Predictors of malignancy and recommended follow-up for patients with negative endoscopic ultrasound-guided fine-needle aspiration of suspected pancreatic lesions

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BACKGROUND: Endoscopic ultrasound (EUS) with fine-needle aspiration (FNA) can characterize and diagnose pancreatic lesions as malignant, but cannot definitively rule out the presence of malignancy. Outcome data regarding the length of follow-up in patients with negative or nondiagnostic EUS-FNA of pancreatic lesions are not well-established.

OBJECTIVE: To determine the long-term outcome and provide follow-up guidance for patients with negative EUS-FNA diagnosis of suspected pancreatic lesions based on imaging predictors.

METHODS: A retrospective review of patients undergoing EUS-FNA for suspected pancreatic lesions, but with negative or nondiagnostic FNA results was conducted at a tertiary care referral medical centre. Patient demographics, EUS imaging characteristics and follow-up data were examined.

RESULTS: Seventeen of 55 patients (30.9%) with negative/nondiagnostic FNA were subsequently diagnosed with pancreatic malignancy. The risk of cancer was significantly higher for patients who had associated lymph nodes on EUS (P<0.001) and vascular involvement on EUS (P=0.001). The mean time to diagnosis in the group with false-negative EUS-FNA diagnosis was 66 days. The true-negative EUS-FNA patients were followed for a mean of 403 days after negative EUS-FNA results without the development of malignancy.

CONCLUSION: For patients undergoing EUS-FNA for a suspected pancreatic lesion, a negative or nondiagnostic FNA does not provide conclusive evidence for the absence of cancer. Patients for whom vascular invasion and lymphadenopathy are detected on EUS are more likely to have a true malignant lesion and should be followed closely. When a patient has been monitored for six months or more with no cancer being diagnosed, there appears to be much less chance that a pancreatic malignancy is present.

Key Words: *Endoscopic ultrasound; Fine-needle aspiration; Pancreas*

Les prédicteurs de malignité et le suivi recommandé des patients recevant un diagnostic négatif de lésions pancréatiques présumées au moyen d'une endoscopie échographique par aspiration à l'aiguille

HISTORIQUE : L'endoscopie échographique (EÉ) par aspiration à l'aiguille (AA) peut permettre de caractériser et de diagnostiquer les lésions malignes du pancréas, mais elle ne peut écarter complètement la possibilité de malignité. Les données d'issue au sujet de la durée du suivi des patients après une EÉ-AA négative ou non diagnostique des lésions pancréatiques ne sont pas bien établies.

OBJECTIF : Déterminer l'issue à long terme des patients recevant un diagnostic négatif de lésions pancréatiques présumées au moyen de l'EÉ-AA d'après les prédicteurs d'imagerie.

MÉTHODOLOGIE : Les auteurs ont effectué une analyse rétrospective des patients ayant subi une EÉ-AA en raison de lésions pancréatiques présumées, mais ayant obtenu des résultats négatifs ou non diagnostiques de l'AA à un centre de soins tertiaires. Ils ont examiné la démographie des patients, les caractéristiques d'imagerie de l'EÉ et les données de suivi.

RÉSULTATS : Dix-sept des 55 patients (30,9 %) ayant obtenu des résultats négatifs ou non diagnostiques de l'AA ont reçu un diagnostic de malignité du pancréas par la suite. Le risque de cancer était beaucoup plus élevé chez les patients présentant des ganglions lymphatiques connexes à l'EÉ (P<0,001) et une atteinte vasculaire à l'EÉ (P=0,001). Le délai moyen avant le diagnostic était de 66 jours dans le groupe ayant reçu un diagnostic faux-négatif par EÉ-AA. Les patients ayant reçu un diagnostic vrai-négatif par EÉ-AA ont été suivis pendant une moyenne de 403 jours après l'obtention des résultats négatifs, sans apparition de malignité.

CONCLUSION : Chez les patients qui subissent une EÉ-AA en raison d'une lésion pancréatique présumée, un EÉ négative ou non diagnostique ne fournit pas des données probantes concluantes d'absence de cancer. Les patients chez qui on dépiste une invasion vasculaire ou une lymphadénopatie par EÉ sont plus susceptibles de présenter une véritable lésion maligne et doivent être suivis de près. Lorsqu'un patient est suivi pendant au moins six mois et qu'aucun cancer n'est diagnostiqué, le risque de malignité pancréatique semble bien moindre.

Noninvasive, yet accurate diagnosis and staging of malignant pancreatic lesions remains a challenge. The advent of endoscopic ultrasound (EUS) combined with fine-needle aspiration (FNA) has contributed significantly to the advancement in this field and is the most accurate method to characterize pancreatic lesions without surgery. While cross-sectional imaging with computed tomography (CT) or magnetic resonance imaging (MRI) may be more beneficial for distant staging of pancreatic malignancy, EUS alone has proven to be superior for the diagnosis and local staging of pancreatic cancer, particularly for tumours of less than 2 cm in size (1-6). Furthermore, the most important advantage that EUS (when combined with FNA) has over CT or MRI is the ability to simultaneously provide local staging and a tissue diagnosis. When EUS-FNA provides tissue that indicates malignancy, the positive predictive value is reported to be close to 100% (1).

