



Prognostic and predictive value of microsatellite instability status among patients with colorectal cancer

Firas Baidoun^{*,1} , Anas M Saad² & Omar Abdel-Rahman³ 

¹Department of Hospital Medicine, Cleveland Clinic Foundation, Cleveland, OH 44195, USA

²Heart & Vascular Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, USA

³Department of Oncology, University of Alberta, Cross Cancer Institute, Edmonton, AB, T6G 1Z2, Canada

*Author for correspondence: Tel.: +1 609 225 0910; firasb2@hotmail.com

Objectives: Compare overall survival (OS) between microsatellite instability (MSI) high and MSI-stable and analyze the effect of chemotherapy on OS. **Methods:** National cancer database was queried for patients diagnosed with colorectal adenocarcinoma between 2010 and 2016. We evaluated the OS and the chemotherapy effect using Kaplan–Meier estimates and multivariate Cox regression analyses. **Results:** Total of 30,436 stage II patients and 30,302 stage III patients were included. In stage II with high-risk features and MSI-high, patients who received chemotherapy had better OS compared to patients who didn't receive chemotherapy. The same was found in stage II with no high-risk features and MSI-high group. **Conclusion:** Stage II colorectal cancer patients with high-risk features and MSI-high who received chemotherapy have better OS compared to patients who didn't receive chemotherapy.

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Colorectal cancer (CRC) is the third most common diagnosed malignancy and the second leading cause of cancer-related death in the USA. In 2020, 147,950 new CRC cases (accounting for 8.2% of all new cancer cases) are estimated to be diagnosed; and 53,200 CRC-related death (accounting for 8.8% of all cancer deaths) are estimated to happen [1–3]. About 15% of CRC cases have deficient DNA mismatch repair system (dMMR) which causes microsatellite instability (MSI) in the cancer cells' DNA and those cases are referred to as dMMR, or MSI-high (MSI-H) CRC, whereas 85% of CRC cases has proficient mismatch repair mechanism system (pMMR) which results in a microsatellite stable (MSI-S) pattern and those cases are referred to as pMMR, MSS or MSI-stable (MSI-S) CRC [4,5]. MSI-H CRC is more common in stage II followed by stage III [6].

The DNA MMR mechanism is responsible for repairing the errors in the mismatched bases that occur during DNA replication and synthesis and a defect in this mechanism results in the accumulation of these mismatched bases which are referred to as MSI [7]. In CRC, dMMR can occur as a result of a germline mutation in one of the MMR genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*) or more commonly as a result of sporadic inactivation of the *MLH1* gene caused by DNA hypermethylation [6,8].

The MSI status in CRC has been under the spotlight in the last few years with an extensive effort to assess the MSI status as a prognostic and a predictive tool to determine the benefit of adjuvant chemotherapy, especially in nonmetastatic CRC. Until today, the role of MSI status in CRC prognosis is not well-established and its usage as guidance in adjuvant chemotherapy is controversial [9–11]. In this study, we aimed to evaluate the difference in overall survival (OS) between MSI-H and MSI-S CRC and analyze the effect of chemotherapy on OS based on the MSI status and high-risk features.

Materials & methods

Data source

The National Cancer Database (NCDB) is a nationwide database sponsored by the American Cancer Society and the Commission on Cancer (CoC) of the American College of Surgeons. It includes about 70% of all diagnosed cancer cases in the United States from more than 1,500 cancer centers. The database provides details of patients' demographics, malignancy staging and histological characteristics in addition to treatment and outcome information [12]. The CoC's NCDB and the hospitals participating in the CoC's NCDB are the sources of the de-identified data used herein; they have not verified and are not responsible for the statistical validity of the data analysis or the conclusions derived by the authors.

After we obtained the approval of our proposed protocol and the letter of support from the cancer committee chair at Cleveland Clinic, the NCDB was queried for patients diagnosed with CRC between 2004 and 2016.

Patient selection

For our study, we selected the patients who were diagnosed with CRC after 2010, at age of 18 or older, and have a radical surgical resection. We excluded patients who didn't have pathologic stage II (defined as T3 or T4 with N0) or stage III (defined as T1–4 with N1 or N2) and patients who had a clinical metastatic disease or unknown clinical metastatic status. Also, we excluded patients who lost follow up.

Variables

Using the NCDB, we included the following patient demographic variables: age, gender, race, median income, insurance status, education level, facility type (which includes community cancer program, comprehensive community cancer program, academic/research program and integrated network cancer program) in addition to the geographic area which was classified as metropolitan, urban or rural location. Also, we collected the following disease-related variables: tumor side, pathologic tumor and nodal stage and clinical metastatic stage based on American Joint Committee on Cancer (AJCC) staging, number of examined lymph nodes, perineural invasion, lymphatic/vascular invasion, MSI status, Charlson–Deyo comorbidity score and chemotherapy. For the sake of the current study, MSI status will be classified into MSI-H and MSI-S.

Outcome

The primary outcome of this study was OS in months defined as the time from diagnosis to the time of death from any cause (stratified by the high-risk features and the MSI status of the disease).

Statistical analysis

We compared the baseline demographics and characteristics between MSI-H CRC and MSI-S patient groups using descriptive statistics and we compared the frequency of various characteristics according to MSI status by using the Pearson Chi-square test.

For OS comparisons, we split the cohort into two separate groups according to pathologic TNM stage, CRC stage II and CRC stage III. Then, we compared the OS between MSI-high and MSI-S patients in each of these groups.

We also did subgroup analyses. In the first subgroup analysis, we included patients with stage II CRC who had high-risk features (defined as pathologic T4, examined lymph node <12, lymphatic/vascular invasion or perineural invasion according to the National Comprehensive Cancer Network and European Society for Medical Oncology guidelines [13,14]), and we split them according to MSI status into MSI-high and MSI-S groups and compared the OS between patients who received chemotherapy and patients who didn't receive chemotherapy in each MSI group.

In the second subgroup analysis, we included patients with stage II CRC who had highest risk features (defined as pathological T4 or examined lymph node <12) and we split them according to MSI status into MSI-high and MSI-S groups and compared the OS between patients who received chemotherapy and patients who didn't receive chemotherapy in each MSI group.

In the third subgroup analysis, we included patients with stage II CRC who didn't have any of the high-risk features (defined as pathologic T4, examined lymph node <12, lymphatic/vascular invasion or perineural invasion) and we split them according to MSI status into MSI-high and MSI-S groups and compared the OS between patients who received chemotherapy and patients who didn't receive chemotherapy in each MSI group.

In the fourth subgroup analysis, we included patients with stage III CRC and MSI-high status and we split them into high-risk group (defined as T4 or N2) and low-risk group (defined as T1–T3 and N1 according to the National Comprehensive Cancer Network and European Society for Medical Oncology guidelines [13,14]) and compared the OS between patients who received chemotherapy and patients who didn't receive chemotherapy in each risk group.

OS analyses were evaluated by using the Kaplan–Meier survival method and Kaplan–Meier curves for survival were generated. A log-rank test was used to evaluate survival differences between groups.

In the first subgroup analysis, we used nearest neighbor, 1:1, propensity score matching to reduce the possible bias and effect of the variation in age, Charlson–Deyo comorbidity score, TNM pathological T stage and number of examined lymph node between the patient group that received chemotherapy and the one that didn't receive chemotherapy, while in the third subgroup analysis we used nearest neighbor, 1:1, propensity score matching to reduce the possible bias and effect of the variation in age and Charlson–Deyo comorbidity score between the patient group that received chemotherapy and the one that didn't receive chemotherapy using RStudio Version 1.3.1093 (MA, USA).

We used Cox regression analysis to conduct multivariate analyses to evaluate the factors associated with improved OS; hazard ratios (HR) with associated 95% CIs were accordingly generated, statistical significance was defined as a p-value less than 0.05 for all analyses. All statistical analyses were performed using the IBM SPSS Statistics for Macintosh, Version 27.0 (NY, USA).

