

Metabolic Activity Via 18 F-FDG PET/CT is Predictive of Microsatellite Instability Status In Colorectal Cancer

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Research Article

Keywords: colorectal cancer, microsatellite instability, metabolic tumour volume, positron emission tomography

Posted Date: December 15th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-839816/v1>

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Abstract

Purpose: Identification of microsatellite instability high (MSI-H) colorectal cancer (CRC) is crucial for screening patients most likely to benefit from immunotherapy. We aim to investigate whether the metabolic characteristics is related to MSI status and can be used to predict the MSI-H CRC.

Methods: A retrospective analysis was conducted on 420 CRC patients who were identified via [^{18}F]fluorodeoxyglucose (^{18}F -FDG) positron emission tomography (PET)/computed tomography(CT) prior to therapy. Maximum standardised uptake (SUV_{max}), mean standardised uptake (SUV_{mean}), metabolic tumour volume (MTV) and total lesion glycolysis (TLG) of the primary tumour were calculated and compared between MSI-H and microsatellite stability (MSS). Predictive factors of MSI status were selected from metabolic parameters and clinicopathological profiles via a multivariate analysis.

Results: Of 420 colorectal cancers, 44 exhibited a high incidence of MSI. Both MTV and TLG were significantly higher in MSI-H group compared with the MSS group ($P=0.004$ and $P=0.010$, respectively). Logistic regression analysis indicated that CRC with MSI-H were related to younger age ($P=0.013$), primary lesion located at right hemi-colon ($P<0.001$) and larger MTV on PET/CT imaging ($P=0.019$). MTV more than 32.19 cm^3 of colorectal cancer was linked to the presence of MSI ($P=0.019$).

Conclusion: Tumor metabolic burden were higher in MSI-H CRC which may be useful for predicting the MSI status of CRC patient and thus aid in determination of immunotherapy for patients with CRC.

Introduction

Colorectal cancer (CRC) is a major cause of cancer death worldwide[1, 2]. Despite significant improvements in screening and treatment, the prognosis of patients with metastatic CRC (mCRC) remains poor, with a median overall survival (OS) of approximately 30 months[3]. The development of novel and more effective therapeutic strategies are an urgent unmet need for patients with CRC. Several advances from the past few years indicate the potential to improve the effectiveness of treatments through patient stratification based on an individual's tumor-specific biomarkers[4-6]. Arguably, the most prominent examples are provided by the successes with anti-programmed cell death 1 (PD-1) in CRC with microsatellite instability high (MSI-H)[7]. Several prospective clinical trials in chemotherapy-resistant MSI mCRC have demonstrated a high disease control rate (DCR) and a favorable progression-free survival (PFS) with immune checkpoint inhibitors (ICPIs)[6, 8]. However, reported response rates (RR) of 23%-69% and DCR of 47%-89% to ICPIs in MSI-H CRC are likely reflecting patient and/or tumor heterogeneity[6, 9, 10]. Selecting CRC patients with MSI-H will be exceptionally useful in screening out patients most likely to benefit from immunotherapy to prolong patient survival and improve prognosis.

Currently, there are three major methods for detecting MSI status, including immunohistochemistry (IHC), polymerase chain reaction (PCR) and next-generation sequence (NGS)[11]. Although these analyses of CRC samples have been incorporated into routine clinical practice for the purpose of treatment algorithms, the heterogeneity of MSI-H status, poor DNA quality of biopsy samples, the invasive

procedure, or/and the long experiment period may become limiting factors in their clinical application[12, 13]. Therefore, alternative noninvasive strategies or an MSI-predicting model could therefore be of good choice.

[¹⁸F]Fluorodeoxyglucose (¹⁸F-FDG) positron emission tomography (PET) is an important molecular imaging modality that is a less invasive tool for diagnosis, treatment response monitoring, surveillance, and prognostication of CRC[14]. Despite imaging techniques being critical in the preoperative workup for treatment decisions, some correlation studies exist between pretreatment image findings and genomic expression in patients with malignant disease, such as c-Met status in gastric cancer[15] and PD-L1 expression in lung cancer[16]. Similarly, Chung et al have shown MSI status to be correlated with ¹⁸F-FDG uptake in gastric cancer [17]. Thus, we hypothesized that the pretreatment ¹⁸F-FDG PET/CT might be a helpful tool for non-invasively inferring the MSI-H CRC patients.

Therefore, our purpose was to explore the relationship between metabolic parameters, clinicopathological profiles and MSI expression in CRC patients.

