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Research Article

Diagnostic Accuracy of Pancreatic Cystic Lesions Evaluated by Endoscopic Ultrasound-Guided Fine Needle Aspiration Cytology

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Abstract

Background: Endoscopic ultrasound-guided fine needle aspiration (EUS-guided FNA) of pancreatic lesions is now a routine practice in many hospitals and clinics. Prior reports suggested that cytology alone had only moderate diagnostic accuracy distinguishing pancreatic cysts; therefore, many endoscopists supplement cytology with cyst fluid carcinoembryonic antigen (CEA) levels (> 192 ng/ml is positive). We hypothesized cytology-based diagnoses may be more predictive than previously reported compared with cyst fluid CEA.

Methods: We performed a retrospective review of 44 pancreatic cysts sampled by one endoscopist using EUS-guided FNA and diagnosed by cytopathologists with more than five years of clinical experience. CEA levels were measured in all 44 biopsies and all cases had at least five years of clinical follow-up. To determine the diagnostic accuracy of cytology alone, archived slides were further evaluated by one cytopathologist blinded to the original cytologic diagnoses and clinical outcomes. Cases were classified as i) negative for mucinous neoplasm, ii) cystic mucinous neoplasm, iii) cystic mucinous neoplasm with atypia, or iv) adenocarcinoma.

Results: We observed good agreement between the blinded review of the of the cytologic preparations and the original cytologic diagnosis, which was based on all available clinical information, including cyst fluid CEA levels (kappa statistic = 0.68 [0.46-0.90], p<0.0001). The diagnostic accuracy of distinguishing mucinous tumors by cytology alone was 84%, while CEA levels alone had an accuracy of 57%. The original FNA diagnoses, which utilized clinical data and CEA levels, had an accuracy of 86%.

Conclusions: Experienced endoscopists and cytopathologists provide excellent overall diagnostic accuracy when evaluating pancreatic cystic lesions by EUS-guided FNA.

Keywords: Cytology; CEA; pancreatic cysts; mucinous; IPMN; EUS-guided FNA

Introduction

Cystic lesions of the pancreas are a heterogenous group of entities ranging from non-neoplastic to malignant [1]. Once thought to be rare, pancreatic cysts have proven to be relatively common with current imaging techniques [2]. Many of these lesions require no intervention, but mucinous cysts may warrant surgical resection [3,4], making diagnostic accuracy of these lesions vital.

Endoscopic ultrasound (EUS) has emerged as a safe and useful tool for the diagnosis of cystic pancreatic lesions [5,6]. In addition to imaging the lesion, EUS-guided fine

needle aspiration (FNA) biopsies provide material for cytologic diagnosis and ancillary testing [7]. Older studies suggested that FNA cytology alone may have only low to moderate accuracy when distinguishing cystic pancreatic lesions [8-10]; therefore, many endoscopists supplement the cytologic evaluation with cyst fluid carcinoembryonic antigen (CEA) levels [8]. Since our institution has nearly 20 years of experience performing and evaluating EUS-guided FNAs [11-16], we hypothesized that we may have significantly better diagnostic accuracy than these older studies [8] and compare favorably with the sensitivity and specificity of cyst fluid CEA levels.

