Genetic mutation risk calculation in Lynch syndrome inheritance: Evaluating the utility of the PREMM1,2,6 model in Lyon: The first French study

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Summary

Lynch syndrome is due to germline mutations in mismatch repair genes: MLH1, MSH2, MSH6 and PMS2. It is characterized by an increased risk of various cancers including colorectal and endometrial cancers. Early diagnosis of these patients allows for appropriate surveillance and improves survival rates. Differentiating between patients who should undergo genetic testing and those for whom it is not necessary is difficult despite various established criteria (Amsterdam and Bethesda). Often, health professionals meet in multidisciplinary committees (MDC) to discuss patient cases regarding Lynch syndrome. In this study, we evaluated if the prediction model PREMM1,2,6 could be used to enhance MDC decision-making and whether it should be included in our own routine practice and in those of other French teams. Using the prediction model in our cohort would have avoided 12% of the analyses recommended by our MDC. Furthermore, all patients with a mutation in one of the MMR genes would have been detected. In addition, according to the model, we should have provided 20% more genetic testing, which suggests that the decision-making criteria used by the professionals in our MDC, was too restrictive. These results suggest that PREMM1,2,6 should be used in current practice to validate the decisions of the MDC before genetic testing is performed in complex cases. The model should be added as a major quality criterion for genetic testing, along with somatic tests, as previously reported in the literature.
**Introduction**

Lynch syndrome is one of the most common diseases that predispose to colorectal cancer. A mutation in one or more *MMR* gene is responsible. In order to provide high-quality care management for patients and their family, genetic counseling is imperative. Decision-making for a given case is not always easy. In complex cases, multidisciplinary committees (MDC) are organized to help professionals optimize decision-making regarding genetic analysis and patient/families follow-up. In France, 76% of hospitals have instituted MDCs to consult in digestive cancers predisposition cases. In our center in Lyon, the MDC meets once-monthly [1].

The MDC meetings bring together oncogeneticists, gastro-enterologists, internal medicine physicians, genetic counselors, pathology and genetic biologists, surgeons and gynecologists. They influence medical management decision-making regarding each specific case so that the joint decision is most beneficial for the patient and the family when a predisposition to cancer is suspected.

Many models of genetic mutation risk calculation exist, notably three whose performances seem to be highest: MMRpredict [2], MMRpro [3], and PREMM1,2,6 [4]. MMRpredict can only be used for patients with colorectal cancer. While it takes into account somatic testing, it does not incorporate endometrial cancer or the others cancers of Lynch-spectrum, nor cancer cases from 2nd degree relatives. MMRpro requires more time to implement as it considers the whole family. However, it only takes into account colorectal and endometrial cancers of the Lynch spectrum, and somatic testing.

In our investigation, we chose to use PREMM1,2,6 because of its statistical strength and its rapidity and simplicity of use. Previous studies have shown that the PREMM1,2,6 prediction tool is the only one that captures all cancers of the Lynch spectrum of 1st and 2nd degree relatives. MMRpro requires more time to implement as it considers the whole family. However, it only takes into account colorectal and endometrial cancers of the Lynch spectrum, and somatic testing.

In our investigation, we chose to use PREMM1,2,6 because of its statistical strength and its rapidity and simplicity of use. Previous studies have shown that the PREMM1,2,6 prediction tool is the only one that captures all cancers of the Lynch spectrum of 1st and 2nd degree relatives. It also allows risk calculation in healthy individuals. Contrary to the two other tools, it does not take into account somatic tests (microsatellite instability and immuno-histochemistry). The 5% threshold of a good positive predictive value of mutation detection is still retained. A comparative synthesis of different risk calculation softwares is shown on table 1.

Different strategies for analysing clinical cases have been proposed in the literature, including somatic tests, informatics-based prediction tools and genetic analysis. Our study aimed to evaluate if this kind of tool would be beneficial to our current practice. Would using prediction risk calculation models, especially PREMM1,2,6, enhance decision-making within our oncogenetic MDC? We explored whether and how PREMM1,2,6 could be integrated our practice in Lyon.