Materials And Methods

Study design and patients

The Investigational Review Board of Peking University Cancer Hospital approved the present study. The requirement to acquire informed consent was waived owing to the Ethics Committee of Peking University Cancer Hospital. Retrospective data were collected with consecutive patients between January 2010 and March 2019 at Peking University Cancer Hospital based on the following criteria: (a) histologically proven CRC, (b) no prior treatment before ¹⁸F-FDG PET/CT scan, and (c) the presence of complete medical history and clinicopathological data. The exclusion criteria were (a) secondary malignant disease; (b) serious infection or inflammation; or (c) uncontrolled diabetes mellitus. Figure 1 shows the flowchart of selection criteria.

¹⁸F-FDG PET/CT

Whole-body acquisition was performed in 1 min/bed using a hybrid system (PHILIPS Gemini TF) that covered the area from the base of the skull to the upper thigh after intravenous injection of about 3.7 MBq/kg of ¹⁸F-FDG. All patients fasted for at least 6 h previously and presented with a blood glucose level lower than 10 mmol/L. Attenuation weighted ordered-subsets expectation maximization (AW-OSEM) iterative algorithm with 4 iterations and 8 subsets, Gaussian filter with 4.0 mm full width at half maximum (FWHM) and scatter correction were used for reconstruction[15].

Quantitative PET parameter computation

Maximum standard uptake volume (SUV_{max}), mean SUV (SUV_{mean}) with the threshold of 40%, metabolic tumour volume (MTV) and total lesion glycolysis (TLG) were calculated in 3D mode using vendor-

provided software at a PHILIPS EBW workstation. MTV was estimated using the adaptive threshold method[18] for each primary CRC lesion (for a single fixed threshold may be not well suited for assessing actual tumour volume and an adaptive threshold would be more appropriate for accurate tumour delineation and assessment of the actual tumour volume) by selecting a volume of interest (VOI) on the axial image, and the size of VOI was verified on the corresponding coronal and sagittal images to include the entire tumour in the VOI. TLG was defined as the product of SUV_{mean} and MTV[15].

Immunohistochemistry

Immunohistochemistry was carried out in an automated tissue staining system (ImmunoVision Technologies, Brisbane, CA). Briefly, formalin-fixed, paraffin-embedded blocks were cut into 4- μ m-thick sections, deparaffinized in xylene and rehydrated, which also used for histopathologic determinations and tumor-infiltrating lymphocytes (TIL). Antigen retrieval was performed using EDTA (pH 8.0; Santa Cruz Biochemistry, Dallas, TX) in a pressure cooker for 3 minutes. the sections were the incubated in 3% H_2O_2 solution for 10 minutes at room temperature to block endogenous peroxidase activity.

IHC was conducted using antibody to hMLH1 (ready-to-use, clone GM002; GeneTech, Shanghai, CHINA), hMSH2 (ready-to-use, clone RED 2; GeneTech, Shanghai, CHINA), hMSH6 (ready-to-use, cloneEP49; GeneTech, Shanghai, CHINA), and hPMS2 (ready-to-use, clone EP51; GeneTech, Shanghai, CHINA), CD8 (Leica Biosystems PA0183, mouse clone 4B11, ready-to-use formulation), and CD3 (Leica NCL-L-CD3-565, mouse clone LN10, diluted 1:100). Tumors were designated as a high incidence of MSI when at least one out of the four markers showed instability[12]. Microsatellite stability (MSS)was defined when no loss of expression was observed for any of these markers. ≥ 30 lymphocytes per 100 epithelial cells were considered as intra-epithelial lymphocytosis[19]. All CRC diagnoses were provided by specialty-trained intestinal pathologists.

Statistical analysis

Statistical analysis was performed using SPSS software 23.0, (Inc.). Data are presented as median (range). Significant differences of variable characteristics between groups were compared by Mann-Whitney U-test for the continuous variables, and c^2 tests for the categorical variables. Receiver operating characteristic (ROC) curves were used to define optimal cut-off values for age, MTV and TLG. Logistic regression analysis was performed to determine the independent significant clinicopathological factors that showed a causal relationship with a dependent variable; a forward conditional method was used and the results are reported as hazard ratios (HR) and 95% confidence intervals (CI). Datasets were compared for patient demographic and clinical characteristics. $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 420 patients met our clinical and PET-based inclusion criteria and the details of clinicopathological characteristics are presented in Table 1. The mean age of CRC patients was 60 years (range 18-87 years), and 254 of these patients (60.5%) were men. The primary tumour was localized in the right hemi-colon in 118 (28.1%) patients, and moderately/highly differentiation was the most common histologic grade (approximately 85.9% of patients). The majority of patients were T₂₋₃ (71.2%), N₁₋₂ (58.6%) and M₀ (79.3%) stage according to pathological results. Among those patients who performed molecular tumour markers analysis, 62.5% cases showed Ki-67 index \geq 75%, 60 (14.5%) were Her2+, approximately half were found c-Met positive and 70.6% were EGFR+. On the basis of MSI status analysis of the primary tumour, 420 observational patients were classified into 2 groups: patient with MSI-H (44 patients, 10.5%) and MSS (376 patients, 89.5%). The median SUV_{max}, SUV_{mean}, MTV and TLG values for the primary lesion were 13.775 (4.10-54.73), 7.93 (2.81-22.70), 18.56 cm³ (range 1.34–391.04 cm³) and 141.25 g (range 5.36–2634.39 g), respectively.

