Clinicopathologic Features and Prognostic Value of DNA Mismatch Repair Statues for Stage Ⅱ/Ⅲ Colorectal Cancer in Northeast China.

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Research

Keywords: Immunohistochemistry, Carcinoma Embryonic Antigen

Posted Date: November 8th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1033588/v1

Abstract

Background

MSI CRCs were associated with better prognosis and limited predictive value for adjuvant chemotherapy. However, whether the same is true in Northeastern China is still unclear. The aim of the present study was to evaluate the association of clinicopathologic features and MMR/MSI status determined with immunohistochemistry analysis in Northeast China patients with stage Ⅱ/Ⅲ CRCs. Particularly, we sought to detect the relationship between MMR/MSI status and efficacy of oxaliplatin and fluoropyrimidine based adjuvant chemotherapy.

Methods

In total, 476 pathological specimens from eligible stage Ⅱ/Ⅲ CRCs were analyzed with IHC between 2016 and 2018, of which 63 CRCs were diagnosed with MMR protein deficiency. Clinicopathological features and overall survival (OS) were compared between these above two groups.

Result

The incidence of dMMR CRCs in our cohort was 13.2 % (63/476). Immunohistochemistry (IHC) revealed two common dMMR IHC patterns in 63 dMMR CRCs. And dMMR type1 showed a higher proportion of women (P=0.001) and earlier pathological N stage (P=0.075). In the multivariate Cox regression model, POC and dMMR were associated with a favor prognosis in CRC patients with stage II/III (HR 0.47, 95%CI 0.30-0.74, P=.001; HR 0.34, 95%CI 0.14-0.79, P=.013). However, adjuvant chemotherapy based on oxaliplatin and fluorouracil cannot prolong the OS of dMMR CRCs (P=0.182).

Conclusions

MMR protein appeared distinct associations with tumor staging, serum CEA level and tumor size. And MMR protein was an independent prognostic marker in patients with stage Ⅱ CRC, whereas dMMR CRC patients did not seem to benefit from oxaliplatin combined with fluorouracil-based adjuvant chemotherapy.

Take Home Message

1. In this work, IHC was used to analyze the MMR protein status of surgical specimens obtained from CRC patients, and the clinicopathological characteristics and prognosis were compared between dMMR and pMMR CRCs.
2. In addition, our results suggest that the deficiency of MMR protein in tissue of color rectal cancer was associated with a better prognosis in stage Ⅱ patients, but not to benefit from oxaliplatin/5-FU based adjuvant chemotherapy.
3. This study is of reference for the chemotherapy treatment of dMMR CRCs in the northeast of China.

Background

Following lung cancer and prostate cancer in males and lung cancer and breast cancer in females, colorectal cancer (CRC), the third most commonly diagnosed malignant and the third leading cause of carcinoma-related mortality, continues a major international health problem[1]. There were more than 1.8 million new colorectal carcinoma cases and approximately 900,000 deaths in 2017[2].

Currently, it was wildly accepted that chromosomal instability (CIN) and microsatellite instability (MSI) were the major pathways for CRC development[3]. CIN carcinomas, the most commonly pathway, were characteristic by mutations in genes resulting in chromosomal aneuploidy, loss of heterozygosity, and structural chromosomal rearrangements. However, MSI tumors were usually caused by the deficiency of mismatch repair (MMR) genes including MSH1, PMS2, MSH2 and MSH6 with the morbidity of approximately 15% of all sporadic CRCs[4]. Compared with CIN CRCs, dMMR/MSI colorectal malignancy tend to be proximal and histologically exhibit poor differentiation, a mucinous cell type and a better prognosis[5 6]. Therefore, it's crucial to identify the MMR/MSI status of CRCs.

Although there were currently three approaches for detection including immunohistochemistry (IHC), polymerase chain reaction (PCR) based methods and next generation sequencing (NGS), the sensitivity and specificity among the three methods were with high concordance rate (92-97%)[7].

And the sensitivity to detect dMMR/MSI carcinomas with IHC is approximately 95% and the specificity has reached 100% in most reports[8 9].

