Evaluation of a nationwide Dutch guideline to detect Lynch syndrome in patients with endometrial cancer

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HIGHLIGHTS

• Implementation of Lynch syndrome screening in endometrial cancer can be improved.
• Gynaecologists ought to be aware of Lynch syndrome screening in endometrial cancer and might require additional training.
• Quality assurance protocols should be implemented to ensure adherence to Lynch syndrome screening in endometrial cancer.

ABSTRACT

Objective. In the Netherlands a nationwide guideline was introduced in 2016, which recommended routine Lynch syndrome screening (LSS) for all women with endometrial cancer (EC) <70 years of age. LSS consists of immunohistochemical (IHC) staining for loss of mismatch repair (MMR) protein expression, supplemented with MLH1 methylation analysis if indicated. Test results are evaluated by the treating gynaecologist, who refers eligible patients to a clinical geneticist. We evaluated the implementation of this guideline.

Methods. From the nation-wide pathology database we selected all women diagnosed with EC <70 years of age, treated from 1.6.2016 – 1.6.2017 in 14 hospitals. We collected data on the results of LSS and follow up of cases with suspected LS.

Results. In 183 out of 204 tumours (90%) LSS was performed. In 41 cases (22%) MMR protein expression was lost, in 25 cases due to hypermethylation of the MLH1 promotor. One patient was known with a pathogenic MLH1 variant. The option of genetic counselling was discussed with 12 of the 15 remaining patients, of whom three declined. After counselling by the genetic counsellor nine patients underwent germline testing. In two no
pathogenic germline variant was detected, two were diagnosed with a pathogenic PMS2 variant, and five with a pathogenic MSH6 variant, in concordance with the IHC profiles.

**Conclusion.** Coverage of LSS was high (90%), though referral for genetic counselling could be improved. Gynaecologists ought to be aware of the benefits and possible drawbacks of knowing mutational status, and require training in discussing this with their patients.

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1. Introduction

Endometrial cancer is the most common gynaecological malignancy in the Netherlands [1] and in other high income countries [2,3]. It is the second most common gynaecological cancer worldwide [4], and in recent years there has been an increase in the incidence of endometrial cancer globally [3]. Risk factors for endometrial cancer are nulliparity, late onset of menopause, prolonged oestrogen exposure, obesity and tamoxifen use. There are also genetic predispositions for endometrial carcinoma recognised, as is the case in Lynch syndrome (LS). LS is characterized by early onset of colorectal carcinoma, endometrial carcinoma and other extra colonic cancers [5,6]. A gynaecological malignancy will be the first presentation in more than 50% of women with LS, and 6% of cases of EC >70 are caused by LS [7]. LS is an autosomal dominant condition caused by germline pathogenic variants in DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS2) and TACSTD1 [8] or more rare constitutional epimutation in the promoter regions of MLH1 or MSH2 [9]. Complete inactivation of one of the MMR proteins, as the result of pathogenic mutations in a MMR gene or hypermethylation of the MLH1 promoter, causes instability in highly repetitive DNA sequences, known as microsatellite instability (MSI). Immunohistochemistry (IHC) for MMR proteins and MSI analysis can be used to detect loss of MMR function. Since sporadic MMR deficient (MMRd) tumours can be due to hypermethylation of the MLH1 promoter in the tumour, in the case of absent MLH1 protein expression, MLH1 hypermethylation analysis is carried out to distinguish between sporadic MLH1 deficient tumours and tumours likely to be caused by LS [10,11]. To confirm or exclude the presence of an underlying germline variant referral for genetic testing is indicated for patients suspect of LS. If germline testing does not reveal a pathogenic germline variant, these patients may have a Lynch-like syndrome (LLS). Partially deficient cases may be due to somatic events. [12–15] Although LLS patients have a lower risk of developing CRC compared to LS patients, their risk is higher than patients with sporadic CRC [16].

Identification of subjects carrying a pathogenic MMR gene variant is important, because surveillance colonoscopy in these people is associated with improved overall and colorectal cancer related mortality [17]. For the detection of LS in patients with endometrial cancer, current guidelines and policies show a broad spectrum concerning the age and criteria to commence tumour screening [18,19,20]. Routine tumour screening for LS (LSS) for women with EC <70 years of age has shown to be cost effective, allowing them and their relatives to benefit from surveillance colonoscopy [21]. Since January 2016 this strategy is incorporated into the Dutch national guideline concerning endometrial cancer. This guideline is developed by the Guideline Commission Gynaecologic Oncology (RCGO) and published by the Netherlands Comprehensive Cancer Organization (IKNL) [22]. The guideline recommends LSS by IHC because this can be implemented easily in all pathology laboratories and is most cost effective; MSI analysis is only advised in case of inconclusive IHC results [23]. The primary outcome of this study was to record to what extent the current Dutch national guideline was implemented in daily practice; whether patients under the age of 70 years with an endometrial carcinoma were screened for LS, and whether eligible patients were referred to a clinical geneticist. The secondary outcomes were the technique used to screen for LS (IHC or MSI analysis), the specimen on which the screening was performed (endometrial biopsy or hysterectomy specimen) and the final diagnosis of a germline pathogenic variant.