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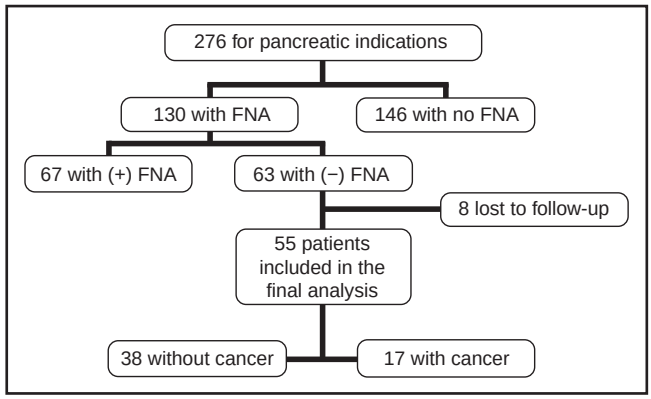

Figure 1) *Selection of study patients. Of the 276 endoscopic ultrasound procedures performed for pancreatic indications, 130 patients underwent fine-needle aspiration (FNA). There were 63 patients with a negative (–) FNA diagnosis and 55 patients were included in the final analysis. + Positive*

Multiple studies have shown that the negative predictive value (NPV) of EUS-FNA can be relatively low, ranging from 16% to 85% in previous studies (1,7-13). A negative FNA cannot definitively rule out the presence of malignant disease because as many as one in 10 EUS-FNA procedures result in false-negative cytology. Thus, a common clinical dilemma presented to the clinician is how to manage a patient who has a negative EUS-guided FNA.

However, while multiple studies have described the accuracy, positive predictive value and NPV of EUS-FNA for pancreatic lesions, no study has described how to manage these patients when the FNA result is negative and how long they should be followed after the negative EUS-FNA diagnosis. The ability to use specific EUS findings as predictors of an eventual cancer diagnosis or to more confidently rule out malignancy, would be efficacious and has not been studied. The first aim of the present study was to identify imaging findings on EUS that could predict a higher likelihood of a cancer diagnosis in patients with a negative cytology FNA diagnosis. Second, we attempt to provide guidance in determining the correct long-term follow-up of patients after a negative EUS-FNA diagnosis of a suspected pancreatic lesion.

METHODS

Study design and patients

The present retrospective study was conducted on a prospectively constructed EUS database at a tertiary care referral medical centre. The database includes patient's name, medical record number, date of procedure and indications for all EUS procedures performed from 2004 to 2006. The institutional review board of the University of Wisconsin School of Medicine and Public Health, Wisconsin, USA approved the study protocol.

A subset of patients from the database was selected for the present study based on the criteria presented in Figure 1. All patients who had EUS for pancreatic indications during a 34-month period from January 2004 to October 2006, were reviewed and included (n=276). All EUS examinations were performed by one of three experienced endosonographers with five to 10 years of EUS experience in a unit that performs approximately 500 EUS examinations per year. Indications for a pancreatic EUS were recorded and included common bile or pancreatic duct dilation/stricture on imaging (CT, endoscopic retrograde cholangiopancreatography [ERCP] or magnetic resonance cholangiopancreatography [MRCP]); acute or chronic pancreatitis determined clinically or by imaging (CT, x-ray or MRCP); pancreatic fullness, mass or cyst on imaging (CT or MRI); obstructive jaundice; elevated carbohydrate antigen (CA) 19-9 (normal range 0 U/mL to 37 U/mL); and/or weight loss. Some patients referred for an EUS examination of the pancreas had more than one of the above indications.

This subset of patients was further restricted to those undergoing EUS-FNA of the pancreas (n=130). At the University of Wisconsin Hospital and Clinics, a gastroenterologist performed every EUS-FNA procedure with the assistance of a member of the cytopathology FNA team. Once the specimen was obtained, the aspirate material was expressed onto glass slides. Both air-dried and alcohol-fixed smears were made. Air-dried smears were stained with Hema-Diff (Anapath, USA) and reviewed immediately to determine specimen adequacy and provide a preliminary diagnosis when possible. An aspirate was considered 'adequate for assessment' if there were abundant cells on the smear(s) that were representative of the target organ or lesion sampled. The specimen was classified as unsatisfactory or nondiagnostic if the aspirated cells were not sufficient to render a diagnosis or not representative of the target organ or lesion. For cystic lesions, the cellularity and morphological characteristics may be insufficient for making a definitive diagnosis; consequently, cyst fluid was also obtained and sent for analysis of tumour markers as well as cytology. On completion of the procedure, the slides were taken to the laboratory for final processing, staining and interpretation by the cytopathologist. For each FNA procedure, the total number of FNA passes was recorded. If an adequate sample was obtained after the initial pass, no further samples were obtained.

The cytopathologists classified the results using one of the following six categories: positive for malignancy; suspicious for malignancy; negative for malignancy; atypical or indeterminate; nondiagnostic; or unsatisfactory based on previously reported criteria (14). Results positive for malignancy and suspicious for malignancy were excluded from the study; all of the other classifications stated above were considered clinically as 'negative FNA' results. Of the 130 patients who underwent EUS-FNA of suspected pancreatic lesions, 67 had cytology consistent with a malignancy. Thus, the remaining 63 patients were the patients included in the study.

The final step of selecting patients for the present study included using only patients for whom follow-up data were available. The clinical course and outcome data were collected from the University of Wisconsin Hospital and Clinics electronic chart as well as documentation sent by the referring physician. For referred patients, letters and telephone messages were sent to their referring physicians in an attempt to obtain complete follow-up, which was performed by two researchers (BS and PP). Eight patients had no clinical course follow-up data available after their initial endoscopic procedure, thus leaving 55 patients for follow-up data collection and analysis. The flowchart depicting these eligibility criteria is shown in Figure 1.

