Article details: 2022-0136
Title: A descriptive study of screening and testing practices for Lynch syndrome Nova Scotians with endometrial cancer
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Reviewer 1: Lesley Roberts
General comments (author response in bold)

1. Suggestions for edits:
- Page 4/16, line 28 - "Risk" (as opposed to risks)
- Page 7/16, line 35 - Close bracket following 72.7%
- Page 8/16, line 3 - Based "on"
Thank you, this was corrected.

2. I would suggest stating in the Methods section that MLH1 promoter hypermethylation testing was not available in NS during the study period. This will make it clearer to the reader moving forward why all MMR deficient tumors were referred.
Thank you, this was added under the method section. (Page 4/14, End of 1st paragraph of the setting section.)

3. When reading the results, I was a bit confused by where the 269 patients eligible for genetics testing comes from. Would suggest clarifying this (page 8/16). It is clear 118 were referred because of MMR deficiency as described on previous page, but the remainder is not clear. If due to family/personal history, please specify.
We have taken care to explain this under the result section and illustrated this in Figure 2. (Page 7/14, Paragraph 2; Figure 2)

4. In the discussion, you mention results may not be generalizable due to the demographic risk factors for endometrial cancer in NS. Would be helpful for the broader readership if you could describe what specifically about the NS population is different from the rest of the Canadian or North American population.
This was edited to ‘’ As our population is geographically restricted to NS, results may not be generalizable to other populations’’. (Page 11/14, under *Limitations*)

Reviewer 2: Cathy Popadiuk
Institution: Department of Women's Health, Memorial University
General comments (author response in bold)

1. There is a great deal of information presented here but at times the manuscript seems to deviate from a focused purpose or from established objectives. For example, in the abstract, the aim of the study was to determine the incidence of Lynch Syndrome in Nova Scotia women with endometrial cancer (EC), and "evaluate the appropriateness of investigation and referrals offered to them." Then at the end of the introduction, the aim is diffused 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."
Our study objectives have been restated as:
1. To described screening and testing practices for LS in Nova Scotia women with endometrial cancer and,

2. To determine the prevalence of LS in Nova Scotia women with endometrial cancer
Objectives are now consistent between the abstract and the introduction. (Page 2/14, Background; Page 4/14, paragraph 1; Page 11/14, last paragraph)

2. The introduction gives an excellent overview of what is known about EC and Lynch syndrome including the various identification and testing strategies. Implementation and translation of the research into clinical and lab practice can be complex. Discouragingly, for half the population studied, the data are incomplete, thus unfortunately limiting confidence in the key outcomes about Lynch in EC patients in NS.
We agree, that having such a low proportion of eligible patients undergo germline testing is a major limitation in our ability to confidently estimate the prevalence of Lynch Syndrome in this population. As such, we have decided to present this as a description of screening and testing practices for Lynch Syndrome in NS women with EC. Even though there is missing data; that in of itself is informative in identifying gaps in our program to hopefully improve access to genetic counseling for patients who need it. (N/A)

3. IHC has been performed in EC patients in NS since April 6, 2017. Is this on endometrial biopsy specimens, hysterectomy specimens or other biopsies? Is there a particular standard that NS follows? How is the determination made to do a 2 versus 4 panel stain and what is the rationale to include these two differing methodologies? Is the choice considered a variable for the study? Is this a lab decision? Did a practice evolve from one or the other?
IHC is performed on endometrial biopsy if specimen is sufficient, otherwise, it is performed on surgical specimen (uterus).
Standard is established by our gynecologic pathologists in Halifax, as they are the only group reviewing gynecologic oncologic pathology in the province.
Performing a 2 vs 4 stain panel is at the pathologist's discretion.
The difference in pick-up between the 2 vs. 4 stain panel is very minimal – in theory all MLH1-deficient tumors will have lost PMS2, and all MSH2-deficient tumors will have lost MSH6, hence it's reasonable to screen with the 2 stain panel. However, occasional cases display unusual patterns of staining.
Co-authors RW and MC have written a paper with regards to this (Wood RK, Offman SL, Murray SK, Carter MD. Lynch syndrome screening in endometrial carcinoma: Lynch syndrome screening in endometrial carcinoma: A two-antibody (PMS2/MSH6) immunohistochemistry panel can lead to underdiagnosis of MSH2-deficient tumours. *Canadian journal of pathology*. 2021;13(3):14.), which showed that there is a small number of MSH2-absent, MSH6-intact tumors that are at high risk for LS and yet screen negative with the 2-stain panel. Their cohort had 6/293 (2%) with this aberrant staining pattern, with 2 of these 6 missed on initial screen due to sufficient MSH6 expression to qualify as "negative" without further assessment (e.g. adding MSH2 and MLH1).
Since the above project has been done 4-stain panel is the standard in NS, but it was still at the pathologist's discretion during our study period. (Page 4/14, 1st paragraph under *setting*)

4. Is there a public site outlining details for establishment of the Tupper Database, data parameter inclusion, and so on? Reference is made to the database being audited for quality, but by whom? A provincial body? department? Is this a local database through

Gynecologic Oncology which may or may not be associated with a provincial cancer registry? The manuscript states that all EC is managed through the Nova Scotia program but the total of 465 patients in the 3 year period of the study, seems low when compared to the Statistics Canada site outlining NS has from 185 to 200 EC patients a year from 2014 to 2018. It is not unreasonable to expect the EC rates have been 200 cases per year or more up to April 2020. It is not clear why the study has an apparent short fall. A comment explaining the place of this database compared to national and provincial statistical reporting would help the reader gain perspective for the NS situation.

