Serum tumour markers combined with clinicopathological characteristics for predicting MMR and KRAS status in 2279 Chinese colorectal cancer patients: a retrospective analysis

Ning Zhao

Wuhan Union Hospital

Yinghao Cao

Wuhan Union Hospital

Jia Yang

Wuhan Union Hospital

Hang Li

Wuhan Union Hospital

Ke Wu

Wuhan Union Hospital

Jiliang Wang

Wuhan Union Hospital

Tao Peng

Wuhan Union Hospital

Kailin Cai (✉ caikailin@hust.edu.cn)

Wuhan Union Hospital <https://orcid.org/0000-0002-2572-0907>

Research article

Keywords: KRAS, MMR, Serum Tumour Markers, Clinicopathological Characteristics, Prediction

Posted Date: December 22nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-132443/v1>

License: (cc) (i) This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Although serum tumour markers (STMs), clinicopathological characteristics and the status of KRAS and MMR play an important role in optimizing the treatment and improving the prognosis of colorectal cancer, their interrelationships remain largely unknown.

Methods

A retrospective analysis of 2279 patients who underwent KRAS or MMR status testing and STM measurements prior to treatment over the past four years was conducted. Univariate and multivariate logistic regression were performed to identify independent predictive factors of KRAS and MMR status. The area under receiver operating characteristic (ROC) curve (AUC) was used to evaluate the predictive value of individual and combined factors.

Results

Of the 784 patients tested for KRAS and 2279 patients tested for MMR status, KRAS mutations and dMMR were identified in 276 patients (35.20%) and 177 patients (7.77%), respectively. Logistic regression analysis demonstrated that right colon, well and moderate differentiation and negative CA19-9 were independent predictors for KRAS mutations. The ROC curve yielded an AUC of 0.609 for the combination of the three factors. Age < 65 was an independent predictive factor for dMMR, along with tumour size > 4.6 cm, right colon, poor differentiation, harvested lymph nodes ≥ 22 , no lymph node metastasis, no perineural invasion, negative CEA and positive CA72-4. When the nine criteria were used together, the AUC was 0.849.

Conclusion

Both STMs and clinicopathological characteristics were found to be significantly associated with the status of KRAS and MMR. The combination of these two factors possessed a strong predictive power for targeted genes among CRC patients.

Introduction

Colorectal cancer (CRC) is the third most common type of cancer and the second leading cause of cancer-related death worldwide[1]. CRC imposes a substantial burden on the healthcare system, with the direct costs of CRC accounting for close to 10% and 12% of all direct cancer-related costs across the European Union[2] and the United States[3], respectively. It has been estimated that more than 20% of patients present with metastatic CRC, and approximately half of patients with localized CRC will develop

metastases[4]. In the majority of mCRCs, tumour lesions tend to be unresectable, and chemotherapy is recommended to prolong survival and improve symptoms. Fluoropyrimidine-based chemotherapy regimens and monoclonal antibodies directed against epidermal growth factor receptor are approved for first-line treatment of the disease. Molecular testing for KRAS and mismatch repair (MMR) status are mandatory to optimize the choice and sequencing of therapy[5].

Kirsten rat sarcoma viral oncogene (KRAS) is located downstream of EGFR signals, and KRAS mutation leads to its constitutive activation[6], which makes advanced colon cancer less responsive to anti-EGFR monoclonal antibodies such as cetuximab and panitumumab[7, 8]. Mismatch repair (MMR) proteins are responsible for length alterations in microsatellites as they correct strand alignment and base matching errors during DNA replication[9]. CRC patients with mismatch repair deficiency (dMMR) are not only highly likely to have a better prognosis[10] and higher incidence of Lynch syndrome[11] but are less likely to benefit from 5-FU-based chemotherapy[12, 13] and immune checkpoint blockade[14]. However, translating genetic testing into routine clinical practice is frequently hindered by many barrier factors, such as the high cost of testing, the lack of preoperative tumour tissue and the need for specialized clinical laboratories[15–17]. These difficulties are particularly obvious in developing countries, especially in county-level hospitals. Therefore, there is an urgent need to develop a convenient, noninvasive and cost-effective modality to identify appropriate candidates for genetic testing.

Previous studies have demonstrated that serum tumour markers (STMs) and clinicopathological characteristics are both important prognostic factors as well as indicators of the therapeutic effect and recurrence risk in patients with CRC[18–20], while their association with KRAS and MMR status is largely unknown. In this study, we explored the predictive value of STMs in combination with clinicopathological indicators for KRAS and MMR status across East Asian CRC patients.

Methods

Study design and patient cohort

From January 6, 2016, through December 10, 2019, a total of 5457 patients were diagnosed with CRC in our centre. All patients were subjected to thorough history taking, and their information was collected from “Biological big data platform for individualized diagnosis and treatment of gastrointestinal cancer” (national software copyright 2019SR1267841). This study protocol was approved by the ethics committee of our college.

A flow diagram for the screening of eligible CRC participants is presented in Figure 1. A total of 2521 patients with MMR or KRAS testing were identified. A total of 242 patients with the following conditions were excluded from the study: (1) 211 patients underwent neo-chemoradiotherapy before KRAS and MMR status detection; and (2) 31 patients did not have data for STMs. Tumour stage was classified according to the 8th edition of the American Joint Committee on Cancer Staging System.

KRAS mutation analysis

The primers for the amplification and Sanger dideoxy chain termination sequencing of KRAS gene exon 2 were forward 5'-GTCCTGCACCAGTAATATGC-3' and reverse 5'-ATGTTCTAATATAGTCACATTTTC-3' for exons 3 and 4. Polymerase chain reaction (PCR) was performed using 100 ng of genomic DNA as a template. Each mixture contained 10 pmol of each primer. The reactions were performed in a total volume of 31.5 µL. The amplification reaction was as follows: an initial denaturing cycle of 95 °C for 5 min; 45 cycles of 94 °C for 25 s, 58 °C for 25 s, 72 °C for 25 s; and a final extension cycle at 72 °C for 10 min. The PCR products were then purified and subjected to direct sequencing using an automatic sequencer (ABI-3730 DNA Sequencer; Life Technologies, CA). Tumours with any KRAS mutations were classified as mutant KRAS, whereas the rest were classified as wild-type KRAS. Representative histological images of two patients with KRAS mutant or wild-type CRC are shown in Figure 2.