Dependent variables

The primary study end point included cancer diagnosis (positive or negative) at or before the end of the follow-up period. Cancer diagnosis was coded as positive if patient records indicated either a positive biopsy or positive surgical specimen following removal of the suspicious lesion after a negative EUS-FNA.

One patient was diagnosed with cancer based on the presence of a pancreatic head mass on imaging, elevated CA 19-9 and lung biopsy revealing adenocarcinoma. Cancer diagnosis was coded as negative if surgical resection was negative for malignancy, the lesion stability was seen on sequential imaging and/or on repeat clinic visits. There was no situation in which the lesion had increased in size. Survival status at the end of follow-up and time to cancer diagnosis and/or death were also recorded. Death was confirmed by searching the Social Security Death Index on May 1, 2007 (15).

Independent variables

Patient variables considered as potential predictors of an eventual cancer diagnosis following a negative FNA included patient age at cancer diagnosis, patient sex and EUS findings. Indications for the pancreatic EUS were also used to describe the sample; however, they were not used in the modelling of risk because it could not be determined from the data whether presence or absence of each pancreatic indication had been definitely and accurately determined for each patient before the performance of the EUS-FNA.

The EUS findings were recorded for each of the following: presence of chronic pancreatitis on EUS; lesion size 3 cm or greater; lesion size 2 cm or greater (1); and vascular invasion and lymphadenopathy. The variable 'cyst/mass type' was coded as 0 when no definite mass or cyst was present, coded 1 when a cyst was present, and coded 2 when a mass was detected. Chronic pancreatitis was diagnosed if there were more than five parenchymal (ie, hyperechoic foci, lobularity of the gland, hyperechoic stranding, cysts) or ductal (ie, main ductal dilation of more than 3 mm, duct irregularity, dilated side branches, hyperechoic ductal margins) abnormalities detected (16,17). Vascular invasion was defined as present if there was demonstration of a tumour in the vascular lumen, complete vessel obstruction by tumour or the presence of collateral vessels (6,18,19). Visualization of an irregular tumour-vessel interface without evidence of the echo-rich vascular wall greater than 15 mm was also considered invasion. Lymphadenopathy was recorded if any of the four features predictive of lymph node metastases were present (ie, homogenous and hypoechoic appearance, sharply demarcated borders, rounded shape or size greater than 10 mm) (20). Masses and cysts were classified based on echogeneity with hypoechoic, heterogeneous solid lesions classified as a mass, while cysts tended to be anechoic and may have had micro- or macroseptations (21).

EUS-FNA was performed as previously described (22) with the patient in the left lateral decubitus position and under conscious sedation consisting of intravenous midazolam and fentanyl, and occasionally droperidol or promethazine when appropriate. All patients first underwent EUS examination with a radial echoendoscope (Olympus GF-UM130 or GF-UE160, Olympus, Japan) for evaluation and staging of target pancreatic lesions. A curvilinear echoendoscope (GF-UC140P-ALK5, Olympus, Japan) was subsequently used for further evaluation of the pancreas and FNA of suspicious pancreatic lesions.

For cystic lesions in the pancreas, carcinoembryonic antigen (CEA) measured in the cystic fluid was recorded for the majority of the patients for further stratification of cancer risk. Patients found to have CEA levels of 200 ng/mL or higher were considered higher risk for the presence of a mucinous cyst and malignancy, but not definitively diagnostic (23).

Statistical analysis

A two-step process was used to develop a model for predicting which patients with negative FNA results were at highest risk for a subsequent cancer diagnosis. In the first step, univariate analyses of the patient characteristics and risk factors after EUS were considered. That is, the patient variables listed in the independent variable section were compared across the cancer versus no cancer groups using *t* tests for continuous variables and χ^2 tests for dichotomous variables. These variables, or risk factors, were included for consideration in the second step of the risk modelling process if the univariate analysis of that variable versus cancer status had a $P \leq 0.30$, because variables with $P > 0.30$ in a univariate analysis are highly unlikely to contribute anything to a model that includes other risk factors. Cox proportional hazards regression was used as the second step to determine the significance of continuous predictors while the nonparametric log-rank test of equality across strata was used to test the significance of categorical predictors. Interaction terms were also examined in this step. The Schoenfeld residual test was used to check the proportional hazard assumption after fitting the model using proportional hazard Cox regression. For each potential categorical risk factor, Kaplan-Meier graphs were also used to depict the probability of cancer-free survival over time (by category) and to estimate whether the categories were proportional. Pancreatic indication variables (obtained before FNA) were not included in this phase of the analysis because these variables were not collected systematically across all patients in the database. Analyses were performed using SAS version 8.0 and STATA for windows, version 9.2 (STATA, USA). All CIs and significance tests were significant at $P < 0.05$ and were calculated using robust estimates of the variance.

RESULTS

Patient characteristics

Of the patients with suspicious pancreatic lesions who had a negative EUS-FNA diagnosis (n=55), 17 (30.9%) were eventually diagnosed with cancer. For the diagnosis of pancreatic cancer, EUS-FNA had a sensitivity of 60%, a specificity of 75%, a positive predictive value of 92% and a NPV of 27%. The mean (± SD) age of this group of patients was 64±13.36 years (range 35 to 82 years) and the male to female ratio was 1.2:1. There was no significant difference in the age or sex of the patients who had a false-negative FNA and developed cancer versus those who did not (Table 1).

