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A Model for Predicting DNA Mismatch Repair-deficient Colorectal Cancer

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Abstract. *Background/Aim: Identifying patients with DNA mismatch repair-deficient (dMMR) colorectal cancer (CRC) is vital to improve treatment and identify patients with Lynch syndrome (LS). We developed a prediction model for dMMR CRC using clinicopathologic features. Patients and Methods: We reviewed the medical records of 1,147 patients who underwent resection of stage I-IV CRC in whom universal screening for LS using immunohistochemistry for MMR proteins had performed. Univariate and multivariate logistic regression analyses were used to build a prediction model of dMMR CRC. Results: The prevalence of dMMR CRC was 5.2%. Age (≥75 years), tumor location (right-sided colon), main histologic features (poor differentiation), maximum tumor size (≥65 mm), and stage (I/II) were independent significant variables related to dMMR. We created a formula for predicting the likelihood of dMMR, and the probability ranged from 0.2% to 83%. Conclusion: dMMR CRC can be identified efficiently using clinicopathologic features obtained in daily clinical practice.*

Frequent replication errors in microsatellite repeats caused by germline or somatic alterations in DNA mismatch repair (MMR) genes lead to tumor development. The hallmark of MMR-deficient (dMMR) tumors is loss of MMR proteins as assessed by immunohistochemistry (IHC), and/or high frequency microsatellite instability (MSI-H).

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There are at least three purposes for detecting dMMR/MSI-H colorectal cancer (CRC) in clinical practice. The first is to identify patients with metastatic CRC who are candidates for anti-PD-1 blockade (1). Second, postoperative adjuvant chemotherapy with fluorouracil in stage II dMMR/MSI-H colon cancer has been reported to worsen prognosis (2, 3). As such, identifying dMMR/MSI-H patients before chemotherapy allows them to avoid unnecessary adjuvant chemotherapy. The third purpose is to identify Lynch syndrome (LS) (4, 5).

In the process of the popularization of universal tumor screening (UTS) for LS, several models to efficiently predict LS and dMMR/MSI-H CRC (6-15) have been created. These methods include characterization of the pathologic findings of dMMR/MSI-H CRC (10-14) and usage biomarkers as predictors (15); however, these models do not have good utility in clinical practice.

We have previously performed UTS using IHC for MMR proteins to identify LS in 1234 consecutive patients who had undergone primary tumor resection after an initial diagnosis of CRC (16). In the previous study, we identified 61 cases (4.9%) of dMMR CRC and reported three molecular genetic subtypes; sporadic dMMR, LS, and Lynch-like syndrome (LLS). Of these, sporadic (non-hereditary) dMMR CRC is known to be associated with acquired hypermethylation of the *MLH1* promoter region. LS is caused by germline variants of MMR genes such as *MLH1*, *MSH2*, *MSH6*, and *PMS2*. The prevalence of LS (0.9%) was not as high as that in previous reports from Western countries (17-19). LLS is a recently reported entity which usually shows somatic variants in the MMR genes in the absence of neither germline MMR pathogenic variants nor hypermethylation of MLH1 gene (16, 20, 21).

In the present study, we extracted stage I-IV patients from the previous study cohort and investigated predictive factors to efficiently identify dMMR CRC using clinicopathologic features obtained in clinical practice.

Table I. *Clinicopathologic characteristics of patients with colorectal cancer.*

	n, (%)
Age at diagnosis, years	
Median (range)	69 (24-97)
Gender	
Male	689 (60.1)
Female	458 (39.9)
Main histological features	
Well differentiated adenocarcinoma	324 (28.2)
Moderately differentiated adenocarcinoma	742 (64.7)
Poorly differentiated adenocarcinoma	62 (5.4)
Mucinous carcinoma	16 (1.4)
Signet ring cell carcinoma	3 (0.3)
Tumor location	
Right-sided colon	382 (33.3)
Left-sided colon/Rectum	765 (66.7)
Tumor size, mm	
Mean (range)	45 (2-170)
Depth of tumor invasion[a]	
T1	116 (10.1)
T2	137 (12.0)
T3	552 (48.1)
T4	342 (29.8)
Extramural venous invasion	
Present	828 (72.2)
Absent	319 (27.8)
Extramural lymphatic invasion	
Present	755 (65.8)
Absent	392 (34.2)
Lymph node metastasis	
Present	505 (44.1)
Absent	642 (55.9)
Stage[a]	
I	207 (18.0)
II	371 (32.4)
III	360 (31.4)
IV	209 (18.2)

[a]According to TNM classification (UICC 8th edition). UICC: Union for International Cancer Control.