Immunohistochemical analysis of MMR status

Immunohistochemical staining was performed by the streptavidin-biotin-peroxidase detection method. First, CRC tissues fixed with 4% formaldehyde and embedded with paraffin were cut into 5 µm thick slices that were fixed onto glass slides. After rehydration with ethanol and microwave antigen retrieval, tissue sections were labelled with anti-MSH2 antibody (clone G219, 1:100, Cellmark, Rocklin, CA, USA), anti-MSH6 antibody (clone 44, prediluted; Ventana Medical Systems), anti-MLH1 antibody (clone M1, prediluted; Ventana Medical Systems) or anti-PMS2 antibody (clone mrq-28, 1:200; Cellmark) overnight at 4 °C. After washing with PBS, slides were incubated with the specific HRP-conjugate antibody at 37 °C for 10 min, cleaned with cold PBS and treated with peroxidase-conjugated biotin streptavidin complex for 10 min. Finally, staining was performed with DAB and counterstaining was performed with Mayer's haematoxylin. Binary interpretation was used to determine whether MMR was deficient or proficient as follows: tumours displaying loss of expression of one or more MMR proteins were considered to be dMMR, whereas tumours with intact MMR proteins were classified as pMMR.

STMs measurements

Prior to any anticancer treatment, blood samples were obtained through peripheral venipuncture, and STMs were detected by a commercial chemiluminescence immunoassay kit (Abbott Laboratories, I4000, America). The detected STMs included carcinoembryonic antigen (CEA), alpha fetoprotein (AFP), squamous cell carcinoma antigen (SCC), neuron-specific enolase (NSE), carbohydrate antigen (CA) 72-4, CA 125, CA 15-3, CA 19-9, ferritin (FERR) and soluble fragment of cytokeratin 19 (CYFRA21-1), which had threshold values of 5 µg/L, 8.78 µg/L, 1.5 ng/ml, 16.3 µg/L, 6.9 U/ml, 35 U/ml, 31.3 U/ml, 37 U/ml, 275 µg/L (male) or 204 µg/L (female), 2.5 ng/ml, respectively. Tumour marker values above these thresholds were considered positive.

Statistical analysis

Statistical analysis was performed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). Data are presented as numbers and percentages for categorical variables, and continuous data are expressed as the mean ± standard deviation, unless otherwise specified. Patient characteristics were compared using t tests for

continuous variables and χ^2 or Fisher exact tests for categorical variables. All candidate predictors with a $P < 0.05$ in univariate analysis were included in a multivariate logistic regression model. The discrimination ability of individual and combined factors was measured by the area under the ROC (receiver operating characteristic) curve (AUC). A value of $P < 0.05$ was considered significant.

Results

Patient clinical characteristics

Among the 2279 recruited CRC patients, the number of participants tested for KRAS and MMR was 784 and 2279, respectively. The characteristics are summarized based on whether the patients were tested for KRAS or MMR (Table 1). Of the 784 patients tested for KRAS and 2279 patients tested for MMR status, KRAS mutations and dMMR were identified in 276 patients (35.20%) and 177 patients (7.77%), respectively.

KRAS mutation is correlated with STMs and clinicopathological features

The STMs and clinicopathological features of the CRC patients are summarized in Table 1 based on KRAS status. KRAS mutations were found to be more frequent in non-adenocarcinoma (43.56% vs. 32.30%, $P = 0.005$), right colon (42.04% vs. 32.09%, $P = 0.009$), well and moderately differentiated tumours (36.86% vs. 22.31%, $P = 0.003$), positive CEA (39.76% vs. 32.05%, $P = 0.033$), and positive CA19-9 (44.78% vs. 32.78%, $P = 0.006$).

In addition to the established significance in metastatic colorectal cancer, it was also reported that KRAS mutations were correlated with a worse prognosis in stage II/III CRC[21, 22]. Therefore, we further explored the correlation between KRAS status and STMs and clinicopathological features in stage II/III CRC (Table 2). The results demonstrated that KRAS mutation was highly correlated with non-adenocarcinoma (45.21% vs. 32.54%, $P = 0.003$), right colon (43.04% vs. 32.47%, $P = 0.008$), well and moderately differentiated tumour (38.0% vs. 22.12%, $P = 0.002$), and positive CA 19-9 (45.10% vs. 33.46%, $P = 0.011$) but not associated with CEA (40.13% vs. 32.70%, $P = 0.054$).

DMMR is associated with STMs and clinicopathological features

The STMs and clinicopathological features of the CRC patients are summarized in Table 1 according to MMR status. DMMR was more prone to occur in younger patients (8.72% vs. 5.26%, $P = 0.008$); in non-adenocarcinoma (10.59% vs. 6.81%, $P = 0.005$); and in tumours with larger diameters (14.58% vs. 3.60%, $P < 0.001$), right colon (20.28% vs. 3.67%, $P < 0.001$), low differentiation (13.20% vs. 5.86%, $P < 0.001$), deeper invasion (8.38% vs. 4.94%, $P = 0.025$), more harvested lymph nodes (14.90% vs. 4.62%, $P < 0.001$), fewer positive lymph nodes (10.73% vs. 4.17%, $P < 0.001$), no peripheral nerve invasion (10.33% vs. 2.33%, $P < 0.001$), no lymphovascular invasion (8.42% vs. 5.69%, $P = 0.047$), CEA-negative status (8.82% vs. 6.12%, $P = 0.022$) and CA72-4-positive status (14.47% vs. 6.27%, $P < 0.001$).

MMR status is an important factor when deciding whether to use adjuvant chemotherapy for patients with stage II CRC[5] and is a significant prognostic indicator in stage III CRC patients with recurrence after adjuvant chemotherapy[23]. Therefore, we further analysed whether dMMR was associated with clinicopathological features and STMs in stage II/III CRC (Table 2). The results were similar to those for the whole CRC population, except for T stage, which was not associated with dMMR in stage II/III CRC.

Predictive value of STMs in combination with clinicopathological features for KRAS mutation

For the whole CRC population, univariate logistic regression analysis demonstrated that histology type, tumour location, degree of differentiation, and CEA and CA 19-9 levels were significantly associated with KRAS mutations (Table 3). When these predictive factors were subsequently assessed in the multivariate logistic regression, all except for non-adenocarcinoma and CEA remained highly significant. Therefore, right colon was found to be an independent predictor of KRAS mutations (OR, 1.550; $P = 0.012$), along with well and moderate differentiation (OR, 2.203; $P = 0.001$) and negative CA19-9 (OR, 1.600; $P = 0.022$). The predictive potential of these factors using ROC curves is shown in Figure 2A. When the three indexes were used together, the AUC was 0.609.

For stage II/III CRC, univariate logistic regression analysis showed that histology type, tumour location, degree of differentiation, and CEA and CA19-9 levels were significantly correlated with KRAS mutations (Table 4). In the multivariate logistic regression analysis, non-adenocarcinoma (OR, 1.553; $P = 0.035$), right colon (OR, 1.626; $P = 0.008$), well and moderate differentiation (OR, 2.227; $P = 0.002$) and positive CA19-9 (OR, 1.591; $P = 0.030$) were independent factors for predicting KRAS mutations. In addition, the AUC was 0.622 when the four indexes were used together (Figure 2B).

