Research Article

Cancer Prevention Research

Evaluation of Clinical Criteria for the Identification of Lynch Syndrome among Unselected Patients with Endometrial Cancer

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Abstract

Clinical criteria, primarily young age of cancer onset and family history of signature cancers, have been developed to identify individuals at elevated risk for Lynch syndrome with the goals of early identification and cancer prevention. In 2007, the Society of Gynecologic Oncology (SGO)–codified criteria for women presenting with gynecologic cancers. These criteria have not been validated in a population-based setting. For 412 unselected endometrial cancers, immunohistochemical expression of DNA mismatch repair proteins and *MLH1* methylation were assessed to classify tumors as sporadic or probable Lynch syndrome (PLS). In this cohort, 10.5% of patients were designated as PLS based on tumor testing. The sensitivity and specificity of the SGO criteria to identify these same cases were 32.6% [95% confidence interval (CI), 19.2–48.5] and 77% (95% CI, 72.7–81.8), respectively. With the exception of tumor location in the lower uterine segment, multivariate analysis of clinical features, family history, and pathologic variables failed to identify significant differences between the sporadic and PLS groups. A simplified cost-effectiveness analysis demonstrated that the SGO clinical criteria and universal tissue testing strategies had comparable costs per patient with PLS identified. In conclusion, the SGO criteria successfully identify PLS cases among women with endometrial cancer who are young or have significant family history of signature tumors. However, a larger proportion of patients with PLS who are older and have less significant family history are not detected by this screening strategy. Universal tissue testing may be necessary to capture more individuals at risk for having Lynch syndrome. *Cancer Prev Res; 7(7); 686–97. ©2014 AACR.*

Introduction

Lynch syndrome is an inherited cancer syndrome due to a germline mutation in a DNA mismatch repair (MMR) gene, primarily *MLH1*, *MSH2*, *MSH6*, or *PMS2*. Lynch syndrome is thought to account for 1% to 3% of all endometrial cancers (1). For women with Lynch syndrome, a gynecologic cancer is the sentinel cancer diagnosis in 50% of instances (2). The lifetime risks of developing endometrial cancer and colorectal cancer for these women are 39.4% and 42.7%, respectively, and the lifetime risk of developing either one of these carcinomas is 73.4% (3). After sentinel cancer diagnosis, individuals with Lynch syndrome have a cumulative risk of developing a secondary Lynch syndrome–associated tumor of 1.5% to 3% per year (4). A diagnosis of Lynch syndrome provides the patient with the opportunity to undergo surveillance for these cancers, facilitating their prevention and/or early detection, and allows for the opportunity for cancer prevention in first-degree relatives (FDR).

Computer-based clinical prediction models, $PREMM_{1,2,6}$; MMRPredict; and MMRPro, have emerged to help identify an individual's risk for having a Lynch syndrome mutation (5–7). These models were validated in the colorectal cancer population for the detection of *MLH1*, *MSH2*, and *MSH6* mutations with favorable results. When applied to the endometrial cancer patient population, however, these models fail to perform at a level that would support their use as a clinical screening tool (8).

In 2007, the Society of Gynecologic Oncology (SGO) published a statement with clinical criteria for which patients with gynecologic cancer would benefit from further evaluation for Lynch syndrome. On the basis of a constellation of criteria dominated by features such as young age of cancer diagnosis and family history of Lynch syndrome–associated tumors, SGO established two groups of patients—those with a 5% to 10% probability of having

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a germline mutation in a DNA MMR gene and those with a 20% to 25% probability. The expert panel stated that genetic risk assessment (genetic counseling with genetic testing if appropriate) for individuals with a 5% to 10% likelihood of having a germline mutation is reasonable and asserted that individuals with a 20% to 25% possibility of a germline mutation should undergo risk assessment (9).

Ryan and colleagues investigated the utility of these criteria in a cohort of 76 patients with endometrial cancer with a known germline mutation and found that the SGO 5% to 10% criteria performed the best by correctly identifying 93% of known mutation carriers, whereas the SGO 20% to 25% criteria identified 71%. It should be noted that these patients had a mean age of 47.3 years and were identified through databases from the British Columbia Familial Cancer Registry and Mount Sinai Hospital Familial Gastrointestinal Cancer Registry. In addition, 68 of 76 of these patients had *MLH1* or *MSH2* mutations; only 8 of 76 had an *MSH6* mutation, and there were no *PMS2* mutation carriers (10). The SGO criteria have not yet been validated in a population-based setting.

In addition to clinical screening criteria, molecular diagnostic techniques such as the PCR-based microsatellite instability (MSI) analysis and immunohistochemistry evaluating expression of the DNA MMR proteins can be used to screen for Lynch syndrome–associated cancers. For tumors with immunohistochemical loss of MLH1, the PCR-based *MLH1* promoter methylation assay assists in delineating sporadic (methylated) from suspicious for Lynch syndrome (unmethylated) tumors. The overall sensitivity of MSI and immunohistochemistry for identifying Lynch syndrome germline mutations in colorectal cancers is similar, with rates of 83% and 94%, respectively (11). Current recommendations in the colorectal cancer literature are to perform tissue testing on all newly diagnosed patients with colorectal cancer tumors regardless of personal or family history (12).

The "gold standard" for diagnosing Lynch syndrome is to detect a germline mutation in one of the DNA MMR genes. Sequencing has excellent sensitivity for detecting point mutations and minor insertions and deletions, but large deletions/insertions or gene rearrangements pose limitations of conventional sequencing techniques (13). It is unclear whether individuals with tissue testing results suggestive of Lynch syndrome with negative germline testing are truly negative or whether their mutations have genetic features that make accurate identification of the germline aberration by conventional techniques more difficult.

