Identifying Lynch Syndrome in patients with gynecologic malignancies: Arriving where we started

Lynch Syndrome and Endometrial cancer

Lynch syndrome (LS) is an autosomal dominant cancer susceptibility syndrome in which patients are at significantly elevated lifetime risk for several cancers (colon, endometrium, ovary, stomach, ureteropelvic tract, small bowel and brain). It is attributable to inherited defects in the mismatch repair genes, \textit{MLH1}, \textit{PMS2}, \textit{MSH2}, \textit{MSH6} and \textit{EPCAM}. Lynch syndrome accounts for 2 to 6\% of endometrial cancer (EC) and about 1\% of ovarian cancer (OC). Conversely, women with LS have a 40 to 60\% and a 10 to 12\% lifetime risk of EC and OC respectively. Importantly, in about two-thirds of these women, a gynecologic malignancy will be the sentinel cancer. Thus successful identification of LS at this juncture will allow implementation of highly effective colon cancer screening and prevention programs, reducing morbidity and mortality. Furthermore, cascade testing of family members to identify carriers, will further allow appropriate surveillance and screening and possibly surgery to prevent cancer. Depending on the number of relatives identified per proband, this could ensure benefits for the health care system.

Thus there is growing impetus to identify underlying LS in patients presenting with appropriate cancer types. Optimal strategies to identify LS in gynecologic cancer patients are not settled. The traditional approach has been genealogy based, such as the Amsterdam and Bethesda criteria, which assess a patient’s personal and family history of cancer. However, such criteria lack the requisite sensitivity and specificity to triage patients and importantly are difficult to implement in a busy clinical setting. The colon-centric bias of the Amsterdam and Bethesda criteria mean they perform even more poorly in the setting of gynecologic cancer patients and Hampel and colleagues showed that 62\% of LS-EC patients did not meet either of these two guidelines. This prompted the Society of Gynecologic Oncologists (SGO) to propose two gynecologic-centric guidelines. However, studies have shown that these too lack the required performance characteristics failing to detect
a large proportion of LS patients who are older or have less significant family history.

In the setting of colorectal cancers, pathologists attempted to identify morphologic and other clinical features that could independently predict microsatellite instability. These parameters included tumor-infiltrating lymphocytes, proximal location, mucinous histology, poor differentiation, Crohn's-like reaction, and diagnosis before age 50 years. In population based studies the MsPath scoring system performed well in identifying MSI-H colon cancers. Despite this, reflex testing of colon cancer specimens using mismatch repair immunohistochemistry (MMR-IHC) has received broad support and is emerging as standard of care in North America. According to a recent survey, 71% of National Cancer Institute Comprehensive Cancer Centers, 36% of Community Hospital Comprehensive Cancer Programs, and 15% of Community Hospital Cancer Programs are now performing reflex screening for LS in cases of colorectal cancer. Similar morphologic criteria were applied to EC including the brisk intra- and peri-tumoral immune response, tumor heterogeneity in particular “dedifferentiated” histology as well as location of tumor in the lower uterine segment. While several studies have found utility in these morphologic criteria, others have questioned their efficacy as tools to triage patients for screening. Furthermore, one also needs to consider ease of implementation in a busy pathology service. It has also been recognized that restrictive age based criteria are problematic since many LS patients present with cancer over the age of 50 years, in particular those with MSH6 and PMS2 mutations.

Recognizing the shortcomings of the clinical and morphology based criteria, in 2010, at the Jerusalem Workshop on Lynch Syndrome, a panel of experts recommended reflex testing of all EC and colorectal cancer specimens using MMR-IHC to triage patients for genetic counselling and possible germline testing for Lynch Syndrome. Reflex testing of EC with MMR-IHC is being rapidly adopted in many centers in the United States and Canada. There is still controversy with some centers opting for reflex MMR-IHC testing of EC regardless of morphologic features while some have advocated a combination of patient age and morphologic features to direct screening.

**Lynch Syndrome and Ovarian cancer**

About 15% of ovarian cancers are hereditary and after BRCA1/2 syndrome, Lynch syndrome is the most frequently associated hereditary syndrome. Most studies of
EC in LS have shown a similar distribution of subtypes as the sporadic population. There have been only limited studies of LS associated OC and most of these did not include centralized pathology review. It must also be noted that designation of OC subtype has only recently become reproducible. Despite these limitations, most studies have suggested a predominance of endometrioid and clear cell subtypes in LS cohorts. This is further supported by the finding that most LS associated OCs usually present at early stage (stage I/II) which would be unusual for high grade and low grade serous carcinoma. Several recent tissue microarray based studies of OC cohorts classified according to contemporary criteria have shown that mismatch repair deficiency is almost exclusively seen in the “endometriosis” associated OC tumor types, namely endometrioid, clear cell and undifferentiated subtypes. This has led to recommendations for a histotype specific LS screening approach in OC.

