# FLD-04
# Pancreatic Cyst Fluid

Clinical Scenario:
A 44-year-old female presents to her internist with a 1 month history of dull epigastric pain. Clinical examination of the abdomen reveals mild epigastric tenderness but no masses are palpated; however, a CT scan of the abdomen documents a circumscribed 6.8 cm multiloculated, macrocystic mass in the body of the pancreas. Radiologic evaluation fails to demonstrate a direct communication between the pancreatic cyst and the main pancreatic duct. No other pancreatic, hepatic or biliary masses are noted. Additional medical history raises the possibility of alcohol abuse and a possible bout of acute pancreatitis approximately 6 months ago.

A subsequent endoscopic ultrasound (EUS) guided aspiration of the cystic lesion yields 3.6 mL of slightly thick fluid which was submitted for cytologic examination, CEA analysis and amylase. FLD-04 is the pancreatic cyst fluid aspirate.

**DISCUSSION:**
Over the past 2 decades, technical imaging advances have resulted in the radiologic detection of a substantial number of previously undetectable pancreatic cysts, especially in the older population.[1] Some estimates suggest that approximately 1% of the inpatient population in general medicine have pancreatic cysts, many of them small and asymptomatic. Although many pancreatic cysts are benign, some have a high rate of synchronous or metachronous malignancy. The clinical dilemma is the separation of which cysts require further evaluation and possible resection vs. which can be clinically followed with a low risk of malignancy.[2, 3]

The majority of pancreatic cysts fall into one of the following four categories: pseudocyst (the most common category), intraductal papillary mucinous neoplasm (IPMN), mucinous cystic neoplasm (MCN) and serous cystadenomas. The risk of malignancy is low in pseudocysts and serous cystadenomas but may be substantial in the mucinous cystic lesions (IPMN, MCN). Although a few predominantly solid pancreatic neoplasms may undergo cystic degeneration and there are a small number of uncommon benign cystic lesions (e.g. retention cysts, congenital cysts, etc.), the four categories listed above account for almost all cysts requiring CEA and amylase analysis of the cyst fluid in the clinical laboratory.

The clinical evaluation of a pancreatic cyst includes incorporation of data from a variety of sources including the patient's age, history, size of the cyst, radiologic characteristics, cytological evaluation and chemical assessment of CEA and amylase levels, sometimes accompanied by CA 19-9. Molecular testing may have also found a place in cyst fluid analysis.[4]

Many pancreatic cysts are classified on the basis of the clinical and radiologic data while a minority requires additional evaluation that may include endoscopic ultrasound (EUS) guided drainage of the cyst fluid. Although the aspirated fluid volume is often limited, analysis of the fluid contents can add significant information to the risk assessment of these pancreatic cysts. When cyst fluid is available, cytological assessment for mucin and abnormal cellular content as well as cyst fluid CEA and amylase are often ordered. Molecular analysis may also have a role in the assessment of fluid samples of limited volume (<1 ml) where the volume restriction limits the laboratory's ability to adequately assess CEA, amylase and/or cytology.[4, 5, 6]

*Utility of CEA/amylase*
CEA and amylase have emerged as two of the more useful clinical laboratory tests in the assessment of pancreatic cyst fluid aspirates.[4, 6, 7] While these analytes do not readily separate benign cysts from malignant cysts, CEA is often helpful in separating mucinous cysts (IPMN, mucinous cystic neoplasm) from non-mucinous cysts. In general, the higher the CEA value, the more likely the cyst is a mucinous cyst with the attendant increased risk of malignancy. In contrast, serous cystadenoma is associated with CEA values that are often <5 ng/ml.

Amylase may be helpful in suspected cases of pseudocyst or serous cystadenoma. Cyst fluid amylase is most always substantially elevated in pseudocysts, often in the 1000's to 10,000's (U/ml), but low in serous cystadenomas or in many non-pancreatic cysts that are anatomically close to the pancreas and thus must be separated from true pancreatic cysts. Pseudocyst aspirates generally have low to undetectable CEA values. Intraductal papillary mucinous tumors (IPMN) produce fluid with increased CEA due to the mucinous lining of the cyst but variable amylase levels as many IPMN's communicate with the pancreatic ductal system.