Predictive value of STMs in combination with clinicopathological features for MMR status

For the whole CRC population, univariate logistic regression analysis showed that 12 potential predictors had a significant association with dMMR, including age, histology, tumour location, tumour size, degree of differentiation, T stage, harvested lymph nodes, positive lymph nodes, perineural invasion, lymphovascular invasion, CEA and CA 72-4. All these potential predictors were subsequently entered into a multivariate Cox regression analysis, and nine clinicopathological characteristics (age < 65 (OR, 1.923; $P = 0.006$), tumour size > 4.6 cm (OR, 2.646; $P < 0.001$), right colon (OR, 4.762; $P < 0.001$), poor differentiation (OR, 2.768; $P < 0.001$), harvested lymph nodes ≥ 22 (OR, 1.680; $P = 0.012$), no lymph node metastasis (OR, 2.924; $P < 0.001$), no perineural invasion (OR, 3.205; $P < 0.001$), negative CEA (OR, 1.667; $P = 0.017$) and positive CA 72-4 (OR, 1.901; $P = 0.006$) were finally selected as independent predictive factors for dMMR in the whole CRC patient cohort. When the nine criteria were used together, the AUC was 0.849 (Figure 2C).

For stage II/III CRC, all potential predictors were consistent with those for the whole CRC population, except for T stage, which was not associated with dMMR. In the multivariate logistic regression, non-adenocarcinoma ($P = 0.585$), lymphovascular invasion ($P = 0.354$) and CA72-4 ($P = 0.058$) were found to

be unrelated to dMMR, and the remaining eight indicators were identified as independent predictors for dMMR. When the eight criteria were used together, the AUC was 0.849 (Figure 2D).

A summary of the AUCs of the individual and combined assessments used to predict mutation status is presented in Table 7.

Discussion

CRC is the third most common cancer in men and the second most common cancer in women worldwide, accounting for approximately 10% of all cancer-related deaths.[24] To minimize the side effects of current treatments and achieve better results, tremendous progress has been achieved in targeted therapy for CRC over recent decades. The status of KRAS and MMR was reported to be significantly correlated with the clinical outcomes of target therapy[7, 8, 12–14]. For example, KRAS mutations make CRC less responsive to anti-EGFR monoclonal antibodies[7, 8], and dMMR makes CRC less likely to benefit from 5-FU-based chemotherapy[12, 13] and immune checkpoint blockade[14]. However, the rate of KRAS and MMR detection was far below expected, mainly due to the following aspects: 1) a significant part of the population in developing countries cannot afford the high cost of gene testing; 2) for unresectable mCRC, endoscopic biopsy and cytology of peritoneal washing may not provide high-quality samples or sufficient tumour cells for detection of KRAS mutation and MMR status after their initial use for histologic detection to first confirm the cancer type[15, 16]; 3) as a result of significantly uneven distribution of medical resources in China, the qualified clinical laboratory and professional team required for gene testing are not available in county-level hospitals; and 4) the values of KRAS and MMR status are poorly understood at basic-level hospitals or in underdeveloped areas. In contrast, STMs and histopathological characteristics can be quickly and accurately detected in county-level or province-level hospitals at a low cost.

In this study, we explored the interrelationships among STMs, histopathological characteristics, and MMR and KRAS status using data from 2279 participants. Of the 784 patients tested for KRAS and 2279 patients tested for MMR status, KRAS mutations and dMMR were identified in 276 patients (35.20%) and 177 patients (7.77%), respectively. The discriminative ability of clinicopathological characteristics in combination with STMs was 0.609 for KRAS mutations and 0.849 for dMMR in the whole population. In addition, the combination of STMs and clinicopathological characteristics yielded an AUC of 0.622 for KRAS mutations and 0.849 for dMMR among TNM(II/III) participants.

Previous studies on the correlation between clinicopathological characteristics and KRAS mutations are controversial. Gao et al[18]. reported that no significant difference between KRAS mutations and tumour locations was observed, whereas Wilson et al[25]. and Julien et al[26]. supported that right-side CRC has a higher KRAS mutation rate. In our study, right colon (OR, 1.550; $P = 0.012$) and well and moderately differentiated tumours (OR, 2.203; $P = 0.001$) were independent predictive factors of KRAS mutations. STMs were reported to not be associated with KRAS mutations in several studies[21, 27, 28]. However, negative CA199 was found to be significantly correlated with KRAS mutations in our study. Furthermore,

the AUC was 0.609 when clinicopathological characteristics were combined with CA 19 – 9. The combination exceeds the discriminative ability of individual relevant factors, including right colon (AUC = 0.547), well and moderate differentiation (AUC = 0.545) and CA 19 – 9 (AUC = 0.544).

Previous studies have shown that MMR status was significantly correlated with clinicopathological characteristics of CRC[29–31], including tumour location, degree of differentiation, perineural invasion, and number of harvested lymph nodes, which are consistent with our results. In our study, younger age (OR, 1.923; P = 0.006), larger tumour (OR, 2.646; P < 0.001), and fewer positive lymph nodes (OR, 2.924; P < 0.001) were also independent predictive factors for dMMR. However, the existing evidence of the correlation between MMR status and STMs is controversial. Fan et al[31]. reported that dMMR was not associated with CEA, CA72-4, CA 242 and CA 19 – 9, but Schieman et al[32]. reported that patients with high microsatellite instability had lower preoperative CEA serum levels than those with microsatellite stability. In our study, dMMR was significantly associated with negative CEA and positive CA72-4. When STMs were combined with clinicopathological characteristics, the AUC increased from 0.837 to 0.849. This result indicates that clinicopathological characteristics in combination with STMs can improve the discriminative ability of previously confirmed relevant factors, such as tumour location (AUC = 0.715), degree of differentiation (AUC = 0.57), perineural invasion (AUC = 0.622), and number of harvested lymph nodes (AUC = 0.652).

This study's limitations deserve commentary. First, this was a nonrandomized retrospective analysis from a single centre, and as such, there were potential biases for comparison, such as patient inclusion and sample selection biases. Second, there is a lack of a validation group to further validate our results. Third, we did not evaluate the treatment response or perform a survival analysis according to clinical characteristics or serum tumour marker levels. However, our results demonstrated that clinicopathological characteristics in combination with STMs possessed a strong predictive power for KRAS and MMR status among CRC patients.

Conclusion

In conclusion, this is the largest retrospective study to investigate the interrelationship among KRAS mutations and dMMR, STMs and clinicopathological characteristics. KRAS mutations was significantly correlated with right colon, well and moderate differentiation and negative CA19-9. DMMR was significantly associated with younger age, larger tumours, right colon, poor differentiation, more harvested lymph nodes, fewer positive lymph nodes, no perineural invasion, negative CEA and positive CA72-4. The discriminative ability of clinicopathological characteristics combined with STMs reached 0.609 for KRAS mutations and 0.849 for dMMR. Radiomics signatures based on deep learning features have been used in previous studies to predict KRAS and MMR status and have resulted in remarkable accomplishments[31, 33–35]. Therefore, for those CRC patients who could not undergo genetic testing, our findings will hopefully be integrated with radiomics or other markers to achieve a stronger discrimination ability of KRAS and MMR status.

