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Lynch Syndrome Screening Should Be Considered for All Patients With Newly Diagnosed Endometrial Cancer

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Abstract

Lynch syndrome (LS) is an autosomal dominant inherited disorder caused by germline mutations in DNA mismatch repair (MMR) genes. Mutation carriers are at substantially increased risk of developing cancers of the colorectum and endometrium, among others. Given recent recommendations for universal, cost-effective screening of all patients with newly diagnosed colorectal cancer using MMR protein immunohistochemistry, we evaluated MMR protein expression in a series of endometrial cancers in the general population. A total of 605 consecutive cases of primary endometrial cancer at a single institution (1997 to 2013) were evaluated regardless of age, family history, or histologic features. Evaluation methods consisted of immunohistochemistry for the MMR proteins MLH1, MSH2, MSH6, and PMS2, followed by DNA methylation analysis for cases with MLH1/PMS2 deficiency. Germline mutation testing was performed on a subset of cases. Forty MMR-deficient, nonmethylated endometrial cancers were identified: 3 MLH1/PMS2 and 37 MSH6/MSH2 protein deficiencies. Only 25% occurred in women below 50 years of age (range, 39 to 88 y), 1 of which was in a risk-reducing hysterectomy specimen. Only 15% of patients had a prior history of carcinoma, including only 2 patients with prior colorectal carcinoma. Most (80%) of the endometrial cancers were purely endometrioid; there were 2 mixed endometrioid/mucinous, 1 mucinous, 1 serous, 2 clear cell, and 2 carcinosarcoma cases. When grading was applicable, 40% of the endometrial malignancies were FIGO grade 1, 34% grade 2, and 26% grade 3. Thirteen percent arose in the lower uterine segment, and 23% had tumor-infiltrating lymphocytes. Of the tumors with known germline testing, 41% with a LS-associated germline mutation were not associated with any of the traditional indicators that have been recommended for LS screening (ie, age 50 y or younger,

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personal/family cancer pedigree that meets Bethesda guideline criteria, presence of MMR-associated tumor morphology, or location in the lower uterine segment). These data suggest that a significant number of LS-associated endometrial carcinomas are missed using clinical, histologic, and locational screening parameters and provide support for universal screening of all newly diagnosed endometrial cancers.

Keywords

Lynch syndrome (LS), or hereditary nonpolyposis colorectal cancer syndrome, is caused by germline defects in DNA mismatch repair (MMR) genes and is characterized by a predilection for oncogenesis at a variety of sites including the colon, rectum, small bowel, endometrium, ovary, stomach, pancreas, renal pelvis and ureter, and brain.¹⁻³ Although first identified in patients with familial lower gastrointestinal cancers, the incidence of gynecologic cancers equals or exceeds that of colorectal cancers in LS patients. Endometrial cancer is particularly common in these patients and represents the harbinger or index malignancy in over half of patients.⁴ Germline defects in DNA MMR have been reported in 1.8% to 2.1% of unselected endometrial carcinoma patients^{5,6} and in up to 9% of endometrial carcinoma patients under age 50.^{7,8}

The strong association between endometrial cancer and LS has led to increased screening of endometrial carcinoma specimens for abnormalities in the DNA MMR system.^{7,9-12} LS screening can use a variety of techniques to directly and indirectly identify mutations in MMR genes. The most conclusive method is germline mutation analysis, although germline testing misses a subset of LS. Germline testing is focused on the 4 primary MMR genes implicated in LS: *MSH2*, *MSH6*, *MLH1*, and *PMS2*. In a small subset of patients, mutations in the gene *EPCAM* can also lead to an LS phenotype by causing hypermethylation and inactivation of the *MSH2* promoter.^{13,14}

Immunohistochemical (IHC) staining to identify the loss of *MSH2*, *MSH6*, *MLH1*, and *PMS2* protein expression serves as a more cost-effective screening approach and has been shown to be sensitive and, in the absence of sporadic *MLH1* promoter methylation, specific for underlying germline defects.¹⁵⁻¹⁹ Microsatellite instability testing also serves as a surrogate for abnormalities in the MMR system, but this test has been shown to be less sensitive than IHC, in large part due to failure to detect many *MSH6* germline mutation carriers.¹⁹ In addition, microsatellite instability fails to identify the putative causative gene/protein deficiency. Importantly, loss of *MLH1/PMS2* expression and high microsatellite instability are not necessarily due to germline mutations, as they can occur in tumors with sporadic methylation of the *MLH1* promoter. Approximately 10% to 20% of endometrial carcinomas show loss of *MLH1/PMS2* expression, but this loss is attributable to germline mutations in only a small subset.^{20,21} IHC, microsatellite instability, and germline mutation analysis can be used in varying combinations to screen for and solidify a diagnosis of LS.

