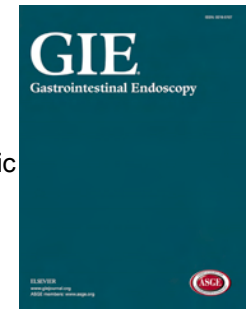

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Pancreatic cyst fluid glucose in differentiating mucinous from nonmucinous pancreatic cysts: a systematic review and meta-analysis

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Pancreatic cyst fluid glucose in differentiating mucinous from nonmucinous pancreatic cysts: a systematic review and meta-analysis

SUMMARY

ABSTRACT

Background and Aims: Recently, low levels of intracystic glucose acquired via EUS-guided pancreatic cyst fluid sampling have been shown to help to differentiate mucinous from nonmucinous cystic neoplasms. The aim of this study was to perform a systematic review and meta-analysis to evaluate the diagnostic characteristics of pancreatic cyst fluid glucose compared with carcinoembryonic antigen (CEA) for pancreatic cystic lesions.

Methods: Individualized searches were developed in accordance with PRISMA and MOOSE guidelines and meta-analysis analyzed according to Cochrane Diagnostic Test Accuracy working group methodology. A bivariate model was used to compute pooled sensitivity and specificity, likelihood ratio, diagnostic odds ratio, and summary receiver operating characteristics curve for intracystic glucose or CEA alone or combination testing.

Results: Eight studies (n=609 lesions; mean age 63.56 ± 2.75 years; 60.36% female) were included. The pooled sensitivity for pancreatic cyst fluid glucose was significantly higher compared with CEA alone (91% [95% CI, 88-94; I^2=0.00] versus 56% [95% CI, 46-66]; I^2=537.14; *P*<0.001) with no difference in specificity (86% [95% CI, 81-90; I^2=24.16] versus 96% [95% CI, 90-99]; I^2=38.06; *P*>0.05). Diagnostic accuracy was significantly higher for pancreatic cyst fluid glucose versus CEA alone (94% [95% CI, 91-96] vs 85% [95% CI, 82-88]; *P*<0.001). Combination testing with pancreatic cyst fluid

glucose and CEA did not improve the diagnostic accuracy compared with glucose alone (97% [95% CI, 95-98] vs 94% [95% CI, 91-96]; *P*>0.05).

Conclusions: Low pancreatic cyst fluid glucose was associated with a high sensitivity and specificity with significantly improved diagnostic accuracy compared with CEA alone for the diagnosis of mucinous versus nonmucinous pancreatic cystic lesions.

Keywords: Pancreatic cystic lesions; Intraductal papillary mucinous neoplasm (IPMN); Mucinous cystic neoplasm (MCN); Pancreas; Intracystic glucose; Carcinoembryonic antigen (CEA); Endoscopic ultrasound (EUS); Fine-needle aspiration (FNA)

INTRODUCTION

The ability to differentiate benign from premalignant or malignant pancreatic cystic neoplasms remains challenging for gastroenterologists, underscored by a host of potential tests available to aid in the clinical diagnosis. Currently, providers may use a variety of potential diagnostic methods including detailed history taking and physical examination findings, high-resolution computed tomography (CT) or magnetic resonance imaging (MRI), endoscopic ultrasound (EUS)-assisted imaging characterization, as well as EUS-acquired ancillary fluid studies.[1] Although traditional EUS sampling includes measurement of carcinoembryonic antigen (CEA), using a threshold of ≥192 mg/dL to aid in the diagnosis of mucinous cystic neoplasms, CEA alone remains a less than ideal test with a modest sensitivity and diagnostic accuracy of 63% and 79%, respectively.[2]

Given the increased identification rate of pancreatic cystic lesions, most commonly due to improvements in high-resolution cross-sectional imaging, effective measures to determine the clinical characteristics of these lesions have become increasingly needed.[3-7] Although a vast majority of these lesions are benign, or associated with a low rate of malignant potential, the last decade has seen a shift in focus to testing to differentiate mucinous versus nonmucinous pancreatic cystic neoplasms.[8-10] More recently, sampling of pancreatic cystic lesions and measurement of pancreatic cyst fluid glucose levels have been shown to potentially distinguish mucinous from nonmucinous lesions.[11-18]

As such, the primary aim of this study was to perform a structured systematic review and meta-analysis to evaluate the diagnostic performance of intracystic glucose sampling of EUS-acquired fluid to differentiate mucinous from nonmucinous pancreatic cysts.

METHODS

Literature Search

Individualized literature search strategies were performed in effort to identify published manuscripts and abstracts evaluating the diagnostic performance of intracystic glucose sampling of cysts and ability to diagnosis mucinous versus nonmucinous pancreatic lesions. Systematic searches of PubMed, EMBASE, Web of Science, and the Cochrane Library databases were performed from available literature from inception through December 15, 2020. The following medical subject heading (MESH) terms included *intracystic glucose* or *pancreatic cyst glucose*. For articles

related to *intracystic glucose* or *pancreatic cyst fluid glucose*, subject heading search terms and title and abstract were reviewed for *pancreatic cystic lesions*, *intraductal papillary mucinous neoplasm (IPMN)*, and *mucinous cystic neoplasm*.

All relevant English language full-text articles regardless of year of publication were included in this systematic review and meta-analysis. From the initial search results, duplicate articles were extracted, and then the titles and abstracts of all potentially relevant studies were screened for eligibility. The reference lists of studies of interest were then manually reviewed for additional articles by cross checking bibliographies as shown in the flow diagram (**Fig. 1)**. Two reviewers (T.R.M. and R.G.) independently screened the titles and abstracts of all the articles according to predefined inclusion and exclusion criteria. In the case of studies with incomplete information, contact was attempted with the principal authors to obtain additional data.

Study Selection Criteria

This study was prospectively submitted in PROSPERO, an international database of prospectively registered systematic reviews in health and social care. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement outline and Meta-Analysis of Observational Studies in Epidemiology (MOOSE) reporting guidelines for reporting systematic reviews and meta-analyses was used to report findings (**Appendix 1** and **Appendix 2)**.[19,20] Full-text manuscripts as well as abstracts were considered for inclusion in this meta-analysis. Studies were only included if there were data available for the construction of a 2 x 2 contingency tables.

The number of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN) were retrieved.

Studies were required to include EUS-acquired sampling of pancreatic cystic lesions and measurement of intracystic glucose. Included studies were also required to have confirmatory testing based upon history or surgical histology/pathology as a reference standard. If surgical resection was not performed (expected for nonmucinous lesions), the diagnosis was based upon additional clinical criteria including established consensus criteria used by the study authors (**Supplementary Table 1)**. Given the novelty of intracystic glucose sampling, a definitive glucose cutoff or threshold was not determined *a priori* though low intracystic glucose levels (typically <50 gm/dL) are believed to be associated with mucinous cystic lesions.

Given this was a diagnostic methods meta-analysis, only studies involving human subjects were enrolled. Animal studies were excluded given limited relevance to clinical outcomes. Additionally, a study was also excluded if deemed to have insufficient data, as were review articles, editorials, and correspondence letters that did not report independent data. Case series and reported studies with <10 cysts were excluded to minimize selection bias. Multiple published work from similar authors was evaluated for overlapping enrollment times to preserve independence of observations.

Outcome Measures

The primary outcome measurement in this study was to assess the diagnostic performance of intracystic glucose sampling of pancreatic cystic lesions to differentiate mucinous versus nonmucinous etiologies. Diagnostic evaluation of individual pancreatic

cyst glucose measurements included diagnostic sensitivity, specificity, positive and negative likelihood ratio, diagnostic odds ratio, and accuracy. Additionally, comparison of intracystic glucose sampling to traditional CEA measurement alone as well as combination testing for either low levels of intracystic glucose (typically ≤50 mg/dL and/or CEA above threshold (≥192 mg/dL) was performed. Other measured outcomes relevant to the study included patient and pancreatic cyst characteristics, as well as threshold cutoffs used to determine accuracy of pancreatic cyst glucose and CEA. These were abstracted manually from the included studies.

Statistical Analysis

This systematic review and meta-analysis was performed according to the Cochrane Diagnostic Test Accuracy working group methodology.[21,22] Two-by-two contingency tables were conducted separately for intracystic glucose, CEA, and combination testing to calculate diagnostic performance (sensitivity, specificity, diagnostic odds ratio, and diagnostic accuracy) for differentiation of mucinous versus nonmucinous lesions. Data on test accuracy and disease prevalence as well as TP, TN, FP, and FN allowed for calculation of diagnostic performance with measures of statistical uncertainty (ie, 95% confidence intervals [CI]). All calculated *P* values were 2-sided, and $P < 0.05$ was considered statistically significant. Diagnostic performance was analyzed using the STATA 15.0 software package (Stata Corp LP, College Station, Tex, USA) with midas user-written command.

A bivariate model was used to compute combined weighted sensitivity, specificity, positive likelihood ratio (LR), negative LR, diagnostic odds ratio (OR), and

summary receiver operating characteristic curve (SROC) and corresponding 95% CI. Overlapping CIs were used to identify nonsignificant differences between diagnostic yield outcomes. If there was no evidence of overlapping CIs, 2-sample t-tests for binomial proportions were used to compare diagnostic characteristics. A random effects model was used based upon heterogeneity inherent to diagnostic accuracy meta-analyses.