Clinicopathological findings and metabolic parameters according to the MSI status

The correlations between patient characteristics and MSI status are summarized in Table 2. More than half of the MSI CRC were located in the right (59.1%), whereas MSS CRC were found predominantly in the left hemi-colon (75.5%) ($P < 0.001$) (Figure 2). And younger patients prone to encounter in MSI-positive CRC [56.5(24-84) vs 61(18-87); $P = 0.048$; Table 2, Figure 2]. MSI colorectal cancers exhibited significantly higher MTV (33.60 cm³) and TLG (229.03 g) values of the primary lesion than MSS ones (18.08 cm³, and 134.26 g, respectively; $P = 0.004$ and 0.010 , respectively) (Figure 2). However, no significant differences between the MSI and MSS groups were found in terms of gender, histologic grade, TNM category, molecular tumour markers, and SUVs (Table 2).

HE-staining results showed intratumoral inflammatory cell infiltration, and the TIL in the MSI-H group was significantly higher than in the MSS group (Figure 3). IHC staining of CRC lesion showed that CD8+ were resided in the tumor stroma and the tumor epithelium (Figure 4). These results can suggest that MSI-H group had higher metabolic burden, possibly by increased density of TILs.

Predictive factors for the presence of microsatellite instability

We sought to determine the threshold of MTV and TLG for optimal differentiation between the MSI-H CRC group and the MSS CRC group. The optimal cut-off values of MTV and TLG were 32.19 cm³ ($P = 0.004$) and 352.72 g ($P = 0.010$), respectively. Logistic analysis for microsatellite instability status revealed that right hemi-colon (HR 4.72, 95% CI 1.89-8.95; $P < 0.001$), age < 52 y (HR 2.22, 95% CI 1.10-4.47; $P = 0.026$) and MTV ≥ 352.72 g (HR 2.34, 95% CI 1.19-4.61; $P = 0.014$) were independent variables predicting MSI status (Table 3, Figure 5).

Discussion

The most important contribution of this study is the evaluation of MSI status of primary CRC lesions with a set of metabolic parameters (SUV, MTV, and TLG) on ^{18}F -FDG PET/CT imaging to guided personalized medicine. The strong predictive value of higher MTV, younger age and right hemi-colon could be confirmed with MSI-H CRC. This is the first study to demonstrate the significance of MSI status in detecting CRCs using PET/CT imaging.

CRC can be categorized into two discrete groups on the basis of mutation patterns: tumors that have an MSI signature with high overall mutation burden (>12 mutations per 10^6 DNA bases) and tumors that have an MSS signature with a much lower mutation burden (<8.24 mutations per 10^6 DNA bases)[20]. MSI-H CRCs, approximately 15% of all sporadic CRCs[21, 22], are more common in younger patients(<50 years), right-sided and poorly differentiated stage 2 CRCs[23-25]. MSI-H colorectal cancers (CRCs) have been shown to have a better overall prognosis compared with MSS cancers[26]. There is also evidence that MS-H is one of these important molecular markers which not only associated with resistance to 5-fluorouracil (5-FU) based chemotherapy particularly in stage II patients but also predicts the response of CRC to PD-1 blockade[9, 26-28]. Several PD-1 blockades, such as pembrolizumab and nivolumab, were granted by FDA for adult and pediatric patients with unresectable or metastatic microsatellite instability–high (MSI-H) tumors. Thus, identifying potentially susceptible subgroups is important and necessary for applying immunotherapy in CRC patients.

In the present study, we have added another piece in the big puzzle of immunotherapy. Indeed, by using ^{18}F -FDG PET/CT, we demonstrated an interesting association between MSI status and elevated metabolic tumor burden.