2. Methods

Via the Dutch Pathology Registry (PALGA; Nationwide network and registry of histopathology and cytopathology in the Netherlands encompassing all histology reports in the Netherlands since 1974) a search was performed to identify all patients with endometrial carcinoma younger than 70 years within two regional oncology networks in the Southwest Netherlands encompassing 14 hospitals, listed in the acknowledgments. Cases from the period July 2016 to July 2017 were included. Patients with a different diagnosis, those that were treated outside the catchment area, or before or after the inclusion period were excluded. A database was compiled containing the anonymous data for the included cases. These data were then analysed for tumour characteristics and MMRd analysis. After identifying patients with loss of expression of a MMR protein, we assessed whether MLH1 hypermethylation analysis was performed if indicated. The hypermethylation analysis was always performed on both the tumour and normal tissue to exclude constitutional epimutations. Once the remaining patients at risk of LS were identified, the gynaecologist who treated the patient was contacted through the PALGA portal. This portal ensured anonymity of the patients but made it not possible to extract data from the patient file personally. The clinician was asked i) whether the patient was informed about the findings, ii) if the patient was referred to a clinical geneticist, iii) if the patient went for counselling and iv) the outcome of genetic tests. These data were included in the database and implementation of the guideline was evaluated.

Statistical analyses were performed using SPSS, version 25.0.0.1 (SPSS Inc., Chicago, IL, USA). This study was approved by the Medical Ethical Commission of the Erasmus MC Cancer Institute (MEC-2018-1292) and all participating hospitals.

3. Results

3.1. Screening for LS

A total of 238 reports coded with a diagnosis of endometrial carcinoma were collected from the PALGA database. After assessment of the reports 34 cases were excluded due to treatment in another region or another diagnosis, leaving 204 cases with endometrial carcinoma. In 21 cases (10%) IHC or MSI analysis was not performed. Follow up of these patients who were not screened revealed a variety of reasons. In some cases it was not yet standard protocol to perform LSS in the pathology laboratory and the screening was not specifically requested by the treating gynaecologist.

After this study had revealed that the information was missing, screening was performed later for two patients. Both cases appeared to have an increased risk for LS, unfortunately one woman died before the screening result became available. The other patient was referred for genetic counselling and germline
testing, which identified a pathogenic MSH6 variant. These patients were not included in the further analyses concerning implementation of the guideline.

The clinicopathological features of all LS screened cases are shown in Table 1. The vast majority of cases showed endometrioid histology (155/183, 85%). Eleven patients showed metastasis in the ovaries. Concomitant epithelial ovarian cancer was not reported. Two patients had a concurrent second tumour; one patient had a granulosa cell tumour in the ovary and one patient an oesophageal carcinoma (T2,N0). MMR analysis was performed by IHC in the majority of cases (180/183), in three cases MSI analyses was carried out, and in two cases both IHC as MSI analyses was conducted. The MMR analyses showed loss of MMR protein expression on immunohistochemistry in 41 cases (22%), subsequent testing showed tumour-specific hypermethylation of the MLH1 promotor in 25 cases (14%) and these tumours were considered to be sporadic tumours. The remaining 16 cases (9%), loss of MLH1 in 5 cases, loss of PMS2 in 5 cases and loss of MSH6 in 6 cases, were considered at risk of a pathogenic MMR gene germline variant. One patient was already known to have a pathogenic germline MLH1 variant, she was not referred to a clinical geneticist. (Fig. 1).

3.2. Referral and germline testing

There were 15 patients at risk of an as yet undiagnosed germline LS pathogenic variant. Three patients were not informed about the tissue screening result and were not referred to a clinical geneticist. We could not establish the reason why the screening results were not discussed with these patients. Twelve patients were advised to consult a clinical geneticist, of whom three declined referral. One patient refrained from genetic counselling because her husband was terminally ill, she also withdrew from gynaecological follow up. The clinical files did not elucidate why the other two patients declined genetic counselling. The remaining nine patients visited the clinical geneticist and underwent germline testing; in two no pathogenic germline variant was detected, two were diagnosed with a pathogenic PMS2 variant and five with a pathogenic MSH6 variant.