In many published studies, the data about Lynch syndrome–associated endometrial cancer are derived from hereditary colorectal cancer registries. These patient populations are biased toward young age at cancer diagnosis and having larger families with many family members affected by Lynch syndrome–associated cancers. The purpose of this study was to evaluate the performance of the SGO 5% to 10% criteria (summarized in Supplementary Table S1) in identifying endometrial cancers with tissue testing results consistent with a diagnosis of Lynch syndrome in a large cohort of unselected patients with endometrial cancer.

Materials and Methods

Patient population and study design

After obtaining the Institutional Review Board approval, cases of endometrial cancer involving women who underwent hysterectomy at the MD Anderson Cancer Center through 2011 were identified. Beginning with the most recent cases, endometrial cancers were included if the patient was 18 years of age or greater and sufficient tissue from the surgery was available for molecular analysis. Endometrioid and nonendometrioid histologies of endometrial carcinoma were included. Relevant clinical data were extracted from physician and genetic counselor notes and patient intake forms, all available in the electronic medical record. All hysterectomies were pathologically reviewed by a gynecologic pathologist (R.R. Broaddus). Pathologic data were derived from the pathology report for each hysterectomy specimen. Surgical stage was derived from the pathology report and the surgeon operative note. These data are summarized in Table 1.

The endometrial cancer cohort from this study is derived from a large NCI-designated cancer center. Comparison with published data from a national epidemiologic analysis of 161,513 patients with endometrial cancer shows that the MD Anderson cohort is comparable in terms of age at diagnosis, proportion of endometrioid versus nonendometrioid histologies, and stage distribution. The only notable difference between the two patient populations is that MD Anderson has a higher proportion of women with grade 2 and 3 endometrioid carcinomas (14).

For this work, patients with sporadic and probable Lynch syndrome (PLS) were defined on the basis of the results of tissue testing studies. Thus, a patient with endometrial cancer with intact immunohistochemical expression of MLH1, MSH2, MSH6, and PMS2 would be considered as having a sporadic endometrial carcinoma. Similarly, a patient with immunohistochemical loss of MLH1, but presence of *MLH1* methylation, was also considered sporadic. Patients with tumors with immunohistochemical loss of MSH2, MSH6, and PMS2 were considered as PLS. Those with tumors with immunohistochemical loss of MLH1 and absence of *MLH1* methylation were also considered PLS. When reviewing medical records of these patients, a family history of a specific cancer was defined as having a first- or second-degree relative with a diagnosis of that specific cancer.

Molecular analyses

All tissue-based analyses were performed using formalin-fixed, paraffin-embedded endometrial carcinoma sections derived from the hysterectomies in Clinical Laboratory Improvement Amendments—and College of American Pathology—approved clinical pathology laboratories. Immunohistochemistry was performed using standard techniques for MLH1 (G168-15, 1:25; BD Biosciences Pharmingen), MSH2 (FE11, 1:100; Calbiochem), MSH6 (44,

Table 1. Clinicopathologic features of sporadic and PLS-associated endometrial cancers

Clinical features	Sporadic EC *N* (%)	PLS EC *N* (%)	*P*
Median age at diagnosis (y) range	61 (18–92)	61 (42–87)	
Age, y			
<50	52 (14.2)	10 (23.3)	0.12
≥50	313 (85.8)	33 (76.7)	
BMI (kg/m^2)			
<30	121 (33.2)	17 (39.5)	0.41
≥30	243 (66.8)	26 (60.5)	
History of diabetes			
Y	89 (24.4)	9 (20.9)	0.62
N	276 (75.6)	34 (79.1)	
History of hypertension			
Y	201 (55.1)	18 (41.9)	0.10
N	164 (44.9)	25 (58.1)	
Family history of EC[a]			
Y	35 (9.8)	4 (9.8)	>0.99
N	323 (90.2)	37 (90.2)	
Family history of CRC[a]			
Y	58 (16.2)	8 (19.0)	0.63
N	301 (83.8)	34 (81.0)	
Family history of CRC or EC			
Y	87 (24.2)	12 (28.6)	0.54
N	272 (75.8)	30 (71.4)	
Pathologic features	**Sporadic EC *N* (%)**	**PLS EC *N* (%)**	***P***
Histology			
Endometrioid	299 (81.9)	37 (86.0)	0.67
Nonendometrioid	66 (18.1)	6 (14.0)	
FIGO Stage			
I and II	289 (79.2)	37 (86.0)	0.42
III and IV	76 (20.8)	6 (14.0)	
FIGO Grade			
1 and 2	267 (73.2)	32 (74.4)	0.86
3	98 (26.8)	11 (25.6)	
Depth of myometrial invasion			
<50%	257 (70.4)	32 (74.4)	0.58
≥50%	108 (29.6)	11 (25.6)	
Tumor location			
Corpus	357 (97.8)	38 (88.4)	**0.007**
Lower uterine segment	8 (2.2)	5 (11.6)	
Largest gross tumor dimension (cm)			
Mean ± SD	4.3 ± 3.2	3.6 ± 2.9	0.13
Median (range)[b]	4 (0–25)	3.5 (0–13)	

Abbreviations: CRC, colorectal cancer; EC, endometrial cancer; FIGO, International Federation of Gynecology and Obstetrics.

[a]Family history of EC or CRC; patient has a first- or second-degree relative with a diagnosis of endometrial cancer or colorectal cancer.

