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Prediction models in Lynch syndrome

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Abstract

Prediction models for the identification of Lynch syndrome have been developed to quantify an individual's risk of carrying a mismatch repair gene mutation and help clinicians decide for whom further risk assessment and genetic testing is necessary. There are diverse clinical settings in which a healthcare provider has the opportunity to assess an individual for Lynch syndrome. Prediction models offer a potentially feasible and useful strategy to systematically identify at-risk individuals, whether they are affected with colorectal cancer or not, and to help with management of the implications of molecular and germline test results. Given the complexity of diagnostic information currently available to clinicians involved in identifying and caring for patients with Lynch syndrome, prediction models provide a useful and complementary aid in medical decision-making. Systematic implementation of prediction models estimates should be considered in routine clinical care and at various stages of cancer risk assessment and prevention. In this manuscript, we review the main prediction models developed for Lynch syndrome, focus on their specific features and performance assessed in several validation studies, compare the models with other clinical and molecular strategies for the diagnosis of Lynch syndrome, and discuss their potential uses in clinical practice.

Keywords

Lynch syndrome; Prediction models; PREMM_{1,2,6}; MMRPro; MMRPredict

Introduction

Lynch syndrome is the most common hereditary colorectal cancer (CRC) syndrome and is associated with underlying mutations in the mismatch repair (MMR) genes, mainly *MLH1* and *MSH2*, but also *MSH6*, *PMS2*, and *EPCAM*. Individuals with Lynch syndrome have an increased risk of developing CRC, often at an early age, as well as other cancers, including those of the uterine tract lining, ovaries, stomach, small intestines, urinary tract, brain, and

cutaneous sebaceous glands [1]. Early identification of individuals with MMR gene mutations is important as it allows for implementation of cancer prevention strategies such as intensified surveillance, prophylactic surgery or chemoprevention.

Several approaches are available to identify at-risk individuals and families with Lynch syndrome, including selecting individuals based on fulfillment of clinical criteria and molecular testing of tumors for evidence of microsatellite instability (MSI) through a universal (testing all CRCs) or targeted approach (based on age or family history criteria), followed by germline genetic testing for those deemed to be at increased risk. Prediction models in Lynch syndrome have been recently developed to quantify the risk of detecting a mutation based on personal and family history and help clinicians decide who should be referred for further risk assessment and/or genetic testing. In this manuscript, we will review the main prediction models developed for Lynch syndrome, focusing on their specific features and performance assessed in several validation studies, compare the models with other clinical and molecular strategies for the diagnosis of Lynch syndrome, and discuss their potential uses in clinical practice.

Identification of individuals with Lynch syndrome

Clinical criteria: Amsterdam criteria and Bethesda guidelines

Common strategies to identify individuals at risk for Lynch syndrome include fulfillment of clinical criteria such as the Amsterdam criteria or the revised Bethesda guidelines, which were developed by a consensus of experts [2, 3]. The Amsterdam criteria were originally developed for research purposes to distinguish families suspected of having hereditary non-polyposis colorectal cancer and determine the prevalence of MMR gene mutations [4]. The Bethesda guidelines were developed as a broader screening tool to identify patients whose tumors should be tested for MSI and were revised in 2004 to include both personal and family history features, including extracolonic malignancies associated with Lynch syndrome, age at diagnosis and pathologic characteristics of the tumor [2, 3]. However, the Amsterdam criteria and some components of the Bethesda guidelines are quite complex to apply and each aspect of personal and family history is weighed equally in both guidelines. Furthermore, these criteria encompass multiple diagnoses across generations and are not designed to determine the likelihood of carrying a genetic mutation for an individual patient. Several studies report that the Amsterdam criteria lack sensitivity and specificity for identification of individuals with Lynch syndrome and some have shown that the Bethesda guidelines may miss between 6 and 25 % of mutation carriers [5–8].

Molecular tumor screening: MSI and IHC testing

Germline mutations in the MMR genes result in the accumulation of mutations during DNA replication, particularly in repetitive sequences known as microsatellites. This microsatellite instability is a hallmark of tumors associated with Lynch syndrome. Mutations in the MMR genes also lead to loss of expression of the corresponding protein in the tumor. Therefore, immunohistochemical (IHC) analysis can be performed to assess which gene has been mutated. Lack of staining of MLH1 indicates either a germline mutation or methylation of the promoter, while lack of staining for MSH2 is a strong indicator for either an *MSH2* or *MSH6* mutation. From a clinical perspective, recognizing the heterodimeric partners *MSH2–MSH6* and *MLH1–PMS2* helps identify the causal mutations in Lynch syndrome by IHC. Nevertheless, approximately 15 % of sporadic cases also exhibit MSI, mainly due to somatic hypermethylation of the *MLH1* promoter. Inclusion of *BRAF* mutation testing and *MLH1* hypermethylation analyses on tumor tissue identifies sporadic microsatellite unstable CRC tumors and thereby may prevent unnecessary genetic evaluations [9].

