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ORIGINAL ARTICLE

A Comparative Analysis of Pancreas Cyst Fluid CEA and Histology with DNA Mutational Analysis in the Detection of Mucin Producing or Malignant Cysts

Jayaprakash Sreenarasimhaiah, Luis F Lara, Saad F Jazrawi, Carlton C Barnett, Shou-jiang Tang

Division of Digestive and Liver Diseases, Clinical Center for Pancreatic Diseases, University of Texas Southwestern Medical Center. Dallas, TX, USA

ABSTRACT

Context The main objective of pancreatic cyst fluid analysis is to differentiate mucin-producing or malignant cysts from other cysts which have a benign course. K-ras-2 point mutation and at least two mutations of allelic imbalance or loss of heterozygosity with good quality DNA has been suggested to predict mucinous cystic neoplasm (MCN). Elevated carcinogenic embryonic antigen (CEA) level in cyst fluid has also been shown to be predictive of mucinous or malignant cysts. **Objective** Identify the clinical impact of DNA mutational analysis of pancreatic cyst fluid with its correlation to cyst fluid chemistry and histologic analysis. **Patients** This retrospective analysis included all consecutive patients with pancreatic cysts who presented for evaluation by endoscopic ultrasound (EUS) with fine needle aspiration (FNA) over an 18 month period until November 2007. **Main outcome measures** DNA analysis performed by Pathfinder TG® (RedPath Integrated Pathology, Inc., Pittsburgh, PA, USA) and fluid CEA exceeding 192 ng/dL were used to suggest mucinous or malignant cysts. These parameters were compared to surgical histology or cytopathology of FNA specimens. **Results** Twenty-seven consecutive patients with cysts had samples submitted for DNA analysis which included 15 men and 12 women (mean age 62.8 and 61.3 years, respectively). In 20 patients, all parameters including cyst fluid, DNA analysis, and histology were available for comparison. Consistent findings were seen in 7/20 (35%) in which all parameters suggested negative benign findings. CEA level was elevated in 7 patients of which 4 had mucinous or malignant histology. In the remaining 13 patients with low CEA levels, 11 had negative histology. The sensitivity and specificity of CEA based on these results was 66% and 78.6% respectively. The positive predictive value (PPV) of CEA was 57% and the negative predictive value (NPV) was 84.6%. K-ras-2 mutation was detected in 3 patients, absent in 17 patients and falsely negative in 4 cases based on histology. The sensitivity and specificity were 33% and 92.6% respectively. The PPV was 66% and NPV was 76%. Detection of loss of heterozygosity mutations was noted in 7 patients, of which 4 were falsely positive. In the remaining 13 patients, 3 were falsely negative. The sensitivity and specificity were 50% and 71% respectively. The PPV was 42.9% and NPV was 76.9%. In a group of 6 patients with available surgical histology demonstrating mucin-producing or malignant cysts, fluid CEA level had a sensitivity of 66.7%. However, K-ras-2 and loss of heterozygosity mutational analysis had a much lower sensitivity at 33% and 50% respectively. **Conclusions** Consistency in histology, CEA levels, and K-ras-2 and loss of heterozygosity mutations was seen in only 35% of cases, all of which were benign cysts. In the detection of malignant cysts, elevated CEA levels were more predictive of histology in comparison to K-ras-2 or loss of heterozygosity mutations. Additionally, false positivity of loss of heterozygosity mutations was noted to be considerably higher than K-ras-2 mutations or even fluid CEA levels. These findings suggest that DNA mutation analysis should not be used routinely but rather selectively in the evaluation of pancreatic cysts.

