

Format for ANSWERING REVIEWERS

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Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13724_revised.doc).

Title: Endosonography in the Diagnosis and Management of Pancreatic Cysts

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The manuscript has been improved according to the suggestions of reviewers:

1) Following revisions made to manuscript:

1. **Abstract (page 3). Sentence** "Rapid advances in radiologic technology and increased use of cross-sectional imaging have led to a sharp increase in incidental discoveries of pancreatic cystic lesions." **replaced with** "Rapid advances in radiologic technology and increased cross-sectional imaging have led to a sharp rise in incidental discoveries of pancreatic cystic lesions."
2. **Introduction (page 4). Sentence** "Endoscopic ultrasound with fine needle aspiration (EUS-FNA) has revolutionized our ability to diagnose, and more recently treat, a variety of gastrointestinal conditions accurately and safely." **replaced with** "Endoscopic ultrasound with fine needle aspiration (EUS-FNA) has revolutionized diagnosis, and more recently treatment, of a variety of gastrointestinal conditions accurately and safely."
3. **Introduction (page 4). Sentence** "Recent studies have estimated the prevalence of pancreatic cysts in the United States as 2.5% overall" **replaced with** "Recent studies from the United States have estimated an overall prevalence of 2.5%"
4. **Introduction (page 4). Sentence** "Pancreatic cysts can generally be classified as non-neoplastic, cystic neoplasms and necrotic degeneration of solid tumors." **Replaced with** "Pancreatic cysts can generally be classified as non-neoplastic, neoplastic and necrosis of solid tumors."
5. **Introduction (page 4). Sentence** "Comprising two-thirds of all pancreatic cysts, cystic neoplasms include mucinous cysts (mucinous cystic neoplasms [MCN] and intraductal papillary mucinous neoplasms [IPMN]), non-mucinous cysts (serous cystadenoma [SCA]) and solid pseudopapillary neoplasms (SPEN) (Table 1)." **Replaced with** "Two-thirds of pancreatic cysts are cystic neoplasms (Table 1); these include mucinous cysts (mucinous cystic neoplasms [MCN] and intraductal papillary mucinous neoplasms [IPMN]), non-mucinous cysts (serous cystadenoma [SCA]) and solid pseudopapillary neoplasms (SPEN)."
6. **Introduction (page 5). Sentence** "MRI/MRCP is preferred as it is better able to evaluate septa, nodules, main pancreatic duct involvement, branch duct involvement, communication with the main pancreatic duct and cyst contents/debris, and may be superior in identifying mucinous



cysts with 79-82% accuracy." **Replaced with** "MRI/MRCP is preferable as it is better able to evaluate septa, nodules, main pancreatic duct involvement, branch duct involvement, communication with the main pancreatic duct and cyst contents/debris; and is 79-82% accurate in identifying mucinous cysts."

