Original Article

Performance Characteristics of Screening Strategies to Identify Lynch Syndrome in Women With Ovarian Cancer

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BACKGROUND: For women with ovarian cancer (OC), the optimal screening strategy to identify Lynch syndrome (LS) has not been determined. In the current study, the authors compared the performance characteristics of various strategies combining mismatch repair (MMR) immunohistochemistry (IHC), microsatellite instability testing (MSI), and family history for the detection of LS. **METHODS:** Women with nonserous and/or nonmucinous ovarian cancer were recruited prospectively from 3 cancer centers in Ontario, Canada. All underwent germline testing for LS and completed a family history assessment. Tumors were assessed using MMR IHC and MSI. The sensitivity, specificity, and positive and negative predictive values of screening strategies were compared with the gold standard of a germline result. **RESULTS:** Of 215 women, germline data were available for 189 (88%); 13 women (7%) had pathogenic germline variants with 7 women with mutS homolog 6 (*MSH6*); 3 women with mutL homolog 1 (*MLH1*); 2 women with PMS1 homolog 2, mismatch repair system component (*PMS2*); and 1 woman with mutS homolog 2 (*MSH2*). A total of 28 women had MMR-deficient tumors (13%); of these, 11 had pathogenic variants (39%). Sequential IHC (with *MLH1* promoter methylation analysis on MLH1-deficient tumors) followed by MSI for nonmethylated and/or MMR-intact patients was the most sensitive (92.3%; 95% confidence interval, 64%-99.8%) and specific (97.7%; 95% confidence interval, 94.2%-99.4%) approach, missing 1 case of LS. IHC with *MLH1* promoter methylation analysis missed 2 patients of LS. Family history was found to have the lowest sensitivity at 55%. **CONCLUSIONS:** Sequential IHC (with *MLH1* promoter methylation analysis) followed by MSI was found to be most sensitive. However, IHC with *MLH1* promoter methylation analysis also performed well and is likely more cost-effective and efficient in the clinical setting. The pretest probability of LS is high in patients with MMR deficiency and warrants universal screening for LS. ***Cancer* 2020;126:4886-4894**.

KEYWORDS: Lynch syndrome, ovarian cancer, screening, tumor testing.

INTRODUCTION

Lynch syndrome (LS) is an autosomal dominant inherited cancer susceptibility syndrome caused by germline mutations in DNA mismatch repair (MMR) genes, primarily mutL homolog 1 (*MLH1*); mutS homolog 2 (*MSH2*); mutS homolog 6 (*MSH6*); PMS1 homolog 2, mismatch repair system component (*PMS2*); and epithelial cell adhesion molecule (*EPCAM*).[1,2] LS is associated with increased lifetime risks of colorectal cancer (CRC; 40%-80%), endometrial cancer (EC; 33%-61%), and ovarian cancer (OC; 9%-12%).[1-3] In women with LS, gynecologic tumors usually present as the sentinel malignancy with a significant lead time of up to 10 years,[4] thereby creating an opportunity to identify and treat premalignant or early-stage cancers at other sites. In addition, identifying first-degree relatives through cascade testing

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offers an opportunity for cancer prevention through cancer screening and risk reduction strategies.

Historically, LS has been identified through analysis of family histories that met Amsterdam II clinical criteria. However, due to the low sensitivity, multiple bodies now recommend universal tumor testing with immunohistochemistry (IHC) for MMR protein expression or microsatellite instability (MSI) testing at the time of diagnosis of EC and/or CRC.[5-7] Due to defects in MMR genes, LS-associated tumors characteristically demonstrate loss of MMR protein expression on IHC (MMR deficient [MMRd]) or MSI.[8] However, because the majority of patients of MMRd EC and/or CRC are sporadic, with MLH1/PMS2 deficiency from epigenetic *MLH1* promoter methylation,[1,9] patients with MMRd tumors without *MLH1* promoter methylation are referred for further genetic counseling and germline testing for LS. In keeping with these recommendations to identify individuals at risk of developing LS, the province of Ontario, Canada, has implemented IHC followed by *MLH1* promoter methylation analysis of all EC and CRC specimens in patients aged <70 years.[6,10,11] Compared with MSI testing, IHC is less expensive, can direct genetic testing, and is easier to operationalize with superior performance characteristics.[10]

Although universal screening is becoming routine in patients with EC and CRC to identify LS, patients with OC appear to be completely neglected by current recommendations.[6,7] OC is the third most common LS-associated cancer in women, with carrier-specific risks of 5% to 20% in *MLH1*, 10% to 38% in *MSH2*, 1% to 11% in *MSH6*, and an indeterminate risk in *PMS2*.[3] Most commonly, LS-associated OCs are nonserous and nonmucinous, with an enrichment in endometrioid histology (>50%).[12] Retrospective studies have estimated that 2% to 29% of nonserous OCs are MMRd,[13-15] but to our knowledge it is unknown how many of these patients are LS carriers. Furthermore, although there is some evidence that supports the need for histotype-specific screening to identify LS in patients with OC,[16] to our knowledge there is no comparative study of various screening approaches. Therefore, the primary objective of the current study was to compare the performance characteristics of various screening strategies to identify LS in a prospective cohort of nonserous and/or nonmucinous OC. The secondary objective was to establish the incidence of LS in this cohort.