Identification of Lynch syndrome patients

The identification of patients and families with Lynch syndrome remains a challenge. Some investigators have recommended identifying individuals based upon fulfillment of the revised Bethesda guidelines followed by MMR deficiency screening, while others recommend performing IHC on all CRC specimens [6, 10, 11]. Studies have consistently shown suboptimal assessment of family cancer history and genetics referrals among different health care professionals [12, 13]. In addition, a recent study carried out in the United States has shown that universal screening for Lynch syndrome among CRC patients based on IHC, with or without MSI testing, is feasible, but not well-adopted by cancer centers [14]. For all of the above reasons, different diagnostic strategies for identification of Lynch syndrome patients continue to be debated in the literature and clinical practice. Quantification of risk based on prediction models offers an additional means to identify those individuals at higher risk of being mutation carriers, whether affected or unaffected by cancer. The numerical estimates that the models provide can also be very helpful in communicating the results of the cancer risk assessment and its implications to patients and their family members.

Development of prediction models

Historical perspective: The Wijnen model and the Amsterdam plus model

In 1998, Wijnen and colleagues were the first to develop a multivariable model for the identification of *MLH1* and *MSH2* point mutations [15]. They identified three predictors in 184 unrelated kindreds at high risk of familial CRC: fulfillment of the Amsterdam criteria, mean age at diagnosis of CRC, and presence of endometrial cancer in the family. In 2004, a prediction model called Amsterdam plus, was developed from a familial cancer clinic population that added five variables to the Amsterdam criteria to improve its ability to predict MMR gene mutations: number of CRC and endometrial cancers in the family, number of individuals with two or more CRC or endometrial cancers, mean age at diagnosis, and number of individuals with five or more adenomas [16]. Both models include rather complex variables within the Amsterdam criteria, were developed using relatively small populations from dedicated high-risk clinics and have not been externally validated, potentially limiting their generalizability to other settings. These models also do not consider other important factors, such as the extracolonic tumors other than endometrial cancer that are part of Lynch syndrome, and have not become widely adopted in clinical practice.

Recent prediction models

In 2006, three prediction models were introduced to quantify an individual's probability of carrying a MMR gene mutation most commonly associated with Lynch syndrome. These models include MMRPredict, MMRPro, and Prediction of Mismatch Repair Gene Mutations in *MLH1* and *MLH2* (PREMM_{1,2}) [17–19]. The latter model has recently been extended to include prediction of *MSH6* gene mutations and has been replaced by the PREMM_{1,2,6} model (Prediction of Mismatch Repair Gene Mutations in *MLH1*, *MSH2*, and *MSH6*) [20]. While the overall purpose of each model is the same, each has been developed very differently, Table 1 provides a comparison of the three prediction models.

MMRPredict

The MMRPredict model was developed using data from unselected patients with CRC diagnosed under the age of 55 years and applies logistic regression methodology [17]. The mutation prevalence in the development cohort of 875 subjects was 4 % and included mutations in *MLH1* (n = 15), *MSH2* (n = 16), and *MSH6* (n = 7). The model gives an overall likelihood of carrying a mutation in *MLH1*, *MSH2* or *MSH6*, but does not provide

gene-specific risk estimates. MMRPredict calculates the overall risk estimate based on information obtained in two stages. Variables in the model's first stage pertain to clinical information and include the patient's age at CRC diagnosis, gender, location of tumor (proximal versus distal), multiple CRCs (synchronous or metachronous), and presence and age(s) of CRC and/or endometrial cancer diagnoses in first-degree relatives (FDR). The second stage incorporates tumor molecular diagnostic information (MSI and IHC testing) to refine the risk estimate provided in the first stage. MMRPredict does not include extracolonic cancer history in the proband. Family history is limited to FDRs, and does not take into account the number of relatives affected with CRC or endometrial cancer. The investigators also validated the model in an independent, retrospective series of patients diagnosed with CRC before the age of 45 years. Overall, the model would perform best in patients with young-onset CRC, as it was developed and validated in patients diagnosed before age 55 and 45 years respectively, and does not generate risk estimates for probands that are affected with extracolonic Lynch syndrome associated cancers but have not had CRC.

