Young age and a positive family history of colorectal cancer are complementary selection criteria for the identification of Lynch syndrome

P. Manders a, L. Spruijt a, C.M. Kets a, H.W. Willems b, D. Bodmer a, K.M. Hebeda b, I.D. Nagtegaal b, J.H.M. van Krieken b, M.J.L. Ligtenberg a,b, N. Hoogerbrugge a,*

a Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
b Department of Pathology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

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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.

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1. Introduction
Lynch syndrome, previously called Hereditary Non-Polyposis Colorectal Cancer (HNPCC), 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 are identified, this will lead to identification of
more family members with Lynch syndrome in a highly
cost-effective way.\textsuperscript{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 pro-
teins MLH1, PMS2, MSH2 and MSH6.\textsuperscript{4,6,8} 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.\textsuperscript{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 identi-
fied.\textsuperscript{12–15} This is due to small families, unawareness by the pa-
tients of their own family history and suboptimal registration
of family history of cancer by doctors.\textsuperscript{12–15}

To improve recognition of patients with a newly diagnosed
tumour, that is known to be associated with Lynch syndrome,
as being at risk for Lynch syndrome, a new guideline was
developed. In this MSI-testing-indicated-by-a-Pathologist
(MIPA)-procedure\textsuperscript{9}, 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.\textsuperscript{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 outpa-
tient 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.\textsuperscript{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 syn-
drome in newly diagnosed patients selected by a pathologist
based on individual characteristics only (MIPA), to that in pa-
tients who are included for MSI testing by a genetic coun-
selling 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](image-url) - 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 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 56 ± 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 fulfilment 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 compared to 95% (74/78) in the MIFH-group (P < 0.001). In families of only 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.
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; \( P < 0.05 \)).

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 12% having a sporadic tumour with somatic MLH1 hypermethylation. In contrast every patient in the MIPA-group with a positive MSI test in a second CRC had a probability of 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

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Table 2 – Family characteristics of patients recognised by individual characteristics (MIPA-group) or family history (MIFH-group) (family characteristics are given of those patients who had a germline DNA mutation analysis).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MIPA-group</th>
<th>MIFH-group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fulfilled the Amsterdam I criteria</td>
<td>8 (26%)</td>
<td>36 (25%)</td>
<td>ns(^a)</td>
</tr>
<tr>
<td>Fulfilled the Amsterdam II criteria</td>
<td>10 (32%)</td>
<td>60 (42%)</td>
<td>ns(^a)</td>
</tr>
<tr>
<td>Fulfilled revised Bethesda criteria</td>
<td>29 (94%)</td>
<td>120 (83%)</td>
<td>ns(^a)</td>
</tr>
<tr>
<td>Two first degree relatives with CRC(^b)</td>
<td>14 (45%)</td>
<td>104 (74%)</td>
<td>&lt;0.001(^f)</td>
</tr>
<tr>
<td>Two first degree relatives with Lynch syndrome assoc. one under 50(^b)</td>
<td>14 (45%)</td>
<td>76 (53%)</td>
<td>ns(^a)</td>
</tr>
<tr>
<td>Mean age of two youngest relatives with Lynch syndrome assoc. cancer(^b)</td>
<td>49 ± 11</td>
<td>46 ± 11</td>
<td>ns(^a)</td>
</tr>
</tbody>
</table>

\(^a\) CRC: colorectal cancer.
\(^b\) Lynch syndrome associated cancer: colorectal cancer, carcinomas of the endometrium, ovaries, small bowel, stomach, sebaceous gland, biliary tract, and upper urinary tract.
\(^c\) Only 31/71 patients tested positive in the PA-lab of the RUNMC were referred for genetic counselling at the RUNMC.
\(^d\) Only 140/144 patients with MSI positive tumour were actually tested.
\(^e\) ns: Not statistically significant.
\(^f\) Pearson Chi-square test.

Table 3 – MSI positive and DNA germline mutation tested.

<table>
<thead>
<tr>
<th>Lynch syndrome</th>
<th>Unexplained MSI</th>
<th>Hypermethylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIPA</td>
<td>MIFH</td>
<td>MIPA</td>
</tr>
<tr>
<td>Number of patients(^a)</td>
<td>13 (100%)</td>
<td>78 (78%)</td>
</tr>
<tr>
<td>Filling one of the MIPA criteria</td>
<td>13 (84%)</td>
<td>61 (78%)</td>
</tr>
<tr>
<td>Criterion 1: CRC &lt;50(^b)</td>
<td>11 (8%)</td>
<td>12 (7%)</td>
</tr>
<tr>
<td>EC &lt;50(^c)</td>
<td>1 (8%)</td>
<td>1 (12%)</td>
</tr>
<tr>
<td>Criterion 2: second CRC &lt;70(^b)</td>
<td>1 (8%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Criterion 3: second extracolonic cancer &lt;70(^d)</td>
<td>–</td>
<td>5 (8%)</td>
</tr>
<tr>
<td>Criterion 4: adenoma HDP age &lt;40(^e)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^a\) MIPA-group: only 31/71 patients tested positive in the PA-lab of the RUNMC were referred for genetic counselling at the RUNMC/MIFH-group: only 140/144 patients with MSI positive tumour were actually tested.
\(^b\) CRC: colorectal cancer.
\(^c\) EC: endometrial cancer.
\(^d\) Extracolonic cancer: carcinomas of the endometrium, ovaries, small bowel, stomach, sebaceous gland, biliary tract, and upper urinary tract.
\(^e\) HDP: high grade dysplasia.
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

Table 4 – Individual and family characteristics of Lynch syndrome and patients with unexplained MSI.

