Diagnosing Lynch syndrome in Nova Scotia endometrial cancer patients: An interdisciplinary audit

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None of the authors have competing interests to declare.

Abstract

Background: Diagnosing Lynch syndrome allows for implementation of risk-reducing strategies for patients and their families. This study aimed to determine the incidence of Lynch syndrome in Nova Scotia women with endometrial cancer, and to evaluate the appropriateness of investigations and referrals offered to them.

Methods: All patients diagnosed with endometrial cancer in Nova Scotia between May 1, 2017 and April 30, 2020 were identified through a provincial gynecologic oncology database. Patients from out-of-province were excluded. The following information was collected on all patients: Age, body mass index, tumor mismatch repair protein immunohistochemistry results, personal and family histories, and germline testing information. Total numbers and percentages were reported for all variables.

Results: During the study period, 465 women were diagnosed with endometrial cancer. Most were ≥ 60 years old, had obesity, and had low grade, early stage, endometrioid tumors. Tumor immunohistochemistry was performed in 95.5% of cases. Based on local criteria, 269 patients were eligible for genetic counseling. Appropriate referral was offered to 66.5% of eligible women. Germline testing was performed in 95 patients. Ten patients were diagnosed with Lynch syndrome.

Interpretation: The incidence of Lynch syndrome was 2.2% in this population. This may be an underestimation given that fewer than half of eligible women underwent germline testing. Creating clinical pathways that ensure genetic counselling access to all eligible patients is necessary for prompt diagnosis of Lynch syndrome. This will allow for implementation of risk prevention strategies, and, in cases of advanced disease, help optimize treatment.

Introduction

Endometrial cancer is the fourth most common malignancy among Canadian women (1). A small proportion of all endometrial cancer cases are associated with hereditary syndromes, most commonly Lynch syndrome (2). Lynch syndrome is an autosomal dominant inherited syndrome caused by inactivating mutations in DNA mismatch repair (MMR) genes. These genes include *MLH1, MSH2, MSH6,* and *PMS2*, as well as large deletions in *EPCAM* which can lead to transcriptional silencing of *MSH2* (3). Harboring a pathogenic germline mutation in a MMR gene confers a high risk of developing a somatic "second hit" mutation, with subsequent genomic instability and development of cancer in affected individuals (3). The incidence of Lynch syndrome in patients affected with endometrial cancer is approximately 2-3% (4,5).

Lifetime risks of endometrial cancer for women with Lynch syndrome is 40-60% (6). Women with Lynch syndrome are also at an increased risk of ovarian cancer and other Lynch-related cancers such as small bowel, stomach, pancreas and urothelial malignancies (6). Identifying affected individuals is crucial to the management of their care and that of their affected relatives, as many cancer risk-reducing strategies are available.

Tumor immunohistochemistry (IHC) testing for expression of *MLH1*, *MSH2*, *MSH6*, and *PMS2* via their protein products has been widely used to triage individuals at risk of Lynch syndrome. Traditionally, patients were triaged for germline testing using clinical criteria, however up to 15-30% of endometrial cancer patients with Lynch syndrome do not fulfill these criteria(7). For this reason, one of the recommended strategies to identify individuals who should be offered germline testing is to perform tumor IHC for all endometrial cancer patients, regardless of age(7).

There are two different approaches to IHC testing. The four-antibody approach targets all four, *MLH1*, *MSH2*, *MSH6*, and *PMS2,* proteins. The two-antibody approach targets only the minor heterodimer partners *PMS2*, which binds to *MLH1*, and *MSH6*, which binds to *MSH2*, under the premise that expression of a minor partner is lost alongside its partner, but not vice versa(8). In other words, positivity for these two markers implies intact expression of their counterparts whereas *PMS2* is lost in virtually all *MLH1*-deficient tumors, and likewise for *MSH6* in *MSH2*-deficient tumors. Loss of either *PMS2* or *MSH6* in this paradigm triggers IHC for the other two immunostains (*MLH1, MSH2*) so as to fully characterize the pattern of MMR loss(8).

In Nova Scotia, tumor IHC has been performed on all endometrial carcinomas, including carcinosarcomas, since April 6, 2017. Women found to have loss of expression of any of the above four MMR proteins are subsequently referred to medical genetics for consideration of germline testing. Other criteria used to identify patients that should be referred for genetic counselling include: Personal history of other Lynch-associated malignancy, family history of Lynch-associated malignancies, and age less than 60. To our knowledge, the incidence of Lynch syndrome in the Nova Scotia endometrial cancer population has never been described. We aimed to characterize our local endometrial cancer population, clarify the incidence of Lynch syndrome in this population and to evaluate how effectively women identified at increased risk of Lynch syndrome were appropriately referred and investigated for the condition.

