Contrast-enhanced EUS for the characterization of mural nodules within pancreatic cystic neoplasms: systematic review and meta-analysis

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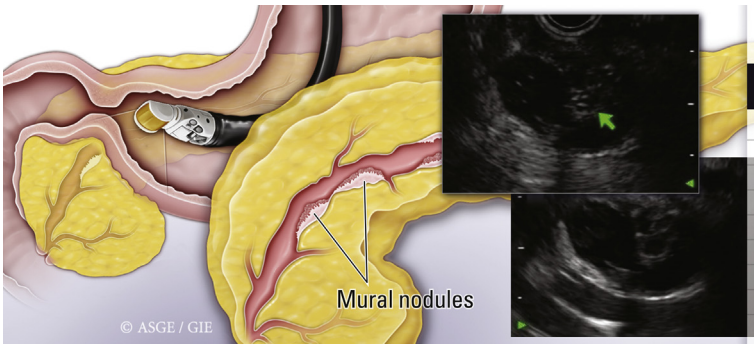
GRAPHICAL ABSTRACT



Pooled diagnostic performance of CH-EUS for the characterization of mural nodules in pancreatic cystic neoplasms

Diagnostic performance	CH-EUS (8 studies, 320 PCNs)
Sensitivity (95% CI)	97.0% (92.5% - 99.2%)
Specificity (95% CI)	90.4% (85.2% - 94.2%)
Positive likelihood ratio (95% CI)	8.89 (4.50 – 17.55)
Negative likelihood ratio (95% CI)	0.06 (0.03 – 0.13)
Estimated prevalence (95% CI)	41.7% (36.3% - 47.0%)
Positive predictive value (95% CI)	87.8% (81.5% – 92.1%)
Negative predictive value (95% CI)	97.7% (94.2% - 99.1%)
Diagnostic accuracy (95% CI)	95.6% (92.6% - 98.7%)
Number needed to diagnose (95% CI)	1.2 (1.3 – 1.1)

Background and Aims: Pancreatic cystic neoplasms (PCNs) carry a considerable malignancy risk. Along with main duct dilation, the presence of enhanced mural nodules represents a significant risk factor for malignancy. Several articles assessed the role of contrast-enhanced EUS (CE-EUS) for the identification of malignant features in mural nodules. We evaluate the pooled diagnostic performance of CE-EUS for the identification of high-grade dysplasia or invasive carcinoma among mural nodules in PCNs.

Methods: A systematic review (Medline, PubMed, EMBASE) and meta-analysis were conducted. Subgroup analysis was used to assess the usefulness of a dedicated contrast-harmonic (CH-EUS). The primary outcome was pooled sensitivity for identification of high-grade dysplasia or invasive carcinoma.

Results: Ten studies (532 patients) were included. Pooled sensitivity of CE-EUS was 88.2% (95% confidence interval [CI], 82.7%-92.5%), specificity 79.1% (95% CI, 74.5%-83.3%), and diagnostic accuracy 89.6% (95% CI, 83.4%-95.8%). Eight studies (320 patients) were conducted using CH-EUS: pooled sensitivity increased to 97.0% (95% CI, 92.5%-99.2%), specificity to 90.4% (95% CI, 85.2%-94.2%), and diagnostic accuracy to 95.6% (95% CI, 92.6%-98.7%). At 42% disease prevalence (pretest probability), a positive CH-EUS increased the disease probability to 88%, whereas a negative test decreased the disease probability to 2%. The number needed to diagnose was 1.5 (95% CI, 1.7-1.3) for CE-EUS and just 1.2 (95% CI, 1.3-1.1) for CH-EUS.

Conclusions: This study provided robust evidence on CE-EUS value for the characterization of mural nodules within PCNs. A dedicated contrast-harmonic mode, namely CH-EUS, provided an increased diagnostic yield in the identification and characterization of malignant mural nodules. (Gastrointest Endosc 2021;94:881-9.)

(footnotes appear on last page of article)

Pancreatic cysts have considerably increased their prevalence over the last 2 decades, mainly because of improvements in imaging techniques, representing 2% to 10% of pancreatic lesions.[1] According to World Health Organization criteria, pancreatic cystic lesions can be histologically classified as non-neoplastic (such as lymphoepithelial cyst, pseudocyst, or retention cyst) and neoplastic, also termed pancreatic cystic neoplasms (PCNs). PCNs include intraductal papillary mucinous neoplasms (IPMNs), mucinous cystic neoplasms, serous cystic neoplasms, and other rare cystic lesions such as solid pseudopapillary neoplasms and cystic neuroendocrine tumors.[2,3]

Estimation of malignancy risk is the main issue concerning PCN assessment.[4] Because serous cystic neoplasms have a negligible risk of becoming an invasive cancer compared with those that do carry a significant malignancy risk such as branch duct IPMNs (6%-46%), mucinous cystadenoma (20%-40%), and main duct IPMNs (>60%), the diagnosis of type of PCN and the evaluation of potential risk factors is crucial for a patient's clinical management.[5-7] However, most available knowledge on malignancy risk are based on surgical series; therefore, the assessment of the risk of cyst progression represents a still-debated issue.

The differential diagnosis of PCNs (either serous or mucinous) is mainly based on cross-sectional imaging, relying on cyst diameter, shape, communication with the main pancreatic duct, and EUS-guided FNA (EUS-FNA) for cytology and biochemical fluid analyses. However, the diagnostic accuracy of conventional imaging, B-mode EUS, and cytology ranges from 56% to 78%.[8-10] In particular cases, the use of ancillary techniques such as confocal laser endomicroscopy and through-the-needle microforceps biopsy sampling could be required to increase diagnostic yield and obtain meaningful information.[11-14]

According to the International Association of Pancreatology guidelines, the presence of an enhancing mural nodule ≥5 mm within PCNs represents a high-risk stigmata for either high-grade dysplasia (HGD) or malignancy together with a main pancreatic duct dilation ≥10 mm and jaundice.[6] A meta-analysis specifically conducted to assess the risk of malignancy associated with each IPMN feature reported a 9.3-fold increased risk of malignancy if mural nodules are present.[15]

Although studies failed to demonstrate a diagnostic benefit for PCN characterization by using contrast agents during EUS examination,[16] contrast-enhanced EUS (CE-EUS) can provide information on tissue microvascularization, contributing to the differential diagnosis between enhanced mural nodules and other nonenhanced solid components (ie, mucous clots or debris). Several studies evaluated CE-EUS for the characterization of mural nodules in PCNs, reporting sensitivity for HGD or invasive carcinoma ranging from 60% to 100%.[17-26] A meta-analysis aiming to identify the best cutoff for mural nodule size as a predictor of malignancy suggested that CE-EUS could be considered the most accurate technique in this field[27]; therefore, CE-EUS was included in the diagnostic flowchart of PCNs in International Association of Pancreatology and European guidelines.[5,6]

Nevertheless, robust evidence on this particular issue is still lacking. The aim of this study was to evaluate the pooled diagnostic performance of CE-EUS for the characterization of mural nodules in PCNs.