7. **Role of EUS in Diagnosis of Pancreatic Cysts (page 5). Sentences** " Whether EUS and EUS-FNA adds useful information beyond radiology was examined recently. EUS with or without (cytology, CEA, and amylase), was more sensitive (76%) than CT or MRI (48% and 34%) for differentiating neoplastic from non-neoplastic cysts.^[22] was the low diagnostic accuracy of radiology contradicts other studies. While these results support the usefulness of EUS in diagnosing neoplastic cysts, the low diagnostic accuracy of radiology contradicts other studies." **Replaced with** "A recent study examined the diagnostic utility of EUS and EUS-FNA beyond that of radiology. EUS with or without cyst fluid aspirate analysis (cytology, amylase and CEA) was more sensitive (76%) than CT or MRI (48% and 34%) for differentiating neoplastic from non-neoplastic cysts.^[22] While these results indicate EUS may be useful in identifying neoplastic cysts, the accuracy of radiologic imaging in this study was far lower than has been demonstrated by others."
8. **Role of EUS in Diagnosis of Pancreatic Cysts (page 6). Sentence** " In addition, the 2012 International Association of Pancreatology (IAP) guidelines for mucinous cysts recommends that patients with these "high risk stigmata" for malignancy should undergo surgical evaluation: obstructive jaundice with a cyst in the head of the pancreas, an enhancing solid component to cyst, or main pancreatic duct ≥ 10 mm.^[23]" **replaced with** "In addition, the 2012 International Association of Pancreatology (IAP) guidelines for mucinous cysts recommends that patients with these "high risk stigmata" for malignancy should undergo surgical evaluation: obstructive jaundice with a cyst located in the pancreatic head, a solid component with post-contrast enhancement, or a main pancreatic duct diameter ≥ 10 mm.^[23]"
9. **Role of EUS in Diagnosis of Pancreatic Cysts (page 6). Sentences** "In patients with cysts >1 cm, further investigation by EUS-FNA would be advised to rule out the presence of concerning features and differentiate between mucinous and non-mucinous cysts. In a recent retrospective study of resected cysts > 3 cm EUS-FNA with cytology and cyst fluid analysis correctly differentiated mucinous and non-mucinous lesions in 88% of cases.^[18]" **replaced with** "In patients with cysts >1 cm, further investigation by EUS-FNA would be advised to rule out the presence of concerning features and determine if the cyst is mucinous. In a recent retrospective study of resected cysts > 3 cm, EUS-FNA with cytology and cyst fluid analysis correctly identified mucinous and non-mucinous lesions in 88% of cases.^[18]"
10. **Role of EUS in Diagnosis of Pancreatic Cysts (page 7). Sentences** "EUS is particularly valuable in assessing diagnostic features and potential predictors of malignancy, including size, shape (lobular versus smooth contour), number of cysts, calcifications, cyst wall structure (thick versus thin wall), septa, nodules, solid masses associated with the cyst, pancreatic duct diameter, communication between the cyst and pancreatic duct, and lymphadenopathy (Table 2, Figures 2-8). In a study of 50 patients EUS was found to be comparable to MRI in its sensitivity for identifying septa (77.8%), mural nodules (58.3%), main duct dilation (85.7%) and communication between the lesion and the main pancreatic duct (88.9%).^[17]" **replaced with** EUS is particularly valuable in assessing diagnostic features and potential predictors of malignancy, including size, shape (lobular versus smooth contour), number of cysts, calcifications, cyst wall structure (thick versus thin wall), septa, nodules, solid masses associated with the cyst, pancreatic duct diameter, communication with the pancreatic duct, and lymphadenopathy (Table 2, Figures 2-8). In a study of 50 patients EUS was found to be comparable to MRI in its sensitivity for identifying septa (77.8%), mural nodules (58.3%), main duct dilation (85.7%) and communication with the