The indications for EUS included common bile or pancreatic duct dilation/stricture (n=8 [14.5%]); chronic pancreatitis (n=5 [9%]); pancreatic fullness, mass or cyst on imaging (n=39 [70.9%]); obstructive jaundice (n=5 [9%]); elevated CA 19-9 (n=3 [5.5%]); and weight loss (n=2 [3.6%]). Seven patients (13%) had more than one of the above listed indications.

A median of 4±2.95 EUS-FNA passes per patient were performed (range one to nine needle passes). The cyst was aspirated in one pass in 15 of the 27 cases with cystic lesions, evacuating the cyst entirely. In 12 cystic lesions, more than one pass was performed. Five patients had a second EUS-FNA at a different date.

There were 22 mass lesions (40%), 27 cystic lesions (49%) and six lesions classified as having no definite mass or cyst. Some patients without a definite mass or cyst underwent FNA because they had abnormal EUS findings (eg, focal or diffuse areas of hypoechoic echogenicity) but the presence of an

TABLE 1
Characteristics of patients with suspected pancreatic lesions and negative endoscopic ultrasound-guided fine-needle aspiration results with comparison of groups with and without subsequent cancer diagnosis

Characteristic	Overall (n=55)	Cancer (n=17)	No cancer (n=38)	P*
Age, years (mean ± SD)	64.04±13.36	60.06±12.64	65.82±13.44	0.141
Women, n (%)	25 (45.45)	6 (35.29)	19 (50)	0.311
Follow-up, days (mean ± SD)	299.16±273.87	66.06±86.27	403.44±264.78	<0.001
Endoscopic ultrasound imaging, n (%)				
Chronic pancreatitis	22 (40.00)	5 (29.41)	17 (44.74)	0.284
Lesion size ≥3 cm	27 (49.09)	9 (52.94)	18 (47.37)	0.702
Lesion size ≥2 cm	42 (76.36)	13 (76.47)	29 (76.32)	0.990
Vascular invasion	11 (20.00)	8 (47.06)	3 (7.89)	0.001
Lymphadenopathy	8(14.55)	7 (41.18)	1 (2.63)	<0.001
Cyst/mass, n (%)				
Cyst	27 (49.00)	5 (29.41)	22 (57.89)	0.051
Mass	22 (40.09)	11 (64.71)	11 (28.95)	0.012
No definite mass or cyst	6 (10.91)	1 (5.88)	5 (13.16)	0.424

**P values based on χ^2 test for variables listing n (%) and* t *test for variables listing mean ± SD*

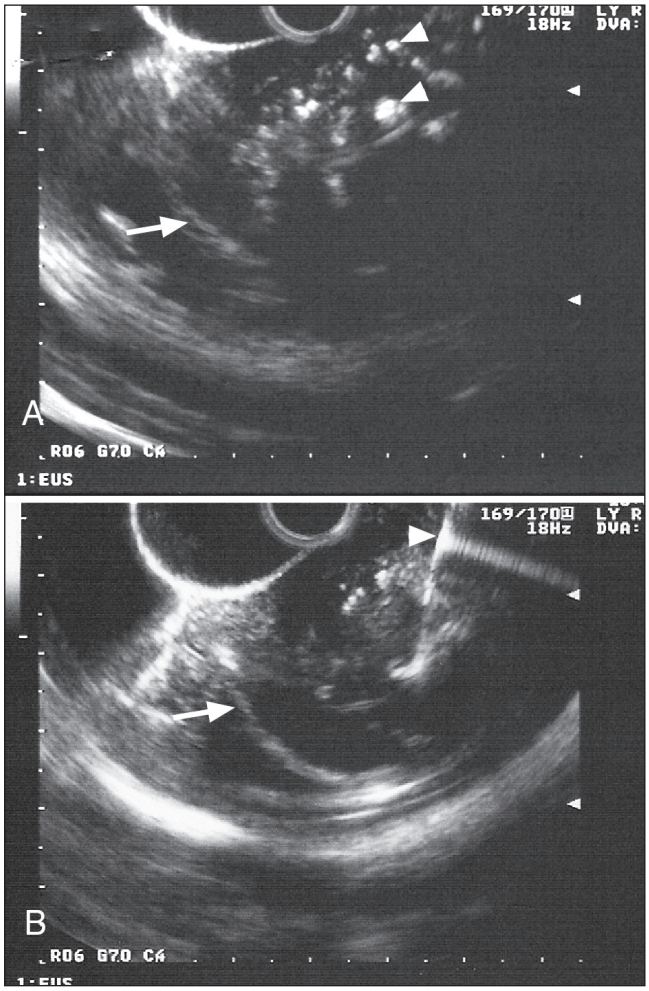

Figure 2) *Endoscopic ultrasound image of a patient with a diffuse hypoechoic irregular pancreatic head and the coexisitng diagnosis of chronic pancreatitis (arrows identify suspected mass; arrowheads indentify calcifications seen in chronic pancreatitis.* **A** *Fine-needle aspiration (FNA) was performed given the difficulty in ruling out the presence of an underlying mass.* **B** *The FNA diagnosis was negative and the patient is cancer-free at two years follow-up*

TABLE 2
Log-rank test of endoscopic ultrasound with fine-needle aspiration-negative patients by risk of eventual cancer diagnosis (n=55)*

Characteristic	P
Age, years†	0.077
Women	0.362
Endoscopic ultrasound imaging	
Chronic pancreatitis	0.343
Lesion size ≥3 cm	0.600
Lesion size ≥2 cm	0.934
Vascular invasion	<0.001
Lymphadenopathy	<0.001
Cyst/mass versus no definite lesion	0.046
Cystic lesion versus mass lesion	0.019

**Values represent Log-rank test unless specified otherwise; †Values represent proportional hazards Cox regression. The Schoenfeld residual test supports the proportional hazard assumption*

underlying mass lesion could not be definitively visualized (Figure 2). Twenty of the cystic lesions were evaluated by CEA analysis of the aspirated fluid.

