Cyst Fluid Carcinoembryonic Antigen Is an Accurate Diagnostic Marker of Pancreatic Mucinous Cysts

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**ORIGINAL ARTICLE**

**Objectives:** Endoscopic ultrasound (EUS) may offer a diagnostic tool through the combination of imaging and guided fine-needle aspiration of pancreatic cysts. The purpose of this investigation was to determine the most accurate test for differentiating mucinous from nonmucinous cysts.

**Methods:** The results of EUS imaging, cytology, and cyst fluid biochemical markers were prospectively collected and compared in a large single-center study (776 patients) using histology or malignant cytology as the final diagnostic standard in 198 patients.

**Results:** The mean cyst fluid carcinoembryonic antigen (CEA) was greater in mucinous cysts (4703.0 ng/mL) compared with nonmucinous cysts (25.8 ng/mL) \((P = 0.008)\). When using the optimal cutoff value of 109.9 ng/mL, the CEA was more accurate (86%, receiver operating characteristic area = 0.928) than EUS imaging (48%) and cytology (58%) in predicting a mucinous cyst \((P < 0.0001)\). Malignant cysts had a mean cyst fluid CEA value (2588.2 ng/mL) similar to benign cysts (4700.2 ng/mL). Cytology (75%) more accurately diagnosed malignant cysts than EUS (66%) and CEA (62%) \((P < 0.05)\).

**Conclusions:** Cyst fluid CEA concentration provides a highly accurate test for the diagnosis of a mucinous cyst, but does not distinguish benign from malignant cysts. Cytology is the most accurate test for the diagnosis of a malignant cyst.

**Key Words:** carcinoembryonic antigen, diagnostic marker, pancreatic mucinous cysts

**Abbreviations:** EUS - endoscopic ultrasound, CEA - carcinoembryonic antigen, MCN - mucinous cystic neoplasm, IPMN - intraductal papillary mucinous neoplasm

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Pancreatic cystic neoplasms were once thought to be rare. Recent computed tomography (CT) imaging studies in asymptomatic adults have demonstrated a prevalence of 2.6%, increasing with age to 8.7% in adults older than 80 years. Although CT scanning is very sensitive for the detection of cystic lesions, the overall diagnostic accuracy is 61% for differentiating between mucinous, nonmucinous, and malignant lesions. The distinction between mucinous and nonmucinous cysts is of great clinical relevance because of the malignant potential of mucinous cysts. Mucinous cysts, composed of mucinous cystic neoplasms (MCNs) and intraductal papillary mucinous neoplasms (IPMNs), are lined by a secretory, columnar epithelium that generates a number of glycoproteins, including carcinoembryonic antigen (CEA). We previously reported the results of a multicenter trial of endoscopic ultrasound (EUS), cyst fluid CEA, and aspiration cytology in distinguishing between mucinous and nonmucinous cysts. Concerns have been raised regarding the variability of assays for measuring cyst fluid CEA concentration. Various centers have reported a wide range of diagnostic cutoff values for CEA in the diagnosis of mucinous cysts. We report the results of a large, prospective, single-center, single-CEA-assay study of the relative accuracy of EUS imaging, cytology, and cyst fluid CEA in differentiating between mucinous and nonmucinous as well as benign and malignant cysts of the pancreas. We have not previously reported the use of cyst CEA in differentiating between malignant and benign cysts.

**MATERIALS AND METHODS**

A single-center trial, with institutional review board approval at Massachusetts General Hospital (MGH), was initiated in July 1999. The institutional review board did not require a patient consent for data collection. All patients found to have a pancreatic cystic lesion on transabdominal ultrasonography or CT scanning who underwent EUS-guided cyst aspiration were entered into the database. The results of a clinical assessment, EUS examination, cyst fluid cytology, and histology were blindly entered on a regular basis into a prospectively maintained database by a study coordinator. Generally, the results of the EUS findings were immediately entered into the database, followed by the results of cytology and CEA. When it was determined that the patient had undergone a surgical procedure, the results of surgical pathology were entered into the database. Refrigerated, undiluted cyst fluid (≥1 mL) was collected for determination of CEA and amylase concentration. The results of the cyst fluid analysis was determined and reported by the MGH Department of Clinical Chemistry without knowledge of the clinical, pathological, or EUS results.

