ORIGINAL ARTICLE

Compliance with mismatch repair testing in pT1 colorectal cancer diagnosed before the age of 70 years

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Abstract

Mismatch repair (MMR) testing is recommended in the Netherlands for all patients under 70 years of age with newly diagnosed colorectal cancer (CRC) in order to identify Lynch syndrome. T1 CRC can be removed by local excision or oncological surgical resection. We evaluated the frequency of MMR testing in pT1 lesions within the Dutch CRC screening cohort. pT1 CRC diagnosed within the Dutch population-based screening program from 2016–2018 were identified by the Dutch pathology registry (PALGA). Pathology reports were evaluated, including registration of MMR testing (by immunohistochemistry and/ or microsatellite instability PCR). Frequency of MMR testing was compared between pT1 tumors that were treated by local (endoscopic or transanal) excision and oncological surgical resections. A total of 3.692 pT1 CRCs were diagnosed (median age 63 years, 61.4% males). MMR testing was performed in 83% and uptake increased over time (71% in 2016 to 92% in 2018, $p<0.01$). MMR testing was significantly more often performed in younger patients and in academic hospitals. When pT1 CRC was treated by oncological surgical resection ($n=1.132$), MMR testing was performed in 89% of cases and was known prior to oncological resection in 51% of cases. MMR testing occurred significantly less often in case of local excision (80% of $n=2.560$) compared to oncological surgical resection ($p<0.01$). MMR testing was performed in 83% of T1 CRCs and uptake increased over time. MMR testing was more frequently performed in pT1 CRC resected by oncological surgical resection compared with local excision.

Keywords Colorectal cancer · Lynch syndrome · Mismatch repair deficiency · Screening

Introduction

Lynch syndrome is detected in 3% of all newly diagnosed colorectal carcinomas (CRC) [1]. Identification of Lynch syndrome is relevant for both patients and their relatives, as surveillance can be offered and subtotal colectomy instead of segmental resection can be advised in order to improve survival and reduce cancer incidence [2–5]. In the Netherlands, mismatch repair (MMR) deficiency testing has been recommended since January 2016 in all newly diagnosed CRC before the age of 70 years [6, 7].

T1 CRC differs from the other CRC stages as complete local (endoscopic or transanal) excision may be sufficient instead of oncological surgical resection. With the introduction of the national CRC screening program in 2014, there is a shift towards increased proportion of pT1 CRC in the Netherlands [8]. However, it is unknown whether adherence to MMR testing is different for pT1 CRC treated by local excision versus oncological surgical resection.

This study aims to determine the compliance to MMR testing in pT1 CRCs diagnosed within the Dutch population-based screening program. Furthermore, we will evaluated whether differences in MMR deficiency testing occur between tumors removed by local (endoscopic or transanal) excision

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and surgical oncological surgical resection in academic and non-academic hospitals.

Materials and methods

Study population

In this retrospective study, patients with newly diagnosed CRC were identified through a search of the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA), registered as LZV2020-44 [9]. Patients with CRC, diagnosed between 55 and 75 years of age within the Dutch population-based screening program, were selected [10, 11]. CRC diagnosed before 2016 (guideline recommended MMR testing since 2016 in the Netherlands for all CRC under the age of 70 [12]), ≥70 years of age at diagnosis and tumor stages pTis, pT2, pT3, and pT4 were excluded. Patients with well differentiated neuroendocrine tumors, neuroendocrine carcinoma, squamous cell carcinoma, or metastases in the colorectum were also excluded. The obtained pathology reports of each potential pT1 CRC case were reviewed. The pathology reports contained information about type of resection, T and N status, if available, and results of immunohistochemistry of MMR proteins (MLH1, MSH2, MSH6 and PMS2 by IHC), microsatellite instability testing (MSI) and *MLH1* promoter hypermethylation if performed [13].

Differences between local excision and segmentally resected pT1 CRC, type of laboratory (academic or non-academic) and year of diagnosis were evaluated. Local excision included endoscopic excision and transanal endoscopic surgery. Oncological surgical resection was performed primarily or secondary after local excision. CRC in the proximal colon was defined as located between cecum and splenic flexure or distally located from descending colon to rectum.