We evaluated the decisions made by our MDC based on the calculation of mutation risk according to PREMM1,2,6. We compared, in our cohort, PREMM1,2,6 analysis (with a 5% PPV threshold of mutation risk) with the decisions made by the MDC and with the genetic result (when known) to look for concordance.

To our knowledge, no previous studies have been conducted to assess the condition and benefits of this tool associated with MDC experts. Our aim is to examine the evaluation of MDC and the improvement of quality decision-making for a family suspected of having Lynch syndrome. This initiative falls within the framework of quality control of the MDC, and assesses the performance of PREMM1,2,6 to determine if it should become a new tool for decision-making in our genetics center.

**Methodology**

**Cohort**

The cases included in this study were patients affected by colon cancer or healthy relatives with a putative genetic predisposition, according to criteria published for the two major consensus conferences focusing on HNPCC-related digestive cancers [5,6], and discussed within our MDC from 2004 to 2012. In total, 240 individual cases were initially selected. Risk calculation using PREMM1,2,6 was possible for 175. The remaining cases were those in which familial polyposis or familial gastric cancers were suspected. Multidisciplinary committees discussed *MMR* gene analysis for 165 patients. Genetic testing was performed via PCR-based sequencing and large rearrangements study of the four *MMR* genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*). *EpCAM* gene testing was also performed. We calculated the gene mutation rate and analyzed the PREMM1,2,6 model’s efficiency in our cohort.

**Genetic risk calculation via PREMM1,2,6**

PREMM1,2,6 is freely available on the following link [http://premm.dfci.harvard.edu/](http://premm.dfci.harvard.edu/).

For each case, this predictive model provides a mutation risk for each gene *MLH1*, *MSH2*, and *MSH6* individually and a cumulative value for an overall risk mutation for all 3 genes combined. We used the 5% relevant threshold for validation of gene analysis as recommended by the model’s designers. Criteria taken into account in this tool are described in box 1.

**Statistical analysis**

For evaluating MDC decisions, statistical analysis was performed on the cohort of patients for whom genetic test indication was discussed (*n* = 165).

For evaluating PREMM1,2,6 mutation rate values (threshold of detecting mutation: 5%), statistical analysis was performed on the cohort of patients for whom genetic results were available (*n* = 55):

- true positive: patients for whom gene test has been validated by PREMM1,2,6 and a mutation has been identified;
- false positive: patients for whom gene test has been validated by PREMM1,2,6 but no mutation has been identified;
- true negative: patients for whom gene test has not been validated by PREMM1,2,6 and no mutation has been identified;
false negative: patients for whom gene test has not been validated by PREMM1,2,6 but a mutation has been identified.

Results
Evaluation of decisions made by MDC vs. PREMM1,2,6
In the 240 selected patients, PREMM1,2,6 calculations were performed for 175 patients. Multidisciplinary committees discussed MMR gene analysis for 165 patients. The 10 remaining cases were discussed for another reason and genetics results were already known. In total, 55 (of 58 performed, validated by MDC according to Amsterdam and Bethesda criteria) genetic results were known.

In 9 cases, lack of familial data meant some cancers were not been taken into account in the risk calculation. In these cases, mutation rates might have been underestimated.

We decided not to take into account unspecified uterine cancers since a significant proportion of these cancers were consistent

