AMP case report: A 48-year-old woman with endometrial cancer. Importance of screening for Lynch syndrome in patients with EC

CAP TODAY and the Association for Molecular Pathology have teamed up to bring molecular case reports to CAP TODAY readers. Here, this month, is the second such case. (See CAP TODAY, February 2013, for the first, on multilocus sequencing for rapid identification of molds.) AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, treatment, and more. Case report No. 2 comes from Dartmouth-Hitchcock Medical Center, Hanover, NH. (If you would like to submit a case report, please e-mail the AMP at amp@amp.org. For more information about the AMP, visit www.amp.org.)

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Abstract

August 2013—Lynch syndrome (LS) is an autosomal dominant syndrome that predisposes patients to multiple malignancies. LS has traditionally been thought of as a colorectal-cancer-dominated syndrome; however, the incidence of endometrial cancer in women with LS actually exceeds that of colorectal cancer. Here we report a case of a woman with metachronous colorectal cancer and endometrial cancer, with the goal of increasing awareness of the need to screen endometrial cancer patients for LS. Identifying these patients is important not only for the patient but also for other family members who would benefit from genetic counseling and surveillance for LS-associated malignancies.

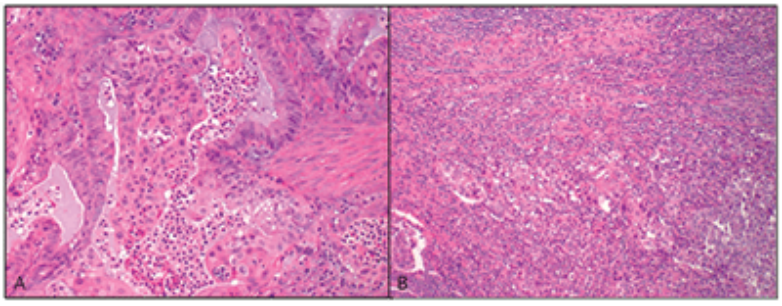

Fig. 1. The patient's endometrial carcinoma showing **(A)** mucinous and squamous differentiation and **(B)** dense peri- and intratumoral lymphocytic infiltration (H&E, magnification 20×).

Introduction

Lynch syndrome, or hereditary non-polyposis colorectal cancer (HNPCC), is an autosomal dominant cancer susceptibility syndrome caused by germline mutations in one of four mismatch repair (MMR) genes: *MLH1, MSH2, MSH6*, and *PMS2*. The MMR proteins function as dimers (MLH1 with MSH2 and MSH6 with PMS2), and mutations in any one of these genes cause inactivation of the MMR system. This allows for the accumulation of unstable mismatched DNA in highly repeated microsatellite sequences, and gradually increasing instability with larger numbers of erroneous DNA segments (microsatellite instability, or MSI) and eventual gene expression alteration and subsequent carcinogenesis. Though Lynch syndrome was originally described as a familial predisposition to colorectal carcinomas, its association with carcinomas of noncolonic organs, such as endometrium, ovary, and stomach, among others, is now well recognized.

Lynch syndrome accounts for approximately two to three percent of CRC and 2.3 percent of endometrial cancers (EC), with an overall risk of developing CRC of 68 percent and EC of 62 percent in Lynch patients. However, when looking at the two genders separately, the risk of CRC for men is 83 percent versus 48 percent for women. Therefore, women with Lynch syndrome are at a substantially greater risk of developing EC than CRC, and in patients with metachronous cancers, 51 percent were diagnosed first with a primary gynecologic malignancy.[1] Thus, while most pathologists and clinicians are aware of the association of CRC with LS, additional education on screening patients with endometrial cancer is needed.

Here we report a case of a woman with metachronous CRC and endometrial cancer who, despite a significant family history, was not evaluated for Lynch syndrome until her second primary tumor was identified. This case raises awareness of the association between LS and endometrial cancer and the modalities used to screen patients for LS, and the significance that identifying this syndrome can have on patients' families.

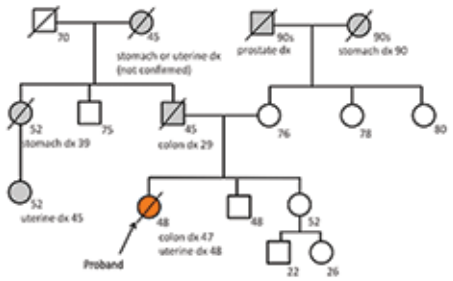

Fig. 2. The patient's (Proband's) family pedigree showing a number of relatives with Lynch syndrome-associated malignancies. The patient's germline *MLH1* mutation was most likely inherited from her father.

