SEMMELWEIS EGYETEM

DOKTORI ISKOLA

# Ph.D. értekezések

## 3344.

# MARIE ANNE ENGH

**Transzlációs és klinikai farmakológia**

című program

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# Equipment and techniques for optimizing diagnostic efficacy after endoscopic ultrasound-guided tissue acquisition from solid pancreatic and abdominal masses


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Budapest

2025

***"And the point is, to live everything. Live the questions now. Perhaps you will then gradually, without noticing it, live along some distant day into the answer."***

*Rainer Maria Rilke*

Contents

## 1. LIST OF ABBREVATIONS

| | |
|---|---|
| **CEH-EUS** | Contrast-enhanced endoscopic ultrasound |
| **CI** | Confidence Interval |
| **CS** | Conventional smear |
| **EUS** | Endoscopic ultrasound |
| **FN** | False Negatives |
| **FNA** | Fine needle aspiration |
| **FNB** | Fine needle biopsy |
| **FP** | False positives |
| **LBC** | Liquid based cytology |
| **MOSE** | Macroscopic on-site evaluation |
| **OR** | Odds Ratio |
| **QUADAS-2** | Quality Assessment of Diagnostic Accuracy Studies, 2nd version |
| **RoB** | Risk of Bias |
| **ROBINS-I** | Risk of bias in non-randomized studies of interventions |
| **ROSE** | Rapid on-site evaluation |
| **RR** | Risk Ratio |
| **TA** | Tissue acquisition |
| **TN** | True Negatives |
| **TP** | True Positives |

## 2. STUDENT PROFILE

### 2.1. Vision and mission statement, specific goals


My vision is a world in which pancreatic cancer is no longer a near immediate death sentence, which patients and physicians dread alike. To achieve this, my mission is to optimize procedures for diagnosis of pancreatic cancer. As a small first step, this includes creating a clear manual for endoscopic procedures. As my specific goals towards this, I aim to investigate the effect of different types of endoscopic ultrasound devices and different cytology methods in the connection with the procedure.

### 2.2. Scientometrics

| | |
|---|---|
| **Number of all publications:** | 37 |
| Cumulative IF: | 155.909 |
| Av IF/publication: | 4.214 |
| Ranking (SCImago): | D1: 9, Q1: 36, Q2: 1, |
| **Number of publications related to the subject of the thesis:** | 2 |
| Cumulative IF: | 5.6 |
| Av IF/publication: | 2.8 |
| Ranking (SCImago): | D1: 0, Q1: 2 |
| **Number of citations on Google Scholar:** | 218 |
| **Number of citations on MTMT (independent):** | 123 |
| **H-index:** | 6 |

The detailed bibliography of the student can be found on pages 71-83.

### 2.3. Future plans

I initially plan to continue the investigation by completing my assessment of needle sizes and design during the procedure. However, as a next step, I consider it of utmost importance that I step into the medical realm and find and dedicate myself to my medical training and involvement. Once I have found my stride in medicine and confidently call myself an expert of my future field, I will return to science to apply the skills and ways

of thinking I have learned over the course of the last 4 years – and I will dedicate my life to improving the lives of my patients with evidence-based medicine.

## 3. SUMMARY OF THE THESIS

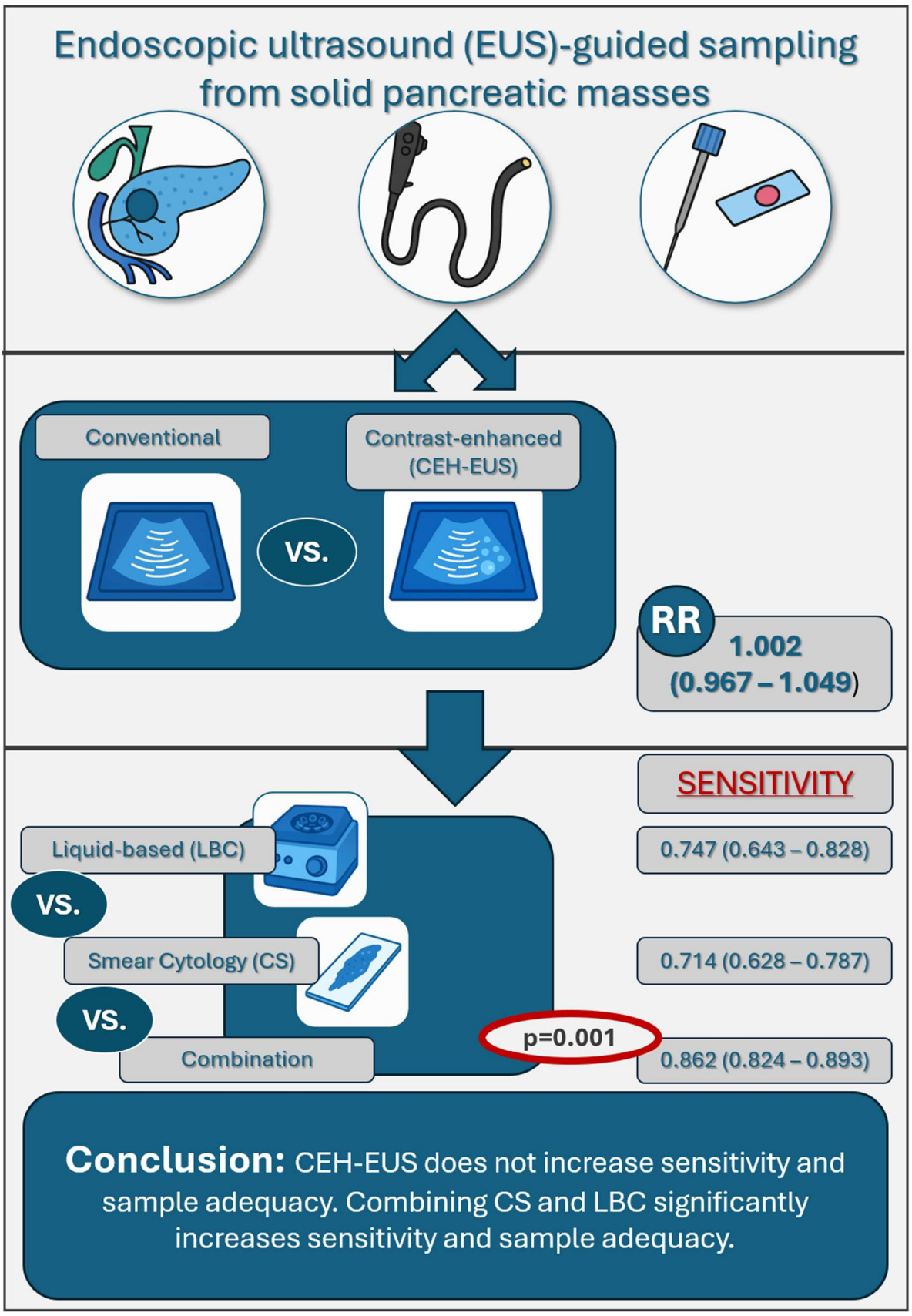
Pancreatic cancer is a cancer that is difficult to diagnose and sample, endoscopic ultrasound-guided tissue acquisition is a prime method for the diagnosis and evaluation of detected masses. Improving the sampling adequacy of the procedure is a key step in reducing delays in treatment once suspicion of cancer has been raised.

In this thesis we investigate the use of contrast-enhanced endoscopic ultrasound versus conventional endoscopic ultrasound during the tissue acquisition procedure, and we investigate the use of conventional smear and liquid-based cytology for the cytology assessment.

According to the results, contrast-enhanced endoscopic ultrasound does not improve adequacy or sensitivity after the procedure. Combining conventional smear and liquid-based cytology on the other hand did significantly improve both adequacy and sensitivity over using either method alone.

We conclude that contrast-enhanced endoscopic ultrasound does not currently have a place in the sampling of pancreatic masses, while a combination of conventional smear and liquid-based cytology is greatly beneficial.

## 4. GRAPHICAL ABSTRACT



# 5. INTRODUCTION

## 5.1. Pancreatic Cancer

Pancreatic cancer is one of the most common and most deadly gastrointestinal cancers in current times, as the third leading cause of cancer-related mortality in the US(1). Due to the pancreas' location and a lack of compressible structures in the vicinity, thereby causing few specific symptoms, pancreatic cancer is usually detected late – once pancreatic function is impaired, biliary drainage impeded, or duodenal compression occurs. At this stage disease is so advanced that only 15-20% have resectable disease(2). Once symptomatic, the course of disease is brief and severe. Median survival is 4 months and the 5-year survival only 13%(3).

Pancreatic cancer is frequently associated with pancreatitis, especially chronic pancreatitis, and shares certain risk factors with other pancreatic diseases, such as alcohol abuse, smoking and dietary factors.

## 5.2.Endoscopic ultrasound guided tissue acquisition

Upon detection of a pancreatic mass, which often indicates pancreatic ductal adenocarcinoma (PDAC) or other pancreatic cancers, if surgery is not directly indicated and the mass is not clearly diagnosable, the suggested diagnostic method is endoscopic ultrasound (EUS)-guided tissue acquisition (TA)(4, 5). Using an EUS to endoscopically target the mass, a needle is used to either aspirate cells, as in the case of fine needle aspiration (FNA), or take a biopsy sample, as in the case of fine needle biopsy (FNB).

The area is frequently vascular and fibrotic if chronic pancreatitis is present, and parts of the mass may be necrotic. This raises the importance of proper targeting during the procedure, to avoid bleeding complications and damaging the sample with blood, and to sample the correct area of the mass, avoiding fibrotic and necrotic areas which may not be suitable for pathological investigations.

## 5.3.EUS-technicalities

EUS may be conducted with several modalities, in addition to conventional ultrasound. Elastography allows for detection of elasticity of tissues and may help in detecting fibrosis. Contrast-enhanced EUS, which involves the injection of gas bubbles allowing

clear visualization of vessels and vascular tissues allows for the hyperenhancement of vascular tissues and hypoenhancement of necrotic and fibrotic tissues(6).

### 5.4.Cytology and Histology

Samples taken by FNA are usually processed for cytological investigations. The two primary cytology methods are conventional smear (CS), by which the sample is smeared between two slides, air-dried or ethanol-fixed and stained for analysis (frequently using Hematoxylin-Eosin, Giemsa or Papanicolaou staining), and liquid-based cytology (LBC), in which a monolayer is produced by one of two methods – either filtration-based (where cells are collected onto a filter and stamped) or sedimentation-based (in which the sample is centrifuged). These two methods (CS and LBC) may also be combined, in which case either a single sample may be split (first smeared for a CS sample, then processed for LBC), or individual samples may be used(4, 7).

Samples taken by FNB aim to trap a tissue core in the needle, utilizing a cutting needle such as the Franseen, Fork-Tip or Reverse Beveled Westcott needles. This retains tissue structure and allows for true histological investigation, possibly complimented by cytological investigations(4).

### 5.5.Sample adequacy and improvement

EUS-guided tissue acquisition is a semi-invasive procedure, necessitating resources, sedation and carrying risks of complications(8). Roughly 10-15% of samples are not adequate for histology and 5-10% percent not adequate for cytology(9). Rapid on-site evaluation (ROSE) and macroscopic on-site evaluation (MOSE) may be applied during the procedure to ensure adequacy of sample before termination. However, when unavailable, the inadequacy of samples may not become certain until reaching the pathologist’s desk, at which point a new procedure may be necessary.

Several investigations have been conducted to investigate factors impacting the success of the sampling procedure, including the sampling approach (needle fanning versus targeting), type and size of needle, number of passes, and presence of ROSE(10-13). Studies have also been conducted to investigate the effect of contrast-enhanced EUS on sample adequacy, and the use of different cytology methods.

### 5.6.Necessary guideline improvements

Current ESGE guidelines(14) aim to give specific instructions for optimizing the EUS procedure to minimize the rate of inadequate samples, and to maximize the sensitivity for malignancy, which is the prime objective to detect. The most recent guidelines highlight the lack of evidence for or against use of CEH-EUS, and also suggest that sample processing for cytology may be best if two types of cytology are combined, but again highlight a lack of evidence(5). Clarifying these two questions may contribute to moving the field of endoscopy closer to a clear procedural protocol that can maximize the outcomes of the procedure.

# 6. OBJECTIVES

## 6.1. Study I. – Comparing CEH-EUS to conventional EUS for tissue acquisition from solid pancreatic masses

This study aimed to compare CEH-EUS to conventional EUS, and to assess the potential use of CEH-EUS to increase adequacy rate during sampling from pancreatic masses.

## 6.2. Study II. – Comparing CS to LBC, and their combination, for EUS-guided tissue acquisition from abdominal masses

This study aimed to compare LBC to CS during EUS-guided tissue acquisition from solid pancreatic masses, in terms of diagnostic test performance such as sensitivity, specificity and accuracy.

# 7. METHODS

## 7.1. Study I.

### 7.1.1. Methodology and protocol

This meta-analysis is reported following the PRISMA 2020 guideline(15) and was conducted according to guidance of the Cochrane collaboration(16). The protocol was prospectively registered on PROSPERO (ID: CRD42022285023). The only protocol deviations were the inclusion of risk ratios for true positive results as an outcome measure, and updated inclusion criteria to include non-randomized studies during peer review.