### Table 1

| MMR (n) | % | MLH1/PMS2 loss and hypermethylated promotor (n) | % | MMRd (n) | % | Total (n) | % |
|---------|----|-------------------------------------------------|----|---------|----|----------|----|
| Age (average, yrs) | 61.2 | 61.3 | 61.2 | 61.2 |
| (42–69) | (50–69) | (53–67) | (42–69) |
| Histology | | | | |
| Endometrioid | 117 | 82 | 24 | 96 | 14 | 88.8 | 155 | 85 |
| Grade 1 | 89 | 10 | 9 | 9 | 10 | 108 |
| Grade 2 | 15 | 11 | 5 | 5 | 31 | |
| Grade 3 | 12 | 3 | 0 | 0 | 15 | |
| Unknown | 1 | | | | 1 | |
| Serous | 13 | 9 | 0 | 0 | 0 | 13 | 7 |
| Clear cell | 4 | 3 | 0 | 0 | 0 | 4 | 2 |
| Carcinosarcoma | 6 | 4 | 0 | 1 | 5.6 | 7 | 4 |
| Undifferentiated | 0 | 0 | 1 | 4 | 0 | 0 | 1 | 0.5 |
| Mixed type | 1 | 1 | 0 | 1 | 5.6 | 2 | 1 |
| Uncertain diagnosis | 1 | 1 | | 0 | 0 | 1 | 0.5 |
| Stage | | | | | | | |
| T1a | 73 | 51 | 17 | 68 | 13 | 82 | 103 | 56 |
| T1b | 38 | 27 | 5 | 20 | 0 | 0 | 43 | 24 |
| T2 | 6 | 4 | | 2 | 11 | 8 | 4 |
| T3a | 7 | 5 | 2 | 8 | 0 | 0 | 9 | 5 |
| T3b | 2 | 1 | | 0 | 0 | 0 | 2 | 1 |
| T4 | 1 | 1 | | 0 | 0 | 1 | 0.5 |
| Unknown | 15 | 11 | 1 | 4 | 1 | 11 | 17 | 9.5 |
| Genetic counselling | 12 | 75 | 12 | 7 |
| Germline testing | 9 | 56 | 9 | 5 |
| Lynch syndrome | 7 | 44 | 4 | 4 |
used was clarified in only one case in which there was an inconclusive result of IHC after which MSI analysis demonstrated that the tumour was microsatellite stable. To ensure uniformity in the execution of the guideline, it is advisable that laboratories initiate screening by IHC and only use MSI analysis in case of an inconclusive IHC result.

Of the screened patients, 8% (15/183) were at increased risk of an as yet undiagnosed LS, however 20% of these patients were not informed about this finding. This is in contrast to the AMA Code of Medical Ethics, in which the right to know is explicitly spelled out. Patients should have been informed about their test results, especially since the result of germline testing can have a major impact on the future health of the patient and her family members. We could not establish why these results were not shared with the patient. Several issues might be relevant: ignorance of the result, a knowledge gap (the gynaecologist does not understand the IHC test results) and unease with genetic counselling have all been reported previously [28,29].

In the Netherlands a standardized format is used for the reporting of IHC test results, including advice on additional testing and when referral to a geneticist is indicated. This standardized reporting should ensure that gynaecologists know what action ought to be taken. It could be argued that universal endometrial cancer screening for LS would increase the uptake of LSS. It would increase awareness of both pathologists and gynaecologists, that a test result is required for optimal patient care. There is no international consensus up to what age and under which criteria routine diagnostics for heritable causes of MMR deficiency is indicated. [18,19,20,23] Due to the fact that LSS has been shown cost effective in patients with EC < 70 years, till now the Dutch guideline has limited the screening up to 70 years of age. Introducing a monitoring system, to check that IHC test results have been communicated to all patients, can prevent that patients are unaware of their LS risk.

We performed a short survey on knowledge about LS, and familiarity with the current guideline, among gynaecologists and residents visiting a regional meeting where the preliminary results of this study were presented (September 2018). See Supplementary Table 1 for the questions asked and the results. This survey revealed a lack of knowledge concerning the indication for LSS. Less than 50% of the respondents answered these questions correctly, the result was worse for gynaecologists than for junior residents. This urges the need for education of gynaecologists regarding LS.