Sensitivity and Subgroup Analyses

Additional sensitivity analyses were performed for only prospective studies, excluding retrospective observations studies. Given variable thresholds used for pancreatic cyst fluid glucose measurement, pooled diagnostic results were calculated for only studies using a glucose cutoff of ≤50 mg/dL. Further analyses were performed based on type of glucose measurement (ie, laboratory testing, glucometer testing, and reagent strip testing). This was done in attempt to standardize the data, reduce the possibility of selection bias, and limit heterogeneity of results.

Appraisal of Clinical Utility

In effort to determine the meaningfulness or clinical utility of glucose testing, a probability modifying plot and Fagan nomogram were constructed. To put the results into context with Bayes' theorem, a Fagan plot with pre- and post-test probability was generated. Briefly, the Fagan nomogram is a graphical tool for estimating how much the result of a diagnostic test changes the probability that a patient has a disease.[23] Furthermore, a probability modifying plot was constructed as a graphical sensitivity

analysis of predictive value across a continuum (ie, low to high prevalence defining low- to high-risk populations).[24] This was performed for studies evaluating cyst fluid glucose sampling alone, CEA alone, or combination testing of pancreatic cystic lesions.

Assessment of Methodologic Quality

To evaluate for methodologic quality of individual studies, quality assessment of diagnostic accuracy studies (QUADAS-2) was performed using Review Manager 5 (RevMan 5.3) to summarize assessments.[3] This is an evidence-based tool for assessment of quality in systematic reviews of diagnostic accuracy studies with each key domain using a set of signaling questions to assess bias and applicability. Disagreement among raters (T.R.M. and R.G.) was resolved by consensus with the third author (T.R.).

Investigation of Heterogeneity

Within diagnostic test accuracy reviews, heterogeneity is presumed to exist. Therefore, random effects models were fitted by default.[25] Heterogeneity was assessed I^2 >50%.[26,27] Further quantification of heterogeneity was categorized based upon I^2 with values of 25%, 50%, and 75% indicating low, moderate, and high amounts of heterogeneity, respectively. To further investigate heterogeneity, a bivariate boxplot was used to demonstrate the degree of interdependence of the sensitivity and the specificity, including the identification of any outliers. Potential sources of between-study heterogeneity were explored by univariable meta-regression and subgroup analyses.[28,29] Covariates were determined a priori and included year of study, sample

size, mean age of included patients, mean cystic lesion size, type of glucose testing (defined as only laboratory testing or other testing), and glucose threshold <50 mg/dL.

Publication Bias

As proposed by The Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy, assessment of publication bias was performed using Deeks' funnel plot asymmetry testing.[30] Deeks' funnel plot asymmetry testing was specifically developed for test accuracy reviews and plots the diagnostic odds ratio against the inverse of the square root of the effective sample size. It is the preferred method for diagnostic accuracy meta-analyses.[31]

RESULTS

Study and Lesion Characteristics

A total of 8 studies (n=609 lesions) were included in this systematic review and meta-analysis.[11-18] The protocolized search results and PRISMA flow diagram is shown in **Figure 1**. Full-text and published abstracts were included in this analysis with publications from 2014 to 2020. Four studies were prospective in design with the remaining studies being retrospective in nature. No randomized controlled trials were included. Mean age of included patients was 63.56 ± 2.75 years, with no difference in patient age between patients with mucinous versus nonmucinous lesions (*P*=0.594). A majority of patients were female (60.36%). The mean size of pancreatic cystic lesions was 36.47 ± 5.60 mm. Two studies included only lesions that had pancreatic cyst diagnosis confirmed on surgical pathology.[12,13] In these studies by Zaikos et al[12] and

Carr and colleagues,[13] all surgical pathology specimens were reviewed by a pancreatic pathologist. All additional studies used additional sampling techniques or consensus criteria as the reference standard (**Supplementary Table 1)**. Individual study and patient characteristics are highlighted in **Table 1**.

Diagnostic Yield of Pancreatic Cyst Fluid Glucose

All included studies reported the diagnostic yield of intracystic glucose to differentiate between mucinous versus nonmucinous cystic neoplasms. Mean intracystic glucose for mucinous lesions was 15.92 ± 6.20 gm/dL and nonmucinous lesions was 94.03 ± 12.23 gm/dL (P<0.001). Five of the 8 studies used an intracystic glucose threshold cutoff of <50 mg/dL with additional studies adopting a threshold even lower than 50 mg/dL to be associated with mucinous cystic lesions. Based on the literature, the pooled sensitivity and specificity of EUS-acquired pancreatic cyst fluid glucose was 91% (95% CI, 88 - 94; I^2=0.00) and 86% (95% CI, 81 - 90; I^2=24.16) (**Fig. 2A)**. Intracystic glucose sampling alone demonstrated a diagnostic accuracy of 94% (95% CI, 91 to 96) (**Fig. 3A)**. A complete breakdown of diagnostic yield for pancreatic cyst fluid glucose measurement is shown in **Table 2**.

Sensitivity analyses limiting data to that only reported in prospective studies was not statistically different and is highlighted in **Table 3**. Of the 8 included studies, a total of 5 studies used a glucose threshold of ≤ 50 mg/dL, finding a sensitivity of 91% (95% CI, 86 - 94), specificity of 87% (95% CI, 80 - 92), and diagnostic accuracy of 94% (95% CI, 92 - 96). When subgroup analyses were performed based upon type of glucose testing, 6 studies reported laboratory testing, 3 studies reporting glucometer testing, and

one study reporting glucose reagent strip testing (**Table 1)**. There was no difference in diagnostic characteristics between different glucose testing modalities based upon overlapping CIs (**Table 3)**. The one study by Zikos et al[12] that reported outcomes of glucose reagent strip testing found a sensitivity of 81% (95% CI, 66 - 91) and specificity of 74% (95% CI, 52 - 90).

Diagnostic Yield of CEA

The mean CEA level for mucinous lesions was significantly higher compared with nonmucinous lesions (997.32 ± 776.38 versus 5.99 ± 3.15 gm/dL; *P*<0.001). Comparing the results of pancreatic cyst fluid glucose to traditional CEA alone, revealed that CEA was associated with a lower sensitivity (56% [95% CI, 46 - 66; I^2=57.14] versus 91% [95% CI, 88 - 94; I^2=0.00]; *P*<0.001) (**Fig. 2B)**. Specificity on the other hand, was not different between CEA alone (96% [95% CI, 90 – 99]; I^2=38.06) compared with intracystic glucose specificity (86% [95% CI, 81 - 90; I^2=24.16]). However, intracystic glucose outperformed CEA alone with regard to diagnostic accuracy (94% [95% CI, 91 - 96] versus 85% [95% CI, 82 – 88]; *P*<0.001) (**Fig. 3B)**. Sensitivity analyses limited to only prospective studies demonstrated a significantly lower diagnostic accuracy for CEA (73%; 95% CI, 69 - 76) compared with all included study data, though no difference in sensitivity or specificity (**Table 3)**.

Combination Testing

Given the improved sensitivity and diagnostic accuracy of pancreatic cyst fluid glucose to CEA alone, combination testing with both strategies was also examined.

Sampling that revealed a low glucose or elevated CEA was reported in 4 studies (n=348 lesions) and resulted in a combined sensitivity and specificity of 97% (95% CI, 90 – 99; I^2=0.00) and 72% (95% CI, 47 - 88; I^2=68.85), respectively (**Fig. 2C)**. The diagnostic accuracy associated with combination testing was 97% (95% CI, 95 - 98; I^2=0.00) (**Fig. 3C)**. Although the sensitivity and diagnostic accuracy of glucose and CEA combination testing was significantly improved compared with CEA alone (*P*<0.001), combination testing did not improve the testing characteristics compared with pancreatic cyst fluid glucose sampling alone (based upon the presence of overlapping CIs) (**Table 2)**.

Clinical Utility

Given the available sampling strategies (ie, independent or combination testing) to differentiate between mucinous versus nonmucinous pancreatic cystic lesions, Fagan plots as well probability-modifying plots for positive and negative results were constructed to determine the clinical meaningfulness. Clinical utility and probability modifying plots for pancreatic cyst fluid glucose measurement, CEA level, and combination testing are shown in **Figure 4A-C**. Assuming a pretest probability of 40% for a mucinous lesion, if the intracystic fluid glucose alone is <50 mg/dL, the post-test probability that the patient truly has mucinous lesion would be approximately 82%. For a similar pretest probability, CEA testing alone >192 mg/dL would produce a post-test probability of approximately 91%. Combination testing, again assuming a 40% pretest probability, would be associated with a post-test probability of 69% for the lesion being mucinous. Alternatively, if a glucose, CEA, or combination test was negative (ie, glucose >50 mg/dL and/or <192 mg/dL), the post-test probability that the patient has a

mucinous pancreatic lesion would be approximately 6%, 23%, and 2%, respectively. Informativeness of independent and combination testing, represented graphically by likelihood ratio scattergrams, is shown in **Supplementary Figure 1**.