### 7.1.2. Inclusion criteria

Studies were eligible for inclusion if they reported on EUS-guided TA for a solid pancreatic mass, comparing CEH-EUS to conventional EUS for targeting on the mass, and reported diagnostic adequacy, adverse events, technical failures, needle passes and tissue yield. Randomized trials and non-randomized studies were eligible for inclusion. For any studies that matched the inclusion criteria (e.g. mentioning having assessed an outcome) but did not report the outcome data, first and corresponding authors were contacted.

### 7.1.3. Outcomes

The primary outcome was diagnostic adequacy, as the contrast-enhancement is rather an improvement to the sampling method rather than the diagnostic test itself. Any definition for diagnostic adequacy applied in papers was eligible, however the definitions were extracted and compared to ensure their comparability.

We also included diagnostic parameters as outcomes, particularly due to their inclusion in previous meta-analyses(17) and the key articles. These were sensitivity, specificity, and accuracy for malignancy, all treated as plain dichotomous outcomes and calculated as proportions and ratios. To calculate these, true negatives (TN), false positives (FP), true positives (TP) and false negatives (FN) were extracted and used for calculation.

Additional outcomes eligible for inclusion in our meta-analysis were adverse events, number of needle passes until adequacy, and technical failures.

As studies were inconsistent in the number of needle passes performed, we decided on a simple algorithm for deciding which analyses to run. Each needle pass for which the

minimum number of studies (3) was satisfied was analyzed. If there was no specific limit to the number of needle passes, the study was designated based on the mean number of needle passes used. Additionally, we analyzed all studies together, using only their final needle pass.

#### 7.1.4. Search and selection

The search was run on April 24th 2022, and updated on November 19th, 2023. The search was conducted in 5 databases (Medline – via PubMed, Embase, Cochrane Controlled Register of Studies – CENTRAL, Scopus and World of Science) using a search key composed of three domains (pancreatic masses, TA and CEH-EUS): *(("contrast" AND "ultrasound") OR ("contrast" AND "EUS") OR "CEH-EUS" OR "sonovue" OR "definity") AND ("FNA" OR "FNB" OR "fine-needle" OR "fine needle" OR "tissue acquisition")*

Duplicates were removed manually in EndNote X9 by MAE, selection was performed in Rayyan by two independent investigators in two stages (first by Title-Abstract, then by Full-text). Any disagreements were resolved by discussion between the two authors. Cohen's kappa(18) was calculated to determine the degree of interrater agreement.

A reference search was performed on May 9th, 2023 using the CitationChaser(19) tool.

#### 7.1.5. Data extraction

Data extraction was done in duplicate by two independent authors. It was performed in a pre-designed excel sheet, and data were extracted on the following items:

Author, year, location, number of centers, age, sex, location, procedural information (sampling, endoscopist/pathologist experience), outcomes: Diagnostic adequacy, adverse events, technical failures, needle passes, tissue yield, rates of accurate diagnoses and diagnostic sensitivity. Data sought for outcomes: Definitions used. For continuous outcomes: Mean/Median/SD/IQR/range, for dichotomous ones: event rates, pre-calculated relative effects with measure of spread. Diagnostic data were also extracted (precalculated rates, TP, FP, TN, FN).

#### 7.1.6. Risk of bias and quality of evidence assessment

The risk of bias was assessed by two independent authors.

For randomized studies, the Cochrane Risk-of-Bias tool for randomized trials v.2 (RoB2)(20) was used, and disagreements were resolved by a third investigator.

For non-randomized studies, the Risk of Bias tool for Non-randomized Studies of Interventions (ROBINS-I)(21) was used, and disagreements were resolved by discussion. Results were visualized using robvis(22).

The quality of evidence was assessed by a single author using the GRADE recommendations(23), applying the GradePro(24) tool.

#### 7.1.7. Synthesis methods

We pooled risk ratios (RR) with 95% confidence interval (CI) for randomized trials, and odds ratios (OR) with 95% CI for non-randomized studies. For continuous outcomes we pooled mean differences with 95% CI. A random-effects model was applied due to an expectation of significant between-study heterogeneity. For the diagnostic outcomes, for direct comparison we pooled RRs/ORs, and proportions separately. The Hartung-Knapp adjustment was used for CIs, and the Paule-Mandel(25) method to calculate $tau^2$, with Q profile for the CI. Analyses were done in R (R Core Team 2022, v4.2.1) using the meta (v5.5.0) package28 and dmetar29 for meta-analysis calculations.

Publication bias/outlier/influential could not be performed due to low number of studies.

### 7.2.Study II.

#### 7.2.1. Methodology and protocol

This systematic review with meta-analysis was conducted in accordance with the guidelines by the Cochrane collaboration(16) and is reported following the PRISMA 2020 guidelines(15). The protocol was registered on PROSPERO (CRD42024612112) and was fully adhered to, except for an additional analysis investigating inadequacy rate of the methods.

#### 7.2.2. Eligibility criteria

Studies were eligible for inclusion if the included patients undergoing EUS-guided TA for abdominal lesions (including pancreatic, gastrointestinal and others). Studies had to report diagnostic parameters of both LBC and CS to minimize confounding in the comparison. Studies reporting on the combined diagnostic value of LBC and CS were

eligible for inclusion regardless of whether the two cytology methods were also reported to maximize available data. Where the combination was not directly reported, attempts were made to deduce the information from the papers.

Both articles published in indexed journals and conference abstracts were eligible for inclusion, as long as they contained the information necessary for analysis.

#### 7.2.3. Information sources and search strategy

Three databases were searched on November 7th, 2024 (Medline – via PubMed, Embase and CENTRAL). The search strategy was composed of three domains, one for the abdominal region, one for TA and one for the tissue preparation types:

*(pancrea* OR gastrointest* OR abdom*) AND (FNA OR FNB OR (tissue AND acquisition) OR (fine AND needle)) AND ("tissue preparation" OR (smear AND "liquid cytology"[tiab:~4]))*

References and citations of included papers were searched using the citationchaser(19) tool on November 17th, 2024.

#### 7.2.4. Study selection and data extraction

Duplicates were removed manually using EndNote. The selection was performed in parallel by two investigators blinded to the other's decisions. Records were screened by Title and Abstract, then by full text, and any disagreements were resolved by discussion. A Cohen's kappa(18) was calculated to quantify the degree of interrater agreement.

Data extraction was done in duplicate by two independent investigators. The data were extracted into a prospectively designed Excel sheet, and data were compared by the primary investigator. Any discrepancies were resolved by consensus. Data were sought on study design, years of patient enrollment, study population (type of lesions, age and gender), sampling procedure (needle type), reference standard, number of benign and malignant cases and the outcomes. For the outcomes, true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) were extracted. If available, precalculated accuracy, sensitivity, specificity, and positive and negative predictive values were extracted with confidence intervals.

### 7.2.5. Quality assessment

The risk of bias was assessed using the QUADAS-2 tool(26), in parallel by two independent reviewers. Disagreements were discussed to resolve conflicts and results were visualized using the robvis(22) online tool.

### 7.2.6. Data synthesis and analysis

Analysis was performed using R software (version 4.12). A random-effect meta-analysis was applied for each outcome, a p-value of less than 0.5 was considered significant.

We only meta-analyzed sensitivity and specificity, as these are independent from the proportion of the malignant cases. We also calculated accuracy from the pooled sensitivity and specificity, using different malignancy prevalence assumptions (w). Due to the high specificity, we analyzed sensitivity and specificity separately, rather than in the usual bivariate model(27, 28).

A three dimensional model was applied using the rma.mv() function of the metafor R package to adjust for crossover studies evaluating the tests in the same studies. We used the Pustejovsky(29) approach using the coef_test() fucntion of the clubSandwich R package, as we did not know the correlations, and applied several within-study correlation assumptions to test this approch for sensitivity. Pooled specificity could not be approached in this way and was instead calculated using the methodology of Stijnen et al.(30), adding 0.1 to zero cell counts as a continuity correction.

Results were visualized on forest plots and $I^2$ values with CI were calculated to assess the heterogeneity.

# 8. RESULTS

## 8.1.Study I: Contrast-enhanced EUS

### 8.1.1. Study selection and data extraction

7200 studies were identified by the search. After removal of duplicates, 3852 studies remained. The interrater agreement of title-abstract selection was substantial (Cohen's kappa 0.79), while that of full-text selection was perfect (1.0). Finally, 9 studies(31-39), enrolling 1160 patients, were included for synthesis. Two protocols of ongoing randomized trials were also identified. Details of the selection are visualized in the PRISMA flowchart in **Figure 1**, while details of the included studies can be seen in **Table 1**.

***Table 1:*** *Baseline characteristics of included studies.*

| Author (Year) | Endoscopist experience | Sampling technique | Ultrasound technique | Needle |
|---|---|---|---|---|
| **Cho (2021)** | "Experienced endosonographers" | 10 mL negative pressure 20 to-and-fro movements | GF-UCT 260; Olympus. CEH-EUS: 2.4 mL SonoVue, 10 mL saline flush | 19-25G FNA or FNB |
| **Facciorusso (2020)** | "Board certified gastroenterologist with 20 years' experience" | 10 mL negative pressure "more than 10 to-and-fro movements" | Pentax FG-36UA<br>CEH-EUS: 4.8 mL SonoVue followed by 20 mL saline flush | 22G FNA |
| **Hou (2015)** | "Experienced Endosonographer" | NA | GFUCT2000(Olympu)<br>CEH-EUS: GFUC-30p (Olympus)<br>4.8 mL SonoVue, 20mL saline flush | 22G needle |
| **Itonaga (2020)** | > 300 EUS-FNA procedures | Negative pressure with 20 mL syringe, 20 to-and-fro movements | GF-UCT260 (Olympus)<br>CEH-EUS: No information regarding contrast agent. | 22G FNA |

| Author (Year) | Endoscopist experience | Sampling technique | Ultrasound technique | Needle |
|---|---|---|---|---|
| **Kuo (2023)** | “Experienced Endosonographers” | No suction. Conventional: 4x4 to-and-fro movement, fanning technique. CEH-EUS: 16 to-and-fro movements | GF-UCT260, (Olympus)<br>CEH-EUS: 0.015 mL/kg body weight Sonazoid, 10 mL saline flush | 22G FNB |
| **Lai (2022)** | "Two endoscopists who achieved the FNA learning curve" | Fanning method from at least 4 areas, slow-pull or low-negative suction | GF-UCT260, (Olympus)<br>CEH-EUS: 0.015 mg/kg Sonazoid | 22G FNB |
| **Seicean (2015)** | No information | No suction, fanning technique used where possible. | GF-UCT180-AL5 (Olympus).<br>CEH-EUS:<br>2.4mL SonoVue followed by 5 mL saline flush- | 22G FNA |
| **Seicean (2020)** | >7000 EUS-FNA and >500 CEH-EUS | Slow-pull, 10 to-and-fro movements | GF-UCT 180 AL5 (Olympus)<br>CEH-EUS:<br>2.4 ml SonoVue, 5 mL saline flush | 22G FNA |

| Author (Year) | Endoscopist experience | Sampling technique | Ultrasound technique | Needle |
|---|---|---|---|---|
| **Sugimoto (2015)** | 1st pass <100 EUS-FNA, 2nd pass >300 EUS-FNA | Negative pressure with 10 ml syringe, 20 to-and-fro movements | GF-UCT 260, GF-UCT24-AL5 (Olympus)<br>CEH-EUS:<br>0.015 ml/kg Sonazoid | CEH-EUS: 22G FNB<br>Conventional: 22G or 25G |

*RCT: Randomized controlled trial, EUS-FNA: Endoscopic Ultrasound-guided Fine Needle Aspiration, CEH-EUS: contrast-enhanced harmonic endoscopic ultrasound, mm: millimeter*