It is well known that dMMR/MSI is associated with a better prognosis in colorectal malignancy. However, whether MSI CRCs can benefit from postoperative adjuvant chemotherapy remains controversial. Multiple previous studies had demonstrated that 5-FU-based adjuvant chemotherapy did not improve 5-year overall survival (OS) in patients with dMMR/MSI tumors[10 11]. Recently, several researches considered that the addiction of oxaliplatin to fluoropyrimidine adjuvant chemotherapy can significantly improve OS and DFS compared to fluoropyrimidine alone treatment in dMMR/MSI CRCs[12 13]. Therefore, further study is needed to verify the predictive significance of MMR/MSI status for adjuvant chemotherapy, especially in Northeast China. After all, few relevant reports were conducted here.

The aim of the present study was to evaluate the association of clinicopathologic features and MMR/MSI status determined with immunohistochemistry analysis in Northeast China patients with stage □/□ CRCs. Particularly, we sought to detect the relationship between MMR/MSI status and efficacy of oxaliplatin and fluoropyrimidine based adjuvant chemotherapy.

Materials And Method

Patients and materials

The ethics committee of Second Affiliated Hospital of Jilin University approved the present retrospective study, and the research was performed in accordance with the Helsinki Declaration of World Medicine Association. Since December 2016, our institution has routinely performed immunohistochemistry staining for the four MMR proteins in all newly diagnosed colorectal carcinoma resection specimens. After rigorous screening, eventually, 476 patients who went through CRC radical resection were considered eligible from December 1st, 2016 to December 1st, 2018 in our institution. The clinical data of the eligible CRCs (such as gender, age, BMI, tumor location, tumor size, Carcinoma Embryonic Antigen (CEA) and postoperative chemotherapy (POC) etc.) was obtained from medical records. The pathologic data (such as T category, pathologic N category, differentiation, tumor pathologic type and vascular invasion) was collected from pathological examination result, and IHC was used to evaluated MMR status in CRC patients. The overall survival (OS) of the CRCs was defined as the time from radical surgery to death. The Follow-up information was obtained at outpatient clinic in our institution or using a telephone questionnaire directly with the final follow-up date of September 1st, 2021. Proximal colon carcinomas were defined as cecum, ascending colon or transverse colon. Pathological stage (TNM) depended on the tumor invasion depth, lymph nodes and metastases status according to the American Joint Committee on Cancer (AJCC) cancer staging manual. Adjuvant chemotherapy was performed postoperatively was determined by the TNM stage and the patient's willingness with the regimen of oxaliplatin combined with capecitabine.

IHC

The immunohistochemistry analyses for surgical specimens obtained from CRC patients who underwent radical resection were performed in Department of Pathology, Second Affiliated Hospital of Jilin University. IHC was performed on formalin-fixed paraffin-embedded tissues using a standard avidin-biotin complex peroxidase procedure with an autostainer (YZB/USA 2016-2012). Here are the brief steps. 2-μm-thinck formalin-fixed and paraffin-embedded tumor tissue sections were heating for two hours at 70 ∘C. The heat-induced epitope retrieval was performed using a PT link machine(California, America) after the sections were deparaffinized and rehydrated. The slides were incubated with primary antibodies for PMS2 (clone: ZA-0542; 1:1; Wuxi), MLH1(clone: ZM-0154; 1:1; Wuxi), MSH2(clone: ZA-0622; 1:1; Wuxi), MSH6(clone: G24072; 1:1; Ventana). The slides were counterstained with hematoxylin and then sealed with neutral balsam. In all the runs, positive and negative controls were included. The tissue sections omitting the primary antibodies were regarded as negative controls, and the tissues known to express the proteins were positive controls.

Evaluation Of IHC

All the IHC stains of the CRC samples were reviewed by two pathologists (Z.Y. Wang and D.W. Huang). The expression of four MMR proteins (PMS2, MLH1, MSH2 and MSH6) was defined as abnormal (loss) when nuclear straining of carcinoma cells was absent whereas the surrounding stromal cells performed positive nuclear straining. Proficient mismatch repair (pMMR) was defined as expression of all the four

MMR proteins in tumor tissues, while nuclear staining absence at least one MMR proteins was regarded as deficient mismatch repair (dMMR) **(Figure 1)**.

Figure1. The evaluation for immunohistochemistry (IHC) analyses of dMMR CRCs.