Patients and Methods

This study was approved by the local ethics committee of the Saitama Medical University, Saitama Medical Center (No.747-VII, No. 860-III, No. 924-VIII, and No.926-V). We conducted the following tests for LS screening in 1234 consecutive patients who underwent primary tumor resection for CRC from March 2005 to April 2014: IHC analysis of four MMR proteins (MLH1, MSH2, MSH6, and PMS2), followed by *BRAF* V600E analysis and *MLH1* promoter C-region methylation analysis where appropriate. Furthermore, to identify LS or LLS, we analyzed MMR gene variants at the germline or somatic level. The experimental procedure for UTS from IHC to LS identification has been described previously (16). Informed consent was obtained from each patient. Of the enrolled patients, 1147 patients were included in the analysis after excluding stage 0 patients. Data on patient clinicopathologic backgrounds were retrieved from the medical records.

Table II. *Molecular characteristics of patients with mismatch repair-deficient colorectal cancer.*

Loss of MMR expression	dMMR (n=60)	LS (n=8)	LLS (n=2)	Sporadic (n=50)
MLH1/PMS2	52	1	1	50
MSH2/MSH6	6	5	1	0
MSH6	2	2	0	0
PMS2	0	0	0	0

MMR: DNA mismatch repair; dMMR: DNA mismatch repair-deficient; LS: Lynch syndrome; LLS: Lynch-like syndrome.

Statistical analysis. Results are expressed as median (range). Comparison between groups was performed by the Mann-Whitney test. The clinicopathologic features related to dMMR were analyzed using a logistic regression model. For dichotomization of continuous variables, the cutoff value was calculated using the Youden Index from the receiver operating characteristic (ROC) curve. Factors with $p<0.05$ in univariate logistic regression analysis were selected as covariables, and factors with $p<0.05$ in multivariate analysis by backward stepwise selection were identified as independent variables. The evaluation of logistic regression was expressed by odds ratio (OR) and 95% confidence interval (CI). The area under the curve (AUC) was calculated from the ROC curve of the logistic regression model. JMP Pro 14.0.0 (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses.

Results

Univariate analysis and multivariate analysis for prediction of dMMR CRC. Table I shows the clinicopathologic characteristics of 1147 cases of stage I-IV CRC. The prevalence of dMMR CRC was 5.2% (n=60). Table II shows the pattern of IHC loss of MMR proteins and the breakdown of sporadic dMMR CRC, LS, and LLS based on the genetic profiles.

In univariate analysis, age (≥75 years), gender (female), tumor location (right-sided colon), main histologic features [poorly differentiated adenocarcinoma/mucinous carcinoma/signet ring cell carcinoma (poor differentiation)], depth of tumor invasion (T3/4), maximum tumor size (≥65 mm), and stage (I/II) were significantly associated with dMMR ($p<0.05$) (Table III).

Multivariate analysis of these seven factors by backward stepwise selection indicated that age (≥75 years old) (OR=2.08, 95%CI=1.14-3.80, $p=0.02$), tumor location (right-sided colon) (OR=12.8, 95%CI=5.62-29.3, $p<0.01$), main histologic features (poor differentiation) (OR=5.30, 95%CI=2.46-11.4, $p<0.01$), maximum tumor size (≥65 mm) (OR=6.31, 95%CI=3.39-11.7, $p<0.01$), and stage (I/II) (OR=3.49, 95%CI=1.76-6.82, $p<0.05$) were independent significant variables that predicted dMMR CRC (Table III).

Based on the results of the multivariate logistic regression analysis, the likelihood of a tumor being dMMR was best predicted by the following formula (11):

Table III. *Univariete and multivariate analysis of factors associated with dMMR.*

