Cyst Fluid Markers for Differential Diagnosis of Pancreatic Cystic Neoplasms: Beyond CEA

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Introduction

Despite the remarkable advances in computed tomography and magnetic resonance imaging, their ability to differentiate pancreatic cystic lesions and to determine the presence of malignancy still remains uncertain.¹ Although endoscopic ultrasound (EUS) provides detailed imaging of the pancreatic cystic lesions, the imaging features alone do not seem sufficient to differentiate benign from malignant lesions.² EUS provides a platform for fine-needle aspiration (FNA), which enables the aspiration and subsequent analysis of pancreatic cyst fluid. In this chapter, we will discuss the utility of pancreatic cyst fluid analysis in the evaluation of pancreatic cystic lesions. We will focus on the use of cyst fluid analysis in the differentiation between mucinous and non-mucinous cysts as well as in the detection of malignancy.

Pancreatic Cyst Fluid Aspiration

In almost all cases, pancreatic cyst fluid aspiration is performed with EUS-guided FNA (EUS-FNA) using a curvilinear echoendoscope. Linear EUS provides high resolution imaging of the entire pancreas and its associated cystic lesions. FNA is an important component of the diagnostic evaluation by EUS. Color Doppler imaging enables the endoscopist to readily differentiate cysts from vascular structures during FNA. Pancreatic cystic lesions in the head/uncinate process of the pancreas are accessed via transduodenal approach; those in the body/tail of the pancreas are approached via transgastric approach. Potential contamination of the aspirated cyst fluid with gastro-duodenal contents is minimized with the use of an occluding needle stylet. Prophylactic antibiotics are given during and after the procedure to prevent cyst infection. Although the risk of pancreatitis and bleeding are rare, minimizing the number of needle aspirations and the size of needles aids in risk reduction (Fig. 1).³⁻⁶ In general, a 22-gauge needle enables the endoscopist to aspirate mucinous fluid with minimal trauma to the pancreas.

The safety profile of EUS-FNA of pancreatic cystic lesions has been well established in a number of retrospective studies. In one study, the complication rate was 2.2% (13 of 603 cases). The reported complications were pancreatitis (n=6), abdominal pain (n=4), retroperitoneal bleeding (n=1), infection (n=1), and bradycardia (n=1).⁶ The use of needle-guided brush cytology seems to increase morbidity (bleeding and pan-
creatitis), and has been associated with a mortality due to retroperitoneal hemorrhage. On the other hand, FNA of the cyst wall for the collection of cytologic material does not seem to increase the risk of pancreatitis.

**Components of Pancreatic Cyst Fluid Analysis**

**Appearance of the Cyst Fluid**

Appearance of the cyst fluid may provide information of the type of pancreatic cystic lesion. Cyst fluid of mucinous pancreatic cystic lesions such as intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN) may show viscous and clear fluid (Figure 2). Aspiration of serous cystic neoplasm may reveal thin and clear yellow fluid that is often bloody (Figure 3). It should be noted that the actual amount of cyst fluid aspirated may be quite scanty, making it difficult to evaluate the appearance of the fluid. Cyst fluid from a pancreatic pseudocyst is thin, dark, and opaque.

**Cyst Fluid Carcinoembryonic Antigen Concentration**

Cyst fluid tumor markers such as carcinoembryonic antigen (CEA), CA 72-4, CA 125, CA 19-9, and CA 15-3 have been tested for their use in the diagnosis of pancreatic cystic neoplasms. Of these, cyst fluid CEA concentration is reported to be the most accurate marker to differentiate mucinous from non-mucinous pancreatic cystic lesions. The cyst fluid CEA concentration of mucinous pancreatic cystic lesions is higher than that of non-mucinous lesions. In the study by Brugge et al., cyst fluid CEA concentration with a cutoff value of 192 ng/mL provided the greatest accuracy (79%) for differentiation between mucinous and non-mucinous pancreatic cystic lesions. The sensitivity and specificity were 73% and 84%, respectively. Increasing the cutoff value of cyst fluid concentration increases the diagnostic specificity at the expense of sensitivity. The 2012 international consensus guideline indicates that a cutoff value of 192 – 200 ng/mL is approximately 80% accurate for the diagnosis of a mucinous pancreatic cystic lesion.

