Reporting Subclonal Immunohistochemical Staining of Mismatch Repair Proteins in Endometrial Carcinoma in the Times of Ever-Changing Guidelines

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Context.—The current College of American Pathologists reporting guideline for mismatch repair protein (MMRP) immunohistochemistry for Lynch syndrome (LS) screening considers the presence of any positive nuclear staining as intact MMRP expression. This would include tumors with combined areas of subclonal retention and loss of MMRP staining.

Objective.—To evaluate the clinical significance of reporting subclonal staining patterns of MMRP immunohistochemistry in endometrial carcinoma.

Design.—We retrospectively reviewed 455 consecutive MMRP immunohistochemistry results of endometrial carcinoma in hysterectomy specimens from 2012 through 2017 and identified cases with subclonal MMRP staining. These results were correlated with the patient’s personal and family history of LS-associated carcinoma, MLH1 promoter methylation status, and LS genetic testing.

Results.—Subclonal staining of MMRP was seen in 48 of 455 cases (10.5%) on review. Thirty cases demonstrated isolated subclonal staining and were reported by pathologists as follows: subclonal (n = 5), complete MMRP loss (n = 4), and intact MMRP (n = 21). Eighteen cases had subclonal staining in combination with complete loss of other MMRP. Cases reported as subclonal or complete MMRP loss had appropriate clinical follow-up. Two of 2 cases with isolated subclonal MSH6 loss tested positive for LS. One of 3 cases with isolated subclonal MLH1/PMS2 loss was negative for MLH1 promoter methylation; LS genetic testing was not performed because of cost.

Conclusions.—Our study reveals that LS germline mutation can be detected in endometrial carcinoma patients whose tumors display sole subclonal MMRP staining. Our results stress the importance of reporting subclonal staining patterns to ensure appropriate clinical follow-up.

(Screening of endometrial carcinomas by mismatch repair (MMR) protein immunohistochemistry (IHC) permits the detection of microsatellite instability (MSI) phenotypes due to mutations in the MMR genes, MLH1, MSH2, MSH6, and PMS2.1–11 Loss of expression of one or more of the MMR proteins by IHC correlates with MMR protein deficiency in approximately 95% of cases and may indicate the presence of a Lynch syndrome–associated germline mutation.2,5,8,12 The use of tumor immunohistochemical screening allows for guided genetic testing in patients deemed at risk for Lynch syndrome.4,6,7,9,11,13 Variable immunohistochemical staining patterns of MMR proteins have emerged through the use of universal screening practices for newly diagnosed endometrial carcinomas.10,12,14–16 Documented patterns of IHC staining include uniform or complete loss of nuclear staining of tumor cells and heterogenous or subclonal loss of MMR protein expression, although reporting of subclonal staining was not recommended10,12,14 until the January 2021 publication by Casey and Singh17 in the Proceedings of the International Society of Gynecologic Pathology companion society session at the United States and Canadian Academy of Pathology 2020 annual meeting. The current reporting guideline issued by the College of American Pathologists18 considers the presence of any positive nuclear staining, albeit weak or focal, as intact MMR protein expression, thus excluding tumors with subclonal immunohistochemical staining.16,19,20

The purpose of this study is to evaluate the clinical significance of reporting subclonal staining patterns of MMR protein IHC in pathology reports from an academic institution with a large volume of endometrial carcinoma cases.

MATERIALS AND METHODS

Selection of Cases and Data Collection

We conducted a retrospective review of all endometrial carcinoma cases from January 2012 through December 2017 at the University of Tennessee Medical Center (Knoxville). Any patient who underwent a hysterectomy and had case slides available for review, including the MMR protein IHC panel, was included in the study.