	Univariate analysis		Multivariate analysis	
	OR (95%CI)	*p*-Value	OR (95%CI)	*p*-Value
Age (≥75 years old)	2.95 (1.74-4.98)	<0.01	2.08 (1.14-3.80)	0.01
Gender (Female)	1.77 (1.05-2.99)	0.03		
Tumor location (Right-sided colon)	17.44 (7.85-38.77)	<0.01	12.84 (5.62-29.31)	<0.01
Histological features (Poor differentiation)	6.97 (3.79-12.79)	<0.01	5.30 (2.46-11.43)	<0.01
Depth of tumor invasion (T3/T4)	2.65 (1.13-6.22)	0.03		
Tumor size (≥65 mm)	5.99 (3.51-10.23)	<0.01	6.31 (3.39-11.75)	<0.01
Lymphatic invasion (Present)	1.33 (0.75-2.37)	0.33		
Venous invasion (Absent)	1.32 (0.76-2.29)	0.33		
Lymph node metastasis (Absent)	1.74 (0.99-3.05)	0.05		
Stage (Stage I/II)	1.88 (1.09-3.24)	0.02	3.44 (1.75-6.78)	<0.01

dMMR: Mismatch repair-deficient; OR: odds ratio; CI: confidence interval.

Clinicopathologic feature	**Score**
Age	
≥75 years old	0.7
<75 years old	0
Tumor location	
Right-sided colon	2.6
Left-sided colon/Rectum	0
Main histological features	
Poorly differentiated adenocarcinoma/Mucinous/Signet ring cell carcinoma	1.7
Well/Moderately differentiated adenocarcinoma	0
Tumor size	
≥65 mm	1.8
<65 mm	0
Stage	
Stage I/II	1.2
Stage III/IV	0
PredictionScore	**Total:**

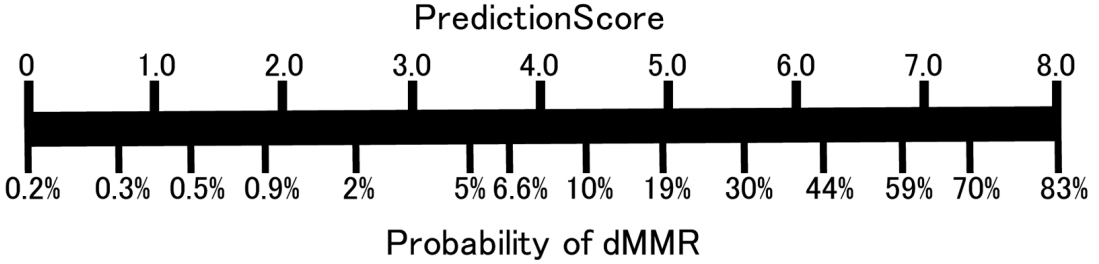

Figure 1. *Application of significant clinicopathological predictors of colorectal cancer patients to the scoring system and the model with corresponding coefficients. All scores were added to get the prediction score, and the conversion scale was used to obtain the estimated probability that the tumor is dMMR CRC. dMMR CRC: Mismatch repair-deficient colorectal cancer.*

Probability of dMMR=exp(-6.448 + PredictionScore)/
[1 + exp(-6.448 + PredictionScore)]
where the prediction score was calculated as follows:
PredictionScore=0.7339 (Age under 75) + 2.5526 (Right-sided tumor) + 1.6680 (Poor differentiation) + 1.8427 (Tumor size over 65 mm) + 1.2367 (Stage I/II)

Using this model, a total dMMR prediction score was calculated and used to determine the probability of dMMR, as shown in Figure 1. For example, a prediction score of 0 indicated a 0.2% probability that the tumor was dMMR, whereas a prediction score of 8, which was the maximum score, indicated an 83% probability that the tumor was dMMR (Youden Index=0.62, cutoff: 3.8 points, probability: 6.6%). The ROC curve for this model is shown in Figure 2. The AUC was 0.89.

Prediction score by molecular characteristics. Figure 3 shows the actual prevalence of dMMR CRC for each prediction score range. Patients with LS/LLS were evenly distributed from low to high scores. Therefore, the prediction scores were compared after subclassifying the patients into sporadic (*MLH1*-hypermethylated) dMMR CRC patients and patients with LS/LLS. The median prediction scores were 4.7 (1.9-7.3) for LS/LLS and 5.1 (1.2-8.0) for sporadic dMMR, and there was no significant difference between molecular genetic subtypes (p=0.73).

