Q&A column, 12/16

Editor: Frederick L. Kiechle, MD, PhD

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Submit a Question

Q. Are there guidelines on microsatellite instability analysis by immunohistochemistry on colorectal adenocarcinomas? Specifically, should immunohistochemical stains for the mismatch repair enzymes be performed on all colorectal adenocarcinomas regardless of the clinical or pathological findings? A medical group recently requested these studies on all colorectal adenocarcinomas.

A. Lynch syndrome (LS) is a hereditary cancer predisposition syndrome, usually owing to germline mutation of a DNA mismatch repair (MMR) gene: MLH1, PMS2, MSH2, or MSH6. With a population prevalence of 1:500 to 1:2,000, LS is the most common hereditary cause of colorectal cancer (CRC), accounting for two to four percent of all CRCs. CRCs in LS tend to be relatively early onset, to involve the right colon, and to have characteristic though not diagnostic histologic features, including mucinous or medullary histology and a prominent tumor-associated lymphoid response. Synchronous and metachronous tumors are not uncommon. Patients with LS are also at significant risk for other tumors, especially endometrial adenocarcinoma but also upper tract urothelial carcinoma, upper gastrointestinal and nonserous ovarian adenocarcinoma, glioblastoma, sebaceous neoplasm, and keratoacanthoma.

LS-associated CRCs nearly always demonstrate immunohistochemical loss of one or more of the DNA MMR proteins. Deficient DNA MMR (dMMR) function leads to microsatellite instability (MSI), the latter detected by molecular testing. The results of these two assays, MMR IHC and MSI testing, are thus highly concordant. In addition to LS, 15 percent of sporadic CRCs are dMMR/microsatellite unstable owing to silencing of MLH1 by promoter methylation.

In the past, CRCs were selected for LS screening based on clinical criteria, histologic features, patient age, or, frequently, on an ad hoc basis. These strategies have been shown to underperform in clinical practice. Performing MMR IHC (and/or MSI testing) on all CRCs is referred to as "universal testing," and this strategy is gaining traction. The Evaluation of Genomic Applications in Practice and Prevention Working Group and the Association for Molecular Pathology Mismatch Repair Defective CRC Working Group have endorsed universal testing.[1,2] National Comprehensive Cancer Network guidelines co-endorse either universal testing or testing all patients 70 with a family history concerning for LS.[4] The USMSTF guideline states that "universal testing is likely to become the future national standard of care."

Universal LS screening in CRC has been shown to be cost-effective, with an incremental cost-effectiveness ratio similar to that for screening colonoscopy.[5] Cost-effectiveness is largely attributable to the subsequent identification of at-risk family members, who benefit from intensive colonoscopic surveillance. LS-specific surveillance also includes annual endometrial sampling, transvaginal ultrasound, and urine cytology. Given a significant risk for metachronous CRC, patients with LS may be counseled to undergo a subtotal (rather than segmental) colectomy; as such, it is preferable to perform LS-screening on initial biopsy material, if available.

Furthermore, the determination of MMR/MSI status in CRC is prognostic and predictive.6 This is an additional motivator for universal testing. dMMR/MSI is prognostically favorable (hazard ratio, 0.65) and tumors show relative resistance to 5-fluor-ouracil-based chemotherapy. Oncologists are less likely to give adjuvant chemotherapy to patients with dMMR/microsatellite unstable stage II CRC. Recently, in patients with progressive metastatic CRC,

dMMR/microsatellite unstable status has been shown to predict response to anti-PD-1 immunotherapy.[7]

Universal testing strategies may be MMR IHC and/or MSI-based. Among cancer centers responding to a national survey on LS screening practices, 48 percent used IHC, 38 percent used combined IHC and MSI testing, and 14 percent used MSI testing.[8] The advantages of immunohistochemistry include its more widespread availability, rapid turnaround time, requirement of only limited amounts of tumor tissue, lack of need for matched non-tumor DNA, and ability to suggest which MMR gene may be abnormal, which directs the subsequent patient workup.[6]

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Q. Why does the CAP use a Wright-Giemsa (or Wright) stain for the urine eosinophils on proficiency testing if the Hansel stain is supposedly the preferred method of staining?

A. Eosinophils in the urine are difficult to identify without the Wright, Wright-Giemsa, or Hansel stains. Studies comparing performance of these stains date back to the late 1980s. The Hansel stain (methylene blue and eosin-y in methanol) was hailed as superior to the Wright stain for identifying urine eosinophils in a 1986 New England Journal of Medicine article. In studies, the reported sensitivities for the Hansel stain for urine eosinophil detection were 63 to 91 percent versus 18 to 85 percent for the Wright stain.[1-3]

Yet, before I address the question, it would be productive to first discuss the clinical implications of finding urine eosinophils (UEs). The finding of eosinophils in the urine, or eosinophiluria, was initially touted as a useful, noninvasive test in the diagnosis of acute interstitial nephritis. AIN is clinically suspected in a patient who presents with acute renal failure, sterile pyuria, and exposure to a drug known to cause AIN. Commonly implicated in drug-induced AIN are methicillin, cephalosporins, rifampin, sulfonamides, anticonvulsants, nonsteroidal anti-inflammatory agents, cimetidine, allopurinol, azathioprine, alpha-methyldopa, and interferon. For years, detection of UEs has been used as the biomarker of choice for general internists in diagnosing AIN. However, eosinophiluria is also associated with myriad other conditions, including (but not limited to) transplant rejection, prostatitis, parasitic infections (such as schistosomiasis), rapidly progressive glomerulonephritis, atheroembolic disease, and ileal conduits.[4] Thus, eosinophiluria must be interpreted in the appropriate clinical context.

The UE test is considered positive when eosinophils compose greater than one percent of total white cells. This is further categorized as low-grade (one to five percent of WBCs) or high-grade (greater than five percent) eosinophiluria, with high-grade eosinophiluria considered a strong indicator of acute interstitial nephritis.[1,3,5] For the purposes of CAP proficiency testing, the choices for urine eosinophils are present versus absent. In a recent informal poll, it was found that some large reference laboratories perform the Hansel stain, while Wright and/or Wright-Giemsa stains are used successfully in the majority of laboratories. To answer the reader's question: We suspect that the majority of laboratories chose to use the more familiar Wright/Wright-Giemsa stain rather than have two separate stains to maintain and validate. For this reason, proficiency testing images used by the CAP are stained with the more familiar Wright/Wright-Giemsa stain.

Having said this, a study published in 2013 from the Mayo Clinic (which uses the Hansel stain) found that UEs performed poorly in distinguishing AIN from other kidney diseases. Urine eosinophils were positive in 29 percent of cases of AIN, essentially equivalent to UE positivity found in 31 percent of acute tubular necrosis cases. Furthermore, about 70 percent of biopsy-confirmed acute interstitial nephritis cases were UE negative. The positive predictive value was 15.6 percent and the negative predictive value was 83.7 percent at the greater than one percent UE cutoff.[6] Another author bemoans the use of urine eosinophils and cites an even more dismal PPV of three percent for AIN in his institution.[7] In aggregate, these show that urine eosinophil testing (even those detected with the Hansel stain) performs poorly compared with the gold standard of renal-biopsy–proven AIN. With these test performance characteristics, one may wonder if there is any clinical utility to UE testing. As the authors of a nephrology journal editorial posit: Is it time to say "farewell to an old biomarker?"[8]

Nevertheless, it would be of great interest to know what stains laboratories are, in fact, currently using for identification of urine eosinophils in a more formal manner. To address this issue, we will attempt to collect data by including this as a supplemental question in an upcoming urine eosinophil CAP proficiency test.

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