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Abbreviations LOH: loss of heterozygosity; MCN: mucinous cystic neoplasm

Correspondence Jayaprakash Sreenarasimhaiah
Department of Medicine, Division of Digestive and Liver Diseases, University of Texas Southwestern Medical Center, 5323 Harry Hines, MC 9083, Dallas, Texas, USA 75390
Phone: +1-214.645.6415; Fax: +1-214.645.0596
E-mail: jayaprakash.sree@yahoo.com

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INTRODUCTION

Cystic lesions of the pancreas are often found incidentally during abdominal radiographic imaging performed for other purposes. Some cysts have distinguishing features such as size, debris, septations, and communication with the pancreatic duct which are sometimes noted on standard imaging studies such as computerized axial tomography or magnetic resonance imaging. However, in the majority of cases, small pancreatic cysts are better visualized by higher resolution imaging modalities such as endoscopic ultrasound, which also enables simultaneous sampling of the pancreas fluid for analysis by fine needle

aspirate biopsy [1, 2]. This information helps differentiate the cyst as benign, pre-malignant or malignant, which is the major clinical dilemma in such patients, and guides subsequent management.

Analysis of cystic fluid is useful in distinguishing between mucinous cystic neoplasms and non-mucinous benign cysts which has significant implications for clinical intervention or follow-up of such patients. Previous studies have determined the utility of fluid carcinogenic embryonic antigen (CEA) elevation combined with low levels of amylase as a marker for mucinous cysts. A CEA level exceeding 192 ng/dL has been shown to be consistent with mucinous cysts and is a widely accepted standard by many experts in the workup of pancreatic cysts [3, 4, 5]. Mucin, periodic acid-Schiff stains as well as detection of ovarian stroma and other histologic features of mucinous cysts can sometimes be obtained during fluid analysis [6, 7, 8, 9].

Recent studies have suggested that DNA mutational analysis of cyst fluid can further enhance the ability to identify mucinous cystic neoplasms. Previous investigators have demonstrated that the presence of K-ras-2 point mutations and allelic imbalance or loss of heterozygosity (LOH) with good quality DNA can aid in differentiating mucin producing cysts from benign cysts [10, 11, 12].

The traditional approach to the workup of pancreatic cysts includes evaluation of clinical history, imaging of cyst features such as septations and viscosity of fluid aspirate by EUS, cyst fluid analysis of CEA and amylase levels and cytology evaluation. The absolute role of DNA mutational analysis in this workup is still being defined. Thus, the objective of this study is to identify the clinical impact of DNA analysis of pancreatic cyst fluid with its correlation to cyst fluid chemistry and histologic analysis.

METHODS

This retrospective analysis included all pancreatic cysts that were examined consecutively by endoscopic ultrasound over an 18-month period until December 2007. The subgroup of patients in which DNA fluid analysis was performed constituted the study population.

Subjects

During the study period, 60 patients presented for EUS evaluation of pancreatic cysts. In 29 patients, clinical, radiographic, and sometimes endosonographic appearance was highly suggestive of pancreatic pseudocyst and thus DNA mutational analysis was not applied to this group. In 31 patients, diagnosis was not certain by clinical or imaging characteristics. Of these, 27 patients qualified for insurance coverage of fluid analysis from EUS-FNA for DNA analysis. This included 15 men and 12 women (mean age of 62.8 and 61.3 years, respectively). This analysis was contingent on sample volume, which was first sent for chemistry and histology (at least 2 mL) with the remainder being submitted for DNA analysis. In 7 patients, there was inadequate volume for fluid CEA testing or cytology. Thus, the final study group consisted of 20 patients, in whom cyst fluid CEA level, DNA analysis, and histology were available.

Endoscopic Ultrasound Exam

Examination with endoscopic ultrasound was performed with a linear echoendoscope (GFUC140P, Olympus Inc., Tokyo, Japan) to facilitate fine needle aspiration (FNA) of fluid. Fluid aspiration was performed with a single pass into the cyst with either a 22-gauge or 19-gauge Wilson-Cook needle (Winston-Salem, NC, USA). Complete aspiration of cystic contents was accomplished when possible. Antibiotic prophylaxis was administered prior to FNA with either cefazolin or vancomycin intravenously. All cases were performed by a single endoscopist (J.S.).