Table 1
Comparative table of 3 mains known predictive risk calculation softwares

<table>
<thead>
<tr>
<th></th>
<th>PREMM1,2,6</th>
<th>MMRpredict</th>
<th>MMRpro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model's basis cohort</td>
<td>4539 patients (2526 with CRC)1</td>
<td>870 patients (with CRC &lt; 50 years old only)</td>
<td>Meta-analysis6</td>
</tr>
<tr>
<td>Sex</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Colorectal cancer (CRC) in proposant</td>
<td>X (and number if many CRC)</td>
<td>X3</td>
<td>X</td>
</tr>
<tr>
<td>Endometrial cancer in proposant</td>
<td>X (and age at diagnosis)</td>
<td>0</td>
<td>X</td>
</tr>
<tr>
<td>Proposant's age at diagnosis</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Lynch's large spectrum cancers included in the model</td>
<td>X (but number and age at diagnosis not taken into account)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Relatives included in the model</td>
<td>1/2: affected relatives2</td>
<td>1/4: Genealogic tree2</td>
<td></td>
</tr>
<tr>
<td>RER/IHC results included in the model</td>
<td>0</td>
<td>X5</td>
<td>X</td>
</tr>
<tr>
<td>Mutation risk evaluation in affected proposants</td>
<td>X (not for PMS2)</td>
<td>X (not for PMS2)</td>
<td>X (not for PMS2)</td>
</tr>
<tr>
<td>Mutation risk evaluation in non affected proposants</td>
<td>X</td>
<td>0</td>
<td>X</td>
</tr>
<tr>
<td>Cancer risk evaluation</td>
<td>0</td>
<td>0</td>
<td>X6</td>
</tr>
<tr>
<td>Time spent</td>
<td>Quick</td>
<td>Quick</td>
<td>Longer</td>
</tr>
<tr>
<td>Availability</td>
<td>Free, available online</td>
<td>Free, available online</td>
<td>Free online but require pre-registration and installation of &quot;R&quot; operating system</td>
</tr>
</tbody>
</table>

Three main softwares were described: PREMM, MMRpredict, MMRpro. CRC: colorectal cancer; RER: replication error: microsatellite stability study; IHC: immuno-histochemistry.  
1Other patients have personal or familial history for Lynch syndrome.  
2But don’t take care of several cancers in the same related.  
3Youngest age at diagnosis for CRC and endometrial cancer presence asked.  
4Include also the MMR genetic result if already performed.  
5Chen S, et al. [3]. Study based on mutations frequencies, penetrance and MSI test's predictive value.  
6AFFECTED, non affected, and relatives' age, ages at diagnosis.  
7Colorectal and endometrial cancer risk estimated taking into account the MMR genetic result if already performed.

Box 1
Description of PREMM1,2,6 model score

For each proband, the model takes the following criteria into account:

• sex;
• number of colorectal cancers (0; 1; 2 or more) and age at youngest diagnosis (mandatory);
• endometrial cancer and age at youngest diagnosis (mandatory);
• other cancers of the large spectrum of Lynch syndrome (ovary, stomach, small intestine, urinary tract; kidney, biliary tract, multiform glioblastoma (brain) tumors of the sebaceous glands, pancreas).

For 1st and 2nd degree relatives:

• number of relatives with colorectal cancer and age at youngest diagnosis (mandatory) – number of relatives with endometrial cancer and age at youngest diagnosis (mandatory);
• other Lynch Syndrome-associated cancers (large spectrum).
with cervical cancer [7]. This occurred in 5 cases and may have resulted in an underestimation of their mutation rates (appendix 1).

One case of appendix carcinoma that was reported in a proband was also not taken into account. A study by Taggart et al. [8] showed that of this type of carcinoma may not have the same specifications as the colorectal cancers linked to Lynch syndrome (few MSI-h phenotype and no constitutional MMR gene mutation).

In our cohort, 38% ($n = 66$) were male and 62% ($n = 109$) were female. The Male/Female ratio was 0.6. The average age of patients was 56 years. Among the 165 cases discussed, the MDC recommended MMR gene analysis in only 35% ($n = 58$). Genetic test results were known for 55/58 of this population. PREMM1,2,6 indicated an analysis would have been appropriate in 55% of cases ($n = 91/165$). Somatic testing results are the key for indicating whether genetic testing is appropriate. Table II represents the somatic test results obtained in our cohort. When microsatellite instability (MSI-high) and/or non-expression of MMR proteins was reported by immuno-histochemistry, analyzing MMR genes was recommended. In some cases with MSS or negative IHC phenotype, MMR gene was recommended by MDCs because that corresponded to families that met Amsterdam or Bethesda criteria, or according to the youngest age of cancer diagnosis. The MDC’s decision to recommend genetic testing was concordant with PREMM1,2,6 in 63% of cases ($n = 104$). Discordance between MDC and PREMM1,2,6 was shown in 37% of cases ($n = 61$) (appendix 2).