The mean follow-up period for all patients who underwent an EUS-FNA procedure with negative or nondiagnostic results was 299 days (median 227 days; range nine to 1074 days). There was a longer follow-up period for patients who never developed cancer (403 days) versus patients in whom an eventual cancer diagnosis was made (66 days).

Analysis of risk factors

A significant risk of cancer was found in patients in whom there was vascular invasion by the suspected mass (P<0.001) and patients with associated lymphadenopathy seen on EUS examination (P<0.001) (Table 2). Patients with a cyst or mass versus those with no definite mass or cyst were more likely to have an eventual cancer diagnosis made (P=0.046). On univariate analysis, the presence of a mass versus cystic lesion was found to be a significant risk factor for the eventual diagnosis of cancer (P=0.019). However, on multivariate analysis, the presence of a pancreatic cyst versus mass was not a risk factor for the eventual diagnosis of malignancy (P=0.43). Neither the lesion size (2 cm or larger, or 3 cm or larger) nor evidence of chronic

TABLE 3
Multivariate analysis hazard ratio (HR) and 95% CI for cancer diagnosis (n=55)*

Risk factor	HR	P	95% CI
Vascular invasion	11.28	<0.001	2.66–47.91
Lymphadenopathy	17.62	<0.001	2.99–103.67
Vascular invasion and lymphadenopathy	0.09	0.03	0.01–0.78

**Controlled for patients' age and sex*

pancreatitis significantly increased or affected the risk for an eventual diagnosis of cancer after a negative FNA diagnosis (Table 2).

Variables included in the multivariate model consisted of the standard covariates of patient age and sex, and all variables that met the P<0.30 criteria in Table 2 (ie, vascular invasion, lymphadenopathy, and cyst/mass type). Results of the multivariate analysis revealed that patients with vascular invasion or lymphadenopathy seen on the EUS examination, were at higher risk for being diagnosed with cancer following a negative FNA result (Table 3). Specifically, patients with a negative FNA but with a lesion with vascular invasion on the EUS examination (Figure 3) were eventually diagnosed with cancer at approximately 11 times the rate of those without vascular invasion (P<0.001). Patients with lymphadenopathy on the original negative EUS-FNA examination were eventually found to have cancer at approximately 18 times the rate of those without lymph nodes (P<0.001) (Table 3).

Outcome and survival data

Follow-up and outcome data of the 17 patients who had false-negative FNA is presented in Table 4. The eventual cancer diagnosis in these patients was adenocarcinoma in 12 patients (70.6%), mucinous cystadenocarcinoma in four patients (23.5%) and intraductal papillary mucinous neoplasia in one patient (5.9%). The mean time to a cancer diagnosis was 66 days (range nine to 360 days) (Figure 4). In 82.4 % of patients (14 of 17), cancer was diagnosed in the first 90 days after EUS, with only one patient (5.9%) diagnosed with cancer more than 200 days after EUS with negative FNA (adenocarcinoma diagnosed at autopsy). Of the 17 patients with original negative EUS-FNA results, 11 were diagnosed with cancer at the time of subsequent surgery, two diagnosed by radiological-guided biopsy, one by repeat EUS-FNA, one diagnosed by a positive bile duct brushing, one diagnosed on autopsy and one patient by biopsy of a metastatic lung lesion.

Of the original 55 patients, 43 were alive at the termination of the study. Nine of the 43 patients who were alive had a cancer diagnosis. Of the 12 patients who had died, eight had cancer. One patient was diagnosed with pancreatic adenocarcinoma at autopsy after undergoing no further testing.

Classification and analysis of cystic lesions

There were 27 cystic lesions identified. Twelve (44%) were simple cystic structures without septation, debris or other solid component; six (22%) had a single septation or debris, but no solid component; seven (26%) had multiple septations; and two (7%) had solid components. Of the two lesions with solid components noted, one was biopsied with negative results and did not require surgery because the lesion was stable on repeat imaging. The other lesion had stability noted on repeat imaging.

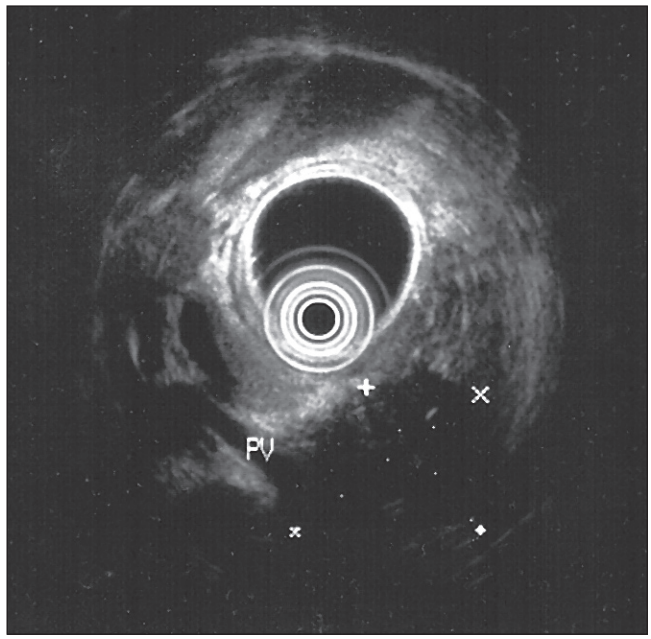

Figure 3) *Endoscopic ultrasound image of a mass invading the portal vein (PV). Despite a negative fine-needle aspiration diagnosis, the patient was diagnosed with pancreatic adenocarcinoma 43 days after the procedure*