Statistical analysis

SPSS Statistics (version 22) was used for statistical analysis. Data were summarized using descriptive statistics. Categorical and continuous data between groups were compared by chi-square tests and independent t-test, respectively. Values of $p<0.05$ were considered significant. All authors had access to the study data and reviewed and approved the final manuscript.

Results

Patient characteristics

A total of 3.692 pT1 CRCs were diagnosed within the Dutch CRC screening program between January 2016 and December 2018 (Fig. 1). The median age at CRC diagnosis was 63 years (range 55–69 years) and the majority were male (61%). Most CRC were located in the distal colon (84%, Table 1). pT1 CRC were removed by local excision in 2.560 (69%) and oncological surgical resection in 1.132 (31%) of the cases (Table 1). Oncological surgical resection was performed after a local excision in 291 of 1132 (26%) of the pT1 CRC.

MMR testing

MMR testing was performed in 3.050 (83%) of newly diagnosed pT1 CRC. MMR testing increased over time (71% in 2016, 87% in 2017 and 92% in 2018, $p<0.01$). MMR testing was most frequently tested by IHC (71% of 3.050, Fig. 2). The majority of IHC MMR testing occurred by staining for the four proteins (2.792 out of 2.842, 98%). MSI PCR only was performed in 6% of cases.

In 207 cases, both IHC MMR and MSI PCR were performed on the same tumor material with a concordance of 97%. The concordance was 99% when MMR testing was performed on biopsy or local excision in comparison with oncological surgical resection material ($n = 83$).

Among the pT1 CRC treated by local excision only, MMR testing was performed in 2.048 cases (80%) compared with 1.002 cases (89%, $p<0.01$) treated by oncological surgical resection. Because MMR status could influence the type of surgery, we evaluated how often MMR status was known before oncological surgical resection. In 552 (51%) patients that underwent oncological surgical resection, the MMR result was performed on biopsy or local excision samples and known prior to surgery. Over time the uptake of MMR testing increased from 67.3% to 90.6% for local excision ($p<0.01$) and 79.1% to 96.7% for surgical resection ($p<0.01$, Fig. 3).

MMR testing was more often performed in academic setting compared to non-academic setting (90% vs 81%, $p<0.01$) and more often for patients at younger age (88% vs 80%, $p<0.01$, Table 2). MSI PCR only was performed in 1% in academic laboratories versus 8% of non-academic laboratories (p-value <0.01).

MMR deficiency

MMR deficiency was detected in 186 of the 3.050 T1 CRCs of cases were MMR status was evaluated (6%). Of those cases loss of MLH1 and/or PMS2 staining was found in 81%. In cases with MLH1 deficiency, *MLH1* promoter hypermethylation status was evaluated in 133 (89%) of the cases. In 15/150 cases with MLH1 deficiency both MLH1 promoter hypermethylation status and BRAF was evaluated. In 16/150 cases, neither *MLH1* promoter hypermethylation nor BRAF was determined.

Fig. 1 Study flowchart
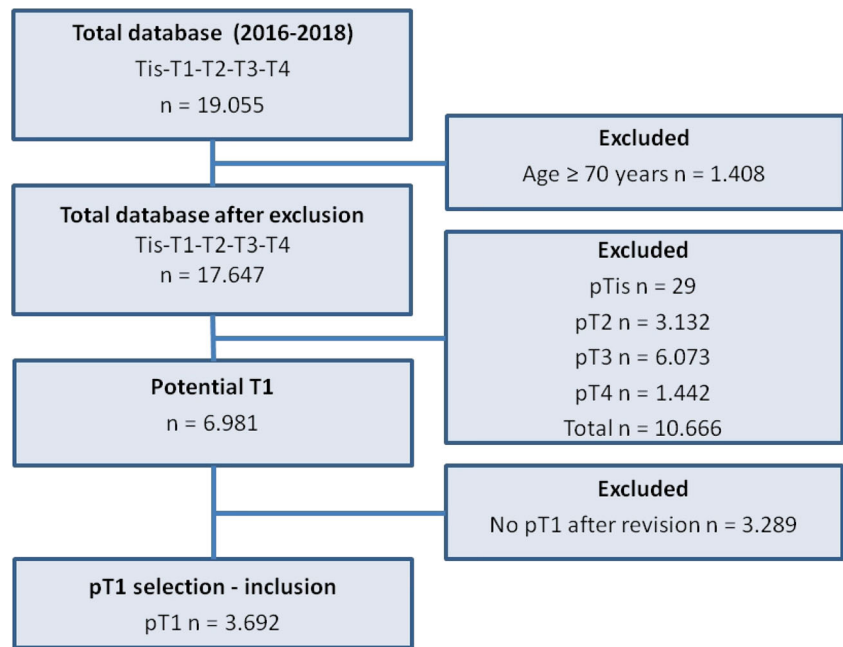
MMR deficient pT1 CRC were diagnosed more frequently in females, in the age category 61–69 years and were more