Regardless of the testing approach utilized, it is incumbent upon gynecologic oncologists and pathologists to appropriately screen endometrial cancer patients for defects in the DNA MMR system. At present, screening is predominantly based on patient age (below 50 y),

family history, and/or patient history of prior or concurrent malignancies²²⁻²⁴; however, it is clear that clinical screening criteria have imperfect efficacy in identifying MMR-deficient cases.²⁵ Family history-based screening is inadequate in part because there are inconsistencies in patient and clinician reporting of family cancer history.^{26,27} Some screening recommendations give importance to histologic features such as tumor-infiltrating lymphocytes and high-grade histology^{28,29} or anatomic location,³⁰ although these have not been standardized. A similar approach was initially used for colon cancer patients; however, subsequent work has shown that limiting screening on the basis of these criteria misses a substantial number of LS patients.^{16,31} For instance, restricting MMR testing to colorectal carcinoma patients under age 50 fails to identify 56% of LS patients.³¹ On the basis of these findings, many institutions, including our own, now routinely test all colorectal carcinomas for loss of MMR protein expression on IHC irrespective of patient age, patient history, and tumor histology. It has been well established that endometrial cancer often precedes colorectal and other LS-associated malignancies in women, and it follows that age-based screening is also likely to be inappropriate in this patient population. *MSH6* mutations, in particular, have been implicated in endometrial cancers arising in LS patients above 50 years of age^{21,32} and are known to be missed using current clinical screening criteria.³³ Buchanan et al³⁴ recently demonstrated that restricting MMR testing to women under 50 years of age misses a significant proportion of MMR mutation-positive cases and proposed expanding testing recommendations to endometrial cancers arising in women below 60 years of age. Here, we evaluate a large number of unselected endometrial carcinomas for loss of DNA MMR protein expression and, on the basis of the results herein, propose universal screening of endometrial carcinomas for LS.

MATERIALS AND METHODS

The internal files of the Stanford University Department of Pathology were searched for endometrial cancers diagnosed between 1997 and 2013. A total of 605 cases of primary endometrial cancer were identified and were evaluated for LS regardless of age, family history, or histologic features (IRB-11663). All cases were assessed using IHC for the MMR proteins MLH1, MSH2, MSH6, and PMS2. Primary monoclonal antibodies against MLH1 (clone G168-728; BD PharMingen, San Diego, CA; 1:200), MSH2 (clone FE11; Oncogene Research Products, Cambridge, MA; 1:100), MSH6 (clone 44; BD Transduction, San Jose, CA; 1:200), and PMS2 (clone MRQ-28; Cell Marque, Rocklin, CA; 1:10) were applied to 4-mm-thick formalin-fixed, paraffin-embedded sections. Antigen retrieval was performed using Leica's proprietary antigen retrieval solution at pH 6.0 (MLH1, PMS2, and MSH2) or pH 9.0 (MSH6). Normal expression was defined as nuclear staining within tumor cells, using nuclei of infiltrating lymphocytes, and/or normal stromal cells as positive internal control. Negative protein expression was defined as complete absence of nuclear staining within tumor cells with concurrent positive labeling of the internal control.

Mutational analysis for *BRAF* V600E was performed on an early, initial subset of 30 cases but discontinued due to absence of detectable *BRAF* mutation in the tumors tested. For these cases, DNA was extracted from paraffinembedded tissue at the Stanford Molecular Laboratory. In brief, sections cut from paraffin blocks underwent paraffin removal using Histoclear (National Diagnostics, Atlanta, GA). This was followed by ethanol washing,

complete drying, and submersion in Protein K buffer solution. The tissue specimens were incubated overnight, and digestion was then stopped by immersion in boiling water. Agarose gel electrophoresis was performed to assess the DNA quality and concentration. Real-time polymerase chain reaction (PCR) (Methylight) for DNA methylation was performed on all cases with *MLH1/PMS2* deficiency in the retrospective cohort. The prospective cohort was assessed for promoter hypermethylation by Mayo Medical Laboratories (Rochester, MN) and (for a subset of cases) GoPath Laboratories (Chicago, IL).