MATERIALS AND METHODS

Participants

Participants were recruited prospectively from 3 Ontario gynecology oncology centers between September 2015 and June 2019. Institutional research ethics board approval and written informed consent were obtained. Eligibility criteria included histologically confirmed nonserous and/or nonmucinous invasive epithelial OC of all histologic grades and International Federation of Gynecology and Obstetrics (FIGO) stages. All participants were asked to complete family history questionnaires (FHQs), underwent testing of their ovarian tumors with IHC and MSI, and provided blood samples for germline mutation testing using a next-generation sequencing (NGS) gene panel of MMR genes. Clinicodemographic information was extracted from the electronic patient records.

Family History

Participants were asked to complete an extended FHQ (eFHQ), which was developed to create a 3-generation pedigree as previously described.[17] Details from the eFHQ then were used to determine whether the patients met the criteria for referral for genetic assessment based on Amsterdam II clinical criteria, Society of Gynecologic Oncology (SGO) 20% to 25%, or Ontario Ministry of Health (OMOH) family history criteria.[18] For the purposes of the current study, OMOH family history criteria (see Supporting Table 1) was chosen as the family history variable of interest to calculate the performance characteristics because it encompasses the Amsterdam II clinical criteria and SGO 20% to 25% criteria and is the current family history criteria used in Ontario for LS genetic testing.[11]

Tumor Testing: IHC and MSI

All OC specimens were reviewed by a pathologist experienced with ovarian pathology and MMR IHC and who was blinded to the germline results. IHC was used to test for expression of MLH1, MSH2, MSH6, and PMS2 proteins on 4-μm paraffin sections of all tumors as described previously (see Supporting Information).[11] The tumors were considered to be MMRd if there was an absence of staining in the tumor cell nuclei compared with adjacent normal tissue.[11] For MSI testing, DNA was extracted from tumor and normal tissue and amplified using polymerase chain reaction as previously described (see Supporting Information).[11] Tumors were considered MSI-high (MSI-H) if ≥2 of 5 markers were unstable, and were considered to be microsatellite stable (MSS) if <2 markers were unstable. Tumors were considered equivocal if <3 loci could be amplified unless ≥2 markers demonstrated stability.[19] For the purpose of sensitivity and specificity calculations, tumors with focal or heterogeneous loss of MMR protein expression

by IHC were considered to be MMRd[20] and any equivocal case on IHC or the MSI test was considered to be MMR intact and/or MSS.

Germline Targeted Panel Sequencing

All participants were offered germline testing using a NGS panel we developed with hybrid capture probes tiling: 1) all exons, introns, and flanking regions of MMR genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*; 2) all exons of *EPCAM*; and 3) the intergenic region between *EPCAM* and *MSH2*.[21] The panel has been validated to recapitulate the results of clinical testing.[21,22] Description of the panel (see Supporting Table 2), laboratory workflow, bioinformatics workflow, and variant interpretation are available in the Supporting Information. Briefly, normal DNA was extracted from either blood buffy coat or adjacent normal tissue that was macrodissected from formalin-fixed, paraffin-embedded tumor slides. After extraction, DNA was sheared and target-enriched genomic libraries were prepared on each sample. Samples were sequenced on an Illumina NextSeq 500 device with the resulting reads aligned to the human reference genome (UCSC Genome Browser hg38). Our bioinformatics pipeline queries the MMR genes for germline single-nucleotide variants, insertions and deletions, copy number alterations, and structural rearrangements (see Supporting Fig. 1). Germline variant filtration and interpretation were performed according to American College of Medical Genetics and Genomics guidelines, blinded to tumor testing results.[23] For all MLH1-deficient patients, *MLH1* promoter methylation analysis was performed using our panel as described in the Supporting Information. For the calculation of performance characteristics, patients with pathogenic or likely pathogenic variants were considered to have LS, whereas those with a variant of unknown significance (VUS) were considered to have a negative germline result.