Arch Pathol Lab Med

Subclonal IHC Staining of MMR Proteins—Scheiderer et al
Clinicopathologic data, including patient age, final pathologic diagnosis, reported MMR protein IHC status, and MLH1 promoter methylation testing results, were collected from the pathology reports. Personal and family history of carcinoma and Lynch syndrome genetic testing results were collected from medical records. Carcinoma history involving the gastrointestinal, gynecologic, upper urinary, and pancreaticobiliary tracts, as well as the brain and sebaceous glands, was considered a Lynch syndrome–associated tumor.9,13,21–24

**MMR Protein IHC Reevaluation**

Microscopic reevaluation of tumor hematoxylin-eosin–stained slides and corresponding MMR protein IHC slides (MLH1, MSH2, MSH6, PMS2) was performed. The presence of subclonal MMR protein staining, defined as combined areas of retention and loss of nuclear staining; the MMR protein(s) affected; and the presence of complete loss of immunohistochemical staining of other MMR proteins, if any, were recorded. We determined the morphologic staining pattern of heterogeneity based on classifications previously described by Watkins et al14 and Joost et al25; pattern 1, subclonal nuclear staining within glands; pattern 2, complete loss of nuclear staining in a small subset of entire glands; and pattern 3, complete loss of nuclear staining across large areas. The examples of this classification may be seen in Figure 1: abrupt loss of nuclear expression of MSH6 MMR protein is seen within glands (Figure 1, A), in a small group of glands (Figure 1, B), or as complete regional (geographic) loss of nuclear expression (Figure 1, C), with intervening stromal positivity serving as an obligatory internal control. We also estimated the percentage of tumor area with loss of staining.

Once subclonal staining was detected, IHC for the MMR protein(s) that demonstrated subclonal staining was repeated on 2 tumor blocks to ensure IHC quality and reproducibility including exclusion of potential fixation artifact: (1) the block on which the original panel was performed and (2) an additional block of tumor. Cases with subclonal staining were recorded as subclonal MMR protein staining only if subclonal staining was reproducible on at least 1 of the 2 repeated IHC-stained slides and if positive nuclear staining of intervening stromal cells, inflammatory cells, or nonneoplastic epithelial cells serving as internal control(s) was adequate.

**Methods for MMR Protein IHC**

Immunohistochemistry for the MMR proteins MLH1, MSH2, MSH6, and PMS2 was performed using the Dako Autostainer Link 48 and the EnVision FLEX+ detection method (Dako, Santa Clara, California). Four-micrometer-thick sections were obtained from formalin-fixed, paraffin-embedded blocks of selected tumor sections. The assay was performed with a manufacturer-supplied kit with modifications: the tissue was pretreated with heat-induced epitope retrieval (Dako Target Retrieval Solution pH 9) and incubated at 97°C for 20 minutes. Peroxidase activity was blocked for 5 minutes prior to antibody application. Prediluted, ready-to-use monoclonal mouse (MLH1, MSH2) and monoclonal rabbit (MSH6, PMS2) immunoglobulin G antibodies (Dako, Santa Clara, California) were used for IHC analysis: MLH1 (clone ES05) was incubated for 20 minutes for both the primary and linker antibodies, MSH2 (clone FE11) primary antibody was incubated for 20 minutes and the linker antibody for 15 minutes, MSH6 (clone EP49) primary antibody was incubated for 20 minutes, and PMS2 (clone EP51) primary antibody was incubated for 45 minutes. There were no linker antibodies for MSH6 and PMS2. The tissue was counterstained with hematoxylin for 5 minutes. Positive and negative controls were performed appropriately throughout the process.

**Statistical Analysis and Institutional Review Board Approval of the Study**

Descriptive and frequency statistics were performed using SPSS version 25 (IBM Corp, Armonk, New York). Approval was obtained from the Institutional Review Board of the Graduate School of Medicine at the University of Tennessee Medical Center prior to initiation of this study.

**RESULTS**

Subclonal staining of MMR protein expression was seen in 48 of 455 endometrial carcinoma cases (10.5%) (Figure 2).
Thirty of these cases displayed isolated (ie, pure) subclonal staining of MMR protein(s), whereas 18 had subclonal staining in combination with complete loss of staining of other MMR protein(s). The cases showing isolated subclonal staining of MMR protein(s) (n = 30) were reported by pathologists as (1) subclonal in 5 cases, (2) complete loss of staining in 4 cases, and (3) intact nuclear staining in 21 cases.