Abbreviations

CRC: colorectal cancer; KRAS: Kirsten rat sarcoma viral oncogene; MMR: mismatch repair proteins; pMMR: proficient MMR; dMMR: deficient MMR; STMs: serum tumour markers; ROC: receiver operating characteristic; AUC: the area under ROC curve; CEA: carcinoembryonic antigen; AFP: alpha fetoprotein; SCC: squamous cell carcinoma antigen; NSE: neuron-specific enolase; CA: carbohydrate antigen; FERR: ferritin; CYFRA21-1: soluble fragment of cytokeratin 19; TNM: tumour-node-metastasis classification

Declarations

Acknowledgements

Not applicable.

Authors Contributions

Kailin Cai and Tao Peng conceived and designed the study. Ning Zhao, Yinghao Cao, Jia Yang, Hang Li, Ke Wu, and Jiliang Wang collected and analyzed data. Ning Zhao and Yinghao Cao wrote the paper. Kailin Cai and Tao Peng reviewed and edited the manuscript. All authors read and approved the manuscript.

Availability of data and materials

The data used or analysed during this study are included in this article and available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Ethics approval was obtained from the Huazhong University of Science and Technology, and written informed consent was obtained from study participants.

Consent for publication

Not applicable.

Funding

This study was supported by grants from the Science and Technology Department of Hubei Province (No.2018CFC884) and Free innovation pre-research fund and platform scientific research fund (02.03.2019-111).

Competing interests

The authors have declared that no competing interests exist.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394-424.
2. Luengo-Fernandez R, Leal J, Gray A, Sullivan R: Economic burden of cancer across the European Union: a population-based cost analysis. *Lancet Oncol.* 2013;14(12):1165-1174.
3. Ansa BE, Coughlin SS, Alema-Mensah E, Smith SA: Evaluation of Colorectal Cancer Incidence Trends in the United States (2000-2014). *J Clin Med.* 2018;7(2):
4. Siegel RL, Miller KD, Jemal A: Cancer Statistics, 2017. *CA Cancer J Clin.* 2017;67(1):7-30.
5. Benson AB, Venook AP, Al-Hawary MM, Cederquist L, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Engstrom PF *et al*: Rectal Cancer, Version 2.2018, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw.* 2018;16(7):874-901.
6. Yarden Y, Sliwkowski MX: Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol.* 2001;2(2):127-137.
7. Venook AP, Niedzwiecki D, Lenz HJ, Innocenti F, Fruth B, Meyerhardt JA, Schrag D, Greene C, O'Neil BH, Atkins JN *et al*: Effect of First-Line Chemotherapy Combined With Cetuximab or Bevacizumab on Overall Survival in Patients With KRAS Wild-Type Advanced or Metastatic Colorectal Cancer: A Randomized Clinical Trial. *JAMA.* 2017;317(23):2392-2401.
8. Stintzing S, Wirapati P, Lenz HJ, Neureiter D, Fischer von Weikersthal L, Decker T, Kiani A, Kaiser F, Al-Batran S, Heintges T *et al*: Consensus molecular subgroups (CMS) of colorectal cancer (CRC) and first-line efficacy of FOLFIRI plus cetuximab or bevacizumab in the FIRE3 (AIO KRK-0306) trial. *Ann Oncol.* 2019;30(11):1796-1803.
9. Karran P: Microsatellite instability and DNA mismatch repair in human cancer. *Semin Cancer Biol.* 1996;7(1):15-24.
10. Wright CM, Dent OF, Newland RC, Barker M, Chapuis PH, Bokey EL, Young JP, Leggett BA, Jass JR, Macdonald GA: Low level microsatellite instability may be associated with reduced cancer specific survival in sporadic stage C colorectal carcinoma. *Gut.* 2005;54(1):103-108.
11. Liu B, Parsons R, Papadopoulos N, Nicolaides NC, Lynch HT, Watson P, Jass JR, Dunlop M, Wyllie A, Peltomaki P *et al*: Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. *Nat Med.* 1996;2(2):169-174.
12. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, French AJ, Kabat B, Foster NR, Torri V *et al*: Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol.* 2010;28(20):3219-3226.
13. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, Hamilton SR, Laurent-Puig P, Gryfe R, Shepherd LE *et al*: Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med.* 2003;349(3):247-257.

14. Diaz LA, Jr., Le DT: PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med*. 2015;373(20):1979.
15. Eriksson J, Amonkar M, Al-Jassar G, Lambert J, Malmenas M, Chase M, Sun L, Kollmar L, Vichnin M: Mismatch Repair/Microsatellite Instability Testing Practices among US Physicians Treating Patients with Advanced/Metastatic Colorectal Cancer. *J Clin Med*. 2019;8(4):
16. Sperber NR, Andrews SM, Voils CI, Green GL, Provenzale D, Knight S: Barriers and Facilitators to Adoption of Genomic Services for Colorectal Care within the Veterans Health Administration. *J Pers Med*. 2016;6(2):
17. Wood ME, Kadlubek P, Pham TH, Wollins DS, Lu KH, Weitzel JN, Neuss MN, Hughes KS: Quality of cancer family history and referral for genetic counseling and testing among oncology practices: a pilot test of quality measures as part of the American Society of Clinical Oncology Quality Oncology Practice Initiative. *J Clin Oncol*. 2014;32(8):824-829.
18. Gao Y, Wang J, Zhou Y, Sheng S, Qian SY, Huo X: Evaluation of Serum CEA, CA19-9, CA72-4, CA125 and Ferritin as Diagnostic Markers and Factors of Clinical Parameters for Colorectal Cancer. *Sci Rep*. 2018;8(1):2732.
19. Thomsen M, Skovlund E, Sorbye H, Bolstad N, Nustad KJ, Glimelius B, Pfeiffer P, Kure EH, Johansen JS, Tveit KM *et al*: Prognostic role of carcinoembryonic antigen and carbohydrate antigen 19-9 in metastatic colorectal cancer: a BRAF-mutant subset with high CA 19-9 level and poor outcome. *Br J Cancer*. 2018;118(12):1609-1616.
20. Pesta M, Kucera R, Topolcan O, Karlikova M, Houfkova K, Polivka J, Macanova T, Machova I, Slouka D, Kulda V: Plasma microRNA Levels Combined with CEA and CA19-9 in the Follow-Up of Colorectal Cancer Patients. *Cancers (Basel)*. 2019;11(6):
21. Deng Y, Wang L, Tan S, Kim GP, Dou R, Chen D, Cai Y, Fu X, Wang L, Zhu J *et al*: KRAS as a predictor of poor prognosis and benefit from postoperative FOLFOX chemotherapy in patients with stage II and III colorectal cancer. *Mol Oncol*. 2015;9(7):1341-1347.
22. Auclin E, Zaanani A, Vernerey D, Douard R, Gallois C, Laurent-Puig P, Bonnetain F, Taieb J: Subgroups and prognostication in stage III colon cancer: future perspectives for adjuvant therapy. *Ann Oncol*. 2017;28(5):958-968.
23. Zaanani A, Shi Q, Taieb J, Alberts SR, Meyers JP, Smyrk TC, Julie C, Zawadi A, Tabernero J, Mini E *et al*: Role of Deficient DNA Mismatch Repair Status in Patients With Stage III Colon Cancer Treated With FOLFOX Adjuvant Chemotherapy: A Pooled Analysis From 2 Randomized Clinical Trials. *JAMA Oncol*. 2018;4(3):379-383.
24. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A: Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65(2):87-108.
25. Gonsalves WI, Mahoney MR, Sargent DJ, Nelson GD, Alberts SR, Sinicrope FA, Goldberg RM, Limburg PJ, Thibodeau SN, Grothey A *et al*: Patient and tumor characteristics and BRAF and KRAS mutations in colon cancer, NCCTG/Alliance N0147. *J Natl Cancer Inst*. 2014;106(7):