Methods

Ethics approval for the study was obtained from the Nova Scotia Health Research Ethics Board (File #1026033).

This was a population-based descriptive study using Nova Scotia data. In this province, all women affected with a gynecological malignancy have their pathology reviewed and are typically treated in one centre – the Nova Scotia Cancer Centre (NSCC) in Halifax. The gynecologic oncologists at the NSCC enter data on all patients with a diagnosis of gynecological malignancy in a database called the Tupper Gynecology Oncology Database. The database regularly gets audited for accuracy. Data collected includes patient demographics such as age and body mass index (BMI), primary diagnosis, histology, staging and treatments.

We used the Tupper Gynecology Oncology Database to generate a list of all patients diagnosed with endometrial cancer between May 1, 2017 and April 30, 2020. Pertinent demographic and clinical information (i.e. age and BMI at time of diagnosis, histology, FIGO grade and FIGO stage) were also extracted from this database. A chart review was then performed for each patient to confirm eligibility and collect any missing information. Tumor MMR IHC testing results for each patient, was extracted from pathology reports. Germline testing results, and information regarding family history and/or personal history of Lynch-associated malignancies for all eligible patients, based on above mentioned criteria, were extracted from medical genetics records. Patients with a health card number from any province other than Nova Scotia were excluded.

Age and BMI are reported using mean and range. Total number and percentage are reported for: histologic subtype and grading, staging (i.e. stage I, stage II, stage III, and stage IV), personal history of any other Lynch syndrome-associated cancer, presence of high-risk family history (i.e. first-degree relatives with Lynch syndrome-associated cancer diagnosed before the age of 50, or more than two first or second-degree relatives with Lynch syndrome-associated cancer diagnosed at any age)**,** testing performed (i.e. no testing performed, tumor

MMR testing only, both tumor MMR and germline MMR gene mutation testing, or germline MMR gene mutation testing only), response to offered germline testing (i.e. accepted, declined, not offered**),** indication for germline testing (i.e. tumor MMR defects, personal or family history of Lynch-related malignancy, or other), tumor IHC staining results for *MLH1*, *MSH2*, *MSH6*, and *PMS2*, and germline MMR gene mutation testing results, specifically for Lynch syndrome (i.e. positive, negative, or unknown).

Results

A total of 475 women were identified through the Tupper Gynecology Oncology Cancer Database as having received a new diagnosis of endometrial cancer between May 1, 2017 and April 30, 2020. Ten patients were excluded – (three from outside Nova Scotia and seven who did not have a diagnosis of endometrial cancer), yielding a final cohort of 465 women. Most were older than 60 years at time of diagnosis, however 35.7% were aged 60 or less. Most women were affected with obesity (65%) and the majority of tumors were of endometrioid histology (85.6%), FIGO grade 1 (65.4%) and FIGO stage 1 (72.7%. Personal and family history was unknown in 314 and 321 cases, respectively (Table 1).

IHC staining was performed in 444 patients (95.5%), using a two-stain panel for MSH6 and PMS2 in 267 patients and a four-stain panel for MLH1, PMS2, MSH2 and MSH6 in 177 patients (Figure 1). A total of 118 patients (26.6 %) were found to have loss of expression of at least one protein. Different patterns of protein expression loss included: loss of both *MLH1* and *PMS2* (83.1 %), loss of both *MSH2* and *MSH6* (less than 5%), isolated *PMS2* loss (less than 5%), isolated *MSH6* loss (less than 5%), isolated *MSH2* loss (less than 5%) and a loss of *MLH1*, *PMS2*, and *MSH6* (less than 1%).

Based criteria for referral in Nova Scotia, 269 women were eligible for a medical genetics consultation. At least 179 women (66.5% of eligible patients) were referred for consultation with medical genetics, with 166 having received genetic counseling at time of the study. Ninety patients were not offered a referral to medical genetics. Following consultation, germline testing was offered to 142 women (52.8% of eligible patients), 95 (66.9% of patients offered testing) of whom accepted the offered testing (Figure 1). Ten patients were confirmed to have Lynch syndrome based on germline testing results, which corresponds to 10.5% of women tested and to 2.2% of the study population.