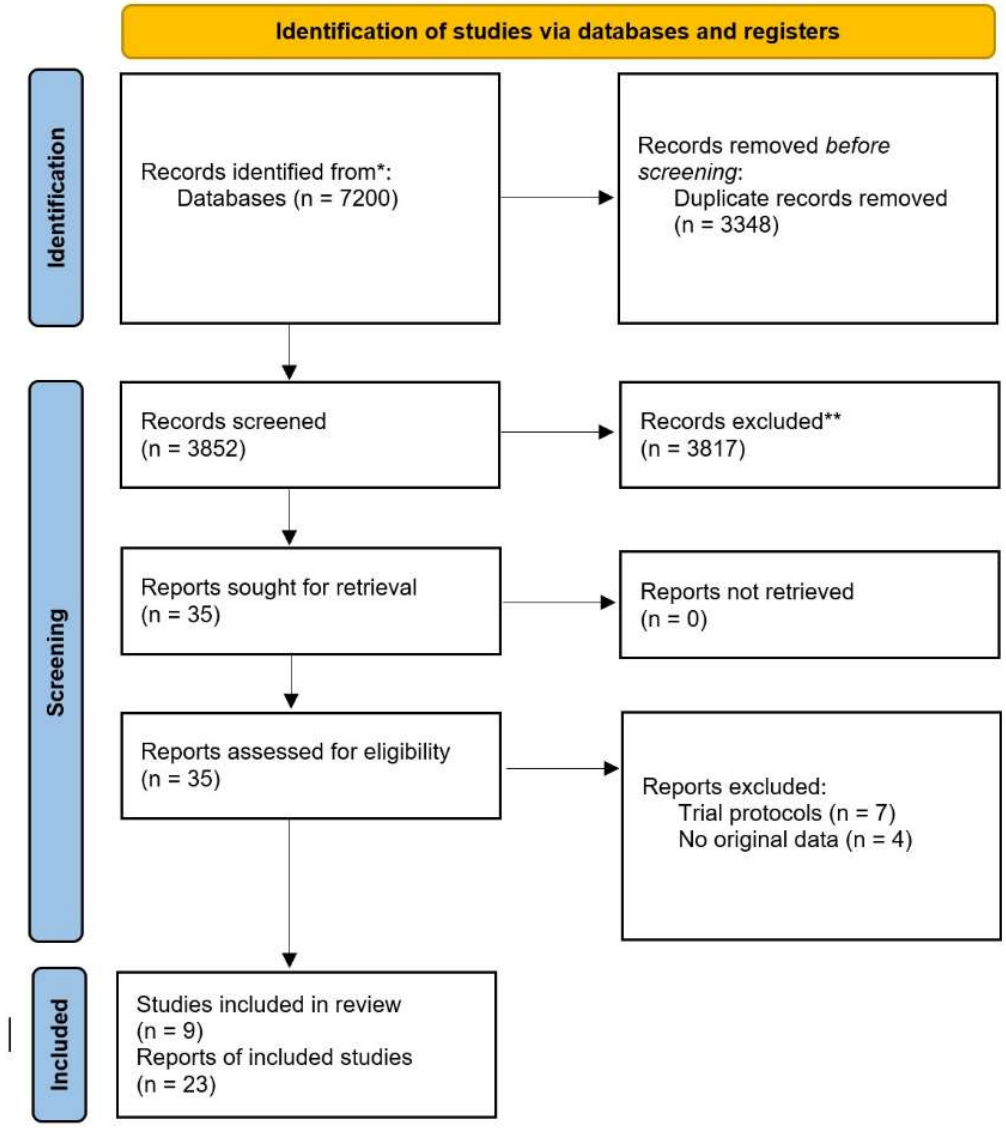



***Figure 1:*** *PRISMA Flowchart detailing the selection process*

### 8.1.2. Diagnostic adequacy

Diagnostic adequacy was reported by six studies, of which three(34, 36, 38) were RCTs and three(31, 34-38) were non-randomized. An additional RCT(33) did not report the outcome but responded to our request and provided the data.

Final pass

After the final pass, the pooled OR for sample adequacy was 1.467 (CI: 0.850-2.533), **Figure 2**. The RCT subgroup showed a OR of 0.902 (CI: 0.541 – 1.505) while the non-randomized subgroup showed an OR of 2.396 (CI: 0.916-6.264), with significant subgroup differences (p = 0.0045).

A subset analysis of RCTs only yielded an RR of 1.002 (CI: 0.81-1.39), seen in **Figure 3**.

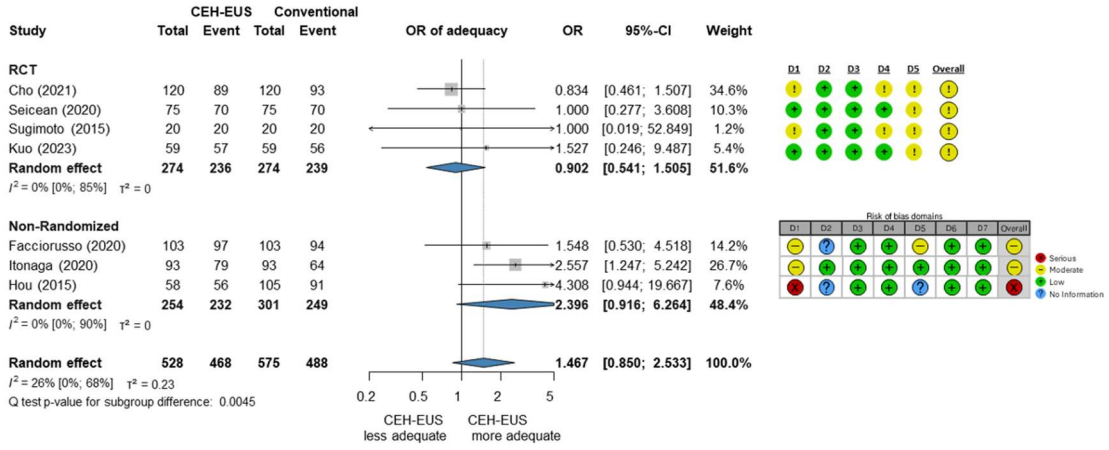



***Figure 2:*** *Forest plot of pooled odds ratios for an adequate sample following tissue acquisition (FNA/FNB) using contrast-enhanced versus conventional ultrasound. Data for final needle pass used in each study. Results of the risk of bias assessment are summarized on the right by study, domain and overall. For Cochrane risk-of-bias tool v.2 (RoB2): Domains: D1: Risk arising from the randomization process, D2: Bias due to deviations from intended intervention, D3: Bias due to missing outcome data, D4: Bias in the measurement of the outcome, D5: Bias in the selection of the reported result. For Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) tool: D1: Bias due to confounding, D2: Bias due to selection of participants, D3: Bias in classification of interventions, D4: Bias due to deviations from intended interventions, D5: Bias due to missing data, D6: Bias in measurement of outcomes, D7: Bias in selection of the reported results. CEH-EUS: Contrast-enhanced harmonic endoscopic ultrasound, OR: Odds Ratio*

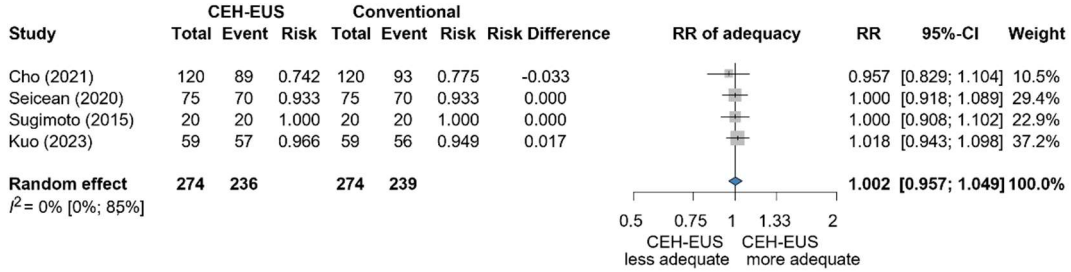



***Figure 3:*** *Forest plot of pooled risk rations for an adequate sample following tissue acquisition (FNA/FNB) using contrast-enhanced versus conventional ultrasound, including only randomized trials. Data for final needle pass used in each study. Results of the are summarized on the right by study, domain and overall. CEH-EUS: Contrast-enhanced harmonic endoscopic ultrasound, OR: Odds Ratio*

1st pass

Additionally, an analysis of diagnostic adequacy after the first pass was feasible, having been reported by four studies (three RCTs(33-35, 38) and one non-randomized trial(37)). The pooled OR was 2.263 (CI: 0.960 – 5.334), seen in **Figure 4**. Pooling only RCTs, the RR was 0.988 (CI: 0.959 – 1.017), seen in **Figure 5.**

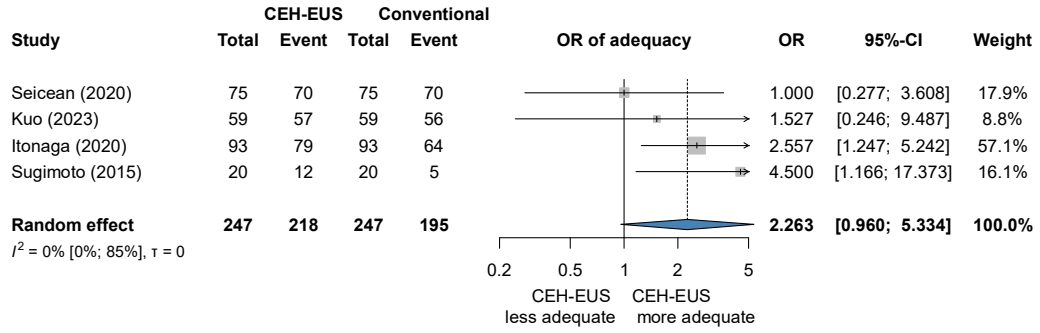


***Figure 4:*** *Forest plot of pooled odds ratios for diagnostic adequacy after the first pass, including all study designs. CEH-EUS: Contrast-enhanced harmonic endoscopic ultrasound, OR: Odds Ratio*

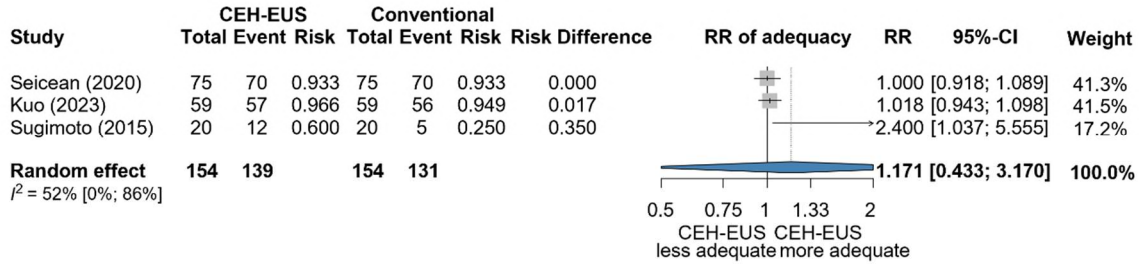



***Figure 5:*** *Forest plot of Risk Ratios for diagnostic adequacy after the first pass, including only randomized trials. CEH-EUS: Contrast-enhanced harmonic endoscopic ultrasound, OR: Odds Ratio*

### 8.1.3. Diagnostic accuracy

Final pass

Accuracy was reported in seven studies, four RCTs(33, 34, 36, 38) and three non-randomized studies(31, 32, 35). The pooled OR for diagnostic accuracy (**Figure 6**) was 1.326 (CI: 0.890 – 1.977), with significant subgroup differences (p = 0.0467) between the RCT subgroup at 0.997 (CI: 0.593 – 1.977) and a non-randomized subgroup OR of 1.928 (CI: 1.096 – 3.393). A subset analysis of RCTs only (**Figure 7**) yielded an RR of 0.988 (CI: 0.959 – 1.017).

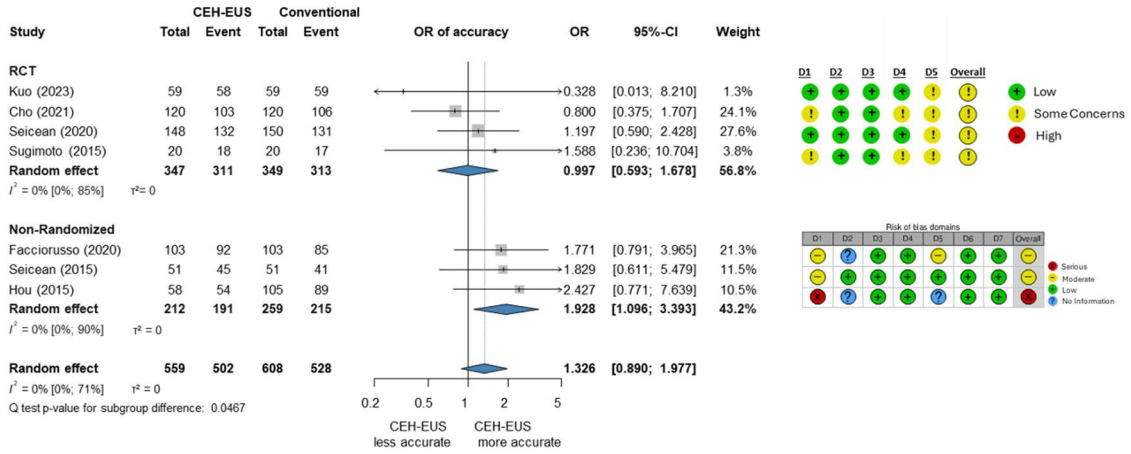



***Figure 6:*** *Forest plot of pooled odds ratios for accurately diagnosing both negative and positive cases (diagnostic accuracy). Results of the Risk of Bias assessment are summarized on the right by study, domain and overall. For Cochrane risk-of-bias tool v.2 (RoB2): Domains: D1: Risk arising from the randomization process, D2: Bias due to deviations from intended intervention, D3: Bias due to missing outcome data, D4: Bias in the measurement of the outcome, D5: Bias in the selection of the reported result. For Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) tool: D1: Bias due to confounding, D2: Bias due to selection of participants, D3: Bias in classification of interventions, D4: Bias due to deviations from intended interventions, D5: Bias due to missing data, D6: Bias in measurement of outcomes, D7: Bias in selection of the reported results. CEH-EUS: Contrast-enhanced harmonic endoscopic ultrasound, OR: Odds Ratio*

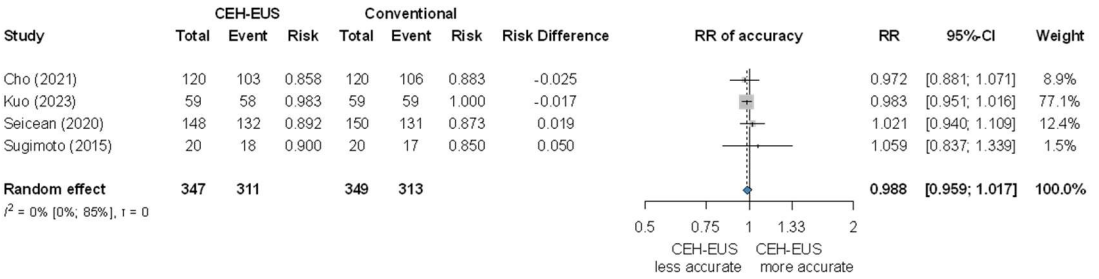



***Figure 7:*** *Forest plot of Risk Ratios (RR) for accurately diagnosing both malignancy and benign cases (Diagnostic accuracy), including only randomized controlled trials, between Contrast-Enhanced Endoscopic Ultrasound (CEH-EUS) and conventional.*

1st pass

The accuracy after the first pass could be pooled, as it was reported by three studies(33, 36, 38). The pooled OR was 1.182 (CI: 0.806 – 1.733), **Figure 8**.