26. Taieb J, Le Malicot K, Shi Q, Penault-Llorca F, Bouche O, Tabernero J, Mini E, Goldberg RM, Folprecht G, Luc Van Laethem J *et al*: Prognostic Value of BRAF and KRAS Mutations in MSI and MSS Stage III Colon Cancer. *J Natl Cancer Inst*. 2017;109(5):
27. Connell LC, Boucher TM, Chou JF, Capanu M, Maldonado S, Kemeny NE: Relevance of CEA and LDH in relation to KRAS status in patients with unresectable colorectal liver metastases. *J Surg Oncol*. 2017;115(4):480-487.
28. Gao XH, Yu GY, Gong HF, Liu LJ, Xu Y, Hao LQ, Liu P, Liu ZH, Bai CG, Zhang W: Differences of protein expression profiles, KRAS and BRAF mutation, and prognosis in right-sided colon, left-sided colon and rectal cancer. *Sci Rep*. 2017;7(1):7882.
29. Nakayama Y, Iijima T, Wakaume R, Takahashi K, Matsumoto H, Nakano D, Miyaki M, Yamaguchi T: Microsatellite instability is inversely associated with type 2 diabetes mellitus in colorectal cancer. *PLoS One*. 2019;14(4):e0215513.
30. Baek DW, Kang BW, Lee SJ, Kim HJ, Park SY, Park JS, Choi GS, Baek JH, Kim JG: Clinical Implications of Mismatch Repair Status in Patients With High-risk Stage II Colon Cancer. *In Vivo*. 2019;33(2):649-657.
31. Fan S, Li X, Cui X, Zheng L, Ren X, Ma W, Ye Z: Computed Tomography-Based Radiomic Features Could Potentially Predict Microsatellite Instability Status in Stage II Colorectal Cancer: A Preliminary Study. *Acad Radiol*. 2019;26(12):1633-1640.
32. Schieman U, Gunther S, Gross M, Henke G, Muller-Koch Y, Konig A, Muders M, Folwaczny C, Mussack T, Holinski-Feder E: Preoperative serum levels of the carcinoembryonic antigen in hereditary non-polyposis colorectal cancer compared to levels in sporadic colorectal cancer. *Cancer Detect Prev*. 2005;29(4):356-360.
33. Wu X, Li Y, Chen X, Huang Y, He L, Zhao K, Huang X, Zhang W, Huang Y, Li Y *et al*: Deep Learning Features Improve the Performance of a Radiomics Signature for Predicting KRAS Status in Patients with Colorectal Cancer. *Acad Radiol*. 2020;
34. Yang L, Dong D, Fang M, Zhu Y, Zang Y, Liu Z, Zhang H, Ying J, Zhao X, Tian J: Can CT-based radiomics signature predict KRAS/NRAS/BRAF mutations in colorectal cancer? *Eur Radiol*. 2018;28(5):2058-2067.
35. Lovinfosse P, Polus M, Van Daele D, Martinive P, Daenen F, Hatt M, Visvikis D, Koopmansch B, Lambert F, Coimbra C *et al*: FDG PET/CT radiomics for predicting the outcome of locally advanced rectal cancer. *Eur J Nucl Med Mol Imaging*. 2018;45(3):365-375.

Tables

Table1 Associations of clinical characteristics with MMR and KRAS status in all participants.

	MMR			KRAS		
	pMMR	dMMR	P-Value	Wild-Type	Mutant-Type	P-Value
Age (years)			0.008			0.205
<65	1508 (91.28)	144 (8.72)		364 (66.30)	185 (33.70)	
>=65	594 (94.74)	33 (5.26)		144 (61.28)	91 (38.72)	
Gender			0.914			0.43
Female	840 (92.31)	70 (7.69)		197 (63.14)	115 (36.86)	
Male	1262 (92.18)	107 (7.82)		311 (65.89)	161 (34.11)	
Pathological type			0.005			0.005
non-adenocarcinoma	515 (89.41)	61 (10.59)		114 (56.44)	88 (43.56)	
adenocarcinoma	1587 (93.19)	116 (6.81)		394 (67.70)	188 (32.30)	
Tumor size			<0.001			0.447
<=4.6cm	1364 (96.40)	51 (3.60)		288 (63.58)	165 (36.42)	
>4.6cm	738 (85.42)	126 (14.58)		220 (66.47)	111 (33.53)	
Tumor location			<0.001			0.009
Right	448 (79.72)	114 (20.28)		142 (57.96)	103 (42.04)	
Left	1654 (96.33)	63 (3.67)		366 (67.90)	173 (32.09)	
Degree of differentiation			<0.001			0.003
Poor	263 (86.80)	40 (13.20)		94 (77.69)	27 (22.31)	
Well/Moderate	1719 (94.14)	107 (5.86)		382 (63.14)	223 (36.86)	
T stage			0.025			0.229
I/II	385 (95.06)	20 (4.94)		72 (70.59)	30 (29.41)	
III/IV	1717	157 (8.38)		436	246	

