

GASTROENTEROLOGY

Biochemical analysis of pancreatic fluid collections predicts bacterial infection

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Abstract

Background and Aims: Despite our understanding of the pathophysiology of different types of pancreatic fluid collections (PFC), few studies have attempted to correlate the biochemical analysis of PFC contents with clinical and radiological characteristics. The aim of this study was to assess the predictive value of fluid analysis for discerning collection type (pseudocyst *vs* acute fluid collection with necrosis), presence of infection or communication with the pancreatic duct in the setting of acute and chronic pancreatitis.

Methods: Pancreatic fluid from 34 consecutive patients undergoing endotherapy of PFC was prospectively analyzed for seven variables: lactate dehydrogenase (LDH), total protein, albumin, glucose, amylase, lipase and specific gravity.

Results: In multivariate analysis, adjusting for age and gender, high intracystic levels of protein (OR 6.2; 95% CI 1.3–37.0), LDH (OR 6.8 [2.3–38.3]), and albumin (OR 7.8 [1.3–67.4]), and low levels of glucose (OR 0.2 [0.03–0.9]) predicted the presence of PFC infection. The optimal threshold value for protein was 1000 g/dL, which achieved a sensitivity of 73% and specificity of 75% for detecting infection; the optimal cut-off for LDH was 1000 U/L (sensitivity 64%, specificity 85%), and the cut-off for albumin was 500 g/dL (sensitivity 75%, specificity 85%). There were no statistically significant differences in biochemical fluid analysis with respect to fluid collection type (pseudocysts *vs* acute fluid collection with necrosis) and the presence of pancreatic duct communication.

Conclusions: Biochemical analysis of PFC fluid is clinically helpful in detecting fluid infection in patients with bacteria on Gram stain or positive fluid cultures. Our findings fail to support the utility of fluid analysis in characterizing cyst type, and we caution against its use in distinguishing pseudocysts from acute fluid collection with necrosis.

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Key words: analysis, biochemical, infection, pancreatic pseudocysts, pancreatitis.

INTRODUCTION

Pancreatic fluid collections (PFC) generally arise from acute or chronic pancreatitis.¹ An accepted nomenclature for classifying PFC that occur as a complication of acute pancreatitis has been developed to include acute fluid collection, pancreatic necrosis, pancreatic abscess, and acute pseudocyst. These terms have been explicitly defined according to the Atlanta criteria.² Chronic pancreatitis may lead to pseudocyst formation, primarily

because of pancreatic ductal leaks arising from pancreatic duct obstruction.³ Despite our understanding of the pathophysiology and the biochemical composition of pancreatic fluid collections, few studies have attempted to correlate analysis of biochemical parameters of PFC with clinical and radiological characteristics.^{4,5}

In the present study, we prospectively performed a biochemical analysis of fluid obtained from various PFC in the setting of acute and chronic pancreatitis during therapeutic endoscopic drainage. The aim of this

study was to assess the predictive value of fluid analysis for: (i) clinical characteristics of fluid collections, namely collection type (pseudocyst or acute fluid collection with necrosis); (ii) presence of infection; or (iii) communication of fluid collection with the pancreatic duct.

METHODS

Patient population

During a 3-year period (March 1997 to February 2001), pancreatic fluid from 34 consecutive patients undergoing transmural endotherapy of PFC was prospectively analyzed. Informed consent was obtained from all patients prior to endoscopic retrograde cholangiopancreatography (ERCP). The size and relationship of the collection to the stomach or duodenal wall was defined by contrast-enhanced computed tomography (CT). Pancreatograms were attempted in all patients during the initial ERCP and after removal of the drainage catheters on follow-up examination. The anatomy of the pancreatic duct (PD), including caliber, side branches, presence or absence of stricture, and communication with the PFC were recorded.

