PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/97526

Please be advised that this information was generated on 2019-04-21 and may be subject to change.
Young age and a positive family history of colorectal cancer are complementary selection criteria for the identification of Lynch syndrome


ARTICLE INFO

Article history:
Received 7 September 2010
Received in revised form 16 December 2010
Accepted 22 December 2010
Available online 25 January 2011

Keywords:
Colorectal cancer
HNPPC
Lynch syndrome
MSI testing
Bethesda criteria
Family history

ABSTRACT

Families at high risk for Lynch syndrome can effectively be recognised by microsatellite instability (MSI) testing. The aim of the present study is to compare the effectiveness of a MSI test for the identification of Lynch syndrome in patients selected by a pathologist mainly based on young age at diagnosis (MSI-testing-indicated-by-a-Pathologist; MIPA), with that of patients selected by a clinical geneticist mainly based on family history (MSI-testing-indicated-by-Family-History; MIFH).

Patients with a Lynch syndrome associated tumour were selected using MIPA (n = 362) or MIFH (n = 887). Germline DNA mutation testing was performed in 171 out of 215 patients (80%) with a MSI positive tumour.

MSI was tested positive in 20% of the MIPA-group compared to 16% in the MIFH-group (P = 0.291). In 91 of 171 patients with MSI positive tumours tested for germline mutations were identified as Lynch syndrome patients: 42% in the MIPA-group and 56% in the MIFH-group (P = 0.066). Colorectal cancer (CRC) or endometrial cancer (EC) presenting at an age below 50 years would have led to the diagnosis of Lynch syndrome in 89% of these families (CRC below 50 years: 88% and EC below 50 years: 12%). Families detected by MIPA were characterised more often by extracolonic Lynch syndrome associated malignancies, especially EC (P < 0.001).

Our results indicate that recognition of Lynch syndrome by CRC or EC below 50 years is as effective as a positive family history. Families from patients selected by individual criteria more often harbour extracolonic Lynch syndrome associated malignancies.

1. Introduction

Lynch syndrome, previously called Hereditary Non-Polyposis Colorectal Cancer (HNPPC), is the most common type of hereditary colorectal cancer (CRC) and is caused by a germline mutation in one of the mismatch repair (MMR) genes. Lynch syndrome accounts for up to 5% of CRCs. The recognition of Lynch syndrome is highly relevant, because surveillance substantially reduces morbidity and mortality in family members carrying a MMR gene mutation. Once patients with Lynch syndrome have been identified, germline DNA testing should be performed to confirm the diagnosis.
syndrome are identified, this will lead to identification of more family members with Lynch syndrome in a highly cost-effective way.9,10

Tumours that develop as a result of Lynch syndrome can effectively be recognised by microsatellite instability (MSI) testing or immunohistochemical analysis of the MMR proteins MLH1, PMS2, MSH2 and MSH6.4,6,9 MSI is a hallmark of a defective MMR system, which can also be caused by an acquired non-hereditary hypermethylation of the MLH1 promoter. This is the main cause of MSI in CRC diagnosed at relatively high age, but hardly occurs in patients with a MSI-positive tumour diagnosed before the age of 50.11

Traditionally, patients are included for MSI testing after referral to a genetic counselling unit (MSI-testing-indicated-by-Family-History; MIFH) mostly because of occurrence of multiple CRC in the family (Bethesda criteria). However, by using family history only a small proportion of the expected number of patients at risk for Lynch syndrome is identified.12–15 This is due to small families, unawareness by the patients of their own family history and suboptimal registration of family history of cancer by doctors.12–15

To improve recognition of patients with a newly diagnosed tumour, that is known to be associated with Lynch syndrome, a new guideline was developed. In this MSI-testing-indicated-by-a-Pathologist (MIPA)-procedure9, pathologists initiate MSI testing based on one of the following criteria, called MIPA criteria: (1) CRC or endometrial cancer (EC) diagnosed before age 50; (2) second CRC before age 70; (3) CRC and a Lynch syndrome associated cancer before age 70; or (4) a colorectal adenoma with high grade dysplasia before age 40.9,16,17 Pathologists report the MSI test result to the surgeon or gastroenterologist with the advice to consider referral for genetic counselling in an outpatient clinic for hereditary cancer if the MSI test was positive. This new guideline was proven to be feasible, cost-effective and could easily be implemented.9,18,19

The aim of the present study is to compare the predictive value of a positive MSI test for the presence of Lynch syndrome in newly diagnosed patients selected by a pathologist based on individual characteristics only (MIPA), to that in patients who are included for MSI testing by a genetic counseling unit mainly based on family history (MIFH).