METHODS

Search strategy

A comprehensive electronic systematic research was carried out through Medline using PubMed, Google Scholar, and Embase databases at the end of January 2021. Search queries are available in Appendix 1 (available online at www.giejournal.org). References from the selected articles were also analyzed to retrieve any additional study that eluded the primary search.

Study selection

Original studies assessing the diagnostic performance of CE-EUS for the characterization of mural nodules in PCNs were included; true-positive, false-positive, false-negative, and true-negative cases were calculated. In case of missing data, these numbers were extracted from the reported results or queries were sent to the corresponding authors. Only full-text articles were included. Studies with unavailable, incomplete, duplicated, or updated data were excluded as well as case reports and studies with ≤10 patients.

Two authors (A.L. and A.C.) independently screened the literature, excluding duplicates and overlapping and irrelevant studies. The same 2 authors evaluated the full texts of the selected studies, excluding those that did not meet inclusion criteria. Any disagreements or doubts were solved through discussion with a third author (P.F.). For studies including either both solid and cystic pancreatic neoplasms or PCNs without mural nodules, raw data were extrapolated and/or a personal email was sent to the corresponding author to obtain missing data.

The following data were collected: first author, publication year, affiliation and country, study design, study period, US contrast mode (either color Doppler or dedicated harmonic mode), US contrast agent, study population, patient age and gender, PCN size, reference standard for final diagnosis (either surgery or EUS tissue acquisition and clinical follow-up), criteria for "positive case" (either HGD or carcinoma), and prevalence of positive cases. A validated score (Qualitative Assessments of Diagnostic Accuracy Studies-2 system) based on 4 domains (patient selection, index tests, reference standard, and flow and timing) was used.[28]

Definitions

The diagnostic reference standard was the final diagnosis of the nature of the mural nodule based on histologic

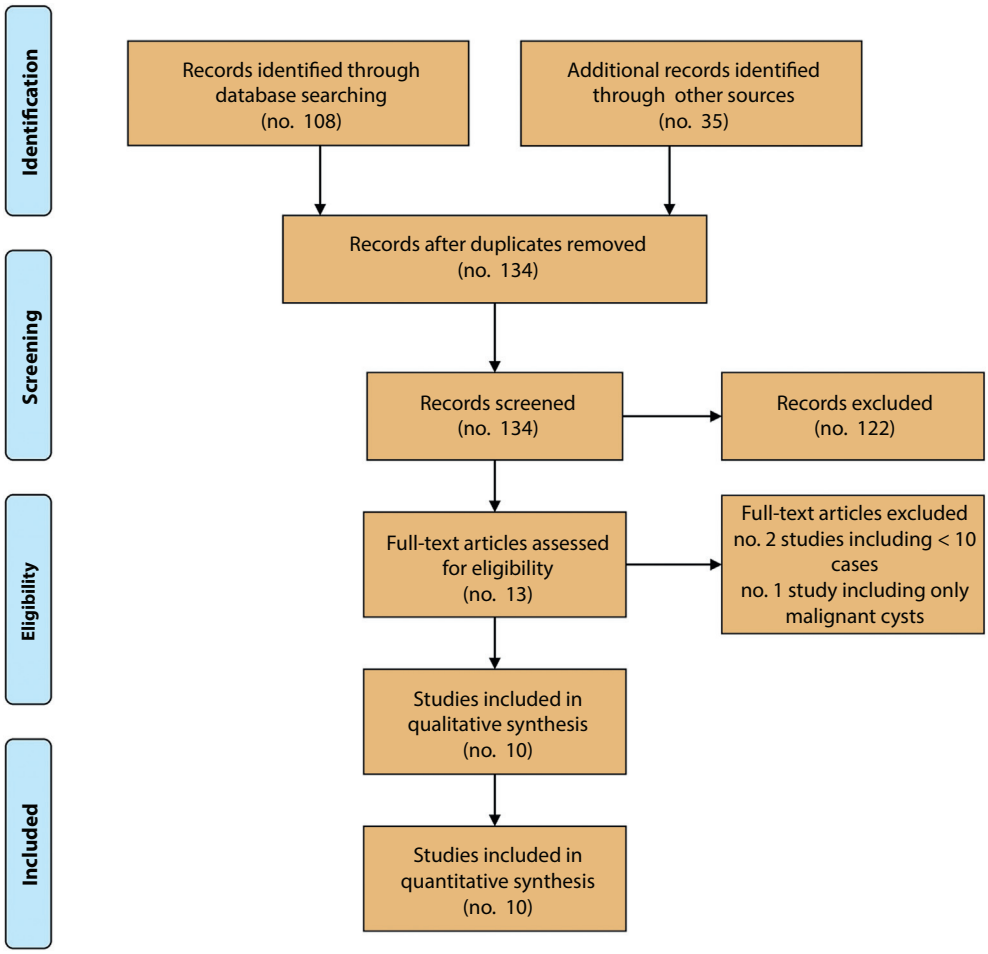


Figure 1. Study flowchart of the systematic literature search.

evaluation on surgical or EUS-guided tissue acquisition specimens, together with adequate clinical follow-up. Pathologic malignant features within mural nodules were the presence of either HGD or invasive carcinoma on pathology.

US contrast agents are drugs composed of microbubbles enclosed in a lipid shell that allow the real-time visualization and enhancement of microvascularization during US visualization. EUS real-time visualization could be conducted either using color Doppler mode or with a dedicated second harmonic dedicated contrast mode. These contrast agents depict different enhancement phases, such as arterial (10-30 seconds after administration) and venous (30-120 seconds) phases.

CE-EUS was defined as the real-time EUS evaluation after the peripheral intravenous administration of a US contrast agent. CE-EUS could be performed either with color Doppler mode or contrast-harmonic mode. Contrast-enhanced harmonic EUS (CH-EUS) was defined as the real-time EUS evaluation after the peripheral intravenous administration of a US contrast agent performed under a dedicated contrast-harmonic mode. For quantitative CH-EUS, the quantitative analysis of different CH-EUS parameters was evaluated on the time-intensity curve analysis. After recording a 2-minute video of CH-EUS examinations, a software-based analysis of the enhancement pattern was obtained.

A positive CE-EUS evaluation was defined as the presence of hyperenhancement or inhomogeneous enhancement detected on mural nodules after intravenous administration of US contrast agents. The primary outcome of the study was the pooled sensitivity for the diagnosis of HGD or invasive carcinoma within mural nodules.