Pancreatic fluid collection definitions

Pancreatic fluid collections were classified according to definitions set forth at the International Symposium on Acute Pancreatitis (Atlanta, Georgia, 1992).²

- Acute pseudocyst: collection of pancreatic juice enclosed by a wall of fibrous or granulation tissue, which arises as a consequence of acute pancreatitis, of more than 4 weeks duration.
- Chronic pseudocyst: collection of pancreatic juice enclosed by a wall of fibrous or granulation tissue, which arises as a consequence of chronic pancreatitis, lacking an antecedent episode of acute pancreatitis.
- Pancreatic abscess: circumscribed intra-abdominal collection of pus, usually in proximity to the pancreas, containing little or no pancreatic necrosis, which arises as a consequence of acute pancreatitis or pancreatic trauma. Although infected PFC were drained in this study, by strict definition no pancreatic abscess was drained.
- Pancreatic necrosis: diffuse or focal area of non-viable pancreatic parenchyma, which is typically associated with peripancreatic fat necrosis. Pancreatic necrosis was defined as areas of nonenhanced pancreatic parenchyma larger than 3 cm or involving more than 30% of the pancreas on dynamic contrast-enhanced CT.⁶
- Acute pancreatic fluid collection: fluid collection occurring early in the course of acute pancreatitis (less than 4 weeks) located in or near the pancreas. The critical clinical distinction between an acute fluid collection and a pseudocyst or pancreatic abscess is the lack of a defined wall. In the present study, drainage was performed in those patients with an acute

pancreatic fluid collection in the setting of pancreatic necrosis. No patients with acute fluid collections without necrosis were drained in the present study.

The following characteristics were recorded on ERCP: anatomy of pancreatic duct, including caliber, side branches, presence or absence of stricture, disruption and communication with the PFC. The diagnosis of chronic pancreatitis was made based on the Cambridge Classification.^{7,8}

Patients with PFC were eligible for transmural (transgastric and transduodenal) drainage if they had symptoms (i.e. abdominal pain, fever, jaundice, gastric outlet obstruction) of an enlarging fluid collection in close proximity (<1 cm) to the duodenal or gastric wall. Similarly, patients were eligible for transpapillary drainage if they had symptoms of an enlarging PFC with PD communication. The decision for endoscopic therapy was made after discussion of the various treatment options with each patient. The study was approved by the institutional review board of our institution.

Instruments and technique

Transmural drainage of PFC was performed with a therapeutic side viewing videoduodenoscope (TJF-100, Olympus America, Melville, NY, USA) as previously described.⁹ The fluid collections were then entered and drained using the Seldinger technique through a single entry site using a 200-cm 18G needle (GAN-18 or Howell needle and, after 1999, a Baron needle; Wilson-Cook Medical, Winston-Salem, NC, USA). After the needle was inserted through the gastrointestinal wall into the collection, fluid was aspirated and then placed in red-top tubes, and the remaining fluid in the syringe was transported immediately to the microbiology laboratory. Following endoscopic drainage, serial CT scans were obtained to document resolution. The transmurally placed endoscopic stents were removed endoscopically 2–4 weeks after resolution of the collection. If required, endoscopic re-intervention was performed 2–4 weeks after the first procedure.

Fluid biochemistry

The fluid was analyzed for seven variables: lactate dehydrogenase (LDH), total protein, albumin, glucose, amylase, lipase and specific gravity. All biochemical tests were determined using a selective discrete multi-channel analyzer (Ektachem, Kodak, Rochester, NY, USA).

Fluid microbiology

Fluid samples were taken immediately to the microbiology laboratory. Each sample was analyzed with Gram stain and cultures. Upon receipt of the transport vial, the specimens were placed into an anaerobe glove box incubator and opened under strict anaerobic conditions in an atmosphere of 95% nitrogen and 5% hydrogen.

Colony counts were determined on agar individually for each different isolate. A diagnosis of infected fluid collection was based on the presence of bacteria on Gram stain and positive fluid cultures.

Statistical analysis

Descriptive statistics were used to summarize baseline demographic data. All data are expressed as mean (\pm standard error) or median (range) for parametric and non-parametric data, respectively. The statistical significance of differences between means of the various biochemical parameters was estimated by using the Wilcoxon Sign Rank test. A *P*-value of <0.05 was considered statistically significant for all analyses.

Seven biochemical parameters (LDH, total protein, albumin, glucose, amylase, lipase and specific gravity) from the pancreatic collection aspirate were analyzed with respect to three clinical outcomes: fluid collection type, presence of infection and communication with PD. Multiple logistic regression analysis was performed to take account of the possible confounding effect of age and gender on the analysis. Odds ratios and their 95% confidence intervals served to describe the influence of each biochemical parameter on the clinical outcome of interest.