Results

Baseline characteristics

Supplementary Figure 1A provides a flowchart for patient selection within the current study. The initial cohort had 1,294,632 patients who were diagnosed with CRC between 2004–2016. Patients were excluded if they were diagnosed with CRC before 2010 ($n = 590,294$), who didn't have radical surgical resection ($n = 593$), and patients who lost the follow-up for OS ($n = 16,698$). We then excluded patients who didn't have pathologic stage II (defined as T3 or T4 with N0) or stage III (defined as T1–4 with N1 or N2; $n = 404,245$) and patients who had a clinical metastatic disease or unknown clinical metastatic status ($n = 6998$). Next, we excluded all patients who didn't have a known MSI status ($n = 215,066$), which left us with 60,738 patients who had MSI known status stage II and stage III CRC.

A total of 30,436 patients had stage II CRC; 7078 (23.26%) were MSI-high and 23,358 (76.74%) were MSI-S. The baseline characteristics' comparison between MSI-H and MSI-S patients is summarized in [Table 1](#).

A total of 30,302 patients had stage III CRC; 5442 (17.96%) were MSI-high and 24,860 (82.04%) were MSI-S. The baseline characteristics' comparison between MSI-high and MSI-S patients is summarized in [Table 2](#).

Survival outcomes

MSI-H versus MSI-S

We initially split the entire cohort that included 60,738 patients into two groups; stage II CRC that has 30,436 (50.11%) patients and stage III CRC that has 30,302 (49.89%) patients. We compared the OS between MSI-H and MSI-S and found no statistically significant difference in the OS between MSI-H and MSI-S patients in stage II CRC (mean OS 72.8 vs 71.9 months; $p = 0.323$). Whereas in stage III CRC, MSI-S had better OS than MSI-H (mean OS 65.1 vs 59.8 months; $p < 0.001$) as illustrated in the Kaplan–Meier survival curves ([Supplementary Figures 1A & B](#)).

Subgroup analysis

We initially deleted the cases that had unknown chemotherapy status. In the stage II group, we deleted 1157 patients and ended up with 29,281 patients. In the stage III group, we deleted 822 patients and ended up with 29,480 patients.

Stage II CRC

We initially split the stage II CRC that included 29,281 patients into two groups according to MSI status; stage II CRC MSI-H that has 6796 (23.21%) patients and stage II CRC MSI-S that has 22,485 (76.79%) patients and compared the OS between patients who received chemotherapy and patients who didn't receive chemotherapy in each MSI group.

Table 1. Baseline characteristics of stage II colorectal cancer patients according to MSI status as found in the National Cancer Database between 2010 and 2016.

Characteristics	All cases (n = 30,436)	MSI-high (n = 7078) (23.26%)	MSI-S (n = 23,358) (76.74%)	p-value and test
Mean age in years	66.91 ± 13.92	68.28 ± 14.77	66.49 ± 13.62	<0.001; t-test
Sex				<0.001; Chi-square
– Male	15,369 (50.5%)	3077 (43.5%)	12,292 (52.6%)	
– Female	15,067 (49.5%)	4001 (56.5%)	11,066 (47.4%)	
Race				<0.001; Chi-square
– White	25,730 (75.6%)	6270 (88.6%)	19,460 (83.3%)	
– African American	3145 (10.3%)	517 (7.3%)	2628 (11.3%)	
– Others	1342 (4.4%)	239 (3.4%)	1103 (4.7%)	
– Unknown	219 (0.7%)	52 (0.7%)	167 (0.7%)	
Facility type				0.375; Chi-square
– Community cancer program	2458 (8.3%)	596 (8.8%)	1862 (8.2%)	
– Comprehensive Community Cancer Program	12,123 (41.1%)	2763 (40.8%)	9360 (41.2%)	
– Academic/Research Program	9721 (33.0%)	2250 (33.2%)	7471 (32.9%)	
– Integrated Network Cancer Program	5188 (17.6%)	1171 (17.3%)	4017 (17.7%)	
Median household income 2012–2016				<0.001; Chi-square
– Unknown	351 (1.2%)	65 (0.9%)	286 (1.2%)	
– Less than US\$40,227	5110 (16.8%)	1027 (14.5%)	4083 (17.5%)	
– \$40,227–US\$50,353	6449 (21.2%)	1514 (21.4%)	4935 (21.1%)	
– US\$50,354–US\$63,332	7152 (23.5%)	1727 (24.4%)	5425 (23.2%)	
– US\$63,333+	11,374 (37.4%)	2745 (38.8%)	8629 (36.9%)	
Education level 2012–2016 (percentage of not graduated from high school)				<0.001; Chi-square
– Unknown	302 (1.0%)	55 (0.8%)	247 (1.1%)	
– 17.6% or more	5686 (18.7%)	1185 (16.7%)	4501 (19.3%)	
– 10.9%–17.5%	7510 (24.7%)	1663 (23.5%)	5847 (25.0%)	
– 6.3%–10.8%	8734 (28.7%)	2146 (30.3%)	6588 (28.2%)	
– Less than 6.3%	8204 (27.0%)	2029 (28.7%)	6175 (26.4%)	
Insurance status				0.249; Chi-square
– Yes	29,089 (95.6%)	6790 (95.9%)	22,299 (95.6%)	
– No	1031 (3.4%)	221 (3.2%)	810 (3.5%)	
– Unknown	316 (1.0%)	67 (0.9%)	249 (1.1%)	
Area				0.418; Chi-square
– Metro counties	25,553 (84.0%)	5944 (84.0%)	19609 (83.9%)	
– Rural counties	487 (1.6%)	110 (1.6%)	377 (1.6%)	
– Urban counties	3670 (12.1%)	872 (12.3%)	2798 (12.0%)	
– Unknown	726 (2.4%)	152 (2.1%)	574 (2.5%)	
Pathologic T stage				0.063; Chi-square
– T3	25,865 (85.0%)	5966 (84.3%)	19,899 (85.2%)	
– T4	4571 (15.0%)	1112 (15.7%)	3459 (14.8%)	
Primary site				<0.001; Chi-square
– L colon	13,238 (43.5%)	1710 (24.2%)	11,528 (49.4%)	
– R colon	16,479 (54.1%)	5176 (73.1%)	11,303 (48.4%)	
– Unknown	719 (2.4%)	192 (2.7%)	527 (2.3%)	
Regional lymph node examined				<0.001; Chi-square
– 12 or more	27,847 (91.5%)	6683 (94.4%)	21,164 (90.6%)	
– <12	2549 (8.4%)	388 (5.5%)	2161 (9.3%)	
– Unknown	40 (0.1%)	7 (0.1%)	33 (0.1%)	
Lymph-vascular invasion				<0.001; Chi-square
MSI: Microsatellite instability.				

Table 1. Baseline characteristics of stage II colorectal cancer patients according to MSI status as found in the National Cancer Database between 2010 and 2016 (cont.).

Characteristics	All cases (n = 30,436)	MSI-high (n = 7078) (23.26%)	MSI-S (n = 23,358) (76.74%)	p-value and test
– No	24,394 (80.1%)	5531 (78.1%)	18,863 (80.8%)	
– Yes	4279 (14.1%)	1149 (16.2%)	3130 (13.4%)	
– Unknown	1763 (5.8%)	398 (5.6%)	1365 (5.8%)	
Perineural invasion				<0.001; Chi-square
– Yes	2387 (7.8%)	464 (6.6%)	1923 (8.2%)	
– No	26,652 (87.6%)	6301 (89.0%)	20,351 (87.1%)	
– Unknown	1397 (4.6%)	313 (4.4%)	1084 (4.6%)	
Chemotherapy				<0.001; Chi-square
– Yes	7891 (25.9%)	1262 (17.8%)	6629 (28.4%)	
– No	21,390 (70.3%)	5534 (78.2%)	15,856 (67.9%)	
– Unknown	1155 (3.8%)	282 (4.0%)	873 (3.7%)	
Charlson–Deyo score				0.003; Chi-square
– 0	20,531 (67.5%)	4676 (66.1%)	15,855 (67.9%)	
– 1	6924 (22.7%)	1633 (23.1%)	5291 (22.7%)	
– 2	1993 (6.5%)	515 (7.3%)	1478 (6.3%)	
– 3	988 (3.2%)	254 (3.6%)	734 (3.1%)	

MSI: Microsatellite instability.

We found that patients who received chemotherapy had a better OS than those who didn't receive chemotherapy in both groups as illustrated in the Kaplan–Meier survival curves (Figure 1A & B).