The cancer cells revealed nuclear staining absence of MLH1 (A), PMS2 (B), MSH2 (C), and MSH6 (D).

The Inclusion And Exclusion Criteria

The inclusion criteria were showed as follows: 1) Age between 18-75 y. 2) Pathologically diagnosed as CRC. 3) TNM □/□. 4) colorectal R0 resection was applied. The following exclusion criteria applied to patients in the present research were: 1) History of malignant carcinoma. 2) Severe respiratory tract, liver, kidney or cardiovascular disease. 3) Underwent preoperative neoadjuvant therapy, which was considered likely to affect MMR protein expression[14]. 4) Clinicopathological data cannot be collected accurately.

Statistical analysis

Statistical analyses were performed using SPSS for MAC, version 26.0 (IBM Corporation). Mann–Whitney U test or t test was used for continuous variables, while $\chi 2$ test or Fisher exact test was used for comparing categorical data. Multivariate analyses were evaluated with Cox proportional hazards models and survival curves were created using the Kaplan-Meier method. All P values calculated in the analysis were 2-sided, and P values less than 0.05 were considered statistically significant.

Result

Table 1 Patients' clinicopathologic characteristics with respect to mismatch repair protein expression.

Characteristic	Patients, No. pMMR (n=413)	dMMR (n=63)	P value
gender			
male	264	42	
female	149	21	0.672[a]
age, y	61.4(±9.8)	60.7(±10.5)	0.602[c]
BMI, Kg/m^2	23.0(±3.5)	22.3(±3.0	0.114[c]
Tumor site			
Proximal colon	114	38	
Distal colon or rectum	299	25	0.000[a]
Tumor size, cm			
CEA, ng/ml	4.7(±1.7	5.8(±2.1)	
pT status	4.05(2.26-7.54)	2.75(1.33-4.40)	0.000[c]
pT1			0.004[d]
pT2	6	0	
pT3	15	1	
pT4	366	55	
pN status	26	7	
pN0			0.469[b]
pN1	192	45	
pN2	154	13	
TNM stage	67	5	
			0.001[a]
	192	44	
Differentiation	221	19	
Well			0.001[a]
Moderate	10	0	
Poor	383	55	
Pathological type	20	8	

Tubular			0.040[b]
Mucinous	389	47	
Mixed	11	16	
Vascular invasion	13	0	
Yes			0.000[b]
No	160	18	
	253	45	
			0.120[a]

Abbreviations: dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; BMI, Body Mass Index; CEA, Carcinoma Embryonic Antigen.

a, χ^2 test; b, Fisher exact test; c, t test; d, Mann–Whitney U test.

The clinicopathological features of 29 type 1 and 24 type 2 dMMR CRCs are presented in **Table 2**. A loss of MSH2/MSH6 protein expression was associated with gender, where the MMR-protein negative carcinomas occurred more frequently in male (P=0.011).

However, there was no significant differences between the two groups among the other clinicopathological features (P>0.05).

Table 2 Clinicopathological features of 29 type 1 and 24 type 2 dMMR CRCs

Characteristic	Type 1 PMS2/MLH1 (n=29)	Type 2 MSH2/MSH6 (n=24)	P value
gender			
male	16	21	
female	13	3	0.011[a]
age, y	60.14(±11.26)	60.33(10.21)	0.948[c]
BMI, Kg/m^2	22.35(3.43)	22.66(2.45)	0.722[c]
Tumor site			
Proximal colon	18	15	
Distal colon or rectum	11	9	0.974
Tumor size, cm			
CEA, ng/ml	6.09(2.41)	5.54(1.89)	0.372[c]
pT status	2.75(1.33-4.32)	2.78(1.33-4.40)	0.308[d]
pT3			
pT4	27	21	
pN status	2	3	
pN0			0.649[b]
pN1	22	16	
pN2	7	4	
TNM stage	0	4	
□			0.075[b]
□	21	16	
Differentiation	8	8	
Moderate			0.650[a]
Poor	25	21	
Pathological type	4	3	
Tubular			1.000[b]
Mucinous	20	19	
Vascular invasion	9	5	

Yes			0.402[a]
No	7	8	
	22	16	
			0.459[a]

Abbreviations: BMI, Body Mass Index; CEA, Carcinoma Embryonic Antigen.

a, χ^2 test; b, Fisher exact test; c, t test; d, Mann–Whitney U test.