For those parameters that demonstrated statistically significant predictive value, receiver operator characteristic (ROC) curves were constructed to illustrate the changing sensitivity and specificity as the threshold defining an abnormal parameter value was varied. This allowed determination of the optimal cut-off value that maximized sensitivity and specificity.

RESULTS

Patient cohort

A total of 34 patients with PFC were analyzed. The baseline demographic features of this patient cohort are illustrated in Table 1. Acute pseudocysts were present in three patients, chronic pseudocysts in 16 and acute fluid collection with necrosis in 15 patients. For the

purposes of analysis, we combined patients with pseudocysts (either acute or chronic) and compared them to patients with acute fluid collection with necrosis. Twelve patients (38%) had infection of the fluid collection, whereas pancreatograms demonstrated PD communication in 17 patients (55%). Of the 12 patients with infected collections, bacterial organisms were isolated in eight: *Pseudomonas* in five patients and *Klebsiella*, *Enterobacter* and *Stenotrophomonas* in the other three. Of the other four patients, two had Gram-negative rods and two had Gram-negative bacilli. The median values of each biochemical parameter, with ranges, are shown in Table 1.

Pancreatic fluid analysis

Table 2 illustrates the comparison of each of the biochemical parameters in the aspirated fluid with respect to the three clinical outcomes of interest. Of note, there were no statistically significant differences between cyst contents with respect to fluid collection type

Table 1 Baseline clinical characteristics of 34 patients with pancreatic fluid collections

Mean age (years, SD)	49.3 (12.5)
No. male (%)	22 (64.7)
Fluid collection type (<i>n</i> , %)	
Acute pseudocyst	3 (8.8)
Chronic pseudocyst	16 (47.1)
Acute fluid collection with necrosis	15 (44.1)
Infection present	12 (37.5)
PD communication	17 (54.8)
Biochemical parameters (median, range)	
Protein (mg/dL)	900 (30–5000)
LDH (U/L)	200 (1–34 160)
Glucose (mg/dL)	65 (2–237)
Albumin (mg/dL)	400 (100–2700)
Amylase (U/L)	17 870 (5–120 948)
Lipase (U/L)	23 262 (1–61 680 000)
Specific gravity	1.02 (1.01–1.07)

LDH, lactate dehydrogenase; PD, pancreatic duct.

Table 2 Comparison of median values for each biochemical parameter in the aspirated fluid with respect to the clinical outcomes (*n* = no. of patients)

	Protein (mg/dL)	LDH (U/L)	Glucose (mg/dL)	Albumin (mg/dL)	Amylase (U/L)	Lipase (U/L)	Specific gravity
Pseudocyst (<i>n</i> = 19)	756	196	65	400	22 060	27 492	1.02
Pancreatic necrosis (<i>n</i> = 15)	1719	581	62	550	11 675	13 033	1.02
Infection (<i>n</i> = 12)	2118*	2456*	35	750**	11 090	26 224	1.02
No infection (<i>n</i> = 22)	630*	151*	76	350**	22 060	26 381	1.03
PD communication (<i>n</i> = 17)	1105	203	70	400	22 000	23 262	1.02
No PD communication (<i>n</i> = 17)	831	198	48	500	16 247	24 161	1.01

LDH, lactate dehydrogenase; PD, pancreatic duct. **P* = 0.04 comparing parameters among patients with and without infection (Wilcoxon rank sum test). ***P* = 0.01 comparing parameters among patients with and without infection (Wilcoxon rank sum test).

(pseudocysts *vs* acute fluid collection with necrosis) and PD communication.