Discussion

This study describes characteristics of Nova Scotia women diagnosed with endometrial cancer between 2017 and 2020, the incidence of Lynch syndrome in this population, and the appropriateness of investigations and medical genetics referral offered to them. The average age at time of diagnosis was 63, almost two thirds had a BMI $\geq$ 30 kg/m^2 (65%) and the most common histological subtype was endometrioid (85.6%). Personal and family history of Lynch-associated malignancies were unknown in more than two thirds of women. Tumor IHC staining was performed in 95.5% of the population to screen for Lynch syndrome. Of all screened tumors, 26.6% (n = 118) were found to have MMR protein defects. Using both IHC and clinical criteria, 90 of 269 eligible women (33.1%) were not referred for genetic counselling. Of women referred for genetic counseling (n = 179), 24 were not offered germline testing (13.4%); 10 did not meet criteria for germline testing, 9 were previously known to medical genetics for other reasons, and the remainder were lost to follow up. Germline testing confirmed 10 cases of Lynch syndrome.

Demographics of our population were similar to other populations previously described (4,9–14). Our assessment of clinical risk factors was limited due to inconsistent recording of

patients' personal and family history of Lynch-related malignancies in medical records. Tumor IHC has been demonstrated to be the most effective screening strategy for Lynch syndrome(7,11,14,15), however, family history remains clinically relevant. A small number of patients may be missed when relying on IHC staining alone(11,14), as IHC staining may be intact for some non-functioning mutant proteins(16). Highlighting this potential pitfall, intact MMR protein expression was observed in 3 out of 10 patients who were subsequently found to have Lynch syndrome. The incidence of Lynch syndrome in the population was 2.2%, which is similar to that described in an American study of women with endometrial cancer(4,5) but, interestingly, less than half the prevalence of 5.0% recently reported in a cohort of Quebec women (17) .The positive predictive value of germline testing for pathological variants in *MLH1, MSH2, MSH6, PMS2* and *EPCAM* in our population was 10.5%.

Germline status remains unknown in 174 women; 90 were not referred to medical genetics, 24 were not offered testing, 13 have a pending appointment for genetic counseling and 47 declined germline testing (Figure 1). We therefore suspect the results to be an underestimate of the true prevalence of Lynch syndrome in Nova Scotia women presenting with endometrial cancer. It was not feasible as part of this study to determine the reason patients did not receive referrals for genetic counselling. While the reasons for lack of referral to medical genetics are unknown, some patients may have been too unwell, and/or declined referral for consultation with medical genetics. It is also possible that some patients may not have been referred to medical genetics because of suspected somatic inactivation of *MLH1*, since loss of *MLH1* expression by IHC can often be observed in sporadic cases of endometrial cancer due to methylation of the *MLH1* promoter (21). The NSCC has recently begun routinely performing hypermethylation

testing on all *MLH1*-deficient tumors to help differentiate between sporadic and germline pathological variants, however this practice was not in place during the study period.

In addition to playing an important role as a screening strategy for Lynch syndrome, tumor IHC for MMR status is becoming a key factor in identifying treatment options for patients with recurrent or advanced disease. Emerging evidence demonstrates that patients with tumor MMR protein defects, regardless whether of somatic or germline origin, tend to show response to the immunotherapeutic agent Pembrolizumab and Dostarlimab in the setting of recurrent or advanced disease(18). Patients with advanced endometrial cancer have a poor prognosis(19) and limited treatment options. Pembrolizumab has been approved in Canada since 2019 for unresectable or metastatic endometrial carcinoma with MMR defects that progressed after prior therapy and for which no other treatment options exist(20). We support the use of universal up-front tumor IHC testing in endometrial cancer patients, regardless of clinical risk factors for Lynch syndrome, in order to maximize long-term treatment options.

The use of province-wide data and a relatively large population were strengths of this study. This is also the first description of the endometrial cancer population in Nova Scotia. Some limitations include inaccuracy in data collected retrospectively through databases, particularly data pertaining to personal and family history and BMI. Given the demographic risk factors for endometrial cancer in Nova Scotia, the results of this study may not be generalizable to other populations. Due to the small number of patients with Lynch syndrome, it was also beyond the scope of this study to explore the exact MMR protein defects and germline mutations in this population to see how they compare to other populations. This may be feasible as part of a larger project in the future. *MLH1* promoter hypermethylation data would have helped improve triaging women for referral to medical genetics.

This study highlighted the importance of both IHC and clinical criteria in screening for Lynch syndrome in endometrial cancer patients. While the incidence of Lynch syndrome in patients with endometrial cancer found in this study are consistent with published literature, it is suspected to be an underestimate given the low rate of high-risk women who ultimately underwent germline testing. Creating clinical pathways that ensure that all eligible patients can access genetic counselling is necessary for prompt diagnosis of Lynch syndrome in patients and their family members, to implement risk prevention strategies, and, in cases of advanced disease, help optimize treatment.

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Table 1. Characteristics of 465 Nova Scotia women diagnosed with endometrial cancer between 2017 – 2020.

