ORIGINAL RESEARCH

Integrated Molecular Pathology as a Predictor of Malignant Transformation of Pancreatic Cysts With up To 11-Year Follow-Up

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ABSTRACT

Background and Study Aims The 2018 ACG guidelines recommend surgical evaluation for pancreatic cysts with high-risk features. Integrated Molecular Pathology (IMP) combines molecular and biochemical analysis with cytology to assess malignant potential of pancreatic cysts. We aim to characterize the long-term diagnostic utility of IMP for pancreatic cysts. **Patients and Methods** Pancreatic cystic lesions from prospective patients with EUS-FNA and IMP (PancraGEN, Interpace Diagnostics) from 9/2010 to 9/2013 were retrospectively analyzed in this study for maximum 11 years of follow-up. Data on patient demographics, EUS and cross-sectional imaging, cyst fluid biochemical and molecular analysis, and cytology were collected. Final diagnosis was based on surgical pathology, repeat biopsy or imaging, and on length of clinical follow up. **Results** 69 patients with mean age 69.9; 36.2% male were included. Median follow-up was 72.3 months (IQR 89.67); 7 patients were lost to follow-up. Average cyst size was 2.3cm (0.2-15.3). Using 2018 ACG EUS criteria 43/69 (62.2%) of cysts were classified as benign and 13/69 (18.8%) were classified as high-risk. All 5 malignancies were detected for a sensitivity of 100% and specificity of 82% combining IMP results with 2018 ACG EUS high-risk criteria. Among cysts followed long term, no additional transformations into adenocarcinoma were detected within the cohort**. Conclusions** The absence of high-risk features based on ACG guidelines and IMP is a strong negative predictor of malignant transformation of pancreatic cysts, based on up to 11-years of follow-up. In our study, high-risk features based on ACG guidelines or IMP identified all malignancies.

INTRODUCTION

Technological developments that have reduced cost and improved the resolution of abdominal imaging have led to increased incidental findings of pancreatic cysts [1]. Recent meta-analyses have estimated prevalence of asymptomatic, incidentally discovered pancreatic cysts to be about 8% [2]. The 2018 ACG guidelines [3] recommend surgical evaluation for pancreatic cysts with high-risk features. Cysts include pseudocysts, cystic neuroendocrine tumors, Serious cystadenomas (SCNs), and mucinous lesions like Mucinous Cystic Neoplasms (MCNs) and Intraductal Papillary Mucinous Neoplasms (IPMNs). EUS (Endoscopic Ultrasound) with Fine-Needle Aspiration (FNA) and cyst fluid analysis are the standard-of-care when assessing cysts with high-risk features. More recently, EUS-Guided Fine Needle Biopsy (EUS-FNB) using endoscopic forceps is becoming more popular and may overcome limitations that EUS FNA has with the potential of inadequate sampling. With EUS-FNB clinicians are better able to analyze cell wall architecture resulting in enhancement in diagnosis of solid lesions [4]. Nonetheless, balancing higher incidence of incidental cysts, high mortality with pancreatic cancer, and lack of highly predictive markers for malignant potential has given rise to a multitude of guidelines to assist with risk stratification and clinical decision-making. These guidelines include, but are not limited to, The European Study Group guidelines, the American College of Gastroenterology (ACG) guidelines, the American Gastroenterological Association (AGA) guidelines, the American College of Radiology, and the International Association of Pancreatology guidelines. In general, the guidelines recommend resection of most cysts with high-risk structural features and/or cytology. However, most pancreatic cyst surgeries reveal nonmalignant disease and are associated with significant risk of morbidities [5].

The recent advent of genetic analysis on pancreatic cyst fluid has contributed to the risk stratification profile of cysts

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previously limited to EUS w/ FNA for cytology and cyst fluid chemistry. Specific point mutations, chromosomal deletions of tumor suppressor genes, and high DNA content have been shown to correlate with malignancy [6, 7, 8]. Integrated Molecular Pathology (IMP) combines molecular and biochemical analysis with EUS and cytology findings to assess the malignant potential of pancreatic cysts. Molecular profiling of pancreatic cyst fluid includes assessing for high-quality DNA, loss of heterozygosity of tumor suppressor genes, and KRAS point mutations, which all can correlate with malignancy [6]. Similarly, the biochemical analysis involves guideline-recommended tests of imaging, cytology, and fluid chemistry (for amylase and/or carcinoembryonic antigen). While none of these features can individually determine the risk of malignancy (except a definitive malignant cytology result), the integration of these molecular and biochemical test results can provide an enhanced level of diagnostic and predictive information. We aimed to characterize the long-term diagnostic utility of IMP for pancreatic cysts using a population of patients with pancreatic cysts who were subsequently followed for up to eleven years.