MMR expression and clinical outcomes

In the present study, 89 CRCs patients had experienced a dead (dMMR CRCs, N=82; pMMR CRCs, N=7). To further analyze the association of MMR status and prognosis in patients with CRC, Kaplan-Meier analyses were performed (**Figure 3**). The survivorship analysis (Kaplan-Meier) showed 86% OS rate in dMMR group and a 68% OS rate in pMMR group after 5 years (P=.004, Kaplan-Meier log-rank). In stage Ⅱ CRC patients, the estimated OS rate for patients with loss of MMR protein was 89% and patients without deficiency was 74% after 5 years, which demonstrated that dMMR was associated with a favorable prognosis in stage Ⅱ patients (P=.014, Kaplan-Meier log-rank). However, OS did not differ from the two groups in patients with stage Ⅲ colorectal carcinomas (P=.353, Kaplan-Meier log-rank).

Notes: (A) the association of OS and MMR protein status in all patients. (B) the association of OS and MMR protein status in patients with stage Ⅱ. (C) the association of OS and MMR protein status in patients with stage Ⅲ. The red line is pMMR. The blue line is dMMR.

Abbreviation: pMMR, mismatch repair proficient. dMMR, mismatch repair deficient. OS, overall survival.

To determine whether dMMR was independent prognostic factor associated with CRC clinical outcomes, a univariate and multivariate analysis was performed using the Cox proportional hazard model (**table 3**). The risk variables included age, gender, tumor location, tumor size, pathological N stage, pathological type, CEA level, differentiation, vascular invasion, TNM stage, MMR status, and postoperative chemotherapy(POC), which were generally considered to be associated with prognosis of CRC. In the univariate analysis, TNM stage (HR 2.25, 95%CI 1.43-3.54, P=.000), CEA level (HR 2.08, 95%CI 1.37-3.16, P=.001), and dMMR (HR 0.32, 95%CI 0.14-0.72, P=.006) were significantly associated with survival, while vascular invasion, gender, age, tumor size, POC, tumor location, pathological type, and differentiation were not. In the final multivariate Cox regression model, POC and dMMR independent of other factors were associated with a favor prognosis in CRC patients with stage II/III (HR 0.47, 95%CI 0.30-0.74, P=.001; HR 0.34, 95%CI 0.14-0.79, P=.013).

Table 3 Univariate and multivariate associations between covariates and the composite primary endpoint of dead in stage II/III CRC patients

	Univariate		Multivariate	
characteristic	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
Gender				
Female	1.0(Reference)			
Male	1.39(0.88-2.19)	0.161		
Age				
<50	1.0(Reference)			
≥50	1.78(0.82-3.85)	0.143		
Tumor site				
Proximal	1.0(Reference)			
Distal	1.33(0.84-2.11)	0.223		
Tumor size				
<5 cm	1.0(Reference)			
≥5cm	1.14(0.75-1.73)	0.539		
TNM stage				
□	1.0(Reference)		1.0(Reference)	
□	2.25(1.43-3.54)	0.000	2.52(1.50-4.24)	0.001
Pathological type				
Tubular	1.0(Reference)		1.0(Reference)	
Mucinous	1.24(0.57-2.70)	0.585	2.58(1.14-5.85)	0.023
Mixed	1.59(0.50-5.04)	0.435		
Differentiation				
Well	1.0(Reference)			
Moderate	1.55(0.21-11.17)	0.663		
Poor	1.43(0.17-12.29)	0.745		
Vascular invasion				
No	1.0(Reference)			
Yes	1.26(0.83-1.92)	0.285		
CEA				

<5.2ng/ml	1.0(Reference)		1.0(Reference)	
≥5.2ng/ml	2.08(1.37-3.16)	0.001	1.93(1.25-2.98)	0.003
POC				
Did not receive	1.0(Reference)		1.0(Reference)	
Receive	0.68(0.45-1.04)	0.075	0.47(0.30-0.74)	0.001
MMR				
pMMR	1.0(Reference)		1.0(Reference)	
dMMR	0.32(0.14-0.72)	0.006	0.34(0.14-0.79)	0.013

Abbreviations: dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; CEA, Carcinoma Embryonic Antigen; POC, postoperative adjuvant chemotherapy.