This is different from a recent study of Chung and colleagues[17] in gastric cancer, we find a positive relationship between MSI status and MTV in colorectal cancer, the specific mechanism behind the correlation between metabolic tumor burden and MSI status is still unknown. Numerous publications have identified histologic features and heterogeneity which are more commonly seen in MSI-H CRCs. MSI-H tumor have high level of CD3+ and CD8+ TILs and a prominent inflammatory reaction at the advancing edge of the tumor (Crohn-like reaction)[25, 29]. Arguably, tumors characterized by MSI-H mechanisms harbor a high level of somatic mutations, resulting in the generation of multiple neopeptides (also referred to as neoantigens), which elicits a robust host immune response, associated with increased density of TILs and upregulation of immune checkpoint expression, including PD-1, PD-L1, CTLA-4, and IDO[30, 31]. Upregulation of PD-L1 has been linked to the activation of mitogen-activated protein-kinase (MARK) and phosphoinositide 3-kinase (PI3K) signaling pathways as well as the hypoxia-inducible factor-1 α (HIF-1 α), an essential factor linked to the elevated FDG uptake[32]. In present study, we found MSI-H CRC had large tumor size with a circumscribed/expansile growth pattern. Histology results revealed abundant lymphocytes infiltrate CRC lesions by conventional H&E stain. Staining with antibodies against CD3 and CD8 revealed the presence of T cells both within and at the invasive margin of the tumor. Increased density of TILs, a prominent inflammatory reaction, and activated HIF-1 α may explain that significantly higher MTV (33.60cm^3) and TLG (229.03g), reflecting the number of cells with

abnormal metabolism, of primary MSI-H CRC than MSS ones (18.08 cm³, and 134.26 g, respectively; $P=0.004$ and 0.010, respectively) in present study.

¹⁸F-FDG PET may play a pivotal role in guiding ICI treatment since there is a strong rationale suggesting that abnormally increased glucose metabolism is a hallmark of cancer associated with tumor aggressiveness and immune response[33-35]. In this study, we reviewed 12 clinicopathological indicators and 4 metabolic parameters of 420 CRC patients, and tried to explore their correlation with MSI status and establish a predicting model based on hazard ratio in multivariate analysis. We found approximately 11% of all CRC and 8% of mCRC with MSI-H, predominantly located in the right hemi-colon (59.1%) among younger people(age<52) in our cohorts. This is in line with reported studies[24, 36-38]. These results may be of significance for patients who would like to receive PD-1/PD-L1 blockades but are unable or unwilling to undergo biopsy for MSI status testing, and provide reference value to the immunotherapy.

Despite these strengths, our study has some notable limitations. First, this was a single-centre study; therefore, a future large-scale multicentred study should be conducted to validate our results. Second, our cohort included mainly locally advanced or mCRC, and it remains unclear whether the conclusion can apply in early stage CRC patients. Finally, even though our study found ¹⁸F-FDG PET/CT may have good prediction performance, it cannot replace pathologic testing for examining MSI status. Nevertheless, our study is the first to demonstrate that the ¹⁸F-FDG PET/CT metabolic parameters in combination with clinicopathologic features is clinically relevant for guiding therapeutic strategies in patients with CRC.

Conclusion

The MSI CRCs have higher MTV and younger age than the MSS types and mostly located in in the right hemi-colon in our large patient cohort. Our founding may serve as powerful indicators that identify patients with MSI status, those who cannot tolerate platinum-containing regiments but may benefit from immunotherapy such as anti-PD-1 monoclonal antibody.

List Of Abbreviations

Full name	Abbreviation
Colorectal cancer	CRC
microsatellite instability high	MSI-H
[¹⁸ F]Fluorodeoxyglucose positron emission tomography/computed tomography	¹⁸ F-FDG PET/CT
maximum standardised uptake	SUVmax
mean standardised uptake	SUVmean
metabolic tumour volume	MTV
total lesion glycolysis	TLG

Declarations

Ethics approval and consent to participate: This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of Peking University Cancer Hospital approved this study. The requirement to acquire informed consent was waived owing to the Ethics Committee of Peking University Cancer Hospital.

Funding:

This work was supported by National Natural Science Foundation of China (82071957), Capital's Funds for Health Improvement and Research (2018-2-1024), Beijing Municipal Science & Technology Commission (No. Z171100001017101), and Beijing Municipal Administration of Hospitals Incubating Program (PX2017028).

Conflicts of interest/Competing interests:

The authors declare that they have no conflict of interest.

Consent to participate:

The requirement to acquire informed consent was waived owing to the Ethics Committee of Peking University Cancer Hospital.

Consent for publication:

The authors affirm that human research participants provided informed consent for publication of the images in Figure(s)1-5.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions:

All authors read and approved the final manuscript. MMW analyzed and interpreted the patient data regarding the colorectal cancer. JLS performed the Image interpretation and statistical calculation, and was a major contributor in writing the manuscript. XJW performed quality control of imaging and article modification and correction.