Screening Strategies

We compared the performance characteristics (sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]) of the following strategies compared with the germline test as the gold standard: 1) IHC only (any case that is IHC deficient is considered as testing positive); 2) IHC with *MLH1* promoter methylation analysis of MLH1-deficient patients (any case that is IHC deficient without *MLH1* promoter hypermethylation is considered as testing positive); 3) MSI only (any case that is MSI-H is considered as testing positive); 4) family history (any case that meets OMOH criteria is considered as testing positive); 5) IHC plus MSI (sequential testing with IHC followed by the MSI test on any IHC-intact patients; any case that is IHC deficient and/or MSI-H is considered as testing positive); and 6) IHC with *MLH1* promoter methylation analysis plus MSI (sequential testing with IHC [with *MLH1* promoter methylation analysis for all *MLH1*-deficient patients] followed by MSI testing on any nonmethylated and/or IHC-intact case; any case that is IHC deficient without *MLH1* promoter hypermethylation and/or MSI-H is considered as testing positive).

Statistical Analysis

Categorical variables were summarized using counts and percentages whereas continuous variables were summarized using medians and ranges. Groups were compared using the Fisher exact test or Wilcoxon rank sum test. The sensitivity, specificity, PPV, and NPV of various screening strategies were calculated using germline testing as the gold standard. Exact binomial confidence intervals were calculated with 95% confidence intervals (95% CIs) for estimation of proportions and the McNemar test was used to compare sensitivities and specificities. All analyses were performed using SAS statistical software (version 9.4), and statistical significance was set at $P = .05$.

RESULTS

Baseline Demographics

In total, 278 consecutive patients were approached for the study, of whom 215 with nonserous and/or nonmucinous OCs provided consent (Fig. 1)[11]: 185 had OC alone (86.1%) and 30 had synchronous OC and EC (13.9%) (Table 1). The median age at the time of diagnosis was 53 years (range, 21-71 years). The most common histology was endometrioid (48.8%), followed by clear cell (40.9%). The majority of patients had stage I disease (66.5%). Women with MMRd and/or MSI-H OCs had more patients of synchronous OC and EC (37.9% vs 10.2%; $P < .001$) and endometrioid histology (65.5% vs 46.2%; $P = .011$) when compared with those with MMR-intact and/or MSS tumors.

Germline results were available for 189 of 215 patients (87.9%) (Table 1). There were 17 patients who declined germline testing and 9 patients without any blood or normal tissue available for testing. Overall, 13 of these 189 patients (6.9%) were found to have a pathogenic and/or likely pathogenic variant in 1 of the MMR

