Research paper

Development and validation of MMR prediction model based on simplified clinicopathological features and serum tumour markers ☆

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ABSTRACT

Background: Although simplified clinicopathological features and serum tumour markers (STMs) were reported to be associated with the status of mismatch repair (MMR) in colorectal cancer (CRC) patients, their predictive value alone or in combination for MMR status remains unknown.

Methods: A retrospective analysis of 3274 participants with MMR testing and STMs measurements from two institutions was conducted. The prediction model was developed in the primary cohort that consisted of 1964 participants. Best subset regression was applied to select the most useful predictors from the primary dataset. The performance of the nomogram was evaluated with respect to its calibration, discrimination, and clinical usefulness. External validation was performed in an independent validation cohort of 1310 consecutive CRC patients.

Findings: Among the ten simplified clinicopathological features, seven variables were selected as the best subset of risk factors to develop pathology-based model, including age, tumour diameters, histology, tumour location, perineural invasion, the number of sampled lymph nodes (LNs) and positive LNs. The model showed good calibration and discrimination, with an AUC of 0.756 (95% CI, 0.722 to 0.789) in the primary cohort and 0.754 (95% CI, 0.715 to 0.793) in the validation cohort. After the addition of CEA and CA 72-4, the performance of pathology-based model was significantly improved in in both the primary cohort (AUC: 0.805 (0.774-0.835) vs. 0.756 (0.722-0.789), P < 0.001) and validation cohort (AUC: 0.796 (0.758-0.835) vs. 0.754 (0.715-0.793), P < 0.001). The results of decision curve analysis revealed that using our models to predict the status of MMR would add more benefit than either the detect-all-patients scheme or the detect-none scheme.

Interpretation: The models based on simplified clinicopathological features alone or in combination with STMs can be conveniently used to facilitate the postoperative individualized prediction of MMR status in CRC patients.

Research in context

Evidence before this study

Though several prediction models based on pathological features have been developed, the complex pathological characteristics included in previous models required detailed pathological diagnosis, which not only caused a huge burden

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on the pathologists, but also lacked of instructive value for clinicians.

Added value of this study

To our knowledge, our study is the first to investigate the predictive value of STMs and incorporate them into the prediction model for MMR status. In addition, we extremely simplify previously complex pathological features and make the MMR risk prediction model available to both clinicians and pathologists.

Implication of all the available evidence

The nomograms based on simplified clinicopathological features alone or in combination with STMs can be conveniently used to facilitate the postoperative individualized prediction of MMR status in CRC patients.

1. Introduction

Colorectal cancer (CRC) is the third most common type of cancer and the second leading cause of cancer-related deaths worldwide [1]. Though complete surgical resection is the primary treatment for patients with locoregional CRC, it has been estimated that approximately half of patients with localized CRC will develop metastases [2]. Adjuvant chemotherapy and immune checkpoint inhibitors has been recommended to improve prognosis of patients with high-risk stage II and stage III/IV CRC [3, 4]. However, accumulating evidence demonstrated that individual treatment response of CRC patients is significantly associated with its molecular characteristics [5-7].

Microsatellite instability (MSI) is the abnormal shortening or lengthening of DNA by 1-6 repeating base pair units, which is caused by the inactivation of the DNA mismatch repair (MMR) system [8]. An increasing body of evidence suggested that CRC patients with MSI are not only highly correlated with a better prognosis [9], higher incidence of Lynch syndrome [10] and a high response to immune checkpoint blockade [11], but less likely to benefit from 5-fluorouracil-based chemotherapy [12]. As a result, genetic testing for MMR or MSI in all CRC patients is recommended by the National Comprehensive Cancer Network (NCCN) guidelines to optimize the treatment and management of CRC [13, 14]. However, the recommendation is not feasible in clinical practice, especially in developing countries. It is reported that only approximately 5%-15% CRC patients presented with deficient MMR (dMMR) and have high levels of microsatellite instability [15, 16], which means 85%-95% CRC patients could not benefit directly from genetic testing. Therefore, there is an urgent need to develop a model for the prediction of MMR status in CRC patients based on phenotypic characteristics.

Previous studies have demonstrated that MMR status of CRC is significantly correlated with their clinicopathological features [17, 18] and serum tumour biomarkers (STMs) [19, 20]. Though several prediction models based on pathological features have been developed, the complex pathological characteristics included in previous models required detailed pathological diagnosis [21–23], which not only caused a huge burden on the pathologists, but also lacked of instructive value for clinicians. STMs are both important prognostic factors and the indicators of therapeutic effect and recurrence in patients with CRC [19, 24], whereas their predictive value for MMR status has not been assessed in previous studies. The aim of our study was to develop MMR prediction models based on simplified clinicopathological features alone or in combination with STMs.