2. Patients and methods

2.1. Patients

The study cohort consisted of patients from the Departments of Human Genetics and Pathology of the Radboud University Nijmegen Medical Centre in The Netherlands, who had a MSI test. A series of 1249 patients with a tumour type known to be associated with Lynch syndrome (colorectal cancer (n = 1141), carcinomas of the endometrium (n = 67), ovaries (n = 6), small bowel (n = 8), stomach (n = 9), sebaceous gland (n = 6), and upper urinary tract (n = 12)) were prospectively selected for the current study. The enclosed patients were divided into two groups: patients with a MSI test indicated by a pathologist based on individual patient characteristics, the so-called MIPA-group (n = 362, collected between January 2005 and November 2009), and patients with a MSI test indicated by a clinical geneticist predominantly based on family history, the so called MIFH-group (n = 887, collected between June

Fig. 1 – The flow of patients following the MIPA-strategy or the MIFH-strategy.
In total, 171 pedigrees were available of the patients with a tumour type known to be associated with Lynch syndrome and were included in the current study of whom 1038 were tested negative for MSI and 215 (17%) had a MSI positive tumour. The mean age at CRC diagnosis was younger in the MIPA-group, compared to the MIFH-group, 46 ± 10 years and 50 ± 11 years, respectively (P = 0.006). At least one of the individual MIPA criteria was fulfilled in 100% of the MIPA-group and in only 56% (499/887) of the MIFH group (P < 0.001). Twenty percent of the patients in the MIPA-group based on individual characteristics (71/362) and 16% in the MIFH-group based on family history (144/887) were tested positive for MSI. This difference was not statistically significant (P = 0.291). The flow of both groups is illustrated in Fig. 1.

Overall, a majority of 58% patients (724/1249) fulfilled the MIPA criteria 1: having a CRC or EC diagnosed below 50 years (CRC below 50: 96% (696/724) and EC below 50: 4% (28/724)). Very few patients, 4% (56/1291), fulfilled MIPA criterion 3 being diagnosed with CRC below 70 years with a history of an extracolonic Lynch associated tumour below 70 years (n = 45), or MIPA criterion 4 having an adenoma diagnosed below age 40 years (n = 11). As illustrated in Table 1, the fulfillment of the various MIPA criteria was equally distributed among the MSI positive and the MSI negative group.

For the MIPA-group 96% of the MSI tests were performed on CRC (352/362) and 3% on EC (9/362), while in the MIFH-group 89% was performed on CRC (789/887) and 7% on EC (58/887). In the MIPA-group less females were index than in the MIFH-group (48% and 59%, respectively), however, there was no statistical significant difference (P = 0.272).

In total, 171 pedigrees were available of the patients with a MSI positive tumour (MIPA-group n = 31 and MIFH-group n = 140). The characteristics of the pedigrees are summarised in Table 2. As compared to the MIFH-group patients from the MIPA-group were less likely to have a family history with two first degree relatives with a CRC (i.e. with the inclusion of the index patient; P < 0.001).

In the MIPA-group 69% (9/13) patients identified as having Lynch syndrome by germline mutation analysis had a family with at least two first degree relatives with a CRC and in four Lynch syndrome patients from the MIPA-group (31%) and of four Lynch syndrome patients from the MIFH-group (5%) Lynch syndrome associated tumours other than CRC, especially EC, were present (P < 0.001). In three of them, a combination of patients with CRC or EC was present and in one family only EC’s were diagnosed.

### Table 1 – Distribution of MIPA criteria among patients recognised by individual characteristics (MIPA) and by family history (MIFH).

<table>
<thead>
<tr>
<th>Microsatellite instability (MSI) positive</th>
<th>MSI negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MIPA</strong> N = 71</td>
<td><strong>MIFH</strong> N = 144</td>
</tr>
<tr>
<td>Fulfilling one of the MIPA criterion</td>
<td></td>
</tr>
<tr>
<td>Criterion 1:</td>
<td></td>
</tr>
<tr>
<td>CRC &lt;50a</td>
<td></td>
</tr>
<tr>
<td>EC &lt;50b</td>
<td></td>
</tr>
<tr>
<td>Criterion 2: second CRC &lt;70c</td>
<td></td>
</tr>
<tr>
<td>Criterion 3: second extracolonic cancer &lt;70c</td>
<td></td>
</tr>
<tr>
<td>Criterion 4: adenoma HDP age &lt;40d</td>
<td></td>
</tr>
</tbody>
</table>

| **MIPA** N = 291                          | **MIFH** N = 743 |
| 71 (100%)                                | 90 (63%)        |
| 291 (100%)                               | 409 (55%)       |

| **MIPA** N = 144                          | **MIFH** N = 743 |
| 68 (76%)                                 | 359 (88%)       |
| 9 (10%)                                  | 11 (3%)         |
| 5 (5%)                                   | 27 (6%)         |
| 7 (8%)                                   | 8 (2%)          |
| 7 (1%)                                   | 4 (1%)          |

a CRC: colorectal cancer.
b EC: endometrial cancer.
c Extracolonic cancer: carcinomas of the endometrium, ovaries, small bowel, stomach, sebaceous gland, biliary tract, and upper urinary tract.
d HDP: high grade dysplasia.
3.2. Outcome of DNA mutation and somatic MLH1 hypermethylation analysis