Assessment of Heterogeneity and Meta-Regression

A bivariate boxplot for all included studies assessing intracystic glucose revealed 2 studies to be outliers (ie, providing indirect evidence of the threshold variability) (**Supplementary Figure 2)**.[11,17] However, exclusion of these outlier studies did not significantly change the diagnostic characteristics for intracystic glucose (**Table 3)**. Quantitatively, univariable meta-regression for a priori covariates did not reveal any significant sources of heterogeneity for pancreatic cyst fluid glucose measurement (**Supplementary Figure 3)**.

Study Quality and Publication Bias

The quality of the eligible studies assessed by QUADAS-2 criteria is reported in **Figure 5**. In a majority of studies, there was a low risk of bias for patient selection with no bias issues or concerns regarding applicability of the selection of patients. There was no significant risk of bias regarding the index test as defined by the studies as well. In several studies, however, flow and timing in regard to the use of the reference standard was found to have an unclear risk of bias (**Supplementary Table 1**). The risk of bias in all but two studies was unclear given lack of confirmatory surgical pathology for all included lesions. With regard to publication bias, Deeks' funnel plot was used and demonstrated no evidence of publication bias (P>0.05) (**Fig. 6A-C)**.

DISCUSSION

Based on the results of this systematic review and meta-analysis, pancreatic fluid cyst glucose measurement provided a significantly improved diagnostic accuracy compared with CEA alone for the differentiation of mucinous versus nonmucinous pancreatic cystic neoplasms. In this study, the pooled sensitivity, specificity, and accuracy associated with intracystic glucose testing was 91%, 86%, and 94%, respectively. There was no difference noted in specificity between pancreatic cyst fluid glucose versus traditional CEA testing; however, the sensitivity and diagnostic accuracy was improved for intracystic glucose. Furthermore, combination testing with cyst fluid glucose and CEA did not appear to improve diagnostic accuracy of mucinous pancreatic cystic neoplasms.

Pancreatic cystic lesions have become increasingly detected as a result of high-resolution cross-sectional imaging with an estimated 70% of lesions discovered incidentally.[3-7] Several alternative or complimentary sampling modalities exist, including various need types (fine-needle aspiration [FNA] or fine-needle biopsy [FNB] sampling), use of rapid on-site cytopathology examination (ROSE), addition of molecular analysis including G-*ras* and K-*ras* mutations, use of EUS-guided confocal laser endomicroscopy (nCLE), and through-the-needle (TTN) microforceps biopsy with goal to identify premalignant neoplasms and avoid overtreatment of lesions with no malignant potential [30,32-40]

The use of intracystic glucose sampling to assist in differentiating mucinous versus nonmucinous cystic lesions was first described by Park and colleagues in

2013.[41] In this initial study, glucose levels were significantly lower for mucinous cysts when compared with nonmucinous cysts (5 vs 82 mg/dL; *P*=0.002). This study was excluded from this systematic review and meta-analysis given overlapping patient enrollment with the study by Zikos et al.[12] Based upon this systematic review and meta-analysis the mean intracystic glucose for mucinous lesions was 15.92 gm/dL versus nonmucinous lesions at 94.03 gm/dL (*P*<0.001). This lower level of glucose within the pancreatic cyst may reflect more metabolic activity mucinous lesions, thus allowing for thresholds of <50 mg/dL to be considered a reasonable cutoff to identify mucinous lesions. Furthermore, glucose measurement may be particularly useful in pancreatic cystic lesions with CEA levels between 5 and 192 ng/mL and nondiagnostic cytology. In the study by Fais et al, this represented 34.1% (28/82) of samples that may have been erroneously classified as nonmucinous and excluded from the surveillance program.[14]

When intracystic glucose is compared with CEA testing, glucose measurements offer several potential advantages. One important advantage relates to rapid on-site availability and accessibility of testing. Fluid glucose testing is readily available in most laboratories across healthcare institutions in the United States and around the globe, without the need for the fluid sample to be sent to a reference laboratory. Although CEA remains an important test for EUS sampling of cystic lesions, commercially available lab testing remains highly variable with a lack of validated methods for measurement in pancreatic cystic fluid (validated in serum or plasma).[42] Although cost-effectiveness studies have not been performed to date, the low cost associated with glucose testing compared with traditional CEA (U.S.$1.89 versus $142) likely translates into a more cost-efficient diagnostic approach to EUS sampling.[13]

Sampling also requires a much lower volume of pancreatic intracystic fluid for glucose testing compared with CEA assay (50 µL versus 500 µL) with some studies reporting as little volume of 2 µL needed for glucose measurement.[12,14] This requirement for a limited sample to effectively measure glucose is potentially very meaningful for clinical practice as in some cases, very limited amount of fluid can be obtained via EUS-FNA precluding standard biochemical analysis, making the small amount of pancreatic cystic fluid required for glucometer analysis a major advantage. Additionally, the glucose test (laboratory essay, reagent strip, or glucometer) possesses a rapid turnaround time making it an immediate on-site test.

Yet, despite these potential advantages, variability in glucose testing remains a limiting factor to more widespread adoption in clinical practice. In the study by Zikos and colleagues,[12] laboratory glucose <50 mg/dL was associated with a sensitivity of 95% and a specificity of 57%, whereas glucometer glucose testing <50 mg/dL had a sensitivity and specificity of 88% and 78%, respectively. Furthermore, reagent strip glucose measurement demonstrated a sensitivity of 81% and a specificity of 74% demonstrating a lack of standardization, but also underscoring easy universal availability of glucose testing. Similar to CEA, varying glucose levels have varying sensitivity and specificity and differ according to testing method used; however, on subgroup analyses, there was no statistically significant difference in diagnostic characteristics between the various glucose measuring tests.

Given the current limitations of CEA testing alone, combination testing is often used with the addition of other biomarkers along with CEA to differentiate mucinous versus nonmucinous pancreatic cystic lesions.[43-46] Though again, the availability of

these various biomarker tests, especially next-generation molecular analyses including K-*ras* and G-*nas* mutations, may limit their use in clinical practice. In this systematic review and meta-analysis, combination testing with CEA and pancreatic cyst fluid glucose did outperform independent measurement of CEA alone; however, the diagnostic characteristics were similar to pancreatic cyst fluid glucose testing alone. Combining intracystic glucose with additional biomarkers such as cytology, imaging, and/or molecular testing may further impact diagnostic performance and the ability to differentiate between mucinous versus nonmucinous pancreatic cystic lesions; however, no diagnostic algorithms yet exist, nor have studies been performed to date to assess this clinically relevant question.

Although this is the first systematic review and meta-analysis to investigate the role of pancreatic cyst fluid glucose to accurately diagnose pancreatic mucinous cystic lesions, this study is not without limitations. Most notably, this study included multiple thresholds/cutoffs for glucose measurement and multiple modalities to measure pancreatic cyst fluid glucose. Importantly, sensitivity and subgroup analyses, along with univariable meta-regression did not reveal variable cutoffs to be a source of between-study heterogeneity. Although multiple glucose testing strategies were included, subgroup analyses performed did not reveal significant differences. Additionally, it is important to acknowledge that surgical histopathology to confirm mucinous versus nonmucinous pancreatic lesions were performed for a minority of samples with variation in reference standard criteria used by individual study (**Supplementary Table 1**), although surgical resection should ideally not occur for benign lesions with no features to suggest malignant or premalignant potential.

Despite these study limitations, our review has several strengths. This study was performed in accordance with the Cochrane Diagnostic Test Accuracy handbook methodology and included a systematic literature search with well-defined inclusion criteria, careful exclusion of redundant studies, inclusion of high-quality studies with detailed extraction of data, and rigorous evaluation of study quality. The pooled sensitivity, specificity, and diagnostic accuracy of CEA as measured in this study in similar to that reported in multiple previous studies, serving as an appropriate reference when evaluating the true impact of pancreatic cyst fluid glucose measurement.[2,47-50] Furthermore, this study constructed Fagan plots as well as probability-modifying plots for positive and negative results to determine the clinical utility or meaningfulness of intracystic glucose sampling. Modifying plots and likelihood ratio scattergrams are able to plot assessment over a spectrum of probabilities, making data more generalizability to individual patient care scenarios.

In conclusion, pancreatic cyst fluid glucose is a valid, simple, less expensive, and rapid test for differentiating mucinous from nonmucinous pancreatic cystic lesions. Pancreatic cyst fluid glucose was found to be more accurate than measurement of CEA level alone, currently the most widely used pancreatic cyst fluid biomarker. Future larger, prospective, ideally randomized studies are needed before more widespread clinical implementation.

FIGURE LEGENDS

Figure 1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow chart of literature search results

Figure 2. Forest plots of sensitivity and specificity of intracystic glucose, CEA, and combination testing for pancreatic cystic lesions. A, Pancreatic cyst fluid glucose. B, CEA. C, Intracystic glucose and/or CEA combination testing.

Figure 3. Diagnostic accuracy of intracystic glucose, CEA, and combination testing for pancreatic cystic lesions. A, Pancreatic cyst fluid glucose. B, CEA. C, Intracystic glucose and/or CEA combination testing.