MMRPro

The MMRPro model was developed using published values for MMR mutation prevalence and penetrance for *MLH1*, *MSH2*, and *MSH6* gene mutations and unlike MMRPredict and PREMM, estimates risk based on a Bayesian approach [18]. Independent, external validation was performed by the investigators involved in its development using data from 279 patients presenting to familial cancer registries where the mutation prevalence was 43 % with 51 *MLH1*, 63 *MSH2*, and 7 *MSH6* gene mutation carriers. The model includes data for the proband and for each FDR and second-degree relative (SDR) on the presence of CRC and/or endometrial cancer, age at diagnosis, and current age or age at last follow-up for those unaffected by these cancers. The MMRPro model does not take into account the presence of multiple CRCs in the proband or any Lynch syndrome associated cancers other than endometrial cancer. The model incorporates molecular tumor testing results including MSI and IHC testing. It calculates the overall risk of carrying any one of the three gene mutations and provides separate risk estimates for each of the genes. The model's unique features are that it can calculate the residual probability of finding a mutation if molecular tumor testing is negative and provide estimates of future colorectal and endometrial cancer risks for unaffected individuals (including known mutation carriers), untested individuals, and individuals in whom no mutation is found.

PREMM_{1,2,6}

The PREMM_{1,2,6} model was developed using genotype and phenotypic data from 4,539 individuals who underwent genetic testing based on either personal or family history of cancer using multivariable polytomous logistic regression methodology [20]. The mutation prevalence was 12 % and the cohort represents the largest number of unrelated gene mutation carriers used for model development and validation (204 *MLH1*, 250 *MSH2*, and 71 *MSH6* gene mutations). Proband specific variables include gender, the occurrence and age at CRC diagnosis (including multiple CRC diagnoses), endometrial cancer and other Lynch syndrome-associated cancers (including cancers of the ovary, stomach, kidney, ureter, bile duct, small bowel, brain (glioblastoma multiforme), pancreas, or sebaceous gland). Variables related to family members are limited to FDR and SDR cancer histories and include the number of relatives with CRC, endometrial cancer, or other Lynch syndrome-associated cancers as well as the minimum age at diagnosis of each cancer among relatives. The PREMM_{1,2,6} model replaces the original PREMM_{1,2} model which calculated risk of *MLH1* and *MSH2* gene mutations based on the same aforementioned variables except for gender and omits the presence of polyps in the proband. The model does not include molecular tumor data in risk prediction or data on unaffected family members. The

PREMM_{1,2,6} model was externally validated by the investigators involved in its development among 1,827 population-based CRC cases and high-risk patients recruited through an international consortium.

Performance of prediction models compared to clinical criteria

With the introduction of Lynch syndrome prediction models, their ability to identify gene mutation carriers as compared to existing clinical criteria has also been studied. The performance of each model compared to Amsterdam and Bethesda guidelines has been assessed in each respective development cohort and in most cases, found to exceed the clinical criteria's ability to discriminate carriers from noncarriers. MMRPredict performed similarly to the Bethesda guidelines at a cut-off of 0.005 with sensitivity of 95 % for both strategies, specificity of 14 and 38 %, and positive predictive value of 5 and 6 % respectively. MMRPredict performed better than the Amsterdam criteria alone and in combination with MSI testing [17]. The PREMM_{1,2} model selected 20 % more individuals than the revised Bethesda guidelines at a 5 % cut-off value, yielding higher sensitivity (94 vs 74 % respectively) but with 10 % lower specificity [19]. At this cut-off, the miss rate of mutation carriers was 6 % for PREMM_{1,2} compared with 26 % for Bethesda and 37 % for Amsterdam II criteria. In an independent, population-based study of 1,222 CRC cases that compared the PREMM_{1,2} and MMRPredict models for the identification of *MLH1* and *MSH2* gene mutation carriers, at a cut-off score of 5 %, the sensitivity of the revised Bethesda guidelines and PREMM_{1,2} was 100 % (95 % CI 71–100 %) compared to 87 % sensitivity (95 % CI 51–99 %) for MMRPredict [21, 22]. Use of the Amsterdam II criteria alone yielded 50 % sensitivity while specificity was highest at 98 %. Lastly, in the sample used to validate MMRPro's performance as determined in the development cohort, MMRPro better identified carriers from noncarriers than the Bethesda guidelines, with and without MSI testing [18].

