Differentiating Neoplastic From Benign Lesions of the Pancreas: Translational Techniques

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There has been substantial recent progress in our ability to image and sample the pancreas leading to the improved recognition of benign and premalignant conditions of the pancreas such as autoimmune pancreatitis (AIP) and mucinous lesions (mucinous cystic neoplasms [MCN] and intraductal papillary mucinous neoplasms [IPMN]), respectively. Clinically relevant and difficult situations that continue to be faced in this context include differentiating MCN and IPMN from nonmucinous pancreatic cysts, the early detection of malignant degeneration in MCN and IPMN, and accurate differentiation between pancreatic cancer and inflammatory masses, especially AIP. These challenges arise primarily due to the less than perfect sensitivity for malignancy utilizing cytological samples obtained via EUS and ERCP. Aspirates from pancreatic cysts are often paucicellular further limiting the accuracy of cytology. One approach to improve the diagnostic yield from these very small samples is through the use of molecular techniques. Because the development of pancreatic cancer and malignant degeneration in MCN and IPMN is associated with well studied genetic insults including oncogene activation (eg, k-ras), tumor suppressor gene losses (eg, p53, p16, and DPC4), and genome maintenance gene mutations (eg, BRCA2 and telomerase), detecting these molecular abnormalities may aid in improving our diagnostic accuracy. A number of studies have shown the utility of testing clinical samples from pancreatic lesions and bile duct strictures for these molecular markers of malignancy to differentiate between cancer and inflammation. The information from these studies will be discussed with emphasis on how to use this information in clinical practice.

Pancreatic Cystic Neoplasms

To optimize the evaluation and management of pancreatic cystic neoplasms (PCN), an accurate differentiation between benign, premalignant, and malignant PCN is required. Benign PCN, eg, serous cystadenomas, when asymptomatic should be followed conservatively, while resection should be considered for mucinous PCN, including mucinous cystadenomas (MCN) and intraductal papillary mucinous neoplasia (IPMN) due to the risk of malignancy developing.[1,2] Recently, asymptomatic pancreatic cysts are coming to attention with increasing frequency.[1–3] Of concern, most of these represent premalignant PCN.[2,3] Unfortunately, the natural history of these often small, asymptomatic lesions remains unclear, as does therefore the optimal management. Emerging data suggest that the risk of malignancy being present in an asymptomatic PCN less than 3 cm in size with no concerning features on imaging may be less than 4%.[4,5] Of course, most such evolving information is based on data abstracted from patients that have undergone resection, interjecting various biases. In other words, it remains unclear what proportion of patients with PCN who do not undergo resection have malignancy or how long will it take for malignancy to develop. As such, clinicians use multiple different sources of information, eg, historical (patient age, symptoms, comorbidities), imaging (primarily cross-sectional and EUS), and cyst fluid analysis (primarily cytology and carcinoembryonic antigen [CEA] level), for clinical decision-making. This information is affected by and combined with a number of factors including available expertise, practice patterns, and patient preference and anxiety level that then lead to a compromise treatment or surveillance strategy. Additional issues pertaining to the evaluation of PCN include the less than perfect performance characteristics of the various tools used in the process. For example, EUS is one of the most accurate imaging techniques to evaluate the pancreas, but is inadequate in differentiating benign and malignant PCN in the absence of a mass[6] and suffers from poor interobserver agreement (eg, κ statistic [measure of agreement against which might be expected by chance] of 0.24 for malignancy).[7] Another example is cyst fluid CEA level. Currently, an elevated cyst fluid CEA level (>192 ng/mL) is the most accurate (79%) test to diagnose a mucinous cyst.[8] However, (1) this cutoff value and its accuracy may differ across laboratories depending on the CEA assay employed; (2) the test typically requires 1 mL of fluid, limiting its use in situations where the cyst is either small or the aspirated fluid thick and therefore limited in quantity; (3) cyst fluid CEA level does not correlate with the presence of malignancy; (4) CEA level suffers from extreme values; and (5) the performance characteristics ascribed to CEA level are based on data abstracted from resected pancreatic cysts and thus may be subject to bias. While highly specific for malignancy, EUS-guided fine needle aspirate (FNA) cytological evaluation also remains suboptimal for the diagnosis of MCN.[8] This results primarily from the often-acellular cyst aspirates. Due to the current difficulties in evaluating PCN, efforts to improve the

Abbreviations used in this paper: AIP, autoimmune pancreatitis; AUC, area under the curve; CEA, carcinoembryonic antigen; ERCP, endoscopic retrograde cholangiopancreatography; EUS, endoscopic ultrasonography; FAL, fractional allelic loss; FMR, fractional mutational rate; FNA, fine needle aspirate; IPMN, intraductal papillary mucinous neoplasms; MBG, microdissection-based genotyping; MCN, mucinous cystic neoplasms; OR, odds ratio; PCN, pancreatic cystic neoplasms; PET, Pancreatic endocrine tumors; PCR, polymerase chain reaction.