2. Methods

2.1. Study design and patient cohort

This study retrospectively reviewed 3251 who were diagnosed with CRC and underwent curative surgical resection at Union Hospital, Tongji Medical College, Huazhong University of Science and Technology between May 2017 and December 2019. Patients with the following conditions were excluded from the study: i) no results about MMR status; ii) no results about STMs; iii) the history of chemoradiotherapy before MMR status detection. A total of 1964 patients met these criteria and were included in the primary cohort (1169 males and 795 females; mean age, 57.1 ± 11.7 years, range from 17 to 92 years). From March 2018 to December 2019, an independent validation cohort of 1310 patients (785 males and 525 females; mean age, 57.3 ± 11.8 years, range from 16 to 89 years) was screened from 1947 patients using the same criteria at China-Japan Union Hospital of Jilin University. The flow diagram of developing and validating the prediction model was demonstrated in Fig. 1. This study protocol was approved by the ethics committee of Tongji Medical College, Huazhong University of Science and Technology (No. 2018-S377). All patients signed an informed consent regarding their understanding of the procedure and its potential complications as well as their approval of participation in the research.

Baseline clinicopathologic data was obtained from medical records, including age, gender, tumour diameters, tumour location, pathological type, histology, T-stage, the number of sampled lymph nodes (LNs), the number of positive LNs, and perineural invasion (PNI). Proximal colon cancers were defined as those occurred in the cecum, ascending colon, and transverse colon; distal colon cancers as those in the descending and sigmoid colon; and rectal cancers as those in the rectosigmoid junction and rectum [17]. The depth of invasion and lymph node metastasis were classified according to the 8th AJCC tumour-node-metastasis classification. Perineural invasion was defined as tumour cells found within the perineural space or the infiltration of cancer cells into the endoneurium [25]. Laboratory analysis of STMs was done via routine blood tests within one week before surgery. The normal upper limits of STMs were as follows: carcinoembryonic antigen (CEA), 5 μg/L; carbohydrate antigen (CA) 19-9, 37 U/ml; CA 72-4, 6.9 U/ml. Tumour marker values above these thresholds were considered positive, otherwise, the sample was deemed as negative. The assessment of MMR status was performed through immunohistochemical (IHC) staining as described previously [8]. MMR status was determined by four markers, including MSH2, MSH6, MLH1, PMS2. Tumours displaying loss of expression of one or more MMR proteins was considered to be dMMR, whereas tumours with intact MMR proteins were classified as proficient MMR (pMMR).

2.2. Predictor selection and the development of prediction model based on clinicopathological features alone or in combination with STMs

Best subset regression was applied to select the most useful predictive factors from the primary dataset, using Akaike's information criterion (AIC) and the likelihood ratio test as the stopping rule [26]. The AIC value for the final model was minimized with the fewest number of variables. A predictive score calculated for each patient via a linear combination of selected features that were weighted by their respective coefficients [27]. To provide clinicians with a quantitative tool to predict individual probability of MMR status, we built the nomogram on the basis of selected variables.
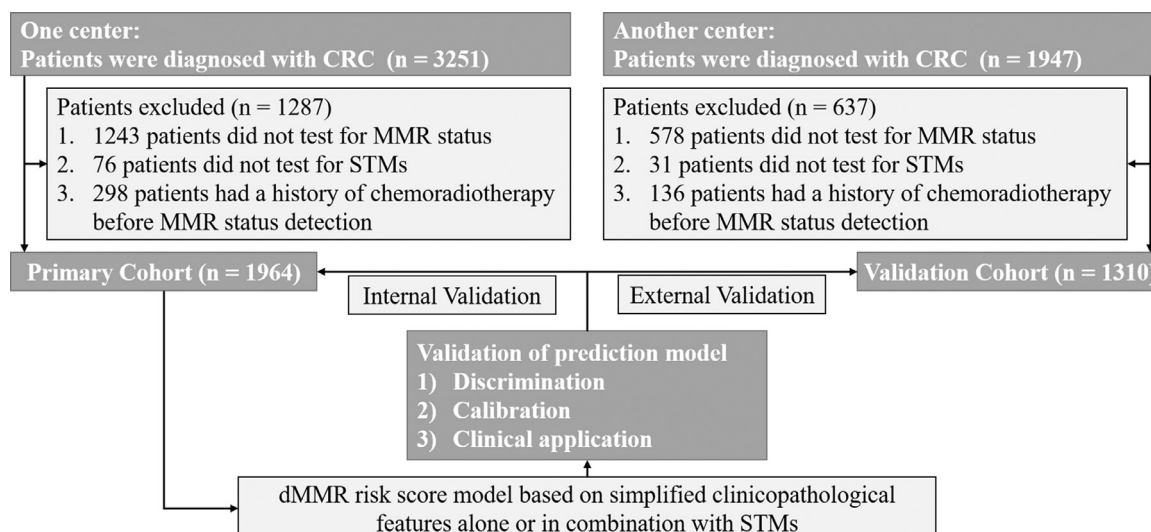
One center:
Patients were diagnosed with CRC (n = 3251)

Patients excluded (n = 1287)
1. 1243 patients did not test for MMR status
2. 76 patients did not test for STMs
3. 298 patients had a history of chemoradiotherapy before MMR status detection

Another center:
Patients were diagnosed with CRC (n = 1947)

Patients excluded (n = 637)
1. 578 patients did not test for MMR status
2. 31 patients did not test for STMs
3. 136 patients had a history of chemoradiotherapy before MMR status detection

Primary Cohort (n = 1964)

Internal Validation

External Validation

Validation Cohort (n = 1310)

Validation of prediction model
1) **Discrimination**
2) **Calibration**
3) **Clinical application**

dMMR risk score model based on simplified clinicopathological features alone or in combination with STMs

Fig. 1. The flow diagram of developing and validating the prediction model.

