REVIEWS

Pancreatic Cyst Fluid Analysis – A Review

Manoop S. Bhutani[1], Vikas Gupta[1], Sushovan Guha[1], Dan Ionuț Gheonea[2], Adrian Săftoiu[2]

1) Department of Gastroenterology, Hepatology and Nutrition, MD Anderson Cancer Center, Houston, USA; 2) Department of Gastroenterology, Research Center of Gastroenterology and Hepatology Craiova, University of Medicine and Pharmacy Craiova, Romania

Motto

"Only those who can see the invisible, can accomplish the impossible."
Patrick Snow

Abstract

An increased number of pancreatic cysts are being diagnosed due to the increased usage of cross-sectional imaging. Endoscopic ultrasound (EUS)-guided fine needle aspiration (FNA) cytology and molecular analysis of these cystic lesions have led to their better detection and characterization. The aim of this review is to assess the value of cyst fluid analysis for the differential diagnosis of pancreatic cystic lesions, in view of the recent progresses of molecular analysis methods.

Pancreatic cysts can be either simple (retention) cysts, pseudocysts and cystic neoplasms, while these are further subdivided into serous cystadenomas, mucinous cystic neoplasms (MCNs) or intraductal papillary mucinous neoplasms (IPMNs). EUS is now being used to investigate cystic pancreatic lesions, particularly by means of EUS-guided cyst aspiration and sampling of the cyst wall or septa, as well as mural nodules. Cyst fluid can be further studied after aspiration in order to analyze cytology, viscosity, extracellular mucin, other tumor markers (CEA, CA 19-9, CA 15-3, Ca 72-4, etc.), enzymes (amylase, lipase), as well as DNA analysis of DNA quality/content or mutational analysis to study allelic imbalance/LOH (loss of heterozygosity) and K-ras mutations. After careful review of the published studies, a conclusion was reached that the use of tumor and molecular markers in conjunction with multimodality detection methods such as CT, MR and EUS-FNA allows risk stratification, while being also cost-effective.

Key words

Endoscopic ultrasound (EUS)–guided fine needle aspiration (FNA) – pancreatic cystic neoplasms – serous cystadenoma – mucinous cystic neoplasms (MCNs) – intraductal papillary mucinous neoplasms (IPMNs).

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Address for correspondence: Manoop S. Bhutani, MD
Professor of Medicine
Department of Gastroenterology,
Hepatology and Nutrition-Unit
Texas, USA 77030-4009
manoop.bhutani@mdanderson.org

Introduction

The extensive use of abdominal imaging has resulted in the diagnosis of pancreatic cysts in a large number of patients. Technological advances like endoscopic ultrasound (EUS)-guided fine needle aspiration (FNA) cytology and molecular analysis of the cysts have led to better detection and understanding of these lesions. Majority of pancreatic cysts are detected incidentally when abdominal imaging is performed for unrelated indications. Due to the possibility of pancreatic cancer in some of these lesions, it is imperative for the physicians to make an accurate diagnosis whenever a cyst is detected, before surgical resection or conservative management.

Background, classification and clinical significance

Pancreatic cystic lesions are found in approximately 1% of abdominal computed tomographic (CT) scans, with prevalence increasing with age [1]. Up to 24% of patients have pancreatic cystic lesions at autopsy [2]. Up until recently, most pancreatic cysts were considered inflammatory in nature. Nevertheless, pancreatic cysts can be either simple (retention) cysts, pseudocysts or cystic neoplasms. Cystic neoplasms are further subdivided into serous, mucinous, IPMNs and papillary cystic neoplasms. Pseudocysts are most commonly detected after pancreatitis or trauma, while a lack of epithelial lining distinguishes them from cystic pancreatic lesions. However, the consequences of mistaking a cystic tumor for a pseudocyst can be serious [5, 6]. Thus, over the past two decades, experience has shown that the majority of pancreatic cystic lesions are neoplasms, including serous cystadenomas, mucinous cystic neoplasms (MCNs) or intraductal papillary mucinous neoplasms (IPMNs) [3, 4]. The majority of pancreatic cysts detected today are mucinous cystic neoplasms (MCNS). Beside being detected with

higher frequency, pancreatic cystic neoplasms present a diagnostic challenge, considering the varied prognosis. The clinical significance of pancreatic cysts lies in the fact that accurate diagnosis can lead to current standard of treatment and consequently it can significantly impact prognosis.

