enter), MSI: microsatellite instability, MSS: microsatellite stability

the overall survival rate of patients with male sex (55.9%), pathological N2 (39.6%) and N1 (59%) involvement, stage IV (44.6%), lymphatic invasion (51.8%), pericapsular invasion (54.7%) and perineural invasion positivity (51.1%) was found to be significantly lower ($P < 0.05$).

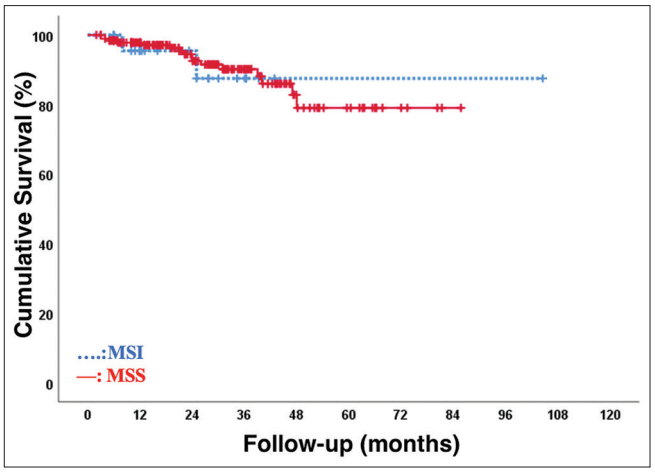

Figure 3: Cancer-specific survival curve of the patients according to MSI status

DISCUSSION

In our study, CRC with MSS and MSI was compared with recurrence, mortality, and survival parameters. Recurrence and mortality were higher in the MSS group, although the difference was not statistically significant. All survival parameters in stage I and II and stage III tumors were longer in MSI CRCs, and this did not change with adjuvant treatment. Compared with MSI tumors, MSS negatively affected disease-free survival HR = 2.08 (0.53–8.26) times, cancer-specific survival HR = 1.83 (0.31–10.84) times, and overall survival HR = 2.17 (0, 55–8.60) times.

When the demographic data were analyzed in the study, the median age of MSS tumors was 66 (30–89), and the median age of MSI tumors was 62 (33–88). Gryfe *et al*.[12] showed in their study that MSI tumors were diagnosed at an earlier age. However, this study was limited to patients under 50 years of age, so it would not be appropriate to generalize the findings to all CRC patients. Other studies have shown that there is no difference between the groups in terms of the patient's age at the time of diagnosis.[13-15]

In our study, 15% of stage I and II tumors and 11% of stage III tumors were MSI CRC. Among the stage IV patients, there was no MSI CRC. However, in their study, Kawakami *et al*.[16] showed that 4% of stage IV CRCs were MSI CRC.

Some studies in the literature state that there is no difference in the number of lymph nodes harvested between MSS and MSI tumors.[17,18] However, our study found that the number of lymph nodes harvested was higher in MSI tumors, consistent with studies involving large patient groups.[19,20] It is known that MSI tumors show more lymphocytic antitumor properties, and it is thought that the number of lymph nodes formed will increase accordingly.[21] More studies are needed on this subject to examine the conflicting results in the literature.

Table 4: Independent factors associated with patient survival (multiple Cox regression analysis results)