**EUS METHODS**

All patients (under conscious sedation) underwent an EUS examination performed using a linear echoendoscope as previously described. Briefly, the echoendoscope provided high-resolution imaging of the head, body, and tail of the pancreas, from the duodenum and stomach respectively. The results of the examination prospectively reported the location, size, and morphology (see below) of the cystic lesion without knowledge of the results of cytology or cyst fluid CEA. The cystic lesion was aspirated under EUS guidance using 1 passage of a 19- or 22-gauge needle (Wilson Cook Inc, Winston-Salem, NC) occluded with a stylet. An oral quinolone was administered for 2 to 3 days after the procedure to prevent cyst infection.

**Diagnostic Criteria**

**EUS Morphology**

The EUS examination provided the size, location, and the presence of associated findings in the pancreas. The specific
EUS morphological findings of the cystic lesion that were prospectively recorded included the presence or absence of (1) an adjacent mass or nodule, (2) macrocystic morphology (defined by the presence of discrete locules), (3) honeycombed septations (microcystic morphology), and (4) a diffusely thickened cyst wall. The official hospital EUS reports were used as the data source, and the attending endosonographer provided the findings of mass or nodule, septations, and wall thickness in the EUS report. Cysts with an adjacent mass-nodule or macrocystic morphology were classified as mucinous cysts by EUS morphology. Endoscopic ultrasound morphology was not used to differentiate between MCNs and IPMNs. Cysts with honeycombed septations (microcystic morphology) or a thickened cyst wall were considered nonmucinous (serous cystadenomas or pseudocysts) by EUS morphology.

**Cytology**

All cytological analyses were carried out by MGH staff cytologists, and the official reports were used as the data source. On-site cytology was not performed. Samples were reported to be diagnostic or nondiagnostic (defined as acellular, nonmucinous material or the presence of gastrointestinal [GI] contaminant cells by the staff cytologists in the MGH cytology report). Diagnostic samples were classified as containing cytological evidence of (1) mucinous epithelium (clusters of benign, atypical/suspicious, or malignant glandular cells with variable quantity of thin or thick extracellular mucin) or (2) nonmucinous epithelium (flat monolayers of small cuboidal cells or inflammatory cells such as pigment-laden macrophages, histiocytes, or leukocytes). The official cytology reports were used for the basis of the study, and the results were entered into the database on a regular basis without review by the staff cytologists. The results of cytology were not used to differentiate between MCNs and IPMNs.

**Histology**

Primary histological interpretations of the resected specimens were performed by specialized GI pathologists at MGH. The official reports were used as the data source. The cysts were classified as (1) a mucinous cystic lesion (benign [low-grade dysplasia], moderate dysplasia, high-grade dysplasia/carcinoma in situ, or invasive carcinoma) or (2) a nonmucinous cyst including serous, inflammatory, and cystic endocrine neoplasms. Although the histology reports differentiated between MCN and IPMN, both lesions constituted “mucinous cysts” for the study. Cysts that could not be classified into the categories stated above were classified as “others.”

**Cyst Fluid Biochemical Markers**

Cyst fluid concentrations of CEA and amylase were measured using a specific radioimmunoassay or a chemical analysis as previously reported. The CEA concentration (in nanograms per milliliter) was determined on an Abbott Diagnostics IMX-MEIA immunodiagnostics analyzer (Abbott Laboratory, Diagnostics Division, Abbott Park, Ill). Cyst fluid was not analyzed for mucin concentration, DNA, or viscosity.

**Data Collection and Analysis**

Analyses were restricted to patients with histological confirmation of the type of cystic lesion or with malignant cytology results. Although many cytology reports were descriptive, only cytology reports with a conclusive interpretation regarding cyst type using the previously outlined criteria were interpreted as being diagnostic. All other cytology reports were classified as nondiagnostic. The sensitivity, specificity, and accuracy rates (percentage) were calculated for the diagnostic criteria. “EUS morphology,” “cytology,” and “CEA,” for mucinous (benign and malignant) and nonmucinous cysts (serous, inflammatory, or endocrine). Separate receiver operating characteristic (ROC) curves were plotted using each CEA value to predict a mucinous or a nonmucinous cyst. The area under each ROC curve, a measure of predictive power, was calculated. For CEA, a cutoff value was selected to maximize the proportion of correct classification of the cyst. Sensitivity and specificity were determined using these values. The CEA cutoff value with the greatest area under the ROC curve was selected for comparison with morphology and cytology. Sensitivity, specificity, and accuracy rates were compared using 2-sample tests of proportions.