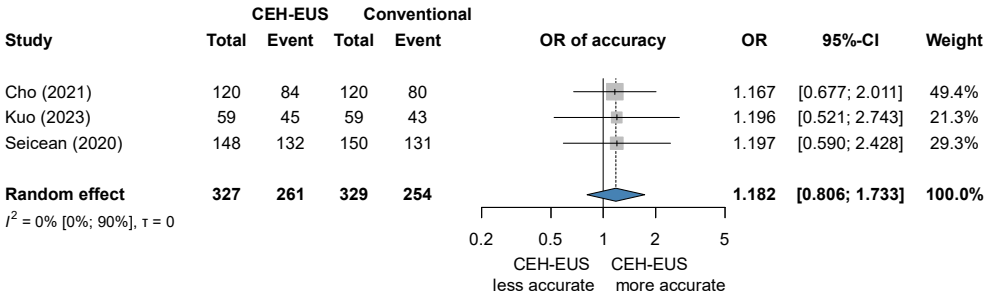



***Figure 8:*** *Forest plot of Odds Ratios (OR) for accurately diagnosing both malignancy and benign cases (diagnostic accuracy) with the first pass, including all study designs, between Contrast-Enhanced Endoscopic Ultrasound (CEH-EUS) and conventional.*

2nd pass

Accuracy after the second pass was reported by three studies(33, 36, 38). The pooled OR was 1.123 (CI: 0.340 – 3.706), **Figure 9**.

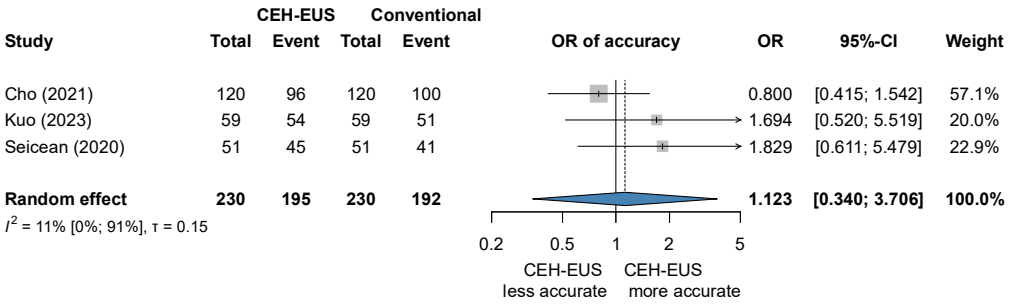


***Figure 9:*** *Forest plot of Odds Ratios for accurately diagnosing both malignancy and benign cases (diagnostic accuracy) with the second pass, including all study designs, between Contrast-Enhanced Endoscopic Ultrasound (CEH-EUS) and conventional.*

### 8.1.4. Sensitivity and Specificity

Sensitivity

Nine studies were included for sensitivity, yielding a pooled OR of 1.494 (CI: 1.052 – 2.121). In the RCT subgroup, including four studies(33, 34, 36, 38), the OR was 0.968 (CI: 0.535 – 1.753) while the non-randomized subgroup of five studies(21, 31, 33, 35, 37, 39) showed an OR of 1.950 (CI: 1.294 – 2.940), a signficant subgroup difference (p = 0.0125), seen in **Figure 10**.

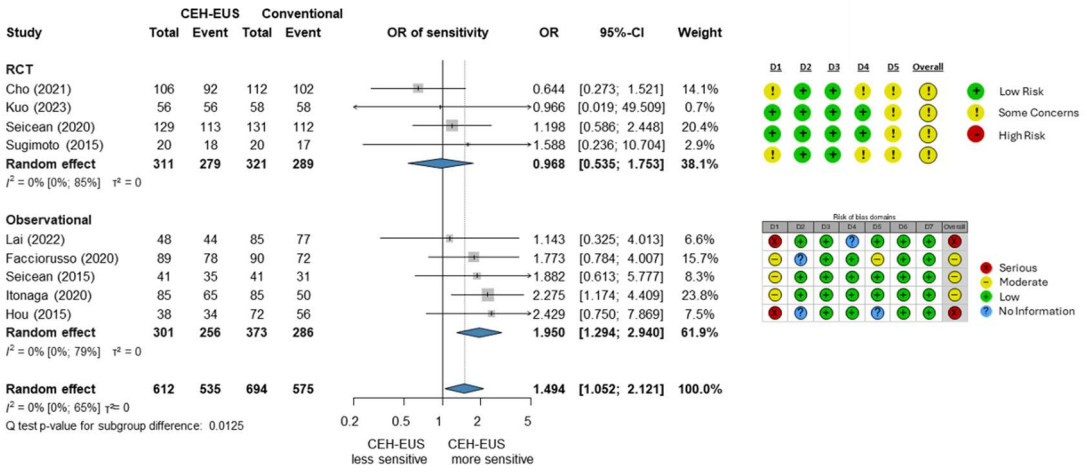



***Figure 10:*** *Forest plot of pooled odds ratios for accurately identifying positive cases (diagnostic sensitivity). Results of the Risk of Bias assessment are summarized on the right by study, domain and overall. For Cochrane risk-of-bias tool v.2 (RoB2): Domains: D1: Risk arising from the randomization process, D2: Bias due to deviations from intended intervention, D3: Bias due to missing outcome data, D4: Bias in the measurement of the outcome, D5: Bias in the selection of the reported result. For Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) tool: D1: Bias due to confounding, D2: Bias due to selection of participants, D3: Bias in classification of interventions, D4: Bias due to deviations from intended interventions, D5: Bias due to*

*missing data, D6: Bias in measurement of outcomes, D7: Bias in selection of the reported results. CEH-EUS: Contrast-enhanced harmonic endoscopic ultrasound, OR: Odds Ratio*

The same nine studies also reported data necessary for proportions of detected malignant cases (sensitivity ratio, **Figure 11**). The pooled proportion in the CEH-EUS group was 0.887 (CI: 0.826 – 0.928), in RCTs the proportion was 0.923 (CI: 0.694 – 0.985) while in non-randomized studies it was 0.858 (CI: 0.766 – 0.918) with no significant differences between the groups (p = 0.2281). In the conventional EUS analysis the proportion was 0.854 (CI: 0.740 – 0.924), with a proportion of 0.923 for RCTs (CI: 0.696 – 0.985) and a proportion of 0.780 (CI: 0.630 – 0.885) in non-randomized studies, with a significant difference between subgroups (p = 0.0384).

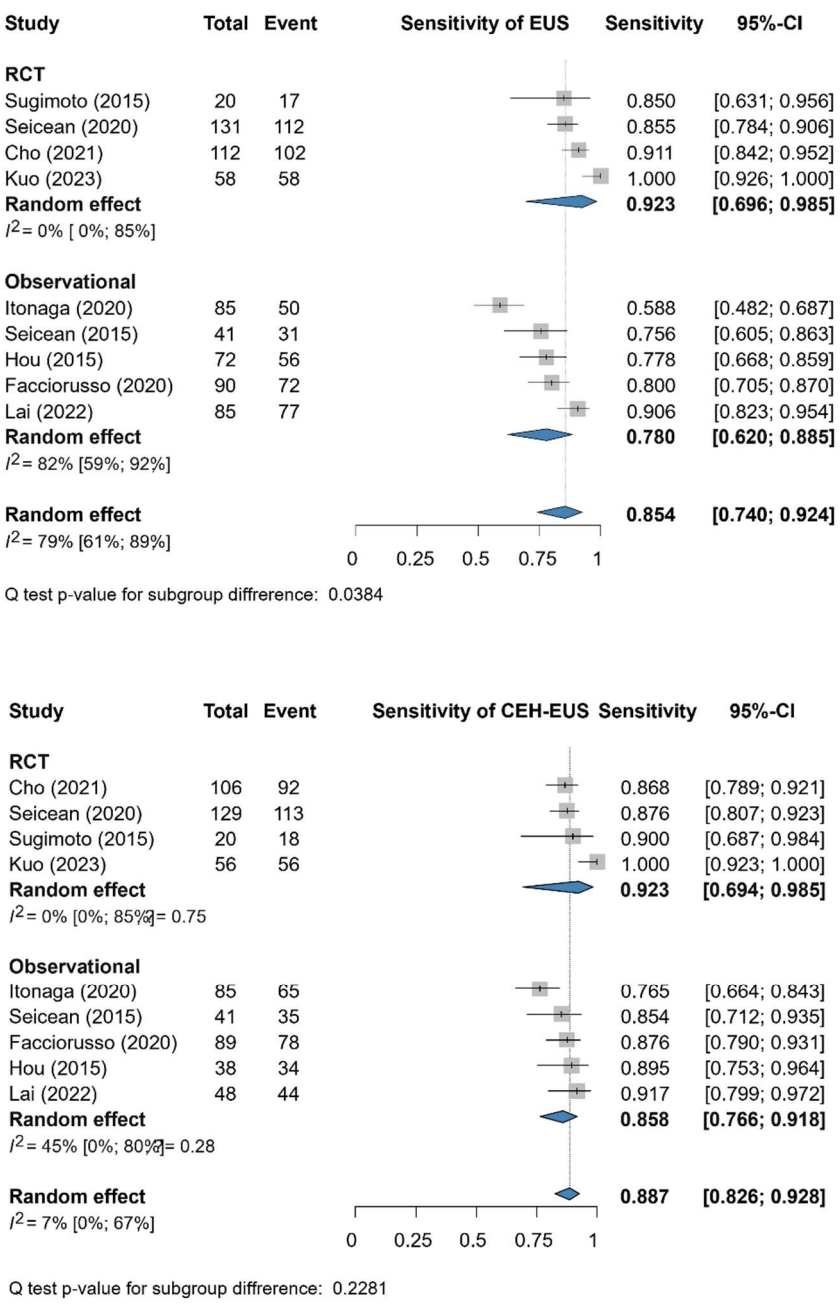



***Figure 11:*** *Forest plot of proportion of detected malignancy cases (Sensitivity ratio). Conventional endoscopic ultrasound on top, contrast-enhanced endoscopic ultrasound on the bottom.*

Specificity

Six studies(31-33, 35, 37, 38) reported data necessary to calculate specificity, which was 100% in all but one study. Due to the high rate of specificity, neither pooling of proportions nor of ratios was feasible.

Bivariate Diagnostic Meta-Analysis

Six studies(31-33, 35, 37, 38) reported data necessary to calculate specificity in a bivariate analysis (**Figure 12**).

For conventional EUS, sensitivity was 0.835 (CI: 0.673 – 0.926) and specificity was 1.000 (CI: 0.000 – 1.000), no subgrouping was feasible.

For CEH-EUS, sensitivity was 0.892 (CI: 0.807 – 0.942), and specificity was 0.998 (CI: 0.476 – 1.000). No subgrouping was feasible.