[b]Some tumors were not apparent grossly, but were identified by microscopic examination of the entire endometrium. These tumors were designated with a largest gross tumor dimension of 0.

1:300; BD Biosciences Pharmingen), and PMS2 (Alb-4, 1:125; BD Biosciences Pharmingen; ref. 15). MLH1, MSH2, MSH6, and PMS2 immunohistochemistry was scored as protein intact or deficient using light microscopic examination. Complete absence of MMR protein expression was required in order for a case to be designated as MMR

Table 2. Technical and professional costs associated with tumor testing, genetic counseling, and germline testing of endometrial carcinomas

	Medicare reimbursement amounts	Cost with SGO screening $N = 97$	Cost for universal screening $N = 412$
Initial genetic counseling consultation (1 h)[a]	$192.67	$18,688.99	$8,862.82
Follow-up genetic counseling visits (30 min)[a]	$105.20	$11,151.20	$4,628.80
IHC for MLH1, MLH2, MSH6, and PMS2[b]	$422.08	$40,941.76	$173,896.96
MLH1 promoter methylation assay for tumor with IHC loss of MHL1[c]	$90.25	$1,173.25	$8122.50
Single gene germline testing[d]	$1,300	$19,500	$57,200
Single-site testing[e,f]	$475	N/A	N/A

Abbreviation: IHC, immunohistochemistry.

[a]Medicare does not reimburse for genetic counseling. Costs shown are estimates for genetic counseling before genetic testing.

[b]CPT code 88342 used for each individual IHC DNA MMR protein.

[c]CPT stack codes 83900, 83909, 83912 used in 2012 (no longer effective 01/2013).

[d]Cost of germline testing was obtained from Myriad ABN worksheet.

[e]Cost of single-site testing was obtained from Myriad ABN worksheet.

[f]Single-site testing intended for FDRs of patients with endometrial cancer who were identified as having Lynch syndrome based upon positive germline test results.

deficient. Stromal cells served as an internal positive control. Tumors with immunohistochemical loss of MSH2, MSH6, or PMS2 were designated as PLS. For tumors with loss of MLH1 protein expression, PCR-based *MLH1* promoter methylation analysis was performed. DNA was isolated from mapped formalin-fixed, paraffin-embedded tissue sections that were dissected with a scalpel blade to provide relatively pure tumor samples for analysis. Isolated DNA was treated with bisulfite to convert unmethylated cytosine nucleotides to uracil using the Zymo EZ DNA Methylation-Gold Kit according to the manufacturer's instructions (Zymo Research). Methylation of *MLH1* was assessed using a modified version of methylation-specific PCR followed by capillary electrophoresis using fluorescein amidite (FAM)-labeled reverse primer and -unlabeled forward primers (Integrated DNA Technology). The primer sequences were the following: methylated forward, 5′-GAT AGC GAT TTT TAA CGC-3′, unmethylated forward, 5′-AGA GTG GAT AGT GAT TTT TAA TGT-3′, and labeled reverse primer, 5′- FAM-TCT ATA AAT TAC TAA ATC TCT TC-3′. The forward primers were designed to distinguish the methylated amplicon from the unmethylated by difference in size. The bisulfite-treated DNA was then amplified by PCR using primers specific for methylated and unmethylated DNA. The methylated PCR product of 85 bp was separated from unmethylated PCR product of 91 bp by capillary electrophoresis using an ABI Prism 3130 Genetic Analyzer. Chromatograms for tumor were compared with those generated for the RKO colon carcinoma cell line (positive control known to have loss of MLH1 protein due to *MLH1* promoter methylation) and the leukemia cell line K562 (negative control with no *MLH1* methylation). Tumors with MLH1 immunohistochemical loss and presence of *MLH1* methylation were designated as sporadic, whereas tumors with MLH1 loss and absence of *MLH1* methylation were designated as PLS (16).

Cost analysis

A simplified cost-effectiveness analysis was performed to compare the direct costs of using the SGO 5% to 10% clinical criteria with universal tissue testing (immunohistochemistry for all and *MLH1* methylation analysis when indicated) for the 412 cases included in this study. The analysis was conducted from a third-party payer perspective. Two approaches were used to assess cost-effectiveness. The first method assessed the direct costs associated with identifying patients with PLS among women diagnosed with endometrial cancer for the SGO 5% to 10% clinical criteria and universal tissue testing. The second method evaluated direct costs associated with identifying cases of PLS among women with endometrial cancer as well as their potentially affected FDRs. Because actual germline mutation results were unknown and tissue testing results do not always correlate with identification of a germline mutation, the percentage of tumors identified as PLS that would have corresponding germline mutations was varied between 25% to 75% to represent the range of germline mutations found in previously published studies (17, 18). Costs included were hospital and professional costs associated with identifying PLS cases. Costs were estimated for initial genetic counseling and follow-up visits, immunohistochemistry for MLH1, MSH2, MSH6, and PMS2, *MLH1* promoter methylation assay for tumors with loss of MLH1, and single gene germline mutation testing. Cost estimates were based on using Current Procedural Terminology (CPT) codes and Medicare reimbursement fees, which were

obtained from the Physician Fee Schedule and Laboratory Fee Schedule (Table 2; ref. 19). All cost amounts were calculated in 2012 U.S. dollars. The following clinical assumptions were made for the cost-effectiveness analysis: (i) 25% to 75% of patients with endometrial cancer with immunohistochemical loss of expression of an MMR protein would have a germline mutation detected; (ii) all women with PLS identified by tissue testing would undergo genetic counseling and recommended germline testing; (iii) all FDRs would undergo recommended genetic counseling and germline testing; (iv) for the purposes of costs associated with testing FDRs, it was assumed that 50% of individuals with immunohistochemical loss of a DNA MMR protein would have an identifiable germline mutation.