Cyst Fluid Analysis

The fluid aspirate was submitted routinely to chemistry laboratory for measurement of carcinogenic embryonic antigen (CEA) and amylase levels. The fluid was also examined by a cytopathologist by standard methods after placement into Cytolyt® (Cytyc Corporation, Marlborough, MA, USA). DNA analysis was performed using Pathfinder TG® (RedPath Integrated Pathology, Inc., Pittsburgh, PA, USA).

The diagnosis of a benign cyst (pseudocyst or serous cystadenoma), pre-malignant cyst (mucinous cystic neoplasm or intra-papillary mucinous neoplasm), or malignant cyst (pancreas ductal adenocarcinoma with cystic degeneration) was established using a combination of cyst appearance on EUS imaging, cytopathology results, chemistry and DNA analysis. CEA level exceeding 192 ng/dL was used to define a mucin containing cyst or malignant cysts. Mucinous or malignant cysts are considered to be present by Pathfinder TG® if the quality of DNA was determined as either good or excellent in addition to the presence of either K-ras-2 mutation or at least two or more LOH allelic mutations. It should be noted that the DNA mutations that are considered to be indicative of pre-malignant or malignant lesions by RedPath Integrated Pathology are proprietary and thus are not divulged. This laboratory does suggest a possible diagnosis in its final report (such as the presence of a mucinous neoplasm) and estimates the likelihood that malignant transformation has occurred based on the number and sequence of allelic mutations, which has been described for pancreas malignancy similar to colon neoplasia [12, 13, 14].

These findings were compared to surgical pathology specimens which were available in patients who underwent operative management, or to diagnostic cytopathology results of EUS-FNA aspirates. A final analysis on patients with histology from surgical specimens suggesting malignant cysts or cystic neoplasms with mucin compared to cyst fluid CEA level and DNA mutations was performed.

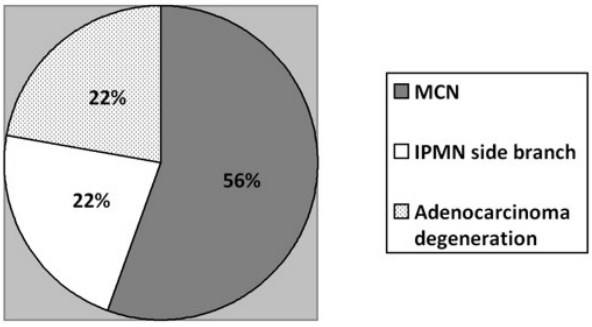

Figure 1. Distribution of malignant cysts. Six of these nine malignant cysts were detected by surgical resection and the remainder was identified by cytopathologic analysis of fluid aspirates alone.
MCN: mucinous cystic neoplasm
IPMN: intraductal papillary mucinous neoplasm

ETHICS

The study was conducted with approval from the Institutional Review Board. Informed consent from human subjects was acquired to collect and analyze data in a confidential manner. The study methods conformed to the ethical guidelines of the "Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects".

STATISTICS

Frequencies were used as descriptive statistics. No statistical comparisons of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were made among the three diagnostic methods because of the small sample size.