Figure 4. Fagan nomogram and probability modifying plot of intracystic glucose, CEA, and combination testing for pancreatic cystic lesions. A, Pancreatic cyst fluid glucose. B, CEA. C, Intracystic glucose and/or CEA combination testing.

Figure 5. QUADAS-2 quality assessment of included studies.

Figure 6. Deeks' funnel plot asymmetry testing for publication bias of intracystic glucose, CEA, and combination testing for pancreatic cystic lesions. A, Pancreatic cyst fluid glucose. B, CEA. C, Intracystic glucose and/or CEA combination testing

Supplementary Figure 1. Likelihood ratio scattergram. A, Intracystic glucose testing alone. B, CEA testing alone. C, Combination testing with intracystic glucose and CEA testing.

Supplementary Figure 2. Bivariate boxplot to investigate heterogeneity with most studies clustering within the median distribution. Two studies were noted to be outliers.

Supplementary Figure 3. Forest plot of multiple univariable meta-regression and subgroup analyses.

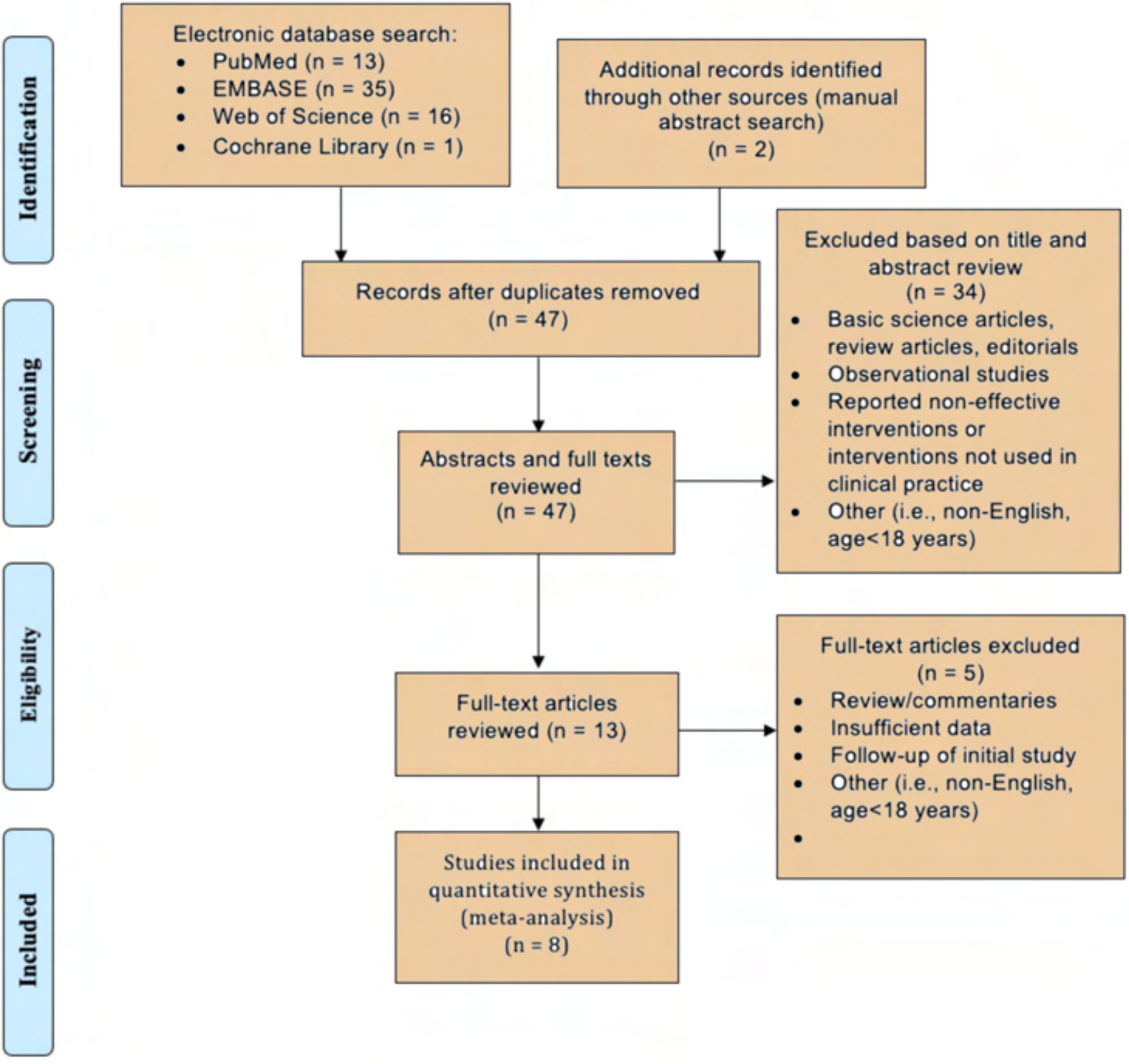
References

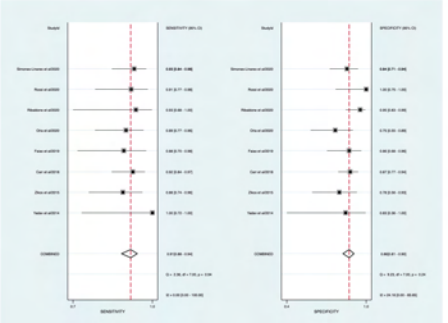
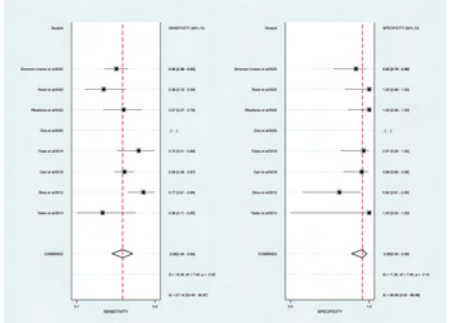
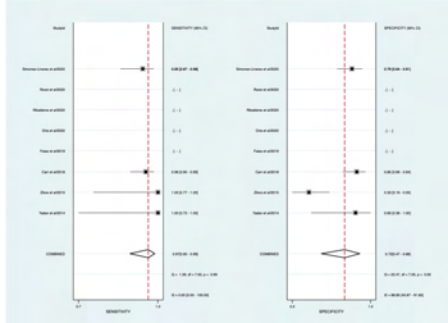
1. Thiruvengadam N, Park WG. Systematic Review of Pancreatic Cyst Fluid Biomarkers: The Path Forward. Clinical and translational gastroenterology 2015;6:e88.
2. Thornton GD, McPhail MJ, Nayagam S, Hewitt MJ, Vlavianos P, Monahan KJ. Endoscopic ultrasound guided fine needle aspiration for the diagnosis of pancreatic cystic neoplasms: a meta-analysis. Pancreatology 2013;13:48-57.
3. de Jong K, Nio CY, Hermans JJ, et al. High prevalence of pancreatic cysts detected by screening magnetic resonance imaging examinations. Clin Gastroenterol Hepatol 2010;8:806-11.
4. Laffan TA, Horton KM, Klein AP, et al. Prevalence of unsuspected pancreatic cysts on MDCT. AJR Am J Roentgenol 2008;191:802-7.
5. Sarr MG, Murr M, Smyrk TC, et al. Primary cystic neoplasms of the pancreas. Neoplastic disorders of emerging importance-current state-of-the-art and unanswered questions. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract 2003;7:417-28.
6. Ferrone CR, Correa-Gallego C, Warshaw AL, et al. Current trends in pancreatic cystic neoplasms. Archives of surgery (Chicago, Ill : 1960) 2009;144:448-54.
7. Assifi MM, Nguyen PD, Agrawal N, et al. Non-neoplastic epithelial cysts of the pancreas: a rare, benign entity. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract 2014;18:523-31.
8. Horvath KD, Chabot JA. An aggressive resectional approach to cystic neoplasms of the pancreas. American journal of surgery 1999;178:269-74.