Statistical analysis

Pooled results were analyzed using a fixed-effects model (Mantel-Haenszel method) when significant heterogeneity was not present and a random-effects model (DerSimonian-Laird method) when significant heterogeneity was detected; results are expressed as rates and 95% confidence intervals (CIs). A subgroup analysis was used to assess the usefulness of a dedicated contrast-harmonic (CH-EUS). The presence of heterogeneity was calculated using I^2 tests with I^2 <20% interpreted as low-level heterogeneity. Any

TABLE 1. Characteristics of included studies

Reference	Affiliation, country	Study design	Study period	US contrast mode	US contrast agent
Ohno et al 2009[17]	Nagoya University, Japan	Retrospective	2001-2007	Color Doppler	Levovist (Nihon Schering)
Yamashita et al 2013[18]	Wakayama University, Japan	Prospective	2009-2011	CH-EUS	Sonazoid (Daiichi-Sankyo)
Hocke et al 2014[19]	Klinikum Meiningen, Germany	Prospective	—	Color Doppler	SonoVue (Bracco)
Harima et al 2015[20]	Yamaguchi University, Japan	Retrospective	2009-2014	CH-EUS	Sonazoid (Daiichi-Sankyo)
Fusaroli et al 2016[21]	University of Bologna, Italy	Retrospective	2008-2011	CH-EUS	SonoVue (Bracco)
Kamata et al 2016[22]	Kinki University, Osaka-Sayama, Japan	Retrospective	2007-2012	CH-EUS	Sonazoid (Daiichi-Sankyo)
Yamamoto et al 2016[23]	Okayama University, Japan	Prospective	—	Quantitative CH-EUS	Sonazoid (Daiichi-Sankyo)
Fujita et al 2016[24]	Tokyo Medical University, Japan	Retrospective	2010-2014	CH-EUS	Sonazoid (Daiichi-Sankyo)
Zhong et al 2019[25]	Chinese General Hospital, Beijing, China	Prospective	2015-2017	CH-EUS	SonoVue (Bracco)
Buxbaum et al 2020[26]	Un. Southern California, Los Angeles, US	Prospective	2016-2019	Quantitative CH-EUS	Definity (Lantheus)

CH-EUS, Contrast-enhanced harmonic EUS; *EUS-TA*, EUS-guided tissue acquisition; *HGD*, high-grade dysplasia; —, not available.
*Values are mean ± standard deviation or median (range).

potential publication bias was verified through visual assessment of funnel plots.[29-33]

A sensitivity analysis was performed according to study design (whether prospective or retrospective), region (East vs West), US contrast mode (color Doppler or contrast-harmonic mode), US contrast agent (Sonazoid or SonoVue), and reference standard for final diagnosis (either surgery or surgery and EUS tissue acquisition). To explore the potential impact on the main study outcome (sensitivity), we used meta-regression to estimate the effect of cystic and mural nodule size and contrast-enhancement patterns.

All statistical analyses were conducted using Meta-DiSc version 1.4 software (Unidad de Bioestadística Clínica, Hospital Ramón y Cajal, Madrid, Spain). For all calculations, a 2-tailed $P < .05$ was considered statistically significant.

RESULTS

Literature search results and quality assessment

One hundred thirty-four studies were identified through database searching. A study flowchart reporting the detailed selection process is shown in Figure 1. One hundred twenty-two studies were excluded because they did not meet inclusion criteria. After extensive evaluation of the full texts, 2 studies with ≤10 cases of PCNs[34,35] and 1 study[36] comparing CE-EUS behavior of invasive IPMNs to ductal adenocarcinoma were excluded. Therefore, 10 studies (532 patients) were finally included in the qualitative and quantitative analysis. Studies characteristics are summarized in Table 1.

Among the studies included in qualitative and quantitative analysis (10 studies, 532 patients), all showed high quality in terms of risk of bias and applicability of patient selection; 1 study[19] presented an unclear risk of bias and applicability in terms of index test, because the authors stated that they introduced tridimensional CE-EUS evaluation during the study period. Four studies[19,20,21,26] showed an unclear risk of bias and applicability for the reference standard; indeed, these studies included both surgery and EUS tissue acquisition as the reference standard. Finally, 5 studies[17,19,21,23,26] presented an unclear risk of bias for flow and timing, because of their retrospective design, an unclear study period, or undefined timing between EUS and surgery (Supplementary Fig. 1A and B, available online at www.giejournal.org).

Diagnostic performance

Ten studies reported the diagnostic performance of CE-EUS for the characterization of mural nodules among 532 PCNs, whereas 8 studies (320 PCNs) were conducted with a dedicated contrast-harmonic mode (CH-EUS). The pooled diagnostic performance of the 10 studies conducted either with color Doppler or dedicated contrast-harmonic mode are reported in Appendix 2 (available online at www.giejournal.org) and Supplementary Figure 2A through D (available online at www.giejournal.org).

Among the included studies, 8 studies (320 PCNs) were conducted using a dedicated contrast-harmonic mode (CH-EUS). Among these studies, pooled sensitivity was 97.0% (95% CI, 92.5%-99.2%) with no heterogeneity ($I^2 = 0$), specificity 90.4% (95% CI, 85.2%-94.2%) with moderate heterogeneity ($I^2 = 66.1\%$), positive likelihood ratio was 8.89 (95% CI, 4.50-17.55) with low heterogeneity ($I^2 = 46.1\%$), and negative likelihood ratio was .06 (95% CI, .03-.13) with no heterogeneity ($I^2 = 0$). (Supplementary Fig. 3A-D, available online at www.giejournal.org). The pooled diagnostic accuracy was 95.6% (95% CI, 92.6%-98.7%) with moderate heterogeneity ($I^2 = 59.4\%$) (Table 2).

The impact of CH-EUS, performed with a dedicated contrast-harmonic mode; results on pretest probabilities, defined as the presence of enhanced mural nodules with

TABLE 1. Continued

Study population	Age* (y)	Gender, male (%)	Cyst size* (mm)	Mural nodule size* (mm)	Reference standard	Prevalence (%)	Positive cases
87	66.5 ± 9.5	60.9	30.5 ± 16.0	11 (2-20)	Surgery	51.7	HGD/carcinoma
17	74	64.7	28 (20-50)	10.7 ± 5.3	Surgery	70.6	HGD/carcinoma
125	64 ± 11	54.4	Range 10-25	—	Surgery, EUS-TA, follow-up	7.2	Carcinoma
50	67.7 ± 9.8	58.0	27.9 ± 10.9	3.5 ± 2.2	Surgery, EUS-TA, follow-up	32.0	HGD/carcinoma
22	63 (40-82)	43.4	30 (7-130)	8.5 ± 3.5	Surgery, EUS-TA, follow-up	18.2	HGD/carcinoma
70	62 (37-82)	44.3	33 (10-82)	—	Surgery	42.9	HGD/carcinoma
30	70 ± 6	60.0	32 (16-48)	7.5 (5-11.3)	Surgery	53.3	HGD/carcinoma
21	65.6 ± 11.5	76.2	29.8 ± 16.8	9.5 ± 5.7	Surgery	33.3	HGD/carcinoma
82	67.6 ± 14.3	19.5	45 ± 15	—	Surgery	43.9	HGD/carcinoma
28	63.2 ± 17.4	—	—	—	Surgery, EUS-TA, follow-up	42.9	HGD/carcinoma