Of the patients who had cystic lesions and a cyst fluid CEA level of higher than 200 ng/mL (but negative cytology), an eventual cancer diagnosis was made in 40% (four of 10 – all mucinous adenocarcinoma) at the time (Table 5, Figure 5). A CEA level of less than 200 ng/mL with associated negative cytology was accurately negative (no cancer developed to the point of follow-up) in 90% (9 of 10) of the patients. Fluid from cystic lesions that that yielded CEA levels of less than 200 ng/mL were at significantly lower risk of a cancer diagnosis (P=0.03).

DISCUSSION

Multiple previous studies (1,7-12) that examined the NPV of EUS-directed FNA for pancreatic lesions have been performed with varied results, but all have at least suggested that a negative FNA cannot effectively rule out pancreatic malignancy. Agarwal et al (1) retrospectively evaluated 81 patients with EUS-FNA who were referred for clinical suspicion of pancreatic cancer and found EUS-FNA to have a 56% NPV that increased to 78% if patients had no definite mass lesions on spiral CT. Raut et al (8) limited their analysis to patients with low-density masses in the pancreas on CT images or a malignant biliary stricture on percutaneous cholangiopancreatography or ERCP, and reported an NPV of only 44%. Eloubeidi et al (13) prospectively evaluated 101 patients with suspected pancreatic neoplasm based on clinical and/or imaging studies, but included only solid pancreatic mass lesions in patients who had failed to obtain a tissue diagnosis by ERCP, CT-guided biopsy, and/or ultrasound-guided biopsy. Using these criteria, this group reported an NPV of 85% and stated that EUS-FNA can be used as a rescue modality when other techniques fail to obtain a diagnosis.

Varadarajulu et al (24) evaluated the role of chronic pancreatitis in EUS-FNA of pancreatic lesions. They reported the significant reduction in sensitivity of a procedure to detect the presence of cancer when there was coexistent,

TABLE 4
Patients with false-negative cytopathology results

Patient age, years (sex)	Indication	Lesion size, cm	Type of lesion, cyst versus mass	Chronic pancreatitis, Yes/No	Nodes, Yes/No	Vascular invasion, Yes/No	Time to cancer diagnosis, days	Cytology	Malignancy diagnosis
56 (Female)	Dilated PD on MRCP and ERCP	1.8×1.4	Mass	Yes	No	No	152	Negative for malignancy	Adenocarcinoma
62 (Female)	Abdominal pain, jaundice, weight loss; CA 19-9 >3000 U/mL	3×3.5	Mass	No	No	Yes	9	Atypical cells	Adenocarcinoma
44 (Male)	Pancreatic mass and lymphadenopathy on imaging	3×3	Mass	No	Yes	No	23	Negative for malignancy	Adenocarcinoma
71 (Male)	Pancreatic cystic lesion on imaging	3.9×4.2	Cyst	Yes	No	Yes	29	Negative for malignancy	Mucinous cyst adenocarcinoma†
39 (Male)	Jaundice and CA 19-9 >3000 U/mL	3.3×2.7	Mass	No	Yes	Yes	13	Nondiagnostic	Adenocarcinoma
61 (Female)	Pancreatic cystic lesion on imaging	5.2×3.6	Cyst	No	No	Yes	9	Negative for malignancy	Mucinous cyst adenocarcinoma†
70 (Male)	Dilated PD on imaging	1.4×1.9	Mass	No	Yes	No	87	Nondiagnostic	Adenocarcinoma
68 (Male)	Jaundice and dilated CBD on ERCP	2.4×1.9	No discrete mass	Yes	No	No	9	Nondiagnostic	Adenocarcinoma
57 (Male)	Pancreatic mass on imaging	1.3×0.8	Mass	No	Yes	No	64	Nondiagnostic	Adenocarcinoma
52 (Male)	Jaundice and CBD stricture on ERCP	1.5×1.9	Mass	Yes	No	No	16	Negative for malignancy	Adenocarcinoma
54 (Female)	Pancreatic mass on imaging	4.2×2.5	Mass	No	Yes	Yes	43	Negative for malignancy	Adenocarcinoma
80 (Female)	Pancreatic cyst on imaging	2.3×1.8	Cyst	No	No	No	119	Negative for malignancy	Mucinous cyst adenocarcinoma†
73 (Male)	Pancreatic cyst on imaging	1.8×2	Cyst	Yes	No	No	43	Atypical cells	Papillary mucinous cyst adenocarcinoma
75 (Male)	Pancreatic mass on imaging	5.3×4.4	Mass	No	Yes	Yes	15	Nondiagnostic	Adenocarcinoma
35 (Female)	Pancreatic cyst on imaging	2.3×1.9	Cyst	No	No	No	71	Nondiagnostic	Mucinous cyst adenocarcinoma†
59 (Male)	Pancreatic mass on imaging and CA 19-9 =1900 U/mL	3×3	Mass	No	No	Yes	360*	Nondiagnostic	Adenocarcinoma
65 (Male)	Dilated CBD and PD with mass on imaging	4.2×2.4	Mass	No	Yes	Yes	61	Nondiagnostic	Adenocarcinoma