9. European Study Group on Cystic Tumours of the P. European evidence-based guidelines on pancreatic cystic neoplasms. Gut 2018;67:789-804.
10. Greer JB, Ferrone CR. Spectrum and Classification of Cystic Neoplasms of the Pancreas. Surg Oncol Clin N Am 2016;25:339-50.
11. Yadav D, Chahal P, Vargo J et al. Clinical Utility of Intracystic Glucose Levels in Differentiating Mucinous From Non-mucinous Pancreatic Cysts. Am J Gastro. 2014;109(Suppl 2):S70.
12. Zikos T, Pham K, Bowen R, et al. Cyst Fluid Glucose is Rapidly Feasible and Accurate in Diagnosing Mucinous Pancreatic Cysts. Am J Gastroenterol 2015;110:909-14.
13. Carr RA, Yip-Schneider MT, Simpson RE, et al. Pancreatic cyst fluid glucose: rapid, inexpensive, and accurate diagnosis of mucinous pancreatic cysts. Surgery 2018;163:600-5.
14. Faias S, Pereira L, Roque R, et al. Excellent Accuracy of Glucose Level in Cystic Fluid for Diagnosis of Pancreatic Mucinous Cysts. Dig Dis Sci 2020;65:2071-8.
15. Oria I, Larino-Noia J, Villaverde A, et al. Cyst Fluid Glucose Obtained by EUS-FNA is Accurate for the Diagnosis of Mucinous Pancreatic Cysts. Experience From Two Tertiary Care Centers. Gastroinest Endosc 2020;91(Suppl 6):AB178-9.
16. Ribaldone DG, Bruno M, Gaia S, et al. Differential diagnosis of pancreatic cysts: A prospective study on the role of intra-cystic glucose concentration. Dig Liver Dis 2020;52:1026-32.
17. Rossi G, Petrone M, Capurso G, et al. Glucose Levels in EUS-Aspirated Cyst Fluid Have a High Accuracy for the Diagnosis of Mucinous Pancreatic Cystic Lesions. Gastrointest Endosc. 2020;91(Suppl 6):AB181.
18. Simons-Linares CR, Yadav D, Lopez R, et al. The utility of intracystic glucose levels in differentiating mucinous from non-mucinous pancreatic cysts. Pancreatology 2020;20:1386-92.
19. Farrell JJ. Prevalence, Diagnosis and Management of Pancreatic Cystic Neoplasms: Current Status and Future Directions. Gut Liver 2015;9:571-89.
20. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000;283:2008-12.
21. Leeflang MM, Deeks JJ, Takwoingi Y, Macaskill P. Cochrane diagnostic test accuracy reviews. Syst Rev 2013;2:82.
22. Cochrane Systematic Reviews of Diagnostic Test Accuracy (DTA) Handbook. Cochrane Screening and Diagnostic Tests Methods Group (SDTM). Available at: https://methods.cochrane.org/sdt/welcome. Accessed 11 November 2020.
23. Mittal C, Obuch JC, Hammad H, et al. Technical feasibility, diagnostic yield, and safety of microforceps biopsies during EUS evaluation of pancreatic cystic lesions (with video). Gastrointest Endosc 2018;87:1263-9.
24. Zhang ML, Arpin RN, Brugge WR, Forcione DG, Basar O, Pitman MB. Moray micro forceps biopsy improves the diagnosis of specific pancreatic cysts. Cancer Cytopathol 2018;126:414-20.
25. Khan MA, Grimm IS, Ali B, et al. A meta-analysis of endoscopic ultrasound-fine-needle aspiration compared to endoscopic ultrasound-fine-needle biopsy: diagnostic yield and the value of onsite cytopathological assessment. Endosc Int Open 2017;5:E363-E75.

26. Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol 2005;58:982-90.
27. Kovacevic B, Karstensen JG, Havre RF, et al. Initial experience with EUS-guided microbiopsy forceps in diagnosing pancreatic cystic lesions: A multicenter feasibility study (with video). Endosc Ultrasound 2018;7:383-8.
28. Lijmer JG, Bossuyt PM, Heisterkamp SH. Exploring sources of heterogeneity in systematic reviews of diagnostic tests. Stat Med 2002;21:1525-37.
29. Petitti DB. Approaches to heterogeneity in meta-analysis. Stat Med 2001;20:3625-33.
30. Committee ASoP, Muthusamy VR, Chandrasekhara V, et al. The role of endoscopy in the diagnosis and treatment of cystic pancreatic neoplasms. Gastrointest Endosc 2016;84:1-9.
31. van Enst WA, Ochodo E, Scholten RJ, Hooft L, Leeflang MM. Investigation of publication bias in meta-analyses of diagnostic test accuracy: a meta-epidemiological study. BMC Med Res Methodol 2014;14:70.
32. Khalid A, Zahid M, Finkelstein SD, et al. Pancreatic cyst fluid DNA analysis in evaluating pancreatic cysts: a report of the PANDA study. Gastrointest Endosc 2009;69:1095-102.
33. Nakai Y, Iwashita T, Park DH, Samarasena JB, Lee JG, Chang KJ. Diagnosis of pancreatic cysts: EUS-guided, through-the-needle confocal laser-induced endomicroscopy and cystoscopy trial: DETECT study. Gastrointest Endosc 2015;81:1204-14.
34. Singhi AD, Nikiforova MN, Fasanella KE, et al. Preoperative GNAS and KRAS testing in the diagnosis of pancreatic mucinous cysts. Clin Cancer Res 2014;20:4381-9.
35. Iglesias-Garcia J, Larino-Noia J, Abdulkader I, Dominguez-Munoz JE. Rapid on-site evaluation of endoscopic-ultrasound-guided fine-needle aspiration diagnosis of pancreatic masses. World J Gastroenterol 2014;20:9451-7.
36. de Moura DTH, McCarty TR, Jirapinyo P, et al. Evaluation of endoscopic ultrasound fine-needle aspiration versus fine-needle biopsy and impact of rapid on-site evaluation for pancreatic masses. Endosc Int Open 2020;8:E738-E47.
37. Klapman JB, Logrono R, Dye CE, Waxman I. Clinical impact of on-site cytopathology interpretation on endoscopic ultrasound-guided fine needle aspiration. Am J Gastroenterol 2003;98:1289-94.
38. McCarty T, Rustagi T. Endoscopic ultrasound-guided through-the-needle microforceps biopsy improves diagnostic yield for pancreatic cystic lesions: a systematic review and meta-analysis. Endosc Int Open 2020;8:E1280-E90.
39. de Pretis N, Mukewar S, Aryal-Khanal A, Bi Y, Takahashi N, Chari S. Pancreatic cysts: Diagnostic accuracy and risk of inappropriate resections. Pancreatology 2017;17:267-72.
40. McCarty T, Paleti S, Rustagi T. Molecular analysis of EUS-acquired pancreatic cyst fluid for K-ras and G-nasmutations for diagnosis of intraductal papillary mucinous neoplasia and mucinous cystic lesions: a systematic review and meta-analysis. Gastrointest Endosc 2020.
41. Park WG, Wu M, Bowen R, et al. Metabolomic-derived novel cyst fluid biomarkers for pancreatic cysts: glucose and kynurenine. Gastrointest Endosc 2013;78:295-302 e2.
42. Boot C. A review of pancreatic cyst fluid analysis in the differential diagnosis of pancreatic cyst lesions. Ann Clin Biochem 2014;51:151-66.
43. Park J, Yun HS, Lee KH, Lee KT, Lee JK, Lee SY. Discovery and Validation of Biomarkers That Distinguish Mucinous and Nonmucinous Pancreatic Cysts. Cancer Res 2015;75:3227-35.

44. Al-Haddad M, DeWitt J, Sherman S, et al. Performance characteristics of molecular (DNA) analysis for the diagnosis of mucinous pancreatic cysts. Gastrointest Endosc 2014;79:79-87.
45. Oppong KW, Dawwas MF, Charnley RM, et al. EUS and EUS-FNA diagnosis of suspected pancreatic cystic neoplasms: Is the sum of the parts greater than the CEA? Pancreatology 2015;15:531-7.
46. Bick BL, Enders FT, Levy MJ, et al. The string sign for diagnosis of mucinous pancreatic cysts. Endoscopy 2015;47:626-31.
47. Brugge WR, Lewandrowski K, Lee-


Identification
Screening
Eligibility
Included
Electronic database search:
PubMed (n = 13)
EMBASE (n = 35)
Web of Science (n = 16)
Cochrane Library (n = 1)
Additional records identified through other sources (manual abstract search) (n = 2)
Records after duplicates removed (n = 47)
Abstracts and full texts reviewed (n = 47)
Excluded based on title and abstract review (n = 34)
Basic science articles, review articles, editorials
Observational studies
Reported non-effective interventions or interventions not used in clinical practice
Other (i.e., non-English, age<18 years)
Full-text articles reviewed (n = 13)
Full-text articles excluded (n = 5)
Review/commentaries
Insufficient data
Follow-up of initial study
Other (i.e., non-English, age<18 years)
Studies included in quantitative synthesis (meta-analysis) (n = 8)


Study
SENSITIVITY (95% CI)
Simonsz Lhoeou et al2020
Rossi et al2020
Ribaldone et al2020
Ohs et al2020
Fosso et al2019
Carl et al2019
Zhou et al2015
Yadav et al2014
COMBINED
SENSITIVITY
Study
SPECIFICITY (95% CI)
Simonsz Lhoeou et al2020
Rossi et al2020
Ribaldone et al2020
Ohs et al2020
Fosso et al2019
Carl et al2019
Zhou et al2015
Yadav et al2014
COMBINED
SPECIFICITY

Study
SENSITIVITY (95% CI)
Simonsz Lhoeou et al2020
Rossi et al2020
Ribaldone et al2020
Ohs et al2020
Fosso et al2019
Carl et al2019
Zhou et al2015
Yadav et al2014
COMBINED
SENSITIVITY
Study
SPECIFICITY (95% CI)
Simonsz Lhoeou et al2020
Rossi et al2020
Ribaldone et al2020
Ohs et al2020
Fosso et al2019
Carl et al2019
Zhou et al2015
Yadav et al2014
COMBINED
SPECIFICITY