TABLE 2. Pooled diagnostic performance of contrast-enhanced harmonic EUS for the characterization of mural nodules in pancreatic cystic neoplasms (8 studies, 320 pancreatic cystic neoplasms)

Diagnostic performance	Contrast-enhanced harmonic EUS
Sensitivity, %	97.0 (92.5-99.2)
Specificity, %	90.4 (85.2-94.2)
Positive likelihood ratio	8.89 (4.50-17.55)
Negative likelihood ratio	.06 (.03-.13)
Estimated prevalence, %	41.7 (36.3-47.0)
Positive predictive value, %	87.8 (81.5-92.1)
Negative predictive value, %	97.7 (94.2-99.1)
Diagnostic accuracy, %	95.6 (92.6-98.7)
Number needed to diagnose	1.2 (1.3-1.1)

Values in parentheses are 95% confidence intervals.

HGD or carcinoma; and results on post-test probabilities are reported in Figure 2. Based on the pretest probability of HGD or invasive carcinoma among patients included in the meta-analysis (42%), a positive CH-EUS performed with dedicated contrast-harmonic mode increased the disease probability to 88% (95% CI, 82%-92%), whereas a negative result decreased the disease probability to 2% (95% CI, 1%-6%). Pooled diagnostic performance of CE-EUS (10 studies, 532 PCNs) is shown in Supplementary Table 1 (available online at www.giejournal.org).

Sensitivity analysis, meta-regression, and publication bias assessment

Heterogeneity between sensitivity of the included studies was high (I^2 = 78.9%). A sensitivity analysis is shown in Table 3. Study design, region, US contrast mode, US contrast agent, and the reference standard for the final diagnosis seemed to be responsible for the observed heterogeneity. Prospective studies (I^2 = .0%), Western studies (I^2 = .0%), use of contrast-harmonic mode (I^2 = .0%), use of either Sonazoid (GE Healthcare, Waukesha, Wisc, USA) (I^2 = .0%) or SonoVue (Bracco, Milan, Italy) (I^2 = .0%), and studies using both surgery and EUS tissue acquisition as diagnostic reference standard (I^2 = .0%) showed no heterogeneity.

Results of the meta-regression are shown in Supplementary Table 2 (available online at www.giejournal.org). The meta-regression did not show any relationship between cystic and nodule size and contrast-enhancement pattern with CE-EUS sensitivity. A visual representation of the meta-regression for the 2 continuous variables, namely cystic size and mural nodule size, are shown in Supplementary Figure 4A and B, respectively. Visual inspection of funnel plots for sensitivity and specificity (Supplementary Fig. 5A and B) suggested no publication bias among studies.

DISCUSSION

We reported the first evaluation of the pooled diagnostic performance of CE-EUS for the characterization of mural nodules in PCNs. Our results demonstrated that CE-EUS had good sensitivity (88.2%) with relatively high

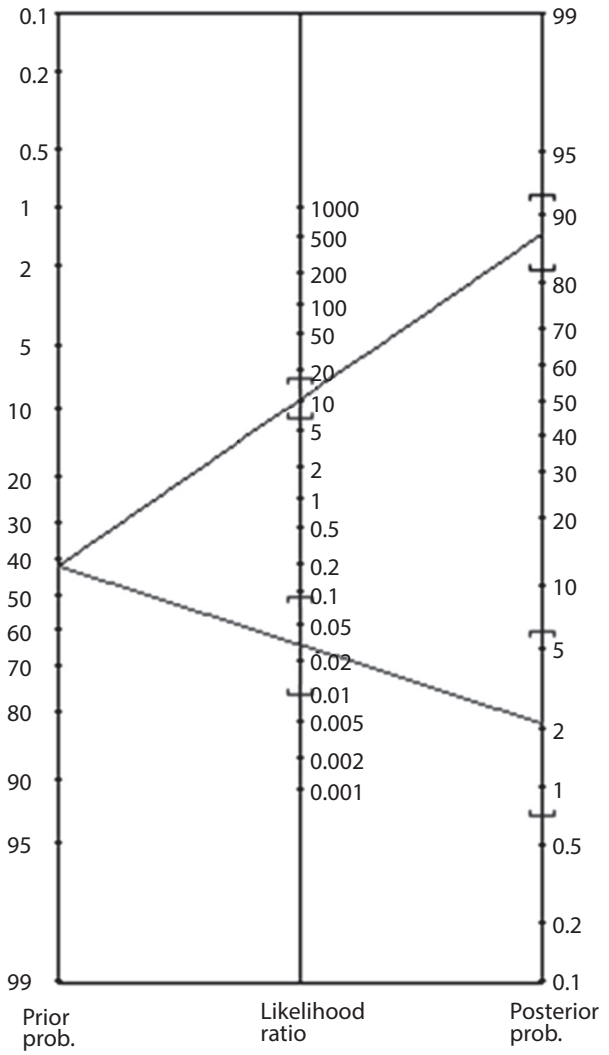


Figure 2. Fagan plot (nomogram) representing the impact of contrast-enhanced EUS with a dedicated contrast-harmonic mode for the identification of high-grade dysplasia or invasive carcinoma within mural nodules of pancreatic cystic neoplasms. Pretest probability was set at 42% as the pooled prevalence of high-grade dysplasia or invasive carcinoma within mural nodules.

specificity (79.1%) for the diagnosis of mural nodules harboring HGD or invasive carcinoma. Interestingly, these results showed a dramatic improvement when only studies conducted with a dedicated contrast-harmonic mode (CH-EUS) were considered: The pooled sensitivity increased to 97.0% and the pooled specificity to 90.4%. Because the use of a dedicated contrast-harmonic mode has been demonstrated to increase diagnostic accuracy in several indications, the use of color Doppler mode has been almost abandoned.[37] We maintain that CH-EUS finally represents an optimal tool for the detection of malignancy in mural nodules within PCNs.

Several clinical, biochemical, and morphologic factors have been identified as direct or indirect signs of "malignant" PCNs.[5,15] Available guidelines pay particular attention to the presence of mural nodules within PCNs because the risk of HGD or invasive carcinoma appears to be 4- to 6-fold higher.[6] To date, mural nodule size (≥5 mm) is the main determinant of surgical referral, although there is a consensus on the role of CE-EUS in the discrimination between mural nodules and mucous clots. However, these data are based on small studies and are not corroborated by strong evidence.