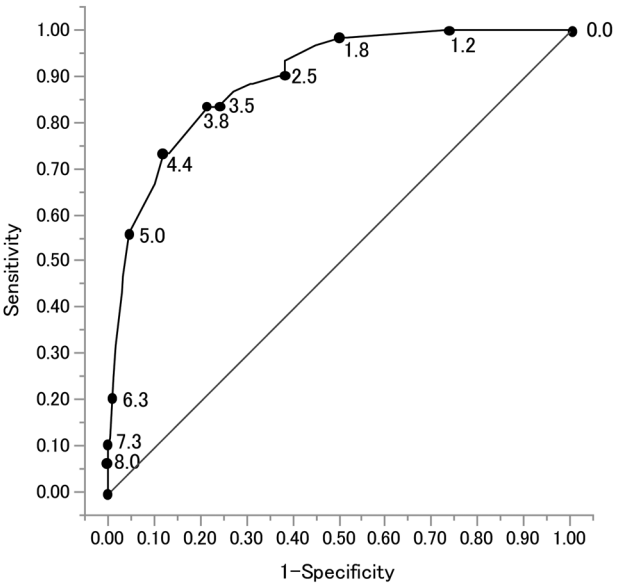

Figure 2. *Receiver operator characteristic curve displaying the sensitivity and specificity for each prediction score. The area under the curve was 0.89. Note that for a dMMR probability score of 3.8 (cutoff), the sensitivity was 83.3% and the specificity was 78.4% (Youden Index 0.62). dMMR: Mismatch repair-deficient; AUC: area under the curve.*

Discussion

We developed a prediction model for efficiently and accurately predicting dMMR/MSI-H CRC in daily clinical practice based on clinicopathologic features of 1234 consecutive patients who underwent primary tumor resection after an initial diagnosis of CRC. The prevalence of dMMR/MSI-H CRC may differ based on ethnicity, race, and location. Because of a lack of sufficient data, it may be influenced by selection biases in studies or by the age distribution of the study cohort. The incidence of dMMR/MSI-H CRC are 10-15% in Western countries (22-26) and 5-6% in Asia, including Japan (16, 27-30). In Western countries, UTS by IHC of MMR proteins or MSI testing are recommended for the identification of LS in CRC (31, 32). However, UTS is not efficient in countries where the prevalence of dMMR/MSI-H in CRC is not as high as that of Western countries. Additionally, that method has also a very high cost.

Some prediction models for MSI-H/dMMR CRC have been reported. To identify LS, Jenkins *et al*. (10) developed the "MsPath" model, an MSI prediction model for under 60 years old, using Crohn's-like reaction, and tumor-infiltrating lymphocytes(TILs) as predictors. These pathological findings are considered to be characteristic of MSI-H CRC (33-35). To predict the probability of MSI-H in patients with CRC regardless of age, Greenson *et al*. (11) developed the "MSI Probability scoring system", which is a prediction model using clinicopathologic factors including lack of dirty necrosis, Crohn's-like reaction, and TILs. Román *et al*. (12) have also developed the "RER test6 model" that includes pathological features such as solid pattern, Crohn's-like reaction, and TILs. High accuracy has been reported for all models; however, evaluating pathological features such as TILs, Crohn's-like reaction, lack of dirty necrosis, and solid pattern in excised specimens imposes a heavy burden on pathologists. Moreover, these models have not been widely used. In addition, findings characteristic of MSI-H, such as TILs and Crohn's-like reaction, are not specific in identifying MSI-H. Evaluations of these findings differ based on the pathologist, which seems to be the reason why these tests are not widely applied clinically (36). Fujiyoshi *et al*. (15) have developed an MSI-H "prediction score model" for Japanese patients aged 50 years and older. Although they did not examine specific pathologic features of MSI-H as predictors, they adopted *BRAF* V600E mutation as a predictor. MSI-H and *BRAF* V600E mutants have a strong link with *MLH1*-hypermethylated (sporadic) MSI-CRC, and *BRAF* V600E mutation is found in about 40% of MSI-H CRC (37). However, testing for *BRAF* V600E mutation in all CRCs to predict MSI-H is not cost effective.

Here, we examined the predictors of dMMR to build an efficient and sensitive model to predict dMMR CRC. However, we did not include an MSI testing in our model. IHC for MMR proteins is generally preferred to MSI testing because of its lower cost, faster turnaround time, wider availability in routine diagnostic laboratories, and ability to perform direct germline mutation testing. An additional advantage of IHC is that the