**Cases With Isolated Subclonal MMR Protein Expression**

**Cases Reported by Pathologists as Subclonal MMR Protein Expression.**—Five endometrial carcinoma cases demonstrating isolated subclonal staining (5 of 30; 16.7%) were reported as subclonal by pathologists in our institution, 3 involving MLH1/PMS2 and 2 involving MSH6 MMR proteins (Table 1).

The 3 cases with subclonal loss of MLH1/PMS2 displayed loss of staining in entire glands comprising between 10% and 40% of tumor area. Two of these cases underwent MLH1 promoter methylation testing and 1 was found to be negative for MLH1 promoter methylation, a result indicative of high probability for Lynch syndrome. Confirmatory genetic testing for Lynch syndrome was not performed on this case because of insurance/cost issues. The third case with subclonal staining of MLH1/PMS2 was sent for Lynch syndrome genetic testing without MLH1 promoter methylation testing and was negative for Lynch syndrome.

The 2 cases with subclonal staining of MSH6 displayed loss of staining across large areas of tumor and demonstrated loss in 60% and 75% of tumor cells (Figure 3, A through D). One of the cases was sent for MSI testing by polymerase chain reaction with a result of MSI low. Both cases underwent genetic testing for Lynch syndrome, and both demonstrated mutations in the MSH6 gene. No personal or family history of Lynch syndrome–related
carcinoma was identified upon review of medical records in any of the 5 cases.

**Cases Reported by Pathologists as Complete Loss of MMR Protein Expression.**—Four cases demonstrating isolated subclonal staining (4 of 30; 13.3%) were reported by pathologists from our institution as exhibiting complete loss of staining of MMR protein(s). On retrospective review, these cases were classified as displaying isolated subclonal staining (Table 2). All 4 cases involved subclonal loss of MLH1/PMS2. Loss of staining was seen in 10% to 60% (median, 45%) of tumor area and encompassed a range from complete loss of nuclear staining in a small subset of entire glands to complete loss of nuclear staining across large areas of tumor (Figure 4, A through D). Two of 4 cases were sent for MLH1 promoter methylation analysis and in both cases MLH1 promoter methylation was detected. The remaining 2 cases underwent genetic testing for Lynch syndrome without MLH1 promoter methylation testing.

### Table 1. Cases Reported by Pathologists as Subclonal Mismatch Repair (MMR) Protein Expression

| Case | Age, y | MMR Protein Expression | % of Loss in Tumor | History of LS-Related Cancer | Genetic Testing Results |
|------|--------|-------------------------|--------------------|-----------------------------|------------------------|
|      |        | MLH1 | MSH2 | MSH6 | PMS2 |                          | Personal | Family | MLH1 PM | Results |
| 1    | 67     | S    | I    | I    | S    | 25                          | No       | No     | NEG     | NP |
| 2    | 59     | S    | I    | I    | S    | 10                          | No       | No     | NP      | NEG |
| 3    | 83     | S    | I    | I    | S    | 40                          | No       | No     | POS     | NP |
| 4    | 44     | I    | I    | 5    | I    | 60                          | No       | No     | NA      | POS |
| 5    | 74     | I    | I    | 5    | I    | 75                          | No       | No     | NA      | POS |

Abbreviations: I, intact; LS, Lynch syndrome; NA, not applicable; NEG, negative; NP, not performed; PM, promoter hypermethylation; POS, positive; S, subclonal.

Figure 3. Case displaying isolated subclonal staining of MSH6 mismatch repair protein by immunohistochemistry. MLH1 (A), MSH2 (B), and PMS2 (D) all show intact nuclear expression of respective mismatch repair proteins by immunohistochemistry. MSH6 (C) displays subclonal staining of MSH6 mismatch repair protein by immunohistochemistry within large area of tumor. An area with negative tumor cell nuclei has positive nuclear staining of stromal cells and tumor-infiltrating lymphocytes (original magnification ×20 [A through D]).
Neither case harbored Lynch syndrome–related genetic mutations.

