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SHORT REPORT

Deficient mismatch repair screening of advanced adenomas in the population screening program for colorectal cancer is not effective

Elisa Vink-Börger,[1] Parag D Dabir,[2] Joyce Krekels,[1] Mariëtte C A van Kouwen,[3] Marjolijn J L Ligtenberg,[1,4] Rachel S van der Post[1] & Iris D Nagtegaal[1]

[1]*Department of Pathology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands,* [2]*Institute of Pathology, Randers Regional Hospital, Randers, Denmark,* [3]*Department of Gastroenterology and Hepatology, Radboud Institute for Health Sciences, Radboud University Medical Center and* [4]*Department of Human Genetics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands*

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Aim: Currently, screening of colorectal cancers (CRC) by assessing mismatch repair deficiency (dMMR) or microsatellite instability (MSI) is used to identify Lynch syndrome (LS) patients. Advanced adenomas are considered immediate precursor lesions of CRC. In this study we investigate the relevance of screening of advanced adenomas for LS in population screening.
Methods and results: Advanced adenomas (n = 1572) were selected from the Dutch colorectal cancer population screening programme, based on one or more of the criteria: tubulovillous (n = 848, 54%) or villous adenoma (n = 118, 7.5%), diameter ≥ 1 cm (n = 1286, 82%) and/or high-grade dysplasia (n = 176, 11%). In 86 cases (5%), all three criteria were fulfilled at the same time. MMR–IHC and/or MSI analyses were performed on all cases. Only five advanced adenomas (0.3%) showed dMMR and MSI, including two cases with hypermethylation. In at least two patients a germline event was suspected based on allelic frequencies. No pathogenic explanation was found in the last case.
Conclusion: Timely testing of precursor lesions would be preferable to detect new LS patients before CRC development. However, standard assessment of dMMR of advanced adenomas from the population screening is not effective.

Keywords: adenomas, colorectal carcinoma, Lynch syndrome, microsatellite instability, population screening

Introduction

Population screening for colorectal cancer (CRC) is aimed on both detecting early CRC and prevention of CRC by removing precursor lesions. Detection of patients with a hereditary cancer risk followed by adequate surveillance is another way of preventing CRC. Lynch syndrome (LS), the most common hereditary syndrome causing CRC, is currently detected by assessing microsatellite instability (MSI) or mismatch repair deficiency (dMMR) on tissue samples of CRC.[1] The question is whether this method is also effective

Address for correspondence: I D Nagtegaal, Department of Pathology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands. e-mail: iris.nagtegaal@radboudumc.nl

on precursor lesions. Previously, we described in a meta-analysis and systematic review of the literature that dMMR/MSI was present in 69.5% of conventional adenomas in LS patients, compared with 2.8% in unselected patients.[2] The highest risk of dMMR/MSI in LS patients was observed in large adenomas (≥ 1 cm, 81%), villous adenomas (84%) and adenomas with high-grade dysplasia (88%). These characteristics are present in advanced adenomas, and are considered the immediate precursor lesions of CRC.[3] To study the relevance of MSI screening of advanced adenomas in the population screening programme, we collected a large cohort and evaluated whether standard assessment of dMMR in this population is useful.

Materials and Methods

Using the local pathology database, we selected 1572 advanced adenomas, identified from 1215 patients who participated in the Dutch colorectal cancer population screening programme between 2014 and 2018 in the Radboud University Medical Center (Nijmegen, the Netherlands).[4] All patients were between 55 and 75 years of age and underwent a colonoscopy after a positive iFOBT test. All adenomas fulfilled one or more of the following criteria: villous component, size ≥ 1 cm and/or presence of high-grade dysplasia. Serrated lesions were excluded, as these are not considered precursors for Lynch-associated CRC, but rather for microsatellite instable CRC due to MLH1 hypermethylation.[5] This study was approved by the Radboud University Nijmegen Medical Center research ethics committee (2022–16134).