Methods

Study Subjects: Using an institutional review board (IRB) approved protocol, we retrospectively identified 44 subjects who had EUS-guided FNAs for cystic lesions of the pancreas at Oregon Health and Science University (OHSU), cyst fluid CEA levels at the time of biopsy, and at least five years of clinical followup. Notably, the endoscopist's clinical impression of all 44 cases was "likely pancreatic mucinous neoplasm", which at our institution prompts cyst fluid CEA level testing. All pancreatic biopsies had been performed using a linear echoendoscope and aspirated in [1-3] passes using a 22-gauge needle by a single experienced endoscopist (dof) with adequacy assessment by cytopathologist at the time of biopsy. At the time of adequacy assessment, air-dried smears were Diff-Quik stained and reviewed on-site. To be considered "adequate", the air-dried smears were required to show at least one group of ductal mucosa (without goblet cells), although all 44 cases in this study showed numerous ductal groups, which is standard in our practice. 100% ethanol fixed smears were Pap stained, and formalin fixed paraffin-embedded histologic sections of the cell block were stained with hematoxylin and eosin. Notably, our practice routinely makes cell block sections from pancreatic FNAs and all of these cases had cell block sections. The original cytopathologic diagnoses were made by three separate cytopathologists, all with more than 5 years of experience evaluating at least three (3-10 on average) EUS-guided pancreatic FNAs per month. They also had the benefit of knowing the endoscopist's clinical impression, imaging findings, and cyst fluid CEA levels at the time of final cytologic diagnosis. For retrospective analysis, all 44 included cases had either followup surgery at OHSU, or long-term clinical followup (5 to 10 years). To test whether cytology alone by an experienced cytopathologist was comparable to CEA level predictive values, the original cytologic preparations (including cell block sections) were re-screened by an experienced cytotechnologist (sw) and classified by a single experienced cytopathologist (tkm) while blinded to the original cytologic diagnosis, CEA levels, and clinical outcomes. Both of these blinded reviewers had 5 years of clinical experience evaluating EUS-guided FNAs.

Diagnostic Criteria: Cases were categorized as either i) negative for mucinous neoplasm, ii) cystic mucinous lesion, iii) cystic mucinous lesion with atypia, or iv) adenocarcinoma. A cytologic diagnosis of negative included those aspirates that lacked thick background mucin with embedded muciphages, mucinous ductal mucosa, or features of malignancy (Figure 1A). Aspirates were diagnosed as mucinous cystic lesion without atypia if the FNA yielded background mucin and cytologically bland mucinous ductal mucosa (resembling endocervical mucosa, ie, Figure 1B). Atypia was defined as mild nuclear pleomorphism (<3-fold variance) not diagnostic of adenocarcinoma (Figure 1C). Malignant aspirates were diagnosed based on greater than 3-fold nuclear pleomorphism, irregular nuclear membranes, and increased nuclear to cytoplasmic ratios (Figure 1D).

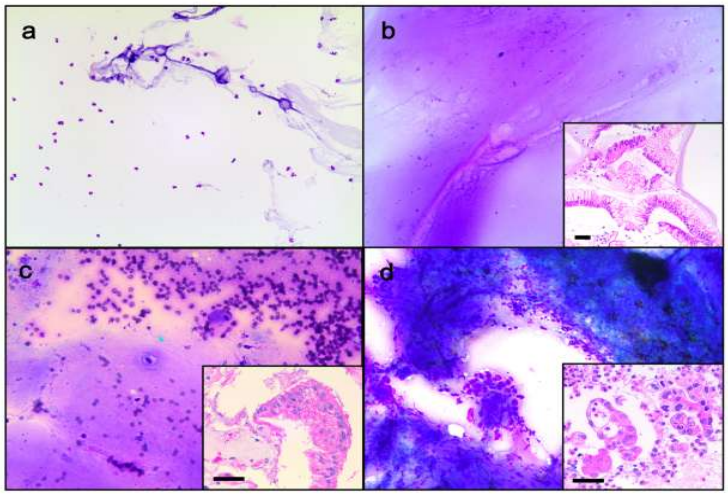

Figure 1. Pancreatic cyst cytopathology. (a) Pancreatic pseudocysts and serous cysts were categorized as negative and had a serous background with debris and benign ductal cells. (b) Mucinous lesions were characterized by background mucin (b-c) and mucinous ductal mucosa either without (b, inset), or with mild nuclear atypia (c, inset). (d) In contrast, ductal adenocarcinoma was diagnosed with 4-fold nuclear pleomorphism, irregular nuclear membranes, and increased nuclear to cytoplasmic ratios. Cytology photomicographs were taken using the 20x objective. Bar = 100 μm in inset hematoxylin and eosin stained paraffin sections of the cell block.

Cyst Fluid Carcinoembryonic Antigen Levels: Cyst fluid CEA concentration was performed in a CLIA approved laboratory using a standard electrochemiluminescent immunoassay and the Roche modular E170/Cobas E 602 analyzer (Roche Diagnostics Corp, Indianapolis IN). The assay is calibrated compared with known standards to provide results in ng/ml.