Characteristics	Disease-free survival		Cancer-specific survival		Overall survival	
	HR (95%CI)	*P*	HR (95%CI)	*P*	HR (95%CI)	*P*
Microsatellite Instability Status						
MSI	Reference (1)		Reference (1)		Reference (1)	
MSS	2.08 (0.53-8.26)	0.297	1.83 (0.31-10.84)	0.505	2.17 (0.55-8.60)	0.268
Age group						
≤65	Reference (1)		Reference (1)		Reference (1)	
>65	1.81 (0.78-4.17)	0.165	1.62 (0.51-5.12)	0.413	1.73 (0.73-4.10)	0.214
Sex						
Female	Reference (1)		Reference (1)		Reference (1)	
Male	2.23 (0.94-5.28)	0.067	6.56 (1.64-26.26)	0.008*	3.92 (1.51-10.16)	0.005*
Depth of invasion						
T 1-2	Reference (1)		Reference (1)**	-	Reference (1)	
T 3-4	4.21 (0.49-36.11)	0.190	-		1.60 (0.18-14.01)	0.670
Lymph node metastasis						
N0	Reference (1)		Reference (1)		Reference (1)	
N1	1.60 (0.56-4.53)	0.379	2.12 (0.45-9.94)	0.340	2.58 (0.94-7.10)	0.067
N2	4.10 (1.37-12.21)	0.011*	9.32 (2.00-43.37)	0.004*	3.68 (1.15-11.75)	0.028*
Tumor size (millimeter)						
<50	Reference (1)		Reference (1)		Reference (1)	
≥50	1.45 (0.63-3.35)	0.388	2.36 (0.76-7.34)	0.138	1.50 (0.69-3.27)	0.306
Differentiation						
Grade I	Reference (1)		Reference (1)		Reference (1)	
Grade II-III	1.86 (0.39-3.06)	0.875	1.14 (0.34-3.85)	0.837	1.05 (0.69-1.59)	0.818
Lymphatic invasion						
No	Reference (1)		Reference (1)		Reference (1)	
Yes	7.05 (1.96-25.35)	0.003*	6.39 (1.12-36.34)	0.037*	2.06 (0.56-7.62)	0.277
Vascular invasion						
No	Reference (1)		Reference (1)		Reference (1)	
Yes	3.43 (0.97-12.10)	0.056	4.71 (0.91-24.53)	0.065	2.45 (0.73-8.20)	0.147
Perineural invasion						
No	Reference (1)		Reference (1)		Reference (1)	
Yes	3.06 (1.21-7.75)	0.019*	4.82 (1.36-17.11)	0.015*	3.34 (1.37-8.15)	0.008*
Adjuvant Chemotherapy						
No	Reference (1)		1.22 (0.32-4.73)	0.774	1.47 (0.59-3.64)	0.411
Yes	1.82 (0.59-5.60)	0.294	Reference (1)		Reference (1)	

*P<0,05; Cox regression analysis (method=enter) **The T-stage factor was not included in the model because there were no events in the reference group.
HR: Hazard ratio, CI: Confidence interval, MSI: Microsatellite instability, MSS: Microsatellite stability

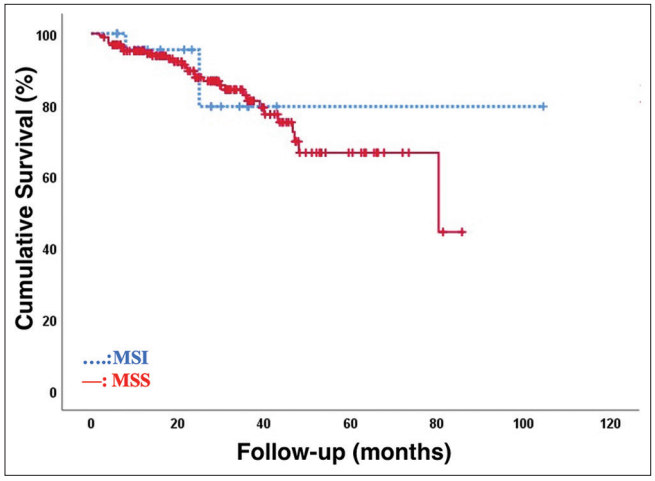

Figure 4: Overall survival curve of the patients according to MSI status

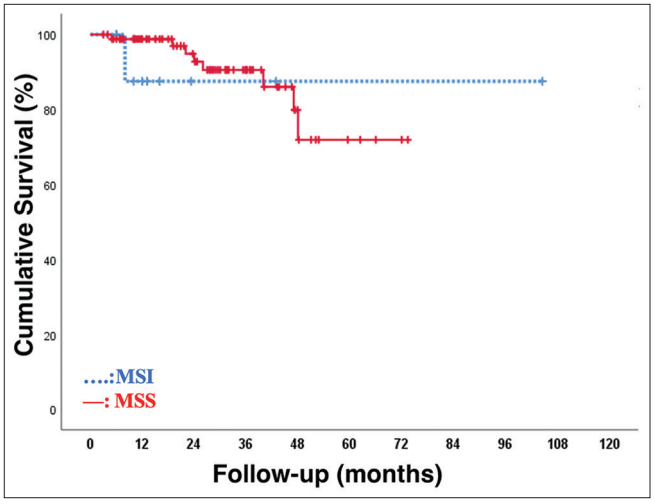

Figure 5: Cancer-specific survival curve of stage III tumors according to MSI status

The mean tumor diameter in the MSI group was larger than that in the MSS group, and our results were compatible with the literature.[22,23] The relationship between lymphovascular and perineural invasion and MSI is controversial in the literature.[24,25] We found no difference between the MSI and MSS groups in our study.