Table 1 Baseline characteristics of pT1 colorectal cancer (CRC)

| Characteristic	$n = 3.692$
Gender	
Male (*n*, %)	2266 (61%)
Female (*n*, %)	1426 (39%)
Age at diagnosis (median, min–max)	63 (55–69)
Year of diagnosis	
2016	1395 (38%)
2017	1096 (30%)
2018	1197 (32%)
Location CRC	
Proximal* (*n*, %)	575 (16%)
Distal (*n*, %)	2995 (84%)
Unknown	122
Type of carcinoma	
Adenocarcinoma (*n*, %)	3589 (97%)
Mucinous carcinoma (*n*, %)	91 (3%)
Signet cell carcinoma (*n*, %)	11 (0%)
Medullar carcinoma (*n*, %)	1 (0%)
Type of procedure	
Local excision (*n*, %)	2560 (69%)
Oncological surgical resection	841 (23%)
Local excision followed by oncological surgical resection	291 (8%)
Type of hospital	
Academic (*n*, %)ntd	702 (19%)
Non-academic (*n*, %)	2990 (81%)

*Proximal: cecum to splenic flexure

frequently located proximal. MMR deficient tumors were more frequently removed by oncological surgical resection (56%) compared with MMR proficient tumors (31%, *p*<0.01) (Table 3).

Discussion

The aim of this study was to evaluate the adherence to MMR testing in pT1 CRC diagnosed within the Dutch CRC screening program before the age of 70 years and whether differences in MMR testing exists between pT1 CRC after local excision and oncological surgical resection. We showed that MMR testing was performed in 83% of pT1 CRC cases diagnosed within the CRC screening program between 2016 and 2018, and testing increased over time. MMR testing was significantly more often performed after oncological surgical resection.

To reinforce the detection of Lynch syndrome, MMR testing has been recommended as routine diagnostics in newly diagnosed CRC diagnosed <70 years of age [12, 14–17]. MMR testing has been shown to be cost-effective when performed in all CRCs diagnosed before age of 70 years [18]. In one English hospital the compliance to MMR testing in newly diagnosed CRCs was 100% [15], while in 19 Dutch hospitals compliance to MMR testing was 84% in all newly diagnosed CRCs including stage I to IV and diagnosed from January 2016 to July 2017 [19]. Our study evaluated specifically pT1 CRC. MMR testing is more conventionally performed on oncological surgical resection specimen, and therefore MMR testing may be forgotten when only local excision has

Fig. 2 Mismatch repair (MMR) testing in pT1 colorectal carcinomas (CRC, $n = 3.692$)
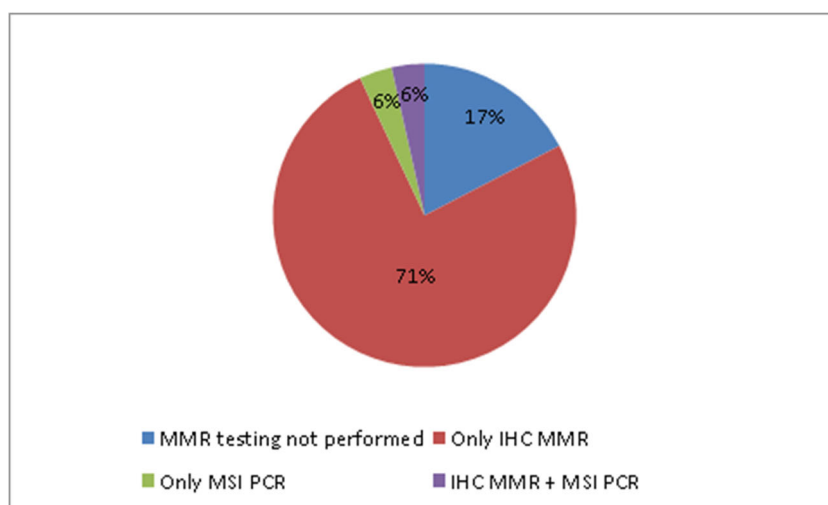
been performed. This may explain the relatively high rate of local excision specimen without MMR testing in up to 20%.