PATIENTS/ MATERIALS AND METHODS

Eligible subjects were > 18 years of age who underwent EUS with FNA and had pancreatic cystic fluid analysis consistent with a mucinous or indeterminate cyst or had complex cysts by EUS. Subjects were excluded if they had pseudocyst, overt cancer invasion or metastasis by EUS or imaging, or presence of other malignancies. Patients were contacted every 6-12 months for any change in GI symptoms, repeat imaging or biopsy and outcomes. The study was approved by the Institutional Review Board at Torrance Memorial Medical Center in Torrance, California (IRB# 2021.09.01).

EUS-FNA and Integrated Molecular Pathology (IMP) were performed as previously described [9]. EUS was performed with either a GF UE160 radial echoendoscope, Olympus Inc or a GF UTC 180 linear array echoendoscope, Olympus Inc; the latter was used for FNA. Aspirated cyst fluid was analyzed for the presence of CEA, amylase, cytology as well as molecular indicators of aggressiveness including DNA quantity, DNA quality, KRAS point mutations, and allelic imbalance (LOH) using PathFinderTG® (Interpace Integrated Pathology, Inc aka PancraGEN®, Interpace Diagnostics). Samples were shipped at 2-8°C with cold packs.

Based on EUS, cyst CEA and amylase levels, pancreatic cysts were categorized as serious (SCN), Mucinous (MCN), Intraductal Papillary Mucinous Neoplasm (IPMN), or indeterminate using the following criteria: SCN: CEA <5 ng/ml, no cyst nodules or cyst wall thickening on EUS, and non-viscous aspirate; MCN: CEA >192 ng/ml and/or viscous aspirate; IPMN: CEA >192 ng/ml, connection with the pancreatic duct by EUS and watery or viscous aspirate, high amylase levels; Indeterminate cyst: CEA<192 ng/ml, no mucinous aspirate, and no connection to the pancreatic duct. IMP classified cysts based on previously validated criteria considered low malignant potential were reported as benign or statistically indolent and cysts with high malignant potential as Statistically Higher Risk (SHR), or aggressive.

Patient follow-up data from the time of the initial EUS to July 2013 included surveillance with EUS or MRI. Cysts deemed to be non-benign were followed every six months or referred for surgery. Patients with benign cysts were contacted by telephone every 6 to 12 months. Questionnaires were administered to patients to determine if they exhibited any symptomatology that could indicate malignant transformation of their pancreatic cysts. A final diagnosis was based on combined evidence of EUS features (presence of cysts with solid nodules, thick septations, wall thickness), fluid CEA, fluid IMP, and amylase level, and final pathology specimens obtained either endoscopically or surgically.

RESULTS

One-hundred and fourteen (114) patients were screened, 45 patients were excluded based on exclusion criteria and 69 were included in the study. Data were collected from sixty-nine patients (44 women and 25 men) with pancreatic cysts who underwent EUS with FNA and IMP by a single operator (VE) from 2010 to 2013. Follow-up data on the enrolled patients was obtained over a range of 1 to 11 years with a median follow-up time of 76.9 months (IQR 88.26). Seven patients in the cohort were lost to follow-up **(Table 1).** Based on the EUS assessment, 56 patients had cysts with malignant potential - 41 cysts were IPMNs, 12 were MCNs, and had 3 were cysts based on final diagnosis. 13 patients had SCN cysts. Overall, the average cyst size was 2.3cm (0.2-15.3). 22/69 (31.9%) cysts were in the head of the pancreas, 13/69 (18.8%) in the neck, 23/69 (33.3%) in the body, and 11/69 (15.9%) in the tail of the pancreas. 10 patients underwent resection or enucleation and of these 10 patients, five were diagnosed as adenocarcinoma. We continued to track patients for

Table 1. Patient Demographics (n=69).

Average age (years)	69.9
Length of follow up in months (IQR)*	76.9 (88.26)
Sex, *n(%)*	
Male	25 (36.2)
Female	44 (63.8)
Average cyst size	2.3 cm (0.2 -15.3)
Pancreatic cyst location, *n(%)*	
Head	22 (31.9)
Neck	13 (18.8)
Body	23 (33.3)
Tail	11 (15.9)

***7 patients with follow-up <1 year- 4 patients with malignancy (Diffuse Large B-Cell Lymphoma, pancreatic adenocarcinoma, HCC with a lung mass, and adenocarcinoma w/ metastasis) and three patients with benign cysts were lost to follow-up.