We found a statistically significant difference between the MSS and MSI groups regarding tumor location. While MSI tumors were mainly located in the right colon, CNS tumors were mainly located in the sigmoid colon and rectum. The literature

Table 5: Survival rates according to clinicopathologic characteristics in patients

Characteristics	Disease-free survival			Cancer-specific survival			Overall survival		
	%	HR (95%CI)	*P*	%	HR (95%CI)	*P*	%	HR (95%CI)	*P*
Microsatellite Instability Status									
MSS	68.1	1.3 (0.4-4.2)	0.680	79.0	1.2 (0.3-5.1)	0.840	66.6	1.1 (0.3-3.8)	0.824
MSI	82.7	1 (Ref)		87.5	1 (Ref)		79.5	1 (Ref)	
Age group									
≤65	80.4	1 (Ref)		83.4	1 (Ref)		76.0	1 (Ref)	
>65	66.7	1.4 (0.7-2.8)	0.411	74.3	1.3 (0.5-3.4)	0.546	57.8	1.8 (0.9-3.6)	0.112
Sex									
Female	87.5	1 (Ref)		90.8	1 (Ref)		82.7	1 (Ref)	
Male	62.0	2.04 (0.9-4.6)	0.078	71.4	3.9 (1.1-13.5)	0.021*	55.9	2.8 (1.2-6.5)	0.012*
Depth of invasion									
T 1-2	94.1	1 (Ref)		100	1 (Ref)	0.153	90.0	1 (Ref)	
T 3-4	67.1	3.6 (0.5-26.4)	0.177	77.0	23.7 (NA)		64.5	3.5 (0.5-25.8)	0.185
Lymph node metastasis									
N0	88.3	1 (Ref)		90.4	1 (Ref)		84.6	1 (Ref)	
N1	76.9	1.7 (0.7-4.2)	0.222	74.2	2.1 (0.7-7.0)	0.209	59.0	2.8 (1.3-6.1)	0.011*
N2	44.2	4.5 (1.9-10.5)	0.001*	47.6	5.8 (1.9-17.4)	0.002*	39.6	3.4 (1.4-8.4)	0.008*
TNM Stage									
Stage I-II	93.0	1 (Ref)		92.9	1 (Ref)		85.5	1 (Ref)	
Stage III	65.2	4.2 (1.8-9.7)	<0.001*	72.8	2.5 (0.8-7.9)	0.136	56.6	2.0 (0.9-4.4)	0.079
Stage IV	-a	NA		44.6	10.5 (2.8-39.1)	<0.001*	44.6	4.4 (1.5-13.0)	0.007*
Tumor size (millimeter)									
<50	73.2	1 (Ref)		83.4	1 (Ref)		68.0	1 (Ref)	
≥50	65.5	1.7 (0.8-3.5)	0.153	82.4	1.3 (0.5-3.4)	0.552	65.5	1.1 (0.6-2.2)	0.737
Differentiation									
Grade I	88.3	1 (Ref)		92.4	1 (Ref)		80.8	1 (Ref)	
Grade II-III	64.1	2.2 (0.8-5.7)	0.104	70.3	1.7 (0.6-5.4)	0.321	58.3	1.4 (0.6-2.9)	0.422
Lymphatic invasion									
Yes	76.9	1.01 (0.5-2.1)	0.904	69.9	1.7 (0.7-4.5)	0.244	51.8	2.2 (1.1-4.5)	0.030*
No	82.2	1 (Ref)		88.0	1 (Ref)		84.0	1 (Ref)	
Vascular invasion									
Yes	74.5	1.6 (0.8-3.2)	0.220	74.2	2.4 (0.9-6.5)	0.067	54.7	2.4 (1.2-4.9)	0.015*
No	84.0	1 (Ref)		83.2	1 (Ref)		78.9	1 (Ref)	
Vascular invasion									
Yes	67.5	3.1 (1.5-6.3)	0.001*	60.5	4.1 (1.6-10.8)	0.002*	51.1	3.1 (1.6-6.2)	0.001*
No	85.4	1 (Ref)		87.2	1 (Ref)		74.8	1 (Ref)	
Adjuvant Chemotherapy									
Yes	73.7	2.8 (1.1-7.3)	0.030*	76.2	1.7 (0.6-5.2)	0.344	63.0	1.2 (0.6-2.6)	0.653
No	93.0	1 (Ref)		85.8	1 (Ref)		74.5	1 (Ref)	