2.3. The validation of prediction model

The nomogram's accuracy was required to be validated by 1000 times bootstrapping and cross-validation measures internally and externally. The fitting degree was evaluated by the area under the receiver operating characteristic (ROC) curve (AUC) and calibration plots. Calibration curves were plotted to assess the calibration of the nomogram, which consisted of two lines: one was a 45- degree reference line, and the other line represented the actual line [28]. The interval between the two lines reflected the accuracy of the nomogram. Hosmer-Lemeshow test was used to evaluate the calibration of prediction model and a significant test statistic implies that the model does not calibrate perfectly. In addition, decision curve analysis (DCA) was conducted to determine the clinical usefulness of the nomogram by quantifying the net benefits at different threshold probabilities [29, 30].

2.4. Statistical analysis

Statistical analysis was conducted with STATA 15.0 (StataCorp, Texas, USA) and R version 4.0.0 (R Foundation for Statistical Computing; http://www.r-project.org/). The R packages used in our study were demonstrated in the Supplementary Table 1. Data are presented as number and percentages for categorical variables, and continuous data were expressed as mean $\pm$ standard deviation, unless otherwise specified. The continuous variables were transformed into binary variables by applying inflexion points of ROC curves as the cut-offs. Patient characteristics were compared using t tests for continuous variables and x^2 or Fisher exact tests for categorical variables. All statistical tests were two-sided, with statistical significance set at 0.05.

2.5. Role of funding source

3. Results

3.1. Patient clinical characteristics

The clinical characteristics of CRC patients in the primary and validation cohort were given in Table 1. Although the detection rate of MMR status in the primary cohort was significantly lower than that in the validation cohort (60.4% vs. 67.3%, P = 0.023), there was no significant difference between both cohorts in the incidence of dMMR (10.9% vs. 11.0%, P = 0.937). In both cohorts, dMMR was found to be significantly associated with age, tumour diameters, tumour location, the number of sampled LNs, the number of positive LNs, PNI, CEA and CA 72-4. Though pathological type (P = 0.010) and T-stage (P = 0.003) were found to be significantly correlated with MMR status in the validation cohort, no significant differences were observed in the primary cohort.

3.2. The development of prediction model based on simplified clinicopathological features

Among the ten simplified clinicopathological features, seven variables were selected as the best subset of risk factors to develop prediction model, including age, tumour diameters, histology, tumour location, the number of sampled LNs, the number of positive LNs and PNI (Table 2). Using the regression coefficients of multivariate logistic regression models to weight each feature in our models [27], we developed a risk score formula to predict MMR status: risk score = -4.281 - 0.581 (if Age < 53 years old) + 0.511 (if tumour diameters $\geq$ 4.6 cm) + 0.652 (if well or moderate differentiation) - (0.097 $\times$ number of positive LNs) + 0.455 (if number of sampled LNs $\geq$ 23) - 0.496 (if perineural invasion is negative) + (1.242, if primary location is distal colon; 1.705, if primary location is proximal colon). Predicted risk = $1/(1 + e^{-\text{risk score}})$. The model that incorporated the above predictors was developed and presented as the nomogram (Fig. 2a).