Patient case

A 48-year-old woman was referred to the gynecologic oncology outpatient clinic at our institution for evaluation of three weeks of vaginal bleeding. Per the patient, the vaginal bleeding was bright red and necessitated the use of pads. She had no further complaints and remained active, working at a local ski resort. Her gynecologic history was unremarkable with no pregnancies, normal Pap smears, and menopause five years prior. Exam revealed bright red blood in the vaginal vault, an exophytic lesion at the cervical os, and a large posterior uterine nodule appreciated on bimanual exam.

She had had an unremarkable personal health history until 10 months prior to this presentation. At that time, she presented to the ED for acute abdominal pain, ultimately determined to be a perforated colonic malignancy at the splenic flexure. A laparoscopic left hemicolectomy was performed at an outside institution and showed an acutely perforated, invasive, moderately differentiated mucinous adenocarcinoma (stage pT4 N0). Adjuvant chemotherapy (FOLFOX) was initiated after followup PETs demonstrated retroperitoneal nodal metastasis. The increasing adenopathy was followed via CT, which also showed "a mass arising in the endometrial cavity, infiltrative into the myometrium." It had increased in size since it was first noted on prior imaging, prompting a referral to the gynecologic oncology clinic.

An endocervical biopsy showed endometrioid type adenocarcinoma (CK7 positive, CK20 and CDX2 negative). Subsequent TAH-BSO confirmed endometrial adenocarcinoma endometrioid type (FIGO grade II) with squamous and mucinous differentiation and foci of secretory change; the tumor was deeply invasive, involved the lower uterine segment and cervix, was associated with peri- and intratumoral lymphocytes, and had lymphovascular space invasion and metastasis to periaortic lymph nodes and one fallopian tube (pT3a N1 [IIIC]) (**Fig.1**).

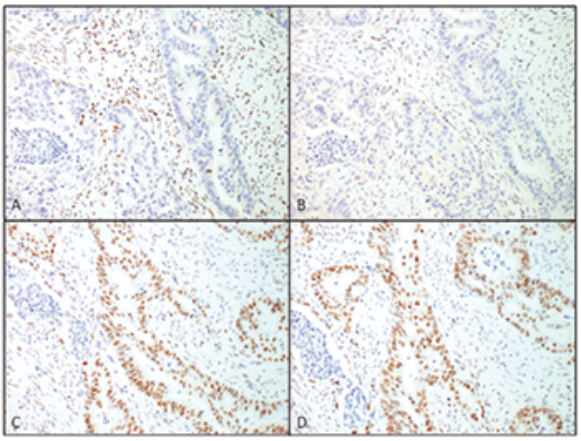

Fig. 3. Immunohistochemistry for the MMR proteins in this patient's endometrial carcinoma showing complete loss of staining for MLH1 **(A)** and PMS2 **(B)** while staining is retained for MSH2 **(C)** and MSH6 **(D)** (magnification 20×).

Her family history was significant for multiple family members with endometrial, stomach, and colon cancers, including her father who was diagnosed with colon cancer at 29 and died of disease at 45 (**Fig.2**). Considering her family history and the diagnosis of two primary cancers, the question of Lynch syndrome was raised. In addition to routine H&E staining, immunohistochemistry was performed for DNA mismatch repair proteins MLH1, MSH2, MSH6, and PMS2. These stains showed a loss of MLH1 and PMS2 nuclear staining in tumor cells while MSH2 and MSH6 staining remained intact (**Fig.3**). Although most tumors exhibiting loss of MLH1 are associated with gene silencing through sporadic promoter methylation, this patient's strong familial history suggested a possible germline mutation and further genetic testing was indicated. Gene sequencing performed at Myriad Laboratories revealed a *MLH1* p.E13X (c.37G>T) deleterious nonsense mutation, confirming the diagnosis of Lynch syndrome likely inherited from her father.

Despite chemotherapy, the patient's two metastatic diseases continued to spread rapidly and she died due to overwhelming tumor burden only a year after her initial presentation to the ED. Though the patient had no children, she had two siblings, a niece and nephew, and several cousins who will receive appropriate genetic counseling and subsequently be tested for this familial germline mutation.

Discussion

Gynecologic malignancies, especially endometrial cancer, are often the initial cancer diagnosis in women who harbor the germline mutations in the MMR genes associated with Lynch syndrome. Therefore, our awareness needs to be heightened when faced with EC patients. During recent decades, multiple criteria and guidelines have been issued in an attempt to identify patients who warrant screening for LS. The Amsterdam criteria were compiled in 1991 and revised in 1999, advocating the classification of Lynch families based heavily on pedigree patterns coupled with emergence of cancer at a relatively young age. Although addressing the inheritance aspect of Lynch syndrome, these criteria were found to be inadequate as they focused too much on pedigree and excluded those who fell outside of the classical Lynch presentation of CRC at a young age.