MMR expression as predictor of benefit from adjuvant chemotherapy

Further assessment was performed to analysis the effect of POC in both pMMR and dMMR CRC patients using Kaplan-Meier analyses (**Figure 4**). Among the 413 pMMR CRCs, the overall 5 years survival rates of patients with POC and without POC were 78.1% and 57.2% respectively(P=.026, Kaplan-Meier log-rank). In the subgroup of 192 stage CRCs, POC didn't seem to make any sense to promote a better prognosis(P=.254, Kaplan-Meier log-rank), whereas did in subgroup of 221 stage CRCs(P=.000, Kaplan-Meier log-rank). However, among 63 dMMR CRCs, POC did not improve the outcome of patients with either stage or .

Notes: (A, B and C) the association of OS and MMR protein status in patients with pMMR CRCs. (D, E and F) the association of OS and MMR protein status in patients with pMMR CRCs. The red line is POC. The blue line is without POC.

Abbreviation: pMMR, mismatch repair proficient. dMMR, mismatch repair deficient. POC, postoperative adjuvant chemotherapy, OS, overall survival.

Declarations

Availability of data and materials

The datasets generated and analyzed during the current study available from the corresponding author on reasonable request.

Acknowledgements

None.

Funding

This study was not funded by any outside source.

contributions

AS and MW conceived the study design. ZZ and DL acquired the data for the study. YG, YY, and RQ analyzed and interpreted the data. ZW read the pathological section. AS drafted the manuscript. YY and ZZ revised the manuscript critically. The authors read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate

The study protocol was approved by the institutional review board of The Second Hospital of Jilin University. And the ethics approval number was 2021111. Due to the retrospective design of the study, the local ethic committee confirmed that informed consent was not necessary from participants. The demand of patient informed consent was deserted because of the retrospective nature of this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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We thank Shuang Wang, Yongbo Zou and Jian Shi, from the second Hospital of Jilin University for their support in the clinical phase of the study.