	(91.62)		(63.93)	(36.07)	
Harvested lymph nodes			<0.001		0.842
<22	1508 (95.38)	73 (4.62)		318 (65.16)	170 (34.84)
>=22	594 (85.10)	104 (14.90)		190 (64.19)	106 (35.81)
Lymph nodes metastasis			<0.001		0.448
No	1115 (89.27)	134 (10.73)		279 (66.11)	143 (33.89)
Yes	987 (95.83)	43 (4.17)		229 (63.26)	133 (36.74)
Peripheral nerve invasion			<0.001		0.471
No	1389 (89.67)	160 (10.33)		351 (65.73)	183 (34.27)
Yes	713 (97.67)	17 (2.33)		157 (62.80)	93 (37.20)
Lymphovascular invasion			0.047		0.755
No	1588 (91.58)	146 (8.42)		402 (65.15)	215 (34.84)
Yes	514 (94.31)	31 (5.69)		106 (63.47)	61 (36.53)
CEA			0.022		0.033
Negative	1189 (91.18)	115 (8.82)		301 (67.95)	142 (32.05)
Positive	875 (93.88)	57 (6.12)		197 (60.24)	130 (39.76)
AFP			1		1
Negative	2041 (92.27)	171 (7.73)		64.66	35.34
Positive	23 (95.83)	1 (4.17)		66.67	33.33
SCC			1		0.337
Negative	1943 (92.30)	162 (7.70)		460 (64.16)	257 (35.84)
Positive	121 (92.37)	10 (7.63)		38 (71.70)	15 (28.30)

NSE		0.559			0.419
Negative	1352 (92.04)	117 (7.96)	295 (63.44)	170 (36.56)	
Positive	712 (92.83)	55 (7.17)	203 (66.56)	102 (33.44)	
CA 72-4		<0.001			0.855
Negative	1733 (93.73)	116 (6.27)	396 (64.92)	214 (35.08)	
Positive	331 (85.53)	56 (14.47)	102 (63.75)	58 (36.25)	
CA 125		0.479			0.179
Negative	1865 (92.46)	152 (7.54)	441 (63.82)	250 (36.18)	
Positive	199 (90.87)	20 (9.13)	57 (72.15)	22 (27.85)	
CA 15-3		0.214			1
Negative	2062 (92.34)	171 (7.66)	497 (64.63)	272 (35.37)	
Positive	2 (66.67)	1 (33.33)	1 (100)	0 (0)	
CA 19-9		1			0.006
Negative	1671 (92.32)	139 (7.68)	408 (67.22)	199 (32.78)	
Positive	393 (92.25)	33 (7.75)	90 (55.21)	73 (44.78)	
FERR		0.143			0.181
Negative	1942 (92.08)	167 (7.92)	467 (64.06)	262 (35.94)	
Positive	122 (96.06)	5 (3.94)	31 (75.61)	10 (24.39)	
CYFRA21-1		1			0.9
Negative	1525 (92.31)	127 (7.69)	366 (64.89)	198 (35.11)	
Positive	539 (92.29)	45 (7.71)	132 (64.08)	74 (35.92)	
Values presented are n (%) unless otherwise noted.					

Table 2. Associations of clinical characteristics with MMR and KRAS status among TNM (II/III) participants.

Characterization	MMR			KRAS		
	pMMR	dMMR	P-Value	Wild-Type	Mutant-Type	P-Value
Age (years)			0.023			0.172
<65	1255 (90.94)	125 (9.06)		317 (65.77)	165 (34.23)	
>=65	507 (94.24)	31 (5.76)		126 (60.00)	84 (40.00)	
Gender			0.976			0.162
Female	692 (91.78)	62 (8.22)		165 (60.66)	107 (39.34)	
Male	1070 (91.92)	94 (8.08)		278 (66.19)	142 (33.81)	
Pathological type			0.021			0.003
non-adenocarcinoma	444 (89.33)	53 (10.66)		103 (54.79)	85 (45.21)	
adenocarcinoma	1318 (92.75)	103 (7.25)		340 (67.46)	164 (32.54)	
Tumor size			<0.001			0.212
<=4.6cm	1072 (96.75)	36 (3.25)		233 (61.80)	144 (38.20)	
>4.6cm	690 (85.19)	120 (14.81)		210 (66.67)	105 (33.33)	
Tumor location			<0.001			0.008
Right	411 (79.96)	103 (20.04)		131 (56.96)	99 (43.04)	
Left	1351 (96.23)	53 (3.77)		312 (67.53)	150 (32.47)	
Degree of differentiation			<0.001			0.002
Poor	241 (87.32)	35 (12.68)		88 (77.88)	25 (22.12)	
Well/Moderate	1408 (93.87)	92 (6.13)		323 (62.00)	198 (38.00)	
T stage			0.077			0.732
I/II	82 (97.62)	2 (2.38)		16 (69.57)	7 (30.43)	
III/IV	1680	154 (8.40)		427	242	

	(91.60)		(63.83)	(36.17)	
Harvested lymph nodes			<0.001		0.976
<22	1229 (95.27)	61 (4.73)		267 (64.18)	149 (35.82)
>=22	533 (84.87)	95 (15.13)		176 (63.77)	100 (36.23)
Lymph nodes metastasis			<0.001		0.78
No	798 (87.40)	115 (12.60)		218 (64.69)	119 (35.31)
Yes	964 (95.92)	41 (4.08)		225 (63.38)	130 (36.62)
Peripheral nerve invasion			<0.001		0.56
No	1086 (88.51)	141 (11.49)		294 (64.90)	159 (35.10)
Yes	676 (97.83)	15 (2.17)		149 (62.34)	90 (37.66)
Lymphovascular invasion			0.028		1
No	1286 (91.01)	127 (8.99)		341 (64.10)	191 (35.90)
Yes	476 (94.26)	29 (5.74)		102 (63.75)	58 (36.25)
CEA			0.013		0.054
Negative	926 (90.52)	97 (9.48)		249 (67.30)	121 (32.70)
Positive	807 (93.73)	54 (6.27)		185 (59.87)	124 (40.13)
AFP			1		1
Negative	1713 (91.95)	150 (8.05)		431 (63.85)	244 (36.15)
Positive	20 (95.24)	1 (4.76)		3 (75.00)	1 (25.00)
SCC			1		0.379
Negative	1630 (91.99)	142 (8.01)		402 (63.41)	232 (36.59)
Positive	103 (91.96)	9 (8.04)		32 (71.11)	13 (28.89)

NSE		0.68			0.569
Negative	1124 (91.76)	101 (8.24)	260 (62.95)	153 (37.05)	
Positive	609 (92.41)	50 (7.59)	174 (65.41)	92 (34.59)	
CA 72-4		<0.001			0.57
Negative	1439 (93.44)	101 (6.56)	346 (64.55)	190 (35.45)	
Positive	294 (85.47)	50 (14.53)	88 (61.54)	55 (38.46)	
CA 125		0.424			0.133
Negative	1557 (92.18)	132 (7.82)	379 (62.85)	224 (37.15)	
Positive	176 (90.26)	19 (9.74)	55 (72.37)	21 (27.63)	
CA 15-3		0.222			1
Negative	1731 (92.03)	150 (7.97)	433 (63.86)	245 (36.14)	
Positive	14 (66.67)	7 (33.33)	8 (61.5%)	5 (38.5)	
CA 19-9		1			0.011
Negative	1378 (91.99)	120 (8.01)	350 (66.54)	176 (33.46)	
Positive	355 (91.97)	31 (8.03)	84 (54.90)	69 (45.10)	
FERR		0.124			0.316
Negative	1628 (91.72)	147 (8.28)	407 (63.40)	235 (36.60)	
Positive	105 (96.33)	4 (3.67)	27 (72.97)	10 (27.03)	
CYFRA21-1		0.776			1
Negative	1253 (92.13)	107 (7.87)	309 (63.84)	175 (36.16)	
Positive	480 (91.60)	44 (8.40)	125 (64.10)	70 (35.90)	
Values presented are n (%) unless otherwise noted.					