RESULTS

Correlation of Histology and DNA Mutational Analysis

All parameters that were measured including cyst fluid CEA level, DNA analysis, and histology were available for comparison in 20 patients. Of these, 9 cysts (45.0%) were cystic neoplasms with mucous (5 mucinous cystic neoplasms, 2 side-branch intraductal papillary mucinous neoplasms, and 2 cystic degeneration of pancreas ductal adenocarcinoma) (Figure 1). In 3 (33.3%) of these patients, the diagnosis was established by cytopathology of EUS-FNA aspirates, and in the other 6 (66.7%) the diagnosis was established by surgical specimens. The remaining 11 cysts (55.0%) were benign or of non-mucinous variety (serous, pseudocyst or indeterminate). Consistency among all parameters was seen in only 7/20 (35.0%) patients, and this was only noted in patients who had a benign cyst.
CEA level was found to exceed 192 ng/dL in 7/20 patients (35.0%). Four of these 7 patients were diagnosed with a mucinous cystic neoplasm (MCN) by histology (positive predictive value: 57.1%). CEA was less than 192 ng/dL in 13/20 patients (65.0%). Eleven of these 13 patients had negative histology (negative predictive value: 84.6%). The sensitivity and specificity of CEA based on these results was 66.7% (4/6) and 78.6% (11/14), respectively.
In the same group, K-ras-2 point mutation was detected in 3 patients (15.0%). Two of the patients had cystic degeneration of pancreas adenocarcinoma or an MCN (positive predictive value: 66.7%). K-ras-2 was absent in 17/20 patients (85.0%), but four of 17 actually had cystic degeneration of pancreas adenocarcinoma or an MCN (negative predictive value: 76.5%). The sensitivity and specificity of K-ras-2 point mutation based on the data was 33.3% (2/6) and 92.9% (13/14), respectively.
In this group of 20 patients, at least two LOH allelic mutations were detected in 7 patients (35.0%) and histology demonstrated MCN or malignant cystic degeneration in three of the 7 patients (positive predictive value: 42.9%). In the remaining thirteen patients, DNA analysis was reported as negative, as patients exhibited none or less than two LOH allelic mutations. Three of the 13 patients with a negative DNA report had an MCN or malignant cystic degeneration (negative predictive value: 76.9%). Thus, the sensitivity of LOH allelic mutations was 50.0% (3/6) with a specificity of 71.4% (10/14) (Figure 2).

Detection of Malignant or Mucinous Cysts

A comparison of CEA, K-ras-2, and LOH mutational analysis including only mucin producing cystic neoplasms (MCN, IPMN and cystic degeneration of pancreas adenocarcinoma) confirmed by histology was performed. Nine patients had malignant lesions of which six (30.0% of the overall group) were confirmed by surgical specimens. Fluid CEA level exceeded 192 ng/dL in four of six patients, thus demonstrating a sensitivity of 66.7%. K-ras-2 point mutation was detected in two of six malignant cysts, producing a sensitivity of 33.3%. LOH allelic mutations were detected in three of six malignant cysts demonstrating 50.0% sensitivity for this test (Figure 3). The sensitivities in this surgically-proven malignant or mucinous cyst subgroup are identical to those determined for each parameter in the overall study group.

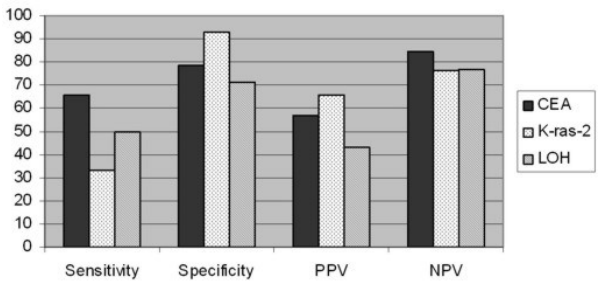

Figure 2. Comparative analysis. This demonstrates that fluid carcinogenic embryonic antigen (CEA) level has the highest sensitivity and negative predictive value (NPV). Detection of K-ras-2 point mutation has the highest specificity and positive predictive value (PPV). Loss of heterozygosity (LOH) mutational analysis has less value than fluid CEA level in all measurements.

DISCUSSION

Among cystic lesions of the pancreas, the identification of the mucinous variety (MCN and IPMN), and of cystic degeneration of malignant lesions (ductal adenocarcinoma, neuroendocrine tumors, etc) is imperative as the clinical approach and prognostic implications differ from non-mucinous lesions [15, 16]. Mucinous cysts, unlike their counterparts, require close surveillance for progression into malignant states and often are subjected to surgical resection [17].