**Diagnosis of pancreatic cancer made at autopsy; †Carcinoembryonic antigen in body fluid >200 ng/mL. CA Carbohydrate antigen; CBD Common bile duct; ERCP Endoscopic retrograde cholangiopancreatography; MRCP Magnetic resonance cholangiopancreatography; PD Pancreatic duct*

TABLE 5
Data for 10 patients with pancreatic cystic lesions and carcinoembryonic antigen (CEA) >200 ng/mL

Patient age, years (sex)	Lesion size, cm	Cytology	CEA, ng/mL	Further evaluation	Follow-up if no cancer, days	Time to cancer diagnosis, days	Diagnosis
71 (Male)	3.9×4.2	Negative for malignancy	324,937	Liver biopsy	–	29	Mucinous adenocarcinoma
61 (Female)	5.2×3.6	Negative for malignancy	819.5	Hemipancreatectomy	–	9	Mucinous adenocarcinoma
80 (Female)	2.3×1.8	Negative for malignancy	1037	Distal pancreatectomy	–	119	Mucinous adenocarcinoma
35 (Female)	2.3×1.9	Nondiagnostic	231.7	Laparoscopic distal pancreatectomy	–	71	Mucinous adenocarcinoma
46 (Female)	6.1×3.8	Negative for malignancy	471.3	Pancreaticoduodenectomy	49	–	Chronic pancreatitis
80 (Male)	1.2×1.2	Nondiagnostic	283	CT showing stable lesion	359	–	–
61 (Female)	1.9×1.4	Negative for malignancy	44,560	Laparoscopic distal pancreatectomy	70	–	Chronic pancreatitis
80 (Female)	2×1.5	Negative for malignancy	215.8	Clinic	175	–	–
82 (Male)	3.9×3.3	Negative for malignancy	31,300	Clinic	1015	–	–
52 (Female)	4×3.1	Insufficient tissue	485	CT showing stable lesion	321	–	–

CT Computed tomography

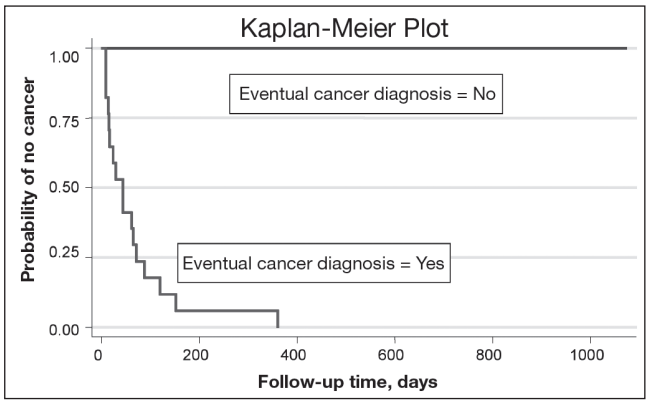

Figure 4) *Time to cancer diagnosis after negative endoscopic ultrasound-guided fine-needle aspiration examination*

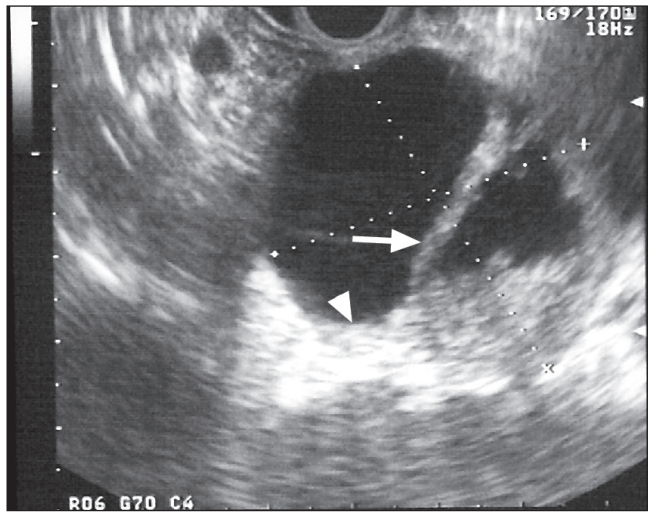

Figure 5) *Endoscopic ultrasound image of a complex, septated cystic lesion (arrow identifies the septation within the cyst; arrowhead identifies debris). Despite a negative diagnosis on fine-needle aspiration, the patient had a carcinoembryonic antigen level of 324,937 ng/mL in the cystic fluid and was diagnosed with pancreatic mucinous adenocarcinoma 29 days after the procedure*

chronic pancreatitis, and that significantly more EUS-FNA needle passes were required to establish a diagnosis compared with those without chronic pancreatitis. Subjects with benign lesions (final diagnosis) were more likely to have concomitant chronic pancreatitis compared with those who had adenocarcinomas.