Formalin-fixed paraffin embedded (FFPE) tissues were available from all selected adenomas and used for constructing tissue microarrays (TMAs). The most atypical or high-grade area of the adenoma on HE was selected. One core punch of 2 mm of those selected areas was obtained from each FFPE block using the Tissue-Tek Quick-Ray System (Sakura Finetek USA, Inc., CA, USA). TMAs were stained automatically (Dako Omnis, Agilent, Carpinteria, CA, USA) with antibodies against MLH1 (clone G168-15; BD Biosciences, San Jose, CA, USA), MSH2 (clone GB12; Calbiochem/Merck, Darmstadt, Germany), MSH6 (clone EPR3945; Abcam, Cambridge, UK) and PMS2 (clone A16-4; BD Biosciences, San Jose, CA, USA).

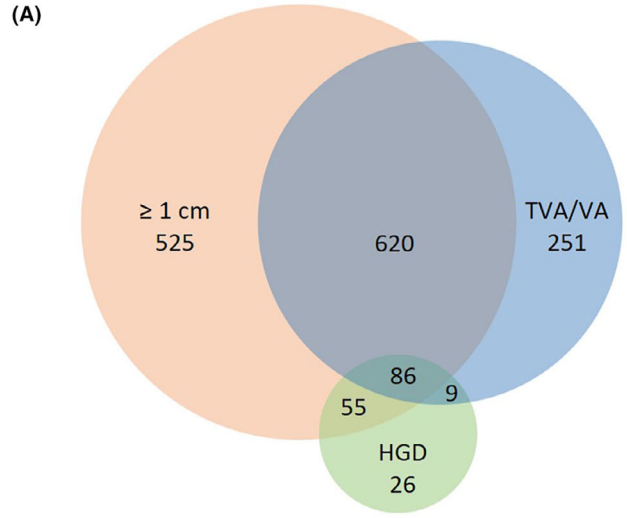
An expert pathologist (P.D.D.) and trained specialist (E.V.B.) independently scored all TMA slides. In case of a discrepancy, an additional expert pathologist (I.D.N.) evaluated the slide. Cases were considered mismatch repair proficient if nuclear staining of neoplastic cells was present. Loss of staining with positive internal control cells was considered dMMR. These cases, together with those that were inconclusive, were further analysed for MSI. DNA was isolated (using TET-lysis buffer with 5% Chelex-100 (Bio-Rad, Hercules, CA, USA) and 400 μg proteinase K (Qiagen, Valencia, CA, USA) for 16 h at 56°C), followed by inactivation at 95°C for 10 min. After centrifugation, the supernatant was used to perform MSI analyses. The MSI status was determined using five microsatellite markers (NR-21, NR-24, NR-27, BAT25 and BAT26) in a pentaplex polymerase chain reaction (PCR). An adenoma was defined as MSI if at least two markers showed instability. If MSI was present or the MSI status remained unclear because one or more markers were inconclusive, the whole slide was then immunohistochemically stained for all MMR proteins.

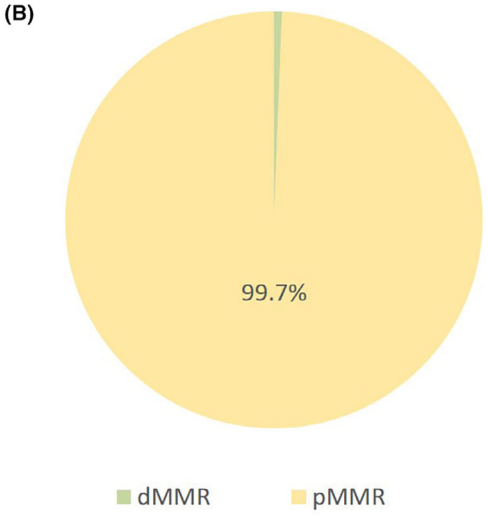
MSI adenomas were further analysed. Hypermethylation status of *MLH1*/*PMS2*/*MSH2*/*MSH6* gene promoter was assessed using a methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) kit (MRC-Holland, Amsterdam, the Netherlands), according to the manufacturer's protocol. If hypermethylation was not detected, mutation analysis of the remaining MSI cases was performed using single-molecule molecular inversion probes-based targeted next-generation sequencing (smMIP-based NGS), as described previously.[6,7] If necessary, MLPA was performed to detect MMR gene deletions or duplications (MRC-Holland).