Statistical analysis

Clinical and pathologic criteria were compared across a variety of groups. STATA v 12 was used to perform statistical analyses. The Fisher, χ^2, Mann–Whitney, or *t* test was conducted to test association across groups depending on the distribution of the data. Clinical and pathologic criteria were compared between the sporadic and PLS groups. Classification and Regression Tree (CART) analysis was performed to identify a set of variables that would possibly predict Lynch syndrome in the absence of tissue testing. Sensitivity and specificity were calculated along with their 95% binomial exact confidence intervals (CI) for the SGO 5% to 10% clinical criteria in its ability to predict PLS tumors.

Results

Comparison of sporadic and PLS endometrial cancers

Of note, 412 patients with endometrial carcinoma met inclusion criteria, and their tumors were subjected to tissue testing. Immunohistochemistry was uninterpretable in one case (lack of positive staining in the internal positive control), and the *MLH1* methylation testing failed in three cases because of insufficient DNA amplification. Of the 411 cases with complete immunohistochemistry results, 293 showed intact nuclear staining for all four DNA MMR proteins, and 118 had loss of at least one mismatch protein (90 MLH1 + PMS2 loss; 12 MSH2 + MSH6 loss; 9 MSH6 loss; 7 PMS2 loss). Of those with loss of MLH1 + PMS2, 72 of 90 (80%) had associated methylation of *MLH1* and were, thus, considered sporadic (Fig. 1). The total number of PLS endometrial carcinomas in this series was 43 (10.5%). These tissue testing data were used to stratify the patients with endometrial cancer into either the sporadic category or the PLS category in Table 1.

Demographic and pathologic information for the 408 endometrial cancer cases with complete tissue testing results are summarized in Table 1. The median age at diagnosis in this cohort was 60.5 years with a range of 18 to 92. The majority of women were older than age 50, obese [body mass index (BMI) $\geq$ 30 kg/m^2], and had unremarkable family histories for endometrial or colorectal cancer. The median largest tumor dimension was 4.3 cm. Most tumors were endometrioid histology, grade 1 or 2, early stage, and located in the uterine corpus.

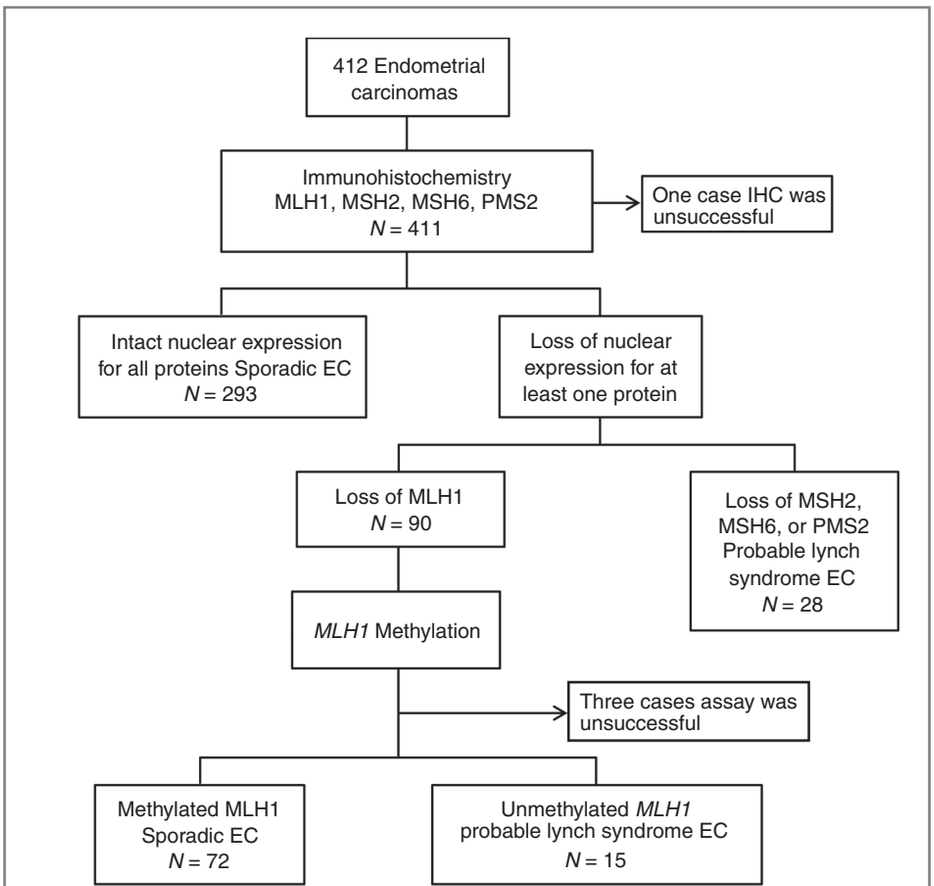
A comparison between patients with sporadic endometrial cancer and PLS-associated endometrial cancer was made using a variety of clinical and pathologic criteria (Table 1). With the exception of tumors arising from the lower uterine segment, there were no pathologic or clinical variables that could distinguish the two groups. Endometrial carcinomas arising in the lower uterine segment were previously identified to be associated with Lynch syndrome. However, lower uterine segment localization was found to occur in only 3.5% of cases in a large study of 1,009 patients (20). Because of its rarity, lower uterine segment localization cannot solely be used to clinically distinguish sporadic endometrial carcinoma from Lynch syndrome–associated endometrial carcinoma.