Study
SENSITIVITY (95% CI)
Simonsz Lhoeou et al2020
Rossi et al2020
Ribaldone et al2020
Ohs et al2020
Fosso et al2019
Carl et al2019
Zhou et al2015
Yadav et al2014
COMBINED
SENSITIVITY
Study
SPECIFICITY (95% CI)
Simonsz Lhoeou et al2020
Rossi et al2020
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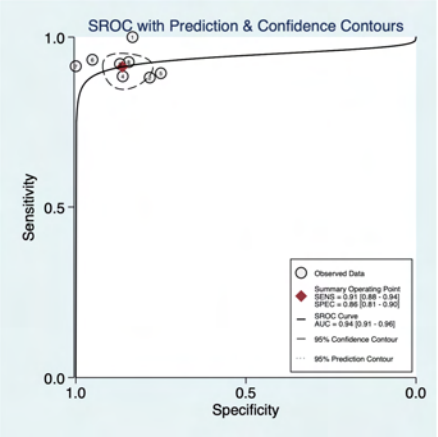
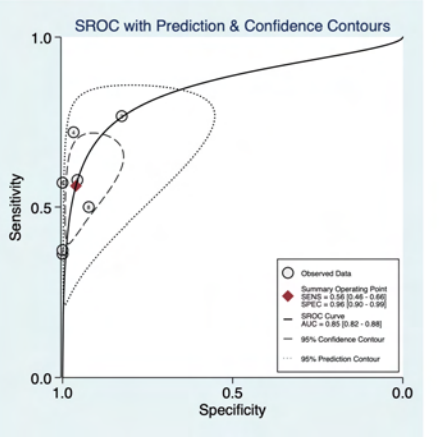
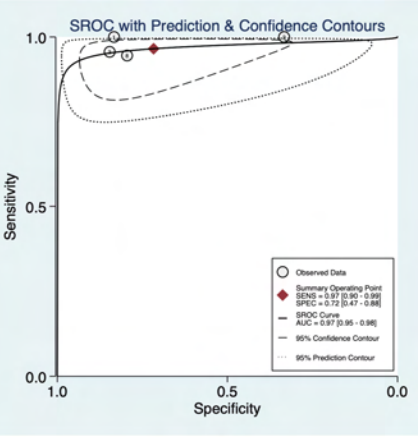
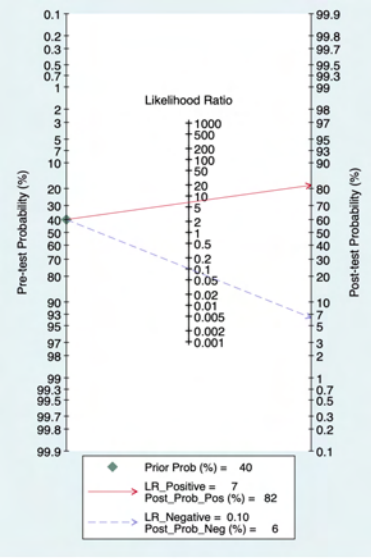
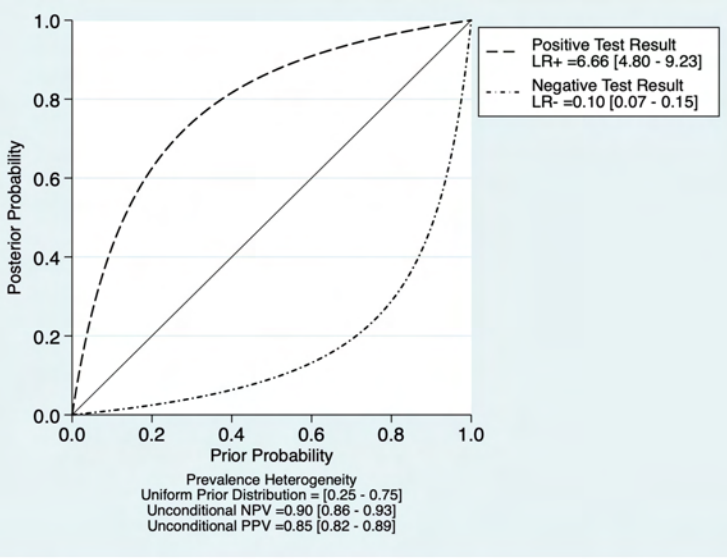
Carl et al2019
Zhou et al2015
Yadav et al2014
COMBINED
SPECIFICITY

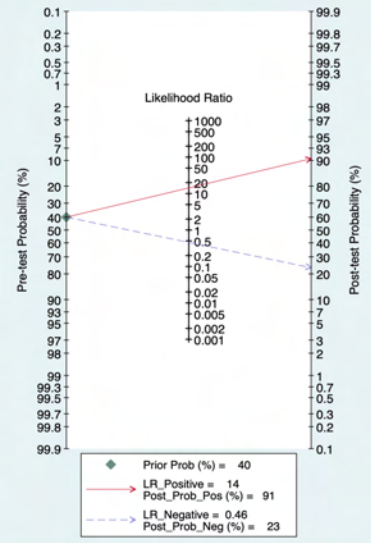

SROC with Prediction & Confidence Contours
Sensitivity
Specificity
1.0
0.5
0.0
Observed Data
Summary Operating Point
SENS = 0.91 [0.88 - 0.94]
SPEC = 0.86 [0.81 - 0.90]
SROC Curve
AUC = 0.94 [0.91 - 0.96]
95% Confidence Contour
95% Prediction Contour

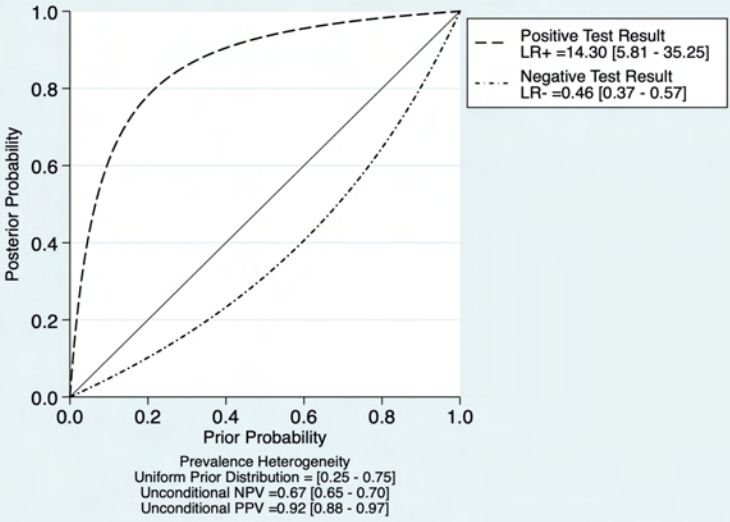

SROC with Prediction & Confidence Contours
Sensitivity
Specificity
1.0
0.5
0.0
Observed Data
Summary Operating Point
SENS = 0.56 [0.46 - 0.66]
SPEC = 0.96 [0.90 - 0.99]
SROC Curve
AUC = 0.85 [0.82 - 0.88]
95% Confidence Contour
95% Prediction Contour

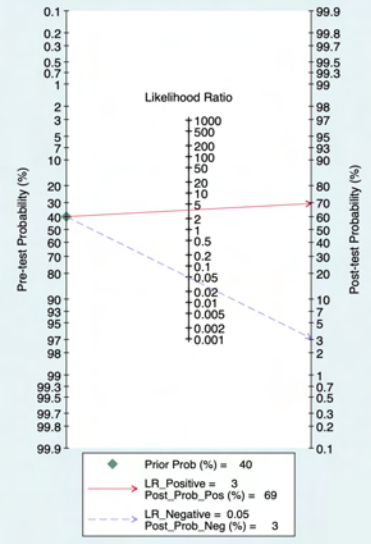

SROC with Prediction & Confidence Contours
Sensitivity
Specificity
1.0
0.5
0.0
Observed Data
Summary Operating Point
SENS = 0.97 [0.90 - 0.99]
SPEC = 0.72 [0.47 - 0.88]
SROC Curve
AUC = 0.97 [0.95 - 0.98]
95% Confidence Contour
95% Prediction Contour

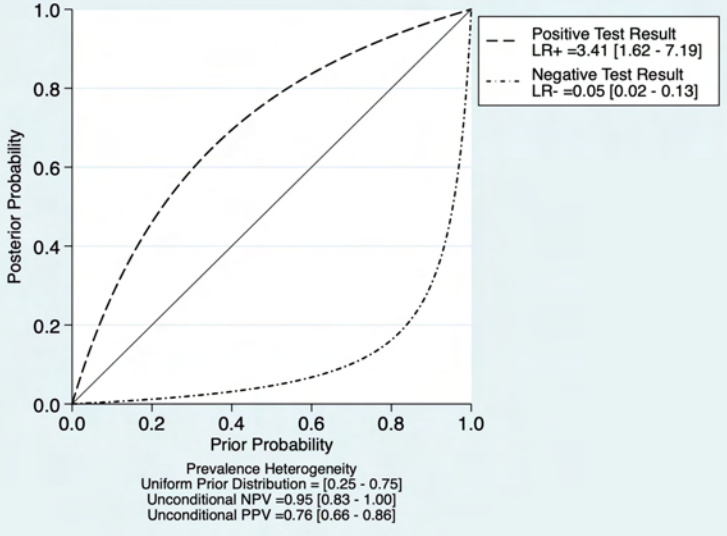

Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 40
LR_Positive = 7
Post_Prob_Pos (%) = 82
LR_Negative = 0.10
Post_Prob_Neg (%) = 6