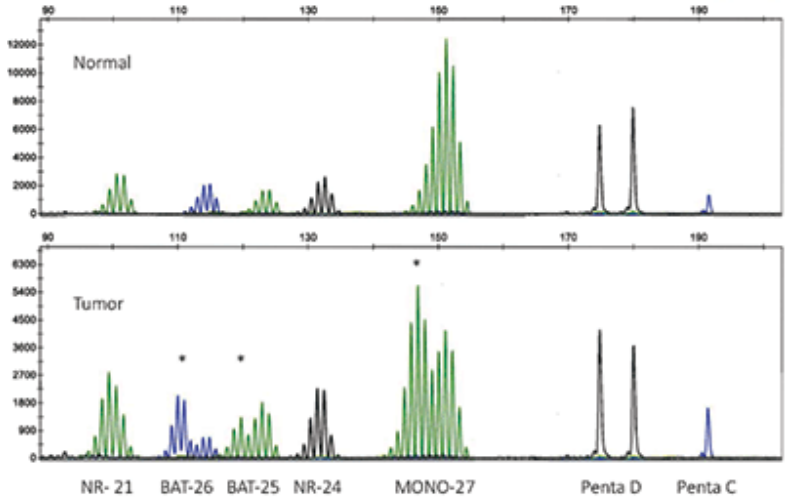

Fig. 4. In MSI testing, genomic DNA from a patient's tumor is compared with genomic DNA from a blood sample. Seven MSI markers are evaluated in this assay (five mononucleotide on the left and two pentanucleotide on the right); the asterisk (*) indicates markers that show instability when comparing the two samples. MSI at two or more of the five mononucleotide markers indicates MSI-H.

Around the same time as the Amsterdam revision, a second set of criteria, the Bethesda guidelines, emerged, also emphasizing pedigree but additionally advocating the use of MSI testing via PCR to evaluate individuals whose presentation is suggestive of a cancer syndrome (such as relatively young age of presentation and synchronous Lynch-associated tumors). According to these guidelines, pedigree was certainly important, but the focus was shifted to the individual and his or her specific presentation, a more effective screening tool for those with a limited family history. Although found to be a much more sensitive mode of screening as compared with the Amsterdam criteria, the Bethesda guidelines still were found to be inadequate, especially for EC patients.

Recently, several organizations including the Association for Molecular Pathology,[2] the EPICOLON Consortium,[3] the National Society of Genetic Counselors and Collaborative Group of the Americas on Inherited Colorectal Cancer,[4] and EGAPP5 have put forth guidelines that recommend universal screening for all individuals with newly diagnosed CRC using MMR IHC and/or MSI, though no particular algorithm is favored. This approach appears to be cost-effective.[6, 7]

Genetic defect	IHC pattern
MLH1	MLH1 (–/+)* / PMS2–
PMS2	MLH1 (+/–) / PMS2–
MSH2	MSH2 – / MSH6–
MSH6	MSH2 + / MSH6–

Table 1. Genetic defect in one of the four MMR proteins (either germline mutation or, in the case of MLH1, somatic promoter hypermethylation also has a gene silencing effect) and the corresponding expected IHC patterns. Because the proteins form dimers, loss of MLH1 is almost always coupled with loss of PMS2, and loss of MSH2 is

accompanied by MSH6 loss. Complete loss of expression in the setting of a positive internal control is interpreted as a positive result. The IHC pattern can guide subsequent genetic testing for specific MMR gene sequencing.
*Occasionally, interpretation of IHC can be problematic; some mutations in MLH1 or abnormal methylation may result in false normal MLH1 IHC staining.

The screening guidelines for endometrial cancer are not quite as straightforward. The Society of Gynecologic Oncologists issued recommendations in 2007 for screening patients at risk for LS-associated gynecologic malignancies; however, these again focused on personal/family history and development of cancer before 50 years of age.[8] However, in one study, based on age alone, six of 10 EC patients would not have been identified using the under 50 years of age criterion for screening.[9] Also, in comparison to CRC, an increased number of endometrial cancers in LS is due to mutations in *MSH6*, which tend to develop after age 50. Several institutions are moving to universal screening of EC or using strict criteria based on patient history and tumor histology with MMR IHC.[9,10] Histopathologic features of EC that seem to correlate with LS cases include peritumoral lymphocytes, tumor infiltrating lymphocytes (TILs), presence of tumor heterogeneity, and undifferentiated/dedifferentiated morphologies, lower uterine segment localization, and synchronous ovarian clear cell carcinoma.[10,11]