**Recent advances:**

**Biallelic somatic mutations in mismatch repair genes**

There is a subset of cases of EC showing loss of expression of mismatch repair protein(s), for which neither a germline mutation nor MLH1 methylation can be identified. While this may be attributable to incorrect interpretation of the immunohistochemical staining or a missed germline mutation, recent studies have highlighted that more than half of such cases can be explained by either biallelic somatic mutations or a somatic mutation and loss of heterozygosity in the tumor. As a result, tumor DNA sequencing of the mismatch repair genes should be performed in those cases with immunohistochemical deficiency but lacking a germline mutation.

**Interpretation of immunohistochemical staining patterns**

The typical expression patterns of the mismatch repair proteins are intact/retained expression in tumor cell nuclei or complete absence of expression in tumor cell nuclei with retained staining of lymphocytes or adjacent non-tumor tissue as an internal control. The implementation of reflex testing has led to observations and molecular correlation of less common patterns of staining typically referred to as heterogeneous staining. Typically, one may see patchy staining of the mismatch repair markers in which tumor cells lacking expression are closely admixed with tumor cells showing strong expression. This pattern is typically regarded as retained. However, the heterogeneous patterns encountered below refer to abrupt...
loss of expression in regions/groups of glands within a tumor otherwise showing retained expression.

Pai and colleagues recently described a pattern of abrupt loss of MLH1 and PMS2 expression in a portion of the tumor in 6 cases of EC. In half of these cases, the tumor areas of retained and absent staining were morphologically distinct. They showed that the areas with MLH1/PMS2 loss were more likely to be microsatellite unstable and demonstrate \textit{MLH1} promoter methylation. Although areas with retained expression might also show \textit{MLH1} promoter methylation, this was typically less compared to areas of absent expression. Thus they attributed this pattern of abrupt loss of MLH1/PMS2 in only a portion of the tumor as clonal evolution of \textit{MLH1} methylation.

Heterogeneous loss of MSH6 expression has also been described both in colorectal cancer and EC.

This can be seen in two settings:
1. In cases with intact MLH1, PMS2 and MSH2 but which have undergone neo-adjuvant chemotherapy or chemoradiation.
2. In cases with absence of expression of MLH1/PMS2, either attributable to somatic or germline events.

Shia and colleagues demonstrated that this heterogeneous pattern in the second setting is attributable to somatic mutation in the coding region microsatellite of the \textit{MHS6} gene. The same phenomenon in the two scenarios listed above, has recently been reported by the Mayo clinical group (Graham et al) in 3 cases of endometrial endometrioid carcinoma. Two of these cases were status post neoadjuvant chemoradiation, were intact for the other three MMR markers (MLH1, PMS2 and MSH2) and were microsatellite stable. The third case showed heterogeneous MSH6 expression and complete absence of expression of MLH1/PMS2. Although not interrogated in the paper, based on the age of this patient, the MLH1/PMS2 loss is most likely due to \textit{MLH1} promoter methylation. Importantly, it must be remembered that although MSH6 heterogeneous expression is a secondary somatic event, it may be associated with a germline event in another of the MMR genes.

More widespread use of MMR-IHC in EC, has also highlighted mechanisms accounting for isolated PMS2 loss of expression. Dudley and colleagues showed that in 8 EC patients with isolated loss of PMS2 expression, who underwent germline testing, 3 had deleterious mutations in \textit{PMS2}, 2 had deleterious mutations in \textit{MLH1} and 3 lacked mutations in either gene. Thus they identified the presence
of “immunohistochemically stable” mutations in MLH1. Such mutations resulted in retained MLH1 protein expression but loss of PMS2 expression which is most likely attributable to instability of the MLH1-PMS2 heterodimer with degradation of PMS2.

Overall in this study when considering colorectal cancer and EC, 24% of patients with isolated loss of PMS2 expression harbored germline MLH1 mutation. The authors recommend that patients with colorectal or EC with isolated loss of PMS2 should have germline analysis of MLH1 performed if no mutation is identified in PMS2. Similar recommendations for testing of MLH1 in the setting of isolated PMS2 loss with no PMS2 mutation are made by the National Comprehensive Cancer Network guidelines and the Guidelines from the National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Colorectal Cancer.

Another explanation for isolated PMS2 immunohistochemical expression in EC patients proposed by Kato and colleagues is that of heterogeneous MLH1 promoter methylation. They identified 8 cases of EC with isolated PMS 2 loss on IHC. Heterogeneous MLH1 staining and MLH1 promoter methylation were detected in 4 of 8 (50%) of isolated PMS2 deficient tumors. Of the remaining 4 cases lacking MLH1 promoter methylation, one was found to harbor a germline PMS2 mutation, in one cases no mutation was found in either MLH1 or PMS2 and in the remaining 2 cases, patients did not undergo germline testing.

Thus when dealing with isolated PMS2 loss of expression, if one has excluded a PMS2 germline mutation, one should consider the possibility of mutation or promoter methylation in MLH1. The latter scenario is often accompanied by heterogeneous MLH1 expression.

Select References