**RESULTS**

Seven hundred sixty-one consecutive patients were enrolled in the trial over 9 years (Table 1). All subjects underwent EUS and fine-needle aspiration. The subjects were predominantly female (60.9%, $P < 0.01$). A majority of the cysts examined (n = 413, 54.3%) were greater than 2 cm in diameter, and half were located in the head of the pancreas (n = 355, 46.6%). One hundred ninety-eight of the enrolled subjects underwent a resection (n = 166), a biopsy (n = 26), fine-needle aspiration that revealed malignant cytology in nonresected tumors (n = 4), or an autopsy (n = 2) that yielded histological confirmation of the cyst type and is the basis of this study. The next most common type of “cyst” was a pseudocyst. There were only 8 serous cysts and 7 neuroendocrine cysts.

The histological characteristics of the mucinous cysts were divided by the stage of malignancy (benign adenoma, borderline, carcinoma in situ, and invasive carcinoma). Many (66/136,
value for distinguishing between a mucinous and nonmucinous cyst was 109.9 ng/mL. The area under the ROC curve was 0.928, indicating a highly accurate test (96%). Using our previously identified cutoff value for cyst fluid CEA (192 ng/mL) in differentiating between mucinous and nonmucinous cysts, the overall accuracy was 82% (126/154). Carcinoembryonic antigen values of 8 patients fell between 109.9 and 192 ng/mL, and there was no significant difference between the accuracy of the current (109.9 ng/mL) and the previously reported (192 ng/mL) cutoff values in diagnosing mucinous cysts ($P = 0.179$).

A comparison between the accuracies of EUS (93/194, 48%), cytology (112/194, 58%), and cyst fluid CEA (132/154, 86%) demonstrates that CEA is most accurate in differentiating between mucinous and nonmucinous cysts (Table 2). Using combination testing, a cyst was included as mucinous (or malignant) if any of the component diagnostic tests classified it as mucinous (or malignant). Combination testing including CEA (EUS morphology or cytology or CEA; cytology or CEA) demonstrated significantly greater accuracy in detecting mucinous cysts ($P < 0.0001$) than combination testing with EUS morphology or cytology, which was further supported by calculated areas under the ROC curves (Table 3).

There was no difference in the mean cyst fluid amylase concentration when comparing mucinous (29,832 U/L) and nonmucinous cysts (26,496 U/L). However, when using cyst fluid amylase in the subgroup analysis of IPMN compared with MCN, there was a modest yet insignificant difference (mean amylase of 24,299 U/L in IPMN compared with MCN cysts [7821 U/L]; $P = 0.12$).

Because cyst fluid CEA is elevated in mucinous lesions, we also examined the possibility that CEA could act as a marker of malignancy. However, there was no difference between the mean cyst fluid CEA concentration in benign mucinous (2558 ng/mL; CI, 623.2–4493.2; n = 102) compared with malignant mucinous cysts (4700.2 ng/mL; CI, 2091.2–7309.2; n = 55) ($P = 0.258$). Similar results were observed in the comparison of all benign and all malignant cysts, including a comparison between benign, moderate, carcinoma in situ, and invasive carcinoma.

When examining the same diagnostic operating characteristics for malignant cysts, we generally found lower accuracy rates of 66%, 75%, and 62%, respectively for EUS, cytology, and cyst CEA (Table 4). Comparing the accuracies of EUS morphology, cytology, and cyst CEA, cytology more accurately

### TABLE 2. Relative Accuracy of EUS Morphology, Cytology, and Cyst Fluid CEA in the Diagnosis of a Mucinous Cyst

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUS Morphology</td>
<td>50/141 (35.5%)</td>
<td>61/141 (43.3%)</td>
<td>89/110 (80.9%)</td>
</tr>
<tr>
<td>Cytology</td>
<td>43/53 (81.1%)</td>
<td>51/53 (96.2%)</td>
<td>43/44 (97.7%)</td>
</tr>
<tr>
<td>CEA*</td>
<td>93/194 (47.9%)</td>
<td>112/194 (57.7%)</td>
<td>132/154 (85.7%)</td>
</tr>
</tbody>
</table>

*Using 109.9 ng/mL as cutoff.