Diagnosis

Pancreatic cysts can be diagnosed and assessed by using computer tomography (CT) and magnetic resonance (MR), but these imaging modalities have been inconsistent in differentiating them. Efforts to differentiate among these tumors from imaging tests have met with mixed success, with up to 40% of neoplastic cysts misdiagnosed as pseudocyts [7, 8]. Moreover, the reported overall accuracy has been highly variable from 20% to 80% [9, 10]. Endoscopic ultrasound (EUS) is now being used to investigate cystic pancreatic lesions, particularly as a means of EUS-guided cyst aspiration [11, 12]. The diagnostic accuracy of EUS alone for detection of malignant or premalignant cysts reaches 95% [13], although it has important limitations for the differential diagnosis of benign and malignant cysts with overall accuracy rates of 40 to 93% [14]. A large multi-center study found an accuracy for the diagnosis of mucinous versus non-mucinous cysts of only 51% [11]. Several EUS features of pancreatic cysts have been proposed as associated with an increased risk of malignancy, including thick wall, septations, presence of intramural nodules and masses [15]. However, recent studies indicated that pancreatic cyst appearance during EUS is not enough as an independent predictor of malignancy [16, 17]. After exclusion of IPMNs which clearly have an indication for surgery, a combination of criteria including age > 50 years, weight loss and size > 1.5 cm indicate a high likelihood of malignancy, near six fold greater [17]. Another study did not confirm that size represents an independent predictor of malignancy, although all malignant tumors were larger than 1.5 cm [18]. Other independent risk factors of malignancy include biliary ductal dilatation or pancreatic ductal dilatation [18], mural nodules or increased wall thickness [19, 20].

EUS has the added advantage of allowing aspiration of the cyst contents (Fig. 1) and sampling of the cyst wall or septa, as well as mural nodules [21,22]. Cyst fluid can be further studied after aspiration in order to analyse cytology, tumor markers (CEA, CA 19-9, CA 15-3, Ca 72-4, etc.), enzymes (amylase, lipase), as well as DNA analysis of DNA quality/content or mutational analysis to study allelic imbalance / LOH (loss of heterozygosity) and K-ras mutations [11, 23].

Biochemical analysis

A number of studies have attempted to study the viscosity, extracellular mucin and other tumor markers in cyst fluid. Cyst fluid contains glycoproteins like CEA, CA 19-9, CA 125, CA 15-3, CA 72-4, which are secreted from the epithelial lining. Various studies have tried to distinguish

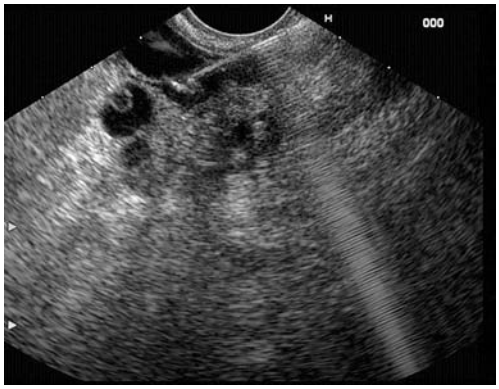

Fig 1. Complex IPMN with both cystic and solid parts, as well as mural nodules. EUS-guided FNA of the fluid content, is followed by puncture and sampling of the solid part.

mucinous and non mucinous lesions by measuring the levels of these tumor markers after aspiration. It is important to ascertain the size of the lesion as smaller lesions will yield lower fluid volumes. An estimate of the cyst fluid volume can be made from cyst size by the formula $4r^3$, r being the radius of the cyst [24].

In the initial studies performed on cystic pancreatic lesions, the combination of viscosity with other markers (CEA, CA 125) and cytology can reliably distinguish mucinous from nonmucinous cystic tumors [25]. **Viscosity** is usually lower in pseudocysts (mean 1.3) and serous cystadenomas (mean 1.27) when compared with mucinous cystadenoma (1.84) and mucinous cystadenocarcinoma (1.90) [26]. All mucinous neoplasms had viscosity levels higher than 1.6. **CEA** levels were higher in mucinous cystadenoma (878 ng/ml), mucinous cystadenocarcinoma (27,581 ng/ml) compared to pseudocyst (189 ng/ml) and serous cystadenoma (121 ng/ml). The study concluded that elevated CEA ≥ 480 ng/ml and viscosity > 1.6 accurately predict mucinous cysts.