Stage II CRC with high-risk features (pathological T4, examined lymph node <12, lymphatic/vascular invasion or perineural invasion)

We initially split the stage II CRC with high-risk features group that included 10,445 patients into two groups according to MSI status; stage II CRC with high-risk features and MSI-H that has 2364 (22.63%) patients and stage II CRC with high-risk features and MSI-S that has 8081 (77.37%) patients and compared the OS between patients who received chemotherapy and patients who didn't receive chemotherapy in each MSI group.

We found that patients who received chemotherapy had better OS than those who didn't receive chemotherapy in both groups as illustrated in the Kaplan–Meier survival curves ($p < 0.001$ for all; Figure 2A & B).

In stage II CRC with MSI-H and high-risk features, propensity score matching yielded 1458 patients for analysis: 729 patients in the no chemotherapy group and 729 patients in the chemotherapy group (Supplementary Figure 1B shows the histogram for raw data and matched data). Baseline characteristics comparison between the groups after propensity score matching is shown in Supplementary Table 1A.

We compared the OS between patients who received chemotherapy and patients who didn't receive chemotherapy using Kaplan–Meier analysis on the propensity score-matched groups and found that patients who received chemotherapy had better OS than those who didn't receive chemotherapy as illustrated in the Kaplan–Meier survival curves ($p = 0.002$; Figure 3).

In another subgroup analysis, we split the stage II CRC with high-risk features group into two groups according to the chemotherapy status; stage II CRC with high-risk features who received chemotherapy and stage II CRC with high-risk features who didn't receive chemotherapy. Then, we compared the OS between patients with MSI-H and patients with MSI-S in each group using Kaplan–Meier analysis on the propensity score-matched groups and found no statistically significant difference in the OS in both groups as illustrated in the Kaplan–Meier survival curves.

Also, we found that patients with MSI-H who received chemotherapy had better OS compared with patients with MSI-S who didn't receive chemotherapy, and patients with MSI-S who received chemotherapy had better OS compared with patients with MSI-H who didn't receive chemotherapy as illustrated in the Kaplan–Meier survival curves (Supplementary Figure 2).

Table 2. Baseline characteristics of stage III colorectal cancer patients according to MSI status as found in the National Cancer Database between 2010 and 2016.

Characteristics	All cases (n = 30,302)	MSI-high (n = 5442) (17.96%)	MSI-stable (n = 24,860) (82.04%)	p-value and test
Mean age in years	63.60 ± 14.61	66.49 ± 15.53	62.97 ± 14.33	<0.001 t-test
Sex				<0.001; Chi-square
– Male	15,398 (50.8%)	2432 (44.7%)	12,966 (52.2%)	
– Female	14,904 (49.2%)	3010 (55.3%)	11,894 (47.8%)	
Race				<0.001; Chi-square
– White	24,950 (82.3%)	4641 (85.3%)	20,309 (81.7%)	
– African American	3531 (11.7%)	537 (9.9%)	2994 (12.0%)	
– Others	1588 (5.2%)	217 (4.0%)	1371 (5.5%)	
– Unknown	233 (0.8%)	47 (0.9%)	186 (0.7%)	
Facility type				0.001; Chi-square
– Community cancer program	2425 (8.5%)	485 (9.5%)	1940 (8.2%)	
– Comprehensive Community Cancer Program	11,420 (39.8%)	2072 (40.5%)	9348 (39.7%)	
– Academic/Research Program	9771 (34.1%)	1730 (33.8%)	8041 (34.1%)	
– Integrated Network Cancer Program	5068 (17.7%)	828 (16.2%)	4240 (18.0%)	
Median household income 2012–2016				<0.063; Chi-square
– Unknown	377 (1.2%)	58 (1.1%)	319 (1.3%)	
– Less than US\$40,227	5310 (17.5%)	902 (16.6%)	4408 (17.7%)	
– US\$40,227–US\$50,353	6298 (20.8%)	1161 (21.3%)	5137 (20.7%)	
– US\$50,354–US\$63,332	7137 (23.6%)	1334 (24.5%)	5803 (23.3%)	
– US\$63,333+	11,180 (36.9%)	1987 (33.5%)	9193 (37.0%)	
Education level 2012–2016 (percentage of not graduated from high school)				0.061; Chi-square
– Unknown	327 (1.1%)	51 (0.9%)	276 (1.1%)	
– 17.6% or more	5801 (19.1%)	995 (18.3%)	4806 (19.3%)	
– 10.9%–17.5%	7555 (24.9%)	1320 (24.3%)	6235 (25.1%)	
– 6.3%–10.8%	8661 (28.6%)	1662 (29.8%)	7039 (28.3%)	
– Less than 6.3%	7958 (26.3%)	1454 (26.7%)	6504 (26.2%)	
Insurance status				0.073; Chi-square
– Yes	28,915 (95.4%)	5166 (94.9%)	23,749 (95.5%)	
– No	1063 (3.5%)	219 (4.1%)	844 (3.4%)	
– Unknown	324 (1.1%)	57 (1.0%)	267 (1.1%)	
Area				0.730; Chi-square
– Metro counties	25,373 (83.7%)	4570 (84.1%)	20,803 (83.7%)	
– Rural counties	493 (1.6%)	89 (1.6%)	404 (1.6%)	
– Urban counties	3721 (12.3%)	666 (12.2%)	3055 (12.3%)	
– Unknown	715 (2.4%)	117 (2.1%)	598 (2.4%)	
Pathologic T stage				<0.001; Chi-square
– T1	1222 (4.0%)	170 (3.1%)	1052 (4.2%)	
– T2	3412 (11.3%)	468 (8.6%)	2944 (11.8%)	
– T3	19,342 (63.8%)	3401 (62.5%)	15,941 (64.1%)	
– T4	6326 (20.9%)	1403 (25.8%)	4923 (19.8%)	
Pathologic N stage				<0.001; Chi-square
– N1	20,290 (67.0%)	3509 (64.5%)	16,781 (67.5%)	
– N2	10,012 (33.0%)	1933 (35.5%)	8079 (32.5%)	
Primary site				<0.001; Chi-square
– L colon	15,739 (51.9%)	1621 (29.8%)	14,118 (56.8%)	
– R colon	13,904 (45.9%)	3666 (67.4%)	10,238 (41.2%)	
– Unknown	659 (2.2%)	155 (2.8%)	504 (2.0%)	

MSI: Microsatellite instability.

Table 2. Baseline characteristics of stage III colorectal cancer patients according to MSI status as found in the National Cancer Database between 2010 and 2016 (cont.).

Characteristics	All cases (n = 30,302)	MSI-high (n = 5442) (17.96%)	MSI-stable (n = 24,860) (82.04%)	p-value and test
Regional lymph node examined				<0.001; Chi-square
– 12 or more	27,846 (91.9%)	5148 (94.6%)	22,698 (91.3%)	
– <12	2414 (8.0%)	288 (5.3%)	2126 (8.6%)	
– Unknown	42 (0.1%)	6 (0.1%)	36 (0.1%)	
Lymph-vascular invasion				<0.001; Chi-square
– No	13,708 (45.2%)	2251 (41.1%)	11,457 (46.1%)	
– Yes	14,265 (47.1%)	2837 (52.12%)	11,428 (46.0%)	
– Unknown	2329 (7.7%)	354 (6.5%)	1975 (7.9%)	
Perineural invasion				0.003; Chi-square
– Yes	6186 (20.4%)	1036 (19.0%)	5150 (20.7%)	
– No	22,575 (74.5%)	4096 (75.3%)	18,479 (74.3%)	
– Unknown	1541 (5.1%)	310 (5.7%)	1231 (5.0%)	
Chemotherapy				<0.001; Chi-square
– Yes	22,703 (74.9%)	3732 (68.6%)	18,971 (76.3%)	
– No	6777 (22.4%)	1537 (28.2%)	5240 (21.1%)	
– Unknown	822 (2.7%)	173 (3.2%)	649 (2.6%)	
Charlson–Deyo score				0.001; Chi-square
– 0	21,364 (70.5%)	3735 (68.6%)	17,629 (70.9%)	
– 1	6302 (20.8%)	1165 (21.4%)	5137 (20.7%)	
– 2	1794 (5.9%)	361 (6.6%)	1433 (5.8%)	
– 3	842 (2.8%)	181 (3.3%)	661 (2.7%)	

MSI: Microsatellite instability.