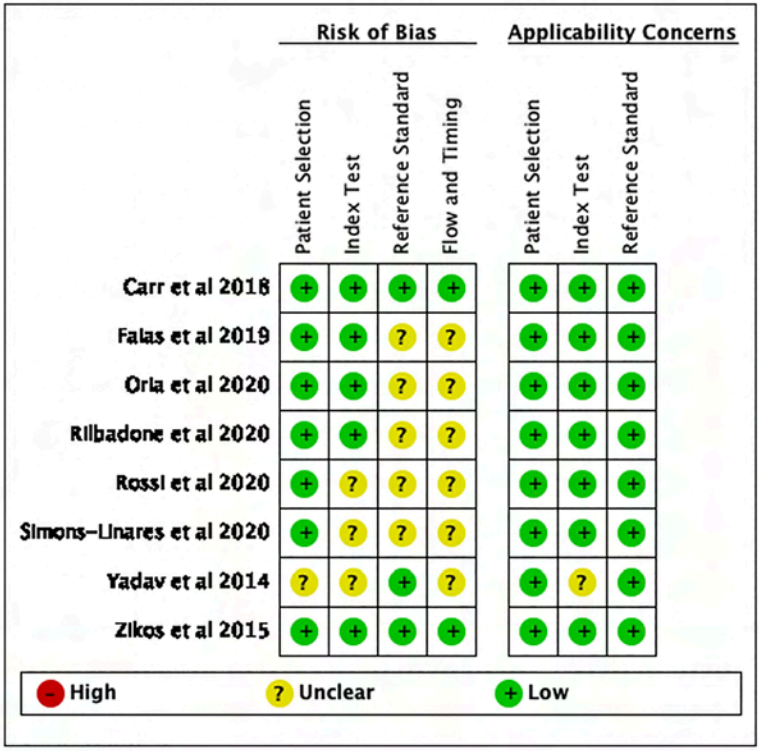

Positive Test Result
LR+ =6.66 [4.80 - 9.23]
Negative Test Result
LR- =0.10 [0.07 - 0.15]
Posterior Probability
Prior Probability
Prevalence Heterogeneity
Uniform Prior Distribution = [0.25 - 0.75]
Unconditional NPV =0.90 [0.86 - 0.93]
Unconditional PPV =0.85 [0.82 - 0.89]

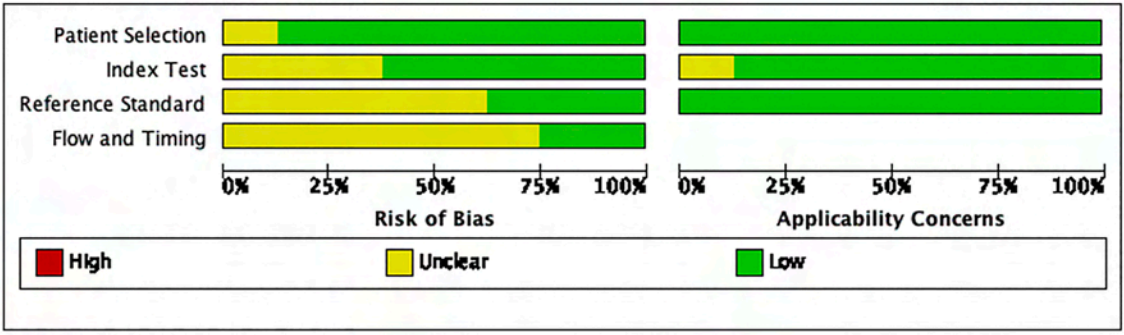

Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 40
LR_Positive = 14
Post_Prob_Pos (%) = 91
LR_Negative = 0.46
Post_Prob_Neg (%) = 23

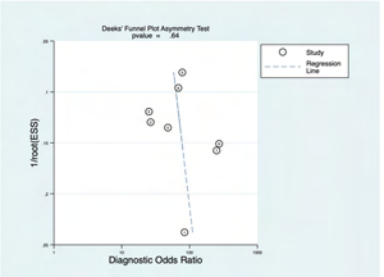

Positive Test Result
LR+ =14.30 [5.81 - 35.25]
Negative Test Result
LR- =0.46 [0.37 - 0.57]
Posterior Probability
Prior Probability
Prevalence Heterogeneity
Uniform Prior Distribution = [0.25 - 0.75]
Unconditional NPV =0.67 [0.65 - 0.70]
Unconditional PPV =0.92 [0.88 - 0.97]

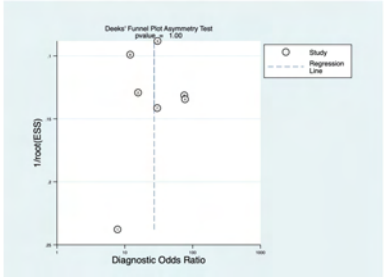

Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 40
LR_Positive = 3
Post_Prob_Pos (%) = 69
LR_Negative = 0.05
Post_Prob_Neg (%) = 3

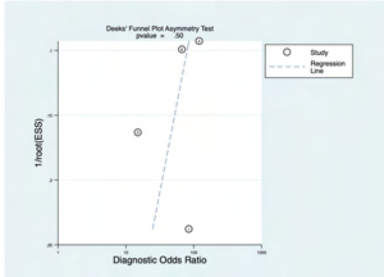

Positive Test Result
LR+ =3.41 [1.62 - 7.19]
Negative Test Result
LR- =0.05 [0.02 - 0.13]
Posterior Probability
Prior Probability
Prevalence Heterogeneity
Uniform Prior Distribution = [0.25 - 0.75]
Unconditional NPV =0.95 [0.83 - 1.00]
Unconditional PPV =0.76 [0.66 - 0.86]

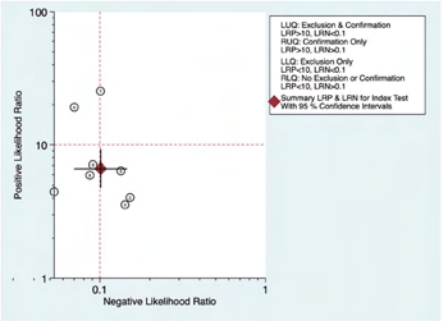

Risk of Bias
Applicability Concerns
Patient Selection
Index Test
Reference Standard
Flow and Timing
Patient Selection
Index Test
Reference Standard
Carr et al 2018
Falas et al 2019
Oria et al 2020
Ribadone et al 2020
Rossi et al 2020
Simons-Linares et al 2020
Yadav et al 2014
Zikos et al 2015
High
Unclear
Low

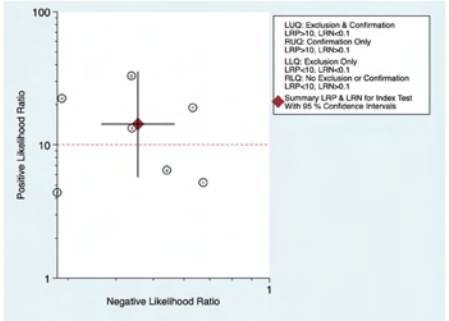

Patient Selection
Index Test
Reference Standard
Flow and Timing
0%
25%
50%
75%
100%
Risk of Bias
0%
25%
50%
75%
100%
Applicability Concerns
High
Unclear
Low

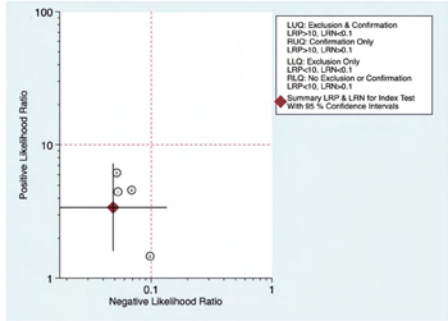

Deeks' Funnel Plot Asymmetry Test
pvalue = .64
Study
Regression Line
1/root(ESS)
Diagnostic Odds Ratio