A recent paper has studied the diagnostic value of EUS findings, serum and cyst fluid tumor markers (CA 19-9 and CEA) and for the first time has examined the role of cyst fluid viscosity "string sign" in differentiating pancreatic cysts [26]. The string sign was performed by the endosonographer by placing a drop of fluid between the thumb and index finger and measuring the maximum length of stretch before disruption of the mucus string. It was used as a surrogate marker of cyst fluid viscosity as viscosity was not directly measured. The results of this study showed that fluid CEA had a median of 1 ng/ml in benign cysts and 471.1 ng/ml in PMN cysts (P< 0.0001). Cyst fluid CA 19-9 was not statistically significant (P=.22). Increased cyst fluid viscosity was associated with malignant or potentially malignant cysts (P<.0001). Median string sign was 0 mm in benign cysts and 3.5 mm in potentially malignant/malignant cysts. The study concluded that a thick cyst wall or intracystic growth, elevated cyst fluid CEA and a long 'string sign' were associated with PMN cysts. The string sign is cost

effective and can be performed quickly. Further studies can shed more light on cyst fluid 'string sign' and its utility but it appears like a promising tool in the diagnostic arsenal for pancreatic cysts.

The presence of **extracellular mucin** in aspirated cyst fluid is moderately predictive of a mucinous neoplasm [28]. Several studies concluded that the combination of CEA and mucin with cytology yielded the best results for differentiation of mucinous lesions [29-31]. Cytological identification of extracellular mucin and CEA are thus considered predictors of mucinous neoplasia and malignancy, as recently proven by a multivariate analysis in 43 patients, which confirmed CEA levels > 300 ng/ml (P=0.0007) and identification of mucin (P<0.001) as significant predictors [30]. Another approach described recently is the use of glycosylation variants of mucin 1, 5AC, 16, as well as CEA or other proteins implicated in pancreatic neoplasia like CA 19-9, although this has to be validated in larger studies with clinical emphasis [32].

In a study by Frossard et al [21], a **CA 19-9** value greater than 50,000 U/ml in the cyst fluid had a 15% sensitivity and 81% specificity to distinguish mucinous cysts from other cystic lesions, whereas it had an 86% sensitivity and 85% specificity to distinguish cystadenocarcinoma from other cystic lesions. **CA 72-4** cyst fluid levels were found to be significantly higher in mucinous cystic tumors (P< 0.005), with 80% sensitivity and 95% specificity in detecting mucinous or malignant cysts [33]. A subsequent study thus found that a CA 72-4 level over 40 U/ml had a 63% sensitivity and 98% specificity for distinguishing mucinous cystadenomas and cystadenocarcinomas from serous cystadenomas and pseudocysts. A CEA level of > 400 ng/ml had 57% sensitivity and 100% specificity for distinguishing mucinous tumors and cystadenocarcinomas from pseudocysts, while a CEA level of < 4 ng/ml had a 100% sensitivity and a 93% specificity for distinguishing serous cystadenomas from other cystic lesions [34].

A comprehensive study by van der Waaij et al [35] comprised a pooled analysis of 12 studies (450 patients) to investigate the value of cyst fluid analysis in differential diagnosis of pancreatic cystic lesions. CEA levels below 5 ng/mL or a CA 19-9 below 37 U/mL suggested a serous cystadenoma or pseudocyst (sensitivity 50%, specificity 95%). CEA levels over 800 ng/ml strongly suggested mucinous cystadenoma or mucinous cystadenocarcinoma (sensitivity 48%, specificity 98%). The study concluded that the pooled analysis of individual patients showed that the determination of cyst fluid concentrations of CEA and amylase, as well as cytologic examination, may help in the differential diagnosis of the most common benign (SCA, PC) vs premalignant or malignant (MCA, MCAC) pancreatic cystic lesions.