Stage II CRC with highest risk features (pathologic T4 or examined lymph node <12)

We initially split the stage II CRC with highest risk features group that included 6491 patients into two groups according to MSI status; stage II CRC with highest risk features and MSI-H that has 1375 (21.18%) patients and stage II CRC with highest risk features and MSI-S that has 5116 (78.82%) patients and compared the OS between patients who received chemotherapy and patients who didn't receive chemotherapy in each MSI group.

We found that patients who received chemotherapy had better OS than those who didn't receive chemotherapy in both groups as illustrated in the Kaplan–Meier survival curves ($p < 0.001$ for all; [Supplementary Figures 3A & B](#)).

Stage II CRC without high-risk features

We initially split the stage II CRC without high-risk features group that included 17,141 patients into two subgroups according to MSI status; stage II CRC without high-risk features and MSI-high that has 4027 (23.49%) patients and stage II CRC without high-risk features and MSI-S that has 13,114 (76.51%) patients and compared the OS between patients who received chemotherapy and patients who didn't receive chemotherapy in each MSI group.

We found that patients who received chemotherapy had better OS than those who didn't receive chemotherapy in both groups as illustrated in the Kaplan–Meier survival curves ($p < 0.001$ for all; [Figure 4A & B](#)).

In stage II CRC with MSI-H and without high-risk features, propensity score matching yielded 966 patients for analysis: 483 patients in the no chemotherapy group and 483 patients in the chemotherapy group ([Supplementary Figure 3C](#) shows the histogram for raw data and matched data). Baseline characteristics comparison between the groups after propensity score matching is shown in [Supplementary Table 1B](#).

We compared the OS between patients who received chemotherapy and patients who didn't receive chemotherapy using Kaplan–Meier analysis on the propensity score-matched groups and found no statistically significant difference in the OS between patients who received chemotherapy and those who didn't receive chemotherapy as illustrated in the Kaplan–Meier survival curves ($p = 0.834$; [Figure 5A](#)).

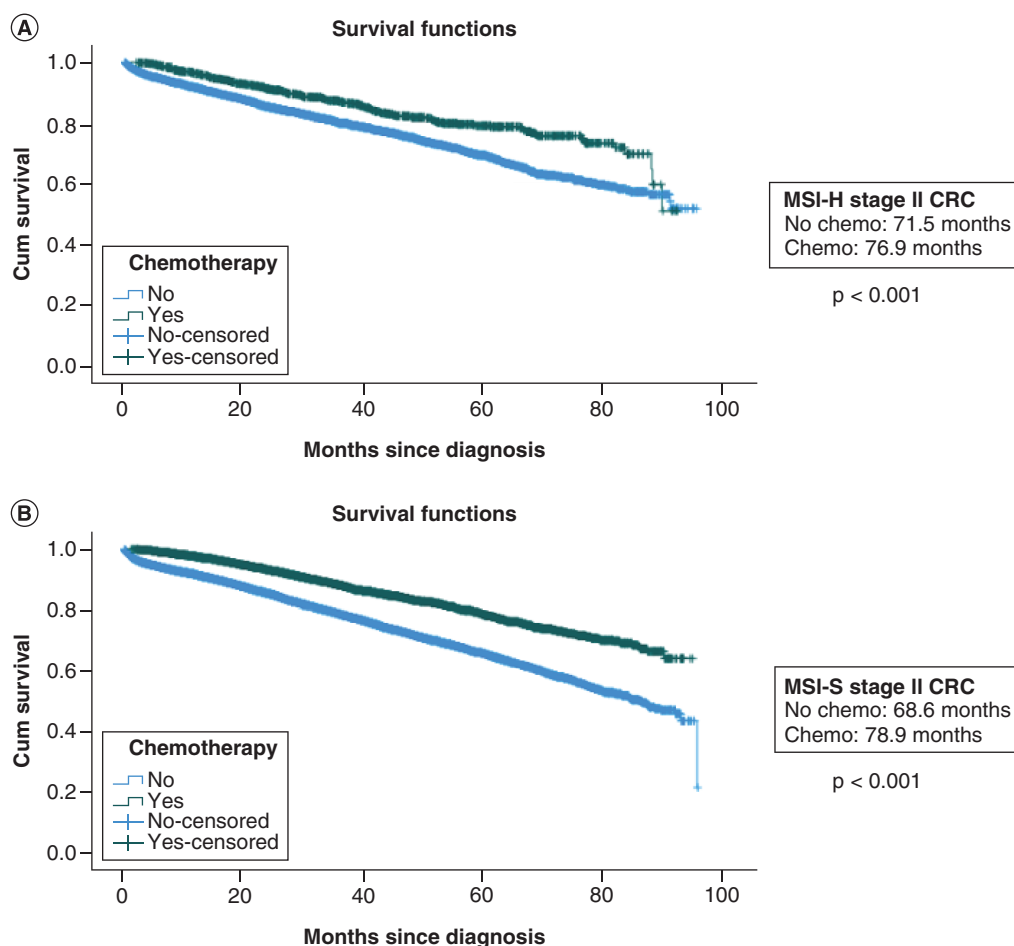


Figure 1. Overall survival in (A) MSI-H and (B) MSI-S stage II CRC patients according to chemotherapy. CRC: Colorectal cancer; MSI: Microsatellite instability; NCDB: National Cancer Database.

In stage II CRC with MSI-S and without high-risk features, propensity score matching yielded 5476 patients for analysis: 2738 patients in the no chemotherapy group and 2738 patients in the chemotherapy group (Supplementary Figure 3D shows the histogram for raw data and matched data). Baseline characteristics comparison between the groups after propensity score matching is shown in Supplementary Table 1C.

We compared the OS between patients who received chemotherapy and patients who didn't receive chemotherapy using Kaplan–Meier analysis on the propensity score-matched groups and found that patients who received chemotherapy had better OS than those who didn't receive chemotherapy as illustrated in the Kaplan–Meier survival curves ($p < 0.001$; Figure 5B).

In another subgroup analysis, we split the stage II CRC without high-risk features group into two groups according to the chemotherapy status; stage II CRC without high-risk features who received chemotherapy and stage II CRC without high-risk features who didn't receive chemotherapy. Then, we compared the OS between patients with MSI-H and patients with MSI-S in each group using Kaplan–Meier analysis on the propensity score-matched groups and found patients with MSI-L had better OS compared with patients with MSI-H in the group that didn't receive chemotherapy, and no statistically significant difference in the OS between MSI-H and MSI-S patients in the group that received chemotherapy as illustrated in the Kaplan–Meier survival curves.

Also, we found that patients with MSI-S who received chemotherapy had better OS compared with patients with MSI-H who didn't receive chemotherapy, and no statistically significant difference in the OS between patients with MSI-H who received chemotherapy and patients with MSI-S who didn't receive chemotherapy as illustrated in the Kaplan–Meier survival curves (Supplementary Figure 4).