3.3. The validation of prediction model based on simplified clinicopathological features

Internal validation: The calibration curve of the nomogram for the probability of dMMR showed good agreement between prediction and observation in the primary cohort (Fig. 3a). The Hosmer-Lemeshow test yielded a nonsignificant statistic (P = 0.983), which suggested that there was no departure from perfect fit. The ROC
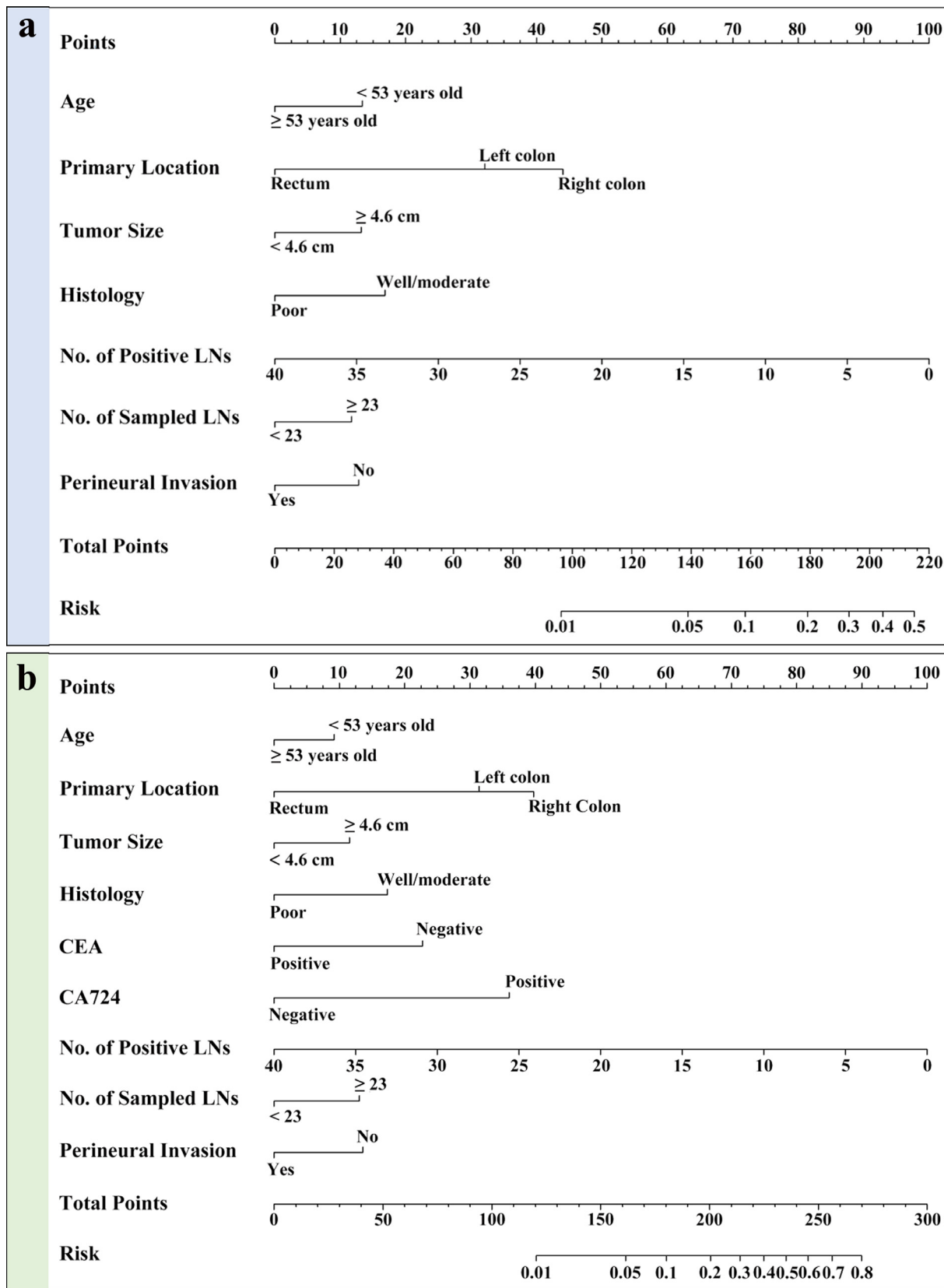
Fig. 2. The nomograms to predict the probability of dMMR in CRC patients from the training cohort. (a) The nomogram based on simplified clinicopathological features alone; (b) The nomogram based on simplified clinicopathological features and serum tumour markers.

Table 1
The clinical characteristics of CRC patients in the primary and validation cohort.