### TABLE 3. Relative Accuracy of a Combination of Tests for the Diagnosis of a Mucinous Cyst

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>Area under ROC curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUS Morphology or Cytology</td>
<td>81/141 (57%)</td>
<td>128/141 (91%)</td>
<td>113/141 (80%)</td>
<td>0.68</td>
</tr>
<tr>
<td>EUS Morphology or Cytology or CEA</td>
<td>42/53 (79%)</td>
<td>41/53 (77%)</td>
<td>50/53 (94%)</td>
<td>0.84</td>
</tr>
<tr>
<td>Cytology or CEA</td>
<td>123/194 (63%)</td>
<td>169/194 (87%)</td>
<td>163/194 (84%)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

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detected malignancy than EUS \((P < 0.05)\) and CEA \((P < 0.01)\). In general, cytology excelled in providing higher specificity (96%), whereas CEA provided a highly sensitive (78%) test. Combination testing of EUS morphology or cytology (70%) demonstrated low levels of accuracy compared with a combination of all 3 diagnostic tests (62%) \((P < 0.05)\) and failed to demonstrate greater accuracy than cytology or CEA (65%) for differentiating between benign and malignant mucinous cysts.

**DISCUSSION**

This study confirms and refines the diagnostic usefulness of cyst fluid CEA for the differentiation between mucinous, non-mucinous pancreatic, malignant, and nonmalignant cysts. Because pancreatic cystic lesions span the full spectrum of benign, inflammatory, premalignant, and malignant lesions of the pancreas, it is important to provide an accurate diagnosis. Serous cystadenomas are characterized by a simple, glycogen-rich, cuboidal epithelium. Because the epithelium of serous cystadenomas does not secrete CEA or mucin, the cyst fluid is characteristically lacking in CEA. There is no epithelium lining pseudocysts, and as such, the CEA concentration of pseudocyst fluid is also generally low. The cyst fluid from mucinous lesions contains variable concentrations of CEA, reflecting the presence of epithelial CEA, as previously documented by immunohistochemical studies.

The traditional approach to the diagnosis of pancreatic cysts is the use of aspirated cyst fluid for cytological examination. In general, stained exfoliated cells and the extracellular mucin in cyst fluid are examined by light microscopy. The passage of the EUS needle across the gastroduodenal epithelium, however, often results in contamination of the cyst fluid with mucin and gastroduodenal epithelial cells. With experience, cytologists can often identify gastrointestinal epithelia, although low-grade dysplasia in mucinous cyst specimens may be difficult to distinguish from gastrointestinal epithelial cells. In our study, the presence of mucinous epithelium in cyst fluid was a highly specific finding for a mucinous cyst, but lacked sensitivity. The finding of malignant cells in cyst fluid was equally important. The presence of malignant cells had a specificity of 96% for the diagnosis of a malignant cyst, indicating the high diagnostic power of the finding of malignant cells. Although cytology has a major role in the diagnosis of mucinous cysts, the ability of cyst fluid cytology for providing a diagnosis of a serous cystadenoma remains a challenge. Cyst fluid cytology had an accuracy of only 58% for differentiating between mucinous and nonmucinous cysts because of the high number of acellular, nondiagnostic cyst fluid specimens.

Carcinoembryonic antigen, a large, cell surface adhesion molecule and a member of the immunoglobulin superfamily, is an oncofetal antigen secreted in identical forms from intestinal, breast, lung, and pancreatic malignancies. Although CEA is present in malignant pancreatic glandular (adenocarcinoma) tissue, serum levels are inaccurate for the diagnosis of malignancy. In benign pancreatic mucinous cysts, CEA is secreted from the luminal surface of the glandular cells and is present in the cytoplasm of malignant cells. With the use of immunohistochemical analysis, CEA appears absent in serous cystadenomas. Cyst fluid protein expression profiling has identified CEA and CA-72-4 as unique markers in the fluid from mucinous cysts. In the original description of cyst fluid CEA analysis, the pancreatic fluid was collected surgically with needle aspiration, and the CEA values were greater than 367 ng/mL in benign and malignant mucinous cystic lesions. Subsequently, studies using radiologically guided cyst fluid aspiration demon
an outside institution, it was difficult to determine the diagnostic accuracy of CT or magnetic resonance imaging in predicting a mucinous cyst. Lastly, 40 subjects did not have sufficient fluid for CEA analysis and were excluded from the CEA data analysis.

In summary, we have established the high diagnostic accuracy of cyst fluid CEA. In selected patients in whom a diagnostic test is important for management, cyst fluid should be aspirated and analyzed for CEA to establish the diagnosis of a mucinous cyst. Cytology should be used to establish the presence of a malignancy arising in a mucinous cyst.

REFERENCES