The most noteworthy differences were observed when fluid contents of patients with infection were compared with those without infection: median levels of protein (2118 *vs* 630 g/dL; $P=0.04$), LDH (2456 *vs* 151 g/dL; $P=0.04$) and albumin (750 *vs* 350 g/dL; $P=0.01$) were all significantly higher in the patients with infection (Table 2). Figure 1 illustrates the differences in median levels of protein (Fig. 1a), LDH (Fig. 1b), albumin (Fig. 1c) and glucose (Fig. 1d) among infected and non-infected fluid collections, for chronic pseudocysts and acute fluid collection with necrosis. For chronic pseudocysts, infected collections contained higher median levels of protein (2730 *vs* 510; $P=0.03$; Fig. 1a), LDH (6162 *vs* 160; $P=0.08$; Fig. 1b), albumin (1200 *vs* 350; $P=0.02$; Fig. 1c) and lower glucose (38.5 *vs* 80; $P=0.006$; Fig. 1d) compared with non-infected chronic pseudocysts. Amylase, lipase and specific gravity levels did not differ significantly. Among patients with acute fluid collections with necrosis, similar trends were seen, although differences were not statistically significant. Higher median levels of protein (2100 *vs* 752; $P=0.2$; Fig. 1a), LDH (1334 *vs* 116; $P=0.3$; Fig. 1b), albumin (600 *vs* 400; $P=0.4$; Fig. 1c) and lower glucose (36 *vs* 69.5; $P=0.7$; Fig. 1d) were observed in infected acute collections with necrosis. Again, amylase, lipase and specific gravity levels did not differ significantly. In multivariate analysis, adjust-

ing for age and gender, high levels of protein (OR 6.2; 95% CI 1.3–37.0), LDH (OR 6.8 [2.3–38.3]), albumin (OR 7.8 [1.3–67.4]) and low levels of glucose (OR 0.2 [0.03–0.9]) demonstrated ability to predict the presence of infection, as shown in Table 3.

Receiver operator characteristic curves were constructed for protein (Fig. 2a), LDH (Fig. 2b) and albumin (Fig. 2c) to illustrate the varying sensitivity and specificity as the threshold defining an abnormal parameter value was changed. As shown, the optimal threshold value for protein was 1000 mg/dL (Fig. 2a), which achieved a sensitivity of 73% and specificity of 75% for detecting infection; the optimal cut-off value for LDH was 1000 U/L (Fig. 2b), with sensitivity 64%, and specificity 85%, whereas the optimal cut-off for albumin was 500 mg/dL (Fig. 2c), with sensitivity 75%, and specificity 85%. As illustrated in Figure 2a–c, threshold values can be varied to maximize either sensitivity or specificity.

DISCUSSION

The term PFC refers to a heterogeneous group of processes in which encapsulation of pancreatic juices occurs. The composition and mechanism of formation of a PFC depends on the presence and degree of underlying acute or chronic pancreatic ductal damage, the presence and severity of acute pancreatitis, and