characteristics	Primary Cohort			Validation Cohort		
	pMMR (n = 1749)	dMMR (n = 215)	P value	pMMR (n = 1166)	dMMR (n = 144)	P value
Age (years)			<0.001			<0.001
< 53	573 (32.8)	98 (45.6)		362 (31.1)	67 (46.5)	
>= 53	1176 (67.2)	117 (54.4)		804 (68.9)	77 (53.5)	
Gender			0.464			0.189
Male	1046 (59.8)	123 (57.2)		706 (60.6)	79 (54.9)	
Female	703 (40.2)	92 (42.8)		460 (39.4)	65 (45.1)	
Primary location			<0.001			<0.001
Proximal colon	343 (19.6)	95 (44.2)		221 (19.0)	60 (41.7)	
Distal colon	512 (29.3)	83 (38.6)		365 (31.3)	53 (36.8)	
Rectum	894 (51.1)	37 (17.2)		580 (49.7)	31 (21.5)	
Tumor diameters (cm)			<0.001			<0.001
< 4.6	1266 (72.4)	121 (56.3)		807 (69.2)	44.4)	
>= 4.6	483 (27.6)	94 (43.7)		359 (30.8)	80 (55.6)	
Pathological type			0.145			0.010
non-adenocarcinoma	274 (15.7)	42 (19.5)		184 (15.8)	35 (24.3)	
adenocarcinoma	1475 (84.3)	173 (80.5)		982 (84.2)	109 (75.7)	
Histology			0.324			0.621
poor	83 (4.8)	7 (3.3)		38 (3.3)	6 (4.2)	
Well/moderate	1666 (95.3)	208 (96.7)		1128 (96.7)	138 (95.8)	
T-stage			0.096			0.003
I/II	365 (20.9)	30 (13.9)		295 (19.8)	19 (10.9)	
III/IV	1384 (79.1)	185 (86.1)		1194 (80.2)	156 (89.1)	
No. of sampled LNs (n)			<0.001			<0.001
<23	1381 (78.9)	133 (61.9)		928 (79.6)	95 (66.0)	
>=23	368 (21.1)	82 (38.1)		238 (20.4)	49 (34.0)	
No. of Positive LNs (n)	2.1 ± 3.8	1.2 ± 2.8	<0.001	2.0 ± 3.8	1.2 ± 3.1	0.011
Perineural invasion			<0.001			<0.001
No	1232 (70.4)	177 (82.3)		812 (69.6)	122 (84.7)	
Yes	517 (29.6)	38 (17.7)		354 (30.4)	22 (15.3)	
CEA			<0.001			<0.001
Negative	944 (53.9)	156 (72.6)		591 (50.7)	105 (72.9)	
Positive	805 (46.1)	59 (27.4)		575 (49.3)	39 (27.1)	
CA 19-9			0.211			0.966
Negative	1451 (83.0)	171 (79.5)		941 (80.7)	116 (80.6)	
Positive	298 (17.0)	44 (20.5)		225 (19.3)	28 (19.4)	
CA 72-4			<0.001			<0.001
Negative	1471 (84.1)	125 (58.1)		976 (83.7)	89 (61.8)	
Positive	278 (15.9)	90 (41.9)		190 (16.3)	55 (38.2)	

Abbreviations: LNs, lymph nodes; Categorical variables, n (%); Continuous data, mean ± standard deviation.

Table 2
Risk factors for deficient MMR in Colorectal Cancer.

Intercept and variable	Model 1			Model 2		
	β	95% OR	P value	β	95% OR	P value
Intercept	−4.281		<0.001	−4.090		<0.001
Age	−0.518	0.595 (0.440 to 0.805)	<0.001	−0.367	0.693 (0.505 to 0.951)	0.023
Tumor size	0.511	1.667 (1.225 to 2.268)	0.001	0.463	1.588 (1.148 to 2.198)	0.005
Histology	0.652	1.920 (0.845 to 4.365)	0.119	0.693	2.000 (0.864 to 4.632)	0.106
No. of positive LNs	−0.097	0.908 (0.855 to 0.964)	0.002	−0.100	0.905 (0.852 to 0.961)	0.001
No. of sampled LNs	0.455	1.576 (1.141 to 2.177)	0.006	0.521	1.683 (1.200 to 2.362)	0.002
Perineural invasion	−0.496	0.609 (0.414 to 0.895)	0.012	−0.543	0.581 (0.389 to 0.868)	0.008
Primary location						
Rectum	reference			reference		
Distal colon	1.242	3.464 (2.301 to 5.213)	<0.001	1.256	3.511 (2.310 to 5.335)	<0.001
Proximal colon	1.705	5.502 (3.634 to 8.329)	<0.001	1.591	4.906 (3.202 to 7.516)	<0.001
CEA	NA	NA	NA	−0.910	0.403 (0.285 to 0.586)	<0.001
CA 72-4	NA	NA	NA	1.440	4.222 (3.012 to 5.920)	<0.001

model 1: based on simplified clinicopathological characteristics alone.
model 2: based on simplified clinicopathological features and serum tumor biomarkers.
Abbreviations: OR, odds ratio; LNs, lymph nodes; CEA, carcinoembryonic antigen; CA, carbohydrate antigen.

curve yielded an AUC of 0.756 (95% CI, 0.722 to 0.789) for the primary cohort (Fig. 4a).

Independent validation: Good calibration was also observed in the validation cohort (Fig. 3b) and the Hosmer-Lemeshow test yielded a nonsignificant statistic (P = 0.962). The discriminative ability of the nomogram in an independent cohort was 0.754 (95% CI, 0.715 to 0.793) (Fig. 4b).