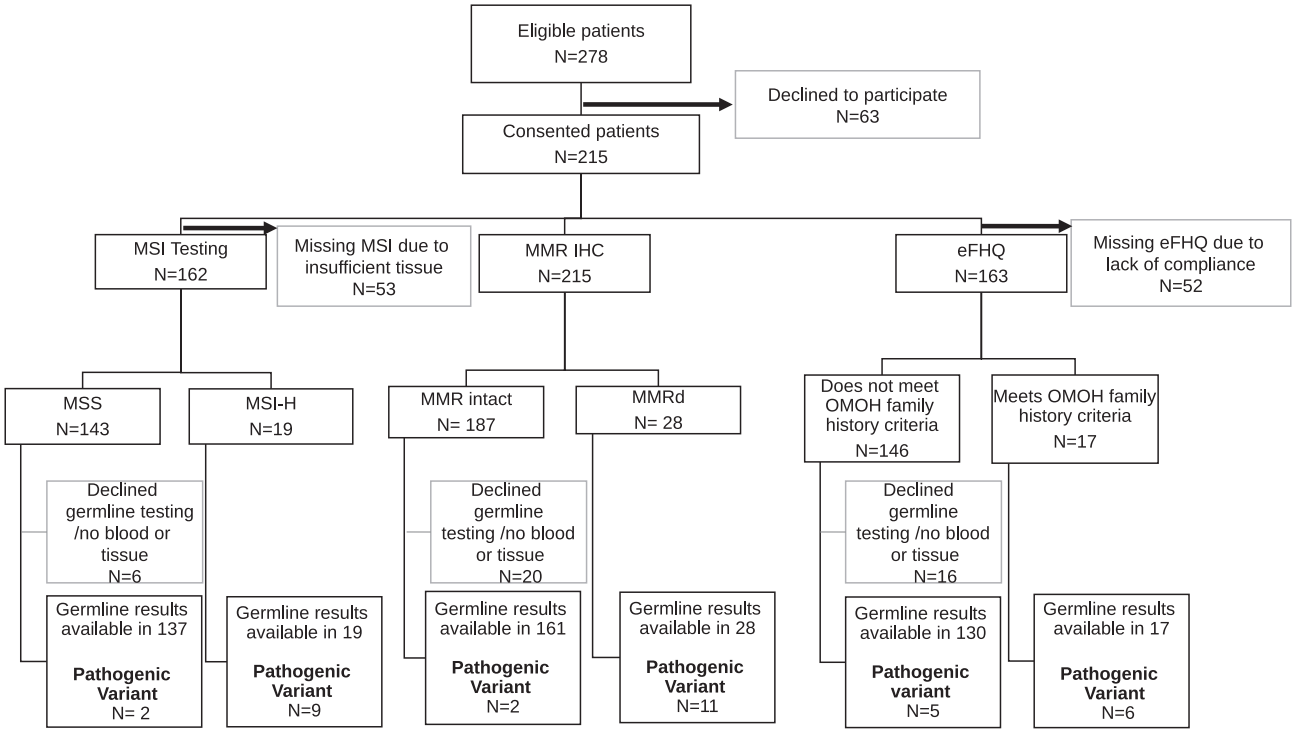

FIGURE 1. Study schema summarizing patient recruitment, screening strategies, and germline pathogenic variant results for Lynch syndrome in women with newly diagnosed nonserous and nonmucinous ovarian cancer. eFHQ indicates extended family history questionnaire; IHC, immunohistochemistry; MMR, mismatch repair; MMRd, mismatch repair deficient; MSI, microsatellite instability; MSI-H, microsatellite instability–high; MSS, microsatellite stable; OMOH, Ontario Ministry of Health.[11]

genes, including 7 with a pathogenic variant in *MSH6* (3.7%), 3 with a pathogenic variant in *MLH1* (1.6%), 2 with a pathogenic variant in *PMS2* (1.1%), and 1 with a pathogenic variant in *MSH2* (0.5%). Thirty-one of the 189 patients (16.4%) had a VUS in an MMR gene (see Supporting Table 3), whereas 145 patients (76.7%) had negative germline results.

Of the 215 patients with IHC results, 28 (13%) were MMRd, with 15 patients deficient in *MLH1/PMS2* (53.6%), 7 patients deficient in *MSH6* (25%), 5 patients deficient in *MSH2/MSH6*(17.9%), and 1 patient deficient in *PMS2* (3.6%) (Table 2). Of these 28 MMRd patients, 11 had pathogenic germline variants (39%) (Fig. 1).[11] Of 215 patients, 162 MSI results were available (75.3%), 19 of which were MSI-H (11.7%); of these 19 MSI-H tumors (of which 18 patients also were found to be MMRd on IHC), 9 had pathogenic germline variants (47%) (Fig. 1).[11] Overall, 29 of the 215 patients demonstrated MMRd and/or MSI-H (13.5%) with concordance of IHC and MSI, with the exception of 1 case that was MSI-H but MMR intact; of these 29 patients, 12 had a pathogenic germline variant (41%). For the assessment of family history, 163 patients had eFHQ results available (75.8%), with 17 having results that met OMOH family history criteria (10.4%), 9 that met SGO 20% to 25% criteria (5.5%), and 2 that met Amsterdam II clinical criteria (1.2%). Of the 17 eFHQ results that met OMOH family history criteria for genetic testing, 6 patients had germline mutations (35%).

MMR Protein-Deficient Patients

Of the 15 patients with tumors that were *MLH1/PMS2* deficient, 2 had *MLH1* pathogenic germline variants (13.3%) (Table 2). Of the 13 patients with either negative or VUS germline results in *MLH1*, 12 had somatic *MLH1* promoter hypermethylation in their tumors and 1 case had biallelic somatic *MLH1* copy number deletion. All 7 patients with *MSH6* deficiency (100%) and 1 of 5 patients (20%) with MSH2/MSH6 deficiency had pathogenic germline variants (Table 2). Of 4 patients with negative germline results in *MSH2*, 1 patient had biallelic single-nucleotide variants in the tumor, whereas the other 3 patients declined tumor sequencing. One patient with PMS2 deficiency in the tumor had a confirmed pathogenic germline variant.