In Table 3, the results are presented of the analysis of the underlying cause of the MMR deficiency in 44% (31/71) and 97% (140/144) of the MSI positive patients from the MIPA- and MIFH-group, respectively. This incompleteness was due to the fact that not all patients with a MSI positive tumour in the MIPA-group have visited our genetic counselling unit yet. Overall, in 91 patients, a pathogenic germline mutation was found. Such a germline mutation was detected in 42% of the patients tested in the MIPA-group (13/31) and in 56% of the patients tested in the MIFH-group (78/140; P=0.066). Overall MIPA-criteria were fulfilled in 81% of the mutation-positive patients (74/91), of whom 89% fulfilled the first MIPA-criterion (66/74), having had a CRC or EC diagnosed below 50 years (CRC below 50 years: 88% (58/66) and EC below 50 years: 12% (8/66)). Every patient in the MIPA-group with a positive MSI test in a CRC or EC diagnosed below 50 years had a probability of 50% having Lynch syndrome and only 15% having Lynch syndrome and 70% having a sporadic tumour with MLH1 hypermethylation.

Overall, hypermethylation was present in approximately a quarter of the MSI-positive tumours from both groups, predominantly from patients with either two CRC’s or a CRC and a second extracolonic Lynch associated tumour. The overall results are illustrated in Fig. 2, and show that 9% of all patients in both the MIPA- and the MIFH-group Lynch syndrome was diagnosed.

3.3. Characteristics of patients with unexplained MSI and their families

Both in the MIPA- and in the MIFH-group a substantial number of patients with a MSI positive tumour could not be explained by the presence of a mutation in one of the MMR genes or somatic hypermethylation of the MLH1 promoter. Overall, 42 of these patients with unexplained MSI were identified.

In Table 4 individual and family characteristics of Lynch syndrome and patients with unexplained MSI are compared. Both the mean age at onset of the index patients (44 and 49 years, P<0.05) and the mean age at diagnosis of the two
youngest relatives (38 and 46 years, \( P < 0.001 \)) were lower in the Lynch syndrome group in comparison to the unexplained MSI group. In 89% of the families from the Lynch syndrome group at least two family members were diagnosed with Lynch syndrome associated cancer (81/91) compared to 52% of the families from the patients with an unexplained MSI (22/42). The clinical Amsterdam II criteria were met in 60% of Lynch syndrome and 14% of patients with unexplained MSI (\( P < 0.001 \)).

4. Discussion

To our knowledge this is the first study among patients with a Lynch syndrome associated tumour showing that selection of

![Fig. 2 – Outcome of the MIPA-method and the MIFH-method.](image)

<table>
<thead>
<tr>
<th>Table 4 – Individual and family characteristics of Lynch syndrome and patients with unexplained MSI.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSI positive and DNA germline mutation detected N = 91</td>
</tr>
<tr>
<td>Age at diagnosis of MSI positive index tumour</td>
</tr>
<tr>
<td>Second Lynch syndrome associated cancers of index patient&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age at diagnosis of first Lynch syndrome associated cancer of index patient&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean age of two youngest relatives with Lynch syndrome associated cancer&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fulfilled Amsterdam II criteria</td>
</tr>
<tr>
<td>Fulfilled Bethesda criteria</td>
</tr>
<tr>
<td>Criterion 1 – one patient CRC &lt; 50 years&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Criterion 2 – one patient multiple Lynch syndrome associated cancers&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Criterion 4 – CRC plus at least one first degree relative with Lynch syndrome associated cancer; one patient &lt; 50 years&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Criterion 5 – CRC plus at least two first or second degree relatives with Lynch syndrome associated cancer&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Lynch syndrome associated cancer: colorectal cancer, carcinomas of the endometrium, ovaries, small bowel, stomach, sebaceous gland, biliary tract, and upper urinary tract.

<sup>b</sup> Lynch syndrome: only 81/91 of the families had at least two family members diagnosed with Lynch syndrome associated cancer/unexplained MSI.

<sup>c</sup> MSI: only 22/42 of the families had at least two family members diagnosed with Lynch syndrome associated cancer.

<sup>d</sup> Student’s t-test.