In case of oncological surgical resection of pT1 CRC, MMR status was evaluated in 89% and was known prior to surgery in 51% of the cases. In a single-center UK study, MMR status was evaluated in 69% on diagnostic biopsies prior to surgery [15]. MMR testing should preferably be performed on biopsy material as the concordance of IHC between biopsies and resection specimen is high [15, 20, 21]. Furthermore, in case of Lynch syndrome the surgical procedure may be adapted: a subtotal colectomy instead of a segmental resection can be considered for patient diagnosed with CRC at a younger age in order to reduce the risk of a second primary CRC [22–24]. Implementation of testing on endoscopic material (biopsy or local excision specimen) would also improve the relatively low testing rate in pT1 CRC (currently 80%).

In case local excision of pT1 CRC, MMR status was known in 80% of the cases. If local excision is feasible, routine biopsies are not advised because this may complicate the endoscopic mucosal resection [6, 7, 25]. In these patients, MMR status will be known after local excision.

The adherence to MMR testing guidelines increased over time for pT1 CRC, suggesting that the awareness increased after implementation of the guideline in 2016 in the Netherlands [12]. There was a clear difference in MMR testing between academic and non-academic hospitals (90% vs. 81%, respectively). Furthermore, MMR testing using MSI PCR only was performed more frequently in non-academic hospitals, lacking information about the loss of the specific protein detected by IHC. In the Netherlands, IHC MMR is the recommended test because of subsequent *MLH1* promoter hypermethylation or germline analyses to identify Lynch syndrome, and because of the easy implementation in the routine diagnostic workflow [12, 19].

Explanations for not performing MMR testing could be costs, delay in completion of pathology report, no quality control (some pathologist would be unaware of the updated
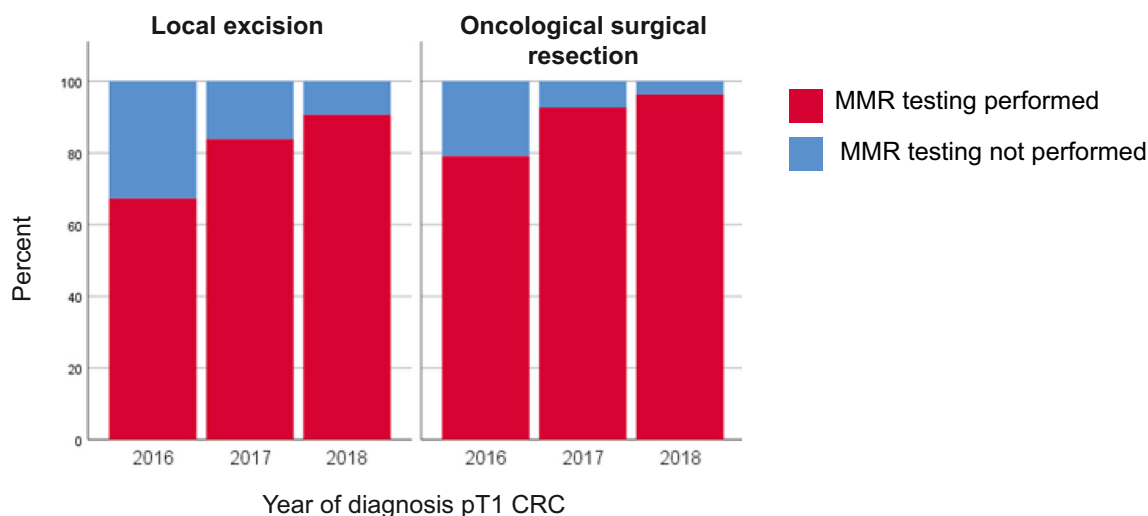

