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“FISHING” FOR AN ACCURATE DIAGNOSTIC TEST FOR CHOLANGIOCARCINOMA

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Navaneethan U, Njei B, Venkatesh PG, et al. Fluorescence in situ hybridization for diagnosis of cholangiocarcinoma in primary sclerosing cholangitis: a systematic review and meta-analysis. Gastrointest Endosc 2014;79:943–950.

Primary sclerosing cholangitis (PSC) is a chronic fibroinflammatory condition epitomized by progressive obliteration of the biliary tree. Although the natural history is variable, secondary biliary cirrhosis and end-stage liver failure occur in 50% of patients. In addition to managing the symptoms of PSC (predominantly pruritus and recurrent acute cholangitis), clinicians and patients remain vigilant for the interval development of cholangiocarcinoma (CCA), the lifetime incidence of which is 6%–20% (Am J Gastroenterol 2007;102:107–114).

With improvements in cross-sectional imaging, PSC diagnosis and surveillance can be accomplished without the routine use of endoscopic retrograde cholangiopancreatography (ERCP). However, individuals with symptoms or signs of disease progression are referred for ERCP to palliate dominant strictures via dilation with or without short-term stent placement, and tissue sampling to evaluate for CCA. In this context, traditional intraductal tissue sampling techniques include brushings for cytology and forceps biopsies for histology. These modalities, alone or in combination, have limited (20%–60%) sensitivity for confirming CCA owing to the desmoplastic nature of these lesions, so clinicians cannot definitively “rule out” CCA with a negative result (Gastrointest Endosc 1995;42:565–572). Because cytopathology and histopathology are essentially qualitative tests that rely on a subjective assessment of cellular morphology, fluorescence in situ hybridization (FISH) has been studied as a supplementary assay. FISH is performed on intraductal brushing samples and uses fluorescently labeled probes that target the centromeres of chromosome 3, 7, and 17, as well as the 9p21 band (P16), evaluating for aneuploidy (abnormal number of chromosomes) by counting the number of affected cells; therefore, it is a quantitative test that requires theoretically fewer cells than cytopathology and removes the subjectivity associated with cytopathologic interpretation.

Navaneethan et al (Gastrointest Endosc 2014;79: 943–950. e3) performed a structured metaanalysis of studies assessing the diagnostic utility of FISH in diagnosing CCA among patients with PSC. The authors included studies that allowed construction of a 2 × 2 contingency table with true-positive, false-negative, false-positive, and true-negative results. The authors used standard metaanalytic techniques to calculate composite sensitivity, specificity, likelihood ratios, and diagnostic odds ratio. There was moderate statistical

heterogeneity among included studies, so the authors used a random effects model. A positive FISH for malignancy was defined as either polysomy (≥ 5 cells with gains of ≥ 2 of 4 probes) or trisomy/tetrasomy (trisomy, ≥ 10 cells with 3 copies of chromosome 7 or 3; tetrasomy, ≥ 10 cells with 4 copies of all probes).

Eight studies of moderate to high methodologic quality (n = 828 individuals with PSC) met eligibility criteria. The pooled analysis from these studies demonstrated that FISH had an overall sensitivity of 68% (95% CI, 61%–74%), and specificity of 70% (95% CI, 66%–73%) for diagnosing CCA. The pooled positive likelihood ratio was 2.69 (95% CI, 1.84–3.97), and the negative likelihood ratio was 0.47 (95% CI, 0.39–0.58). The pooled odds ratio to detect CCA was 7.24 (95% CI, 3.93–13.36). The positive predictive value was 0.42 (95% CI, 0.36–0.47), and the negative predictive value was 0.87 (95% CI, 0.84–0.90). Stated alternatively, an individual with PSC and positive FISH had a 42% posttest probability of CCA and an 87% probability of being cancer free after negative FISH.

Two of the included studies described the accuracy of FISH in the setting of negative cytology, reporting sensitivities of 57% (Gastroenterology 2006;131:1064–1072) and 60% (Am J Gastroenterol 2008;103:1263–1273) and specificities of 87% and 71%. This is the context in which FISH would be considered most valuable: when CCA is present, but routine cytology is negative for malignancy, FISH will be positive in ≥ 60% of cases. However, there remains a possibility that FISH is falsely positive, as reflected its lower specificity. The specificity of FISH seemed to be optimized (93%) by defining a positive test using polysomy alone (6 of 8 included studies), but this compromised the technique's sensitivity (51%). The authors systematically removed 1 dataset at a time and recalculated their outcomes; no single dataset carried sufficient weight to influence significantly the pooled performance of FISH for diagnosing CCA in the setting of PSC.