Evaluation of the SGO 5% to 10% screening criteria

The SGO 5% to 10% screening criteria were next applied to the patients with endometrial cancer who had both informative tissue testing results and evaluable family history information (Fig. 1). Using clinical history exclusively irrespective of tumor-testing results, 96 patients meet the SGO 5% to 10% criteria. Fourteen of these patients had tissue testing results that would be consistent with Lynch syndrome, resulting in 82 women that would potentially undergo unnecessary genetic counseling evaluation and genetic testing. Of these 14 patients, 11 of 14 were diagnosed with endometrial cancer at age less than 50 years and 6 of 11 fulfilled the SGO criteria by age of diagnosis only (no family history of Lynch syndrome–associated cancer). Only 7 of 14 women had family histories that fulfilled the SGO criteria. The specificity of the SGO 5% to 10% criteria was quite favorable (77.3%; 95% CI, 72.7–81.8). Sensitivity, however, was poor (32.6%; 95% CI, 19.2–48.5).

From the data above, we concluded that the established SGO 5% to 10% screening criteria were not optimal for detecting patients with PLS in an unselected endometrial cancer patient population. To determine whether the SGO criteria could be adjusted to be more effective, the age cutoff in these guidelines was incrementally adjusted and a receiver operator characteristic curve (ROC) generated. An area under the ROC curve of 1 indicates perfect predictive ability, and an area under the curve of 0.5 indicates no predictive ability. In this analysis, the area under the curve was 0.5 (data not shown). From the clinical and pathologic data summarized in Table 1, a CART analysis was performed to determine whether alternative screening criteria to detect Lynch syndrome could be generated, but this analysis yielded no superior alternative criteria (data not shown).

The SGO 5% to 10% criteria were then evaluated in the specific PLS groups according to the type of MMR protein loss (Table 3). Approximately 40% of the patients with MLH1+PMS2 loss (and absence of *MLH1* methylation) or MSH2+MSH6 loss fulfilled the SGO 5% to 10% screening criteria. Substantially, fewer patients with tumors with MSH6 loss (22.2%) or PMS2 loss (14.3%) fulfilled these same clinical screening criteria. Lower BMI, which has previously been associated with Lynch syndrome–associated endometrial cancers (21–23), was more common in the



Performance characteristics of SGO 5% to 10% screening criteria

	Probable lynch syndrome EC	Sporadic EC	**Totals**
Fulfills SGO criteria	14	82	96
Does not meet SGO criteria	29	280	309
Totals	43	362	405[a]

[a]Four patients for whom tissue testing did not work are excluded. Three patients who were adopted, so no family history is available, are also excluded. From 412 patients initially studied, 405 are evaluable for sensitivity and specificity calculations.

Sensitivity, 32.6%; 95% CI, (19.2–48.5)

Specificity, 77.3%; 95% CI, (72.7–81.8)

Figure 1. Summary of immunohistochemistry and *MLH1* methylation results with associated sensitivity and specificity of Society of Gynecologic Oncology clinical screening criteria for Lynch syndrome.

MSH2+MSH6 and MSH6 groups, but this was not significantly different from the other MMR loss groups (Table 3). Increasing prevalence of obesity in the United States may be obscuring a previously significant clinical differentiator between patients with sporadic and Lynch syndrome–associated endometrial carcinomas. Of the 43 patients with PLS, only 5 had lower uterine segment tumors, with 3 of 5 of these in the PMS2 loss group (Table 3). Only 9.8% of the patients with PLS had a family history of endometrial cancer, and only 19.0% of these women had a family history of colorectal cancer. Family history statistics were not significantly different among the MMR protein loss groups (Table 3). From Table 3, it was concluded that the SGO 5% to 10% criteria are most predictive for patients with endometrial carcinomas with MLH1+PMS2 loss or MSH2+MSH6 loss, but more than half of the women in these MMR groups would not be accurately identified on the basis of clinical criteria alone.

Table 3. Comparison of select historical risk factors for Lynch syndrome among endometrial cancers by type of immunohistochemical protein loss

	MLH1/PMS2 No *MLH1* methylation	MSH2/MSH6	MSH6	PMS2	
	***N* = 15**	***N* = 12**	***N* = 9**	***N* = 7**	***P***
Median age at diagnosis (y; range)	62 (43–79)	56 (42–71)	62 (50–76)	56 (45–87)	0.50 (—)
BMI <30 kg/m^2	4 (26.7%)	5 (41.6%)	6 (66.6%)	2 (28.6%)	0.29
Family history EC[a]	3 (21.4%)	0	1 (12.5%)	0	0.31
Family history CRC[b]	3 (20%)	3 (25%)	1 (12.5%)	1 (14.3%)	0.95
LUS[c] Tumor	1 (6.7%)	1 (8.3%)	0	3 (42.9%)	0.05
Endometrioid histology	13 (86.7%)	9 (75%)	8 (88.9%)	7 (100%)	0.62
Stage I or II	13 (86.7%)	8 (66.7%)	9 (100%)	7 (100%)	0.14
Meets SGO 5%–10% criteria	6 (40.0%)	5 (41.7%)	2 (22.2%)	1 (14.3%)	0.62

[a]EC, patient has a first- or second-degree relative with a diagnosis of endometrial cancer.
[b]CRC, patient has a first- or second-degree relative with a diagnosis of colorectal cancer.
[c]LUS, Tumor arising from lower uterine segment.