Cases with loss of MMR protein expression by IHC and no evidence of methylation by PCR were reviewed for histologic features including histologic type, grade, and the presence of tumor-infiltrating lymphocytes. Tumor-infiltrating lymphocytes were considered increased when they equaled or exceeded 40/10 high-powered fields. Tumor location was assigned on the basis of the gross description recorded in the pathology report as well as histologic assessment for lower uterine segment. Germline data and history of prior or subsequent carcinoma were obtained, when available. Cases with *MLH1* promoter methylation were reviewed as part of a separate study.

Microsatellite instability testing, when performed, was conducted by Stanford Clinical Laboratories using a commercially available primer set (Promega MSI Analysis System; Promega BioSciences, San Luis Obispo, CA) that includes 5 mononucleotide (BAT25, BAT26, NR21, NR24, and MONO27) and 2 pentanucleotide (Penta C and Penta D) repeats on both tumor and normal tissue for each patient. A semiquantitative expression plot for tumor tissue was obtained and compared with that for normal tissue for each patient in accordance with the NCI guidelines.³⁵

RESULTS

Forty MMR-deficient, nonmethylated endometrial cancers were identified in women with an age range of 39 to 88 years. The vast majority (37/40) of the cases showed loss of *MSH6* and/or *MSH2* (Fig. 1). Isolated loss of *MSH6* was seen in 12 (Fig. 2). Two of the remaining cases had dual loss of *MLH1/PMS2* without evidence of DNA methylation on real-time PCR analysis, whereas 1 showed isolated loss of *PMS2*. In 2 cases, *MSH6* expression was equivocal. Loss of *MLH1/PMS2* was initially identified in 97 cases, which showed methylation of the *MLH1* gene promoter; although some of the patients with these tumors may have LS not detected by the current protocol, these cancers were considered to be sporadic for the purposes of this study and were excluded from further analysis. Only 25% (10/40) of MMR protein-deficient endometrial cancers occurred in women under 50 years of age; one of these cases was identified in a risk-reducing hysterectomy specimen from a woman with a prior diagnosis of LS. A history of prior malignancy was identified in a minority of patients (15%, 6/40): 2 patients had a history of colorectal carcinoma, 1 had a history of transitional cell carcinoma of the renal pelvis/ureters, 2 had a history of ductal carcinoma of the breast (1: in situ, 1: invasive), and 1 had a history of cervical squamous cell carcinoma. One patient had a simultaneous ovarian carcinoma. One patient developed lobular carcinoma of the breast within 1 month of diagnosis, whereas another developed sebaceous skin carcinoma within 2 months of diagnosis. Colorectal adenomas, including 1 with intramucosal carcinoma, were identified in 1 patient who was enrolled in a cancer

surveillance program following diagnosis of LS-associated endometrial cancer (Fig. 3). Lower uterine segment tumors accounted for only 5 cases (13%). High-risk histologies comprised a small proportion of tumors, with only 1 serous carcinoma, 2 clear cell carcinomas, and 2 carcinosarcomas identified. The majority (80%, 32/40) of tumors showed pure conventional endometrioid histology. Mucinous histology was seen in 3 cases; 2 mucinous tumors had admixed endometrioid components, whereas 1 showed pure mucinous morphology. Histologic grading of endometrioid and mucinous tumors showed a similar distribution to MMR-intact carcinomas and consisted of 40% (14/35) FIGO grade 1, 34% (12/35) grade 2, and 26% (9/35) grade 3 cases. Tumor-infiltrating lymphocytes were appreciated in 23% (9/40) of cases. In the tumors studied for microsatellite instability, 85% were microsatellite instability high, whereas 15% were stable.

Germline testing confirmed LS in 17/21 patients. Most had germline mutations in *MSH2* (n = 12), followed by *MSH6* (n = 3) and *MLH1* (n = 2). One tumor with loss of *MLH1/PMS2* was associated with an *MLH1* germline mutation variant of uncertain significance, whereas another tumor with loss of *MSH6* was associated with an *MSH6* germline mutation variant of uncertain significance. No germline mutation was identified in association with 2 tumors with loss of *MSH2/MSH6* expression and 1 tumor with loss of *PMS2*. One of the tumors with equivocal *MSH6* protein expression was not associated with a known germline mutation in *MSH6*. Of the 17 tumors with known germline mutations, 7 (41%) were not associated with any of the traditional screening parameters that have been recommended for LS (ie, age 50 y or younger, personal/family cancer pedigree that meets Bethesda guideline criteria, presence of MMR-associated tumor morphology, or location in the lower uterine segment).