In this population, 77% ($n = 47/61$) of discordances correspond to the situation in which PREMM 1,2,6 recommended MMR genetic testing and MDC excluded it (table III). Non-validation of gene testing by the MDC was generally explained by the observation of negative somatic results ($n = 27/47$) in these cases (table II). In 6 cases, the MDC recommended performing somatic testing before deciding to go forward with MMR analysis. For the 3 cases (out of 14), somatic testing was discordant with the MDC’s decision, PREMM1,2,6 scores. Genetic testing was recommended by the MDCs but did not exceed PREMM1,2,6 threshold in 23% ($n = 14/61$) of the discordant cases. Among them, 7 cases revealed negative genetic results. The remaining 7 test results were still unknown at the time of writing the manuscript. Using the PREMM1,2,6 model would have resulted in at least 7 fewer analysis or 12% ($n = 7/61$) of genetic tests recommended by
the MDC. For these 14 cases, the motivation of the MDC to recommend genetic testing was, primarily, the somatic testing results \((n = 11/14)\) (appendix 3).

**Genetic results of the cohort**

Among the tested patients (validated by MDC according to Amsterdam and Bethesda criteria), a mutation was identified in 25% of cases \((n = 17/68)\), whereas a negative result was obtained in 56% of cases \((n = 38/68)\). The results were unknown in 19% of cases \((n = 13/68)\). Among cases with unknown results, genetic tests were recommended for family members that we have not yet been seen in clinic. Among the available results, we observed a 31% rate of MMR mutation detection. We did not identify MMR mutation for some with MSI and/or positive IHC. This can be explained by the limits of the technique of sequencing used in our laboratory, or even by the possibility of another gene concerned by the suspected predisposition. Nevertheless, these cases were considered "at high risk" and were recommended as Lynch syndrome for digestive surveillance.

Among the known genetic test results \((n = 55)\), a mutation was detected for 17 patients, whereas not for 38. In our cohort, the mutation rate is 23% \((n = 4)\), 59% \((n = 10)\), and 18% \((n = 3)\), for MLH1, MSH2, and MSH6 genes, respectively. No mutations were identified in the PMS2 gene of any proband.

**Evaluation of PREMM\(_{1,2,6}\) in our cohort**

The PREMM\(_{1,2,6}\) model correctly identified every patient who was found to carry an MMR mutation. However, among patients who were recommended for gene analysis by PREMM\(_{1,2,6}\), 62% had no mutation identified \((n = 28/45)\) on subsequent gene testing.

All these results are summarized in table III. Targeted mutated genes are not necessarily those predicted by PREMM\(_{1,2,6}\); the concordance between identifying a patient with a specific mutated gene and PREMM\(_{1,2,6}\) prediction was found to be 53% \((n = 9/17)\). This does not support the effectiveness of PREMM\(_{1,2,6}\) towards orienting analysis to a targeted gene. Past studies also confirm this point [9].

For 62% of cases, the software validated genetic analysis (PREMM+) results were negative (i.e. the model lead to a false positive). This would result in a loss of cost and time spent by laboratories and cancer genetic teams. Multidisciplinary committees, for this reason, provide clinics with a filtering and cost-saving analysis.

Among the known results, PREMM\(_{1,2,6}\) could have save 18% of unnecessary testing \((n = 10/55, negative results)\). If this informatics tool had been used before analysis, 10 expensive and time-consuming analysis would have been avoided (appendix B). This key point supports the usefulness of PREMM\(_{1,2,6}\) before genetic testing validation by an MDC, and can provide the committee with an important tool for gene test validation in MDC.

**Discussion**

The aim of the study was to evaluate the effectiveness of PREMM\(_{1,2,6}\) in identifying genetic predisposition risk in a cohort of patients with a suspected diagnosis of Lynch syndrome whose cases were evaluated by a multidisciplinary committee.

It is clear that somatic testing is the first element to consider prior to recommending genetic testing and represents a very good positive predictive value for mutation identification (table IV). This does not need other tools for validation. It indeed has been recognized that a strategy which includes the PREMM\(_{1,2,6}\) prediction model in association with tumor immuno-histochemistry is more effective than a strategy that does not take into account this second element [10].