**Cyst Fluid Amylase Concentration**

Although cyst fluid CEA concentration is useful in the diagnosis of a mucinous pancreatic cystic lesion, it does not differentiate between IPMN and MCN. Theoretically, one may speculate that the cyst fluid amylase concentration might be higher in IPMNs than MCNs, as IPMNs involve the pancreatic duct and MCNs do not. However, cyst fluid amylase concentration has been shown not to distinguish MCNs from IPMNs.

**Cyst Fluid Cytology**

Cyst fluid cytology is reported to be the most accurate test for the diagnosis of a malignant pancreatic cystic lesion. However, the sensitivity of cytology is often hampered by the low cellular content of the pancreatic cyst fluid. Distinguishing lesional cells from gastrointestinal contamination is crucial to making an accurate interpretation of cyst fluid cytology.

The neoplastic epithelial cells of serous cystic neoplasms are rarely identified on EUS-FNA specimen. If present, serous cystic neoplasms display bland cuboidal cells with round nuclei and scant finely vacuolated but non-mucinous cytoplasm. Periodic acid-Schiff staining confirms the presence of cytoplasmic glycogen. The
cytological features of IPMNs and MCNs are similar, showing mucinous neoplastic epithelial cells. The cellularity of the cyst fluid is seldom sufficient to distinguish IPMN and MCN.\textsuperscript{11}

The FNA cytology of solid-pseudopapillary neoplasm may show small monomorphic cells loosely adherent to thin, branching vessels. The neoplastic cells have round nuclei with grooved nuclear membranes, and have eosinophilic or foamy cytoplasm.\textsuperscript{14}

The cytology of cystic pancreatic neuroendocrine neoplasm is similar to that of its solid counterpart and demonstrates a monotonous population of plasmacytoid cells with granular cytoplasm and round nuclei with ‘salt and pepper’ chromatin. Immunohistochemistry for chromogranin and synaptophysin are positive, when material for immunohistochemistry is adequate.\textsuperscript{15, 16}

**DNA Analysis of Cyst Fluid**

DNA analysis of pancreatic cyst fluid for \textit{KRAS} mutation is reported to be highly specific for mucinous pancreatic cystic lesions. Elevated amounts of cyst fluid DNA, high-amplitude mutations, and specific mutation sequences have been suggested as indicators of malignancy.\textsuperscript{17} The study by Wu et al\textsuperscript{18} reported that mutations in \textit{GNAS}, a gene encoding the guanine nucleotide-binding protein alpha subunit, are present in 61% of IPMN cyst fluid specimens. In this study, 82% of the IPMN cyst fluids were identified to have \textit{KRAS} mutations. When combined with the molecular analysis results from the tissue specimens, 66% of IPMNs harbored \textit{GNAS} mutation, 81% a \textit{KRAS} mutation, and 51% both \textit{GNAS} and \textit{KRAS} mutations. The mutation of at least one of the two genes was present in 96.2%. In contrast, no \textit{GNAS} or \textit{KRAS} mutations were identified in serous cystic neoplasms. No \textit{GNAS} mutations were found in MCNs, although \textit{KRAS} mutations were found in 33% of MCNs.\textsuperscript{18} The cyst fluid specimens analyzed in this study were obtained from surgically resected specimens. It would be interesting to see if the results are reproduced in the cyst fluid specimens obtained during EUS-FNA.

**EUS-FNA of Pancreatic Cystic Lesions: When to Do and What to Test?**

There are no published guidelines on the indication for EUS-FNA of pancreatic cystic lesions. One must remember that the aspirated pancreatic cyst fluid is often scant in amount, making it extremely difficult to perform multiple tests. If a clear diagnosis of the cystic lesion(s) can be made with cross-sectional imaging, there would be little need for EUS-FNA. If there is diagnostic uncertainty, the cyst fluid should be analyzed for CEA and \textit{KRAS} to differentiate mucinous from non-mucinous pancreatic cystic lesions. Each test can be performed with less than 0.3 mL of cyst fluid. If the primary question is whether the cyst is malignant or benign, then the priority should be given to cytology.\textsuperscript{19}

**Summary**

EUS-FNA of pancreatic cystic lesions is a relatively safe procedure. Cyst fluid CEA concentration is reported to be the most accurate marker to distinguish mucinous and non-mucinous pancreatic cystic lesions. Cytology is reported to be the most accurate test for the diagnosis of a malignant pancreatic cystic lesion. DNA analysis of pancreatic cystic fluid may provide additional diagnostic information of the lesions.
References