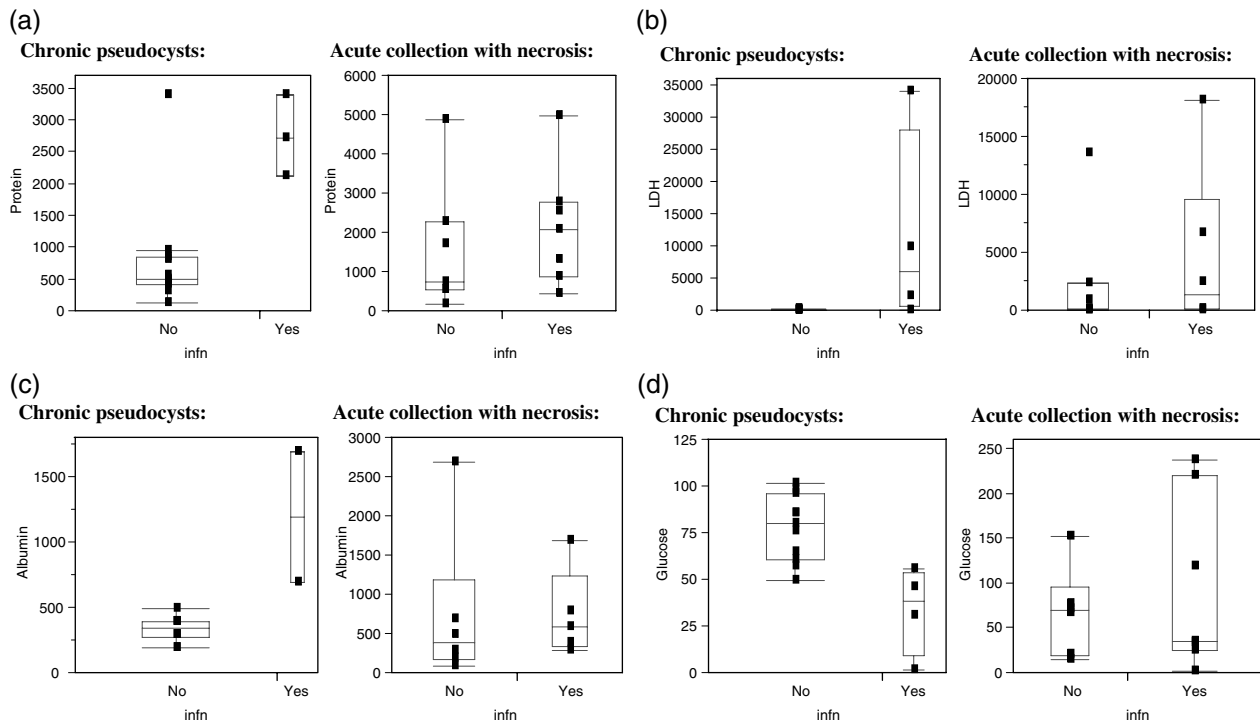


Figure 1 (a) Median protein levels in infected and non-infected pancreatic fluid collections (PFC) according to collection type (chronic pseudocyst, acute collection with necrosis). (b) Median lactate dehydrogenase (LDH) levels in infected and non-infected PFC according to collection type (chronic pseudocyst, acute collection with necrosis). (c) Median albumin levels in infected and non-infected PFC according to collection type (chronic pseudocyst, acute collection with necrosis). (d) Median glucose levels in infected and non-infected PFC according to collection type (chronic pseudocyst, acute collection with necrosis). For all parts, median and interquartile ranges are illustrated.

Table 3 Adjusted odds ratios (with 95% confidence intervals) for each biochemical parameters in the aspirated fluid for various clinical outcomes

	Protein	LDH	Glucose	Albumin	Amylase	Lipase	Specific gravity
Pancreatic necrosis (vs pseudocyst)	2.35 (0.59–10.07)	1.83 (0.46–7.71)	1.11 (0.28–4.50)	2.80 (0.60–14.47)	0.35 (0.08–1.46)	0.36 (0.04–2.61)	1.00 (0.07–13.85)
Infection (vs no infection)	6.22 (1.31–37.01)*	6.78 (2.33–38.34)*	0.19 (0.03–0.90)**	7.80 (1.31–67.40)*	0.45 (0.10–1.98)	0.83 (0.11–6.40)	4.50 (0.30–137.76)
PD communication (vs no communication)	1.33 (0.31–5.82)	1.34 (0.35–6.02)	0.45 (0.10–1.91)	6.00 (1.06–50.03)***	0.49 (0.10–2.12)	1.00 (1.15–6.65)	1.00 (1.15–6.65)

LDH, lactate dehydrogenase; PD, pancreatic duct. * $P = 0.03$; ** $P = 0.04$; *** $P = 0.05$.