Outcome Assessment: Histologic, clinical, and radiographic follow-up data was obtained from the electronic medical record. Surgical diagnoses were reported as negative for mucinous neoplasm (ie, pseudocysts, benign serous cysts, one islet cell tumor, and one solid pseudopapillary tumor), mucinous cystic lesion (mucinous cystadenoma, intraductal papillary mucinous neoplasm [IPMN], and pancreatic intraepithelial neoplasm [PanIN]), or ductal adenocarcinoma. CEA levels exceeding 192 ng/ml were considered a positive CEA test [8].

Analysis: Interobserver agreement between the original cytologic diagnosis and blinded re-screening was calculated using Cohen's Kappa statistic. The sensitivity, specificity, and predictive values of each test were calculated using 2x2 contingency tables with binomial 95% confidence intervals (CI) using either surgical diagnosis or negative long-term followup (more than 5 years) as clinical outcomes.

Results

Patient age ranged from 29 to 83 years at the time of diagnosis, with a mean age of 59 years. 18 subjects were male and 26 were female. 32/44 (73%) subjects had a surgical resection with follow-up tissue histology available for diagnostic confirmation. The remaining 12 patients had at least five years of negative clinical followup at our institution showing no recurrence or progression.

The original cytologic diagnoses rendered at the time of the EUS-guided FNA were aided by the endoscopist's clinical impression, radiographic findings, and cyst fluid CEA levels. 15/44 (34%) of these cases had cyst fluid CEA greater than 192 ng/mL. These non-blinded initial diagnoses included 24 negative (non-mucinous) cases, 16 cystic mucinous lesions, 2 cystic mucinous lesions with atypia, and 2 adenocarcinomas (Table 1). There was good agreement between the original non-blinded diagnoses and the blinded review by one experienced cytopathologist based solely on cytologic criteria (kappa statistic=0.68 [95% confidence interval: 0.46 - 0.89], p<0.0001). The blinded review yielded 23 negative cases, 10 cystic mucinous lesions, 8 cystic mucinous lesions with atypia, and 3 adenocarcinomas (Table 1).

To determine test accuracy of discriminating mucinous versus non-mucinous pancreatic cysts, we grouped the three mucinous diagnostic categories and compared these results to clinical outcomes (Table 2). CEA levels greater than 192 ng/mL provided an overall test accuracy of 57%. Taking into consideration the endoscopist's clinical impression, imaging findings, and cytologic features, the original cytologic diagnoses had an overall test accuracy of 86%. The blinded cytology only-based diagnoses provided a similarly high accuracy of 84% (Table 2).

Table 1. Five Year Clinical Outcomes for 44 Pancreatic Cyst FNA Biopsies

Initial Cytology Diagnosis/ Outcome	Negative	Mucinous	Adenocarcinoma
Negative (n=24)	18	5	1
Mucinous Cystic Lesion (n=16)	0	14	2
Mucinous Cystic Lesion with Atypia (n=2)	0	2	0
Adenocarcinoma (n=2)	0	1	1
Blinded Cytology Review/ Outcome	Negative	Mucinous	Adenocarcinoma
Negative (n=23)	17	5	1
Mucinous Cystic Lesion (n=10)	0	10	0
Mucinous Cystic Lesion with Atypia (n=8)	1	5	2
Adenocarcinoma (n=3)	0	2	1

Negative outcomes: pseudocyst, serous cyst, islet cell tumor, solid and cystic papillary
Mucinous lesion: Intraductal papillary mucinous neoplasm (IPMN); mucinous cystic neoplasm with ovarian-type stroma (MCN), small duct PanIN.
Mucinous lesions with atypia: IPMN grades 2 or 3.