The use of US contrast agents with a dedicated harmonic mode in PCNs with solid components allows avascular components (mucous clots or debris) to be distinguished from neoplastic mural nodules. Several studies tried to characterize CE-EUS behaviors and to correlate them with pathologic features.[38-40] All authors are concordant in considering avascular solid components as non-neoplastic, whereas no agreement was found on the criteria for enhanced nodules, such as the presence of hyperenhancement or inhomogeneous enhancement. However, in a recent study, the authors observed that IPMNs harboring invasive cancer appeared to be isoenhanced in most cases.[36] Sometimes a small portion of normal pancreatic parenchyma surrounded by cysts can be misinterpreted as an enhancing nodule, both on cross-sectional imaging modalities and on CE-EUS. This limit should be considered to account for some "false-positive" results of CE-EUS, leading to a suboptimal positive predictive value of the technique.

Our results showed that CE-EUS, when conducted with a dedicated harmonic mode, presented not only an optimal sensitivity, but also a high specificity and positive predictive value. In other words, a negative CE-EUS performed with dedicated contrast-harmonic mode was able to rule out malignant PCNs with a very low risk of error (2%-2.5%), whereas a positive finding (presence of enhancement within mural nodule) significantly increased the risk of finding HGD and invasive cancer.

Although the optimal sensitivity confirms empirical observations by experts, we acknowledge that the very high pooled specificity could have been partially overestimated because most included studies enrolled only patients who underwent pancreatic surgery. Therefore, these results are likely reproducible in a very-high-risk population. On the other hand, validation with a large, multicenter, randomized trial for intermediate- to low-risk groups (ie, small mural nodule, no main pancreatic duct dilation, smaller size PCN, well-defined time between CE-EUS and surgery, etc) is required.

The precise definition of "malignant" mural nodules is still being debated.[6] Indeed, although there is consensus regarding the presence of invasive carcinoma, the best target for surgical resection is represented by the presence of HGD, because it potentially grants the concept of definitive cure. Most included studies (Table 1) were designed accordingly and included the presence of HGD in the "positive" diagnosis. A significant difference of disease prevalence among patients enrolled in Eastern and Western studies was observed; this difference could

TABLE 3. Sensitivity analysis

	Sensitivity (%)	95% Confidence interval	Heterogeneity (I^2) (%)
Overall (10 studies)	88.2	82.7-92.5	78.9
Study design			
Retrospective (5 studies)	81.4	72.4-88.4	86.1
Prospective (5 studies)	96.5	90.0-99.3	0
Region			
Eastern (7 studies)	87.0	80.9-91.8	84.6
Western (3 studies)	96.0	79.6-99.9	0
US contrast mode			
Color Doppler (2 studies)	66.7	52.5-78.9	87.8
CH-EUS (8 studies)	97.0	92.5-99.2	0
Quantitative CH-EUS (2 studies)	92.9	76.5-99.1	0
US contrast agent			
Sonazoid (5 studies)	97.5	91.4-99.7	0
SonoVue (3 studies)	98.0	89.1-99.9	0
Reference standard			
Surgery (6 studies)	85.6	78.9-90.9	85.4
Surgery + EUS- guided tissue acquisition (4 studies)	97.6	87.1-99.9	0

CH-EUS, Contrast-enhanced harmonic EUS.

partially justify the source of heterogeneity observed according to the region of origin of the included studies.[41]

The authors of the first version of the International Association of Pancreatology guidelines suggested considering only the presence of invasive carcinoma as a pathologic sign of "malignant" PCNs.[42] However, most included studies considered the presence of both invasive neoplasia and HGD as malignant PCNs and only 1[19] considered only invasive carcinoma. The different criteria adopted could explain the lower specificity observed in this study (56%) compared with the others (all ≥75%).

We believe the results of this study may have important applications in clinical practice. Regardless of mural nodule size, the absence of CE-EUS enhancement allows malignant PCNs to be excluded with reasonable confidence. On the other hand, in large (≥5 mm) mural nodules with enhancement at CE-EUS, the indication for surgery appears to be strong and unquestionable. Finally, in a <5-mm mural nodule with pathologic enhancement, the clinical management should be carefully chosen, taking into account the low performance and relative risk of tissue sampling and considering surgery in fit patients. Despite the limitation of a subgroup analysis, meta-regression suggested that the sensitivity of CE-EUS does not seem to be influenced by either the size of the cystic lesion or the mural nodule.

On the other hand, studies included in the meta-analysis considered not only non-neoplastic solid components (such as mucous clots or tissular normal parenchyma) but also mural nodules with low-grade dysplasia as false-negative results; these criteria could account for the relatively suboptimal pooled specificity results. Based on the available data in this study, the pooled prevalence of mural nodules with low-grade dysplasia and non-neoplastic solid components among the so-called false-positive group could not be calculated. Future studies should therefore focus on discriminating neoplastic mural nodules (either low-grade dysplasia, HDG, or invasive carcinoma) from mucous clots and interposed islets of normal pancreatic parenchyma. Moreover, an improved diagnostic yield with CE-EUS and other ancillary techniques in discriminating low-grade dysplasia and HGD from invasive carcinoma should be pursued, because the latter could be considered a sign of "late" diagnosis.[6,27,43]

This study presents some limitations. First, no randomized controlled trial was available in this setting, and the study designs were single-arm retrospective in 50%; a subgroup analysis showed that retrospective studies seem to be partially responsible for the observed heterogeneity. Second, 50% of the studies had an unclear risk of bias in terms of flow and timing because the time between CE-EUS and surgery was not reported. Moreover, 4 studies had an unclear risk of bias in terms of reference standards because both surgery and EUS-guided tissue acquisition were considered; however, the sensitivity analysis showed that the studies conducted with surgery as a reference standard showed more heterogeneity. This aspect could be interpreted as the changing surgical indication for PCNs over time, with consequent heterogeneous populations. On the other hand, no temporal bias was identified,

and all differences observed in sensitivity could be because of the application of dedicated contrast-harmonic mode.

Finally, because highly experienced authors in CE-EUS conducted all the included studies in tertiary referral centers, it is possible that these results could be replicated only in high-volume rather than in low-volume centers. This issue once again confirms that the management of PCNs with worrisome features, such as mural nodules, should be referred to large pancreatic units. On the other hand, it has been demonstrated that the interobserver agreement for CE-EUS in PCNs is high both in expert and beginner EUS operators.[22,44,45] Therefore, CE-EUS should represent another tool to increase the diagnostic yield in PCN risk assessment in the near future; however, the debated and difficult management of patients with PCNs with worrisome features, such as mural nodules, will continue to require the involvement of experts from large pancreatic units.