In a recent study by Green et al., the three models were compared among 725 unselected CRC cases diagnosed before age 75 years and 18 individuals (2.5 %) were identified with MMR gene mutations: eleven carriers met Amsterdam I criteria, one met Amsterdam II criteria, five others met only the Bethesda criteria, and one met none of these criteria [23]. The Bethesda guidelines yielded 94 % sensitivity and in comparing the models at cut-off points that would produce this same sensitivity, specificities were higher for the models, ranging from 61 to 88 % compared to 51 % for the Bethesda criteria (95 % CI 47.2–54.7 %).

Performance of prediction models compared to molecular tumor screening

One study has compared the performance of molecular tumor testing to prediction of MMR gene mutation status using the PREMM_{1,2,6} model [24]. The model's ability to discern gene mutation carriers from noncarriers compared to molecular tumor testing was evaluated in 1,651 patients with CRC recruited through an international consortium of population and clinic-based family registries. There were 239/2,651 (14 %) mutation carriers and when assessed individually, the AUCs for PREMM_{1,2,6}, IHC, and MSI were 0.90, 0.82, and 0.78 respectively. The strategy which most improved the PREMM_{1,2,6} model's discriminative ability among both population and clinic-based cases was adding IHC tumor testing to PREMM_{1,2,6} prediction where AUC improved to 0.94 in the overall and population-based cohorts and 0.92 in the clinic based cohort. There was no additional value of MSI testing to the combination of PREMM_{1,2,6} + IHC. The impact of the proband's age at CRC diagnosis on the strategies' ability to discriminate mutation carriers from noncarriers was assessed and for every 10 year increase in the initial age of CRC diagnosis, IHC's performance decreased with AUC of 0.85 for pro-bands diagnosed by 50 years, 0.84 for CRC diagnosed by 60 years, and 0.82 for CRC diagnoses by 70 years. In contrast, the performance of PREMM_{1,2,6}

increased slightly with every 10 year increase in age at time of CRC diagnosis: AUC of 0.87 to 0.88 to 0.90 for CRC diagnoses made by 50, 60 and 70 years respectively. Similar to IHC, the discriminatory ability of MSI testing was 0.83 for CRC cases diagnosed by ages 50 and 60 years, which decreased to 0.81 for cases diagnosed by 70 years.

Validation of prediction models

A number of studies independently validate the performance and accuracy of the three recently developed prediction models, other than those aforementioned validation studies performed by the investigators involved in the development of each model (Table 2). Only one study provides external validation and comparison of the PREMM_{1,2,6} model to MMRPro and MMRPredict, as the PREMM_{1,2,6} model was most recently introduced in 2011 [25]. In a multicenter US-based study of 230 referred patients at high-risk of CRC, 113 gene mutation carriers were identified where the three models' performance was similar: AUCs were 0.76 for MMRPredict (95 % CI 0.68–0.84), 0.78 for PREMM_{1,2,6} (95 % CI 0.72–0.84), and 0.82 for MMRPro (95 % CI 0.74–0.86). The performance of each model was assessed across a range of sensitivities: to obtain a sensitivity of 90 %, a threshold for mutation testing of >4 % for MMRPro would provide 29 % specificity; a cut-off of >6 % for PREMM_{1,2,6} would be 38 % specific, and for MMRPro, a cut-off score of >7 % would provide 36 % specificity.

Previous validation studies comparing PREMM_{1,2} to MMRPredict and MMRPro have yielded similar results among selected patients at high-risk for CRC. In an early study comparing the models' ability to discern gene mutation carriers from noncarriers identified through a cancer genetics clinic (n = 81), MMRPredict, PREMM_{1,2} and MMRPro performed similarly (AUCs of 0.73, 0.77 and 0.73, respectively) in identifying the 39 carriers [26]. However, predictions were not possible in seven individuals with extracolonic cancers using the MMRPredict model as it requires a CRC diagnosis. The investigators also compared predictions for each model among different risk categories to determine whether there was over- or under-prediction of mutation carriers: all individuals in the highest risk category (75–100 %) according to PREMM_{1,2} were carriers whereas nearly half of the individuals identified by MMRPro were carriers. Within the lowest high-risk category of <10 %, MMRPro and PREMM_{1,2} identified the majority as noncarriers whereas MMRPredict underestimated the carriers, missing eight carriers. MMRPredict overpredicted gene mutation carrier status between 10 and 75 % and had the most variability (dispersion) of probability estimates compared with PREMM_{1,2} and MMRPro. The authors concluded that the PREMM_{1,2} model was more accurate in detecting carriers and MMRPro was more accurate in detecting noncarriers.