Thus, EUS-FNA as used in the diagnosis of malignant pancreatic lesions, is not a perfect test with a not insignificant number of false-negative EUS-FNA results. This creates a clinical problem regarding the appropriate further management of these patients with suspicious lesions on previous pancreatic imaging or a clinical scenario suggestive of pancreatic malignancy, but negative EUS-FNA. Previous studies have not examined what measures should be taken with a patient in whom a risk of pancreatic malignancy remains even after negative FNA. The goal of the present study was therefore twofold. The first goal was to identify EUS imaging characteristics that could predict malignancy even when the FNA was negative or nondiagnostic. Given that no previous study had provided long-term follow-up of negative pancreatic EUS-FNA cases, the second goal was to determine a reasonable follow-up plan when the FNA of a pancreatic lesion is diagnosed negative.

With regard to EUS imaging, our results show that the findings of peripancreatic lymph nodes ($P<0.001$) and suspicion (on EUS) of vascular invasion by the pancreatic lesion ($P=0.001$) determined at the time of EUS, are statistically significant predictors of an eventual cancer diagnosis with negative or nondiagnostic FNA. These are simplistic but very important findings because improving the accurate identification of malignancy in patients with false-negative results on EUS-FNA is crucial. In the presence of a definite mass lesion or cystic lesion with either apparent invasion of the adjacent vessels or if lymph nodes are also present, patients should be considered at high-risk for malignancy and should be considered clinically different from patients without these findings. These findings would suggest a solid adenocarcinoma or in the case of a cystic neoplasm, a mucinous cystadenocarcinoma. These high-risk patients – despite a negative FNA – should be considered as having a false-negative FNA and followed closely with an early repeat attempt at tissue acquisition and repeat imaging, or strong consideration for direct referral to surgery if clinically applicable.

Our results also show that patients with a false-negative EUS-FNA had an eventual cancer diagnosis made in a relatively short time period after the EUS examination (Figure 4). The mean time to a cancer diagnosis was only 66 days and the majority of patients who were diagnosed with cancer had the diagnosis made within 90 days after the EUS examination.

Only one patient was diagnosed with cancer at longer than five months after the initial negative EUS-FNA. This patient had a diagnosis of pancreatic adenocarcinoma made on autopsy almost a year to the day (360 days) after the EUS examination. These data have implications for how patients are monitored and followed after a negative EUS-FNA examination for a suspected pancreatic lesion. Patients who have cancer (the false negatives) will almost always be diagnosed with that malignancy within 90 days of the EUS examination and will tend to have EUS imaging characteristics (solid lesion, lymphadenopathy, vascular invasion) of a malignancy present despite a negative FNA. After three months and even more so after six months post-EUS examination, if a patient has not been diagnosed with cancer despite either clinical suspicion or CT imaging abnormality, and does not have suspicious EUS imaging, it is unlikely that a patient has an underlying malignancy that was missed at the time of EUS-FNA. Patients who do not have evidence of cancer six months after the EUS examination appear to need very little further imaging or surveillance of the pancreas. Thus, after a negative EUS-FNA in patients who do not have suspicious imaging findings on EUS, it is reasonable to perform one or two follow-up examinations such as a CT scan in the first six months after a negative EUS-FNA, but longer-term surveillance is rarely needed.

One weakness of the present paper is the inclusion of patients with all EUS findings such as solid lesions, cystic lesions and those without a definitive mass seen on EUS who still had an FNA. Cystic lesions are less likely to harbour an underlying malignancy than a solid-appearing lesion on EUS and cytopathology of pancreatic cystic lesions does not have the same sensitivity for malignancy as it does for solid lesions. However, we included patients with pancreatic cystic lesions and patients who did not have a clear mass because they still result in the same clinical dilemma of how to manage these

lesions after a negative EUS-FNA diagnosis. With negative cytology on EUS-FNA of cystic lesions, decisions still need to be made on whether the patient is sent to surgery or how the patient's cystic lesion should be followed. In the multivariate analysis, it did not matter if the lesion was cystic or solid because the presence of malignant-appearing nodes or vascular invasion still predicted the presence of a malignancy. Thus, despite negative FNA cytopathology in cystic lesions, the presence of significant findings (vascular invasion and lymphadenopathy) on EUS should prompt the physician to maintain a high suspicion of malignancy followed by close examination of the cyst fluid tumour markers, the clinical situation and the patient's overall suitability for surgery. Furthermore, in our study, negative cytology and a cyst fluid CEA level of less than 200 ng/mL ruled out a cystic neoplasm in 90% of patients.

Close cooperation with the cytopathologists is needed as well as an understanding of the interpretation of the cytology findings on an EUS-guided FNA. For example, the clinician performing the examination needs to be aware of the difference that exists between the cytological diagnosis of atypical versus nondiagnostic. While neither result is 'positive' for malignancy, the patient with atypical cells on FNA obviously needs to be more closely followed after EUS-FNA with a higher suspicion of underlying malignancy than a nondiagnostic or negative cytology result.

CONCLUSION

The performance of EUS-FNA on suspected pancreatic lesions can be improved if the imaging characteristics on EUS are factored into the decision-making process after a negative or nondiagnostic FNA result. Lesions seen on EUS with lymphadenopathy or vascular invasion are at high risk for having malignancy and should be followed very closely after a negative EUS-FNA, while the size of the lesion or presence of chronic pancreatitis does not appear to be predictive of eventual cancer diagnosis. Second, patients with a pancreatic lesion who have a negative EUS-FNA diagnosis should be followed very closely for the subsequent six months to detect a missed cancer diagnosis. However, if a cancer diagnosis has not been made within six months after a negative EUS-FNA, it is very unlikely that a patient has a pancreatic malignancy and extensive surveillance past this point is not recommended.

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