Fig. 3 Mismatch repair (MMR) testing in local excision ($n = 2.560$) and oncological surgical resection ($n = 1.132$) over time

Table 2 Factors associated with mismatch repair (MMR) testing in pT1 colorectal cancer

	MMR testing performed (n = 3.095)	MMR testing not performed (n = 642)	p-value
Gender			0.13
Male	1855 (82%)	411 (18%)	
Female	1195 (84%)	231 (16%)	
Age at diagnosis			<0.01
≤ 60 years	982 (88%)	140 (12%)	
>61 years	2.068 (80%)	502 (20%)	
Hospital			<0.01
Academic	633 (90%)	69 (10%)	
Non-academic	2417 (81%)	573 (19%)	

guideline or do not know on which material should be used for MMR testing) and limited knowledge.

MMR deficiency was detected in 6% of pT1 CRC in our retrospective cohort, while reported frequencies of MMR deficiency in sporadic CRC range from 15 to 20% [26]. This relatively low frequency of MMR deficiency can be explained by our selection of pT1 cases diagnosed in participants of the Dutch screening program. Individuals from 55 to 75 years of age are invited to perform a biennial FIT. The frequency of MMR deficient CRC is associated with age; Lynch syndrome

Table 3 Characteristics of mismatch repair (MMR) proficient (n = 2.864) and MMR deficient (n = 186) pT1 colorectal cancer (CRC)

	MMR proficient (n = 2864)	MMR deficient (n = 186)	p-value
Gender			<0.01
Male	1791 (63%)	64 (34%)	
Female	1073 (37%)	122 (66%)	
Age categories			<0.01
55–60 years	950 (33%)	32 (17%)	
61–69 years	1914 (67%)	154 (83%)	
Location			<0.01
Proximal*	365 (13%)	131 (74%)	
Distal	2418 (87%)	47 (26%)	
Differentiation grade			<0.01
Well	40 (1%)	3 (2%)	
Well/moderately	2613 (94%)	155 (89%)	
Moderately	59 (2%)	4 (2%)	
Poor/moderately	66 (3%)	13 (7%)	
Unknown	86	11	
Tumor budding			0.45
No	196 (27%)	10 (27%)	
Yes (not classified)	86 (12%)	5 (14%)	
Low (Bd1)	342 (48%)	20 (54%)	
Intermediate (Bd2)	63 (9%)	0	
High (Bd3)	33 (4%)	2 (5%)	
Unknown	2144	149	
Lymphangioinvasion			0.38
No	2277 (83%)	149 (87%)	
Yes	373 (14%)	17 (10%)	
Suspect	86 (3%)	5 (3%)	
Unknown	128	15	
Removal pT1 CRC			<0.01
Local excision	1966 (69%)	82 (44%)	
Oncological surgical resection	898 (31%)	104 (56%)	

*Proximal location of pT1 CRC is when tumor is located in the cecum to splenic flexure

is predominately detected in younger patients, while sporadic MMR deficient tumors are more often detected at an older age [27]. In our retrospective population, MMR deficient pT1 CRC were predominately detected at an older age (83% at age 61–69 years), assuming that we mostly detected sporadic MMR deficient tumors. We probably miss the young patients with Lynch syndrome in our cohort.

A limitation of this study is the fact that there might be some missing data, since in less than 2% of cases, it was stated that the MMR status was still under investigation without any follow-up reports. Furthermore, we only included pT1 CRC diagnosed within the Dutch population-based screening program and therefore the minimum age at pT1 CRC diagnosis was 55 years. We do not have information about MMR testing adherence for pT1 CRC outside the screening program. The strength of this study is that we have data on a national level from 42/43 Dutch hospital laboratories, allowing differentiation between academic versus non-academic hospitals.

In conclusion, MMR testing was still only performed in 83% of the pT1 CRCs in the Netherlands between 2016 and 2018. However, in 2018, MMR status was evaluated in almost 92% of the pT1 CRCs. By implementing MMR testing on the first endoscopic specimen obtained (either biopsy or local excision specimen), the uptake can easily be increased.

Declarations

Conflict of interest The authors declare no competing interests.

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