Comment

Indeterminate bile duct strictures—particularly those in the setting of PSC—remain a diagnostic conundrum. Improvements in cross-sectional imaging have done little to improve our ability to detect CCA, and the absence of an accurate blood marker means patients are often obligated to close surveillance and repeated diagnostics. Although highly specific (99%), traditional ERCP-based tissue sampling techniques—intraductal brushings and biopsies for cytology and histology, respectively—have limited sensitivity (20–60%) despite the advent of single operator cholangioscopy. To optimize the diagnostic yield of intraductal brushings, the American Society for Gastrointestinal Endoscopy recommends ≥ 5 passes to and fro across the stricture, removal of the brush and the catheter together, and inclusion of washings from the brush catheter in the specimen (Gastrointest Endosc 2006;63:741–745). This technique helps, but is not the panacea. In comparison with fluoroscopically guided biopsies, the incremental benefit of site-directed forceps biopsies using cholangioscopy is controversial (Gastrointest Endosc 2012;75:347–353; Clin Gastroenterol Hepatol 2012;10:1042–1046). Endoscopic ultrasonography (EUS)-fine needle aspiration (FNA) and intraductal ultrasonography have added marginally to the performance of ERCP, particularly in the setting of a distal bile duct stricture, but neither has adequate sensitivity to rule out the potential for missed cancer (Gastrointest Endosc 2011;73:71–78). Furthermore, EUS-FNA

may compromise subsequent operative resection via tumor tracking through the gastroduodenal wall. Enhanced bile duct imaging techniques, such as digital image analysis and confocal endomicroscopy, remain unproven because requisite criteria to achieve a sensitivity of >90% usually results in such a low specificity that clinical management is unaffected (eg, a positive confocal examination is not sufficiently accurate to prompt referral for chemoradiotherapy, because the false-positive rate is too high; Dig Dis Sci 2013;58:2068–2074; Dig Dis Sci 2013;58: 1784–1789). It is difficult to envision a mucosal-based imaging test that could outperform tissue sampling, given these lesions typically result in a denuded or ulcerated mucosa and are predominantly fibrotic.

Has FISH simplified our role as diagnosticians? Navaneethan et al's structured metaanalysis reminds us that FISH is not the answer, but brings us a little closer. The principal advantages of FISH are its presumed lack of inter-observer variability and technical simplicity from the endoscopist's perspective. Of course, the definition of a positive FISH is a moving target, so that "easier" criteria such as trisomy will result in lower specificity. In any case, positive FISH does not equate to positive cytopathology, because the latter has a specificity of >99% at most institutions. We would argue that, for the patient suffering with PSC, a false-positive FISH is a mixed blessing: Some may progress to more urgent orthotopic liver transplantation—and in most cases be cured of their underlying disease—whereas others may undergo unnecessary chemoradiotherapy for a nonexistent CCA (Dig Dis 2013;31:126–129). Clearly, these decisions require a multidisciplinary team composed of the patient and their family along with experts in transplant, oncology, and gastroenterology. FISH adds to a complex algorithm composed of patient symptoms and clinical course (such as response to endoscopic treatment of a dominant stricture), serum carbohydrate antigen 19-9, cross-sectional imaging, cytology, and histology. Before adding FISH to your diagnostic armamentarium, it would be prudent to discuss the existing evidence with relevant subspecialists in your area to assure that a positive FISH will impact clinical decision making.

To minimize the need for repeat tissue sampling, we routinely perform FISH on all patients with potentially malignant bile duct strictures at the time of initial ERCP, assuming an antecedent, same-day EUS fails to confirm the presence of cancer. Despite the limitations of FISH, for patients who are candidates for operative resection (partial hepatectomy or transplantation), a positive FISH jumpstarts the process of referral to appropriate subspecialists. A negative FISH provides additional reassurance to negative cytology and surgical pathology reports. Because none of these modalities has a sensitivity of >95%, few patients can be fully dismissed from surveillance based on a negative FISH. The added costs to perform FISH should be considered, but this is probably offset by some reduction in the need for or frequency of downstream testing that often includes repeat ERCP; however, this is unproven.

Even when a solid lesion is evident on cross-sectional imaging in the setting of bile duct obstruction, 20% of individuals with cancer remain undiagnosed after the first biopsy attempt (Clin Gastroenterol Hepatol 2014;12: 1717–1723). This is partly responsible for the exponential (>5-fold) increase in EUS utilization in the first decade of the 21st century (Gastroenterology 2012;143:1179–1187 e1–3). Sending patients with unproven cancer to the

operating room is unappealing because neoadjuvant chemoradiotherapy is improving and the 5-year survival for patients who undergo “complete” resection of CCA and other periampullary cancers peaks at 20% (BMC Cancer 2014;14:411). Sometimes, operative exploration is preferred to indefinite close surveillance, but this can only be determined on a case-by-case basis. The ideal diagnostic test for CCA would be a novel biomarker panel derived from blood, bile, urine, or some combination; these specimens are easier to procure than bile duct biopsies and could be analyzed for the differential expression of microRNA (Am J Gastroenterol 2014;109:1942–1952), lipids (Am J Gastroenterol 2014;109:895–902), and proteins (Gut 2013;62: 122–130). The development of these markers is in their nascence, but they have the greatest potential to disrupt the complex diagnostic algorithms currently applied in clinical practice.

The bile duct stricture diagnostic toolbox is growing, with newer tools being FISH, cholangioscopy with or without site-directed biopsies, and “optical” biopsies using confocal endomicroscopy. Of these, the strongest evidence is in support of FISH. Keeping this complex literature in perspective, if there were a multiple choice examination querying the best diagnostic test for indeterminate bile duct strictures, potential answers might include clinical history, cholangiographic features, cytology brushings, forceps biopsies, EUS-FNA, and FISH. Although the options increase in number (every test takers nightmare), the correct answer remains “all of the above.” We should calibrate our practices accordingly.