TABLE 1. Clinicopathologic Characteristics of the Study Participants

Characteristics	Total Cohort N = 215 (%)	MMR IHC deficient/MSI-H N = 29 (%)	MMR Intact/MSS N = 186 (%)	*P*
Median age at diagnosis (range), y	53 (21-71)	52 (34-62)	54 (21-71)	.354
Type				
Ovarian	185 (86.1)	18 (62.1)	167 (89.8)	<.001
Synchronous ovarian and endometrial	30 (13.9)	11 (37.9)	19 (10.2)	
Histology				
Endometrioid grade 1	67 (31.2)	9 (31.0)	58 (31.2)	.012
Endometrioid grade 2	29 (13.5)	8 (27.6)	21 (11.3)	
Endometrioid grade 3	8 (3.7)	2 (6.9)	6 (3.2)	
Endometrioid grade unassigned	1 (0.4)	0	1 (0.5)	
Clear cell	88 (40.9)	5 (17.2)	83 (44.6)	
Mixed carcinoma	14 (6.5)	3 (10.3)	11 (5.9)	
Undifferentiated	1 (0.5)	1 (3.4)	0	
Carcinosarcoma	6 (2.8)	1 (3.4)	5 (2.7)	
Nonserous but NOS	1 (0.5)	0	1 (0.5)	
FIGO stage of disease (2009)				
IA	62 (28.8)	6 (20.7)	56 (30.1)	.116
IB	5 (2.3)	0	5 (2.7)	
IC	76 (35.4)	8 (27.6)	68 (36.6)	
II	38 (17.7)	12 (41.4)	26 (14.0)	
IIIA	8 (3.7)	1 (3.4)	7 (3.8)	
IIIB	3 (1.4)	0	3 (1.6)	
IIIC	20 (9.3)	2 (6.9)	18 (9.7)	
IV	3 (1.4)	0	3 (1.6)	
Germline testing (N = 189)				
Pathogenic or likely pathogenic variant	13	12	1	
Variant of uncertain significance	31	3	28	
Negative	145	13	132	

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; IHC; immunohistochemistry; MMR, mismatch repair; MMS, microsatellite stable; MSI-H, microsatellite instability-high; NOS, not otherwise specified.

TABLE 2. Mismatch Repair Protein IHC Results With Germline Status

Overall MMR IHC Deficient	Total Cohort N = 28 (%)
MLH1/PMS2 deficient	15 (53.6)
Germline status	
MLH1 pathogenic variant	2 (7.1)
MLH1 negative	10 (35.7)
MLH1 VUS	3 (10.7)
MSH6 deficient	7 (25)
Germline status	
MSH6 pathogenic variant	7 (25)
MSH6 negative	0
MSH6 VUS	0
MSH2/MSH6 deficient	5 (17.9)
Germline status	
MSH2 pathogenic variant	1 (3.6)
MSH2 negative	4 (14.3)
MSH2 VUS	0
PMS2 deficient	1 (3.6)
Germline status	
PMS2 pathogenic variant	1 (3.6)
PMS2 negative	0
PMS2 VUS	0

Abbreviations: IHC; immunohistochemistry; *MLH1*, mutL homolog 1; MMR, mismatch repair; *MSH2*, mutS homolog 2; *MSH6*, mutS homolog 6; *PMS2*, PMS1 homolog 2, mismatch repair system component; VUS, variant of unknown significance.

Comparison of Screening Strategies

The performance characteristics of the various screening strategies are presented in Table 3. Compared with other strategies, IHC with *MLH1* promoter methylation analysis plus MSI was found to be the most sensitive (92.3%; 95% CI, 64.0%-99.8% [*P* = not significant]) and specific (97.7%; 95% CI, 94.2%-99.4% [*P* < .05]), with a PPV of 75.0% (95% CI, 47.6%-92.7%) and a NPV of 99.4% (95% CI, 96.8%-99.9%), and missing 1 case of LS with a germline *PMS2* pathogenic variant (study ID 8 in Table 4).[24] IHC with *MLH1* promoter methylation analysis also performed well, with a sensitivity of 84.6% (95% CI, 54.6%-98.1%) and a specificity of 97.7% (95% CI, 94.3%-99.4%), and missing 2 patients of LS. One case was study ID 8 and the other case was study ID 11 (Table 4)[24] with a *MLH1* c.306G>T pathogenic variant with an IHC-intact tumor. Family history assessment alone was found to have the lowest sensitivity at 54.5% (95% CI, 23.4%-83.3%). We performed a subgroup analysis (see Supporting Table 4) of performance characteristics excluding study ID 8 because the OC was found to be MMR intact and/or MSS and therefore likely not MMR driven. Excluding this one case, IHC with *MLH1* promoter methylation analysis plus MSI did not miss any case of LS.