DISCUSSION

Our findings demonstrate that a directed screening approach informed by Bethesda guidelines and recently suggested histopathologic parameters misses a substantial proportion of patients with MMR protein-deficient endometrial cancer and many potential LS patients. Although clinical confirmation and/or germline testing was not performed on all patients in this study, prior work has shown that loss of MMR protein expression is a reasonably reliable proxy for identifying potential LS patients. In addition (and based predominantly on colorectal cancer studies), although *MLH1* promoter hypermethylation may rarely occur in LS, the presence of *MLH1* hypermethylation in tumors with loss of *MLH1* and *PMS2* expression can be used as a surrogate marker for sporadic carcinoma.³⁶

A proposal to extend LS testing to all patients has obvious monetary implications. One approach is to initially screen using microsatellite instability testing. However, using microsatellite instability as the first line of screening has several disadvantages. First, it cannot identify which genes are likely to be mutated, therefore it must either be followed by IHC for the protein products of *MLH1*, *PMS2*, *MSH2*, and *MSH6* to direct gene sequencing, or by direct germline sequencing of all 4 commonly implicated genes, a costly option. Alternatively, methylation testing may be performed as a follow-up to microsatellite instability; however, if methylation is negative it still remains unclear which gene is involved, and IHC and/or germline sequencing remains necessary. Microsatellite instability

testing is also poor at identifying possible *MSH6* germline mutation carriers, which account for a higher proportion of LS endometrial cancer patients when compared with colorectal cancer patients.²¹

A more effective approach that ameliorates cost and streamlines the screening approach is to begin with IHC staining for MMR proteins as the initial screen, followed by directed germline mutation testing in cases with MMR protein loss or strong clinical suspicion despite apparently intact protein expression (Fig. 4). It has been demonstrated that IHC is a highly sensitive technique to identify mutations in MMR genes in colorectal cancer, therefore one would predict that an IHC-based screening approach would not miss significant numbers of LS patients.³⁷ Cost can be further reduced by limiting initial screening to *MSH6* and *PMS2* proteins.^{38,39} Because *MSH6* and *PMS2* are obligate binding partners of *MSH2* and *MLH1*, respectively, they are not expressed at the protein level in the absence of their partner proteins. Thus, loss of *MSH6* expression can detect both defects in *MSH6* and *MSH2*, whereas *PMS2* loss is a stand-in for both *PMS2* and *MLH1* defects. Loss of both *MSH2* and *MSH6* protein expression with preservation of both these genes on germline testing may indicate an underlying *EPCAM* deletion, therefore IHC for *EPCAM* and/or germline testing of *EPCAM* should be considered in this setting.

As loss of *MLH1* and *PMS2* protein expression can also be seen with sporadic methylation of the *MLH1* gene, PCR for *MLH1* hypermethylation must be performed on all cases with apparent *MLH1* defects. Unlike colorectal cancer, *BRAF* mutations do not generally occur in association with sporadic methylation of *MLH1* in endometrial cancer.⁴⁰ In evaluating *MLH1* hypermethylation, it must be borne in mind that rare cases of LS are attributed to germline mutations in promoter methylation. If *MLH1* promoter methylation is identified, but the patient's family or personal history is strongly compelling for a heritable process, both tumor and normal tissue should be tested to exclude germline defects in promoter methylation. Germline testing can also be considered in cases for which there is strong clinical suspicion of LS despite preserved expression for all 4 proteins on IHC.

Although broader LS screening may increase the immediate financial burden on the health care system, in the long term the prevention and early detection of cancers in these patients should decrease net health care costs and transition health care delivery to a more optimal approach of screening and prevention. The fiscal arguments against universal screening become even less tenable in the era of personalized medicine, when individualized testing is increasingly requested and recognized as the standard of care by many patients and treating physicians. LS is the most common heritable cancer syndrome in the United States and Europe, and increased screening will likely disclose a higher prevalence than has been suspected. The emergence of affordable whole-genome sequencing may further impact the approach to heritable disorders.

Identification of LS patients is critical for a variety of reasons. First, they are at substantial increased risk for synchronous and metachronous malignancies when compared with the general population. Intensive cancer surveillance should therefore be used for these patients, and risk-reducing surgeries (eg, hysterectomy, colectomy and oophorectomy) should be considered.^{41,42} If established at the time of initial biopsy, a diagnosis of LS can also

influence the extent of resection; for instance, removal of a larger portion of bowel could be considered in colon cancer patients. Recent work has shown that IHC for MMR proteins is a reliable LS screen in small colorectal biopsy specimens, although staining heterogeneity can rarely lead to false-negative cases.⁴³ Similar studies have not been performed in endometrial biopsies but it has been our experience that some cases of low-grade endometrial carcinoma may be difficult to interpret in small biopsy specimens because of the presence of admixed atypical hyperplasia. Although testing for MMR defects has been suggested in these precursor lesions, the vast majority of precursor lesions in the endometrium and in the colon (with the exception of polyps that harbor a focus of high-grade dysplasia) may retain MMR protein expression even in the presence of a germline mutation.