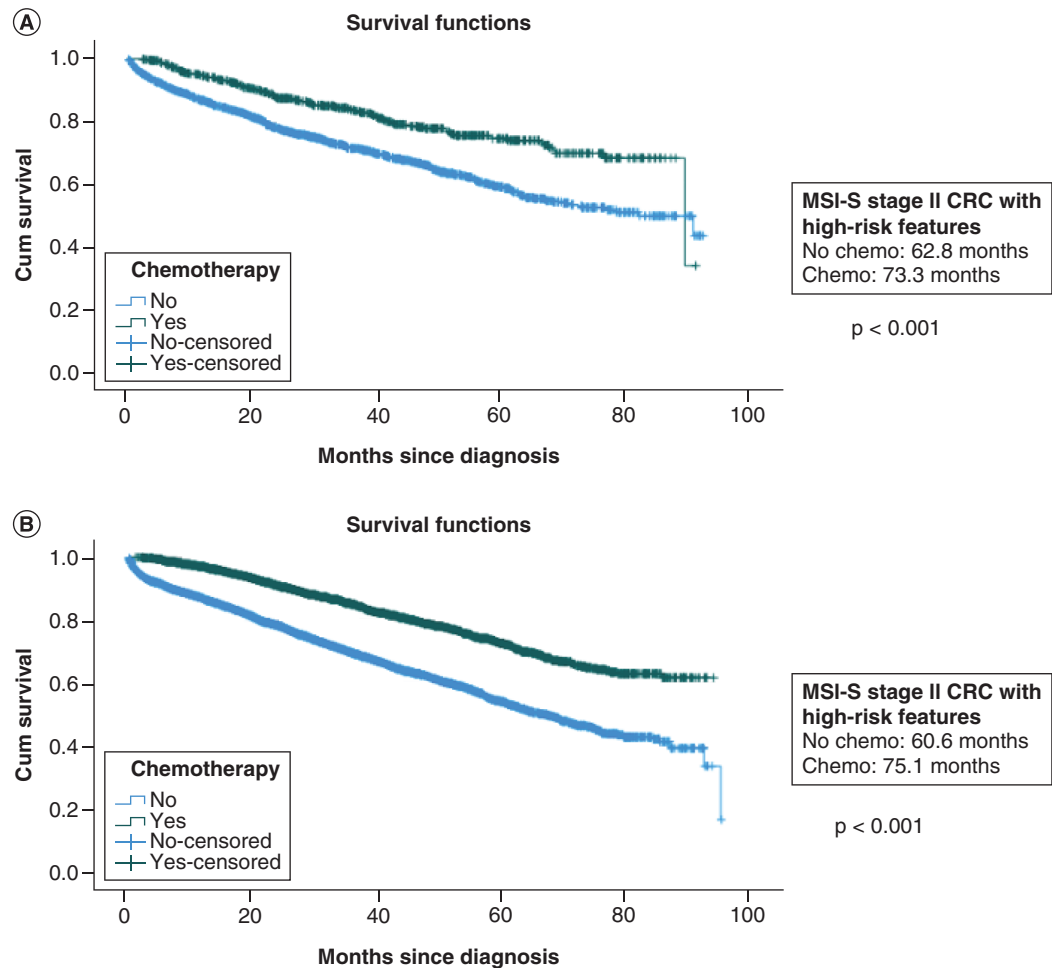


Figure 2. Overall survival in (A) MSI-H and (B) MSI-S stage II CRC patients with high-risk features according to chemotherapy.

CRC: Colorectal cancer; MSI: Microsatellite instability; NCDB: National Cancer Database.

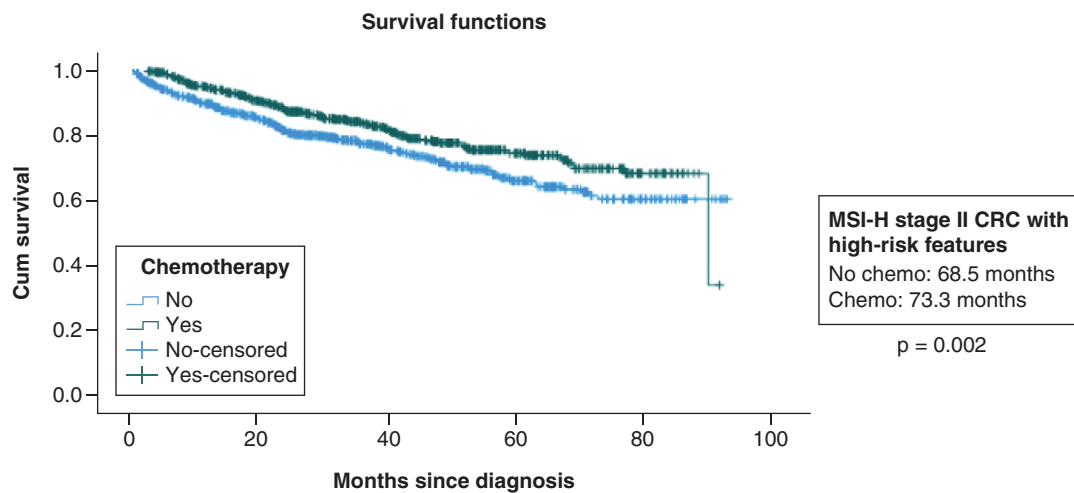


Figure 3. Overall survival in MSI high stage II CRC patients with high-risk features according to chemotherapy in matched groups (after propensity score matching).

CRC: Colorectal cancer; MSI: Microsatellite instability; NCDB: National Cancer Database.

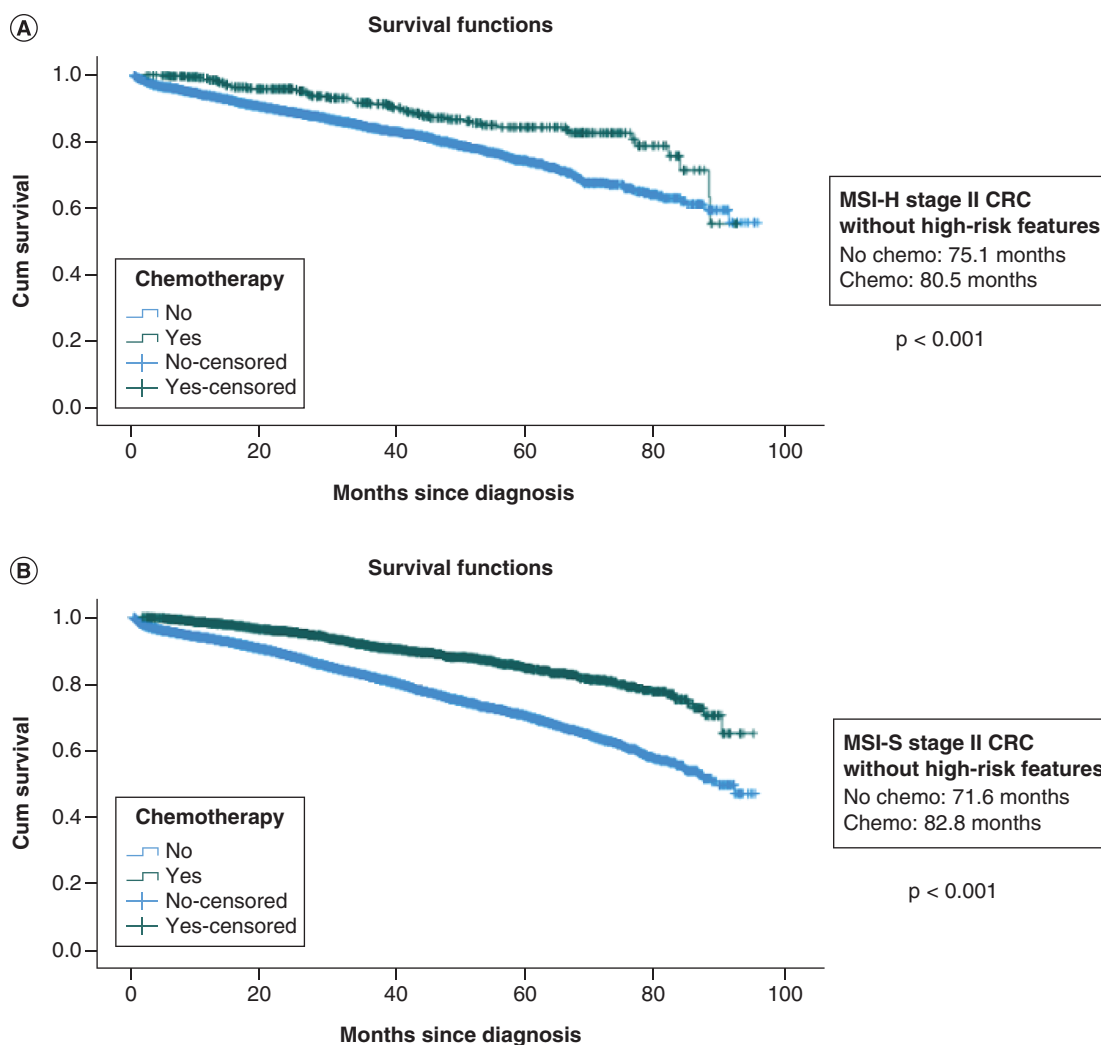


Figure 4. Overall survival in (A) MSI-high and (B) MSI-stable stage II CRC patients without high-risk features according to chemotherapy.