Most laboratories are utilizing serum based assays for the assessment of CEA and amylase in pancreatic cyst fluids. Matrix effects, linearity issues, antigen excess (hook effect), and specimen viscosity may significantly affect analytic results. Thus it is important to assess the validity of the assay in each laboratory. Simply accepting literature values regarding clinical decision points can be clinically misleading. Many of these important issues regarding non-standard samples are reviewed in a CLSI document addressing analysis of body fluids in clinical chemistry.[8] A recent report in Clinical Chemistry cited substantial nonlinearity in 9 of 21 pancreatic cyst fluids analyzed for CEA and CA19-9, highlighting the necessity of thorough test method validation.[9]

*Molecular testing*
Molecular testing of pancreatic cyst fluid may be indicated in specific circumstances as studies have demonstrated that increased DNA levels, the presence of KRAS mutation or loss of heterozygosity are associated with mucinous cystic tumors.[4] These studies have further suggested that KRAS-2 gene mutation with allelic loss is associated with malignancy. Interestingly the combination of molecular testing with CEA testing appears to be complimentary as the combined information is significantly more sensitive for the detection of premalignant/malignant lesions than either in isolation.[10] One of the advantages of molecular testing is that it can often be performed on samples of limited volume that preclude CEA testing and/or cytological evaluation. At this point, the utility of routine molecular testing in cases with sufficient volume for CEA, amylase and cytology of cyst fluid is yet to be determined.

In summary, the clinical laboratory analysis of pancreatic cyst fluid cannot be viewed in isolation. It is the combination of clinical features, radiologic data, EUS findings, cyst fluid analysis (CEA, amylase) and cytological assessment that most accurately classifies a pancreatic cyst and provides support for clinical decisions regarding the appropriate treatment or follow-up of these lesions.

***Supplemental Questions with participant responses and commentary:***
1. Which type of pancreatic cyst is most likely to yield significantly elevated cyst fluid CEA levels?
    Mucinous cyst – 94%
    Pseudocyst – 3%
    Serous cyst – 3%

In general, the higher the CEA value, the more likely the cyst is a mucinous cyst with the attendant increased risk of malignancy, thus the intended response to this question was mucinous cyst. The other choices (pseudocyst, serous cyst) are often associated with low to undetectable cyst fluid CEA.

2. Which type of pancreatic cyst is most likely to yield significantly elevated cyst fluid amylase activity?
    Mucinous cystic neoplasm – 3.9%
    Pseudocyst – 92.2%
    Serous cyst – 3.9%

Pancreatic pseudocysts are commonly associated with high cyst fluid amylase levels, while serous cysts and most mucinous cystic neoplasms usually do not result in high cyst fluid amylase levels. On the other hand, intraductal papillary mucinous neoplasms that communicate with the

pancreatic ductal system are sometimes associated with moderately elevated amylase levels. The intended response to supplementary question 2 was pseudocyst.

3. The CEA cutoff value used in your facility to separate non-mucinous pancreatic cysts from mucinous pancreatic cysts falls into which of the following cyst fluid CEA ranges?

0 - 50 ng/mL – 3.2%
51 - 100 ng/mL – 1.2%
101 - 200 ng/mL – 5.2%
201 - 500 ng/mL – 7.8%
501 - 1000 ng/mL – 1.7%
>1001 ng/mL – 0.3%
Other, specify – 31.2%
Don't know – 49.4%

Of the more than 300 respondents to this question regarding CEA values used to separate non-mucinous from mucinous cysts, almost 80% either did not perform cyst fluid CEA in their laboratory or were not aware of the cutoff values used by clinicians in the assessment of pancreatic cysts. As CEA values may vary substantially by method, the cutoff values for CEA used in cyst assessment are highly dependent upon the analytic system.

4. Based upon the clinical information, radiologic data and your laboratory's CEA and amylase values for the pancreatic cyst aspirate fluid (FLD-04), which of the following pancreatic lesions is the most likely diagnosis:

Intraductal papillary mucinous tumor, main duct type – 3.8%
Invasive ductal adenocarcinoma – 2.3%
Mucinous cystic neoplasm – 35.7%
Pseudocyst – 54.0%
Serous cystadenoma – 4.2%

The intended response was mucinous cystic neoplasm. For pancreatic cyst fluid specimen FLD-04, the target values were CEA of 200 ng/ml with an amylase target of 300 U/L. The most common clinical considerations in a middle aged female with a cyst of the pancreatic body include pseudocyst, mucinous cystic tumors (MCN, IPMN), and serous cystadenoma.

IPMN is often associated with a detectable communication between the pancreatic duct and the cystic lesion and is often located in the pancreatic head. Mucinous cystic neoplasm (MCN) is the most likely diagnosis given the target values of a modestly elevated cyst fluid CEA with an unimpressive amylase, the age and sex of the subject, and location of the non-communicating cystic lesion. With the clinical history of alcohol abuse and possible history of a bout of pancreatitis, pseudocyst is a clinical consideration; however, the CEA value and the relatively low amylase undermine this diagnostic possibility. Although the intended response to the question was mucinous cystic neoplasm, your individual laboratory response may vary due to the spectrum of values seen in the various instrument/reagent groups for the cyst fluid CEA and amylase. The clinical decision points are assay dependent.

**References:**

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