Table 3. Univariate and multivariate analyses of various predictive factors for KRAS status in all participants.

	Univariate Analysis OR (95% CI)	P-Value	Multivariate Analysis OR (95%CI)	P-Value
Age (years)		0.177		
<65	reference			
>=65	1.243 (0.905~1.705)			
Gender		0.43		
Female	reference			
Male	0.887 (0.658~1.196)			
Pathological type		0.004		0.067
non-adenocarcinoma	reference		reference	
adenocarcinoma	0.618 (0.445~0.859)		0.696 (0.472~1.028)	
Tumor size		0.403		
<=4.6cm	reference			
>4.6cm	0.881 (0.653~1.185)			
Tumor location		0.007		0.012
Right	reference		reference	
Left	0.652 (0.477~0.890)		0.645 (0.458~0.909)	
Degree of differentiation		0.002		0.001
Well/Moderate	reference		reference	
Poor	0.492 (0.306~0.768)		0.454 (0.278~0.721)	
T stage		0.190		
I/II	reference			
III/IV	1.354 (0.868~2.158)			
Harvested lymph nodes		0.782		
<22	reference			
>=22	1.044 (0.771~1.410)			
Lymph nodes metastasis		0.404		
No	reference			
Yes	1.133 (0.845~1.520)			

Peripheral nerve invasion		0.423	
No	reference		
Yes	1.136 (0.830~1.551)		
Lymphovascular invasion		0.687	
No	reference		
Yes	1.076 (0.751~1.532)		
CEA		0.027	0.321
Negative	reference		reference
Positive	1.399 (1.038~1.885)		1.184 (0.848~1.650)
AFP		0.918	
Negative	reference		
Positive	0.915 (0.126~4.718)		
SCC		0.270	
Negative	reference		
Positive	0.707 (0.370~1.283)		
NSE		0.376	
Negative	reference		
Positive	0.872 (0.642~1.180)		
CA 72-4		0.783	
Negative	reference		
Positive	1.052 (0.729~1.508)		
CA 125		0.144	
Negative	reference		
Positive	0.681 (0.399~1.125)		
CA 15-3		0.980	
Negative	reference		
Positive	1.254 (0.652~1.674)		
CA 19-9		0.005	0.022
Negative	reference		reference

Positive	1.663 (1.168~2.364)	1.600 (1.068~2.394)
FERR		0.137
Negative	reference	
Positive	0.575 (0.264~1.152)	
CYFRA21-1		0.834
Negative	reference	
Positive	1.036 (0.741~1.443)	
Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval;		
* Items were included in the multivariate analysis only when P value <0.05 in univariate analysis.		

Table 4. Univariate and multivariate analyses of various predictive factors for KRAS status in TNM(II/III) participants.

	Univariate Analysis OR (95% CI)	P-Value	Multivariate Analysis OR (95%CI)	P-Value
Age (years)		0.147		
<65	reference			
>=65	1.281 (0.916~1.787)			
Gender		0.139		
Female	reference			
Male	0.788 (0.574~1.081)			
Pathological type		0.020		0.035
non-adenocarcinoma	reference		reference	
adenocarcinoma	0.584 (0.415~0.824)		0.644 (0.427~0.972)	
Tumor size		0.185		
<=4.6cm	reference			
>4.6cm	0.809 (0.591~1.106)			
Tumor location		0.006		0.008
Right	reference		reference	
Left	0.636 (0.459~0.881)		0.615 (0.430~0.882)	
Degree of differentiation		0.002		0.002
Well/Moderate	reference		reference	
Poor	0.463 (0.282~0.737)		0.449 (0.268~0.729)	
T stage		0.574		
I/II	reference			
III/IV	1.295 (0.545~3.410)			
Harvested lymph nodes		0.911		
<22	reference			
>=22	1.018 (0.741~1.397)			
Lymph nodes metastasis		0.720		
No	reference			
Yes	1.058 (0.776~1.445)			

Peripheral nerve invasion		0.505	
No	reference		
Yes	1.117 (0.806~1.545)		
Lymphovascular invasion		0.936	
No	reference		
Yes	1.015 (0.700~1.462)		
CEA		0.045	0.394
Negative	reference		reference
Positive	1.379 (1.007~1.890)		1.165 (0.819~1.655)
AFP		0.647	
Negative	reference		
Positive	0.589 (0.029~4.627)		
SCC		0.300	
Negative	reference		
Positive	0.704 (0.350~1.338)		
NSE		0.515	
Negative	reference		
Positive	0.899 (0.650~1.239)		
CA 72-4		0.505	
Negative	reference		
Positive	1.138 (0.775~1.661)		
CA 125		0.106	
Negative	reference		
Positive	0.646 (0.373~1.081)		
CA 15-3		0.981	
Negative	reference		
Positive	1.232 (0.631~1.586)		
CA 19-9		0.009	0.03
Negative	reference		reference

Positive	1.634 (1.131~2.355)	1.591 (1.044~2.422)
FERR		0.242
Negative	reference	
Positive	0.641 (0.291~1.308)	
CYFRA21-1		0.949
Negative	reference	
Positive	0.989 (0.697~1.395)	
Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval;		
* Items were included in the multivariate analysis only when P value <0.05 in univariate analysis.		

Table 5. Univariate and multivariate analyses of various predictive factors for MMR status in all participants.