Clinicopathologic Characteristics of Patients With LS

There were 13 LS carriers who were significantly younger than those without LS (aged 50 years vs 53

TABLE 3. Performance Characteristics of Screening Strategies and Family History for Identifying Mismatch Repair Germline Pathogenic Variants (Lynch Syndrome) in Women with Newly Diagnosed Nonserous and/or Nonmucinous Ovarian Cancer

Screening Strategy	Total No.	Sensitivity (95% CI), %	Specificity (95% CI), %	PPV (95% CI), %	NPV (95% CI), %	No. of LS Patients Missed (95% CI)
IHC	189	84.6 (54.6-98.1)	90.3 (85.0-94.3)	39.3 (21.5-59.4)	98.7 (95.6-99.8)	2 (0-6)
IHC with *MLH1* promoter methylation analysis[a]	189	84.6 (54.6-98.1)	97.7 (94.3-99.4)	73.3 (44.9-92.2)	98.9 (95.9-99.9)	2 (0-6)
MSI	156	81.8 (48.2-97.7)	93.1 (87.7-96.6)	47.4 (24.5-71.1)	98.5 (94.8-99.8)	2 (0-6)
OMOH family history	147	54.5 (23.4-83.3)	90.9 (85.6-94.7)	35.3 (14.2-61.7)	96.2 (91.3-98.7)	5 (2-8)
IHC + MSI[b]	188	92.3 (64.0-99.8)	90.9 (85.6-94.7)	42.9 (24.5-62.8)	99.4 (96.6-99.9)	1 (0-7)
IHC with *MLH1* promoter methylation analysis + MSI[c]	188	92.3 (64.0-99.8)	97.7 (94.2-99.4)	75.0 (47.6-92.7)	99.4 (96.8-99.9)	1 (0-7)

Abbreviations: 95% CI, 95% confidence interval; IHC, immunohistochemistry; LS, Lynch syndrome; *MLH1*, mutL homolog 1; MSI, microsatellite instability; NPV, negative predictive value; OMOH, Ontario Ministry of Health; PPV, positive predictive value.

[a] Any case that was IHC deficient without *MLH1* promoter hypermethylation was considered as testing positive.

[b] Refers to sequential testing with IHC followed by MSI testing for all IHC-intact patients; any case that was IHC deficient and/or MSI-high would be considered as a positive test.

[c] Refers to sequential testing with IHC with reflexive *MLH1* promoter methylation analysis for all *MLH1*-deficient patients followed by MSI testing on any IHC-intact case. Any case that was IHC deficient without *MLH1* promoter hypermethylation and/or MSI-high was considered as testing positive.

years; $P = .042$) (Table 4).[24] The subgroup of women with MMRd tumors without LS had a median age of 54 years (range, 43-62 years). The rate of LS was 17% in women with synchronous OC and EC (30 women). Women with LS were more likely to have a higher stage of disease (62% vs 31%; $P = .042$). After excluding patients with synchronous OC and EC, women with LS still demonstrated a trend toward higher stage disease (≥stage III: 40% vs 16%; $P = .196$). Eleven women (84.6%) had a gynecologic malignancy as their sentinel cancers. Four women (30.8%) with LS did not meet Amsterdam II clinical criteria, SGO 20% to 25% criteria, or OMOH family history criteria for genetic assessment and were identified only through IHC or MSI testing.

DISCUSSION

The results of the current study established the incidence of LS as 7% in a prospective cohort of women with nonserous and/or nonmucinous OC. This rate is much higher than what has been reported in the literature (range, 3%-4%), although previous studies were limited by small numbers and their retrospective nature, as well as a lack of central pathology review.[16] The rate of MMRd or MSI-H in the current study was 13% and 11.7%, respectively, and given a tumor demonstrating MMRd or MSI-H, the pretest probability of LS was 41%, which is higher than what is reported for EC and CRC.[25,26] Prospective IHC studies have shown that approximately 25% of unselected ECs are MMRd, with 20% of MMRd patients testing positive for LS.[25] Similarly, 15% to 20% of CRCs are MMRd, with 10% to 15% of all MMRd/MSI-H patients representing LS.[26] Given the high pretest probability of LS in this cohort of patients with nonserous and/or nonmucinous OC, reflex tumor testing for MMR defects should be done routinely as standard of care. We advocate for screening with IHC with *MLH1* promoter methylation analysis because this approach was found to be highly sensitive and specific and is likely to be easiest to implement in the clinical setting.

Various strategies to identify LS have been considered for EC and CRC, with no testing strategy proven to be perfect.[7,27] For example, the current screening algorithm in patients with EC using IHC and *MLH1* promoter methylation analysis leads to a complex cascade of additional tests for the confirmation of LS, and is time-consuming for patients and clinicians.[6,7] Comprehensive NGS approaches may be simpler and more efficient, and are becoming the standard of care in other cancer types such as high-grade serous OC for *BRCA1/2* mutations.[28] Likewise, in patients with CRC, a recent study has established that upfront tumor sequencing with an NGS panel can replace the current sequential tests for LS[29]; based on this study, the National Comprehensive Cancer Network guidelines now list upfront tumor sequencing as a possible testing strategy in patients with CRC.[29] In a similar manner, upfront tumor sequencing should be explored further in patients with EC and nonserous and/or nonmucinous OC because this approach will simplify the current cascade of tumor testing and provide tumor-specific information to open doors to new therapeutics such as pembrolizumab for patients with recurrent MMRd cancers after chemotherapy.[30]