*P<0,05; Kaplan-Meier survival analysis, log rank test, a: Stage IV cases were not included in the disease-free survival calculations. b: Analysis was not performed because the subgroup had inadequate numbers, MSI: microsatellite instability, MSS: microsatellite stability, HR: Hazard ratio, CI: Confidence interval

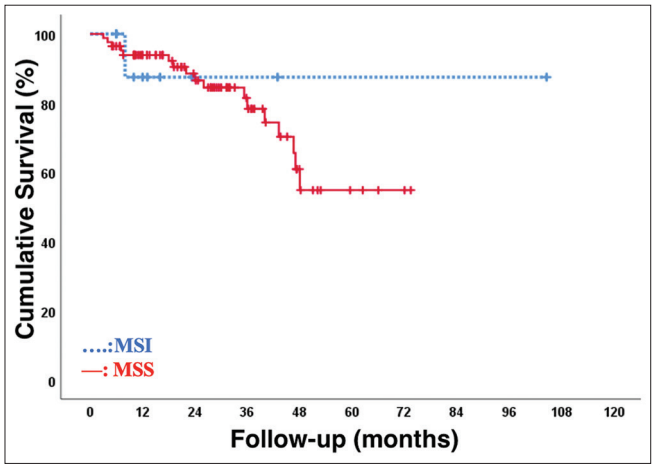

Figure 6: Overall survival curve of stage III tumors according to MSI status

shows that MSI tumors tend to settle in the proximal colon, and our data are compatible with the literature.[20,26,27]

In our study, as a result of Kaplan–Meier survival analysis, although not statistically significant, it was determined that the MSI group had higher cancer-specific, disease-free, and OS rates.

We found that disease-free and overall survival rates were higher in the MSI group in stage I and II tumors, and cancer-specific and overall survival was higher in MSI tumors in stage III tumors; however, these results were not statistically significant. Adjuvant treatment status did not change this situation. In high-volume studies involving stage II and III patients, it was observed that the MSI group had a better prognosis in both stages.[28,29] Some published data show that MSI status does not have a prognostic effect for stage III CRC.[30-32] It is believed that the positive effect of MSI on prognosis is based on the immunological reaction against the tumor. While the prognostic effect of MSI is positive in stage II CRC, it seems to decrease in stage III CRC. It is thought that with the progression of the

disease stage, immune avoidance mechanisms are formed, and the positive effect of MSI on the prognosis is lost.[33] Similar to this hypothesis, it has been shown that the rate of MSI detection in stage IV CRCs decreases, and MSI is not a positive prognostic factor.[34] Our study could not evaluate stage IV CRC since there were no stage IV cases among MSI tumors.

Our study has certain limitations. It is a single-center, low-volume, and retrospective study. Despite our efforts, selection bias may occur due to design. We did not have stage 4 patients, which may be due to the small patient population. The low volume of our cohort may have affected the statistical outcome of our study. Due to the study's retrospective design, we could not compare clinical and pathological stages. Large multicenter studies are needed to better understand the effect of MSI on CRC.

Our study investigated the differences between MSI and MSS CRCs in clinicopathological results. When we grouped the tumors as stages I, II, and III and compared those with MSI and those with MSS, the results of the MSI group were better in terms of all survival parameters; however, these results were not statistically significant. By diagnosing MSI, CRCs can be divided into two groups. The histopathological features of the tumor and the prognosis of the disease differ between these groups. Based on the results of this study, MSI status plays an essential role in terms of the prediction of long-term outcomes.

Ethical policy and Institutional Review board statement

This study was approved by the University of Health Sciences, Sancaktepe Şehit Prof.Dr. İlhan Varank Training and Research Hospital Scientific Research Ethics Committee (Number: 07.04.2021-2021/137) and registered with ClinicalTrials.gov (NCT05162248).

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Conflicts of interest

There are no conflicts of interest.

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