A similar study of 25 gene mutation carriers detected among 72 cases evaluated through a high-risk genetics clinic found comparable performance characteristics between the three models: AUCs of 0.86 (95 % CI 0.7–0.96), 0.89 (95 % CI 0.81–0.98) and 0.93 (95 % CI 0.86–0.99) for the MMR-Predict, MMRPro and PREMM_{1,2} models, respectively where PREMM_{1,2} demonstrated the best performance for predicting carrier status based on the positive likelihood ratios at >10, >20 and >30 % probability thresholds [27].

The three studies that validate the models among unselected, population based CRC cases are limited by the few mutation carriers in each cohort: 8/214, 8/1,222 and 18/725 mutation carriers (Table 2) [21, 23, 28]. The latter study, which assessed patients with CRC diagnosed before age 75 years found that all the models overestimated MMR gene mutation carrier status by 2.1 to 4.3 fold. The investigators further corrected the prediction estimates for bias introduced by family size and age structure on the non-Bayesian derived models (MMRPredict and PREMM_{1,2}) and improvement in discriminating of carriers from

noncarriers was noted for both models. With this correction, the authors concluded that MMRPredict performed best in their dataset, which achieved a sensitivity of 94 % (95 % CI 73–99 %) and a specificity of 91 % (95 % CI 88–93 %), but recommend use of any of the models in identifying patients with CRC who would benefit from molecular tumor testing and further evaluation for Lynch syndrome.

Prediction models and cost-effective strategies for the identification of Lynch syndrome

To the best of our knowledge, only one cost-effectiveness analysis compares different strategies in identifying MMR gene mutation carriers among CRC patients which includes prediction model estimates [29]. Results from a Markov modeling analysis examine clinical criteria (Amsterdam and Bethesda guidelines), the three prediction models (MMRPro, MMRPredict, and PREMM_{1,2,6}), tumor testing or up-front germline mutation testing followed by directed screening and risk-reducing surgery. When clinical criteria were met or prediction model scores were >5 % probability of carrying a gene mutation, IHC testing followed by germline testing was offered or germline testing was directly pursued. Under the assumption of perfect implementation of all strategies, tumor testing strategies were more costly than clinical criteria and prediction model strategies. However, with decreased implementation rates of clinical criteria strategies compared with tumor testing, the latter became more cost-effective. The most cost-effective strategy to evaluate for Lynch syndrome among newly diagnosed patients with CRC diagnosed before age 70 years was IHC testing (plus *BRAF* mutation testing for MLH1 protein loss), followed by targeted MMR gene sequencing; an incremental cost-effectiveness ratio of \$36,200 per life year gained resulted from this strategy. However, the critical determinant of cost-effectiveness of any strategy was highly dependent on the number of relatives per proband who underwent germline testing and opportunities for cancer risk reduction.

A second cost-effective analysis examines screening approaches for Lynch syndrome among individuals without cancer in the general population [30]. In a simulation-based analysis using the PREMM_{1,2,6} model to detect individuals with MMR gene mutations and their at-risk relatives, integrated models of CRC and endometrial cancers with a 5-generation family history to predict health and economic outcomes of 20 primary screening strategies (with a wide range of compliance levels) were analyzed. These strategies were characterized by different screening ages for starting risk assessment and different risk thresholds above which to recommend genetic testing. For each strategy, 100,000 simulated individuals representative of the US population were followed from 20 years and the outcomes were compared with current practice. The results indicate that risk assessment starting at ages 25, 30, or 35 years, followed by genetic testing of those with mutation risks exceeding 5 % assessed by the PREMM_{1,2,6} model, reduced CRC and endometrial cancer incidence in MMR gene mutation carriers by approximately 12.4 and 8.8 %, respectively. For a population of 100,000 individuals with 392 mutation carriers, this strategy increased quality-adjusted life-years (QALY) by approximately 135 with an average cost-effectiveness ratio of \$26,000 per QALY where the cost-effectiveness of screening for MMR gene mutations was comparable to currently well-accepted cancer screening activities for CRC, cervical and breast cancer. These results suggest that assessing unaffected individuals' risk for Lynch syndrome based on their family cancer history starting between the ages of 25–35 years is appropriate and cost-effective.

Summary of comparison of models

The MMRPredict, MMRPro and PREMM_{1,2,6} models all provide a quantitative assessment of the risk of being a MMR gene mutation carrier. There is ample evidence that each of the

models have superior performance characteristics in terms of sensitivity, specificity, positive and negative predictive values to support the use of the models over the existing clinical guidelines for the diagnosis and evaluation for Lynch syndrome.

Deciding which Lynch syndrome prediction model to choose in the risk assessment process depends on both the clinical setting in which it is applied and the patient population that is being evaluated. Validation studies show that the models have similar ability to discriminate gene mutation carriers from noncarriers. While the models were all developed for the same purpose, the populations in which they were validated brings to light each model's specific characteristics that may impact the accuracy of the provided risk estimates and help healthcare providers determine which may be more appropriate to use in their respective clinical settings.