MSI in about 75 percent of endometrial cancer is sporadic, due to *MLH1* promoter methylation, which can be identified with a separate methylation-specific PCR assay. In our laboratory we perform MSI analysis using a clinically available kit (Promega Corp., Madison, Wis.) of seven markers—five mononucleotide repeat markers (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) and two pentanucleotide repeat markers (Penta C and Penta D). The mononucleotide markers are used to determine MSI status, and the pentanucleotide markers confirm that the paired samples (normal and tumor) are from the same person. After PCR, amplicons are run on an ABI capillary electrophoresis instrument; tumors showing instability at two or more markers are defined as MSI-H (high), and MSI-L (low) and MSS (stable) tumors have instability at one repeat or no instability, respectively (**Fig. 4**). Hampel, et al., showed that some Lynch-associated endometrial carcinomas were found to be MSI-L or even MSS, particularly those with *MSH6* mutations, lowering the predictive rate of MSI testing. IHC has been shown to be as accurate as MSI and allows for the additional benefit of targeting a specific MMR gene for sequencing based on staining results (**Table 1**).[9]

Conclusion

This case demonstrates the potential rapidity of events resulting from Lynch syndrome, as the patient went from initial presentation to death in just over one year. Further, this case also shows that while current screening recommendations for LS undergo continuous refinement, testing based on pedigree alone has been proved to be insufficient and should be based on individual case presentations or, as some institutions are adopting, universal screening on all newly diagnosed EC or CRC patients. Finally, this case shows that though there are multiple screening modalities for LS, they are useless unless clinicians think to use them. Thus, it is important to raise awareness of LS in endometrial cancer patients and current screening practices.

References

1. Resnick KE, Hampel H, Fishel R, Cohn DE. Current and emerging trends in Lynch syndrome identification in women with endometrial cancer. *Gynecol Oncol*.

2009;114(1):128–134.
2. Funkhouser WK Jr, Lubin IM, Monzon FA, et al. Relevance, pathogenesis, and testing algorithm for mismatch repair-defective colorectal carcinomas: a report of the Association for Molecular Pathology. *J Mol Diagn*. 2012;14(2):91–103.
3. Moreira L, Balagner F, Lindor N, et al. Identification of Lynch syndrome among patients with colorectal cancer. *JAMA*. 2012;208(15):1555–1565.
4. Weissman SM, Burt R, Church J, et al. Identification of individuals at risk for Lynch syndrome using targeted evaluations and genetic testing: National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Colorectal Cancer joint practice guideline. *J Genet Couns*. 2012;21(4):484–493.
5. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med*. 2009;11(1):35–41.
6. Mvundura M, Grosse SD, Hampel H, Palomaki GE. The cost-effectiveness of genetic testing strategies for Lynch syndrome among newly diagnosed patients with colorectal cancer. *Genet Med*. 2010;12(2):93–104.
7. Ladabaum U, Wang G, Terdiman J, et al. Strategies to identify the Lynch syndrome among patients with colorectal cancer: a cost-effectiveness analysis. *Ann Intern Med*. 2011;155(27):69–79.
8. Lancaster JM, Powell CB, Kauff ND, et al. Society of Gynecologic Oncologists Education Committee statement on risk assessment for inherited gynecologic cancer predispositions. *Gynecol Oncol*. 2007;107(2):159–162.
8. Hampel H, Frankel W, Panescu J, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. *Cancer Res*. 2006;66(15):7810–7817.
9. Garg K, Soslow RA. Lynch syndrome (hereditary non-

polyposis colorectal cancer) and endometrial carcinoma. *J Clin Pathol*. 2009;62(8):679–684.

10. Garg K, Leitao MM Jr, Kauff ND, et al. Selection of endometrial carcinomas for DNA mismatch repair protein immunohistochemistry using patient age and tumor morphology enhances detection of mismatch repair abnormalities. *Am J Surg Pathol*. 2009;33(6):925–933.

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Test yourself: Here are three questions taken from the case report.

Below are three take-home points and questions. Answers to the questions are online now at www.amp.org/casereviews and will be published in CAP TODAY *next month.*

1. What is the mode of inheritance for Lynch syndrome?
A. X-Linked
B. Mitochondrial
C. Autosomal dominant
D. Autosomal recessive

2. What is the expected IHC pattern associated with a genetic defect in MSH6?
A. MSH6 (+) / MSH2 (−)
B. MSH6 (+) / MSH2 (+)
C. MSH6 (−) / MSH2 (−)
D. MSH6 (−) / MSH2 (+)

3. What is the most common cause of microsatellite instability (MSI) in endometrial carcinoma?
A. MLH1 promoter methylation
B. MLH1 germline mutation
C. MSH6 germline mutation
D. PMS2 germline mutation