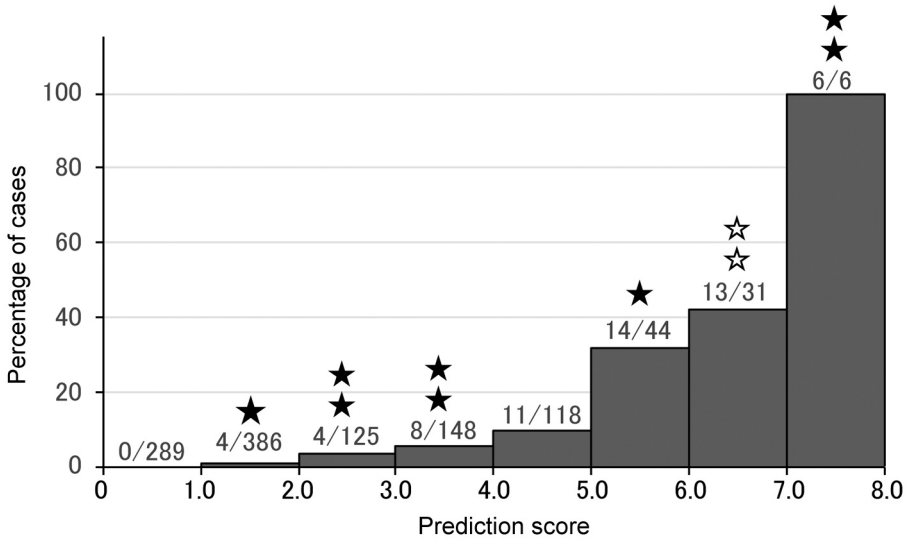

Figure 3. *The distribution of the present study's cases by prediction score categories and the probability of a tumor being dMMR as estimated by the prediction score model. The numerator indicates the total number of patients with dMMR CRC in each prediction score category, and the denominator indicates the total number of CRC patients in that category. ★: Lynch syndrome; ☆: Lynch-like syndrome.*

MMR gene that is likely to be mutated can be pinpointed. The concordance rates of the results of examinations have been reported to be highbetween 92.4% and 97.8% in CRC (38, 39). Therefore, we believe that IHC is sufficient to assess the MMR status in CRC.

The prediction model we proposed in the present study includes only clinicopathologic features of CRC that are available in daily clinical practice. The probability of dMMR CRC can be predicted with a high accuracy (AUC 0.89) by only five factors: age, tumor location, main pathologic feature, maximum tumor size, and stage. In this model, if the prediction score was over 7.0 points, the probability of dMMR was about 70% or more. If the score was 2.0 points or less, the probability was 1% or less. Therefore, there is little need for IHC/MSI testing for these cases. Since scores less than 2.0 accounted for about 60% of cases, IHC/MSI testing might be omitted in these patients as they have an extremely low possibility of dMMR.

The purpose of the present study was to differentiate dMMR CRC from all CRC, but it is likely that this formula also functions as a tool to identify LS/LLS. There were 60 cases of dMMR CRC in this study, including 8 cases of LS and 2 cases of LLS. There was no significant difference between the median prediction scores of LS/LLS and sporadic dMMR (p=0.73). Although the clinicopathologic features of LLS are largely unknown, a recent report indicated several differing features between LLS and LS with CRC, including age (LLS: 66 years *vs*. LS: 44 years, p<0.001), tumor sidedness (right-sided tumors: 50% *vs*. 89%, p=0.086), proportion of poorly differentiated tumors (0% *vs*. 56%), and proportion of mucinous tumors (21% *vs*. 0%) (p=0.009) (40). However, that study included only a small number of cases. The frequency of LLS in CRC was extremely low in the present study (2/1134 cases, 0.17%), and it is difficult to accurately evaluate the suitability of this model for identifying LLS. This should be examined in the future.

Some limitations of the present study must be considered. It is a single-institution study. In addition, we only included patients who underwent primary tumor resection, and patients with unresectable tumors were not included in the present study. However, the present study targeted consecutive patients who underwent primary tumor resection for initially diagnosed CRC, which is considered to fully represent the characteristics of the population in Asia, including Japan, where the prevalence of dMMR/MSI-H is lower than in Western countries.

In conclusion, our prediction model can sufficiently and efficiently identify dMMR/MSI-H CRC using only factors obtained in daily clinical practice. We expect that this model will be used to efficiently identify patients in whom fluorouracil-based adjuvant chemotherapy is not necessary, as well as those patients who might benefit from anti-PD-1 blockade.

Conflicts of Interest

The Authors declare no conflicts of interest associated with this manuscript.

Authors' Contributions

Conception: K. Chikatani, K. Ishibashi, H. Ishida; Collection of data and sample: N. Chika, O. Suzuki, T. Sakimoto; Analysis of sample: H. Eguchi, Y. Okazaki, Supervision: H.Ishida; Final approval of manuscript: All Authors.

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