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Figures

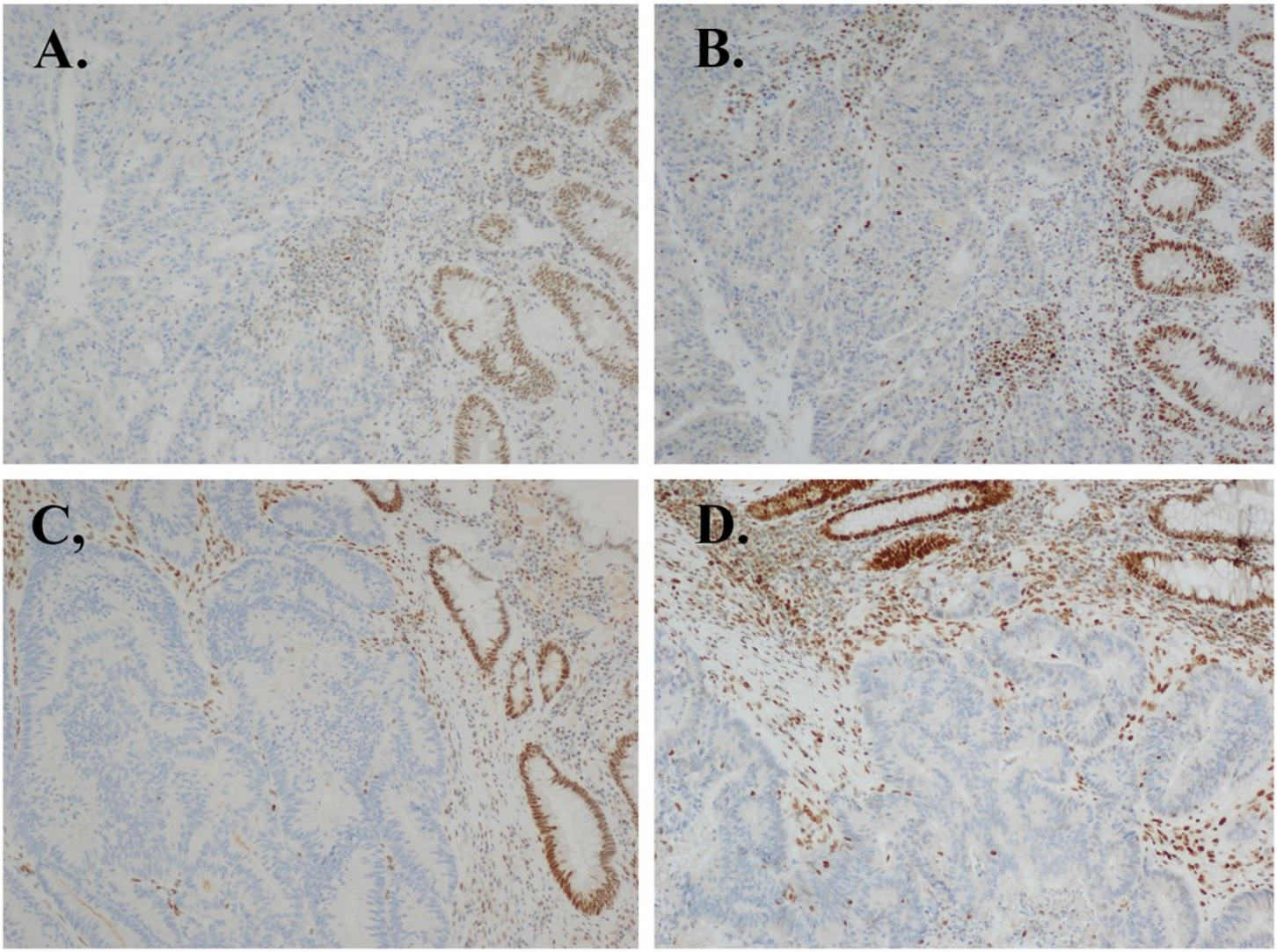

Figure 1

The evaluation for immunohistochemistry (IHC) analyses of dMMR CRCs. The cancer cells revealed nuclear staining absence of MLH1 (A), PMS2 (B), MSH2 (C), and MSH6 (D).