Advanced techniques, such as confocal laser endomicroscopy and through-the-needle microforceps biopsy sampling have been recently proposed to increase the diagnostic yield in PCNs characterization.[11-14] Because both techniques, along with good performances, present high costs and considerable risk of adverse events,[46] the results of the presented study could provide robust evidence to include CE-EUS into algorithms for cyst assessment together with these advanced ancillary techniques.[47]

In conclusion, this study provided reliable evidence on the value of CE-EUS for the characterization of mural nodules within PCNs, corroborating previous empirical findings with robust evidence. Our data suggest that the use of a dedicated contrast-harmonic mode provided an increased diagnostic yield in the identification of malignant features of mural nodules within PCNs.

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Abbreviations: CE-EUS, contrast-enhanced EUS; CH-EUS, contrast-enhanced harmonic EUS; CI, confidence intervals; HGD, high-grade dysplasia; IPMN, intraductal papillary mucinous neoplasms; PCN, pancreatic cystic neoplasm.

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DIVERSITY, EQUITY, AND INCLUSION: We worked to ensure gender balance in the recruitment of human subjects. We worked to ensure ethnic or other types of diversity in the recruitment of human subjects. While citing references scientifically relevant for this work, we actively worked to promote gender balance in our reference list.

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APPENDIX 1. SEARCH STRATEGY

A comprehensive electronic systematic research was carried out through Medline using PubMed, Google Scholar, and Embase interfaces at the end of December 2020.

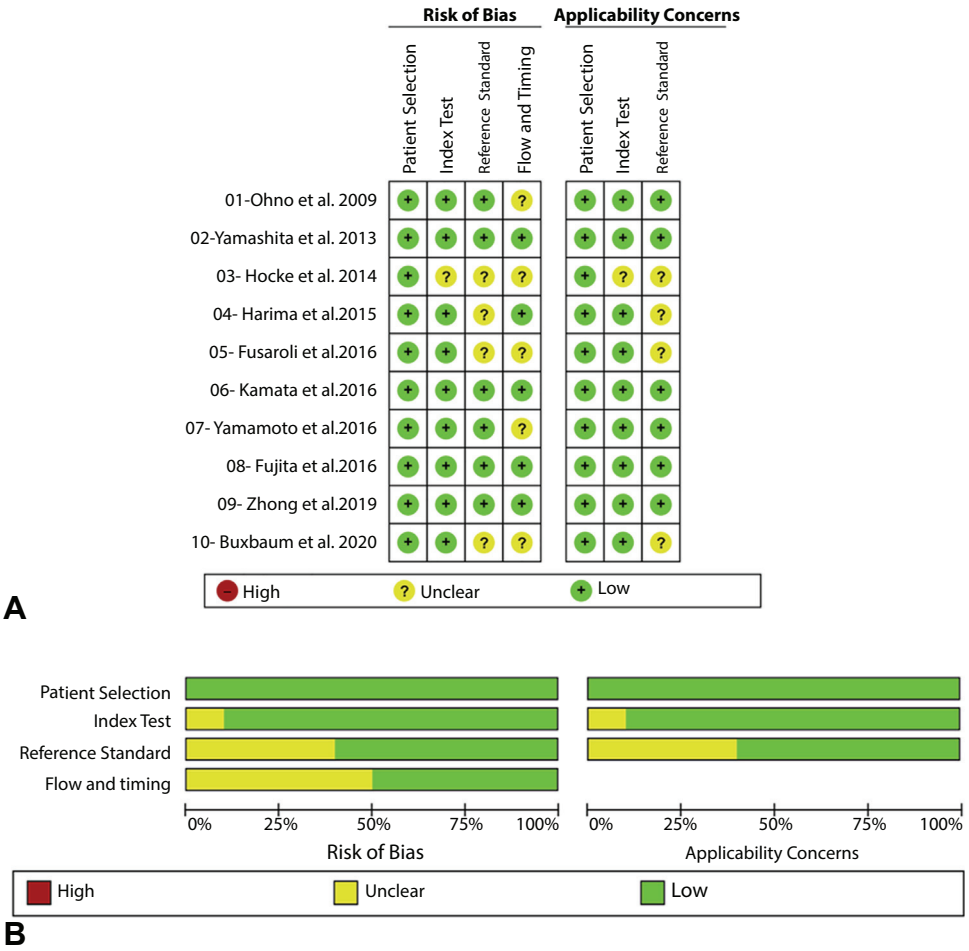
The search queries were ("pancreatic cystic neoplasm"[all fields] OR "PCN"[all fields] OR "pancreatic cyst"[all fields] OR "IPMN"[all fields] OR "intraductal papillary mucinous neoplasm"[all fields] OR "mucinous cystic neoplasm"[all fields] OR "MCN"[all fields] OR "serous cystic neoplasm"[all fields] OR "SCN"[all fields] OR "solid pseudopapillary neoplasm"[all fields] OR "SPN"[all fields] OR "cystic neuroendocrine tumor"[all fields] OR "cNET"[all fields]) AND (“contrast” [all fields] OR "contrast enhanced"[all fields] OR "harmonic"[all fields] OR "CH-EUS"[all fields]) AND ("endoscopic ultrasound"[all fields] OR "EUS"[all fields] OR “endosonography”).

APPENDIX 2. DIAGNOSTIC PERFORMANCE FOR CONTRAST-ENHANCED EUS USING EITHER COLOR DOPPLER OR DEDICATED CONTRAST-HARMONIC MODE

