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IMPROVING THE DIAGNOSTIC ACCURACY OF ENDOSCOPIC ULTRASOUND–GUIDED FINE-NEEDLE ASPIRATION USING MICRORNAS

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Brand RE, Adai AT, Centeno BA, et al. A microRNA-based test improves endoscopic ultrasound–guided cytologic diagnosis of pancreatic cancer. Clin Gastroenterol Hepatol 2014;12:1717–1723.

Owing to its safety profile, diagnostic accuracy, and low risk of tumor seeding, endoscopic ultrasound–guided fine-needle aspiration (EUS-FNA) represents the preferred modality for achieving a tissue confirmation of pancreatic ductal adenocarcinoma (PDAC; J Cancer Res Clin Oncol 2012;138: 1433–1441; Gastrointest Endosc 2003;58:690–595; Endoscopy 2011;43:897–912). In a recent metaanalysis that included 41 studies and 4766 procedures, EUS-FNA had a pooled sensitivity of 87% and specificity 96% for the diagnosis of malignant pancreatic lesions (Pancreas 2013;42: 20–26). However, the sensitivity of EUS-FNA is lower in the setting of chronic pancreatitis (CP), where false-negative rates may be as high as 40% (Gastrointest Endosc 2005;62: 728–736l; Gastrointest Endosc 2009;70:70–79). Thus, there is substantial interest in improving the accuracy of cytopathology for diagnosing PDAC—and potentially informing long-term prognosis of this deadly cancer—using novel molecular markers such as microRNAs (miRNAs; Clin Cancer Res 2011;17:5812–5821). miRNAs are small, noncoding RNAs (17–25 nucleotides long) that regulate gene expression post-transcriptionally. These molecules are stable and easily recovered from tissue or blood, making EUS-FNA specimens suitable for analysis (Clin Chem 2008;54:1716–1724). Unique miRNA “signatures” have been evaluated using formalin-fixed, paraffin-embedded specimens, having >90% accuracy in distinguishing PDAC from CP and normal pancreata in limited studies (Clin Cancer Res 2011;17:5812–5821; Clin Chem 2008;54:1716–1724; Int J Cancer 2012;131:E86–95; Expert Rev Mol Diagn 2011;11:249–257).

Using samples (n = 95) derived from formalin-fixed, paraffin-embedded tissues of PDAC and CP, Brand et al (Clin Gastroenterol Hepatol 2014;12:1717–1723) developed a miRNA panel and then analyzed its utility as a diagnostic test using pancreas tissue specimens (n = 229) derived from EUS-FNA. Starting with 11 candidate miRNAs applied to formalin-fixed,

paraffin-embedded specimens (PDAC, n =52; CP, n = 43), the authors identified a panel of 5 miRNAs (−24, −130b, −135b, −148a, and −196a) having >95% agreement compared with standard histopathology when the miRNA classifier score was ≥0.5. Then, the authors applied this panel using polymerase chain reaction amplification techniques to EUS-FNA specimens prospectively collected at 8 participating centers. Only 1 FNA pass was dedicated for miRNA analysis, yet the rate of failed polymerase chain reaction amplification was only 1 in 229 (0.4%).

Based on standard histopathology, the pretest probability of cancer was 202 in 228 (89%), with 184 of 202 cancers being PDAC (excluding non-PDAC cancers, the pre-test probability of PDAC was 184 of 202 [88%]). Although the prevalence of concomitant CP is not provided—the most common scenario in which the sensitivity of EUS-FNA is diminished—the sensitivity of cytopathology for detecting PDAC was 79% (145/184). By comparison, the miRNA panel correctly diagnosed PDAC in 152 of 184 patients (83%). The combination of cytopathology + miRNA resulted in a sensitivity/specificity of 167 of 184 (91%) and 25 of 26 (96%). Including 1 case of cholangiocarcinoma, the miRNA panel accurately detected adenocarcinoma in 130 of the 146 cases detected by cytopathology, with an 11% false-negative rate. In the setting of negative cytopathology and an miRNA panel score of <0.5, the posttest probability of PDAC (the false-negative rate of EUS-FNA cytopathology + miRNA) was 17 of 43 (40%).

Because the sensitivity of achieving a diagnosis of PDAC increased from 79% using cytopathology alone to 91% when cytopathology was combined with an elevated miRNA panel, the authors concluded that a 5-miRNA classifier panel improves the performance characteristics of EUS-FNA.

Comment

Although EUS-FNA is safe and accurate, cytopathology may be falsely negative in selected cases of PDAC, particularly in the setting of concomitant CP. Strategies to optimize the sensitivity of EUS-FNA include performing ≤7 sequential passes and using on-site cytopathology (Gastrointest Endosc 2004;59:475–481; Clin Gastroenterol Hepatol 2012;10:697–703). Enhanced ultrasound imaging, including elastography and the use of intravenous contrast agents, may improve the endosonographic visualization of pancreas lesions, but have not replaced the need for tissue sampling (Endoscopy 2010;42:564–570; Gastrointest Endosc 2012; 76:953–961). Although cytopathology is generally adequate for confirming a diagnosis of PDAC, cellular morphology/ grade and tumor staging do not adequately inform long-term prognosis in terms of survival and predicted response to medical or surgical therapy.