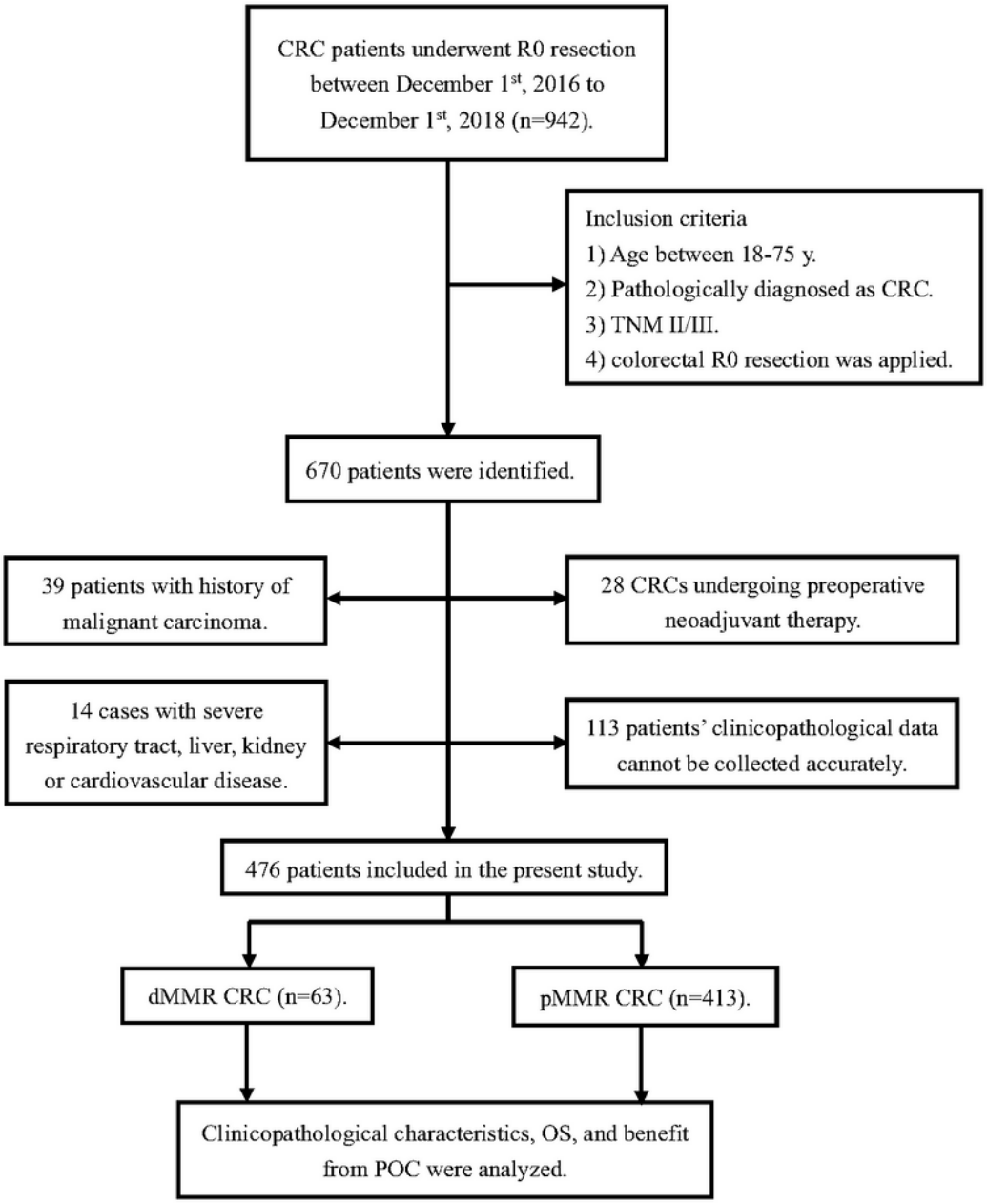

Figure 2

CONSORT Diagram of Patient Flow

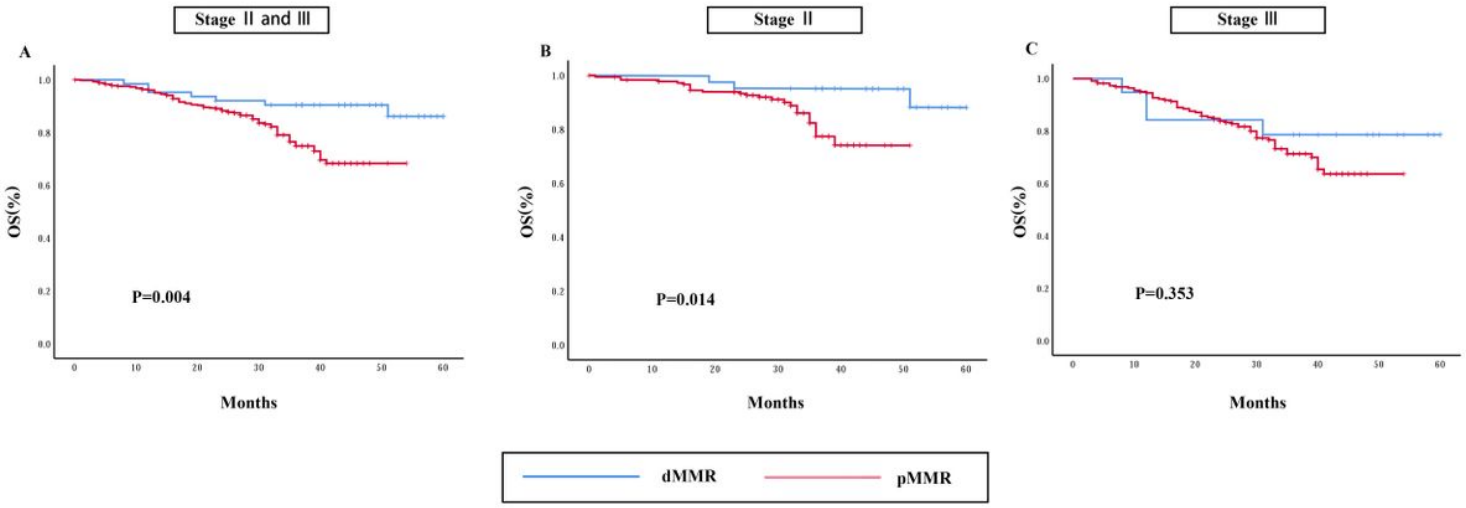

Figure 3

prognosis value for MMR protein status.