	Univariate Analysis OR (95% CI)	P-Value	Multivariate Analysis OR (95%CI)	P-Value
Age (years)		0.006		0.006
<65	reference		reference	
>=65	0.582 (0.388~0.848)		0.520 (0.322~0.815)	
Gender		0.914		
Female	reference			
Male	1.017 (0.745~1.397)			
Pathological type		0.004		0.905
non-adenocarcinoma	reference		reference	
adenocarcinoma	0.617 (0.447~0.859)		1.029 (0.652~1.666)	
Tumor size		<0.001		<0.001
<=4.6cm	reference		reference	
>4.6cm	4.566 (3.279~6.448)		2.646 (1.721~4.123)	
Tumor location		<0.001		<0.001
Right	reference		reference	
Left	0.150 (0.108~0.206)		0.210 (0.138~0.316)	
Degree of differentiation		<0.001		<0.001
Well/Moderate	reference		reference	
Poor	2.443 (1.645~3.566)		2.768 (1.724~4.398)	
T stage		0.020		0.477
I/II	reference		reference	
III/IV	1.760 (1.118~2.923)		0.808 (0.455~1.477)	
Harvested lymph nodes		<0.001		0.012
<22	reference		reference	
>=22	3.617 (2.647~4.965)		1.680 (1.120~2.525)	
Lymph nodes metastasis		<0.001		<0.001
No	reference		reference	
Yes	0.363 (0.252~0.512)		0.342 (0.209~0.545)	

Peripheral nerve invasion		<0.001	<0.001
No	reference		reference
Yes	0.207 (0.120~0.334)		0.312 (0.167~0.552)
Lymphovascular invasion		0.039	0.507
No	reference		reference
Yes	0.656 (0.432~0.965)		1.212 (0.676~2.114)
CEA		0.019	0.017
Negative	reference		reference
Positive	0.674 (0.482~0.932)		0.600 (0.392~0.906)
AFP		0.522	
Negative	reference		
Positive	0.519 (0.029~2.485)		
SCC		0.979	
Negative	reference		
Positive	0.991 (0.479~1.835)		
NSE		0.504	
Negative	reference		
Positive	0.893 (0.636~1.239)		
CA 72-4		<0.001	0.006
Negative	reference		reference
Positive	2.528 (1.789~3.535)		1.901 (1.195~2.979)
CA 125		0.401	
Negative	reference		
Positive	1.233 (0.736~1.965)		
CA 15-3		0.143	
Negative	reference		
Positive	6.029 (0.279~63.251)		
CA 19-9		0.963	
Negative	reference		

Positive	1.009(0.670~1.480)	
FERR		0.110
Negative	reference	
Positive	0.477 (0.167~1.067)	
CYFRA21-1		0.989
Negative	reference	
Positive	1.003 (0.697~1.417)	
Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval;		
* Items were included in the multivariate analysis only when P value <0.05 in univariate analysis.		

Table 6. Univariate and multivariate analyses of various predictive factors for MMR status in TNM(II/III) participants.

	Univariate Analysis OR (95% CI)	P-Value	Multivariate Analysis OR (95%CI)	P-Value
Age (years)		0.019		0.025
<65	reference		reference	
>=65	0.614 (0.402~0.910)		0.572 (0.345~0.919)	
Gender		0.908		
Female	reference			
Male	0.981 (0.703~1.375)			
Pathological type		0.017		0.585
non-adenocarcinoma	reference		reference	
adenocarcinoma	0.655 (0.464~0.933)		1.157 (0.696~1.991)	
Tumor size		<0.001		<0.001
<=4.6cm	reference		reference	
>4.6cm	5.179 (3.563~7.706)		2.892 (1.818~4.710)	
Tumor location		<0.001		<0.001
Right	reference		reference	
Left	0.157 (0.110~0.221)		0.231 (0.149~0.356)	
Degree of differentiation		<0.001		0.001
Well/Moderate	reference		reference	
Poor	2.223 (1.455~3.328)		2.374 (1.435~3.879)	
T stage		0.066		
I/II	reference			
III/IV	3.758 (1.170~22.973)			
Harvested lymph nodes		<0.001		0.037
<22	reference		reference	
>=22	3.591 (2.570~5.052)		1.584 (1.028~2.449)	
Lymph nodes metastasis		<0.001		<0.001
No	reference		reference	
Yes	0.295 (0.202~0.423)		0.359 (0.218~0.578)	

Peripheral nerve invasion		<0.001	<0.001
No	reference		reference
Yes	0.171 (0.096~0.284)		0.278 (0.143~0.503)
Lymphovascular invasion		0.023	0.354
No	reference		reference
Yes	0.617 (0.400~0.923)		1.320 (0.723~2.345)
CEA		0.011	0.04
Negative	reference		reference
Positive	0.639 (0.449~899)		0.637 (0.412~0.975)
AFP		0.586	
Negative	reference		
Positive	0.571 (0.032~2.768)		
SCC		0.993	
Negative	reference		
Positive	1.003 (0.463~1.920)		
NSE		0.616	
Negative	reference		
Positive	0.914 (0.638~1.294)		
CA 72-4		<0.001	0.058
Negative	reference		reference
Positive	2.423 (1.678~3.461)		1.611 (0.974~2.614)
CA 125		0.349	
Negative	reference		
Positive	1.273 (0.746~2.063)		
CA 15-3		0.153	
Negative	reference		
Positive	5.770 (0.267~60.573)		
CA 19-9		0.989	
Negative	reference		

Positive	1.003 (0.564~1.495)
FERR	0.095
Negative	reference
Positive	0.422 (0.128~1.024)
CYFRA21-1	0.705
Negative	reference
Positive	1.073 (0.738~1.538)
Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval;	
* Items were included in the multivariate analysis only when P value <0.05 in univariate analysis.	

Table 7. Summary of AUCs of the individual and combined assessments to predict gene mutation status.

	Whole Population		TNM(II/III) Subgroup	
	dMMR	KRAS (+)	dMMR	KRAS (+)
Individual assessment				
Age	0.548		0.582	
Pathological type				0.554
Tumor size	0.68		0.696	
Tumor location	0.715	0.547	0.717	0.551
Degree of differentiation	0.57	0.545	0.561	0.551
Harvested lymph nodes	0.652		0.673	
Lymph nodes metastasis	0.613		0.666	
Perineural invasion	0.622		0.63	
CEA	0.546		0.554	
CA 72-4	0.583			
CA 19-9		0.544		0.544
Combined assessment	0.849	0.609	0.849	0.622

Figures

Figure 1

Study design and algorithm of patient selection.

Figure 2

Representative histological images with KRAS mutant or wild-type CRC. Findings of a 53-year-old man with KRAS-wild type CRC (A) and a 45-year-old woman with KRAS-mutant type CRC (C) with haematoxylin-eosin staining showing histological type and the ARMS method (B, D) demonstrating KRAS status type.

Figure 3

ROC curves of the combination of features for predicting KRAS or MMR status. The AUC of the KRAS mutation prediction rate was 0.609 in all participants (A) and 0.622 in TNM(II/III) participants (B). The “A” in Figure 3C and 3D means age + tumour size + degree of differentiation + harvested lymph nodes + positive lymph nodes + perineural invasion. The AUC, indicating the KRAS and MMR mutation prediction rate, was 0.849 in all participants (C) and 0.849 in TNM(II/III) participants (D).