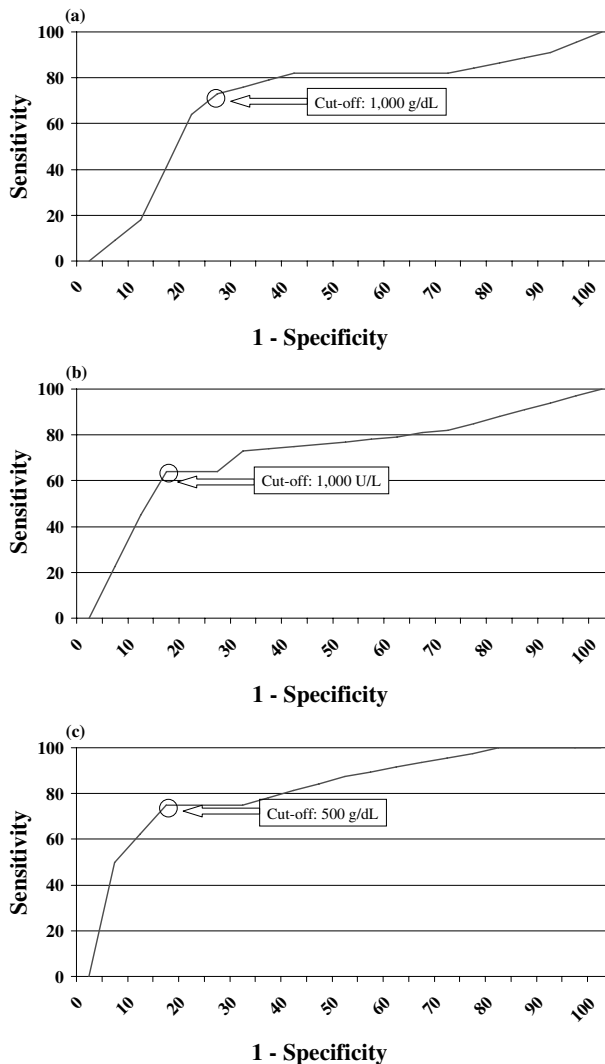


Figure 2 (a) Receiver operator characteristic (ROC) curve for protein levels in infected pancreatic fluid collections (PFC). (b) ROC curve for lactate dehydrogenase (LDH) levels in infected PFC. (c) ROC curve for albumin levels in infected PFC. For all parts, the optimal threshold value that maximizes sensitivity and specificity is illustrated.

maturation of the collection in relation to the onset of acute pancreatitis.^{10–13} Significant advances have been achieved in the radiological characterization of PFC.^{14,15} However, except for the knowledge that these collections are rich in amylase, few data exist regarding their biochemical composition.¹⁶ This is the first attempt to correlate the biochemical contents of these collections with their clinical features.

Prior studies addressing the biochemical analysis of fluid in an attempt to further characterize its origin are most prevalent in the pulmonary literature in the context of pleural effusions. Separation of exudates from transudates using biochemical parameters has received much attention.¹⁷ Historically, specific gravity was used to separate these two entities; later, a pleural fluid protein level of 30 g/L was used.¹⁸ However, many misclassifications were made, resulting in patients enduring

unnecessary investigations. Chandrasekhar *et al.* thus proposed the use of absolute values of pleural fluid LDH in making this distinction.¹⁹ Combining these two entities resulted in the formulation of the well-known 'Light's criteria' published in 1972.²⁰ These characteristics were retrospectively designed to be close to 100% sensitive and 100% specific in identifying pleural exudates.

The most noteworthy finding of the present study was that biochemical analysis of PFC fluid may aid in detecting the presence of infection. Infected cyst fluid appears to behave analogously to pleural effusion exudate, demonstrating elevated levels of protein, LDH and albumin. It has been shown that the pleural microvasculature endothelium is semipermeable, resulting in the protein and albumin content of pleural fluid being lower than that of serum.²¹ Exudative effusions usually involve some type of inflammation and compromise of the pulmonary microvasculature, whereas, in the case of transudative effusions, this microvascular endothelium usually is intact. In the case of exudates, there is an increased leakage of fluid out of the pleural microvasculature, which has a higher concentration of protein and albumin, lowering the gradient between serum and fluid protein and albumin. In the setting of transudates, this gradient is maintained.²¹ Applying these principles to our findings, we may speculate that the inflammatory process involved in infected PFC increases the vascular permeability, leading to leakage of protein and albumin in the cyst cavity. Rabinowitz and Dietz observed that the elevated LDH levels found in exudative pleural effusions were also attributable to inflammatory mechanisms;²² by stimulating normal lymphocytes with phytohemagglutinin, increased amounts of intracellular LDH were produced. Again, similar principles are likely to contribute to elevating LDH levels in infected PFC.

Much of the medical literature to date on pancreatic cyst fluid analysis has addressed its utility in detecting malignancy. Lewandrowski *et al.* measured fluid carcinoembryonic antigen levels in 24 neoplastic and non-neoplastic cysts,²³ using a cut-off level of 24.7 ng/mL, they reported 100% sensitivity and 100% specificity for diagnosing mucinous types of pancreatic neoplasms. The use of pancreatic amylase in cyst fluid has had mixed results. Frossard *et al.* observed a sensitivity of 61% and specificity of 58% for amylase levels >5000 U/L in distinguishing pseudocysts from other cystic lesions.²⁴ Interestingly, in our study, applying a cut-off of 5000 U/L for amylase produced a sensitivity of 89% but a specificity of only 21% for discriminating pseudocysts from acute fluid collection with necrosis. Other investigators have proposed that the varying amylase levels of cystic contents reflect communication of fluid collections with the pancreatic ductal system.²⁵ However, our findings did not corroborate this observation; a significant difference in amylase levels was not detected among those PFC with and without PD communication that was detectable by ERP (Table 2).