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an additional 11 years maximum (median 76.9 months, IQR 88.26) after initial EUS w/ FNA for development of symptoms and transformation of cysts into malignancy. No additional transformations into adenocarcinoma were detected amongst the cohort.

Of the 56 patients with pancreatic cysts with malignant potential, IMP characterized three patients as SHR or Aggressive and 53 patients with benign or statistically indolent cysts **(Table 2).** One of the pancreatic cysts deemed "high risk" by IMP ended up being diagnosed as adenocarcinoma while the other four adenocarcinomas were classified as lower risk, (ie "Benign" or "Stat Indolent"). This resulted in IMP in 2013 having a Sensitivity and Specificity of 20% and 96% with PPV and NPV being 33% and 92% respectively.

There were two cases that IMP deemed higher risk but had final diagnoses of non-malignancy. One of the two patients had high risk EUS and clinical features including a cyst size of 5.5cm x 2.7cm as well as painless jaundice on exam. This patient had a Whipple procedure for chronic pancreatitis. The other patient deemed higher risk by IMP without a final diagnosis of cancer underwent surgery due to high-risk features and was found to have a GI stromal tumor.

Using 2018 ACG EUS criteria [3] for assessing malignancy, 13 of 56 (23%) cysts with malignant potential possessed at least one high-risk endoscopic feature, while 43/56 (77%) did not possess a high-risk feature. All five of the cysts diagnosed as adenocarcinoma (9%) were considered high-risk on EUS with at least one high-risk feature [3]. This approach had a Sensitivity and Specificity of 100% and 84% with PPV and NPV being 38% and 100%, respectively **(Table 3).**

We next assessed the impact of combining our IMP results with 2018 ACG EUS high-risk criteria as diagnostic criteria malignancy. In this analysis, a patient was considered high risk if the patient met ACG-EUS [3] or molecular criteria. Otherwise, the patient was defined as low risk. This approach yielded 100% sensitivity and 82% specificity with a positive-predictive and negative-predictive value of 36% and 100% **(Table 4).**

Table 2. IMP Criteria (n=56).

IMP Criteria	Malignant	Non-malignant
Benign/Stat Indolent	4	49
Aggressive/SHR	1	2
Sensitivity	20%	
Specificity	96%	
Positive predictive value (PPV)	33%	
Negative predictive value (NPV)	92%	

Red Path Molecular Criteria: DNA Quantity, DNA Quality, Allelic Imbalance (LOH) and KRAS point mutation

We further analyzed the data to determine the positive and negative predictive values of different approaches to categorizing patient risk using additional analysis beyond the original IMP evaluation, as well as EUS assessment. This modified method, demonstrated in Al-Haddad et al. [10], combined genetic testing with cytology and imaging. Cysts with <1 molecular criteria (ie., a single high-clonality mutation, elevated level of high-quality DNA, multiple low-clonality mutations, or KRAS mutation) and no concerning clinical features were categorized as low risk [9]. Cysts with ≥ 1 molecular criterion along with 1 or more concerning clinical feature were categorized as high risk [10]. Of the 56 patients with pancreatic cysts with malignant potential (i.e., IPMNs, MCNs, and Indeterminate), 10 patients met criteria for high-risk cysts and 46 patients met criteria for low risk **(Table 5).** Of the 10 patients in the high-risk category, 4 had a malignancy and 6 were benign. Of the 46 patients deemed "low risk", 1 had a malignancy and 45 were benign. This is consistent with IMP combined genetic and clinical features carrying a sensitivity and specificity of 80% and 88%, respectively, with a positive predictive value of 40% and a negative predictive value of 97% **(Table 6).**

Table 3. 2018 ACG EUS Criteria (n=56).

ACG[a]	Malignant	Non-malignant
0 high risk EUS features	0	43
≥1 high risk EUS Feature	5	8
Sensitivity	100%	
Specificity	84%	
Positive predictive value (PPV)	38%	
Negative predictive value (NPV)	100%	

High risk features: cyst size > 3 cm, main pancreatic duct size > 0.5 cm, mural nodule/solid component, change in main duct caliber w/ upstream atrophy, or increase in cyst size > 3mm/yr.

Table 4. 2018 ACG and IMP Criteria (n=56).

ACG EUS or IMP	Malignant	Non-malignant
All Others	0	42
AGA-EUS and/or IMP (+)	5	9
Sensitivity	100%	
Specificity	82%	
Positive predictive value (PPV)	36%	
Negative predictive value (NPV)	100%	

Table 5. IMP Criteria According to Al-Haddad et al.