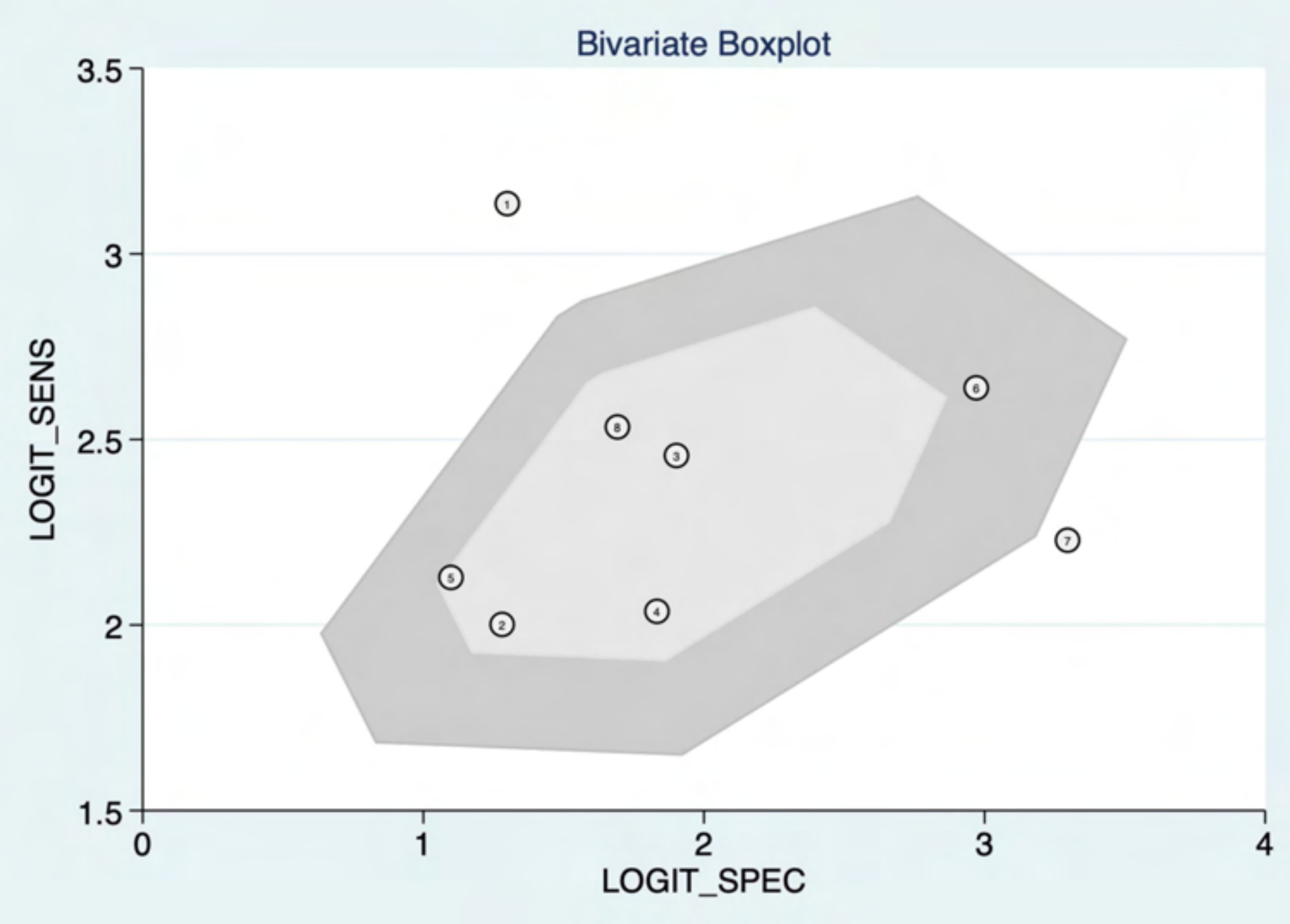

Deeks' Funnel Plot Asymmetry Test
pvalue = 1.00
Study
Regression Line
1/root(ESS)
Diagnostic Odds Ratio

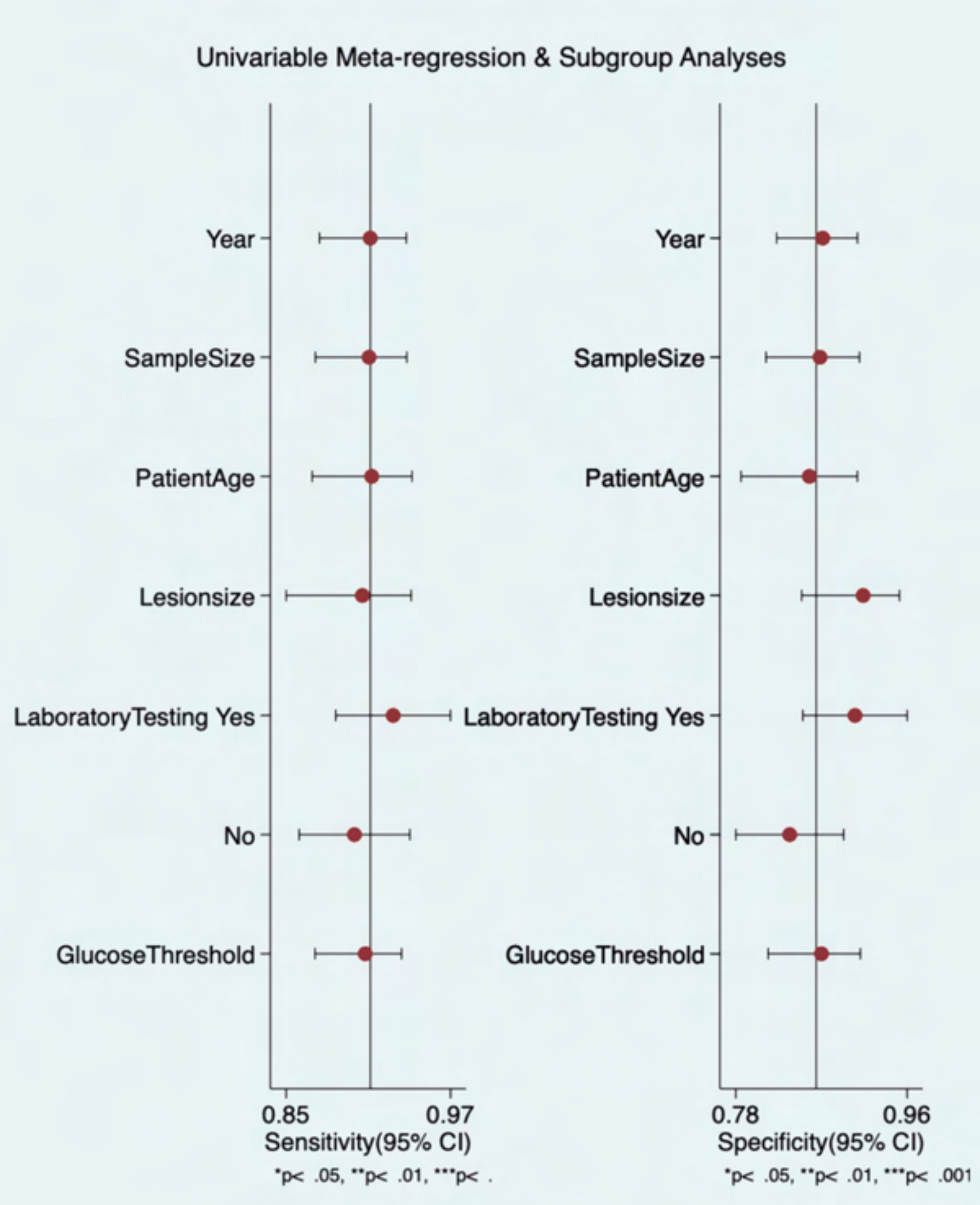

Deeks' Funnel Plot Asymmetry Test
pvalue = .50
Study
Regression Line
1/root(ESS)
Diagnostic Odds Ratio


100
10
1
0.1
1
Positive Likelihood Ratio
Negative Likelihood Ratio
LUQ: Exclusion & Confirmation
LRP>10, LRN<0.1
RUQ: Confirmation Only
LRP>10, LRN>0.1
LLQ: Exclusion Only
LRP<10, LRN<0.1
RLQ: No Exclusion or Confirmation
LRP<10, LRN>0.1
Summary LRP & LRN for Index Test
With 95 % Confidence Intervals


100
10
1
0.1
1
Positive Likelihood Ratio
Negative Likelihood Ratio
LUQ: Exclusion & Confirmation
LRP>10, LRN<0.1
RUQ: Confirmation Only
LRP>10, LRN>0.1
LLQ: Exclusion Only
LRP<10, LRN<0.1
RLQ: No Exclusion or Confirmation
LRP<10, LRN>0.1
Summary LRP & LRN for Index Test
With 95 % Confidence Intervals


100
10
1
0.1
1
Positive Likelihood Ratio
Negative Likelihood Ratio
LUQ: Exclusion & Confirmation
LRP>10, LRN<0.1
RUQ: Confirmation Only
LRP>10, LRN>0.1
LLQ: Exclusion Only
LRP<10, LRN<0.1
RLQ: No Exclusion or Confirmation
LRP<10, LRN>0.1
Summary LRP & LRN for Index Test
With 95 % Confidence Intervals


Bivariate Boxplot
LOGIT_SENS
LOGIT_SPEC
3.5
3
2.5
2
1.5
0
1
2
3
4
1
2
3
4
5
6
7
8


Univariable Meta-regression & Subgroup Analyses
Year
SampleSize
PatientAge
Lesionsize
LaboratoryTesting Yes
No
GlucoseThreshold
0.85
0.97
Sensitivity(95% CI)
*p< .05, **p< .01, ***p< .
Year
SampleSize
PatientAge
Lesionsize
LaboratoryTesting Yes
No
GlucoseThreshold
0.78
0.96
Specificity(95% CI)
*p< .05, **p< .01, ***p< .001