Review of medical records revealed that all 4 patients had at least 1 family member with a history of Lynch syndrome–related carcinoma. No patient had a previously diagnosed carcinoma associated with Lynch syndrome.

**Cases Reported by Pathologists as Intact MMR Protein Expression.**—Twenty-one cases with isolated subclonal staining (21 of 30; 70%) were reported as intact MMR protein staining by pathologists in our institution (Table 3), as per the College of American Pathologists guideline. Eight cases involved MLH1/PMS2, 7 cases involved MSH6/PMS2, and 6 affected PMS2 in isolation. The percentage of tumor cells that demonstrated loss of staining in these 21 cases ranged from less than 5% up to 80%. The staining patterns identified included the loss of staining of a few nuclei within multiple glands, complete loss of nuclear staining in a small subset of entire glands, and complete loss of nuclear staining across large areas of tumor. The cases with subclonal staining of MSH6/PMS2 proteins had the highest percentage of subclonal loss of

| Case | Age, y | MMR Protein Expression | % of Loss in Tumor | History of LS-Related Cancer | Personal | Family | MLH1 PM | Genetic Testing |
|------|--------|------------------------|--------------------|----------------------------|----------|--------|---------|----------------|
| 1    | 67     | S I I S                | 50                 | No                         | Yes      | NP     | NEG     |
| 2    | 41     | S I I S                | 40                 | No                         | Yes      | POS    | NP      |
| 3    | 59     | S I I S                | 60                 | No                         | Yes      | POS    | NP      |
| 4    | 65     | S I I S                | 10                 | No                         | Yes      | NP     | NEG     |

Abbreviations: I, intact; LS, Lynch syndrome; NA, not applicable; NEG, negative; NP, not performed; PM, promoter hypermethylation; POS, positive; S, subclonal.
nuclear staining, with 4 cases displaying loss in 60% to 80% of tumor cells. Within the MLH1/PMS2 group, MLH1 promoter methylation was not performed on any case as the MMR protein status was reported as intact. Furthermore, such reporting implied that clinical follow-up testing was not indicated. Genetic testing for Lynch syndrome was performed on 2 patients from this group based on personal or family cancer history. Both cases had MSH6/PMS2 subclonal staining that involved 20% and 30% of the tumor and both resulted negative for Lynch syndrome germline mutations.

**Cases With Subclonal MMR Protein Staining in Combination With Complete Loss of Staining of Other MMR Protein(s)**

Eighteen of 48 cases (37.5%) with subclonal MMR protein staining displayed subclonal staining of MMR protein(s) in combination with complete loss of staining of other MMR proteins (Figure 2). Fifteen cases showed complete loss of staining of MLH1/PMS2, 13 in combination with subclonal expression of MSH6 and 2 in combination with MSH2/MSH6. The remaining 3 cases presented with complete loss of MSH2/MSH6, 2 in combination with subclonal expression of MLH1/PMS2 and 1 with subclonal expression of PMS2 alone.

All cases had appropriate clinical follow-up testing per the current College of American Pathologists guideline regarding endometrial carcinoma patients with complete loss of expression of MMR proteins. This resulted in identification of 1 case that tested positive for an MLH1 germline mutation. Presence of subclonal staining of MMR proteins in these cases did not influence outcome of the follow-up testing performed for the MMR proteins showing complete loss of nuclear staining.

**DISCUSSION**

Universal screening of endometrial carcinomas for Lynch syndrome, as endorsed by the National Comprehensive Cancer Network guidelines26,27 and joint guidelines from the American College of Obstetricians and Gynecologists and the Society of Gynecologic Oncology, has led to the identification of unusual patterns of immunohistochemical staining of MMR proteins.14,15 From the first version of the guideline issued by the College of American Pathologists for the reporting of MMR protein status by IHC in endometrial carcinoma to the current version of the reporting guideline (version 1.2.0.1, 2019), intact or normal expression of MMR proteins has been defined as any positive nuclear staining of tumor cells.18 Furthermore, it is emphasized that “it is common for intact staining to be somewhat patchy.”18 Therefore, when a case displays subclonal staining, that is, combined areas of retention and loss of nuclear staining, it is reported as intact and thus requires no further follow-up testing for exclusion of Lynch syndrome germline mutations.