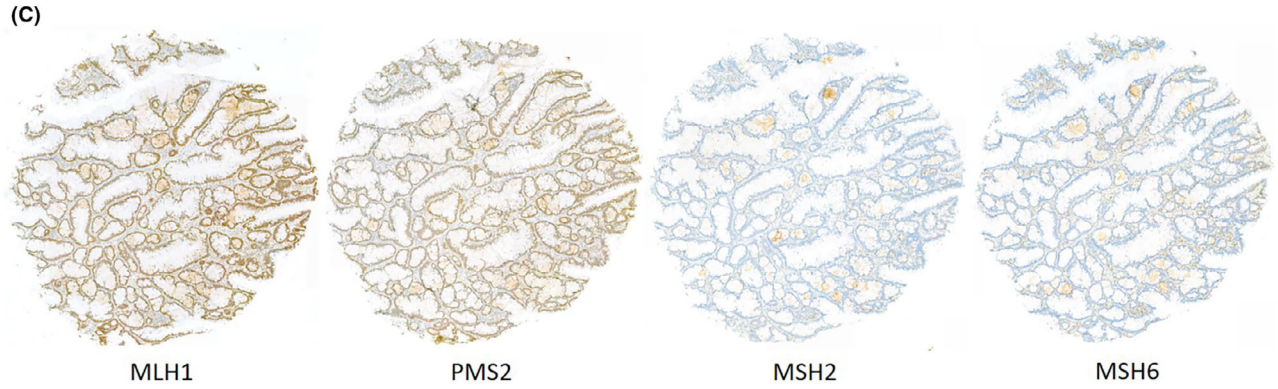
Results

We collected 1572 advanced adenomas from 1215 patients (69% male, 31% female). Most adenomas were located left-sided (66%) versus right-sided (20%), rectum (14%) and unknown (< 0.1%). All adenomas were reviewed and met one or more of the following criteria: tubulovillous (n = 848, 54%) or villous adenoma (n = 118, 7.5%), diameter ≥ 1 cm (n = 1286, 82%) and/or high-grade dysplasia (n = 176, 11%). In 86 cases (5%), all three criteria were fulfilled at the same time (Figure 1A).

MMR–IHC was performed on TMAs of 1572 advanced adenomas; 118 advanced adenomas were further analysed for MSI by pentaplex PCR because of dMMR or inconclusive result. After PCR, the MSI status still remained unclear for 14 advanced adenomas. MMR–IHC of whole slides was performed on those cases, and a conclusive result in all cases was obtained.







(D)

Nr.	Age	Histology	Dysplasia	MMR-IHC	Molecular finding
1	67	TSA	HGD	MLH1/PMS2 -	*MLH1*-promoter hypermethylation
2	69	TA	LGD	PMS2 -	*PMS2*-promoter hypermethylation
3	69	VA	HGD	PMS2 -	NM_000535.5(PMS2):c.1882C>T p.(Arg628*), VAF 54%; NM_000535.6(PMS2):c.756_757dup p.(Glu253fs), VAF 36%
4	69	TVA	LGD	MSH2/MSH6 -	NM_000251.3(MSH2):c.1013G>C p.(Gly338Ala), VAF 50%; NM_000251.2(MSH2):c.2653C>T p.(Gln885*), VAF 20–39%
5	63	TVA	HGD	MSH2/MSH6 -	NM_000179.3(MSH6):c.3261del p.(Phe1088fs), VAF 31%; No pathogenic explanation in MSH2

Figure 1. **A,** Overview of the three criteria in a Venn diagram of all selected adenomas. A total of 802 adenomas met one criterion of either ≥ 1 cm, diagnosed as a (tubulo)villous adenoma (TVA/VA) or having high-grade dysplasia (HGD); 684 adenomas fulfilled two criteria and 86 adenomas fulfilled all three criteria. **B,** Pie-chart, MMR status of 1572 advanced adenomas; green: dMMR (n = 5), yellow: pMMR (n = 1567). **C,** Loss of MSH2/MSH6 immunohistochemical staining (case 4). **D,** Characteristics of the five dMMR advanced adenomas. TSA, traditional serrated adenoma; TA, tubular adenoma; LGD, low-grade dysplasia; VAF, variant allele frequency.