In addition to influencing health care decisions for individual cancer patients, a diagnosis of LS affects screening strategies for related family members. LS demonstrates autosomal dominant inheritance; therefore the identification of one patient can precipitate the unveiling of many affected relatives.⁵ Involvement of genetic counselors is critical for advising both individual cancer patients and family members about the implications of testing.

A secondary function of MMR testing in colorectal cancers is the identification of sporadic carcinomas with *MLH1* promoter methylation. Although not associated with familial cancer syndromes, this defect nonetheless leads to microsatellite instability indistinguishable from what is seen with germline MMR gene mutations. In the colon, microsatellite instability is associated with improved overall prognosis but decreased response to fluorouracil.⁴⁴ The prognostic implications of sporadic *MLH1* methylation have not been well characterized, but as we learn more about the behavior of sporadic endometrial cancer with *MLH1* methylation, it is possible that this information may have independent value for guiding clinical treatment.

In summary, at least 41% with LS-associated germline mutations are not associated with any of the traditional indicators that have been recommended for LS screening in women with endometrial adenocarcinoma (ie, age 50 y or younger, personal/family cancer pedigree that meets Bethesda guideline criteria, presence of MMR-associated tumor morphology, or location in the lower uterine segment). These data suggest that a significant number of LS-associated endometrial carcinomas are missed using these clinical, histologic, and locational screening parameters and provide support for universal screening of all newly diagnosed endometrial cancers. The clinical utility of LS testing depends on the MMR gene mutated. Although it has been suggested that the benefit is lower for those diagnosed at older ages and with less penetrant *MSH6* mutations, data are limited.⁴⁵ In our opinion, a large, prospective study on unselected endometrial cancer patients that includes a rigorous health economic analysis is needed before premature adoption of more limited screening recommendations.⁴⁶