CRC: Colorectal cancer; MSI: Microsatellite instability; NCDB: National Cancer Database.

Stage III CRC with MSI-H

We initially split the stage III CRC with MSI-H group that included 5269 patients into two groups; stage III CRC low-risk and MSI-H that has 2647 (50.24%) patients and stage III CRC with high-risk features and MSI-H that has 2622 (49.76%) patients and compared the OS between patients who received chemotherapy and patients who didn't receive chemotherapy in each group.

We found that patients who received chemotherapy had a better OS than those who didn't receive chemotherapy in both groups as illustrated in the Kaplan–Meier survival curves ($p < 0.001$ for all; [Supplementary Figure 5A & B](#)).

Multivariate analysis

Multivariate analysis was done to assess factors affecting OS in the MSI known status patient group. We initially did the MVA on the entire cohort that included 60,738 patients who had stage II and III CRC. We found that MSI-H status didn't have a significant difference in the OS compared with MSI-S status ([Supplementary Table 1D](#)).

Then, we split the entire cohort that included 60,738 patients into two groups; stage II CRC that has 30,436 (50.11%) patients and stage III CRC that has 30,302 (49.89%) patients.

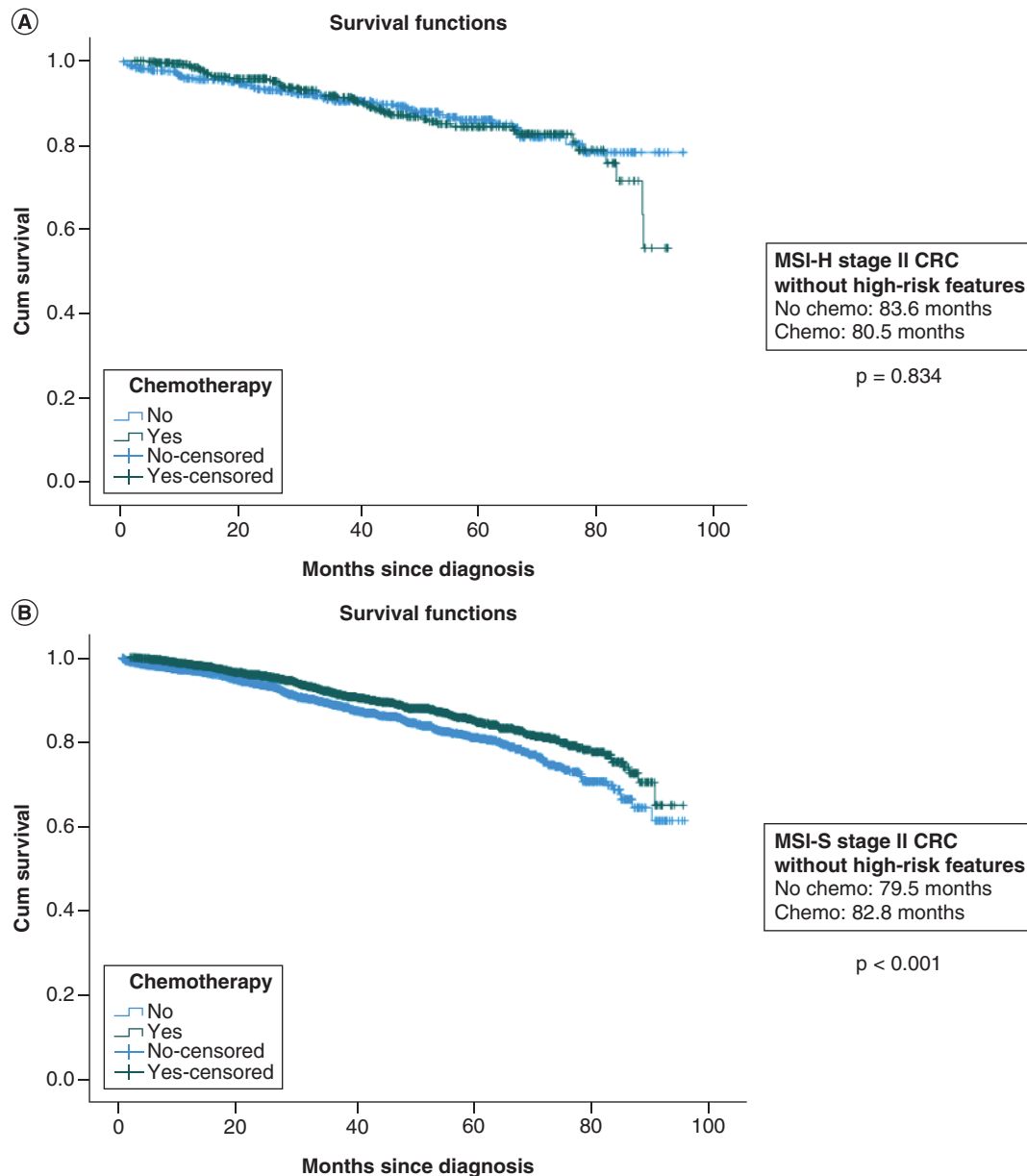


Figure 5. Overall survival in (A) MSI-high and (B) MSI-stable stage II CRC patients without high-risk features according to chemotherapy in matched groups (after propensity score matching).

CRC: Colorectal cancer; MSI: Microsatellite instability; NCCDB: National Cancer Database.

In the stage II CRC group, factors associated with worse OS were age (HR: 1.056; 95% CI: 1.054–1.059; 95% CI: $p < 0.001$), no health insurance (HR: 1.290; 95% CI: 1.091–1.527; $p = 0.003$), L side colon (HR: 1.156, 95% CI: 1.096–1.220, compared with 0.001), lower income levels (HR: 1.293; 95% CI: 1.174–1.424; $p < 0.001$ and HR: 1.191; 95% CI: 1.099–1.390; $p < 0.001$), not receiving chemotherapy (HR: 1.247; 95% CI: 1.160–1.340; $p < 0.001$), male gender (HR for female vs male 0.80; 95% CI: 0.761–0.841; $p < 0.001$), Charlson–Deyo score 3 (HR for score 0, 1 and 2 compared with score 3 was 0.434; 95% CI: 0.393–0.480, HR: 0.543; 95% CI: 0.489–0.603, and HR: 0.702; 95% CI: 0.623–0.791, respectively with $p < 0.001$ for all), poorly differentiated tumor (HR: 1.135; 95% CI: 1.062–1.212; $p < 0.001$), rectal cancer (HR: 1.230; 95% CI: 1.133–1.335; $p < 0.001$), pathologic T4 (HR: for T3 compared with T4 0.547; 95% CI: 0.514–0.582; $p < 0.001$), less than 12 regional lymph node examined (HR: for 12 or more regional lymph node examined compared with less than 12 was 0.669; 95% CI: 0.621–0.720; $p < 0.001$), lymphatic-vascular invasion (HR for no lymphatic-vascular invasion

compared with lymphatic-vascular invasion 0.846; 95% CI: 0.790–0.906; $p < 0.001$), perineural invasion (HR for no perineural invasion compared with perineural invasion 0.815; 95% CI: 0.748–0.887; $p < 0.001$), MSI-S status (HR MSI-H compared with MSI-S 0.924; 95% CI: 0.870–0.981; $p = 0.009$; Table 3).