Acknowledgements: Not applicable

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Tables

Table 1 Clinical characteristics of patients

Variable		Number of the subjects(N=420)
Gender	Male	254(60.5)
	Female	166(39.5)
Age(y)	<52	100(23.8)
	≥52	320(76.2)
Primary lesion	Left hemi-colon	302(71.9)
	Right hemi-colon	118(28.1)
Histologic grade	poorly/moderately-poorly	56(14.1)
	moderately /highly	340(85.9)
Mucinous carcinoma	Yes	34(10.0)
	No	386(90.0)
T stage	T ₂₋₃	299(71.2)
	T ₄	121(28.8)
N stage	N ₀	174(41.4)
	N ₁₋₂	246(58.6)
M stage	M ₀	333(79.3)
	M ₁	87(20.7)
Ki-67 expression	<75%	153(37.5)
	≥75%	255(62.5)
HER2 expression	Negative	355(85.5)
	Positive	60(14.5)
c-Met expression	Negative	199(51.2)
	Positive	190(48.8)
EGFR expression	Negative	120(29.4)
	Positive	288(70.6)
MSI state	Absent	376(89.5)
	Present	44(10.5)

The data presented are number (%) of patients

Table 2 Correlations between patient characteristics and MSI status in CRC patients

	CRC with MSI(n=44)	CRC with MSS(n=376)	P value
Age,years,median,(range)	56.5(24-84)	61(18-87)	0.048*
Male:female ratio	29:15	225:151	0.436 [#]
Primary lesion (right:left hemi-colon)	26:18	92:284	<0.001 [#]
Histologic grade (poorly/moderately-poorly: moderately /highly)	11:31	45:309	0.018 [#]
Mucinous carcinoma (yes: no)	3:50	31:338	0.686 [#]
T stage (T ₂₋₃ : T ₄)	39:12	260:108	0.152 [#]
N stage (N ₀ : N ₁₋₂)	26:25	148:220	0.225 [#]
M stage (M ₀ : M ₁)	45:7	287:80	0.507 [#]
HER2 expression (positive: negative)	5:38	55:317	0.577 [#]
Ki-67 expression (≥75%:<75%)	26:16	229:137	0.933 [#]
c-Met expression (positive: negative)	18:23	172:176	0.503 [#]
EGFR expression (positive: negative)	25:16	263:104	0.154 [#]
SUV _{max} , median(range)	13.81(5.97-47.06)	13.78(4.10-54.73)	0.502*
SUV _{mean} , median(range)	7.29(3.67-16.22)	7.95(2.81-22.70)	0.319*
MTV, cm ³ , median(range)	33.60(1.34-183.42)	18.08(1.86-391.04)	0.004*
TLG, cm ³ , median(range)	229.03(5.36-1540.76)	134.26(7.25-2634.39)	0.010*

SUV_{max}, the maximum standardized uptake values; SUV_{mean}, mean standardized uptake values; MTV, metabolic tumor volume; TLG, total lesion glycolysis; HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor.

p values were calculated using the *Mann–Whitney U-test, [#]c2 test

Table3: Logistic regression analysis for MSI in CRC patients

Variables	Odds ratio	95% confidence interval	P value
Age (<52 vs. ≥52 years)	2.22	1.10-4.47	0.026
Sex (male vs. female)	0.74	0.32-1.71	0.484
Primary lesion (right vs. left hemi-colon)	4.72	1.89-8.95	<0.001
Histologic grade (poorly/moderately-poorly vs. moderately /highly)	0.65	0.24-1.75	0.391
Mucinous carcinoma (yes vs. no)	0.731	0.14-3.85	0.712
T stage (T ₂₋₃ vs. T ₄)	0.518	0.18-1.48	0.219
N stage (N ₀ vs. N ₁₋₂)	0.67	0.30-1.48	0.322
M stage (M ₀ vs. M ₁)	0.95	0.33-2.77	0.928
HER2 expression (positive vs. negative)	0.43	0.11-1.66	0.219
Ki-67 expression (≥75%vs.<75%)	1.32	0.56-3.11	0.530
c-Met expression (positive vs. negative)	1.23	0.50-2.99	0.652
EGFR expression (positive vs. negative)	0.66	0.25-1.77	0.413
SUV _{max} (<14.74 vs. ≥14.74)	0.96	0.37-2.48	0.935
SUV _{mean} (<9.28 vs. ≥9.28)	0.44	0.15-1.31	0.140
MTV (≥32.19 cm ³ vs.<32.19 cm ³)	2.34	1.19-4.61	0.014
TLG (≥352.72 cm ³ vs.<352.72 cm ³)	1.44	0.49-4.26	0.512