pancreatic duct (88.9%).¹⁴⁷

11. **Role of EUS in Diagnosis of Pancreatic Cysts (page 8). Sentence** "An EUS scoring system (0-10) was developed in which points were assigned based on cyst size, height of mural nodules, main pancreatic duct dilation, septal thickening and the characteristic patulous papilla." **Replaced with** "An EUS scoring system (0-10) was developed in which points were assigned based on cyst size, mural nodules, pancreatic duct dilation, thick septa and the characteristic "patulous" papilla.¹²⁸"
12. **EUS-FNA (page 8). Sentences** "Due to the limitations of imaging alone, diagnosis of pancreatic cysts relies on a combination of diagnostic imaging and analysis of cyst fluid. EUS-guidance offers the ability to safely perform FNA to obtain cyst fluid for cytologic and molecular analysis." ¹³² **replaced with** "Due to the limitations of imaging alone, diagnosing pancreatic cysts requires a combination of diagnostic imaging and cyst fluid analysis. Under EUS-guidance, FNA can safely obtain cyst fluid for cytologic and molecular analysis.¹³²"
13. **EUS -FNA (page 9). Sentence** "Occasionally 19-gauge aspiration needles can be advanced into larger cysts with thick fluid although these larger needles are difficult to use in the head or uncinete process of the pancreas." **Replaced with** "Occasionally 19-gauge aspiration needles can be advanced into larger cysts with thick fluid although these larger needles are difficult to use in the pancreatic head or uncinete process."
14. **EUS -FNA (page 9). Sentence** "The "string sign", a marker of viscosity, is performed by placing a drop of fluid between the thumb and index finger and gently pulling apart." **replaced with** "Fluid viscosity may be evaluated by the "string sign": a drop of fluid is placed between the thumb and first finger and slowly pulled apart."
15. **EUS -FNA (page 9 and 10). Sentences** "CEA is the main biomarker used to differentiate mucinous from non-mucinous cysts. Cyst fluid CEA >192ng/mL is 73% sensitive, 84% specific, and 79% accurate for mucinous lesions from the classic study by Brugge *et al.*¹²⁹" **replaced with** "CEA is the main biomarker used to determine if a cyst is mucinous. CEA >192ng/mL is 73% sensitive, 84% specific, and 79% accurate for mucinous lesions from the classic study by Brugge *et al.*¹²⁹"
16. **EUS-FNA (page 10). Sentences** "It is important to note that current CEA assays have been validated in serum, but not in cyst fluid, resulting in up to 85% variation in mean cyst fluid CEA levels among the different assays. However, there is little intra-assay variability in the Roche Elecsys and Bayer Centaur assays. Intra-assay variability is low for the Roche Elecsys and Bayer Centaur assays." **Replaced with** "It is important to note that currently available assays are validated for measuring serum, but not cyst fluid, CEA concentrations. Consequently, there is as much as 85% variation in mean cyst fluid CEA concentrations among the various assays." ¹⁴⁶
17. **EUS-FNA (page 10). Sentences** "Cyst fluid DNA analysis may also help to identify mucinous cysts. A study including 142 surgically resected cysts found that k-ras mutation was 54% sensitive and 100% specific for mucinous lesions.¹⁴⁷" **replaced with** "Molecular analysis of aspirated cyst fluid for DNA mutations may help to distinguish mucinous from non-mucinous cyts. A study including 142 surgically resected cysts found that k-ras mutation was 54% sensitive and 100% specific for mucinous cysts.¹⁴⁷"
18. **EUS-FNA (page 10). Sentence** "Combining CEA and k-ras improved sensitivity for mucinous lesions to 83% but specificity dropped to 85%.¹⁴⁷" **replaced with** "Using a combination of CEA and k-ras improved sensitivity for mucinous lesions to 83% but specificity dropped to 85%.¹⁴⁷".