There is no public site outlining details of the Tupper Database. It is a local, clinical database used by the gynecologic oncologists. It is not associated with a provincial cancer registry. It gets audited by the gynecologic oncologists in Halifax. This is done every few years by comparing its numbers with provincial cancer statistics.

We were unable to find official cancer statistics including years 2017-2020 for comparison. There are always a few cases every year that are diagnosed incidentally on surgical pathology of hysterectomies that were done for benign indications at other Centres, however pathology/case should still have been referred to Halifax and made it to the Tupper Database. (Page 5/14, under *source of data*)

5. The results section is succinct (3 paragraphs) and table and Figure clearly presented. What is striking and perplexing is that so many women required referral for genetics counseling, in many places, a scarce human resource. 118 of 444 women tested with IHC, required genetic counseling referral. It wasn't clear whether the MLH1 patients had hypermethylation testing to further triage genetics referral. And based on full referral criteria (family and personal history), 269 required genetics consultation. It seems like another 151 women were identified on family/personal history alone (but 66+26 = 92 women as per table 1). Yet "personal and family history ... were unknown in more than two thirds of women".

Unfortunately, MLH1 hypermethylation testing was not available during the study period. This was more clearly stated in the method section.

Many women were also eligible for genetic counseling based on age ≤ 60.

Personal and family history was only known for patients who were actually referred to genetics. This is a limitation of database data collection. The genetics database collected information on personal and family histories, but not the gynecologic oncology database. (Page 4/14, End of 1st paragraph of the setting section; Page 7/14, paragraphs 2-4; Page 8/14, paragraph 2)

6. What is surprising, is what appears to be a very high referral rate of endometrial cancer patients to genetics in general. As stated in the introduction: The incidence of Lynch syndrome in patients affected with endometrial cancer is approximately 2-3% (4,5). But over half EC patients in NS are eligible for genetics consultation. What standard is followed to determine appropriateness for referral to genetics? Is this a Canadian standard? It would be interesting to know how such a high genetics referral burden is managed to achieve an acceptable turnaround time. For example, what are the genetics resources in the province and what is the referral process from gyne onc to genetics? These resources vary across the country as do testing capabilities for Lynch; understanding the flow could help answer many unknowns in the manuscript and inform other provinces and jurisdictions.

Criteria used to referral to genetics are:

(1) Any loss of MMR protein expression on tumor IHC,
(2) age ≤ 60 at time of diagnosis,
(3) personal history of other Lynch-associated malignancies, including CRC, ovarian, stomach, pancreas, small bowel, ureter and renal pelvis, and/or biliary tract cancers, and
(4) family history of Lynch-associated malignancies, including EC, CRC, ovarian, stomach, pancreas, small bowel, ureter and renal pelvis, and/or biliary tract cancers, in 2 or more first or second degree relative.

There is a gap between the age criteria used to refer to genetics and the age criteria genetics uses for germline testing. Age appears to be the main criteria that is raising referral rates. Of course, MLH1 hypermethylation testing (now available), will help lower the referral rate. (page 4-5/14; page 7/14, last paragraph; page 8/14, last paragraph; page 10/14, last paragraph)

7. The authors write: "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." This statement seems to challenge the completeness of the Tupper Database, which is regularly audited (paragraph 1 and 2 page 5).
This statement was removed. The issue truly is that *personal/family history* are not variables in the Tupper Database. (page 8/14, paragraph 2)

8. It is interesting that extraordinarily, 3 of the 10 identified Lynch patients had intact MMR proteins and would have been missed on IHC alone. No discussion is provided on the possibility of these three patients' test results being false negatives. In this regard, they should include the sensitivity and specificity of their validated IHC tests in the methods section.
All data was reanalyzed (see details under item 16 explanation). From the 3 patients you are referring to:
-1 had been miscoded and did not have a germline mutation (this has been corrected),
- 1 had a weak expression of MSH6
- 1 truly was reported as having intact protein expression x 4.
Of note, all patients were germline mutations did have a 4-stain panel.

We have added information regarding our lab accuracy in under interpretation -> Main findings (page 9-10/14, last paragraph of page 9, going into page 10)

9. Re comment about NS having half prevalence of Quebec women, Quebec is known to have regions with genetic founder populations. As the study quoted is from a "cohort of Quebec", it would be better to compare carefully whether the "cohort" represents what is expected of the entire province.
We added a brief description of this study in the introduction.
It was cohort study of 261 women treated at the McGill University Health Centre in Montreal.
Wording was therefore modified in the <u>Interpretation</u> section for '' cohort of women treated in Montreal''. (page 3/14, last paragraph; page 10/14, first paragraph)