We began immunohistochemical testing of MMR protein status of all endometrial carcinoma patients who underwent hysterectomy in our academic institution in 2012, per standing clinical order. We noticed that in some cases, the MMR proteins showed unusual patterns of staining, that is, subclonal staining; however, this was not uniformly reported by the pathologists in our department. Upon institutional review board approval, we retrospectively reviewed all consecutive endometrial carcinoma MMR protein immunohistochemical panels (N = 455) in hysterectomy specimens during a 5-year period (2012–2017) to identify cases with subclonal staining patterns. The original pathologist’s interpretation of the MMR protein staining patterns and any additional clinical follow-up studies aimed to exclude Lynch syndrome based on the pathologists’ reporting of MMR protein status were investigated.

Forty-eight of 455 cases were identified as displaying some degree of subclonal staining, ranging from 1% to 80% of loss of staining, and included all 3 patterns of subclonal staining shown in Figure 1, A through C, resulting in 10.5% prevalence of subclonal staining observed in our study. Watkins et al14 reported 7.2% prevalence of subclonal loss of MMR proteins in their prospective study of universal Lynch syndrome screening of 125 endometrial carcinoma cases (9 of 125 with subclonal staining) conducted at Brigham and Women’s Hospital in Boston, Massachusetts, from October 2013 to May 2015. It is worthwhile to emphasize that our rigorous evaluation of adequate nuclear positivity of internal controls such as the nuclei of intervening stromal, inflammatory, or nonneoplastic epithelial cells in areas of subclonal staining assured us that the observed subclonal staining was real and not due to preanalytic artifacts, including poor fixation artifact. In addition, MMR protein IHC was repeated on the same block and an additional block of tumor to further confirm that the observed subclonal staining was real. This approach is in concordance with previously published studies14,28 that also stress the importance of exercising caution in tumors with large degrees of autolysis and around areas of necrosis.

Cases with subclonal staining were categorized based on the absence/presence of concomitant complete loss of other MMR protein(s) and subsequently by the reporting status issued by the pathologists from our institution (Figure 2).

Thirty cases of endometrial carcinoma that showed isolated subclonal MMR protein staining on our review were reported by our institution’s pathologists as (1) subclonal expression of MMR proteins (5 cases), (2) complete loss of expression of MMR proteins (4 cases) and (3) no loss of (intact) expression of MMR proteins (21 cases). In the 5 cases from group 1, the presence of subclonal staining and its uncertain significance at the time of reporting were stressed in the report comment. Because
the unusual subclonal MMR protein staining pattern was flagged in the pathology report, the cases were followed up clinically with appropriate subsequent testing aimed to exclude Lynch syndrome (Figure 2). The follow-up testing resulted in the 2 cases with subclonal MSH6 loss both showing confirmatory Lynch syndrome germline mutations in the MSH6 gene. The tumors in these 2 cases demonstrated pure subclonal loss of entire glands across large areas. In addition, 1 of the cases with subclonal MSH6 staining was sent for MSI testing by polymerase chain reaction simultaneously with genetic testing for Lynch syndrome. Although this patient tested positive for MSH6 germline mutation, as mentioned above, MSI testing by polymerase chain reaction showed an MSI-low result. An MSI-low result by itself would not necessarily warrant Lynch syndrome genetic testing. One case from this group with subclonal MLH1/PMS2 loss tested negative for MLH1 promoter methylation, a result indicative of probable Lynch syndrome. Confirmative genetic testing was declined by the patient because of the insurance/cost issues. However, this patient has been followed clinically, as any patient with a confirmed Lynch syndrome mutation would be. None of these 3 patients had a personal or family history of Lynch syndrome-associated cancer. Our results emphasize the importance of reporting the presence of subclonal MMR protein staining in endometrial carcinomas, especially ones that exhibit loss of MMR protein staining involving entire glands and large areas of tumor (patterns 2 and 3, as described above), because it may carry the same weight as reporting complete loss of nuclear staining of MMR proteins. This manner of reporting in turn triggers the appropriate clinical follow-up and additional testing for identification of patients with Lynch syndrome. In the times of ever-changing guidelines, our results support recommended terminology for reporting of MMR protein IHC, including subclonal cases discussed by Casey and Singh17 in their review paper published very recently (January 2021) in the International Journal of Gynecological Pathology as the Proceedings of the International Society of Gynecological Pathologists companion society session of the United States and Canadian Academy of Pathology 2020 meeting. In this publication,17 reporting of isolated subclonal staining of MMR proteins in endometrial carcinomas was recommended because the subclonal staining may be associated with Lynch syndrome germline mutations.