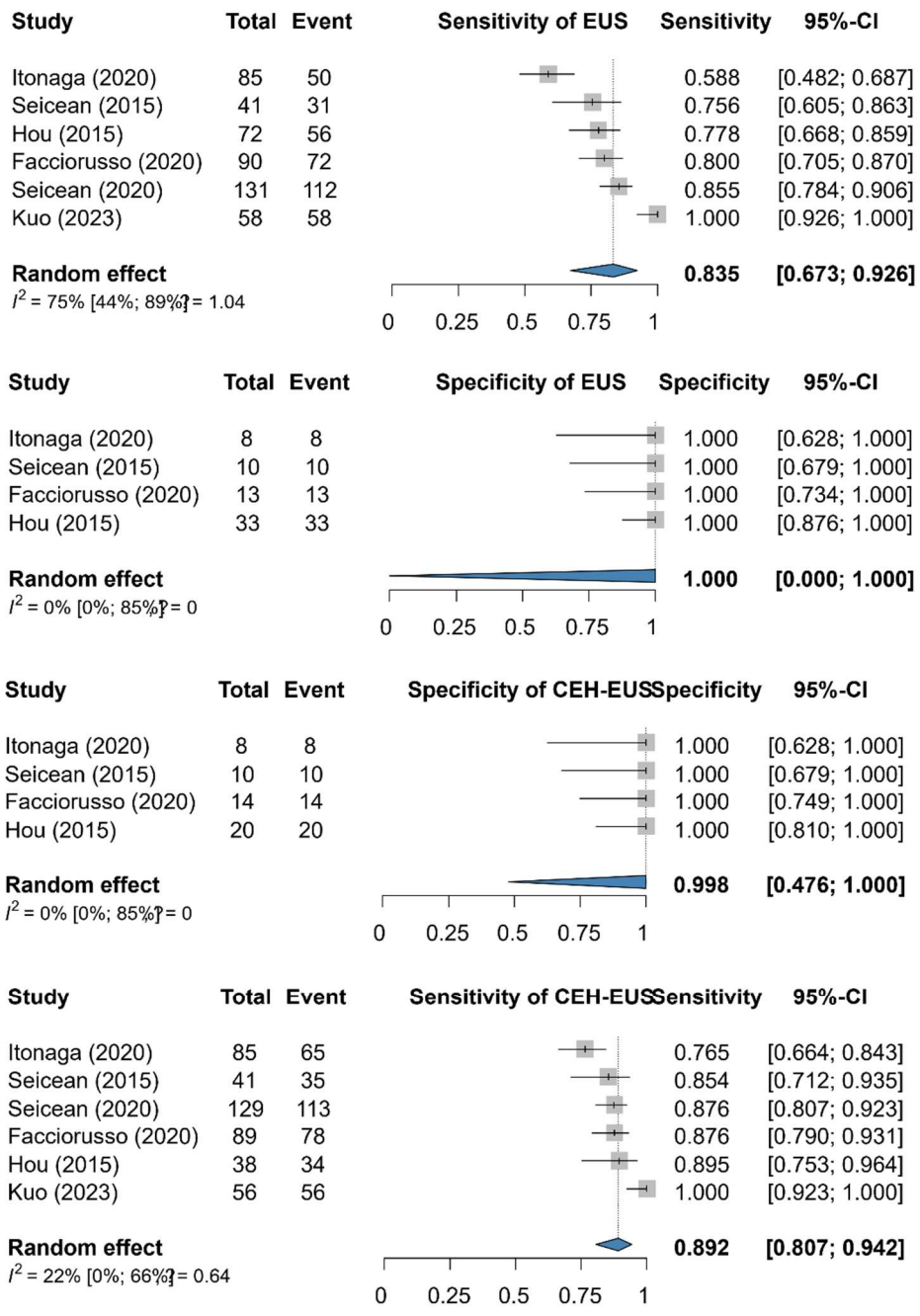



***Figure 12:*** *Forest plot of Bivariate Diagnostic Meta-analysis.*

### 8.1.5. Adverse events

All RCTs reported adverse events, however there were zero events in two of them(33, 34), while the other two(36, 38) observed exactly equal rates of events in both arms. The pooled RR was thus 1.00 (CI: 0.29 – 3.41), seen in **Figure 13**.

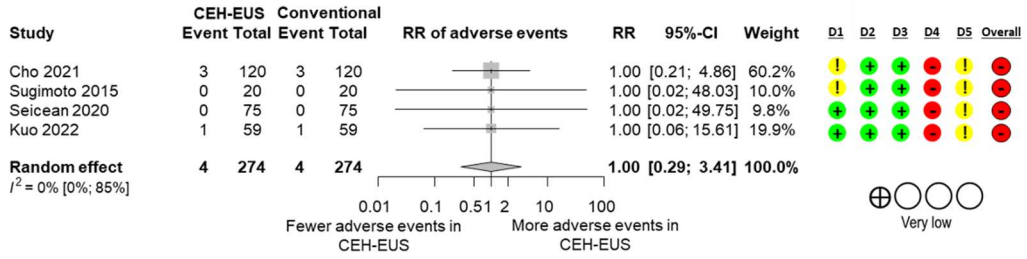



***Figure 13:*** *Forest plot of pooled odds ratios for adverse events. Results of the Risk of Bias assessment are summarized on the right by study, domain and overall. For Cochrane risk-of-bias tool v.2 (RoB2): Domains: D1: Risk arising from the randomization process, D2: Bias due to deviations from intended intervention, D3: Bias due to missing outcome data, D4: Bias in the measurement of the outcome, D5: Bias in the selection of the reported result. For Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) tool: D1: Bias due to confounding, D2: Bias due to selection of participants, D3: Bias in classification of interventions, D4: Bias due to deviations from intended interventions, D5: Bias due to missing data, D6: Bias in measurement of outcomes, D7: Bias in selection of the reported results*

*CEH-EUS: Contrast-enhanced harmonic endoscopic ultrasound, OR: Odds Ratio*

### 8.1.6. Technical failures

No article reported the rate of technical failures, however Seicean et al. provided information on request that no technical failures occurred in either arm.

### 8.1.7. Number of needle passes

The mean number of needle passes needed to achieve an adequate sample was reported in three studies(31, 35, 39), all non-randomized. The mean difference between groups was -0.54 (CI: -2.50 – 1.42), seen in **Figure 14**.

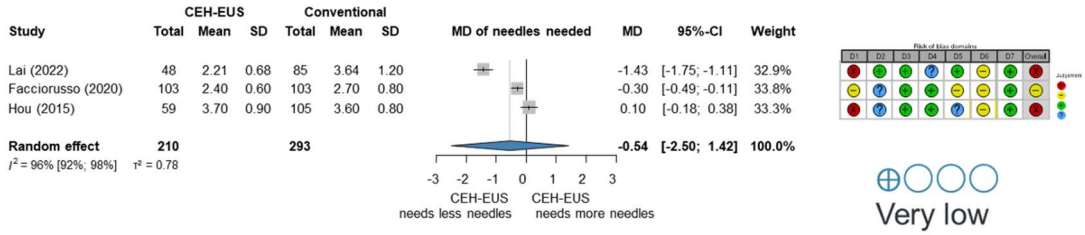



***Figure 14:*** *Forest plot of mean differences of number of needle passes until adequacy. Results of the Risk of Bias assessment are summarized on the right by study, domain and overall. For Cochrane risk-of-bias tool v.2 (RoB2): Domains: D1: Risk arising from the randomization process, D2: Bias due to deviations from intended intervention, D3: Bias due to missing outcome data, D4: Bias in the measurement of the outcome, D5: Bias in the selection of the reported result. For Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) tool: D1: Bias due to confounding, D2: Bias due to selection of participants, D3: Bias in classification of interventions, D4: Bias due to deviations from intended interventions, D5: Bias due to missing data, D6: Bias in measurement of outcomes, D7: Bias in selection of the reported results*
*CEH-EUS: Contrast-enhanced harmonic endoscopic ultrasound*

Kuo et al. reported the cumulative diagnostic accuracy of each needle pass in the CEH-EUS versus conventional groups. This was 76.3% (CI: 63.4-86.4) versus 72.9% (CI: 59.7 – 83.6) after the first pass (p = 0.833), 91.5% (CI: 81.3 – 97.2) versus 86.4% (CI: 75.0 – 94.0) for the second pass (p = 0.558) and 93.2% (CI: 88.3 – 99.6) versus 94.9% (CI: 85.9 – 98.9) for the third pass (p = 1). There was also no difference for the fourth (CEH-EUS: 96.6%, CI: 88.3-99.6 versus Conventional: 94.9%, CI: 85.9-98.9), fifth (CEH-EUS: 96.6%, CI: 88.3-99.6, Conventional: 96.6%, CI: 88.3-99.6) and sixth passes (CEH-EUS: 98.3%, CI: 90.9-100; Conventional: 100%), (p-value: 1).

In Sugimoto et al., the adequacy after each needle pass was reported, up to 5 passes. Adequacy was 60% in the CEH-EUS group, and 25% in the conventional group after the first pass, improving to 75% and 65% with the second pass, and 90% and 95% with the third pass. In the CEH-EUS group, the fourth pass reached 100% adequacy, while the conventional EUS group reached 95% and finally 100% on the fifth pass.

Cho et al. reported the diagnostic sensitivity after each needle pass, with 95% CIs. They found that a 70.0% (CI: 61.2-77.5) sensitivity in the CEH-EUS group and 66.7% (CI: 57.8-74.5) sensitivity in the conventional group after the first pass. This improved to 80.0% (CI: 71.9-86.2) and 83.3% (CI: 75.6-89.0) with the second pass, reaching 85.0% (CI: 77.4-90.3) and 88.3% (CI: 81.3-93.0) with the third pass. For the fourth and fifth needle pass there was little improvement, and both yielded 85.8% (CI: 78.4-91.0) and 88.3% (CI: 81.3-93.0) for CEH-EUS and conventional groups, respectively.

### 8.1.8. Tissue yield

Tissue yield was only reported by Kuo et al., who investigated the median macroscopic visible core size. This was 18 mm (IQR: 10-26) in the contrast-enhanced group, and 18 mm (IQR: 11-30) in the conventional group. There was no difference (p-value: 0.598).

### 8.1.9. Risk of bias assessment

The risk of bias assessment results can be seen on the forest plots, **Figures 2, 5, 6, 10, 13, 14**. Non-randomized studies were mostly rated low to moderate across domains, with lack of information for domain 2 in two studies(31, 35), domain 4 in one study(39) and domain 5 in one study(31). Due to crossover and matching, the intrinsic bias of non-randomized studies was mostly avoided, with two notable examples – one study gave the intervention to patients willing to pay for it(39), and one study was a retrospective review with no clear information of patient selection(31). These studies were both rated as serious risk of bias, while all other non-randomized studies were rated as moderate.

For the randomized studies, two studies received a rating of some concerns for the randomization process due to unclear allocation concealment. All studies lacked information in pre-registered study plans regarding the selection of reported results. There was no information on blinding, definitions or measurement regarding adverse events, leading to a high risk of bias in all included studies.

### 8.1.10. Level of evidence

GRADE assessment was performed only for analyses with pure randomized trials where this was performed, due to the higher level of evidence. A moderate level of evidence was found for adequacy, a low level for accuracy and sensitivity, and a very low level for adverse events, needle passes and technical failures.

### 8.2.Study II: Cytology methods

#### 8.2.1. Search and selection

The search identified 134 records, and 22 reports(40-60) (13 studies were finally included. The citation and reference search yielded 3 further studies(61-63). The selection process is detailed in **Figure 15**. All studies conducted exclusively FNA, the baseline characteristics of included papers are shown in **Table 2**. One paper seemingly meeting inclusion criteria was excluded due to a compound change in sample processing, confounding differences between the types of cytology.