Characteristic	No. (%)	Mean (range)
Age at diagnosis (years)		64 (29 – 95)
≤ 60 years old	166 (35.7)	
> 60 years old	299 (64.3)	
Body mass index (BMI)		34.9 (15.8 – 68)
< 18.5	4 (0.9)	
18.5 – 24.9	58 (12.5)	
25 – 29.9	92 (19.8)	
30 – 34.9	106 (22.8)	
35 – 39.9	64 (13.8)	
≥ 40.0	132 (28.4)	
Not reported	9 (1.9)	
Histology		
Endometrioid	398 (85.6)	
Clear cell	14 (3.0)	
Serous	12 (2.6)	
Mixed carcinoma	12 (2.6)	
MMMT[1]	11 (2.4)	
Other	18 (3.9)	
FIGO Grade		
1	304 (65.4)	
2	58 (12.5)	
3	102 (21.9)	
Not reported	1 (0.2)	
FIGO Stage		
1	338 (72.7)	
2	27 (5.8)	
3	47 (10.1)	
4	25 (5.4)	
Unable to stage	28 (6.0)	
Family history of Lynch-associated cancer		
Yes	66 (14.2)	
No	78 (16.8)	
Unknown	321 (69.0)	
Personal history of other Lynch-associated cancer		
Yes	26 (5.6)	
No	125 (26.9)	
Unknown	314 (67.5)	

1 MMMT: malignant mixed Müllerian tumor

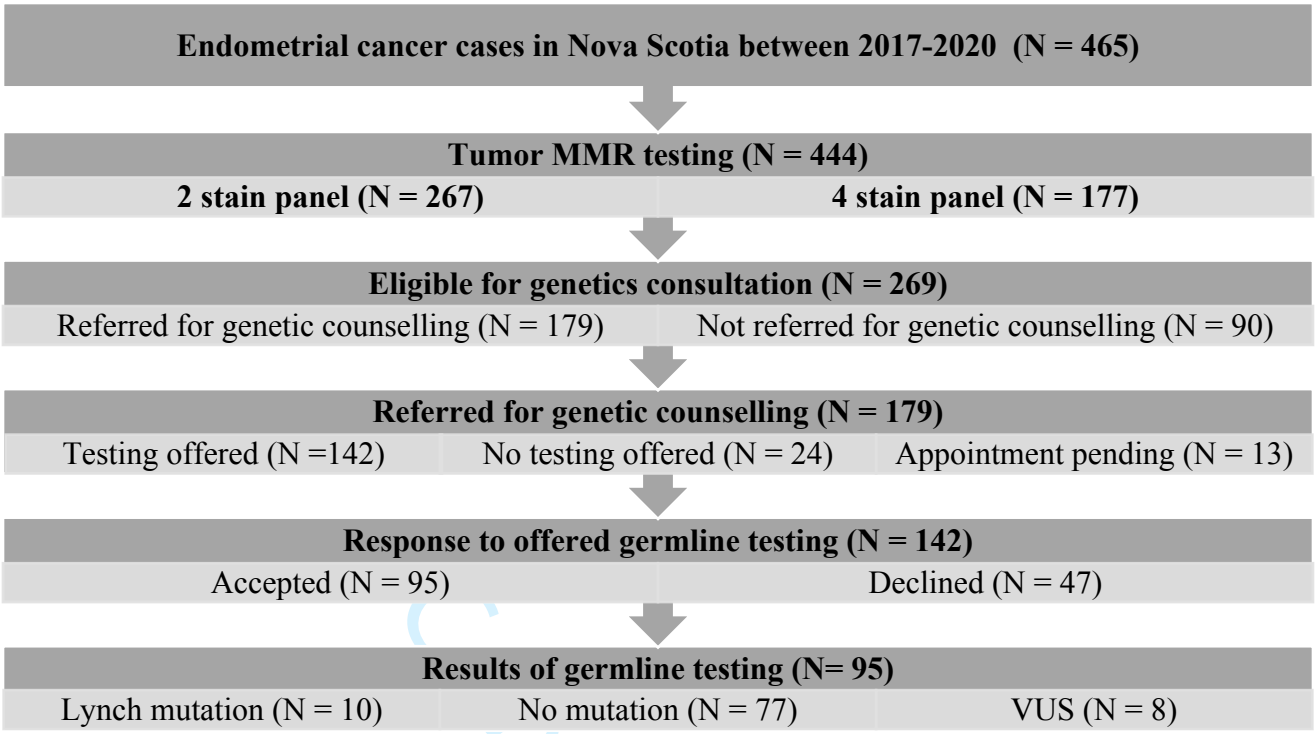

Figure 1. Tumor MMR testing and germline testing for Lynch syndrome in 465 Nova Scotia women from 2017-2020.