With comprehensive tumor NGS panels on the horizon, the costs of the various screening approaches

TABLE 4. Clinical Characteristics of Women with Mismatch Repair Germline Pathogenic Variants (Lynch Syndrome) and Newly Diagnosed Nonserous and/or Nonmucinous Ovarian Cancer

Study ID	Age, Years	Germline Pathogenic Variant	MMR IHC	MSI Testing	Reason for Referral to Genetic Services	Met FH Criteria	History of LS Cancer	Histology	FIGO Stage
1	38	*MSH6* c.3939_3957dup p.(Ala1320Serfs*5)	MSH6 deficient	MSS	IHC	None	None	Mixed ovary	IC
2	56.8	*PMS2* c.(803+1_804-1)_(903+1_904-1)del	PMS2 deficient	MSI-H	IHC/MSI	None	None	Endometrioid grade 3	IIIC
3	52.3	*MSH6* c.1304T>C p.(Leu435Pro)	OC: MSH6 deficient EC: MSH6 deficient	OC: MSI-H EC: MSS	FH and IHC	SGO 20%-25% OMOH	None	OC: Clear cell EC: Endometrioid grade 3	OC: IIB EC: IA
4	49.6	*MSH6* c.766_767delAG p.(Ser256*)	OC: MSH6 deficient EC: MSH6 deficient	OC: MSI-H EC: MSS	FH and IHC	SGO 20%-25% OMOH	None	OC: Undifferentiated EC: Endometrioid grade 2	OC: IA EC: II
5	48.2	*MSH2* c.508C>T p.(Gln170*)	MSH2/MSH6 deficient	MSI-H	IHC/MSI	Missing	None	Endometrioid grade 3	IIB
6	52.3	*MSH6* c.4001+1G>C	MSH6 deficient	MSI-H	IHC/MSI	None	None	Endometrioid grade 2	IIB
7	44.1	*MLH1* c.1731G>A p.Ser577Ser	OC: MLH1/PMS2 deficient EC: MLH1/PMS2 deficient	OC: MSI-H EC: MSI-H	FH and IHC/MSI	AMSII SGO 20%-25% OMOH	Ampullary cancer	OC: Endometrioid grade 1 EC: Endometrioid grade 2	OC: IA EC: IIIC1
8	56.3	*PMS2* c.137G>T p.Ser46Ile	OC: intact EC: intact	OC: MSS EC: MSS	Based on GC discretion	None	None	OC: Endometrioid grade 1 EC: Carcinosarcoma	OC: IC1 EC: IB
9	35	*MLH1* c.546-?_677+?del p.Tyr183_Arg226del	MLH1/PMS2 deficient	Missing	FH and IHC	SGO 20%-25% OMOH	None	Clear cell	IIIA
10	33.6	*MSH6* c.1612dupT p.Tyr538Leufs*4	MSH6 deficient	MSI-H	FH and IHC/MSI	SGO 20%-25% OMOH	None	Endometrioid grade 2	IIA
11	44.4	*MLH1* c.306G>T r.?	Intact	MSI-H	MSI	Missing	Colon	Endometrioid grade 1	IIA
12	52.0	*MSH6* c.3083C>A p.Ser1028*	MSH6 deficient	Missing	IHC	None	None	Endometrioid grade 2	IIB
13	56.3	*MSH6* c.3416dup p.Lys1140Glnfs*24	OC: Equivocal EC: MSH6 deficient	OC: MSI-H EC: MSI-H	FH and IHC/MSI	AMSII SGO 20%-25% OMOH	None	OC: Endometrioid grade 1 EC: Endometrioid grade 1	OC: IC3 EC: II

Abbreviations: AMSII, Amsterdam II clinical criteria; EC, endometrial cancer; FH, family history; FIGO, International Federation of Gynecology and Obstetrics; GC; genetic counsellor; IHC, immunohistochemistry; LS, Lynch syndrome; *MLH1*, mutL homolog 1; MMR, mismatch repair; *MSH2*, mutS homolog 2; *MSH6*, mutS homolog 6; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSS, microsatellite stable; OC, ovarian cancer; OMOH, Ontario Ministry of Health; *PMS2*, PMS1 homolog 2, mismatch repair system component; SGO, Society of Gynecologic Oncology.
Variant nomenclature follows Human Genome Variation Society (HGVS) format.[24]

need to be taken into consideration. A recent microcosting study in patients with EC compared combinations of MSI, IHC, *MLH1* methylation analysis, and NGS and found that initial tumor triage with IHC was the least expensive approach.[31] In the CRC literature, it is well accepted that IHC is approximately 3-fold less expensive than MSI,[32] with the added benefit of IHC being easier to operationalize, with superior sensitivity and the ability to direct germline testing for the affected MMR gene.[10,11] In the current study, although the sequential approach of IHC (with reflex *MLH1* promoter methylation analysis) plus MSI was found to have the best sensitivity, it detected only one additional case of LS when compared with IHC (with reflex *MLH1* promoter methylation analysis). In the long term, tumor triage with IHC is likely to be the most cost-effective approach and will be easier to implement. Until the cost of tumor NGS panels decrease, tumor triage with IHC will need to be the standard of care in the majority of institutions.