3.4. Incremental predictive value of STMs to the above model

To evaluate the additional predictive value of STMs, three STMs, including CEA, CA 19-9 and CA 72-4, together with simplified clinicopathological features, were used to develop MMR prediction model. Finally, nine variables were selected as the best subset of risk factors, including age, tumour diameters, histology, tumour lo-
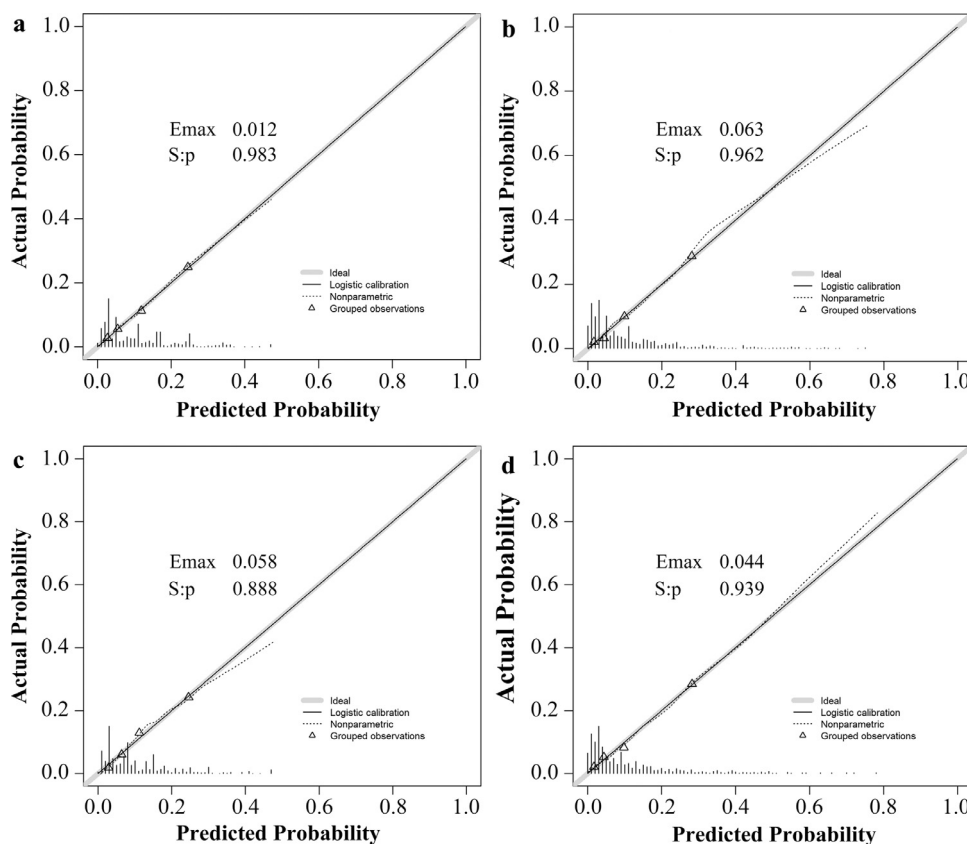
Fig. 3. Calibration curves of the prediction models in each cohort. Calibration curves depict the calibration of prediction models in terms of the agreement between the predicted risks of dMMR and observed outcomes of dMMR. The x-axis represents the predicted dMMR risk and the y-axis represents the actual dMMR rate. The diagonal solid line represents a perfect prediction by an ideal model. The dotted line represents the performance of our prediction models. A closer fit to the diagonal solid line represents a better prediction. (a) and (b) represents the calibration curve of pathology-based model in the primary cohort and validation cohort; (c) and (d) represents the calibration curve of the combined models in the primary cohort and validation cohort.
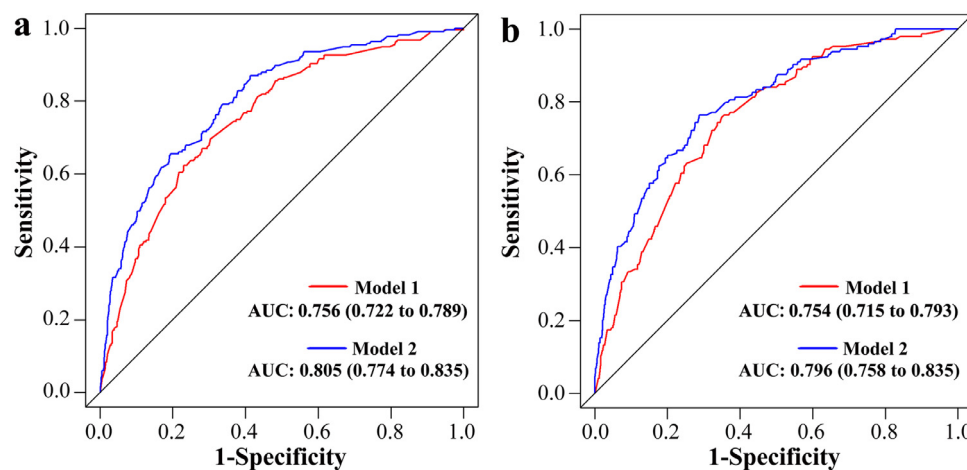

Fig. 4. Receiver operating characteristic (ROC) curve of the prediction models in each cohort. The red line and blue line represent the pathology-based model and the combined model, respectively. (a) represents ROC curve of our prediction models in the primary cohort; (b) represents the ROC curve of models in the validation cohort.