<sup>e</sup> ns: Not statistically significant.

<sup>f</sup> Pearson Chi-square test.
families at risk for Lynch syndrome based on young age at diagnosis is as effective as selection based on family history. These two strategies select slightly different families, for example more families with extracolonic CRC's were found by the MIPA-strategy based on young age. Most likely these two strategies are complementary. Most Lynch syndrome families, carrying mutations in MMR deficiency genes, were found in the group of patients diagnosed with CRC below age 50 years whereas a MSI positive tumour in older patients with a second CRC or extracolonic Lynch syndrome associated cancer, was most often explained by somatic hypermethylation of the MLH1 promoter, and, therefore, sporadic and not hereditary by origin. This study thus shows that the detection of Lynch syndrome facilitated by MSI testing at the initiative of a pathologist, which is mainly based on young age at CRC diagnosis, is an excellent adjunct to family history taking. The conclusion drawn from these data is that MSI testing by pathologists can improve the identification of Lynch syndrome in an easy way and will lead to the recognition of families that are not easily picked up by family history. Especially families with the presence of extracolonic Lynch associated cancers, such as EC, ovarian cancer, gastric cancer, urinary tract cancer and small bowel cancer, are easily overlooked by their family history.

Both the MIPA- and the MIFH-strategies have advantages and disadvantages. The MIPA-procedure, easy to apply in daily clinical practice, is found to be effective and efficient and an electronic reminder system can be used for the identification of the eligible patients. However, not all involved clinicians are being well prepared and informed about the MIPA-procedure. The main advantages of the MIFH-strategy are the selection of patients who were diagnosed with a Lynch associated tumour in the past or who are from a family with remarkable familial clustering. However, by using family history only a small proportion of the expected number of patients at risk for Lynch syndrome is identified due to small families, unawareness by the patients of their own family history and suboptimal registration of family history of cancer by doctors.

Age below 50 years at diagnosis also appeared by far the most prevalently fulfilled MIPA criterion, and the criterion that led most often to the diagnosis of Lynch syndrome. Restriction of the MIPA criteria to one single criterion of age below 50 years, in our population would have led to the recognition of 90% of the families with Lynch syndrome. Further studies are needed to examine which age limit is optimal to detect Lynch syndrome regarding cost effectiveness and feasibility. A restriction of the MIPA criteria to one criterion i.e. young age at diagnosis only, for example age below 50 years, in our population would have led to the recognition of 90% of the families with Lynch syndrome. The main advantages of the MIFH-strategy are the selection of patients who were diagnosed with a Lynch associated tumour in the past or who are from a family with remarkable familial clustering. However, by using family history only a small proportion of the expected number of patients at risk for Lynch syndrome is identified due to small families, unawareness by the patients of their own family history and suboptimal registration of family history of cancer by doctors.

The most important hurdle for diagnosing Lynch syndrome appears to be referral of patients with a positive MSI test for genetic counselling and DNA testing. In the present study, 71 patients in the MIPA-group had a MSI positive tumour, though; only 41% visited our genetic counselling unit for further genetic testing. Although some patients might have visited other genetic counselling units, close to 60% of the patients with a MSI positive tumour in the MIPA-group has not been counselled. Reasons to refrain from genetic counselling or DNA testing may be personal reasons including psychosocial, medical or financial consequences and a lack of adequate information. But this may also be caused by selection bias, i.e. clinicians may only refer patients with a Lynch syndrome associated MSI positive tumour that also have a positive family history for genetic testing. In the present study, 69% of MSI positive patients from the MIPA-group who were referred for genetic testing actually had a positive family history. Previous studies showed that only 12–30% of CRC patients with a high familial risk is referred for genetic counselling. Although improvement of referral is necessary for both the MIPA- and the MIFH-procedures, patients seem to be more motivated to visit a clinical genetic centre when MSI was tested positive (41%) compared to being positive for the Bethesda criteria (12–30%).

Our group investigated the feelings of patients on genetic testing offered directly after the diagnosis of colorectal cancer, which actually is the case in MIPA. The opinion of CRC patients was that the advantages of genetic testing weights-up against the disadvantages. Most of them had wondered why they had got CRC and whether their children were at risk, long before their surgeon had offered genetic counselling. Previous studies by others showed that mutation carriers are able to cope with having a germline mutation on the short as well as on the long term.

In conclusion, our study shows that the identification of Lynch syndrome facilitated by testing for MSI in CRC diagnosed at young age is as effective as MSI-testing based on a positive family history. The families recognised by young age at CRC diagnosis are more often characterised by extracolonic Lynch syndrome associated cancers, than those recognised by family history.

**Conflict of interest statement**

None declared.

**REFERENCES**