On the contrary, many pancreatic cysts are of a non-mucinous variety with a much different course. These may include benign serous cystadenomas which have extremely rare malignant transformation or inflammatory pseudocysts seen most often but not always in clinically identifiable acute or chronic pancreatitis. These cysts may often be observed without intervention. Therefore, differentiating these various types of cysts requires a comprehensive evaluation including patient characteristics (age and gender), different imaging modalities (CT scan, MRI/MRCP, EUS, ERCP), and analysis of cyst fluid.

Commonly utilized tests of pancreas cyst fluid include chemistry analysis of CEA and amylase levels as well as cytopathology from fluid aspirates or specimens from surgical excision. These investigations have their limitations and certain cysts are still deemed of indeterminate classification. DNA mutational analysis has been introduced to improve diagnostic accuracy and more specifically to differentiate mucinous or malignant cysts from non-mucinous cysts. A progression of benign pancreas ductal epithelium to dysplasia (pancreatic intra-epithelial neoplasia) to carcinoma has been shown, similar to the disease progression in colorectal adenocarcinoma. This progression is accompanied by sequential acquisitions of DNA mutations (including loss of heterozygocity) which may mirror the degree of dysplasia or indicate the presence of carcinoma in-situ or frank malignant transformation [18]. Based on this, some have suggested that DNA mutation analysis is an essential part of cyst fluid analysis and deem it as superior to the other testing methods described above. However, this study demonstrates not only the limitations of DNA mutational analysis, but a general inability to establish the presence of pre-malignant or malignant lesions using established cyst fluid parameters.

Our results indicate that there is a large discrepancy between cystic fluid chemistry analysis, histology, and DNA mutational analysis. All of these parameters were found to consistently establish an appropriate diagnosis in only approximately one-third of cases, particularly those that were benign or non-mucin producing cysts. This does not serve the fundamental purpose of these tests which is to identify the mucinous lesions.

Some investigators have suggested that the finding of K-ras-2 mutations in the setting of good quality DNA is highly predictive of mucinous cystic lesions [10, 19]. DNA mutational analysis was examined in a multicenter study of 88 mucinous cysts. It was suggested that high optical density of good quality DNA with elevated amplitude of point mutations in K-ras-2 and LOH alleles has high specificity for mucinous cysts [20]. However, K-ras-2 has been shown by others to also be elevated in benign conditions such as chronic pancreatitis and thus has poor specificity [21]. Furthermore, some studies have demonstrated that rather than K-ras-2, p16 and p53 gene alterations and accumulations are the essential component in the development and malignant progression of IPMN, and thus must be examined further [22]. It has also been demonstrated that in both mucinous cystic neoplasms and IPMN, polyclonal epithelia of the cysts undergo dysplasia with monoclonal expansion which precedes the K-ras-2 mutation [23].

The detection of allelic mutations of loss of heterozygosity has also been suggested to be a predictor of mucinous lesions [10, 24]. The investigators suggest that two or more such allelic mutations have high specificity for differentiating mucinous from non-mucinous lesions. However, in other studies, genetic alterations including both K-ras-2 and LOH frequencies have been shown to be poor predictors of the carcinoma sequence particularly in IPMN [25].

The results of this study demonstrate that standard testing with cyst fluid CEA has a sensitivity and specificity of 67% and 79%, respectively, and this was superior to the DNA analysis which had a sensitivity of 33% (K-ras-2) and 50% (LOH mutations). Although the specificity of K-ras-2 was high (92.9%) for detecting mucin-producing cysts and malignant cystic degeneration, the other component of DNA mutational analysis, LOH allelic mutations, had a specificity of 71% which together with the reduced sensitivity resulted in overall poor positive and negative predictive values. Furthermore, in the subgroup of histologically proven malignancy or mucinous cysts, sensitivity was much lower for overall DNA mutational analysis than the cyst fluid CEA testing.