IMP Diagnostic Category	Genetic Criteria[1]
Benign Cysts (Benign or SI)	All Others
High-Risk Cysts	DNA meets ≥ 1 molecular criterion[1] **and** 1 or more clinical features[2]

1Genetic criteria: i) a single high-clonality mutation, ii) elevated level of high-quality DNA, iii) multiple low-clonality mutations; iv) KRAS mutation.

2 Clinical criteria: cyst size > 3 cm, main pancreatic duct size > 0.5 cm, solid mass, cytologic evidence of high-grade dysplasia.

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Table 6. IPMNs, MCNs and Indeterminant Cysts evaluated by IMP Criteria (n=56).

IMP	Malignant	Non-malignant
Benign or SI	1	45
High-Risk Cysts	4	6
Sensitivity	80%	
Specificity	88%	
Positive Predictive Value (PPV)	40%	
Negative Predictive Value (NPV)	97%	

DISCUSSION

Predicting the malignant potential of pancreatic cysts will continue to be of great importance [2] given their increased detection and asymptomatic nature. The difficulty of balancing the morbidity and mortality of pancreatic surgery [5] on benign lesions with the risk of missing early pancreatic cancer remains a difficult challenge for clinicians. While imaging modalities like CT continue to advance in diagnostic accuracy, they still are lacking in differentiating malignant versus benign pancreatic cystic lesions [11]. CEA levels as well as cytologic analysis also fall short in predicting malignant potential of pancreatic cysts with sensitivities ranging from 59% - 67% and 49% - 59% respectively [12,13]. An enhanced level of prediction is needed to maximize patient outcomes and limit unnecessary surgical complications within this population.

IMP by itself **(Table 2)** yielded a significantly low sensitivity value, only classifying 1/5 malignancies as higher risk. This sensitivity of 20% is especially low when compared to previously published data. Prior retrospective studies and meta-analyses have investigated the diagnostic performance of molecular analysis on pancreatic cyst fluid. The IMP analysis done by Al-Haddad et al., the sensitivity using a sample size of 492 was 83% [10]. Another meta-analysis comprised of six separate studies that included 785 cysts found molecular criteria to have a sensitivity of 94% when predicting the malignant potential of said cysts [14]. Specificities of these prior studies were comparable our findings [10, 14]. The low positive predictive values (33-40%) from our study are limited by the small number of positive cases in our cohort and are lower than those reported elsewhere. This, nonetheless, indicates the need for improvement in the diagnostic testing to rule-in malignancy.

The more recent use of EUS-FNB has proven to be a safe and feasible method to histologically diagnose pancreatic cysts. Unfortunately, the technology for EUS-FNB was not available for use at the time of this study. Published data exists showing this method can result in a diagnostic yield of 87.2% (n=47) [15]. Using EUS-FNB with forceps compared to FNA may have the benefit of increased assurance of adequate sample collection, however, it can only be accomplished using a 19g needle which can be difficult to use for cysts in the uncinate process and possibly pancreas head due to required angulation of the stiff needle. In addition, there is an increased reported risk of pancreatitis.

In our prospectively acquired cohort of patients, we demonstrated that ACG imaging criteria as well as the combination of ACG imaging criteria with IMP provides important additional data which clinicians can use to enhance risk-stratification of their patients. The high NPV and specificity suggests that the combination can reliably predict benign disease and identify patients in whom surgery or active surveillance can be avoided. Using the 2018 ACG imaging criteria, all malignant cysts were detected with a sensitivity of 100%. Additionally, the IMP provided high specificity and only classified two non-malignant cysts as "high risk", compared to the 8 using imaging criteria alone. Thus, we envision IMP serving as an important supplementary analysis to assist with the re-classification of high-risk cysts based on EUS into cysts less likely to transform into malignancy.

The primary limitations in our study are the relatively sample size and the limited number of malignancies. The strong negative predictive outcome is based on up to 11-years of follow-up, one of the longest clinical follow-up studies for pancreatic cysts using EUS with FNA combined with IMP, to our knowledge and provides robustness to the Negative Predictive Values in our analyses. The low PPV may be an acceptable trade-off given the severity of pancreatic disease. The combination of EUS with FNA and IMP therefore presents a useful risk stratification tool to help clinicians with ruling out aggressive disease in patients.

Overall, IMP combined with EUS has favorable sensitivity and specificity with significantly improved diagnostic accuracy compared with other modalities of assessing malignant potential of pancreatic cystic lesions. While additional larger studies are needed to compare 2018 ACG imaging criteria with the combination of imaging and molecular criteria, these findings provide an important first step in evaluating long-term diagnostic utility of IMP and EUS.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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