MMRPredict is best applicable in the assessment of patients with young onset CRC and its use could provide less accurate results when used to evaluate families with Lynch syndrome associated cancers and older individuals affected by CRC. MMRPro's predictions account for family size and unaffected relatives, the possibility of including molecular tumor data in the risk analysis, and the option of predicting gene mutation carrier status following germline testing. The major limitation in the widespread use of MMRPro in routine practice outside the genetics setting is the need to input the entire pedigree which is relatively time consuming. Its best use at the current time is likely to be as a genetic counseling tool in a specialized high-risk clinic or research setting. PREMM's major advantage is that it is easy to use and has been extensively validated. It includes risk prediction based on personal and family cancer history up to second-degree relatives for a broad spectrum of extracolonic cancers. However, the model does not take into account family size and may overestimate the likelihood of a mutation in a pedigree with includes multiple, elderly family members who are unaffected by CRC or endometrial cancer. Furthermore, the model does not incorporate tumor testing results or provide post hoc risk estimates based on gene sequencing results. Given the ease with which one can use the PREMM_{1,2,6} model (it has been deemed less time-consuming than MMRPro in validation studies) [25], it may be best considered by diverse healthcare providers whose aim is primarily to identify those patients who should be referred for genetic evaluation and is likely to be most useful in the pre-testing decision-making process. Further data on the performance of models in diverse populations as well as the accuracy of the gene-specific predictions of MMRPro and PREMM_{1,2,6} are needed.

How can prediction models help clinicians?

The best approach in identifying patients at risk for Lynch syndrome is one that is attainable by a majority of clinical practices. Given the diverse clinical settings in which a healthcare provider has the opportunity to assess an individual for Lynch syndrome, prediction models offer a potentially feasible and useful strategy to systematically identify at-risk individuals, whether they are affected with CRC or not, and to help with management of the implications of molecular and germline test results.

Prediction models and the evaluation of patients with CRC

For the initial assessment of Lynch syndrome among patients with CRC, it is important to determine whether the tumor specimen is available for additional molecular diagnostic testing.

For patients with CRC and available tumor block—Molecular tumor testing, including IHC and MSI testing of the CRC tumor, complemented by *BRAF* testing for cases with loss of *MLH1* protein expression, is an important strategy in the assessment for Lynch

syndrome. Two possible approaches seem to be the most reasonable given emerging data—universal testing of *all* CRCs regardless of age with IHC and/or MSI, or only testing patients diagnosed before age 70 years, as the latter is likely to be more cost-effective [29].

The models can complement molecular testing strategies in both of these scenarios (Fig. 1) [24]. To date, variable cutoff scores have been assessed for the PREMM_{1,2,6} model regarding its use compared to molecular tumor testing; comparable cut-off scores for the MMRPro and MMRPredict models have not yet been explored. A PREMM_{1,2,6} score of 5 % may be used as a first step in the assessment of Lynch syndrome in patients with CRC diagnosed above 70 years. Molecular tumor testing and genetic testing can likely not be pursued with PREMM_{1,2,6} scores <5 % in patients older than 70 years, as the likelihood of Lynch syndrome in the absence of significant family cancer history is unlikely and any abnormal tumor testing results are more likely to be age-related hypermethylation of the *MLH1* gene promoter than a germline mutation. This approach can thereby limit the increased costs related to molecular diagnostic testing. For those patients with PREMM_{1,2,6} scores 5 %, IHC testing, along with *BRAF* testing for those tumors which display loss of *MLH1*, should be performed and results used to direct gene-specific DNA mutation analysis in order to ensure cost-efficiency. In cases where a *BRAF* mutation is detected, no germline testing would be necessary unless the PREMM_{1,2,6} score was >15 %. For patients with normal MSI or IHC, a PREMM_{1,2,6} cut-off of 15 % still necessitates DNA mutation analysis due to the possibility of false negative results. When no gene mutation is detected, MSI testing may be helpful for evaluating these patients as they are high risk for Lynch syndrome based on personal and family cancer history. MSI testing can stratify genetic testing results into either (1) indeterminate negative, where patients with MSI-high tumors should be treated as having as Lynch syndrome or (2) “Familial CRC X,” where patients with microsatellite-stable tumors do not need intensive cancer surveillance as those recommended for Lynch syndrome.