In total, all five adenomas (0.3%) that showed dMMR in IHC (Figure 1B,C) were confirmed with MSI markers; all were > 1 cm in diameter. Four showed a villous component and three showed high-grade dysplasia. One case showed *MLH1*-promoter hypermethylation; revision of the slide showed a clear presence of sessile serrated lesion with conventional dysplasia and one case showed promoter hypermethylation of *PMS2*. Somatic mutation analysis was performed on the remaining three cases. Due to limitations in material and ethical consent, normal tissue was not analysed. However, allelic frequencies suggested that in two cases (one PMS2-deficient case and one MSH2-deficient case) a pathogenic and probably pathogenic germline variant was present, respectively, both in combination with a pathogenic somatic event. In the last case, a pathogenic somatic variant of *MSH6* was present, but no secondary hit was detected. As this variant was present in a stretch of eight repeated Cs, this variant is probably the result of MSI caused by as yet undetected events in *MSH2*.

Discussion

Early detection of LS patients is important to prevent CRC development. The most efficient detection mode for new LS patients is based on testing of the already developed cancers. Timely testing of precursor lesions would be preferable. Indeed, > 80% of the advanced adenomas in LS patients showed dMMR/MSI, as we have previously shown in a systematic review.[2] We performed the current study to evaluate feasibility and relevance in the population screening era. Our data show only 0.3% of all advanced adenomas dMMR and MSI. In 2018 in the Netherlands, 20.805 (35%) participants to the nationwide CRC population screening programme had an advanced adenoma.[8] In the context of our study, it would indicate that approximately 62 of the patients with advanced adenomas would have dMMR or MSI in the total population screening each year. However, the use of tissue microarrays carries a potential sampling bias. Specific for MMR proteins, the concordance with whole slides is particularly high in both CRC,[9,10] as well as in endometrial cancers.[11] The potential presence of heterogeneous staining in adenomas is not well documented, and might still cause false positive results. We tried to overcome this by selecting the most atypical/high-grade area of the adenoma to perform a TMA. In our series, 11 cases showed a heterogeneous staining on TMA; eight of 11 were classified as MSS by PCR. MMR–IHC of whole slides of the three remaining cases indeed showed a heterogenous staining, caused by artefacts (including loss of internal controls).

In our study, two of five cases showed hypermethylation. Histological revision of the *MLH1*-promoter hypermethylation case showed a sessile serrated lesion in which conventional dysplasia developed, indicating the presence of *MLH1* hypermethylation in the serrated pathway. As we included advanced adenomas and not advanced serrated polyps, we did not expect high numbers of *MLH1* hypermethylation.[12–14] Surprisingly, the other case showed *PMS2*-promoter hypermethylation, which is quite rare and only rarely described.[7]

At least two patients (0.13%) were suspected of a germline event (a pathogenic variant in *PMS2* and a probably pathogenic variant in *MSH2*). Both adenomas were left-sided, in line with the literature.[15,16] However, 0.13% is considerably lower than expected, based on both the incidence of LS (3% of all CRC)[17] and the high prevalence of dMMR in LS-associated advanced adenomas (80%).[2] One explanation is that LS patients develop their CRC at a relatively young age (mean age = 40–60 years),[18] and might have already been identified and followed-up outside the setting of the population screening. Therefore, in population screening cohorts with ages between 55 and 75 years, a lower percentage of LS patients might be detected.

Although advanced adenomas are considered to be immediate precursor lesions for CRC, it is unclear to what extent this is also holds true for LS patients. Ahadova *et al.* described three possible pathways to develop CRC in LS patients.[19] The first pathway is the development of MMR proficient adenomas with secondary MMR inactivation. In our study, we could miss these cases as the dMMR may develop at a later stage. Secondly, there is development of MMR-deficient adenomas after early loss of MMR expression. Our data indicate that within the population screening these cases are extremely rare, as a possible germline mutation was found in only 0.13% of adenomas. The third pathway describes CRC developing from non-polypous lesions. It is probable that most CRCs of LS patients follow the third pathway. A certain amount of LS patients have MMR-deficient crypt foci in the normal mucosa that might develop into non-polypous precursors with rapid invasive growth.[19–21] These non-polypous lesions might not be detected by an iFOBT test, because they probably do not bleed.

In conclusion, our study indicates that standard assessment of dMMR to uncover LS patients by

screening advanced adenomas from the population screening is not effective.

Conflicts of interest

The authors report no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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