The 4 cases of isolated subclonal staining (group 2) reported as a complete loss of MMR protein staining all demonstrated patterns 2 and 3 of subclonal staining of MLH1/PMS2 (Figure 2). Because these cases were reported as complete loss of MMR protein staining, the patients received appropriate clinical follow-up, as would cases with complete loss of nuclear staining of MLH1/PMS2. All 4 cases were negative for Lynch syndrome, 2 based on positive MLH1 promoter methylation status and 2 based on negative Lynch syndrome germline testing. The consistent presence of MLH1 promoter methylation in cases of subclonal MLH1/PMS2 staining has been observed in small case series of endometrial carcinomas.16,17 The report by Kato et al16 showed that MLH1 promoter methylation is detected in half of cases with isolated subclonal loss of PMS2 in endometrial carcinoma. No germline mutations were found in any of the above-referenced reports. Results from these small case series are similar to ours. The reporting recommendations for isolated subclonal staining of MLH1 or MLH1/PMS2 are addressed in the recommended terminology for reporting MMR protein IHC proposed by Casey and Singh.17 Per their recommendations, these cases should be reported as such but should require the same follow-up currently recommended for cases that display complete loss of expression for MLH1/PMS2.

Twenty-one cases with subclonal staining patterns of MMR proteins were reported as intact MMR protein expression (group 3) by the pathologists from our institution (Figure 2), concordant with the current College of American Pathologists reporting guideline.18 None of these cases triggered clinical follow-up testing for Lynch syndrome because, per the reporting guideline,18 this result is interpreted as “no loss of nuclear expression of MMR proteins; low probability of microsatellite instability-high (MSI-H).” A caveat following interpretation that “there are exceptions to the above IHC interpretations” meant that these results should not be interpreted in isolation and require clinical correlation, because this test in isolation is not perfect and false-negative results may occur. Therefore, genetic testing was performed on 2 patients, because of the presence of clinical risk factors, and both were negative for Lynch syndrome–associated mutations. The tumors from these 2 patients showed subclonal loss of MSH6/PMS2 in small areas consisting of groups of glands with loss of nuclear staining that made up 20% to 30% of tumor volume. One may wonder, if all cases from this group were reported as subclonal staining of MMR proteins instead of intact MMR protein staining, how many would ultimately result in Lynch syndrome germline mutations. The 4 cases from this group that showed pattern 2 and 3 subclonal MSH6/PMS2 loss of staining in large areas of tumor (60%–80%) become top candidates, because 2 of 5 of our cases with reported isolated subclonal MSH6 protein staining demonstrated similar percentages of expression loss (60% and 75%) and resulted in positive MSH6 germline mutations. We agree with Singh et al,28 who suggested an arbitrary cutoff of 10% for reporting of subclonal staining of MMR proteins. This cutoff allows avoidance of reporting of subclonal staining in cases where the subclonal staining is extremely focal and of unlikely clinical significance. This will also allow for more consistency in everyday pathology reporting of intraglandular subclonal staining (pattern 1), which may be difficult to distinguish from fixation artifact, especially when present in less than 10% of tumor.