To complement cytopathology, previous studies have explored the incremental yield of KRAS staining and fluorescence in situ hybridization analysis using EUS-FNA specimens, both of which improve the sensitivity of EUS-FNA (Gastrointest Endosc 2013;78:596–608; Gastrointest Endosc 2011;74:541–547; J Gastroenterol Hepatol 2014). However, the prevalence of KRAS mutations in the setting of CP lead to false positive results in >10% of cases, and the specificity of fluorescence in situ hybridization in this setting is probably

<95% (Gastroenterology 1996;110: 227–231; Pancreas 2011;40:1057–1062). Consequently, differential miRNA expression in tissue specimens has been explored as a quantitative method of augmenting cytopathology for the diagnosis and prognostication of individuals with PDAC. A previous study measuring miR-10b expression in EUS-FNA tissue samples revealed an association between decreased miR-10b expression in cancer cells with improved survival, response to neoadjuvant radiochemotherapy, and delayed time to metastasis (Clin Cancer Res 2011;17:5812–5821). Brand and colleagues are the first to develop and validate a miRNA panel derived from EUS samples that were prospectively collected at multiple centers. The authors' reported sensitivity of EUS-FNA cytopathology (79%) from pancreatic lesions is lower than expected, although we do not know the prevalence of concomitant CP, the number of EUS passes sent for cytopathologic review, or the use of onsite cytopathology; these factors are associated with a higher diagnostic yield from EUS-FNA (Gastrointest Endosc 2004;59:475–481; Am J Gastroenterol 2011;106:1705–1710). Importantly, the authors only performed 1 dedicated pass for miRNA analysis, yet the failure rate for polymerase chain reaction amplification (1/229) was very low. However, given the heterogeneity of pancreatic lesions, a second FNA pass would have allowed for confirmatory evaluation of the results. Potentially, dedicated passes for miRNA profiling would allow for a reduction in needle passes required for cytopathology, which could reduce procedure complications and the need for multiple needles, and provide faster times to complete EUS-FNA.

The addition of the authors' miRNA panel identified adenocarcinoma in 130 of 146 cases already detected by cytopathology, correctly detected 22 additional cases of PDAC among 39 samples with negative cytology, and resulted in 1 false-positive result. To minimize the likelihood of administering systemic chemotherapy to a patient without cancer, oncologists and cytopathologists expect nearly 100% specificity for cancer diagnostics. Similar to fluorescence in situ hybridization in the setting of indeterminate bile duct strictures, the authors' miRNA panel may be susceptible to occasional false positivity (Cancer Cytopathol 2013;121:708–717). In developing this miRNA panel, the pretest probability of PDAC was 88%; such a high pretest probability minimizes the ramifications of a diagnostic test with lower specificity. For example, in a cohort study that includes all patients with CP undergoing EUS-FNA, where the pretest probability will be much lower, the number of false-positive EUS-FNA samples sent for miRNA analysis will increase. Therefore, although further studies are needed to confirm these findings, a positive miRNA panel may prompt the clinician to pursue more aggressive follow-up testing, as opposed to proceeding directly with cancer treatment.

The utility of miRNA may extend beyond tissue samples. miRNA expression in bodily fluids (blood, urine, feces, bile, and pancreatic juice) may enhance or replace carbohydrate antigen 19-9 for (1) screening for and diagnosing PDAC in high-risk populations (JAMA 2014;311:392–404); (2) monitoring or predicting response to systemic therapy (Clin Cancer Res 2011;17:5812–5821); and (3) enhancing the diagnostic yield of ERCP-based tissue sampling in the setting of concomitant biliary obstruction (Hepatology 2014). The latter would be particularly useful in the setting of proximal bile duct strictures, where the sensitivity of EUS-FNA is <60% (Gastrointest Endosc 2011;73:71–78).

As acknowledged by the authors, a combination of negative cytopathology and miRNA profiling still resulted in a high (40%) posttest probability of cancer. Clearly, these patients cannot be dismissed from cancer surveillance. Currently, clinicians gauge their suspicion of pancreatobiliary malignancy on the clinical presentation (including patient age, and “red flag” symptoms such as unexplained weight loss and obstructive jaundice), imaging presence of an overt mass or duct obstruction, and occasionally serum carbohydrate antigen 19-9 level, despite its diminished sensitivity and specificity in the setting of bile duct obstruction. In our practice, if clinical suspicion is moderate or high, EUS-FNA with inconclusive cytopathology typically prompts a second EUS-FNA. In many cases, cancer is often confirmed during the second intervention (J Gastroenterol Hepatol 2008;23:567–570; J Med Assoc Thai 2012;95 Suppl 2:S68–74), although many of us remain unsettled when the cytopathology is incongruent with our clinical suspicion. This may lead to unnecessary follow-up testing, improper referral for surgical resection, or a delay in diagnosis. For these reasons, miRNA analysis of FNA specimens may inform clinicians to pursue a more or less aggressive surveillance program. Brand and colleagues add to a burgeoning field of miRNA-based cancer diagnostics that is expected to transform and rationalize our approach to patients with suspected pancreatobiliary cancer.