A large multicenter study (Cooperative Pancreatic Cyst Study) [11] investigated the value of various tumor markers in pancreatic cyst fluid collected by EUS.

In this study, the results of EUS imaging, cyst fluid cytology and various cyst fluid markers (CEA, CA 19-9 CA 125, CA 72-4 and CA 15-3) were prospectively collected and compared with histology as the final diagnostic "gold standard". Receiver Operating Curve analysis showed that cyst fluid CEA (cut off 192 ng/ml) demonstrated the greatest area under the curve (0.79) for the differentiation of mucinous versus non-mucinous lesions. The accuracy of CEA (79%) was significantly greater than the accuracy of EUS morphology (51%) or cytology (59%). Also, CEA provided greater accuracy than any other combination of tests. The study concluded that cyst fluid CEA is the most accurate test (among the tested markers) available for diagnosis of mucinous cystic lesions of the pancreas. This was confirmed in a recent study where it was also found fluid CEA concentration to be the best discriminating tool for mucinous and non mucinous lesions of the pancreas [36]. However, the cut-off value of CEA in their study was considerable lower (14.3 ng/mL). The study concluded that fluid tumor markers CEA, CA 72-4 and CA 19-9 in mucinous cystic neoplasms are significantly higher compared with non mucinous lesions.

Tumor marker levels were subsequently analysed in 442 pancreatic cyst fluid patients [37]. The study yielded an optimal cut CEA cutoff of 30 ng/ml; a CEA value ≥ 30 ng/ml had 79% sensitivity, 73% specificity and 84% PPV for detection of a mucinous cyst. For cysts with CEA < 30 ng/ml (i.e. likely non mucinous), amylase > 8500 U/L detected 91% of pseudocysts, while amylase< 350 U/L delineated 85% of serous cystadenomas. In cysts with CEA>= 30 ng/ml (i.e. likely mucinous), CA 19-9 < 8000 U/ml segregated 71% of IPMN from other cyst subtypes. The cutoff value of CEA is considerably lower (30 ng/ml) than of other studies which have reported higher levels. The uniqueness of this study lies in the fact that the algorithm presented involves the use of various analytes sequentially rather than independently.

Not many studies have evaluated the role of pancreatic cyst fluid analysis in IPMN. The level of biochemical and tumor markers in fluid from EUS-FNA in patients with IPMN was used to assess the impact for the diagnosis of IPMN [38]. They found that a CEA level over 200 ng/ml and a CA 72-4 over 40 U/ml had a sensitivity of 44% and 39%, respectively, for diagnosis of IPMN. The levels of CEA, CA 19-9, CA 72-4 were also significantly different between benign and malignant IPMN. The study concluded that CEA and CA 72-4 in pancreatic cyst fluid have excellent negative predictive values in the preoperative differential diagnosis of benign versus malignant IPMN. Another study on IPMN found that cytology and cyst fluid analysis for CEA over 2,500 ng /ml in small branch duct IPMN is slightly more accurate [39]. This might result in improved sensitivity for the detection of malignancy and invasion rather than the management algorithm proposed by international consensuss guidelines [40].

DNA analysis

Other studies have been initiated to look into DNA and mutational analysis in the cystic fluid aspirated by EUS-

FNA. The detection of loss of heterozygosity (LOH) by using microsatellite markers closely linked to key tumor suppressor genes can serve as a surrogate marker for gene inactivation and mutation [41]. The same group [42] studied the role of molecular markers in mucinous cystic lesions of the pancreas. They concluded that malignant cyst fluid contains adequate DNA to allow mutational analysis. A first hit K-ras mutation followed by allelic loss is most predictive of malignancy in a pancreatic cyst.

Evaluation of cyst fluid from IPMNs for genetic abnormalities using K-ras mutational analysis and an appropriate panel of genomic markers for LOH was correlated with the results of surgical resections [43]. Molecular studies for K-ras and LOH using a panel of microsatellite markers were performed on pre and intraoperative cytologic samples. K-ras mutational analysis demonstrated mutations in 2 of 4 carcinomas, 2 of 6 borderline tumors and in none of adenomas. LOH was observed associated with various genetic loci in 3 of 4 carcinomas, 2 of 5 borderline tumors and 2 of 6 adenomas. The study concluded that early detection of solid and cystic pancreatic neoplasia is enhanced by the application of molecular techniques such as LOH analysis and assessment of K-ras mutations.