Our findings have important implications for clinical practice. Biochemical analysis of cyst fluid may provide clinically useful information regarding the presence of infection within a PFC. Recognizing that PFC infection

has been shown to adversely influence patient outcome,¹⁶ early detection in this setting may improve clinical outcome. In the setting of acute fluid collections with necrosis, sterile necrosis can be treated medically without detriment, whereas patients with infected necrosis require effective antibiotic treatment, optimally guided by microbiology results of a fine needle aspiration, and if this approach is not beneficial, drainage or surgery may be required as adjunctive therapy. Similarly, patients with chronic pseudocysts that are sterile may also be managed without drainage, although if they are sterile and symptomatic, pseudocysts are probably best managed with drainage. Infected pseudocysts require drainage. Indeed, one could analyze fluid taken at the time of a percutaneous aspiration of a chronic pseudocyst to perhaps make a decision on whether or not to embark upon endoscopic drainage based on the presence of infection. Therefore, the status of infection is important in determining management. Although it is relatively straightforward to detect PFC infection using Gram staining and culture techniques, we have demonstrated that biochemical analysis provides a further mechanism to confirm the presence of infection. Moreover, although cultures are certainly useful, they require incubation before being identified as positive, unlike biochemical analysis, which can be performed quickly. Indeed, biochemical analysis may prove to be helpful in those situations when infection is clinically suspected but not proven based on standard microbiological analysis. However, considering that the gold standard for identification of infection in the present study was based on the presence of bacteria on Gram stain and positive fluid cultures, our findings do not permit us to comment on the utility of biochemical analysis for the detection of infection in the setting of negative/inconclusive microbiological analysis.

Several limitations of this study deserve comment. First, our sample size was small (34 patients), which likely introduces a type II error and impairs our ability to detect clinically significant differences in the biochemical parameters of cyst fluid among patients with differing clinical characteristics. Nevertheless, we demonstrate the ability to detect statistically significant differences in some parameters, which are probably the most clinically important. Second, our gold standard for detecting PFC infection relied on the presence of bacteria on Gram stain and positive fluid cultures. Therefore, our analysis does not permit us to draw conclusions regarding the utility of biochemical fluid analysis for the detection of infection in the setting of negative/inconclusive microbiological assessment. Antibiotics were instituted in all patients once a diagnosis of collection infection was made, or in some cases when a clinical suspicion of fluid collection infection was entertained. This may have contributed to a false negative Gram stain or culture result in patients with infection who had started empiric antimicrobial therapy prior to collection drainage and fluid retrieval for analysis, and may similarly have impaired the sensitivity of biochemical fluid parameters. Unfortunately, we did not have consistent documentation of the timing of antibiotic administration relative to endoscopic collection. However, reassuringly, the same fluid specimen was used for

Gram stain, culture and biochemical evaluations. Finally, we failed to identify reliable biochemical indicators to distinguish fluid collection types (pseudocyst *vs* acute fluid collection with necrosis). Prior studies have demonstrated differing clinical outcomes in patients with different fluid collection types.¹⁶ Therefore, the ability to discern PFC type based on biochemical analysis would have represented an important clinical advance. However, our findings caution against the interpretation of biochemical fluid characteristics to discriminate pseudocysts from acute fluid collection with necrosis.

In conclusion, this study represents the first attempt to correlate the biochemical contents of PFC with their clinical features. We found biochemical analysis of cyst fluid, specifically protein, LDH and albumin, to be clinically helpful in detecting fluid infection in those patients with the presence of bacteria on Gram stain or positive fluid cultures. Our findings fail to support the utility of fluid analysis in characterizing cyst type, and we caution against its use in distinguishing pseudocysts from acute fluid collection with necrosis.

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