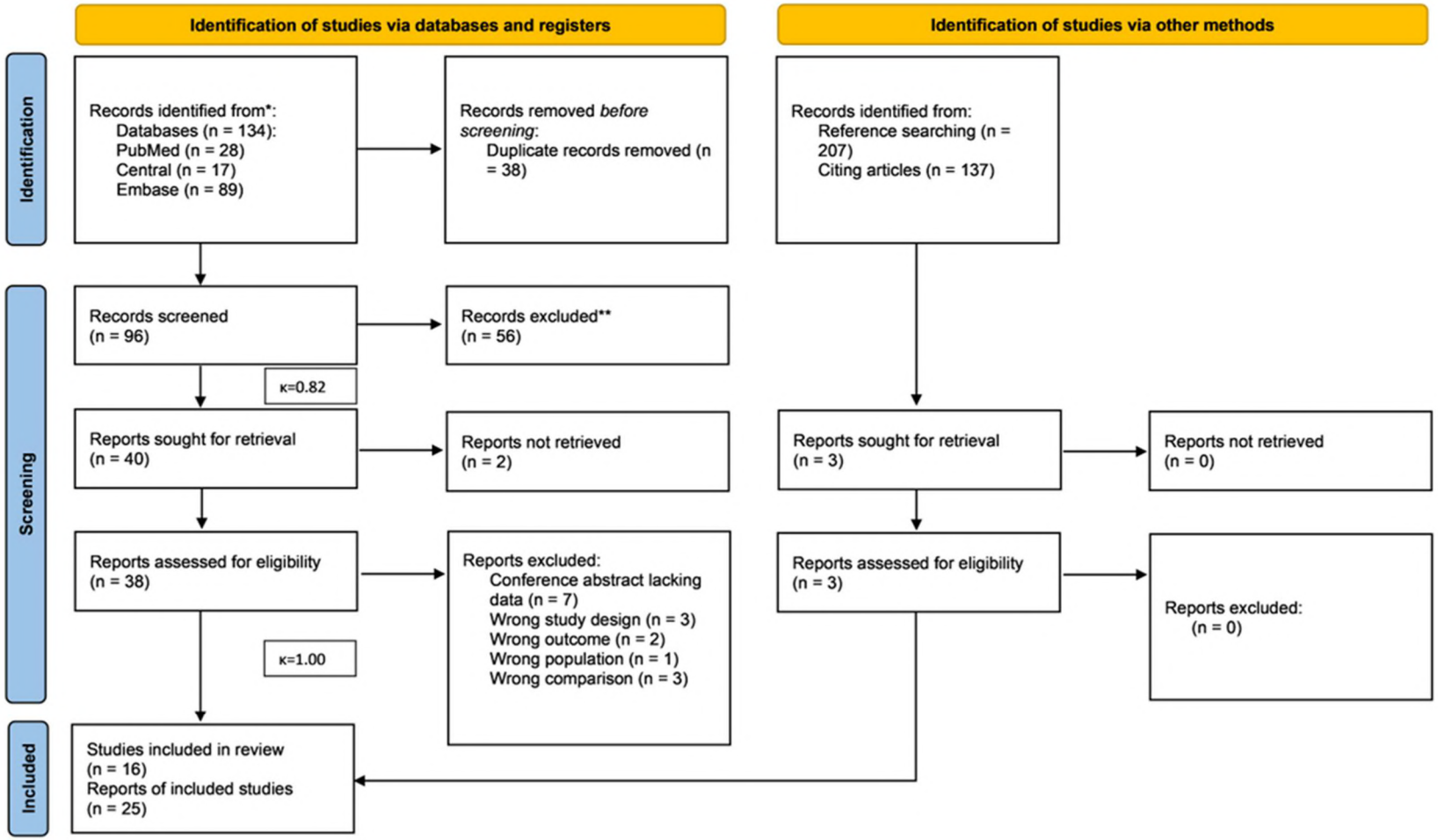



**Figure 15.** PRISMA 2020 flowchart representing the study selection process.

***Table 2.*** *Basic characteristics of included studies.*

| Study | Study Design | Study period | Country | Population | Mass | Needle size | Reference | Assessments | Staining | ROSE | Sample |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chun 2020 | Randomized, crossover | April 2018 - March 2019 | South Korea | N: 170, Female: 44.1%, age: 64.8 +- 10.6 (37-88) | P | 19G 22G | LBC, CS; EUS-FNA core biopsy or surgical specimen, 6-month clinical/radiological follow-up | CS: alcohol, LBC: CytoRich Red, SurePath | Papanicolaou | Unclear/No | I |
| de Luna 2004 | Retrospective, crossover | August 2000 to February 2002 | USA | N: 67, Female: 32.8%, age: 64 (39 - 87) | P | NA | Histologic and clinical follow-up | CS: alcohol/air, LBC: PreservCyt, ThinPrep | Modified Giemsa for air-dried smears, Papanicolaou for alcohol-fixed and LBC samples | Unclear | S |
| Hashimoto 2017 | PSM | January 2009 - August 2014 | Japan | N: 126, Female: 49.2%: , age: CS: 65 (35-93), LBC: 66 (33-85) | P | 19G 22G 25G | Surgical resection, additional EUS-FNA procedure, 6 months clinical/imaging follow-up | CS: alcohol, LBC: SurePath | HE for smears, Papanicolaou for LBC samples | No | I |

| Study | Study Design | Study period | Country | Population | Mass | Needle size | Reference | Assessments | Staining | ROSE | Sample |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Itonaga 2019 | PSM | December 2011 to October 2017 | Japan | N: 311, Female: 42.8%, age: NA | P | 19G 22G 25G | Surgical resection, 12-month clinical follow-up | LBC: ThinPrep, CS: Air/alcohol | Air-dried: Diff-quick, alcohol-fixed and LBC Papanicolaou | Yes | S |
| Jun 2023 | Randomized crossover | January 2019 to August 2022 | South Korea | N: 60, Female: 53.3%, age: 60.7 +- 12.8 (24-85) | A | 19G 22G | Core biopsy, LBC; CS and surgical specimens, plus 6-month follow-up | LBC: CytoRich, SurePath vials, PrepStain processor, CS: alcohol | NA | No | I |
| LeBlanc 2010 | Prospective crossover | April 2005 through April 2007 | USA | N: 50, Female: 36% (whole population), age: mean 63 | A | 22G | Final cytological diagnosis, surgical pathology, or follow-up | CS: air/alcohol, LBC: ThinPrep | Air-dried: Diff-quick, alcohol-fixed and LBC Papanicolaou | Yes | I |
| van Riet 2010 | Prospective crossover | April 2016-2017 | Netherlands | N: 71,Female: NA, age: NA | P | 19G, 22G, 25G | Surgical resection, 12-month clinical follow-up | CS: no information, LBC: Thinprep | Different per center: Hemocolor, no stain, Diff quick, or Giemsa | No | S |

| Study | Study Design | Study period | Country | Population | Mass | Needle size | Reference | Assessments | Staining | ROSE | Sample |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Yan 2023 | Retrospective crossover | January 2014 to February 2022 | China | N: 251, Female: 41%, age: Median 60 (IQR: 13) | P | 20G | Needle biopsy/surgery sample, FNA sample, 6-month clinical/imaging follow-up | CS: alcohol, LBC: ThinPrep | NA | Not all | I |
| Yeon 2018 | Prospective crossover | June 2012 - October 2013 | Korea | N: 43, Female: 32.6%, age: 65.5 +- 12.5 | P | 22G | Biopsy, surgery, or 6-month clinical follow-up | CS: 95% alcohol fixation, LBC: CellPrepPlus | NA | Unclear | I |
| Zhou 2020 | Retrospective crossover | January 2015 to January 2019 | China | N: 514, Female: 37.2%, age: Median 60 (IQR 50-67) | P | 22G 25G | FNA, surgical pathology, 6-month clinical follow-up | CS: alcohol, LBC: CytoRich | CS: HE, LBC: Papanicolaou | Not all | S |
| Qin 2014 | Prospective crossover | January 2011 to January 2014 | China | N: 72, Female: 19.4%, age: 54.6 (24-70) | P | 22G | Surgery, 9-month clinical follow-up | CS: alcohol/air, LBC: ThinPrep | Modified Diff-Quick, Papanicolaou | No | I |
| Cheng 2020* | NA | December 2016 to | China | N: 52, Female: | A | NA | Pathology, follow-up | NA | NA | NA | NA |

| Study | Study Design | Study period | Country | Population | Mass | Needle size | Reference | Assessments | Staining | ROSE | Sample |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | January 2018 | | NA, age: NA | | | | | | | |
| Schmidt 2015* | NA | 2008 to 2011 | Germany | N: 172, Female: 39%, age: 64.8 (+- 12.4) | P | NA | Surgical histology, 12-month follow-up | NA | NA | NA | NA |
| Min 2013* | prospective crossover | November 2010 to February 2013 | China | N: 32, Female: NA, age: NA | P | NA | NA | NA | NA | NA | NA |
| Lee 2016 | Retrospective crossover | July 2010 to June 2015 | South Korea | N: 48, Female: 50%, age: Median 67, range 39-84 | P | 22G | Clinical and imaging follow-up of 12 months, CS, LBC, and pathology from metastatic sites | CS: alcohol, LBC: ThinPrep | Papanicolaou | No | I |

*P: Pancreatic, A: Abdominal, N: Number of patients, CS: Conventional Smear, LBC: Liquid-based cytology, EUS: Endoscopic ultrasound, FNA: fine needle aspiration, IQR: Interquartile range, NA: Not available, HE: Hematoxylin/Eosin, PSM: Propensity score matched. *Conference abstracts*

### 8.2.2. Sensitivity

*Pancreatic masses*

For sensitivity in pancreatic masses, 12 studies(42, 47, 48, 52, 53, 56-62) were eligible for inclusion, six of these investigated the combined methods, seen in **Figure 16**. The sensitivity for detecting malignancy was 0.714 (CI: 0.629 – 0.787, I2: 82%) for CS; 0.747 (CI: 0.643 – 0.828, I2: 84.1%) for LBC; and 0.862 (CI: 0.824 – 0.893, I2: 52.7%) for the combination. There was no significant difference between LBC and CS while the difference between CS/LBC and the combination was significant (p=0.001).

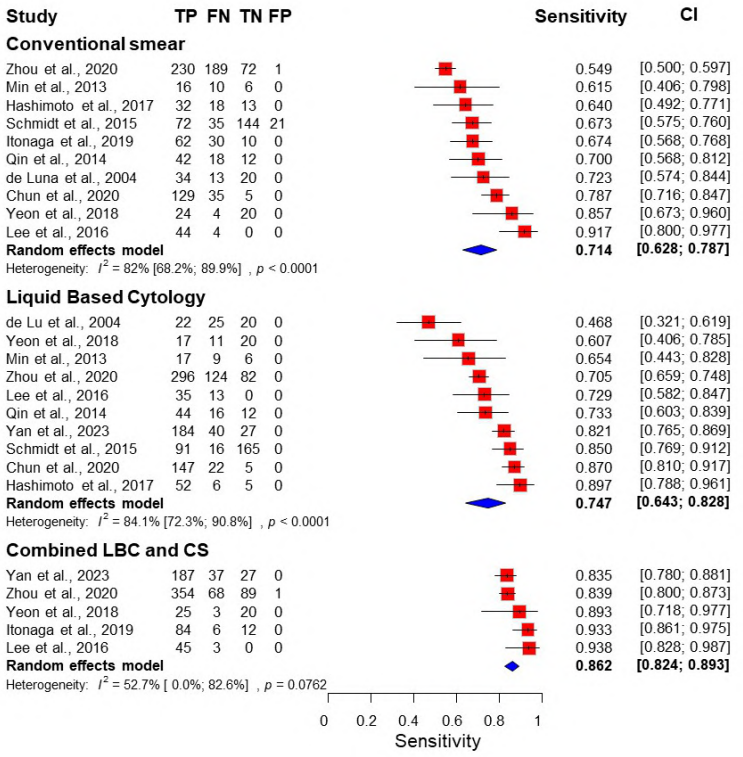



***Figure 16:*** *Forest plot representing the sensitivity of different cytology methods in pancreatic masses. TP: True positives, FN: false negatives, TN: True negatives, FP: False positives. CI: Confidence interval, LBC: liquid-based cytology, CS: conventional smear. There was no significant difference between LBC and CS while the difference between CS/LBC and the combination was significant (p=0.001).*

*All abdominal masses*

Data from thirteen studies(40, 42, 47, 48, 51-53, 56-60, 62) could be analyzed to investigate sensitivity in all abdominal masses, six of these reported on the combined methods, seen in **Figure 17**. The sensitivity in CS was 0.763 (CI: 0.679 – 0.830, I2: 86.5%); in LBC 0.736 (CI: 0.656 – 0.802, I2: 81.8%); and for the combination 0.880 (CI: 0.840 – 0.912, I2: 69.1%). There was no significant difference between LBC and CS, however the difference between conventional smear/LBC and the combination was significant (p=0.001/p=0.006).

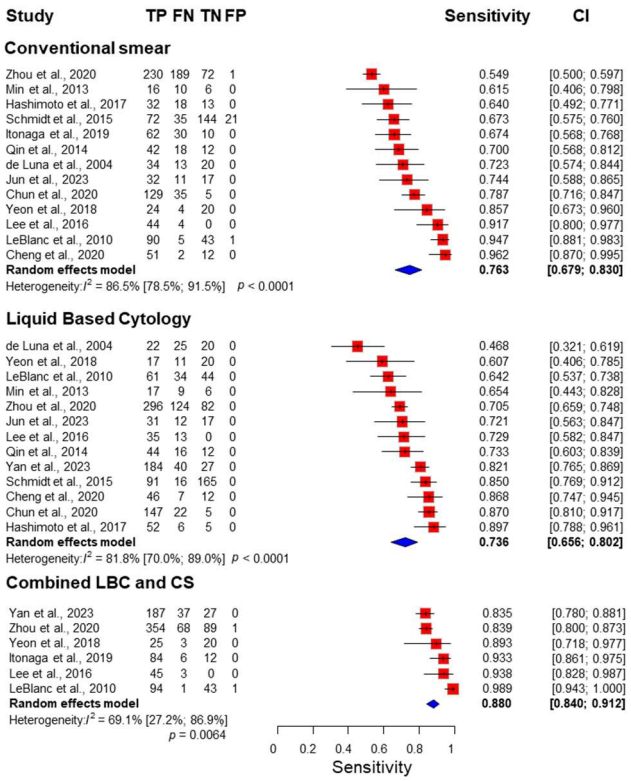



***Figure 17.*** *Forest plot representing the sensitivity of different cytology methods in all abdominal masses. The difference between conventional smear and LBC was not significant (p=0.611). The difference between conventional smear/LBC and the combination was significant (p=0.001/p=0.006). TP: True positives, FN: false negatives, TN: True negatives, FP: False positives. CI: Confidence interval, LBC: liquid-based cytology, CS: conventional smear.*

### 8.2.3. Specificity

Thirteen studies(40, 42, 45, 47, 48, 51-53, 56-61) reported data necessary for calculating specificity (all abdominal masses). There were practically no cases of false positives, with the exception of Schmidt (21 false positives), LeBlanc (1 false positive) and Zhou (1 false positive). Resultingly, the specificity was nearly 100%, a visualization is shown in **Figure 18**.