The remaining 18 cases with subclonal MMR protein expression also displayed complete loss of either MLH1/PMS2 or MSH2/MSH6. The reporting of complete loss of expression of MMR proteins by the pathologists ensured that appropriate clinical follow-up testing was offered to this group of patients. We concur with the recommended terminology for reporting MMR protein IHC reviewed by Casey and Singh17 that in cases where subclonal staining is seen together with complete loss of staining of other MMR proteins, follow-up testing should be determined by the proteins that demonstrate complete loss of MMR protein expression.

We are aware that the POLE mutation, which is present in approximately 10% of endometrial carcinomas, may also be associated with subclonal MMR protein staining,15 and that this mutation is associated with very good endometrial carcinoma prognosis. In fact, based on the proposed algorithm for molecular classification of endometrial carcinomas,11 a positive POLE mutation test performed as the first step in the algorithm would exclude further testing of endometrial carcinomas for p53 and MMR mutations.
Because testing for POLE mutations of endometrial carcinomas is performed in only a very few laboratories in the United States, remains uncovered by most insurance providers, and is still not widely used, we believe that reporting of subclonal MMR staining pattern should be followed by additional testing for exclusion of Lynch syndrome germline mutations. Per Hall and Neumann, experts in the field of Lynch syndrome genetic testing, “ideally, genetic evaluation for Lynch syndrome should begin with a patient affected with a Lynch syndrome cancer.” Furthermore, steps included in their recommendations for a follow-up of an abnormal MMR protein IHC result incorporate tumor sequencing for evaluation of biallelic (double) somatic mutation MMR protein inactivation due to mutation and/or loss of heterozygosity after germline MMR panel testing.

Of note, the College of American Pathologists began offering the DNA MMR survey biannually since 2015 to more than 300 College of American Pathologists–accredited laboratories as part of surveys and anatomic pathology education programs. It is interesting that the recent survey from November 2020 had a question on laboratory approach for “subclonal loss (defined as subclonal positive and negative expression; areas with negative tumor cells should have positive staining stromal cells) of MMR protein staining by IHC.” The possible answer choices included “report the clonal loss of protein staining based on the initial stain; repeat IHC testing on the same block; repeat IHC testing on a different block; perform testing by an orthogonal technique; other; unsure; not applicable—do not perform MMR by IHC.” The summary of the survey results that became available for participating pathologists in February 2021 did not present data on this particular question, and therefore a true picture of the pathology practice regarding subclonal staining of MMR proteins across the United States is unknown at this time.

In summary, our study revealed that Lynch syndrome germline mutations can be detected in endometrial carcinoma patients whose tumors display pure subclonal MMR protein staining. Our results stress the importance of reporting subclonal staining patterns to ensure appropriate clinical follow-up and investigation for the exclusion of Lynch syndrome.