Table 2. Diagnostic Accuracy of Pancreatic FNA Cytology and CEA Levels

Test Accuracy	**Original Diagnosis [95% CI]**	**Blinded Review [95% CI]**	**CEA > 192 ng/mL [95% CI]**
Sensitivity	77% [56-90%]	77% [56-90%]	42% [24-63%]
Specificity	100% [78-100%]	94% [71-100%]	78% [52-93%]
PPV	100% [80-100%]	95% [74-100%]	73% [45-91%]
NPV	75% [53-89%]	74% [51-89%]	48% [30-67%]

PPV: Positive predictive value; NPV: Negative predictive value; CEA: carcinoembryonic antigen

Discussion

Cystic pancreatic lesions are discovered in up to 2.5% of hospitalized patients and the incidence increases with patient age [2,17]. Clinical management of mucinous pancreatic cysts relies mainly on an accurate pathologic

diagnosis [1], because imaging studies alone lack the reproducibility and predictive value necessary to guide patient management [18]. Older studies suggested that cytology alone may not provide sufficient accuracy [8]; therefore, many endoscopists submit a portion of the cyst fluid specimen for CEA levels. However, we have found that at our institution cytomorphologic assessment of the FNA material by experienced cytopathologists is more predictive of outcomes than CEA levels in a series of 44 pancreatic cysts biopsied by EUS-guided FNA.

While cyst fluid CEA levels do provide some value, the interpretation of this assay must be tempered by an understanding of its limitations. In an early study of 341 EUS-guided FNAs reported by Brugge et al., they noted higher cyst CEA levels in mucinous neoplasms of the pancreas [8]. Their receiver operating characteristic (ROC) analysis showed intersection at a CEA level of 192 ng/mL with a sensitivity and specificity of 73% and 84%, respectively [8]. Other studies have failed to show this level of diagnostic accuracy, however. In 2008 Maire et al. reported a CEA sensitivity of only 44% in series of 100 IPMN cases [19]. Importantly, there is considerable overlap in cyst fluid CEA levels between non-mucinous and mucinous cysts as was seen in the Brugge study [8]. As a result, the sensitivity and specificity of cyst fluid CEA levels varies significantly depending on the cutoff levels used [7], and appears to have greatest value at the extremes of measurement. CEA levels can also be affected by the type of assay employed [20], further complicating their interpretation. Finally, CEA levels do not reliably distinguish between benign and malignant neoplasms [21,22].

In addition to CEA levels, there may be other more sensitive and specific antigenic targets in pancreatic mucinous neoplasms [15], but this requires further study. Moreover, recent studies have suggested that molecular analysis may improve the diagnostic accuracy of pancreatic FNAs [23]; but these studies have shown variable sensitivity and specificity in the published literature, and are not currently recommended in the evaluation of pancreatic mucinous lesions [24].

While some of the accuracy of our cytologic assessment may be related to the experience of our endoscopists and cytopathologists, we also performed Diff-Quik stained adequacy assessments on every EUS-guided FNA biopsy. Previous studies have shown the negative impact of inadequate sampling and low specimen cellularity on the ability to provide an accurate cytologic diagnosis. In a study of 143 consecutive EUS-guided FNA biopsies of cystic pancreatic lesions, de Jong et al. found that only 44 (31%) had sufficient material for cytologic diagnosis [23]. Similarly, in a series of 733 cases reviewed by Woolf et al, 17 of 19 false-negative results were due to insufficient aspiration [24]. With experience and on-site adequacy assessment inadequate aspirates are rare [25], and diagnostic adequacy improves. In addition, the clinical impression provided by the endoscopist at the time of the procedure with the knowledge of the patient's imaging findings also facilitates an accurate diagnosis, as evidenced by the slightly higher sensitivity and accuracy of the original diagnosing cytopathologists when compared to the blinded review in our study. The cyst fluid CEA level may also have contributed, although based on its low stand-alone accuracy it was not likely a major consideration during the initial diagnosis.

On-site adequacy assessment, endoscopist impression, CEA levels, and the experience of the cytopathologist compiling this data may all significantly improve diagnostic accuracy. In addition to our retrospective study, a recent meta-analysis of 11 studies by Thosani et al. revealed a considerably higher sensitivity (63%) and specificity (88%) [9] than previously reported for pancreatic cyst fluid cytology. Therefore, we suspect growing experience performing and evaluating EUS-guided FNAs of pancreatic lesions may explain why cytologic diagnoses appear to be more accurate than previously reported.

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