**Analysis of MDC decisions**

MMR gene analysis indication was validated in only 35% of cases compared to 55% of cases that were evaluated by PREMM\(_{1,2,6}\) predictions. Some mutations might have been detected in the 20% of difference shown. Moreover, PREMM\(_{1,2,6}\) could have avoided 12% of unnecessary analysis validated by MDC but in which no mutation was identified in the subsequent testing.
Among the tested patients of our study, we identified 23, 59 and 18% of mutations, respectively for \textit{MLH1}, \textit{MSH2}, and \textit{MSH6} genes. No \textit{PMS2} gene mutations were detected. In the literature, we respectively observe 45, 45 and 5-10% of mutations for \textit{MLH1}, \textit{MSH2}, and \textit{MSH6} genes, and less than 5% of mutations for \textit{PMS2} \cite{11,12}.

**Performance of PREMM\textsubscript{1,2,6} in our cohort**

It detected all mutations identified in patients (true positives) and correctly discouraged testing in nearly 20% of cases (true negatives, \(n = 10/55\)). However, it incorrectly recommended testing in more than 50% of cases (false positives, \(n = 28/55\)). Finally, 33% of genetic testing recommended by the MDC (\(n = 18/55\)), contributed to an incorrect estimation of patient genetic risk in these cases. False negatives could be defined by testing patients who have a PREMM\textsubscript{1,2,6} score > 5. PREMM does not consider more than two colorectal cancers in the proband. In 1st and 2nd degree relatives, PREMM also does not take into account more than two types of Lynch syndrome cancers for risk calculation. It does not consider multiple primary tumors in the relatives either. Additionally, for large spectrum of Lynch-associated cancers, the ages and numbers are not considered. Moreover, it does not consider the size of the family, which can be an important parameter, considered by the MDC, and somatic analysis (MSS and tumor IHC). PREMM does not calculate the \textit{PMS2} risk mutation rate that represents 5 to 15% of mutation in Lynch syndrome \cite{13,14}.

All of these limitations contribute to an underestimation of genetic risk. Therefore, we believe the advice of the experts on an MDC is necessary for appropriate decision-making. In spite of these limits, PREMM\textsubscript{1,2,6} has been shown to have a good positive predictive value and can play a significant role in discussions in the MDC. Other benefits are that it is easy to use and free.

Moreover, this study was performed retrospectively with an evaluation on complete patient field. This is not always the case for prospective cases in every genetics center. In the Lyon region, we use a pre-appointment questionnaire to obtain a maximum of familial history prior to consultation, in order to provide more specific and relevant information to families. A lack of information before consultation would lead to incorrect estimation of risk using PREMM\textsubscript{1,2,6}.

**Perspectives**

In our practice, PREMM\textsubscript{1,2,6} could be used in association with somatic tests for the complex cases where analysis indication is not obvious, in order to help members of the MDC enhance decision-making and patient care management.

IHC studies have a sensitivity of 92% for \textit{MMR} mutation detections, and, when associated with PREMM\textsubscript{1,2,6} predictions this sensitivity jumps to 97.8%. In contrast, when associated with MSS study alone, it is shown to be less powerful \cite{10}. PREMM\textsubscript{1,2,6} can really be useful for genetic risk assessments in a healthy proband, whose family members are concerned about Lynch syndrome-associated cancers but are not available for testing.

In patient follow-up, when the decision to perform gene analysis was not validated by MDC (without available somatic tests) and when PREMM\textsubscript{1,2,6} exceeded the 5% threshold, we recommended continuing the discussion and possibly proposing genetic analysis to these patients through a new consultation.

In conclusion, we found that PREMM\textsubscript{1,2,6} should be used in our MDC as a decision-making tool. Only one French team currently uses PREMM\textsubscript{1,2,6}, and this is primarily to help with deciding whether to recommend genetic analysis and/or when a tumor has an MSS phenotype. We encourage all French teams to use this prediction model.

**Disclosure of interest:** the authors declare that they have no competing interest.

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**Supplementary data**

Supplementary data associated with this article can be found, in the online version of *Bulletin du cancer*, at (http://dx.doi.org/10.1016/j.bulcan.2016.11.017).

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