cation, the number of sampled LNs, the number of positive LNs, PNI, CEA and CA 72-4 (Table 2). The risk score formula of the combined model was as follows: risk score = -4.090 - 0.367 (if Age < 53 years old) + 0.463 (if tumour diameters ≥ 4.6 cm) + 0.693 (if well or moderate differentiation) – (0.100 × number of positive LNs) + 0.521 (if number of sampled LNs ≥ 23) - 0.543 (if perineural invasion is negative) + (1.256, if primary location is distal colon; 1.591, if primary location is proximal colon) - 0.910 (if CEA is negative) + 1.440 (if CA 72-4 is positive). Predicted

risk = $1/(1 + e^{-\text{risk score}})$. The model that incorporated the above predictors was developed and presented as the nomogram (Fig. 2b). The calibration curve for the probability of dMMR demonstrated good agreement between prediction and observation in the primary cohort (P = 0.888) and validation cohort (P = 0.939) (Fig. 3c and Fig. 3d). After the addition of CEA and CA 72-4, the discrimination ability of pathology-based model was significantly improved in the primary cohort (AUC: 0.805 (95% CI, 0.774 to 0.835) vs. 0.756 (95% CI, 0.722 to 0.789), P < 0.001) (Fig. 4a) and valida-
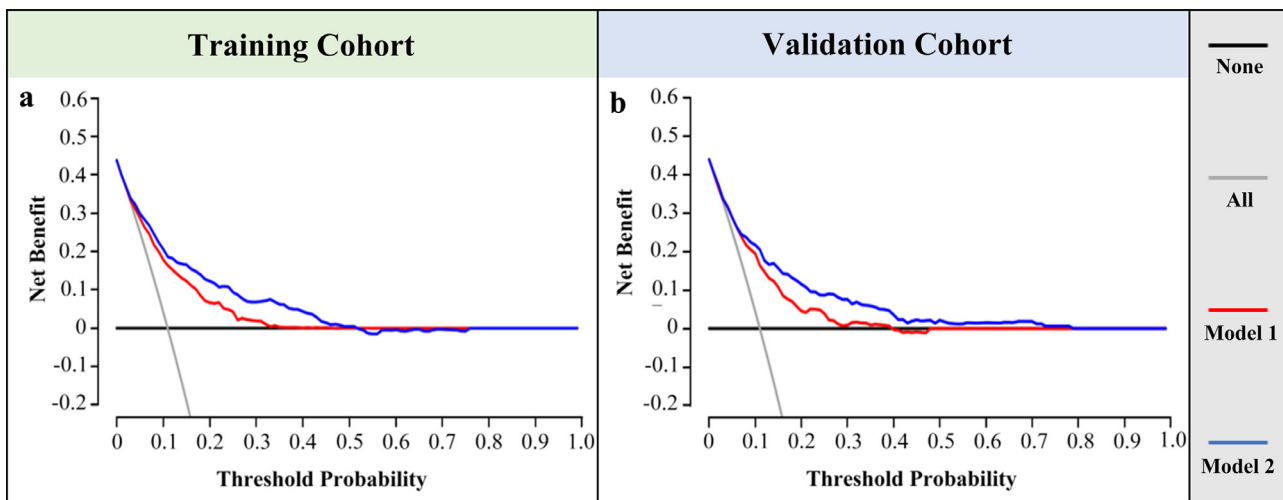
Fig. 5. The decision curve of the nomograms for the prediction of MMR status. The x-axis and y-axis represent the threshold probability and the net benefit, respectively. The red line and blue line represent the pathology-based model and the combined model, respectively. The grey line and black line represent the strategy of conducting IHC-testing for every patient and none. (a) and (b) represent the decision curve of our nomograms in the primary and validation cohort.

tion cohort (AUC: 0.796 (95% CI, 0.758 to 0.835) vs. 0.754 (95% CI, 0.715 to 0.793), P < 0.001) (Fig. 4b).

3.5. Clinical usefulness

The decision curve analysis for the nomogram based on simplified clinicopathological features alone or in combination with STMs was presented in Fig. 5. Decision curve analysis is conducted to determine the clinical usefulness of the nomograms via quantifying the net benefits at different threshold probabilities [29]. The net benefit was calculated by subtracting the proportion of all patients who are false positive from the proportion who are true positive, weighting by the relative harm of forgoing detection compared with the negative consequences of an unnecessary detection [30, 31]. Here, the relative harm was calculated by (Pt/(1-Pt)). "Pt" (threshold probability) is where the expected benefit of detection is equal to the expected benefit of forgoing detection; at which time a patient will opt for IHC testing informs us of how a patient weighs the relative harms of false-positive results and false-negative results ((a-c)/(b-d) = (1-Pt)/Pt) (a, b, c and d represent the value of true positive, false positive, false negative, and true negative, respectively. (a – c) is the harm from a false-negative result; (b – d) is the harm from a false-positive result). By applying our pathology-based prediction models, higher net benefit in the number of false-positives than the strategy of conducting IHC-testing for every patient or none could be achieved when the risk thresholds range from 11% to 37%. Similarly, if the threshold probability of a patient is between 11% and 52%, using the nomogram incorporating STMs and simplified clinicopathological features would add more benefit than either the detect-all-patients scheme or the detect-none scheme. For example, at the 10% risk cutoff in the validation cohort, the net benefit was 4% in the detect-all model, 20% in the pathology-based model, and 22% in the combined model. The net benefit of the pathology-based model was equivalent to performing 20 detection per 100 men without negative results, 16 more than detect-all model. Moreover, the addition of STMs significantly added the net benefit of pathology-based model (22% vs. 20%, P = 0.002).