Seventy-five percent of those patients counselled about genetic testing by the gynaecologist agreed to be referred to the clinical geneticist and all referred patients underwent germline analysis. Previous studies reporting on genetic counselling for LS endometrial carcinoma patients have shown variable percentages for referral to a clinical geneticist: ranging from 44 to 94%. [30–36] This fairly high acceptance rate for genetic counselling could be due to the organization of our health care system. It has been demonstrated that the lack of insurance coverage was one of the major reasons to decline germline testing. [30] The availability of accessible services and the cost structure of genetic counselling and testing within the Dutch health care system lower the barriers for patients to accept genetic counselling. For two of the three patients who refused referral the reason was unclear, and we are unable to determine whether these patients might have been willing to be referred later. Though genetic counselling services are embedded within our health care system, patients may still perceive several barriers to participation [29]. First of all, some patients may not see the benefit of genetic counselling for themselves or their siblings. This could change if the presence of a pathogenic germline variant is shown to lead to adjuvant treatment options. Also patients have reported that there was a paucity of information when they were informed about the genetic counselling, and this limited information may be a reason why they do not participate. This could be due to lack of knowledge and urges the need for education and training of gynaecologists also regarding genetic counselling in case of aberrant MSI results e.g. by e-learning. It has been demonstrated that e-learning can be an effective method for enhancing counselling skills [37]. Thirdly, the timing of the counselling
by the gynaecologist could be important for patients considering referral to the clinical geneticist. Patients might experience an overload of information at the time of diagnosis, and may be less receptive for referral to a clinical geneticist [29]. However, in patients with newly diagnosed colorectal cancer, immediate genetic testing was found to be acceptable [38], without causing high levels of psychological distress [39]. Since the interval between immunohistochemistry and referral is short, it is possible that uptake would increase if counselling is also offered later in follow-up. Since in our study the time frame between implementation and evaluation of the national guideline is rather short it is possible that some patients, who initially refused genetic counselling, are more responsive at a later stage. It might be advisable to discuss the topic again in patients follow-up.

The strengths of our study are that we collected data from 14 hospitals that collaborate within the two cancer networks in the Southwest of the Netherlands and secondly that the central PALGA pathology database covers almost 100% of all diagnoses resulting in a quite large cohort. Though we didn’t have a central pathology review, all associated laboratories perform quality controls concerning their immunohistochemistry analysis. Also the objective of our study was to evaluate the implementation of the guideline in daily practice and not the accuracy of the pathology testing. A weakness of our study is that we have no information on the patients previous or family history (i.e., whether patients had other LS-related cancers) nor on somatic events in the two tumours with aberrant immunohistochemistry, but no pathogenic germline variant. Another pitfall could be that the interval between implementation of the guideline (January 2016) and the opening of the cohort (July 2016) might be too short. Still, up till now in only a few participating clinics a quality assuring protocol has been implemented, therefore it is not certain that the guideline adherence improved. There is also a potential source of bias due to the fact that we had no access to the clinical files of patients because communication with the gynaecologists and pathologists was done via the PALGA portal, which ensured anonymity of all patients. Due to this we were also unable to cross check genetic outcomes of those patients who underwent germline testing.

5. Conclusion

The implementation of the guideline regarding LSS in endometrial cancer has been quite successful though referral to a clinical geneticist could be improved. The use of a standardized national synoptic pathology report that includes results of LS immunohistochemistry should ensure that all patients will be screened. Local protocols should be implemented to confirm that all patients underwent tumour screening for LS.

The rate of referral to a clinical geneticist should be improved, in view of the implications for the patient and her relatives. Gynaecologists ought to be aware of the benefits and drawbacks of knowing mutational status, and require training in discussing this with their patients. The mutational status is not only important for further health risks of the patient and their relatives, but may also provide possibilities for new adjuvant treatment options, such as immune checkpoint inhibitors, PD-L1.

5.1. Future implications

To prevent any barriers for usage of genetic counselling, we propose that LSS should be performed in a uniform manner, and that this should be established in local protocols. This will clarify on which specimen the LSS is to be performed so that gynaecologists know when to expect and discuss the result with the patient. The local protocol should also ensure standard quality controls within the departments involved (pathology, gynaecology, clinical genetics) to check that all patients underwent LSS and appropriate counselling. Those patients declining genetic counselling should be identified, and discussion of the topic for a second time at a later date should be considered. In our region we started to design such local protocols, for each participating clinic, with a (local) quality cycle. This will enable us not only to determine whether the implementation of the guideline over time improved, but also what factors are at stake to raise the uptake to (almost) 100%.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2020.12.028.

Author contributions statement

AST and HCD selected eligible patients from the PALGA database and analysed pathology reports on Lynch syndrome screening. WND and PCE assisted analysis of pathology reports in cases of unclear diagnoses or immunohistochemistry/ molecular testing results. AW provided information concerning genetic counselling. LSA, KEH, ACM, SJM, KS, AAW provided additional histology reports in cases of missing data. They also provided communication to the treating gynaecologist via de PALGA portal ensuring anonymity of the patients. HCD, MEG, MH, ACH, WH, LINH, JK, GMP, HPS, RAS, PJT, PMV, BV provided additional information in case of missing data, information concerning referral to the clinical geneticist and the outcome of counselling and germline testing in cases at risk of Lynch syndrome. All authors were involved in writing the paper and had final approval of the submitted and published version.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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