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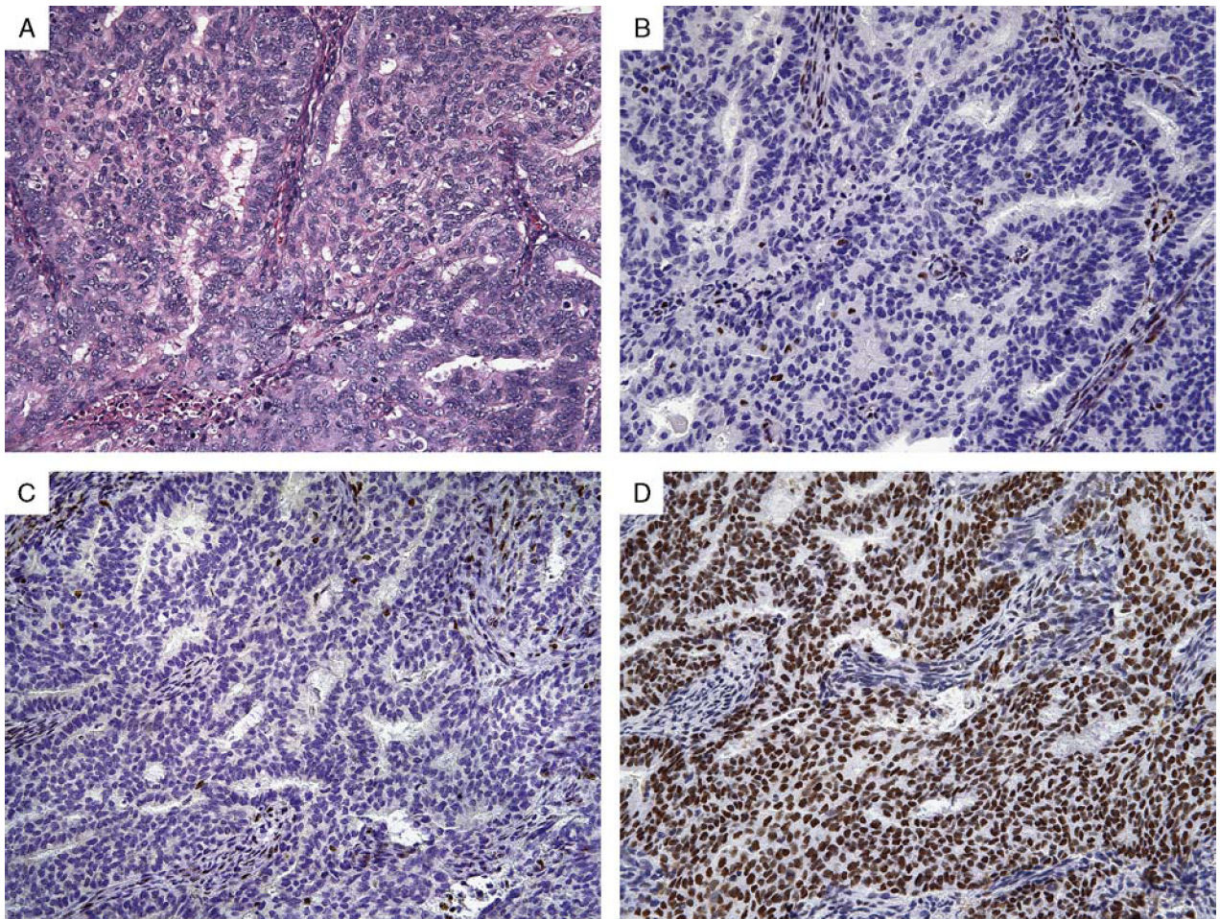
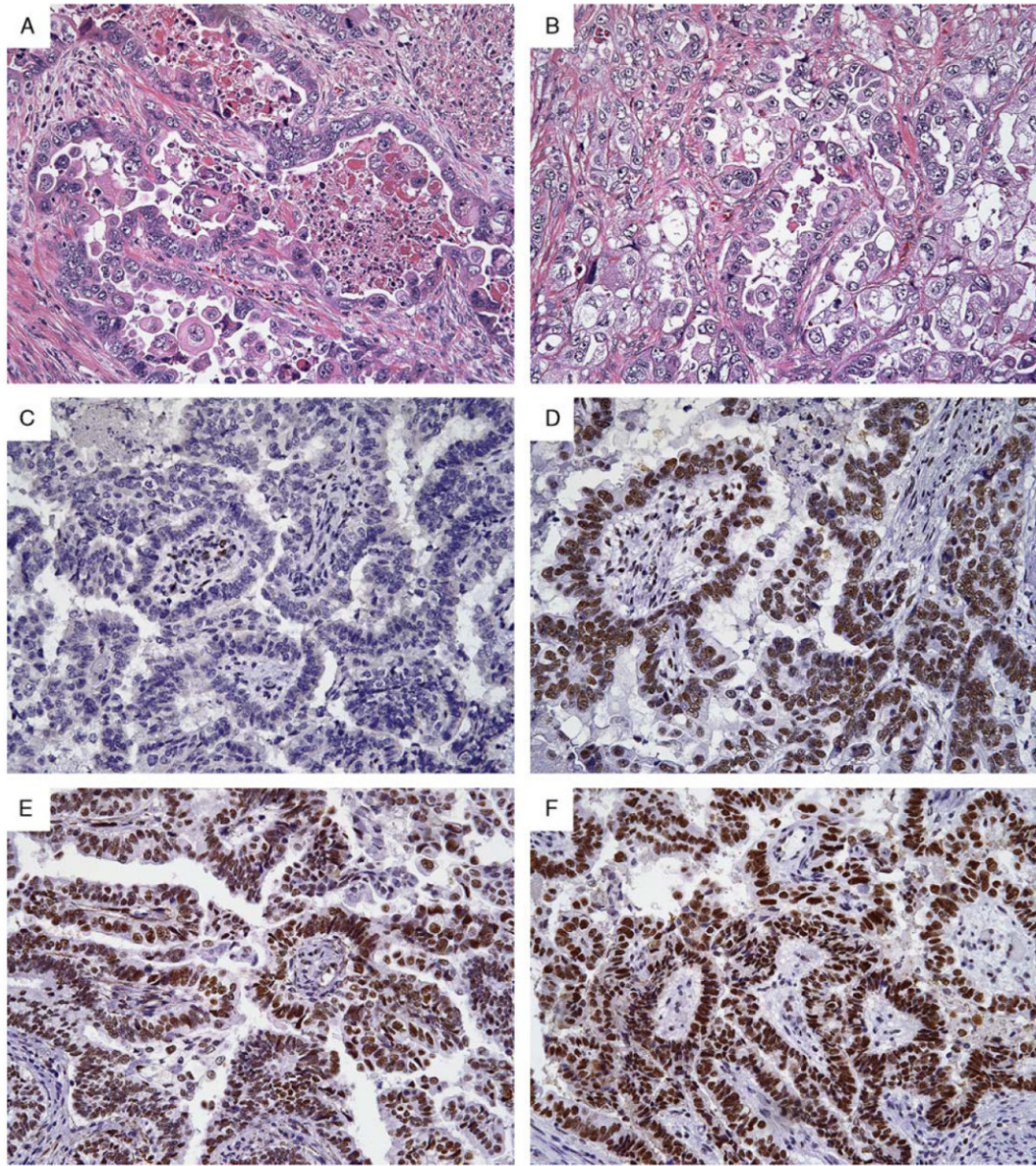