19. **EUS-FNA (page 11). Sentences** "Our group evaluated the diagnostic accuracy for malignant cysts of the 2006 and 2012 IAP guidelines and commercial DNA analysis (k-ras, LOH mutations, and DNA quantity) in 257 pancreatic cysts.¹⁵⁰ The 2012 guideline was most accurately identified malignant cysts with 88% sensitivity and 90% specificity while DNA analysis did not add significantly useful information beyond this." **Replaced with** "Our group evaluated the diagnostic accuracy for malignant cysts of the 2006 and 2012 IAP guidelines and commercially available DNA analyses (k-ras, LOH mutations, and DNA quantity) in 257 pancreatic cysts.¹⁵⁰ The 2012 guidelines were the most accurate for malignant cysts (90% specificity and 88% sensitivity). The addition of DNA mutation analysis contributed no significant improvement in diagnostic performance."
20. **EUS-FNA (page 11). Sentences** "GNAS mutations at codon 201 have been associated with IPMN in pathology specimens, cyst fluid and pancreatic fluid.^{159, 60} The combination of GNAS and k-ras aspirated cyst fluid has been shown to be specific and sensitive for IPMN.¹⁶¹ Our work submitted for publication on resected pancreatic cyst specimens demonstrated a significantly higher prevalence of GNAS mutations in IPMNs (42%) compared to SCAs (10%) and pancreatic adenocarcinomas (0%) and MCNs (0%)." **Replaced with** "GNAS mutations have been associated with IPMNs in resected tissue, cyst aspirates and pancreas fluid.^{159, 60} The combination of GNAS and k-ras aspirated cyst fluid has been shown to be specific and sensitive for IPMN.¹⁶¹ Our own study (accepted for publication) on resected cysts found GNAS mutations to be significantly more prevalent in IPMNs (42%) than in SCAs (10%), adenocarcinomas (0%) and MCNs (0%)."
21. **EUS-FNA (page 12). Sentences** "We evaluated miRNA in 69 pathology specimens of pancreatic cystic neoplasms, and identified several miRNA panels (4 miRNA in each) that differentiated SCA from MCN and IPMN, as well as MCN from BD-IPMN with 85-100% sensitivity and 100% specificity.¹⁶³ These promising miRNA panels require validation in EUS-FNA cyst fluid aspirates. Recent targeted proteomic analysis of mucins in cyst fluid reported 98% accuracy for pre-malignant and malignant lesions.¹⁶⁴" **replaced with** "We evaluated miRNA in 69 pathology specimens of pancreatic cystic neoplasms, and identified several miRNA panels (4 miRNA in each) that differentiated SCAs from MCNs and IPMNs, and MCNs from BD-IPMNs (sensitivity 85-100% and specificity 100%).¹⁶³ These promising miRNA panels now need to be validated in EUS-FNA cyst fluid aspirates obtained during diagnostic evaluation. A recent study of the cyst fluid proteome demonstrated that proteomic profiling of mucin in cyst fluid (obtained by EUS-FNA) was 98% accurate for pre-malignant and malignant cysts.¹⁶⁴"
22. **EUS-FNA (page 12). Sentence** "These molecular biomarkers offer the potential for improved diagnostics, and the ability to perform analyses on small quantities of fluid especially as increasing numbers of smaller cysts are identified." **Replaced with** "These molecular biomarkers may be able to provide improved diagnostic accuracy while requiring only small amounts of fluid, particularly as the number of small cysts identified by imaging continues to increase."
23. **EUS-guided Therapy (page 12). Sentence** "Surgery is currently the standard of care for patients with pancreatic cystic neoplasms that are symptomatic, malignant, or have a high potential for malignant transformation." **Replaced with** "For patients with pancreatic cystic neoplasms that are symptomatic, malignant, or have a high potential for malignant transformation, the current standard of care is surgery."
24. **EUS-guided Therapy (page 13). Sentence** "Ethanol lavage is believed to induce cell membrane lysis, rapid protein denaturation and vascular occlusion.^{170, 71}" **replaced with** "Ethanol lavage is believed to induce cell membrane breakdown, rapid protein degradation and vascular

25. **EUS-guided Therapy (page 13). Sentence** "Long term follow-up of 9 patients with resolved cysts after ethanol lavage found that cyst resolution persisted in all patients over a median 26 month follow-up (range 13-39 months).^[78]" **replaced with** "Long term follow-up of 9 patients who achieved resolution after ethanol lavage found that cyst resolution persisted in all patients over a median 26 month follow-up (range 13-39 months).^[78]"
26. **EUS-guided Therapy (page 13). Sentences** "Without removing the needle, the cyst cavity is then injected with a volume of ethanol equal to the volume of aspirated cyst fluid. The cyst is lavaged for 5 minutes by evacuating and injecting the cyst cavity. This involves 3-4 lavages over 5 minutes when cyst fluid is thick, or 7-8 lavages if the fluid is thin." **Replaced with** "Without removing the needle, the cyst cavity is then injected with ethanol, equal in volume to the aspirated cyst fluid. For 5 minutes by the cyst cavity is repeatedly evacuated and injected. This involves 3-4 lavages over the 5 minutes when cyst fluid is thick, or 7-8 lavages if the fluid is thin."
27. **EUS-guided Therapy (page 13). Sentences** "If paclitaxel is also injected, it is left in the cyst cavity. The total volume injected should not exceed the volume of cyst fluid initially aspirated, i.e. the cyst should not be expanded beyond its original size." **Replaced with** "If used, paclitaxel is then injected into the cyst but not removed. At no point should the cyst be expanded beyond its original size."
28. **EUS-guided Therapy (page 14). Sentence** "Ideally, cysts considered amenable to ablation should be benign with no malignant features, 2-4cm in diameter, unilocular or oligolocular, and without communication to the main pancreatic duct." **Replaced with** "Ideally, cysts considered amenable to ablation should be benign with no malignant features, 2-4cm in diameter, uni/oligolocular, and demonstrate no connection with the pancreatic duct."

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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