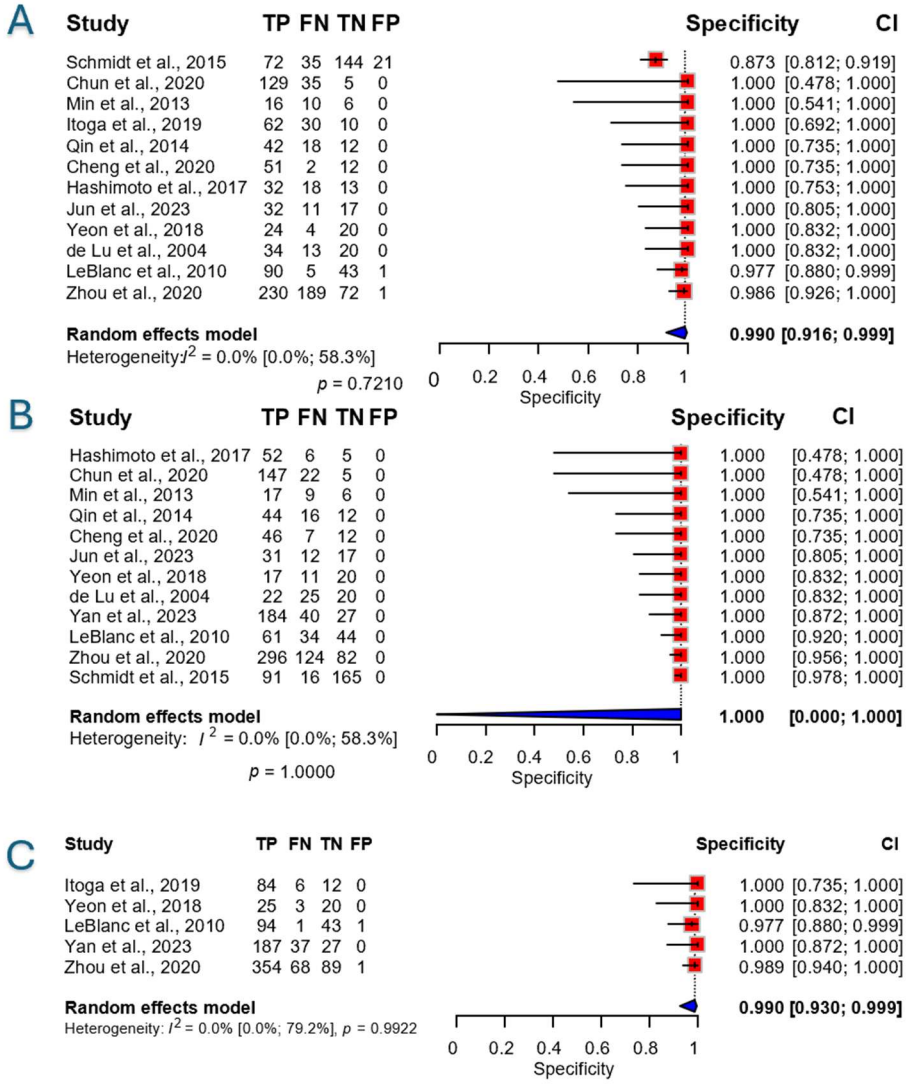


***Figure 18.*** *Forest plot representing the specificity of different cytology methods for all abdominal masses. A: Conventional Smear, B: Liquid-based cytology, C: Combination. TP: True positives, FN: false negatives, TN: True negatives, FP: False positives. CI: Confidence interval.*

### 8.2.4. Accuracy

We calculated accuracy from pooled sensitivity and specificity results, seen in **Table 3.** One study(63) was not included in sensitivity and specificity analysis, but was included in our review due to reporting accuracy. In this study, the accuracy for malignancy was 66.20% (47/71) for conventional smear and 81.70% (58/71) for liquid-based cytology.

*Table 3: Calculations of Accuracy.*

| | **Cases** | | | **Accuracy** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Method** | **Benign** | **Malignant** | **w** | **Actual** | **w=0.2** | **(w=0.4)** | **w=0.6)** | **(w=0.8)** | **(w=1.0)** |
| Combination | **188** | **775** | 0.80 | 0.90 | 0.97 | 0.95 | 0.92 | 0.90 | 0.88 |
| CS | **442** | **1177** | 0.73 | 0.84 | 0.95 | 0.91 | 0.87 | 0.82 | 0.78 |
| LBC | **456** | **1316** | 0.74 | 0.80 | 0.95 | 0.89 | 0.84 | 0.79 | 0.74 |

*CS: Conventional Smear, LBC: Liquid based cytology, w: ratio of malignant/benign cases*

### 8.2.5. Inadequacy rate

*Pancreatic masses*

The rate of inadequate samples was reported in nine studies(42, 47, 48, 52, 56, 58-62) on pancreatic masses. Of these, four reported on the combination of methods (**Figure 19-A).** Using LBC, 7.7% (CI: 2.7 – 20.4, $I^2$*:* 93.7%) of samples were inadequate, with CS only 4.4% (CI:2.4-7.9, $I^2$: 39.1%) were inadequate, and with a combination inadequate samples made up 1.5% (CI: 0%-36.2%, $I^2$: 33.6%).

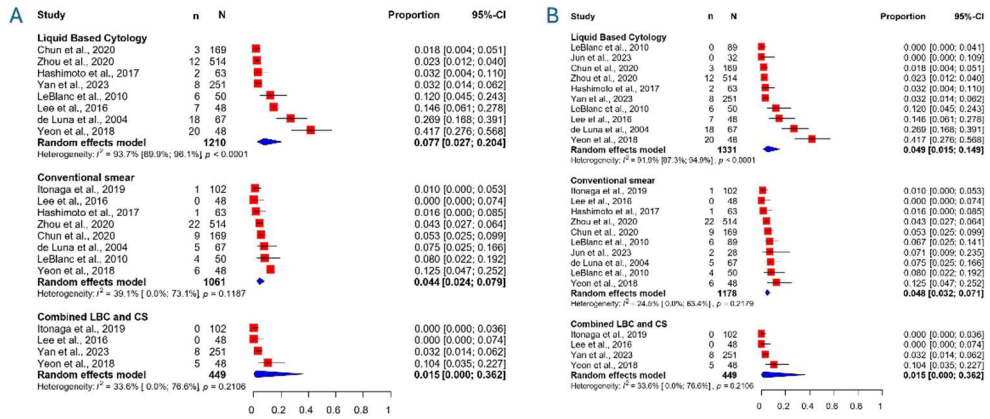



***Figure 19:*** *Proportion of inadequate samples in A: Pancreatic masses and B: All abdominal masses. LBC: Liquid based cytology. CS: Conventional smear. The P-values comparing CS to the combination, LBC to the combination, and CS to LBC were 0.063, 0.15, and 0.66, respectively (in all abdominal masses).*

*All abdominal masses*

The rate of inadequate samples was reported in ten studies(42, 47, 48, 51, 52, 56, 58-62) on abdominal masses. Of these, four reported on the combination of methods (**Figure 19-B).** Using LBC, 4.9% (CI: 1.5-14.9, $I^2$*:* 91.9%) of samples were inadequate, with CS 4.8% (CI: 3.2-7.1, $I^2$*:* 24.5%), were inadequate, and with a combination inadequate samples made up 1.5% (CI: 0-36.2, $I^2$*:* 33.6%).

The P-values comparing CS to the combination, LBC to the combination, and CS to LBC were 0.063, 0.15, and 0.66, respectively.

### 8.2.6. Subgroup results

Two subgroup analyses were conducted for sensitivity, one based on the needle size used, one based on the processing of sample before analysis (one pass split versus individual passes),

*Needle Size*

For investigating needle size, data from 9 studies(42, 47, 48, 51, 52, 56, 59, 60, 62) was eligible (6 on combined methods). Subgroups were defined as mixed needle size versus 20G or 22G. For CS, sensitivity was 0.679 (CI: 0.550-0.786) in the mixed group and 0.902 (CI: 0.781 – 0.959) in the 20G/22G group ($p<0.0001$). For LBC, sensitivity was 0.808 (CI: 0.629-0.912) in the mixed group, and 0.744 (CI: 0.512-0.842) in the 20G/22G group ($p=0.303$). For the combination, sensitivity was 0.882 (0.076-0.999) in the mixed group and 0.934 (0.717-0.987) in the 20G/22G group ($p=0.3267$). Seen in **Figure 20.**

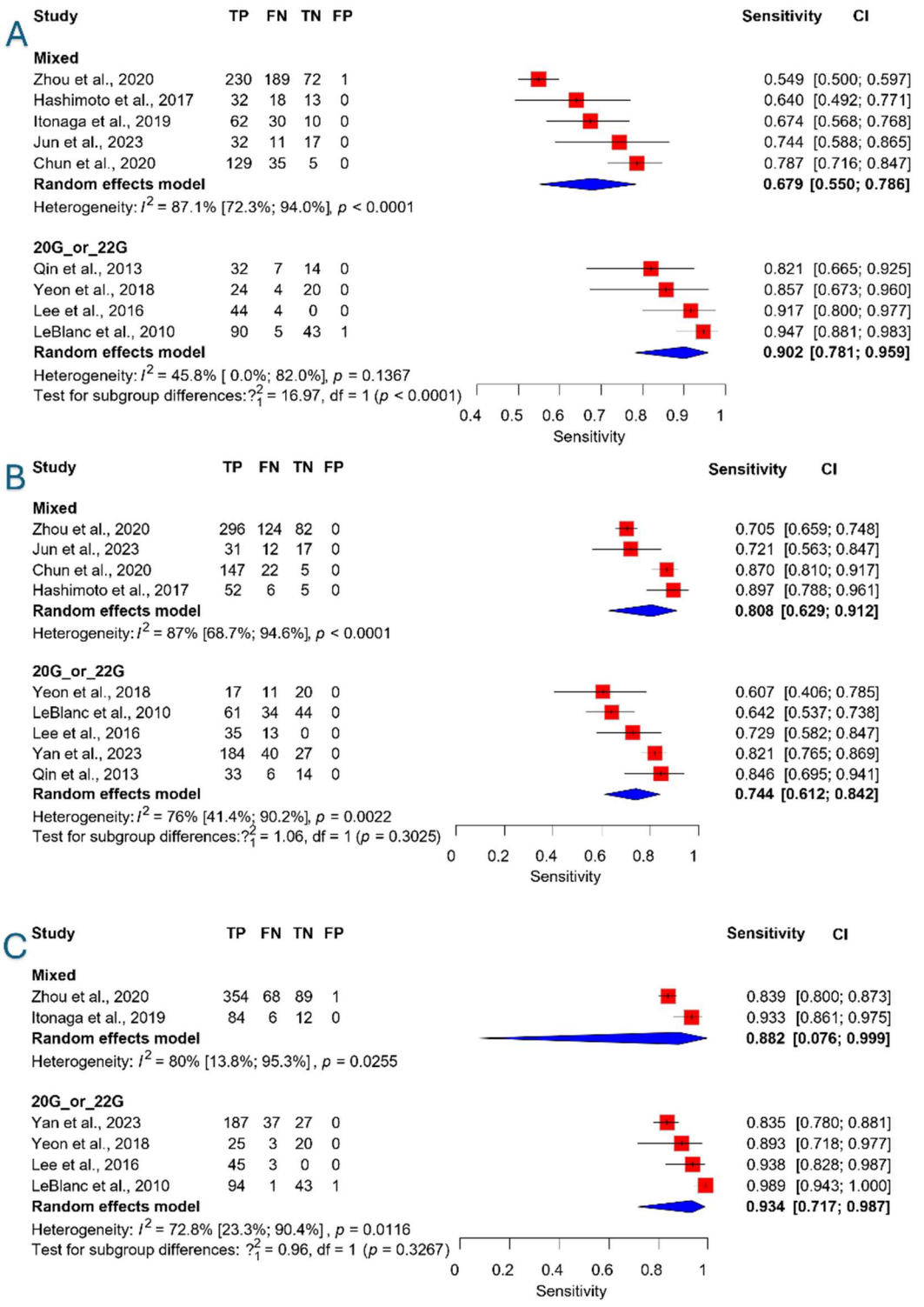



***Figure 20:*** *Subgroup analysis based on needle size (mixed group versus 20G/22G subgroup). A: Conventional Smear, B: Liquid based cytology, C: Combination*

*Split versus individual sample*

For investigating samples, data from 10 studies(42, 47, 48, 51, 52, 56, 59-62) was eligible (6 on combined methods). Subgroups were defined as split sample or individual sample. For CS, sensitivity was 0.835 (CI: 0.716-0.910) in the individual sample and 0.630 (CI: 0.410 – 0.807) in the 20G/22G group (p =0.002). For LBC, sensitivity was 0.785 (CI: 0.692-0.855) in the individual sample group, and 0.610 (CI: 0.017-0.993) in the split sample group (p=0.0387). For the combination, sensitivity was 0.934 (0.717-0.987) in the individual group and 0.882 (0.076-0.999) in the split sample group (p=0.3267). Seen in **Figure 21**.