In the stage III CRC group, factors associated with worse OS were age (HR: 1.026; 95% CI: 1.024–1.028; $p < 0.001$), no health insurance (HR: 1.149; 95% CI: 1.011–1.307; $p = 0.034$), lower income levels (HR: 1.193; 95% CI: 1.099–1.296; $p < 0.001$), higher education level (HR: 1.089; 95% CI: 1.013–1.170; $p = 0.020$ and HR: 1.123; 95% CI: 1.056–1.193; $p < 0.001$) and not receiving chemotherapy (HR: 2.664; 95% CI: 2.534–2.801; $p < 0.001$), male gender (HR: for female vs male 0.803; 95% CI: 0.769–0.838; $p < 0.001$), Charlson–Deyo score 3 (HR: for score 0, 1 and 2 compared with score 3 was 0.536; 95% CI: 0.486–0.592, HR: 0.628; 95% CI: 0.566–0.696 $p < 0.001$, and HR: 0.821; 95% CI: 0.731–0.922; $p = 0.001$, respectively), poorly differentiated tumor (HR: 1.312; 95% CI: 1.252–1.376; $p < 0.001$), rectal cancer (HR: 1.203; 95% CI: 1.128–1.283; $p < 0.001$), pathologic T4 (T1, T2 and T3 compared with T4 was HR: 0.296; 95% CI: 0.251–0.350, HR: 0.415; 95% CI: 0.380–0.454, and HR: 0.574; 95% CI: 0.548–0.602, respectively with $p < 0.001$ for all), pathologic N2 (N1 compared with N2 HR: 0.594; 95% CI: 0.568–0.621; $p < 0.001$), less than 12 regional lymph node examined (HR for 12 or more regional lymph node examined compared with less than 12 was 0.613; 95% CI: 0.572–0.656; $p < 0.001$), lymphatic-vascular invasion (HR for no lymphatic-vascular invasion compared with lymphatic-vascular invasion 0.812; 95% CI: 0.775–0.851; $p < 0.001$), perineural invasion (HR for no perineural invasion compared with perineural invasion 0.787; 95% CI: 0.748–0.829; $p < 0.001$), R side colon (HR for L side compared with R side 0.952; 95% CI: 0.910–0.997; $p = 0.035$). we also found that MSI-H status didn't have a significantly different survival compared with MSI-S patients (HR: 1.041; 95% CI: 0.988–1.098; $p = 0.135$; Table 4).

Discussion

To our knowledge, this is the largest and most detailed study that compared the OS between MSI-H and MSI-S CRC and evaluated the effect of adjuvant chemotherapy on OS in non-metastatic CRC based on MSI status and risk features. Our study shows that chemotherapy improves the OS in stage II CRC with MSI-H and MSI-S. When stratified according to risk features, we found that chemotherapy improves the OS in both MSI-H and MSI-S stage II CRC with high-risk features. Whereas in stage II CRC without high-risk features, chemotherapy only improves the OS in patients with MSI-S but not with MSI-H. Our study also shows that chemotherapy improves the OS in MSI-H stage III CRC with high-risk and low-risk features.

Also, in the multivariate analysis, we found that MSI-H status was not associated with better OS compared with MSI-S in the combined group that had stage II and stage III CRC. But when we analyzed each stage separately, we found that MSI-H status was associated with better OS compared with MSI-S in stage II CRC, but it didn't affect the OS in stage III CRC.

The prognostic effect of MSI status on OS

Despite many studies, the prognostic effect of MSI status in CRC is still controversial and not consistent. While the mechanism in which MSI status affects the CRC prognosis is not well understood, some studies suggest that MSI-H CRC tumors are associated with greater immune response compared with MSI-S tumors [15,16], which results in increasing the apoptosis and reducing cell proliferation by increasing the tumor-infiltrating cytotoxic lymphocytes [17–19]. Lamberti *et al.* did a multivariate analysis to assess the effect of MSI status on OS in a retrospective study of 416 CRC cases and found no correlation between MSI status and OS in the whole cohort and in early-stage or locally advanced cases (stage I, II and III combined) [9]. These findings are consistent with our results that showed no difference in the OS between MSI-H and MSI-S in stage II and III cases combined.

In contrary to our results, Popat *et al.* in a meta-analysis found MSI-H CRC had better OS than MSI-S cases when the analysis was done on stage II and III CRC cases combined, there was no analysis done on each stage separately to determine the effect of MSI status in each stage [10].

Benatti *et al.* assessed the difference in OS based on MSI status in 1,263 CRC cases that included all stages, found the difference in OS only in stage II and III with MSI-H cases had better OS than MSI-S ones [20]. These results align with our results except that in our study we found MSI-H status to be associated with better OS only in stage II but not in stage III.

Table 3. Multivariate analysis for MSI known status stage II colorectal cancer patients.

Variables	HR	95% CI	p-value
Facility type			
– Community cancer program	1.042	0.941–1.154	0.432
– Comprehensive Community Cancer Program	0.983	0.917–1.053	0.617
– Academic/Research Program	0.917	0.853–0.987	0.021
– Integrated Network Cancer Program	Reference		
Age	1.056	1.054–1.059	<0.001
Gender			
– Female	0.800	0.761–0.841	<0.001
– Male	Reference		
Race			
– Unknown	0.797	0.579–1.098	0.165
– African American	1.079	0.987–1.179	0.060
– Others	0.738	0.630–0.865	<0.001
– White	Reference		
Median household income 2012–2016			
Unknown	0.686	0.307–1.532	0.358
– Less than US\$40,227	1.293	1.174–1.424	<0.001
– US\$40,227–US\$50,353	1.191	1.099–1.390	<0.001
– US\$50,354–US\$63,332	1.060	0.986–1.139	0.114
– US\$63,333+	Reference		
Education level 2012–2016 (percentage of not graduated from high school)			
– Unknown	1.682	0.727–3.892	0.224
– 17.6% or more	0.942	0.854–1.039	0.229
– 10.9%–17.5%	1.019	0.939–1.107	0.649
– 6.3%–10.8%	1.056	0.983–1.133	0.135
– Less than 6.3%	Reference		
Insurance status			
– No	1.290	1.091–1.527	0.003
– Unknown	1.166	0.909–1.495	0.227
– Yes	Reference		
Area			
– Unknown	0.986	0.829–1.174	0.877
– Metro counties	0.932	0.863–1.006	0.072
– Rural counties	0.915	0.746–1.122	0.394
– Urban counties	Reference		
Charlson score			
– 0	0.434	0.393–0.480	<0.001
– 1	0.543	0.489–0.603	<0.001
– 2	0.702	0.623–0.791	<0.001
– 3	Reference		
TNM clinical stage			
Pathologic T stage			
– T3	0.547	0.514–0.582	<0.001
– T4	Reference		
Regional lymph node examined			
– Unknown	0.947	0.491–1.829	0.872
– 12 or more	0.669	0.621–0.720	<0.001
– <12	Reference		
Primary site			
– Unknown	1.097	0.940–1.279	0.241

HR: Hazard ratio; MSI: Microsatellite instability.

Table 3. Multivariate analysis for MSI known status stage II colorectal cancer patients (cont.).

Variables	HR	95% CI	p-value
– L colon	1.156	1.096–1.220	<0.001
– R colon	Reference		
Lymph-vascular invasion			
– No	0.846	0.790–0.906	<0.001
– Unknown	0.931	0.828–1.047	0.231
– Yes	Reference		
Perineural invasion			
– No	0.815	0.748–0.887	<0.001
– Unknown	0.865	0.753–0.995	0.042
– Yes	Reference		
MSI status			
– High	0.924	0.870–0.981	0.009
– Stable	Reference		
Chemotherapy			
– No	1.247	1.160–1.340	<0.001
– Unknown	1.026	0.885–1.189	0.737
– Yes	Reference		

HR: Hazard ratio; MSI: Microsatellite instability.

The effect of chemotherapy in MSI-H CRC & the predictive value of MSI status

Despite the growing data showing that stage II CRCs with MSI-H have a better prognosis compared with MSI-S, there's a lack of evidence that chemotherapy is effective in these cases which is reflected in the current recommendation of surgical management alone [6].

Different theories tried to explain why MSI-H CRC is less responsive to chemotherapy compared with MSI-S case. It's suggested that a proficient MMR system plays a significant role in the response of the malignant cells to chemotherapy. One theory suggests that a proficient MMR system facilitates the cell cycle arrest in response to 5-fluorouracil (5-FU). Another theory suggests that a proficient MMR system is crucial in detecting the damaged deoxyribonucleic acid (DNA) caused by some chemotherapeutic agents like cisplatin and carboplatin which leads to programmed cell death. So, in the absence of a proficient MMR system, it seems that tumor cells are more resistant to chemotherapy [21–24].

Kim *et al.* assessed the role of postsurgical resection chemotherapy in 738 patients with stage II colon cancer according to the MSI status, they found that patients with MSI-H status who received adjuvant chemotherapy had better OS than those who didn't. The same was reported in MSI-S cases [25]. These findings are consistent with our results that showed chemotherapy improved the OS in MSI-H and MSI-S stage II CRC. In our study, we did an extra step and further stratified our patient groups according to the presence of high-risk features into four subgroups: MSI-H with high-risk features, MSI-S with high-risk features, MSI-H without high-risk features and MSI-S without high-risk features. Our analysis showed that chemotherapy improved the OS in all subgroup patients, except in stage II CRC with MSI-H without high-risk features.