Although small in number, the 13 patients with confirmed LS in the current study were similar in age to what has been reported in the literature (mean age, 47.6 years vs 45.3 years).[4] A previous review examined the clinical characteristics of 747 women with LS-associated OC; the most frequent mutation identified was *MSH2* (47%) and the most frequent histology was endometrioid and/or clear cell subtype.[4] Unlike that review, the most common germline mutation in the cohort in the current study was found in *MSH6*, with all MSH6-deficient patients found to have a pathogenic variant. Previous studies have shown that the majority of patients with LS-associated OCs present at an early stage (>80% at stage I/II) with excellent survival outcomes, although the LS cohort in the current study had higher stages of disease with poor prognostic features.[4,33]

One of the limitations of the current study was the incomplete information regarding MSI due to the lack of normal adjacent tissue on the formalin-fixed, paraffin-embedded slides. In the clinical setting, MSI testing would occur reflexively at the time of review of the hysterectomy specimen; therefore, pathologists would have access to normal tissue to proceed with MSI testing. Another limitation was the incomplete germline information; given that all 26 patients with missing germline information had MSS-intact and/or MMR-intact tumors, our estimate of the incidence of LS in the current study cohort may be slightly higher than the true incidence. Furthermore, given that the study institutions were specialized cancer centers, there may have been inherent referral bias. However, all gynecologic cancer care in Ontario is regionalized,[34] and the current study is reflective of the real-world scenario. Furthermore, for the purpose of the current analysis, patients found to have focal MMRd on IHC (4 patients) were considered to be MMRd, and 1 case with an equivocal MMR result was considered to be MMR intact. There is evidence that patients with focal MMR deficiency and/or heterogenous loss of MMR protein expression harbor unique molecular aberrations, and these patients should be tested further for germline mutations.[20]

The results of the current study demonstrated that sequential IHC (with reflexive *MLH1* promoter methylation analysis) plus MSI is the most sensitive and specific screening strategy with which to identify LS in women with nonserous and/or nonmucinous OC. Considering the cost of real-world implementation, IHC with reflexive *MLH1* promoter methylation analysis can be a suitable strategy with excellent performance characteristics. Strong consideration should be given to making reflex tumor testing the standard of care for all patients who are newly diagnosed with nonserous and/or nonmucinous OC, with all MMRd and/or nonmethylated patients undergoing confirmatory testing for LS.

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AUTHOR CONTRIBUTIONS

Soyoun Rachel Kim: Data curation, formal analysis, project administration, visualization, writing–original draft, and writing–review and editing. **Alicia Tone:** Conceptualization, data curation, investigation, methodology, and writing–review and editing. **Raymond H. Kim:** Investigation and writing–review and editing. **Matthew Cesari:** Investigation and writing–review and editing. **Blaise A. Clarke:** Investigation and writing–review and editing. **Lua Eiriksson:** Investigation, resources, and writing–review and editing. **Tae Hart:** Resources and writing–review and editing. **Melyssa Aronson:** Resources and writing–review and editing. **Spring Holter:** Resources and writing–review and editing. **Alice Lytwyn:** Investigation and writing–review and editing. **Manjula Maganti:** Formal analysis and writing–review and editing. **Leslie Oldfield:** Formal analysis and writing–review and editing. **Steven Gallinger:** Conceptualization and writing–review and editing. **Marcus Q. Bernardini:** Conceptualization and writing–review and editing. **Amit M. Oza:** Conceptualization and writing–review and editing. **Bojana Djordjevic:** Writing–review and editing. **Jordan Lerner-Ellis:** Investigation and writing–review and editing. **Emily Van de Laar:** Data curation, project administration, and writing–review and editing. **Danielle Vicus:** Investigation, resources, and writing–review and editing. **Trevor J. Pugh:** Formal analysis, investigation, and writing–review and editing. **Aaron Pollett:** Investigation and writing–review and editing. **Sarah E. Ferguson:** Conceptualization, formal analysis, funding acquisition, project administration, investigation, methodology, supervision, and writing–review and editing.

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