Figures

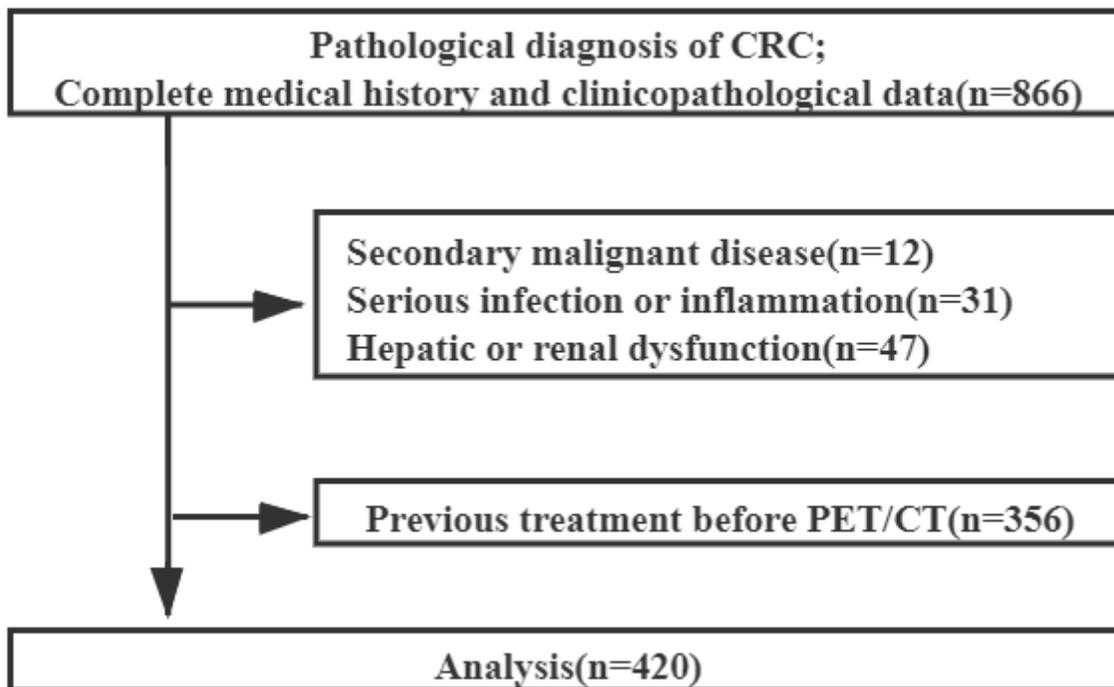


Figure 1

Flow diagram outlining criteria used for patient inclusion and exclusion

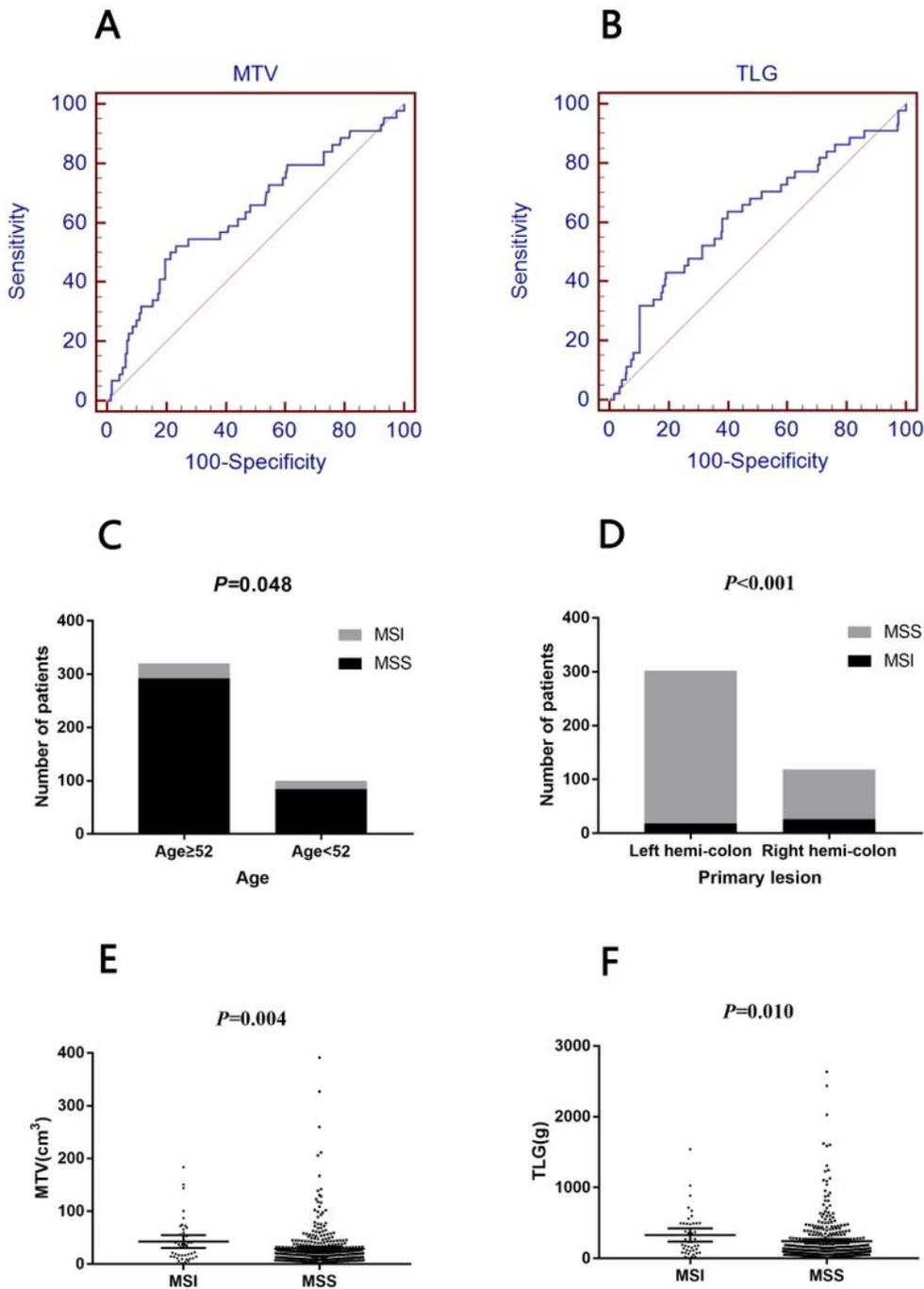


Figure 2

(A) ROC curve of MTV for predicting MSI. Sensitivity and specificity were 52.3% and 76.6% (area under curve (AUC) value=0.633; $P=0.004$). (B) ROC curve of TLG for predicting MSI. Sensitivity and specificity were 43.2% and 80.9% (area under curve (AUC) value=0.619; $P=0.010$). (C) Correlation between Age and MSI status in CRC. Younger patients (age < 52 y) prone to encounter in MSI-positive CRC [56.5(24-84) vs 61(18-87); $P=0.048$]. (D) Correlation between primary lesion and MSI status in CRC. MSS were located

predominantly in the left hemi-colon (75.5 %), whereas more than half of the MSI colorectal cancers were found in the right (59.1%, $P < 0.001$). (E) Correlation between MTV and MSI status in CRC. MTV was significantly higher in tumours with MSI than in those with MSS (33.60 cm³ vs. 18.08 cm³; $P = 0.004$). (F) Correlation between primary tumour TLG and MSI status in CRC. TLG was significantly higher in tumours with MSI than in those with MSS (229.03 g vs. 134.26 g; $P = 0.010$).