Cost analysis

The SGO criteria do not demonstrate a high enough sensitivity to warrant widespread use in the general population of women diagnosed with endometrial cancer. Although universal tissue testing identifies a larger number of patients with PLS endometrial cancer, its costs may be prohibitive. To examine the costs and outcomes associated with identifying PLS cases for each screening strategy, a simplified cost-effectiveness analysis was performed using the entire cohort of 412 unselected endometrial cancer cases.

The SGO 5% to 10% criteria were applied to the cohort, identifying 97 women who would undergo further evaluation through tissue testing and genetic counseling, resulting in a total cost of $91,455 (Table 4). Of the 97 cases identified by the SGO model, only 15 were found by tissue testing to be PLS. The total cost per PLS case detected in the SGO model was $6,097. It is known that positive tissue testing results are not always associated with the identification of a deleterious germline mutation in an MMR gene. To account for the range of germline mutations published in the literature, the proportion of positive tissue tests associated with germline mutations was estimated at 25%, 50%, and 75% in tumors with immunohistochemical loss of a DNA MMR protein resulting in 4, 8, and 11 identifiable germline mutations, respectively. To account for the number of potentially affected FDRs, data from the electronic medical records were used to calculate an average of 5.3 FDRs for patients who met the SGO criteria. On the basis of this number and the range of estimated germline mutation rates among patients with PLS endometrial cancer, 21, 42, and 48 FDRs would be eligible for single-site gene mutation analysis and enhanced Lynch syndrome screening. The estimated cost for screening both the patients with PLS and their FDRs in this strategy is $3,006 to $6,329 per PLS case identified on the basis of germline detection rates of 25% to 75% (Table 4).

The universal tumor-testing model identified 43 patients with endometrial cancer who warranted further work-up through genetic counseling and germline testing. The total cost of this screening strategy was $252,711, with a cost per PLS case identified of $5,877. Identifiable germline mutation detection rates of 25%, 50%, and 75% based on the positive tumor test resulted in 11, 22, and 32 patients who would have a positive germline mutation, respectively (Table 4). The average number of FDRs for those included in the universal tumor-testing model was 5.5. On the basis of this and the range of estimated germline mutation rates among patients with PLS endometrial cancer, 60, 121, and 176 FDRs would be eligible for single-site gene mutation analysis and enhanced Lynch syndrome screening. The estimated cost for screening both patients with PLS and their FDRs in this strategy ranges between $2,970 and $6,455 per case (Table 4). To summarize, although universal tumor testing with immunohistochemistry and *MLH1* methylation analysis when the tumor has loss of MLH1 protein involves greater total dollar costs, it also identifies a greater number of patients with Lynch syndrome and ultimately costs less per identified patient compared with the SGO clinical screening strategy.

Discussion

A major conclusion from this work is that the existing SGO 5% to 10% clinical criteria for identifying patients with endometrial cancer with Lynch syndrome misses most patients who could be captured by universal tissue testing (immunohistochemistry for DNA MMR proteins and PCR-based *MLH1* methylation analysis for tumors with loss of MLH1). The predominance of *MLH1* and *MSH2* mutation carriers within Lynch syndrome registries, including the two registries in which mutation carriers from the Ryan and colleagues study were derived, results in validation of existing criteria for these individuals with *MLH1* and *MSH2* germline mutations (10). The relative paucity of *MSH6* and

Table 4. Comparison of direct Medicare costs associated with the SGO criteria and universal tumor-testing models

Screening strategy	SGO	Universal
Patients with endometrial cancer ($N = 412$)		
who undergo IHC testing	97	412
who have loss of expression of IHC	21	118
who undergo *MLH1* methylation testing	13	90
seen by genetic counselor	97	46
PLS identified by strategy	15	43
PLS with the positive germline test (detection rates of 25%, 50%, and 75% germline detection[a])	4, 8, 11	11, 22, 32
Estimated costs for screening strategies		
Cost to screen 412 patients	$91,455	$252,711
Average cost per PLS case detected	$6,097	$5,877
FDRs		
FDRs eligible for germline testing if 25%, 50%, or 75% of patients have an identifiable germline mutation	21, 42, 58	60, 121, 176
Assuming 50% of PLS cases have an identifiable germline mutation: No. of FDRs who will be germline positive for LS if 25%, 50% or 75% inherit the same mutation[b]	11, 21, 32	30, 61, 91
Estimated costs for screening, including both PLS and FDRs (assuming 50% of patients with PLS have a germline deleterious mutation)		
Cost per LS case identified if 25% of FDRs have a positive germline mutation	$6,329	$6,455
Cost per LS case identified if 50% of FDRs have a positive germline mutation	$4,146	$4,044
Cost per LS case identified if 75% of FDRs have a positive germline mutation	$3,006	$2,970

Abbreviation: IHC, immunohistochemistry; LS, Lynch syndrome.

[a]Estimate of 25% to 75% germline mutation detection rate based upon published data from Hampel et al. and Senter et al. (17, 18).

[b]For SGO and Universal screening methods, we made the assumption that 50% of the PLS group identified in each screening method would have a germline mutation identified. This number was then multiplied by the mean number of FDRs per cohort (SGO, 5.3; Universal, 5.5) and then multiplied by 25%, 50%, and 75% to estimate the number of potentially affected FDRs.