4. Discussion

Though molecular testing for MMR status might significantly improve the treatment and management of CRC patients, the rate of MMR detection was far below expected [32–34]. The consensus criteria for the diagnosis of dMMR proteins are based on selecting CRC patients who fulfill the revised Bethesda guidelines (RBG), followed by MSI testing and/or IHC staining of MMR proteins [35]. Although this method is cost-effective, its age limit and low sensitivity have been shown to miss a substantial number of CRC patients with dMMR [36]. In addition, RBG recommended that all CRC patients between the ages of 50 and 59 years should test for MMR status when they have one or more of the following pathological features: Crohn's-like lymphocytic reaction, mucin/signet ring cell differentiation, medullary growth pattern, tumour-infiltrating lymphocytes [35]. However, it could not provide details on the predictive value of these pathological features, either alone or in combination. On the basis of RBG, Jenkins et al developed the MsPath model to quantify the predictive value of these pathological features in CRC patients before age 60 years [21]. Though MsPath score ≥ 1.0 had a sensitivity of 93% and a specificity of 55% for dMMR, it could not provide guidance for those patients over 60 years. Moreover, the model required detailed pathological diagnosis for complex pathological features, which caused a huge burden on the pathologists. Afterwards, a few prediction models attempted to improve MsPath model by simplifying pathological features [22, 23, 37]. Though several models achieved high sensitivity and specificity for the prediction of MMR status, other molecular targets, such as p53 [37] and BRAF [22], included in their models reduced their practical value in clinical practice. Therefore, the detailed predictive value of simplified clinicopathological features alone for MMR status remains unclear.

Using the data from 3274 participants in two institutions, we assessed the predictive value of simplified clinicopathological features in the CRC patients of all ages. The findings of our study showed that simplified clinicopathological features possessed a strong discrimination ability for MMR status. In addition, our study is the first to explore the predictive value of simplified clinicopathological features in combination with STMs for MMR status. The results demonstrated that the addition of CEA and CA 72-4 could significantly improve the discriminative ability of pathology-based model in the primary cohort (AUC: 0.805 (0.774-0.835) vs. 0.756 (0.722-0.789), P < 0.001) and validation cohort (AUC: 0.796 (0.758-0.835) vs. 0.754 (0.715-0.793), P < 0.001). The information obtained in our study may greatly help clinicians to screen the CRC patients who should conduct IHC staining or MSI testing.

Several clinicopathological features, such as age, tumour diameters, histology and tumour location, has been reported to possess a discrimination ability for MMR status in previous studies [22, 23]. Consistent with these findings, seven simplified clinicopathological features were brought into MMR risk prediction model in our study, including age, tumour diameters, histology, tumour location, number of harvested LNs, number of positive LNs and PNI. Among these variables, number of harvested LNs, number of positive LNs and PNI were the first time to be incorporated into models for the prediction of MMR status. As an independent prognostic factor of CRC, PNI has been recommended by NCCN guidelines as a routine inspection item to assess the risk of stage II CRC [38]. Several retrospective observation studies also reported that PNI was prone to occur in CRC patients with pMMR [39, 40]. In our study, more harvested LNs and less positive LNs were found to be associated with dMMR, which represent a larger extent of LNs dissection and low risk of metastasis. Therefore, our results supported that dMMR is a positive prognostic factor for CRC.

Previous studies have demonstrated that preoperative STMs are important prognostic factor that is independent of clinicopathological parameters [41–43]. However, the predictive value of STMs in combination with clinicopathological features for MMR status in CRC patients remains unknown. Although CEA and MMR proteins belong to different protein superfamilies, Shih-Ching et al. reported a correlation between a normal serum CEA level and dMMR in 213 CRC patients [20]. Johanna et al. also reported that elevated serum CA 72-4 level was correlated with worse survival of CRC [41]. In line with these findings, the results of our study showed that CRC patients with dMMR are prone to have normal serum CEA and elevated CA 72-4. Moreover, after the addition of CEA and CA 72-4, the discrimination ability of pathology-based model was significantly improved.

In conclusion, our study presents two nomograms based on simplified clinicopathological features alone or in combination with STMs, which can be conveniently used to facilitate the postoperative individualized prediction of MMR status in CRC patients. However, its limitations also deserve commentary. First, this was a nonrandomized retrospective analysis, and as such, there were potential biases for comparison, such as patient inclusion and sample selection biases. Second, radiomics, focusing on the relationship between imaging phenotypes and genomics [44], were not considered in this study. However, our findings will hopefully be integrated with radiomics or other markers to achieve a stronger predictive ability of MMR status in the future.

Data Sharing Statement

The dataset generated during the current study is available from the corresponding author on reasonable request.

Declaration of Interests

The authors have declared that no competing interests exist.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2020.103060.

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