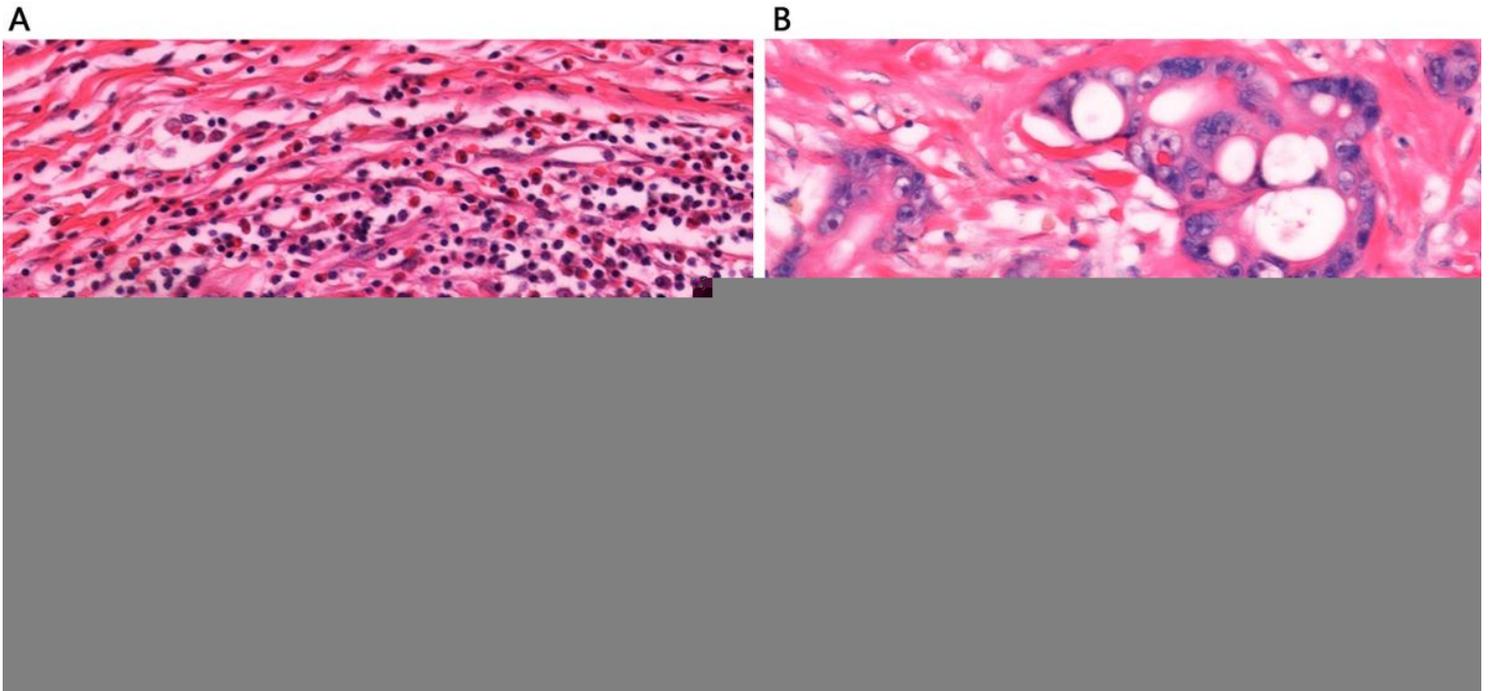


Figure 3

Representative pictures of intratumoral inflammatory cell infiltration by Hematoxylin-Eosin staining ($\times 20$). (A) High density of inflammatory cell infiltration in MSI-H CRC. (B) Low density of inflammatory cell infiltration in MSS CRC.

Figure 4

Representative Immunohistochemical detection of CD8 and CD3 ($\times 20$) expression in slides of MSI-H CRC (A) and MSS CRC (B).

A

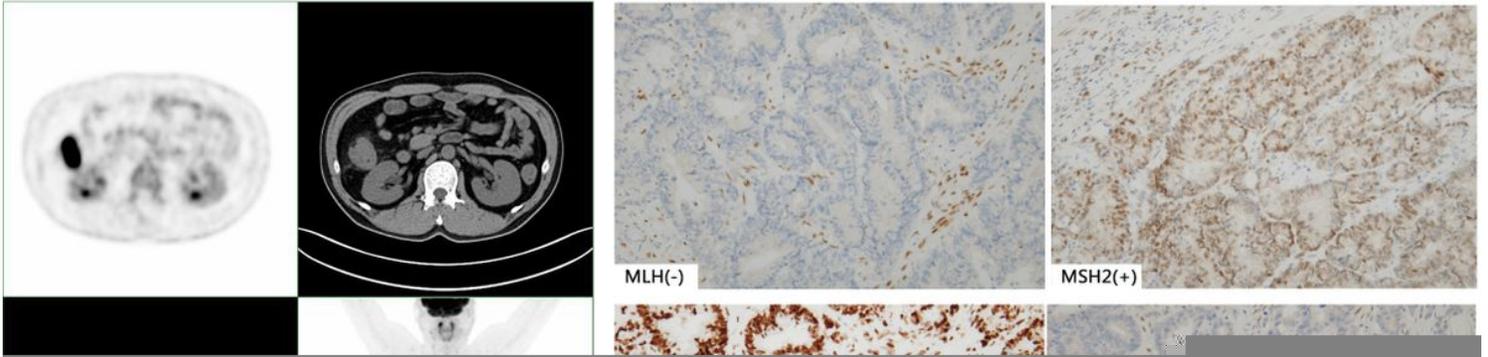


Figure 5

(A) a 45-year-old male had right colon cancer with score of 5.62 using the MSI-predicting model. 18F-FDG-PET/CT scans showed intense accumulation of 18F-FDG in the tumour (arrow; MTV,37.26cm³; TLG,494.44g). IHC analysis revealed MSI-H(MLH1(-) and PMS2(-)) after surgical resection. Scale bars, 100 mm. (B) a 55-year-old male had sigmoid colon cancer with score of 0 using the MSI-predicting model. 18F-FDG-PET/CT scans showed high accumulation of 18F-FDG in the tumour (arrow; MTV, 12.11cm³; TLG,114.91g). IHC analysis revealed MSS after surgical resection. Scale bars, 100 mm.