PMS2 mutation carriers in Lynch syndrome registries suggests that these mutations are rare or that they are underestimated and missed by current screening strategies that rely on young age of cancer onset and family history of signature cancers. Consistent with the idea that these mutations are missed using existing clinical screening strategies, in this study 16 of 43 (37.2%) women had endometrial cancers with MSH6 or PMS2 protein loss. Senter and colleagues investigated 99 individuals with immunohistochemical loss of PMS2 in Lynch syndrome–associated carcinomas (91 colorectal, 5 endometrial, 1 gastric, 1 small bowel, and 1 transitional cell of renal pelvis; ref. 18). They found that 62% of patients with immunohistochemical loss of PMS2 in the tumor had a detectable *PMS2* germline mutation; 25.5% of these patients did not meet any published Lynch syndrome clinical screening criteria. Hendriks and colleagues investigated individuals with *MSH6* germline mutations who met Amsterdam II Criteria and found that *MSH6* mutations carriers typically do not meet standard clinical criteria for identifying patients with Lynch syndrome (24). Additional studies have reported similar findings for *MSH6* mutation carriers (25, 26).

A strong family history of certain cancers played a pivotal role in the initial identification and characterization of Lynch syndrome and continues to be a principal component in widely accepted clinical screening algorithms. In 2009, the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) working group deemphasized the role of family history when evaluating patients with colorectal cancer for risk of Lynch syndrome and recommended a universal tumor-testing approach. This recommendation was due in part to the poor overall sensitivity and specificity profiles of clinic-based screening criteria such as Amsterdam II or Revised Bethesda guidelines as well as suboptimal patient history recording by clinicians (27). Consistent with the EGAPP recommendations, there were no statistically significant differences in family history of endometrial cancer or colorectal cancer between PLS and sporadic cases in this study. Furthermore, the majority of all patients in this cohort did not have a family history of either tumor. Average family size in the 21st century is not the same as it was when Lynch syndrome was first characterized in the early 1900s. In Dr. Warthin original Family G, there was a male proband with 10 children (28). In the cohort examined for this study,

the average number of siblings among the patients with endometrial cancer was 3.4, and the average number of children was only 2.1. It is not that family history lacks relevance in detecting hereditary cancers; rather, our data support the idea that as family sizes decrease, the probability of detecting a family history of cancers also decreases. The clinical utility of family history in identifying an individual patient's risk for hereditary cancer syndromes may be decreasing in the current generations.

The median age of sentinel cancer diagnosis for *MSH6* and *PMS2* mutations carriers has been shown to be older than that of *MLH1* or *MSH2* mutation carriers in several studies (18, 25, 29). The underlying cause for an older median age at diagnosis for *MSH6* or *PMS2* mutations has not been elucidated. Chen and colleagues examined single-nucleotide polymorphisms (SNP) of genes playing key roles in the cell cycle in a population of individuals with identifiable germline Lynch syndrome mutations identified through a colorectal cancer registry (30). CART analysis showed that certain SNPs were associated with either earlier median onset of colorectal cancer diagnosis or a later age of onset. It is possible that genetic variants may also play a role in endometrial cancers in individuals harboring *MSH6* and *PMS2* mutations that might explain the older median age at diagnosis in this subgroup.

Several studies have shown that the prevalence of Lynch syndrome is increased in women diagnosed with endometrial cancer at age younger than 50 years. Lu and colleagues found that 11% (11/100) of women presenting with endometrial cancer at age younger than 50 years had tumor-testing results suggestive of Lynch syndrome, and 9% had an identifiable germline mutation (21). In a similar study performed by Walsh and colleagues, 18% (26/146) of women with endometrial cancer diagnosed at less than age 50 years had tissue testing results (immunohistochemistry, MSI, and *MLH1* methylation) consistent with Lynch syndrome (31). Their study also found an increased rate of PLS endometrial cancer tumors of 16.1% in the population with the age under 50. In this study, the majority of PLS endometrial cancer cases occur at ages greater than 50, and there were no statistically significant different clinical or pathologic variables between those diagnosed before or after age 50.

The endometrial cancer patient population examined for this study had comparable demographics with those previously compiled for the general U.S. population (14). Although the percentage of PLS detected by tissue testing in this study (10.5%) may seem high, it is actually comparable with that of two other studies that also examined unselected patients with endometrial cancer (32, 33). A comparison of the three studies is provided in Table 5. All three studies used immunohistochemistry for the four DNA MMR proteins. The Ohio State group did not perform *MLH1* methylation analysis, so the percentage of sporadic with *MLH1* methylation was estimated on the basis of the data of the two other studies. Despite examining three different endometrial cancer populations, the percentage of PLS based on tissue testing is remarkably similar among the three groups. Note that the percentage of PLS based on tissue testing from Table 5 is higher than the 1% to 3% that is typically attributed to Lynch syndrome–associated colorectal and endometrial cancers (1). The 1% to 3% value represents germline mutations. It is well known that current sequencing strategies do not detect germline mutations for all patients with tumor tissue testing studies suggestive of Lynch syndrome. Rodriguez-Soler and colleagues evaluated 1,705 patients with consecutive colorectal cancer for Lynch syndrome by performing MSI and immunohistochemistry (34). They found that the familial incidence of colorectal cancer was greatest in germline mutation carriers, next highest in patients with positive tumor testing positive but no germline mutation, and lowest in individuals with a sporadic colorectal cancer. Risk of endometrial cancer and other Lynch syndrome–associated tumors was not included

Table 5. Comparison of MD Anderson results to similarly designed, population-based endometrial cancer national and international studies

	MDACC[a]	Ohio State[b]	Netherlands[c]
	***N* = 408**	***N* = 140**	***N* = 179**
% of IHC Loss	28.9	21.4	23.5
% of MLH1 Loss	22.0	17.1	17.9
% of MLH1 methylated	82.7	Not performed	96.9
% of PLS	10.5	6.7–10.1[d]	6 (3–11)

Abbreviation: IHC, immunohistochemistry.