Other studies assessed the effect of chemotherapy based on MSI status on stage II and III as one group. Jover *et al.* in a retrospective study of 505 patients with stage II and III CRC, reported 5-FU based adjuvant chemotherapy improved the OS in MSI-S cases whereas it didn't affect the OS in MSI-H cases [26]. One meta-analysis showed similar results. Popat *et al.* did a meta-analysis to assess the effect of 5-FU based adjuvant chemotherapy in CRC (stage II and III combined cases) with MSI-H status and found no difference in the OS between patients who received chemotherapy and patients who didn't [10].

On the other hand, Sargent *et al.* assessed 457 patients with stage II and III colon cancer previously enrolled in 5 trials, found that 5-FU based adjuvant chemotherapy didn't affect the DFS in MSI-H cases compared with those randomly assigned to surgery alone. But In the pooled data set, patients with stage II disease and MSI-H, adjuvant chemotherapy was associated with reduced OS [27].

In addition to the controversial effect of chemotherapy in MSI-H CRC, there's growing controversy regarding the MSI status as a predictive value for adjuvant chemotherapy efficacy.

Table 4. Multivariate analysis for MSI known status stage III colorectal cancer patients.

Variables	HR	95% CI	p-Value
Facility type			
– Community cancer program	0.987	0.906–1.074	0.759
– Comprehensive Community Cancer Program	0.888	0.838–0.942	<0.001
– Academic/Research Program	0.818	0.818–0.769	<0.001
– Integrated Network Cancer Program	Reference		
Age	1.026	1.024–1.028	<0.001
Gender			
– Female	0.803	0.769–0.838	<0.001
– Male	Reference		
Race			
– Unknown	0.823	0.633–1.071	0.148
– African American	1.058	0.986–1.136	0.116
– Others	0.884	0.789–0.990	0.033
– White	Reference		
Median household income 2012–2016			
– Unknown	1.884	1.222–2.904	0.004
– Less than US\$40,227	1.193	1.099–1.296	<0.001
– US\$40,227–US\$50,353	1.048	0.977–1.125	0.189
– US\$50,354–US\$63,332	1.033	0.971–1.089	0.302
– US\$63,333+	Reference		
Education level 2012–2016 (percentage of not graduated from high school)			
– Unknown	0.551	0.341–0.890	0.015
– 17.6% or more	1.046	0.961–1.139	0.296
– 10.9%–17.5%	1.089	1.013–1.170	0.020
– 6.3%–10.8%	1.123	1.056–1.193	<0.001
– Less than 6.3%	Reference		
Insurance status			
– No	1.149	1.011–1.307	0.034
– Unknown	0.913	0.740–1.127	0.399
– Yes	Reference		
Area			
– Unknown	0.979	0.838–1.143	0.789
– Metro counties	0.957	0.896–1.021	0.184
– Rural counties	1.073	0.909–1.267	0.405
– Urban counties	Reference		
Charlson score			
– 0	0.536	0.486–0.592	<0.001
– 1	0.628	0.566–0.696	<0.001
– 2	0.821	0.731–0.922	0.001
– 3	Reference		
TNM clinical stage			
Pathologic T stage			
– T1	0.296	0.251–0.350	<0.001
– T2	0.415	0.380–0.454	<0.001
– T3	0.574	0.548–0.602	<0.001
– T4	Reference		
Pathologic N stage			
– N1	0.594	0.568–0.621	<0.001
– N2	Reference		
Regional lymph node examined			

MSI: Microsatellite instability.

Table 4. Multivariate analysis for MSI known status stage III colorectal cancer patients (cont.).

Variables	HR	95% CI	p-Value
– Unknown	1.103	0.691–1.761	0.680
– 12 or more	0.613	0.572–0.656	<0.001
– <12	Reference		
Primary site			
– Unknown	1.0985	0.955–1.234	0.211
– L colon	0.952	0.910–0.997	0.035
– R colon	Reference		
Lymph-vascular invasion			
– No	0.812	0.775–0.851	<0.001
– Unknown	0.856	0.795–0.942	0.001
– Yes	Reference		
Perineural invasion			
– No	0.787	0.748–0.829	<0.001
– Unknown	0.875	0.792–0.967	0.009
– Yes	Reference		
MSI status			
– High	1.041	0.988–1.098	0.135
– Stable	Reference		
Chemotherapy			
– No	2.664	2.534–2.801	<0.001
– Unknown	1.301	1.301–1.676	<0.001
– Yes	Reference		

MSI: Microsatellite instability.

Elsaleh *et al.* analyzed 656 stage III CRC cases out of which 272 cases received adjuvant chemotherapy (fluorouracil and levamisole) and found that MSI-H group had better OS than MSI-S group [28]. Whereas Watanabe *et al.* in a study that analyzed patients with stage III colon cancer treated with postoperative adjuvant 5-FU based chemo, who were enrolled in two randomized trials, found no significant difference in the OS between MSI-H and MSI-S in patients [29]. Also, Sargent *et al.* assessed 7803 stage II and III colon cancer patients who were enrolled in 17 trials and found no statistically significant difference in the OS between MSI-H and MSI-S cases who were treated with adjuvant 5-FU monotherapy [30].

Most of the studies that assessed the efficacy of chemotherapy in CRC based on MSI status, have grouped stage II and III together and few studies assessed each stage separately and none has stratified the cases according to the presence of high-risk features like in our study.

Our study draws attention to a very important and controversial topic regarding the role of the MSI status in the efficacy of chemotherapy in stage II CRC, which we believe deserves more research and studies to look at as it might make a changed in the current practice.

Limitations

The current study has some limitations that need to be accounted for; first, this is a retrospective study and thus interpretation of the results can be affected by different types of bias that is incurred by this type of study design. Second, information about the type of chemotherapy and performance status is not available. This is relevant as adjuvant single-agent fluoropyrimidines have been shown to be associated with worse outcomes among patients with MSI-H CRC [31]. Third, some relevant risk features for stage II are not available within NCDB datasets (e.g., preoperative obstruction or perforation). This means that some patients assigned to the group of no high-risk features might have unaccounted for risk features. Fourth, A limiting factor in our study is that the MSI status was reported as being high or low, but detailed information about the testing method and the criteria that was followed to decide who was tested was not available. Fifth, other biomarkers that are promising in this patient group such as circulating tumor DNA (ctDNA) is not provided. Also, the risk of recurrence and cancer-related death were available. In addition, the details whether patients had CRC as sporadic case or from potentially a

lynch syndrome is not provided in the NCDB. Other limitations of this study include the retrospective nature of NCDB and the ability to assess only OS but not cancer-specific survival. It is also notable that MSI-H patients were over-represented in the current dataset compared with the general population (in the majority of previous studies, MSI-H patients accounted for 10–15% of the studied population) [9,27,32,33]. This might have affected the results of the current analysis as well.

Conclusion

Our study shows that chemotherapy improves the OS in stage II CRC with MSI-H and MSI-S. When stratified according to risk features, we found that chemotherapy improves the OS in both MSI-H and MSI-S stage II CRC with high-risk features. Whereas in stage II CRC without high-risk features, chemotherapy only improves the OS in patients with MSI-S but not with MSI-H. Our study also shows that chemotherapy improves the OS in MSI-H stage III CRC with or without high-risk features.

Summary Points

- Colorectal cancer (CRC) is the third most common diagnosed malignancy and the second leading cause of cancer-related death in the USA.
- A total of 15% of CRC cases have microsatellite instability (MSI).
- The role of MSI status in CRC prognosis is not well-established.
- The benefit of chemotherapy in MSI-H stage II CRC is still controversial.
- We found that chemotherapy improved the overall survival in MSI-H stage II CRC with high-risk features.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/cer-2021-0013

Author contributions

F Baidoun: data analysis, data interpretation, manuscript writing. AM Saad: manuscript editing, critical revision. O Abdel-Rahman: study concept, manuscript editing, critical revision.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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