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yield of cyst fluid analysis have led some of us to study molecular markers in cyst fluid. While the rationale behind such an approach is sound, the clinical utility remains in question and has led to confusion and often misinterpretation. From the study of pancreatic cancer precursor lesions or pancreatic intraepithelial neoplasia, it follows that pancreatic cancer develops through the occurrence of various molecular insults including DNA mutations and deletions/chromosomal losses.[9] It appears that this parallel also exists between histological and molecular progression in mucinous cysts, involving the same molecular events including k-ras mutation, p53 overexpression, and loss of p16 and SMAD4.[10–13] Our initial efforts to apply this molecular information in the clinical arena led to a small prospective single center study.[14] EUS-guided pancreatic cyst aspirates were prospectively collected over a 19-month period. The results of cytology, CEA level, and molecular analysis were compared with cyst pathology. The molecular analysis involved DNA quantification (amount and quality), direct sequencing of k-ras point mutation, and broad panel tumor suppressor linked microsatellite marker allelic loss analysis. The sequence of mutation acquisition was calculated depending upon the amount of DNA affected by the mutation. Of the 36 cysts with confirmed histology there were 11 malignant and 15 premalignant cysts. The molecular analysis of malignant cysts was significantly different from the premalignant cysts based on higher DNA quality (lower cycle threshold value on quantitative polymerase chain reaction [PCR]), higher number of allelic loss, and the sequence of a high amplitude k-ras mutation followed by allelic loss in malignant cysts.[14] Encouraged by this data, a prospective 2-year multicenter study involving 7 US centers called the PANDA study was undertaken[15] supported by an American Society for Gastrointestinal Endoscopy Career Development Award. Over the course of 2 years, 391 patients were enrolled and underwent EUS-guided FNA of a pancreatic cyst and the fluid was sent for cytology, CEA, and molecular analysis. Of the 391 patients, 124 reached a final diagnosis based on surgical resection of malignant cytology. The final study cohort consisted of 113 patients including 40 malignant, 48 premalignant, and 25 benign cysts. The presence of a cyst fluid k-ras mutation was most specific (96%) for a mucinous cyst (odds ratio [OR] of 20.9), but not sensitive (45%). An optimized allelic loss amplitude over 65% and CEA of 148 ng/mL yielded similar performance in detecting mucinous cysts (area under the curve [AUC] and OR of 0.79 and 4.2; and 0.74 and 4.7, respectively). In a subgroup analysis in which cysts with a k-ras mutation were excluded, an elevated cyst fluid CEA level remained significantly associated with a mucinous cyst but with poor performance characteristics. While it is difficult to make any firm conclusions from this information due to the small number of cases, the data suggest that an elevated CEA and presence of k-ras mutation may not add substantially and in an independent manner to the overall accuracy of cyst fluid analysis in diagnosing MCN. My approach in this situation is to diagnose an MCN in the presence of a cyst fluid k-ras mutation due to its very high specificity. In the absence of a k-ras mutation, I then turn to the CEA level and if elevated (the exact number and its accuracy depends on which laboratory performed the test), diagnose an MCN, assuming an approximately 20%–25% false positive and false negative rate. This approach assumes that the clinical history and imaging characteristics are not typical for a particular PCN (eg, honeycomb multicystic lesion for serous cystadenomas, or main duct dilation with ampulla extruding mucus for IPMN), and one is completely dependent on the cyst fluid analysis to make a diagnosis. Components of DNA analysis significantly associated with malignant cysts included allelic loss amplitude over 82% (AUC 0.9 and OR 6.2), and high DNA amount (optical density on spectrophotometer >10; AUC 0.79 and OR 7.7). This finding has intuitive appeal since one would expect more amplifiable DNA and most of the DNA with the damage it manifests in a cyst being contributed by the malignant component with high cellular turnover and shedding. A high amplitude k-ras mutation followed by allelic loss was most specific (96%) for malignancy but not sensitive (37%). Of the 40 malignant cysts, 10 were not diagnosed by cytology and these included 6 noninvasive intraductal papillary mucinous carcinomas. All 10 of these malignant cysts with negative cytology evaluation could be diagnosed as such utilizing any 2 of the 3 aforementioned components of molecular analysis associated with malignancy.[15] Though exciting, these results also need to be interpreted with caution due to the inherent selection biases in this study but primarily because the results are based on resected lesions only and have a higher than expected rate of malignant lesions. Additionally, there is the need for validation by other laboratories.

Gene expression profiles have also been shown to differentiate invasive from noninvasive IPMN, albeit in resected specimens. Overexpression of claudin 4, CXCR4, S100A4, and mesothelin were shown to be associated with invasive IPMN in 1 study.[16] Further studies to apply this information in the preoperative setting are needed. Telomerase activity may also serve as a marker for malignancy in PCN. Pancreatic juice aspirated from IPMN at the time of ERCP and analyzed for telomerase activity substantially increased the yield of cytology (from 30% to 84%) in diagnosing malignant IPMN.[17] Telomerase activity was not detected in any of the benign tumors. The high specificity of telomerase activity for malignancy has also been shown previously[18] but requires duplication in larger prospective studies.

Pancreatic Cancer and Autoimmune Pancreatitis

Definitive preoperative diagnosis of pancreatic ductal carcinoma remains challenging in a subset of patients. This difficulty arises due to the less than perfect sensitivity of ERCP brush cytology (less than 60%)[19–23] and EUS-guided FNA cytology (EUS-FNA) (60%–95%).[24–27] This has resulted in an approximately 10% rate of Whipple resections being performed for presumed malignancy that ultimately reveal benign disease. A quarter of these patients have autoimmune pancreatitis and these patients in particular are suspected of harboring pancreatic ductal carcinoma.[28,29] The ensuing discussion will focus on molecular techniques that have been applied in a clinical setting to indeterminate cytology and have the potential to increase its yield. We have studied the role of microdissection-based genotyping (MBG) in this context.[30] MBG involves dissecting individual cell aggregates from existing slides and subjecting them to PCR. The PCR product is analyzed for allelic imbalance (loss of heterozygosity) targeting microsatellites in proximity to known tumor suppressor genes and for k-ras point mutations. The amount of mutational abnormality present is estimated by the fractional mutational rate (FMR), defined as the number of mutations (k-ras point mutation plus number of alleles lost)