[a]MDACC, MD Anderson Cancer Center (4 of the initial 412 patients studied excluded because immunohistochemistry and/or *MLH1* methylation did not work).

[b]Backes et al. investigation of 140 endometrial carcinomas with immunohistochemistry for expression of DNR MMR proteins (32).

[c]Leenen et al. investigation of 179 endometrial carcinomas with immunohistochemistry and *MLH1* methylation analysis in all patients diagnosed at less than age 50 (33).

[d]Ohio State calculation of the percentage of PLS is based on an approximate 82.7% to 96.9% range of *MLH1* methylation rate of tumors with immunohistochemical loss of MLH1 found in the MDACC and the Netherlands studies.

in their study. More investigation is needed to determine the optimal approach to managing patients with positive tumor-testing studies, but no identifiable germline mutation. This is especially important because published guidelines support universal tumor testing of all colorectal cancers, and the incidence of tumor test–positive/germline mutation–negative will increase as these standards become more widely adopted.

There are few published cost analyses that evaluate screening methodologies for Lynch syndrome in women presenting with endometrial cancer. An advantage of the cost analysis used in this study is that calculations were derived from actual patients with endometrial cancer rather than simulated patients. Kwon and colleagues evaluated several different screening strategies using a simulation model to evaluate the costs relative to benefits for multiple screening strategies. They found that triaging all women with endometrial cancer with immunohistochemistry who had an FDR diagnosed with a Lynch syndrome–associated cancer occurring at any age was the most cost-effective method (35). If this screening strategy was applied to the existing population-based cohort of this study, 60 (14.6%) individuals would undergo immunohistochemical analysis, with 9 of these having tumor testing suggestive of Lynch syndrome. This leaves 34 individuals with tumor testing consistent with Lynch syndrome that would go undiagnosed. Although determined cost effective by Kwon analysis, one must determine whether the cost savings are worth the potential health-related implications of missing the diagnosis in 34 individuals and the impact this might have on both the patient and her FDRs. Dinh and colleagues evaluated screening strategies for colorectal and endometrial cancer using a simulation modeled after the U.S. population starting at age 20. They found that risk assessment of all individuals between the ages of 25 to 35 with $PREMM_{1,2,6}$ followed by genetic testing for those with a risk score of $\geq$5% was the most cost-effective strategy (36). In contrast, Mercado and colleagues found that the $PREMM_{1,2,6}$ (93% sensitivity; 5% specificity), MMRPro (57% sensitivity; 85% specificity), MMRPredict proximal (71% sensitivity; 64% specificity), and MMRPredict distal (57% sensitivity; 85% specificity) algorithms do not translate well to the endometrial cancer population (8). These prediction models were attempting to predict the presence of an MMR gene mutation, rather than the presence of tissue testing abnormalities suggestive of Lynch syndrome. It is interesting to note that each of these prediction models, although initially developed to identify Lynch syndrome–associated colorectal cancer, has a better sensitivity than SGO 5% to 10% (32.6% in the present work). Specificity is comparable among all models, except for the very low specificity of $PREMM_{1,2,6}$. These comparative data are summarized in Supplementary Table S2.

In this study, the cost per patient with PLS identified using a universal tumor-testing strategy consisting of immunohistochemistry (and *MLH1* methylation analysis when indicated) is comparable with the cost when the SGO 5% to 10% criteria are used. Creating an ideal cost analysis strategy is difficult. As can be seen by both these data and the work from others described above, there can be immense shift in costs as different assumptions and costs are added or removed from the models. For example, MSI analysis was excluded from the current tissue testing strategy in part because of previous studies documenting substantial subsets of endometrial cancers from patients who have known Lynch syndrome being microsatellite stable or MSI-low (37–39). Exclusion of MSI analysis, however, can result in potentially missing some MSI-high tumors that have intact positive immunohistochemical expression of MMR proteins (15). A two antibody immunohistochemistry panel consisting of MSH6 and PMS2 has been proposed as a means to cut costs associated with tissue testing (40).

The cost-effectiveness model presented here assumes a 100% genetic counseling referral rate for patients with endometrial cancer meeting the SGO criteria, when published rates for such referrals vary from 17% to 48% (41, 42). The model also assumes that all patients either meeting the SGO criteria or with tumor testing suggestive of Lynch syndrome will accept referral for genetic counseling and/or germline testing. Compliance of patients with endometrial cancer with genetic counseling referrals to evaluate for Lynch syndrome may not be 100%. Backes and colleagues surveyed the 47 of 384 patients with endometrial cancer who met institutional criteria for genetic counseling referral through a mailed questionnaire and follow-up telephone call (43). A total of 26 of 47 (55.3%) responded to the questionnaire, and 20 of 26 (77%) stated that they had been referred to see a genetic counselor. Despite referral, only 9 of 20 (45%) actually did see a genetic counselor, and 8 of 9 underwent germline testing. The two most common reasons for not seeing a genetic counselor were lack of insurance/cost and anxiety related to the results.

In summary, this is the first large, single-institution evaluation of the SGO 5% to 10% clinical criteria in their utility for identifying unselected patients with endometrial cancer with Lynch syndrome. Our data show that the SGO criteria identify only a small subset of patients with PLS in the population-based setting. Universal tumor testing of endometrial carcinomas (immunohistochemistry and *MLH1* methylation) is a cost-effective alternative that detects more individuals at elevated risk, providing more opportunity for cancer prevention among these women and their families.

Disclosure of Potential Conflicts of Interest

R.R. Broaddus has honoraria from speakers' bureau from Myriad Genetics sponsored seminar for genetics counselors. No potential conflicts of interest were disclosed by the other authors.

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