<table>
<thead>
<tr>
<th></th>
<th>MSI positive and DNA germline mutation detected</th>
<th>MSI positive, no hypermethylation and no DNA germline mutation detected (“unexplained MSI”)</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 91</td>
<td></td>
<td>N = 42</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis of MSI positive index tumour</td>
<td>44 ± 10</td>
<td>49 ± 11</td>
<td>0.013(^d)</td>
</tr>
<tr>
<td>Second Lynch syndrome associated cancers of index patient(^a)</td>
<td>26 (29%)</td>
<td>9 (22%)</td>
<td>ns(^e)</td>
</tr>
<tr>
<td>Age at diagnosis of first Lynch syndrome associated cancer of index patient(^a)</td>
<td>43 ± 10</td>
<td>48 ± 9</td>
<td>0.007(^d)</td>
</tr>
<tr>
<td>Mean age of two youngest relatives with Lynch syndrome associated cancer(^b)</td>
<td>38 ± 9</td>
<td>46 ± 11</td>
<td>&lt;0.001(^d)</td>
</tr>
<tr>
<td>Fulfilled Amsterdam II criteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fulfilled Bethesda criteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Criterion 1 – one patient CRC &lt; 50 years(^c)</td>
<td>55 (60%)</td>
<td>6 (14%)</td>
<td>&lt;0.001(^f)</td>
</tr>
<tr>
<td>Criterion 2 – one patient multiple Lynch syndrome associated cancers(^b)</td>
<td>78 (86%)</td>
<td>35 (83%)</td>
<td>ns(^e)</td>
</tr>
<tr>
<td>Criterion 4 – CRC plus at least one first degree relative with Lynch syndrome associated cancer, one patient &lt; 50 years(^a,c)</td>
<td>31 (34%)</td>
<td>12 (29%)</td>
<td>ns(^e)</td>
</tr>
<tr>
<td>Criterion 5 – CRC plus at least two first or second degree relatives with Lynch syndrome associated cancer(^a,c)</td>
<td>59 (65%)</td>
<td>8 (19%)</td>
<td>&lt;0.001(^f)</td>
</tr>
<tr>
<td>Criterion 6 – CRC plus at least two first or second degree relatives with Lynch syndrome associated cancer(^a,c)</td>
<td>60 (66%)</td>
<td>13 (31%)</td>
<td>&lt;0.001(^f)</td>
</tr>
</tbody>
</table>

\(^a\) Lynch syndrome associated cancer: colorectal cancer, carcinomas of the endometrium, ovaries, small bowel, stomach, sebaceous gland, biliary tract, and upper urinary tract.

\(^b\) Lynch syndrome: only 81/91 of the families had at least two family members diagnosed with Lynch syndrome associated cancer/unexplained MSI: only 22/42 of the families had at least two family members diagnosed with Lynch syndrome associated cancer.

\(^c\) CRC: colorectal cancer.

\(^d\) Student’s t-test.

\(^e\) ns: Not statistically significant.

\(^f\) 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 pa-
tients with a second CRC or extracolonic Lynch syndrome
associated cancer, was most often explained by somatic
hypermethylation of the MLH1 promoter, and, therefore, spo-
radic 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 recogni-
tion 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 over-
looked by their family history.

Both the MIPA- and the MIFH-strategies have advantages
and disadvantages. The MIPA-procedure, easy to apply in dai-
ely clinical practice, is found to be effective and efficient9
and an electronic reminder system can be used for the identifi-
cation of the eligible patients.21 However, not all involved cli-
nicians 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 his-
tory only a small proportion of the expected number of pa-
tients at risk for Lynch syndrome is identified due to small
families, unawareness by the patients of their own family his-
tory and suboptimal registration of family history of cancer by
doctors.12–15

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 recog-
nition 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 fea-
sibility. A restriction of the MIPA criteria to one criterion i.e.
young age at diagnosis only, for example age below 50 years,
may be more easily applied by pathologists and remembered
by surgeons, gastroenterologist and patients.

A substantial group of patients with a positive MSI test
could not be explained by a mutation or by hypermethylation.
The origin of CRC in this group of patients is still unknown.
The first-degree relatives of such patients are counselled to
follow exactly the same surveillance programme as a patient
with Lynch syndrome. However, cancer risk may not be quite
as high as in Lynch families since the number of involved
family members is low, and mean age at diagnosis is higher
in our data.

The most important hurdle for diagnosing Lynch syn-
drome 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 po-
sitve tumour, though; only 41% visited our genetic counsel-
ling 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 conse-
quences and a lack of adequate information. But this may
also be caused by selection bias, i.e. clinicians may only re-
fer 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 famil-
ial risk is referred for genetic counselling.13,22–26 Although
improvement of referral is necessary for both the MIPA-
and the MIFH-procedures, patients seem to be more moti-
vated 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 can-
cer, which actually is the case in MIPA. The opinion of CRC pa-
tients 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.27
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.28–32

In conclusion, our study shows that the identification of
Lynch syndrome facilitated by testing for MSI in CRC diag-
nosed 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 extraco-
lonic Lynch syndrome associated cancers, than those recogni-
sed by family history.

Conflict of interest statement
None declared.

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