For patients with CRC when a tumor block is unavailable—The opportunity to evaluate a patient for Lynch syndrome should not cease when their CRC tumor block is unavailable for molecular diagnostic testing. This may be the case when (1) the history of CRC was made years prior and tumor specimens can not be retrieved, (2) there are technical limitations to performing IHC or MSI testing, or (3) genetic evaluation and testing is necessary prior to any surgical decision-making. Risk assessment using the prediction models should be the strategy of choice when tumor testing is not feasible and is supported by the National Comprehensive Cancer Network where patients with a risk score of 5 % should be offered germline testing for Lynch syndrome [31].

Prediction models and the evaluation of individuals without CRC

Risk assessment based on family history is necessary in unaffected individuals to ensure appropriate surveillance for CRC and other associated cancers. When a family history of cancer is present, the quantifiable estimates provided by prediction models simplify the risk assessment process, making it easier for patients to understand risk and help them decide the next course of action regarding their genetic evaluation and/or surveillance strategies. Germline testing should be considered for those unaffected individuals with a risk estimate of 5 %, where no affected family member is available for testing, and is an approach that has been shown to be cost-effective [30].

Summary

Given the complexity of the diagnostic information available to clinicians involved in identifying and caring for patients with Lynch syndrome, prediction models provide a useful and complementary aid in medical decision-making. With growing evidence and consensus

that the prediction models outperform existing clinical criteria for Lynch syndrome, systematic implementation of prediction models estimates should be considered in routine clinical care and at various stages of cancer risk assessment and prevention.

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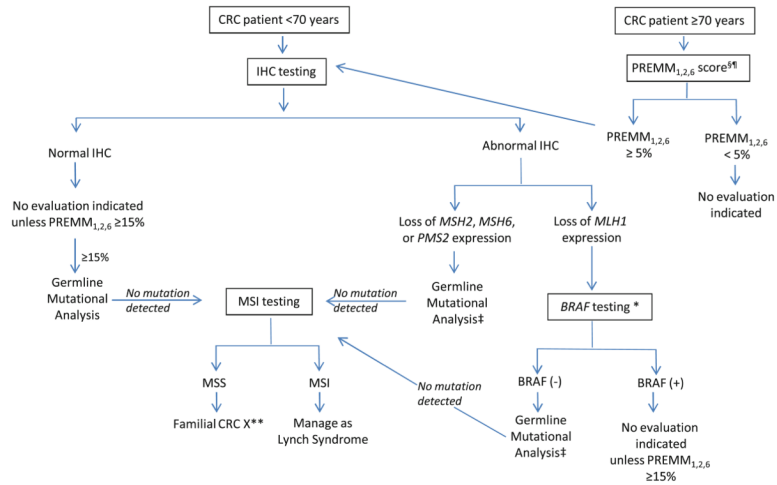


Fig. 1. Proposed Algorithm for Systematic Evaluation for Lynch syndrome in Patients with Colorectal Cancer[‡]. *CRC* colorectal cancer, *MSI* microsatellite instability; *MSS* microsatellite stable, *IHC* immunohistochemistry.[‡] In patients without available tumor for molecular diagnostic testing and in those individuals without colorectal cancer, a $PREMM_{1,2,6}$ score $\geq 5\%$ should be followed by germline mutational analysis.[§] $PREMM_{1,2,6}$ score can be calculated at website <http://www.dfc.org/premm>; [¶] Other models (MMRpro, MMRpredict) may also be used with their own specified cut-off scores; * *BRAF* testing: + mutation present, – mutation absent/wild type; ** Surveillance recommendations based on personal and family history; [‡]Gene-specific germline mutational analysis

Table 1

Summary of the prediction models for the identification of mismatch repair gene mutation carriers