The utility of detailed DNA analysis of pancreatic cyst fluid to diagnose mucinous and malignant cysts has been evaluated in the PANDA study [44]. The study concluded that cyst fluid K-ras mutation was helpful in the diagnosis of mucinous cysts with a 96% specificity. Components of DNA analysis detecting malignant cysts included allelic loss amplitude over 82% and high DNA amount. The criteria of high amplitude K-ras mutation followed by allelic loss showed maximum specificity (96%) for malignancy.

The agreement between CEA and molecular analysis was recently studied for differentiating mucinous from non mucinous cysts [45]. Poor agreement existed between CEA and DNA quantity, K-ras mutation and ≥ 2 allelic imbalance mutations. The authors showed that CEA had a sensitivity of 82% compared with 77% for molecular analysis. However, when CEA and molecular analysis were combined, 100 % sensitivity was achieved.

A recent study analyzed the clinical impact of DNA mutational analysis of pancreatic cyst fluid with its correlation to cyst fluid chemistry and histologic diagnosis [46]. The following statistics were found in the 20 patients who had cyst fluid DNA analysis and histology available for comparison. In 6 patients with available surgical histology demonstrating mucin or malignant cysts, CEA had a sensitivity of 66.7%, k-ras 2 mutation had a sensitivity of 33% and 2 or more LOH had a sensitivity of 50% respectively. The study concluded that consistency in histology, CEA levels and K-ras 2 and LOH mutations were seen in only 35% of cases, all of which were benign cysts. In malignant cysts, elevated CEA was more predictive of histology than K-ras 2 or LOH mutations. The study concluded that DNA mutational analysis should be used rather selectively than routinely.

The correlation between a commercially provided molecular diagnosis with a clinical consensus diagnosis was recently studied, paving the way for extensive clinical testing of molecular diagnostic techniques [47]. The molecular analysis included K-ras gene point mutation, LOH and determination of DNA quantity/quality in cyst fluid. Clinical consensus diagnosis was achieved by EUS features, CEA levels and cytologic examination. This study showed a 83% concordance between molecular and clinical consensus diagnosis. The sensitivity, specificity and PPV of molecular diagnosis were 83%,100% and 100% for a malignant cyst and 86%, 93% and 95% for a benign mucinous cyst. The conclusion reached was that molecular analysis adds diagnostic value to preoperative diagnostics.

Last, but not least, the cost effectiveness of different strategies of management was studied in asymptomatic pancreatic cystic neoplasms [48]. The study has further emphasized the utility of EUS-guided FNA with cyst fluid analysis. The authors compared three approaches in solitary asymptomatic pancreatic cystic neoplasms. The approaches were: 1) no specific intervention; 2) an aggressive surgical intervention; 3) EUS-guided FNA with cyst fluid analysis for risk stratification, with patients with mucinous cysts considered for resection. The results showed that the latter approach yielded the highest quality adjusted life years with an acceptable incremental cost effectiveness ratio.

Conclusion

There is a need for a panel of molecular markers that can help in unraveling the diagnostic conundrum of pancreatic cystic lesions. A cost-effective diagnostic algorithm which would have high accuracy would be very helpful in the work up of pancreatic cystic lesions and could improve prognosis considerably. The use of tumor and molecular markers in conjunction with multimodality detection such as CT, MR and EUS-FNA permits risk stratification and is cost-effective. Further studies require to be conducted to validate the effectiveness of tumor and molecular markers as shown in prior studies.

The reduced number of laboratories available for studying molecular markers is a limitation which needs to be overcome. The role of viscosity, tumor markers such us CEA, CA 19-9, CA 72-4, as well as DNA analysis and genetic mutations such as K-ras have been established by quite a few studies but more studies with larger sample sizes will be useful for the validation of some of these markers, delineating cut-off levels for measuring these tumor markers and establishing a standard protocol of diagnosis and management. Future studies are also required to select the appropriate panel of molecular markers that will best diagnose the pancreatic cystic lesions at low cost and highest diagnostic yield. Let us see what further research will bring, but the future certainly looks promising.

Conflicts of interest

None to declare.

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