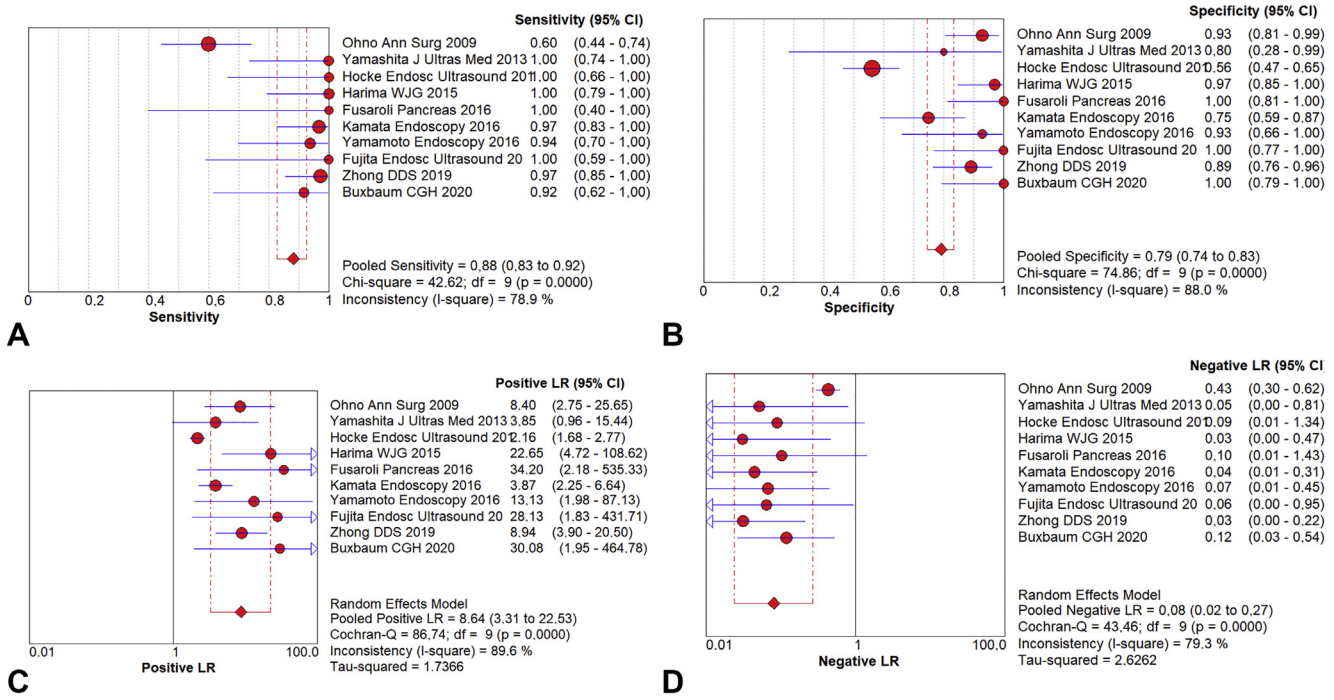
Results

Diagnostic performance of contrast-enhanced EUS. Ten studies reported the diagnostic performance of contrast-enhanced EUS (CE-EUS) for the characterization of mural nodules among 532 pancreatic cystic neoplasms. Summary estimates (random-effects model) were (summarized in Supplementary Table 1) as follows: sensitivity 88.2% (95% confidence interval [CI], 82.7%-92.5%) with high heterogeneity (I^2 = 78.9%), specificity 79.1% (95% CI, 74.5%-83.3%) with high heterogeneity (I^2 = 88.0%), positive likelihood ratio 8.64 (95% CI, 3.32-22.53) high heterogeneity (I^2 = 89.6%), and negative likelihood ratio .08 (95% CI, .02-.27) with high heterogeneity (I^2 = 79.3%). (Supplementary Fig. 2A-D). Pooled diagnostic accuracy was 89.6% (95% CI, 83.4%-95.8%) with high heterogeneity (I^2 = 92.0%).

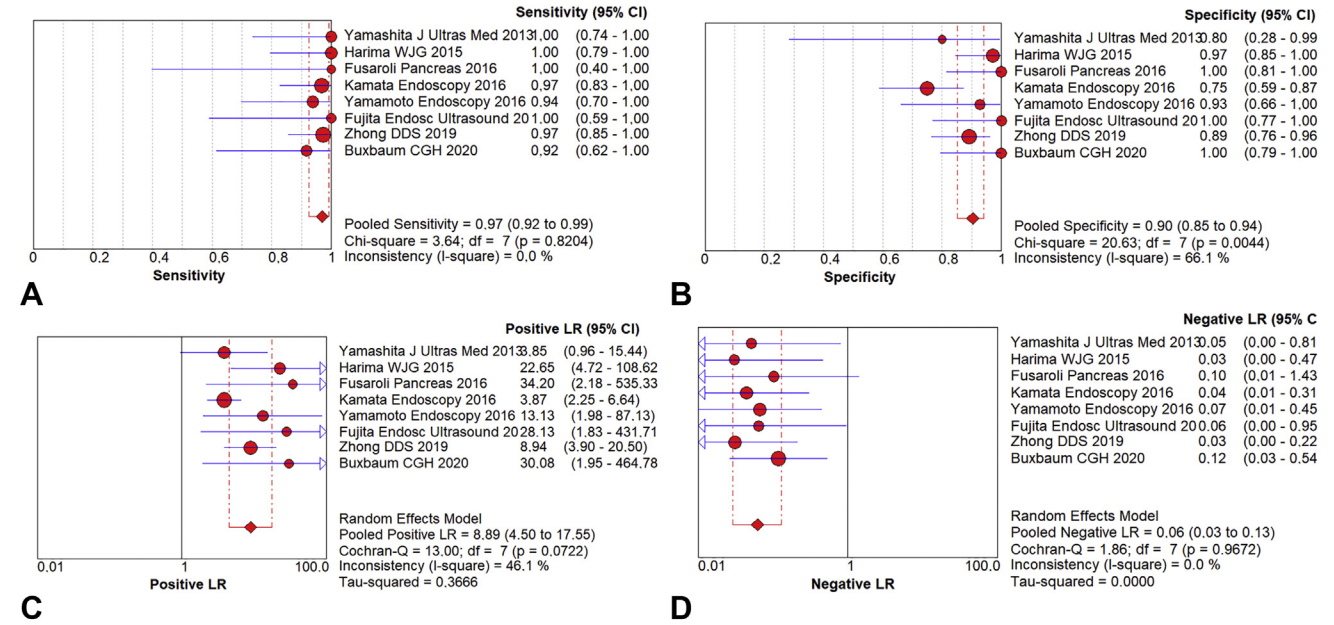
Based on the observed 44.5% disease prevalence (pretest probability), a positive CE-EUS increased the disease probability to 88% (95% CI, 83%-92%), whereas a negative result decreased the disease probability to 10% (95% CI, 8%-14%). The subgroup analysis conducted according to the region of origin (Eastern vs Western) showed that the observed prevalence of malignant features among included patients was different. Among patients (n = 357) included in the 7 studies conducted in Eastern countries, disease prevalence was 45.4%; this rate was lower (14.3%) among the 175 patients included in the 3 studies conducted in Western countries. Among Eastern studies (pretest probability, 45.4%), a positive CE-EUS increased the disease probability to 87% (95% CI, 82%-91%) and a negative result to a probability of 11% (95% CI, 8%-15%). Among Western studies (pretest probability, 14.3%), a positive CE-EUS was correlated to a post-test probability of 32% (95% CI, 27%-37%) and a negative CE-EUS to a post-test probability of 1% (95% CI, 0%-6%).