These results suggest that there may not be an added

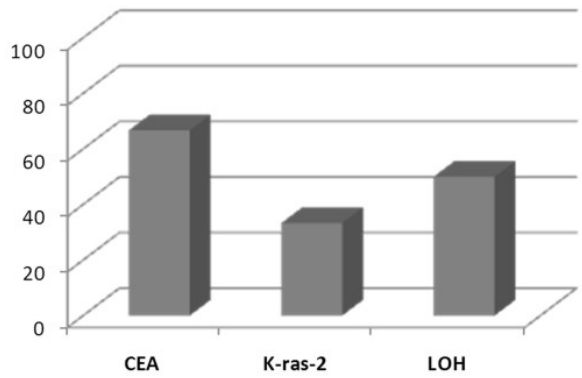

Figure 3. Comparison of tests in subgroup of malignant cysts. In this group of 6 malignant or mucinous cysts determined by surgical histology, fluid carcinogenic embryonic antigen (CEA) level has a higher sensitivity than either component of DNA mutational analysis, K-ras-2 and loss of heterozygosity (LOH).

benefit to analyze pancreatic cyst fluid for DNA mutational alterations. The established practice of testing cyst fluid CEA and histology has been shown in our study to have higher efficacy in detecting mucinous or malignant cysts. Another issue is the added cost of DNA mutational testing which has variations but is considerable in most cases. Understanding the molecular basis of these neoplasms could eventually lead to a test of DNA mutations that may indicate malignant transformation perhaps even prior to morphologically evident changes, and could potentially alter the disease outcome. Translational research should be encouraged, but in our experience the use of this commercially available DNA mutational analysis did not prove beneficial. Not only did it fail to identify neoplasms, but it did not change the management ultimately offered to the patient. As such, we continue to use cyst fluid CEA and cytology plus morphological and patient characteristics in our clinical decision making, particularly to determine if a therapeutic intervention is appropriate for a patient with a pancreas cyst. We are not using the commercially available DNA mutational testing in our institution at this time.

Limitations of this analysis include the small sample size which may have resulted in the larger differences noted between the different tests. Mucinous cysts of the pancreas are slow-growing and shorter follow-up times may limit the detection of malignant transformation. An ideal study would include surgical resection of each cyst, benign and malignant, to identify the true histologic nature of all cysts and the predictive value of each test, or following patients with DNA mutations to learn which ones may be clinically important. However, this has technical and ethical challenges and is difficult to justify when examining the risks and benefits of surgery. Moreover, in our analysis, there was a group of patients with histological confirmation of either malignant cystic degeneration or mucin-producing neoplasms in which fluid CEA level had a greater sensitivity than DNA mutational analysis for detection of these lesions. Nevertheless, a prospective study with a larger number of subjects could help compare these various markers to histology and long-term radiologic observation to detect cyst transformation. Due to the several limitations outlined above and the expense of DNA mutational testing, our institution no longer uses the Pathfinder TG® examination in the evaluation of pancreatic cysts.

SUMMARY

Mucinous cysts of the pancreas require differentiation from nonmucionus cysts due to their potential malignant transformation and aggressive clinical course. Cyst fluid analysis consisting of CEA levels and histology appears to be superior to DNA mutational analysis in the detection of these mucinous pancreas lesions. Overall consistency among mutational analysis, histology, and CEA is poor. Moreover, sensitivity for detecting mucinous cysts is highest for CEA levels. Although the presence of K-ras-2 point mutations has a high specificity for mucinous pancreas lesions, it has a poor sensitivity. This is combined with the overall inferior results of LOH mutational testing demonstrated by poor positive and negative predictive values. Given the challenges of inconsistent findings, reduced sensitivity and high costs of testing, this study suggests that DNA mutational analysis should not be used in the primary evaluation of fluid from pancreatic cysts at this time.

Conflict of interest The authors have no financial disclosures or conflicts of interest

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