FIGURE 1. Conventional endometrioid adenocarcinoma (A) in a 61-year-old woman with dual loss of MSH2 (B) and MSH6 (C) but intact MLH1 (not shown) and PMS2 (D). Subsequent genetic testing confirmed a germline mutation in *MSH2*.

**FIGURE 2.**

High-grade endometrial adenocarcinoma (A and B) in a 77-year-old woman showing isolated loss of MSH6 (C) but intact MSH2 (D), MLH1 (E), and PMS2 (F). This tumor was associated with a germline mutation in *MSH6*.

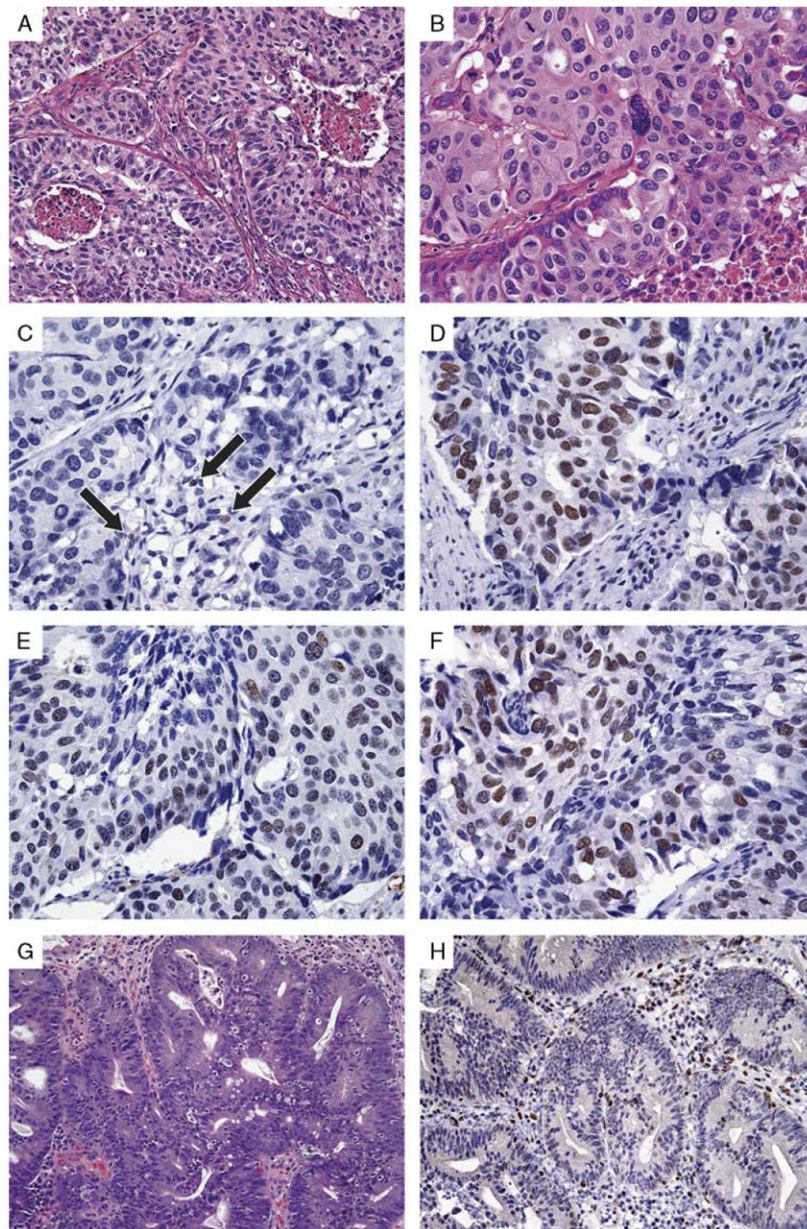


FIGURE 3. Endometrial adenocarcinoma (A and B) in a 58-year-old woman with isolated loss of MSH6 (C) but intact MSH2 (D), MLH1 (E), and PMS2 (F). Subsequent genetic testing confirmed a germline mutation in *MSH6*. Intramucosal carcinoma in a subsequent