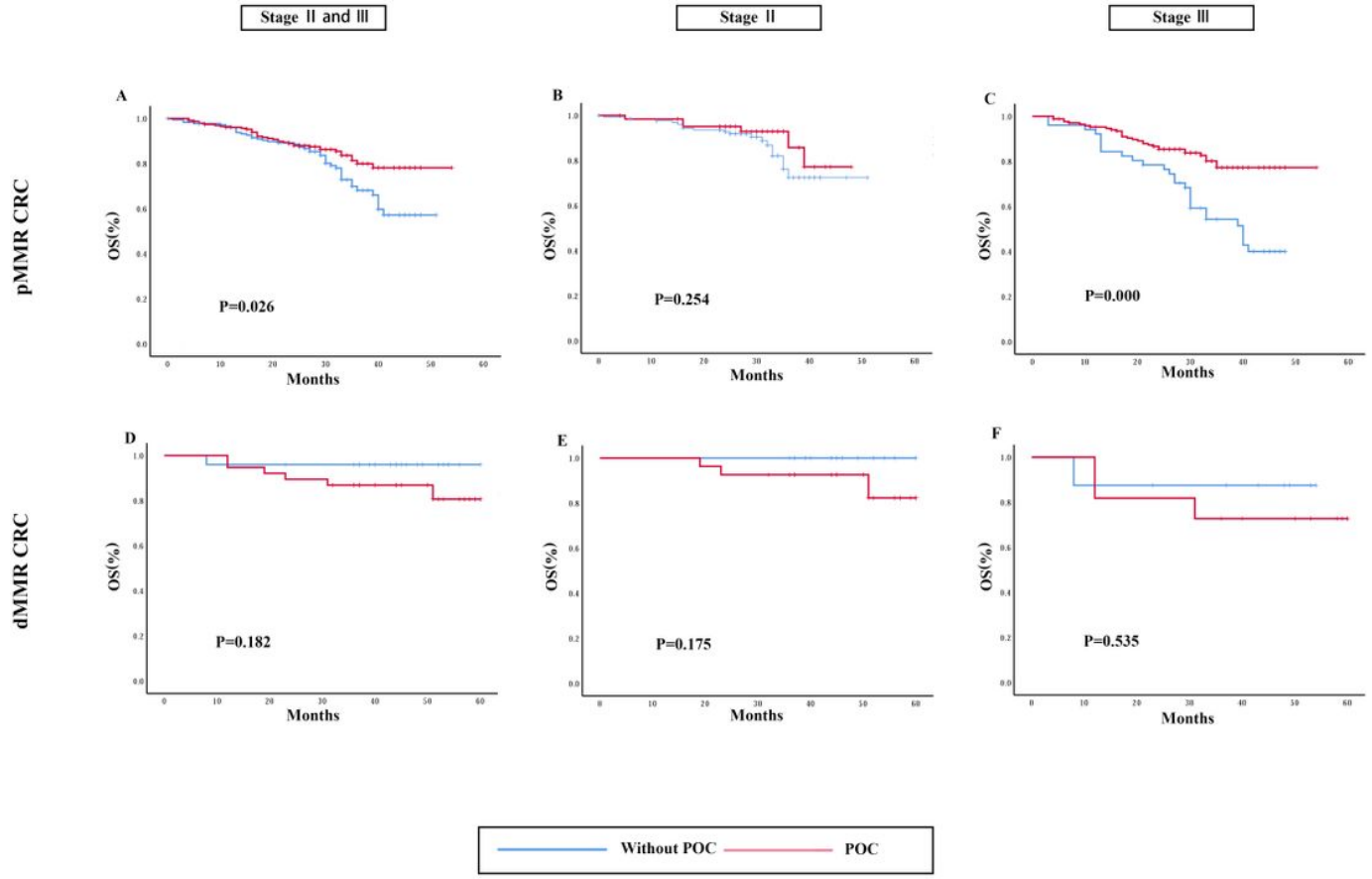

Figure 4

prognosis value for MMR protein status.