References
1. McConechy MK, Talhouk A, Li-Chang HH, et al. Detection of DNA mismatch repair (MMR) deficiencies by immunohistochemistry can effectively diagnose the microsatellite instability (MSI) phenotype in endometrial carcinomas. Gynecol Oncol. 2015;137(2):306–310.
2. Shikama A, Minaguchi T, Matsumoto K, et al. Clinicoopathologic implications of DNA mismatch repair status in endometrial carcinomas. Gynecol Oncol. 2016;140(2):226–233.
3. Bruegl AS, Ring KL, Daniels M, Fellman BM, Ronck SE, Psi RR, Longacre TA. Lynch syndrome screening should be considered for all patients with newly diagnosed endometrial cancer. Am J Surg Pathol. 2014;38(11):1501–1509.
4. Shahgali S, Haeelin K, O’Connell PR, Hanly AM, Martin ST, Winter DC. Lynch syndrome: an updated review. Genes (Basel). 2014;5(3):497–507.
5. Sehgal R, Singh N, POLE, MMR, and MSI testing in endometrial cancer: process recommendation of the ISGCp Companion Society session at the UCASP 2020 annual meeting. Int J Gynecol Pathol. 2021;40(1):5–16.
6. Fitzgibbon PS, Bartley AN, Longacre TA, et al. Template for reporting results of biomarker testing of specimens from patients with carcinoma of the endometrium-biomarker-19-2012.pdf. Accessed February 1, 2021.
7. Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome, part I: the utility of immunohistochemistry. J Mol Diagn. 2008;10(4):293–301.
8. Longacre TA, Broadus R, Chuang LT, et al. Template for reporting results of biomarker testing of specimens from patients with carcinoma of the endometrium. Arch Pathol Lab Med. 2017;141(11):1508–1521.
9. Dillon JL, Gonzalez JL, DeMars L, Bloch KJ, Tafe LJ. Universal screening for Lynch syndrome in endometrial cancers: frequency of germline mutations and identification of patients with Lynch-like syndrome. Hum Pathol. 2017;70:121–129.
10. Abu-Rustum NR, Yashar CM, Bradley K, et al. Uterine neoplasms. Version 1.2021. NCCN Clinical Practice Guidelines in Oncology. Published October 20, 2020. Accessed February 1, 2021.
11. Resnick KE, Hampel H, Fishel R, Cohn DE. Current and emerging trends in mismatch repair immunohistochemistry in endometrial cancer. J Clin Oncol. 2014;32(2):90–100.
12. Ferguson SE, Aronson M, Pollett A, et al. Performance characteristics of screening strategies for Lynch syndrome in unselected women with newly diagnosed endometrial cancer who have undergone universal germline mutation testing. Cancer. 2014;120(24):3932–3939.
13. Goodfellow PJ, Billingsley CC, Lankes HA, et al. Combined microsatellite instability, MLH1 methylation analysis, and immunohistochemistry for Lynch syndrome screening in endometrial cancers from COG210: an NRG Oncology and Gynecologic Oncology Group study. J Clin Oncol. 2015;33(36):4301–4308.
14. Hampel H, Frankel W, Panesu J, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res. 2006;66(15):7810–7817.
15. Mills AM, Liu S, Ford IM, Bonck SE, Psi RR, Longacre TA. Lynch syndrome screening should be considered for all patients with newly diagnosed endometrial cancer. Am J Surg Pathol. 2014;38(11):1501–1509.
16. Shahgali S, Haeelin K, O’Connell PR, Hanly AM, Martin ST, Winter DC. Lynch syndrome: an updated review. Genes (Basel). 2014;5(3):497–507.
17. Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome, part I: the utility of immunohistochemistry. J Mol Diagn. 2008;10(4):293–301.
18. Longacre TA, Broadus R, Chuang LT, et al. Template for reporting results of biomarker testing of specimens from patients with carcinoma of the endometrium. Arch Pathol Lab Med. 2017;141(11):1508–1521.
19. Dillon JL, Gonzalez JL, DeMars L, Bloch KJ, Tafe LJ. Universal screening for Lynch syndrome in endometrial cancers: frequency of germline mutations and identification of patients with Lynch-like syndrome. Hum Pathol. 2017;70:121–129.
20. Abu-Rustum NR, Yashar CM, Bradley K, et al. Uterine neoplasms. Version 1.2021. NCCN Clinical Practice Guidelines in Oncology. Published October 20, 2020. Accessed February 1, 2021.
21. Gupta S, Provenzale D, Llor X, et al. Genetic/familial high-risk assessment: colorectal. Version 1.2020. NCCN Practice Clinical Guidelines in Oncology. https://www.nccn.org/guidelines_CATEGORY_1. NCCN Clinical Practice Guidelines in Oncology. Published October 20, 2020. Accessed February 1, 2021.
22. Singh N, Wong R, Tcharkian N, Allen S-G, Clarke B, Gilks B. Interpretation and reporting terminology for mismatch repair protein immunohistochemistry in endometrial cancer. British Association of Gynaecological Pathologists Web site. https://www.thebagp.org/download/bagp-mmr-ihc-interpretation-june-2020. Published June 11, 2020. Accessed February 1, 2021.
23. Hall MJ, Neumann CC. Lynch syndrome (hereditary nonpolyposis colorectal cancer): clinical manifestations and diagnosis. UpToDate Web site. https://www.uptodate.com/contents/image/print?imageKey=GAST% 2F6J396&topicKey=CARD%2F105&source=see_link. Accessed July 10, 2021.