surveillance polypectomy specimen (G) also demonstrated loss of MSH6 protein (H). Note intact MSH6 expression in admixed lymphocytes (highlighted by arrows in C).

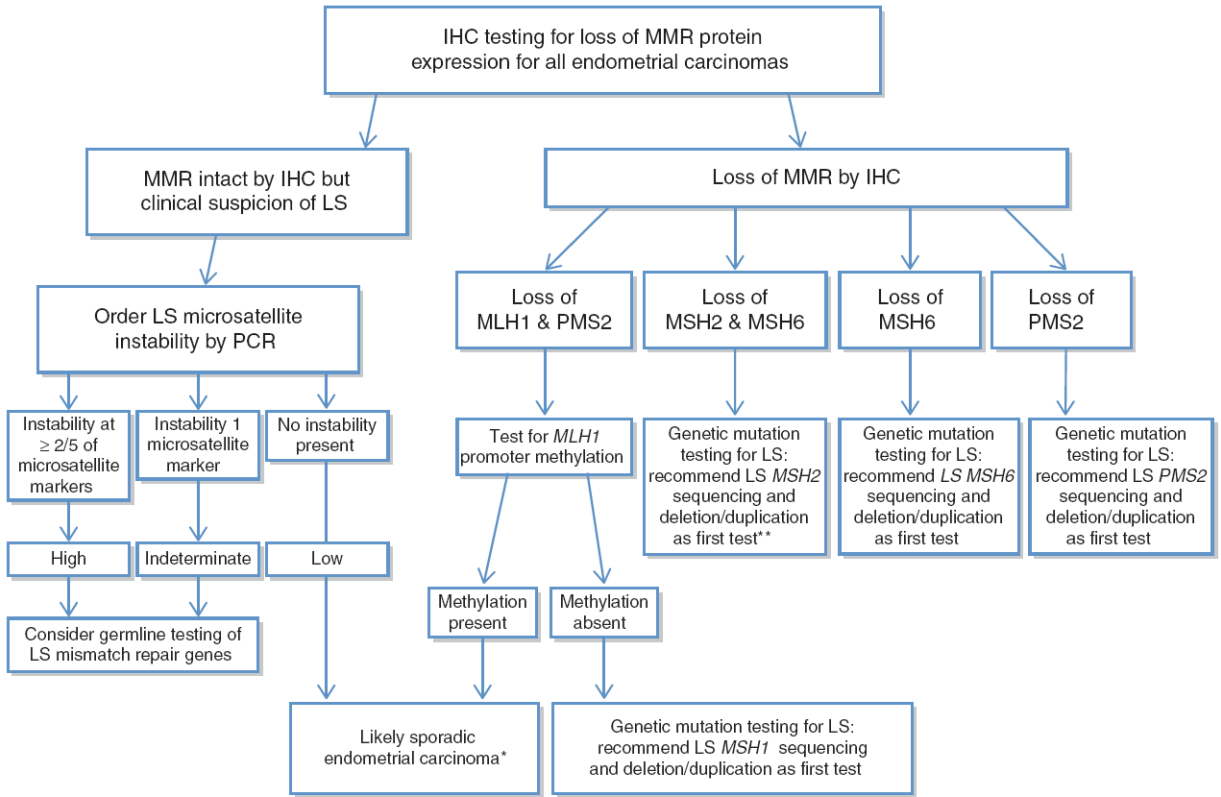


FIGURE 4.

Algorithm for testing of endometrial adenocarcinomas for MMR deficiency. A 2-antibody approach utilizing MSH6 and PMS2 as preliminary screening with subsequent MSH2 or MLH1 IHC for cases that show loss of MSH6 or PMS2 can also be performed.

*If strong clinical suspicion for LS, consider *MLH1* promoter methylation analysis of non-neoplastic tissue/peripheral blood to evaluate for germline epigenetic *MLH1* promoter methylation.

**If *MSH2* and *MSH6* unmutated, consider LS *EPCAM*, sequencing and deletion/duplication.