Model	Ascertainment	# Individuals tested	Outcome	Method	Molecular testing included	Predictors	Strengths	Limitations	Software
Dutch model [15]	Clinic-based	184	Point mutations in <i>MLH1</i> , <i>MSH2</i>	Logistic regression analysis	NO	Young age at CRC diagnosis in the family; fulfillment of Amsterdam criteria; presence of endometrial cancer in the family	1st predictive model developed for Lynch syndrome; simple equation	Developed in a high risk population; Amsterdam criteria as a predictor; not validated; <i>MSH6</i> and large rearrangement analysis not included	http://www5.utsouthwestern.edu
Amsterdam - plus [16]	Derivation and validation: clinic-based	250 and 94	Point mutations in <i>MLH1</i> , <i>MSH2</i> , <i>MSH6</i>	Logistic regression analysis	NO	Fulfillment of Amsterdam criteria; number of relatives with CRC and endometrial cancer; number of relatives with multiple CRC or endometrial cancer; mean age at diagnosis of CRC and endometrial cancer; number of relatives with >5 colonic adenomas	Better accuracy compared to non-individualized Amsterdam criteria	Complex variables; non-individualized risk prediction; software not available; large rearrangement analysis not included	N/A
MMRPredict [17]	Derivation: population-based CRC patients < 55 years Validation: population-based CRC patients < 45 years	870 155	Overall estimate <i>MLH1</i> , <i>MSH2</i> , <i>MSH6</i>	2 stage: Multivariate logistic regression analysis, MSI/IHC data to refine prediction	YES	Proband: age, gender, tumor's location, multiple tumors Family: CRC age (dichotomized at 50 years), endometrial cancer in 1st degree relative)	Population-based cohort; gene-specific estimate and refinement of prediction with molecular data; individualized risk prediction; provides clinical applicability at different cut-offs	Developed and validated in a young onset CRC population; extracolonic Lynch-associated tumors other than endometrial not included	http://www1.hgu.mrc.ac.uk http://www5.utsouthwestern.edu http://astor.som.jhmi.edu/BayesMende/
MMRPro [18]	Derivation: clinic-based Validation: clinic-based	N/A 279	Gene-specific estimate <i>MLH1</i> , <i>MSH2</i> , <i>MSH6</i>	Mendelian and Bayesian analysis	YES	Proband and family: CRC status, age at diagnosis, relation to the proband, current age or at last follow-up, MSI/IHC, germline testing result	Offers pre- and post-sequencing gene-specific estimates; accounts for unaffected relatives and family size; considers molecular data; offers risk prediction of developing cancer in unaffected members	Time consuming process with pedigree drawing; extracolonic Lynch-associated neoplasms other than endometrial cancer not included	http://www5.utsouthwestern.edu http://astor.som.jhmi.edu/BayesMende/
PREMM _{1,2,6} [20]	Derivation: clinic-based Validation: population and clinic-based	4,538 1,827	Gene-specific estimate <i>MLH1</i> , <i>MSH2</i> , <i>MSH6</i>	Logistic regression analysis	NO	Proband: age, gender, number of CRC, other Lynch-associated tumors Family: age and number of 1st and 2nd degree relatives with CRC and other Lynch-associated tumors	Provides gene-specific estimates and individualized risk prediction; provides clinical applicability at different cut offs; easy-to-use	Molecular tumor data not incorporated; family size and unaffected individuals are not considered	http://www.dfci.org/premm

CRC colorectal cancer, IHC immunohistochemistry, MSI microsatellite instability, N/A not available

Table 2
Comparison of prediction models and clinical criteria for the identification of Lynch syndrome

Study	Cohort	Subjects N	MMR Gene Mutation Prevalence N (%)	MMRPredict AUC (95 % CI)	MMRPro AUC (95 % CI)	PREMM AUC (95 % CI)	Revised Bethesda AUC (95 % CI)	Amsterdam Criteria AUC (95 % CI)
Khan et al. [25]	High-Risk	230	113 (49)	0.76 (0.68–0.84)	0.82 (0.74–0.86)	0.78 (0.72–0.84)	AUC: 0.52 (n/a) Sens: 95 % Spec: 10 %	AUC: 0.68 (n/a) Sens: 81 % Spec: 52 %
Pouchet et al. [26]	High-Risk	81	39 (48)	0.73 (0.61–0.86)	0.73 (0.62–0.85)	0.77 (0.65–0.88)	n/a	n/a
Monzon et al. [27]	High-Risk	72	25 (35)	0.86 (0.76–0.96)	0.90 (0.82–0.98)	0.93 (0.86–0.99)	n/a	n/a Sens: 76 % Spec: 74 %
Balmaña et al. [22]	Population-based	1,222	8 (0.7)	0.92 (0.83–1.0)	n/a	0.93 (0.86–0.99)	n/a Sens: 100 % Spec: 77 %	n/a Sens: 50 % Spec: 90 %
Green et al. [23]	Population-based	725	18 (2.5)	0.96 (0.94–0.97)	0.95 (0.93–0.96)	0.91 (0.89–0.93)	n/a Sens: 94 % Spec: 51 %	n/a Sens: 66 % Spec: 96 %
Tresallet et al. [28]	Population-based	214	8 (3.7)	0.76 (0.65–0.88)	0.73 (0.49–0.98)	0.76 (0.59–0.93)	n/a Sens: 75 % Spec: 59 %	n/a Sens: 37 % Spec: 99 %

AUC area under the receiver operating characteristic curve, n/a not available, Sens sensitivity, Spec specificity