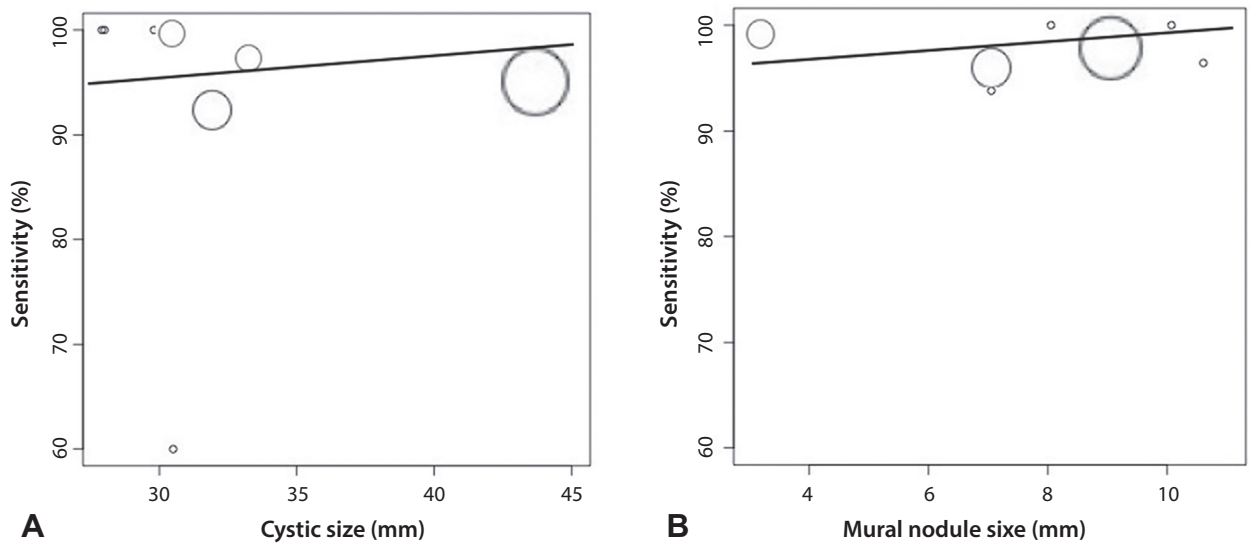
Supplementary Figure 1. A and **B,** Risk of bias of included studies according to the Qualitative Assessments of Diagnostic Accuracy Studies-2 system evaluation tool.



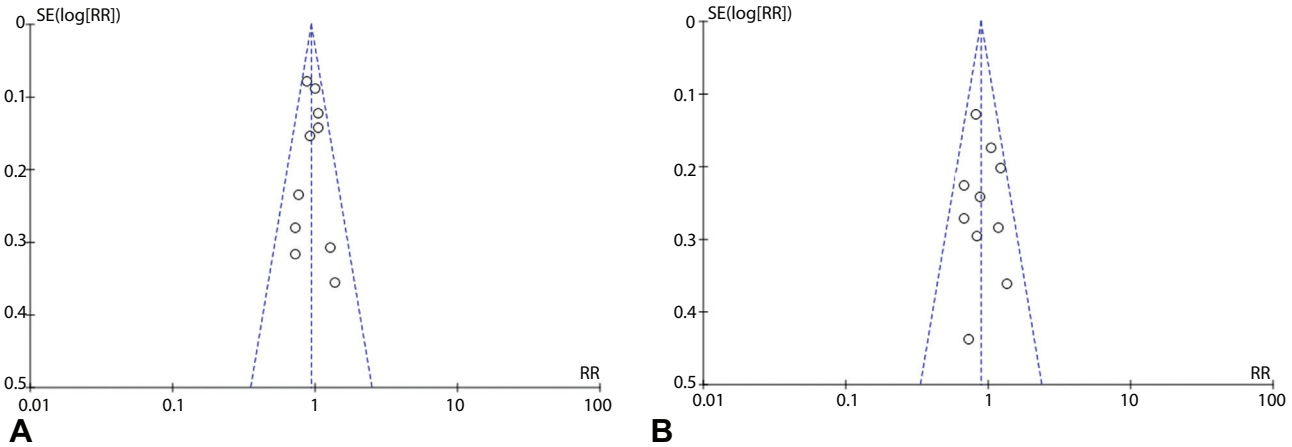
Supplementary Figure 2. Forest plots of diagnostic performance of contrast-enhanced EUS for the identification of high-grade dysplasia or invasive carcinoma within mural nodules of pancreatic cystic neoplasms: (**A**) sensitivity, (**B**) specificity, (**C**) positive likelihood ratio (LR); (**D**) negative LR. *CI*, Confidence interval.



Supplementary Figure 3. Forest plots of diagnostic performance of contrast-enhanced EUS with dedicated contrast-harmonic mode for the identification of high-grade dysplasia or invasive carcinoma within mural nodules of pancreatic cystic neoplasms: (**A**) sensitivity, (**B**) specificity, (**C**) positive likelihood ratio (LR); (**D**) negative LR. *CI*, Confidence interval.



Supplementary Figure 4. A, Meta-regression bubble plot representing the impact of pancreatic cystic neoplasm size (*x* axis) on sensitivity (*y* axis). **B,** Meta-regression bubble plot representing the impact of mural nodule size (*x* axis) on sensitivity (*y* axis).



Supplementary Figure 5. Funnel plots for bias analysis: (**A**) sensitivity and (**B**) specificity. No significant publication bias was shown. *SE*, standard error; *RR*, relative risk.

SUPPLEMENTARY TABLE 1. Pooled diagnostic performance of contrast-enhanced EUS for the characterization of mural nodules in pancreatic cystic neoplasms (10 studies, 532 pancreatic cystic neoplasms)

Diagnostic performance	Contrast-enhanced EUS
Sensitivity, %	88.2 (82.7-92.5)
Specificity, %	79.1 (74.5-83.3)
Positive likelihood ratio	8.64 (3.32-22.53)
Negative likelihood ratio	.08 (.02-.27)
Estimated prevalence, %	35.2 (31.2-39.3)
Positive predictive value, %	69.6 (63.5-75.1)
Negative predictive value, %	92.5 (89.0-95.0)
Diagnostic accuracy, %	89.6 (83.4-95.8)
Number needed to diagnose	1.5 (1.7-1.3)

Values in parentheses are 95% confidence intervals.

SUPPLEMENTARY TABLE 2. Pooled estimates according to the region of origin

Diagnostic performance	Eastern (7 studies, 357 PCNs)	Western (3 studies, 175 PCNs)
Sensitivity, %	87.0 (80.9-91.8)	96.0 (79.6-99.9)
Specificity, %	89.2 (84.0-93.2)	76.0 (67.8-83.5)
Positive likelihood ratio	8.08 (5.37-12)	2.82 (2.23-3.58)
Negative likelihood ratio	.10 (.08-.16)	.06 (.01-.41)
Estimated prevalence, %	45.4	14.3

Values in parentheses are 95% confidence intervals.
PCN, Pancreatic cystic neoplasms.