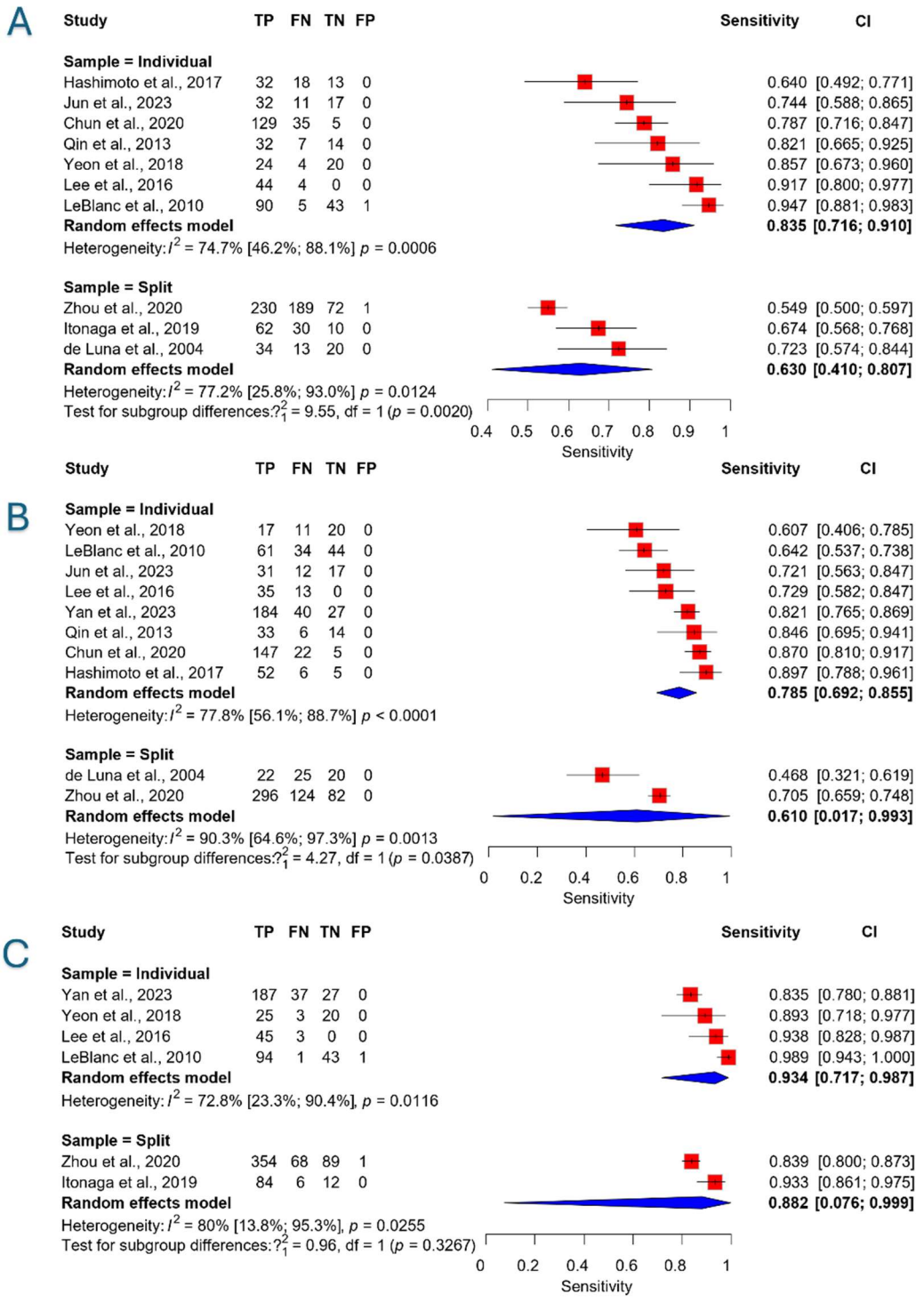



***Figure 21:*** *Subgroup analysis based on sample processing (split versus individual). A: Conventional Smear, B: Liquid based cytology, C: Combination*

### 8.2.7. Risk of bias

The risk of bias was mainly moderate, the most frequently impaired domain was domain 3 (reference domain) (**Figure 22**), the summary can be seen in (**Figure 23**). This was largely due to inclusion of cytology in the reference standard, leading to incorporation bias, and due to different reference standards applied depending on clinical situation. Three studies included were conference abstracts, and "no information" dominated their assessment. An analysis of sensitivity was run excluding these three studies, also to improve data quality, yielding similar results to the primary analysis. Seen in **Figure 24**.

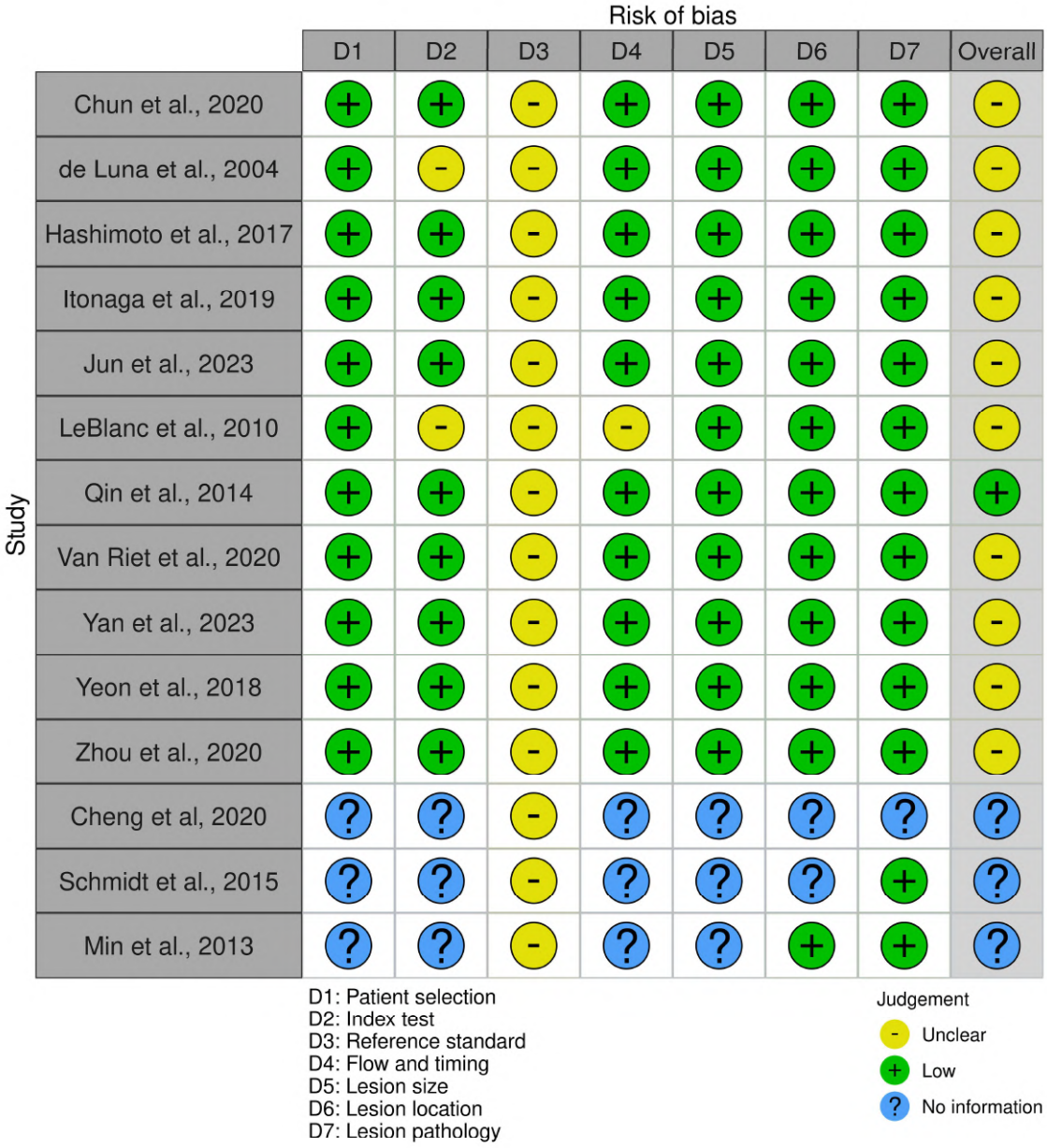


***Figure 22.*** *Result of the risk of bias assessment using the Quality Assessment of Diagnostic Accuracy Studies tool, v2 (QUADAS-2), by study and domain, with overall.*

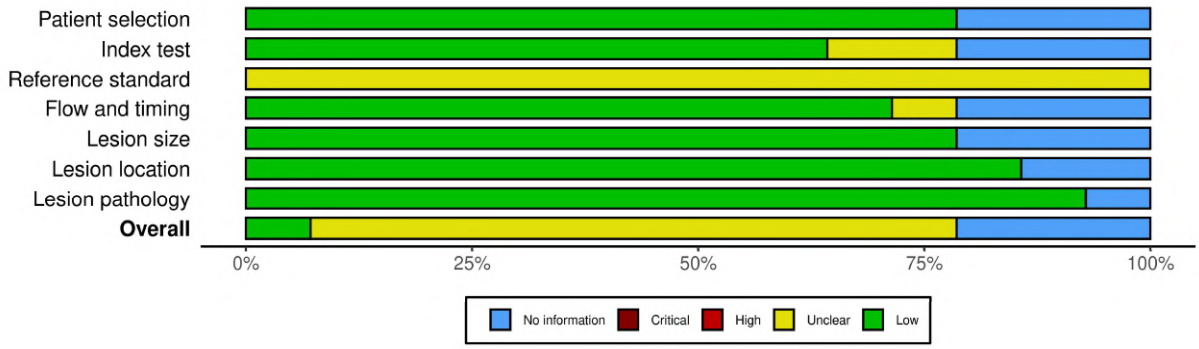



***Figure 23.*** *Overall result of the risk of bias assessment using the Quality Assessment of Diagnostic Accuracy Studies tool, v2 (QUADAS-2) by domain, percentages impaired.*

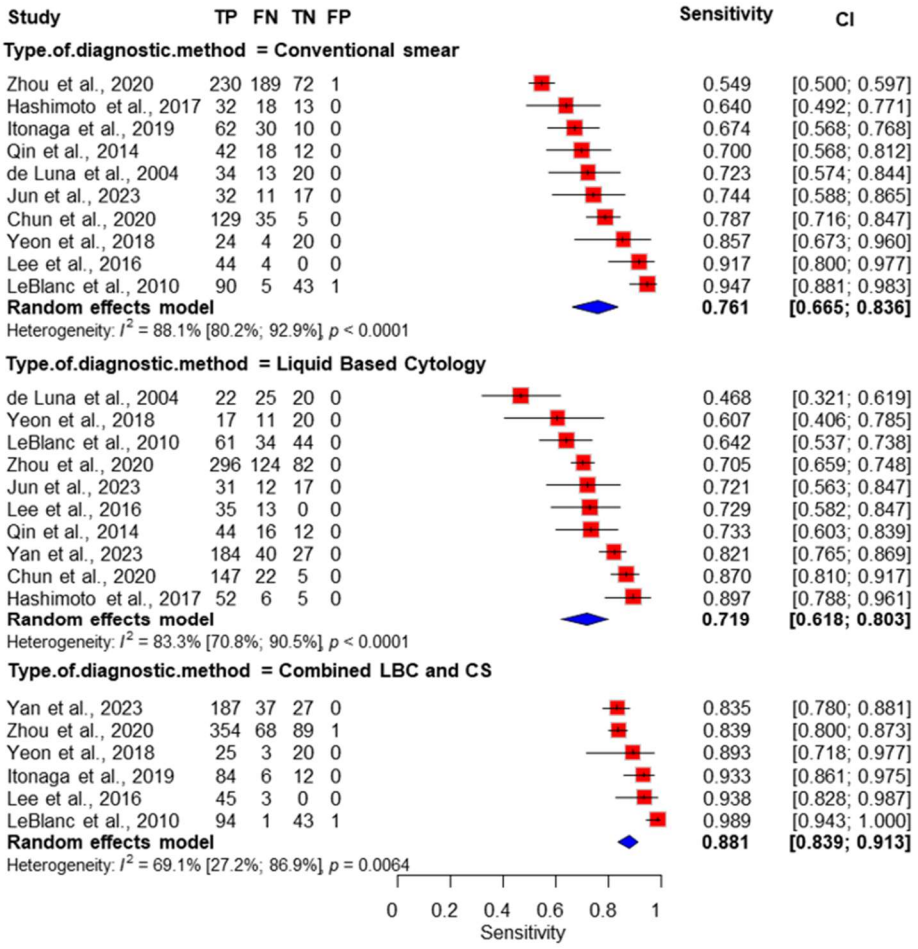



***Figure 24:*** *Forest plot representing the sensitivity of different cytology methods in all abdominal masses, excluding conference abstracts.*

# 9. DISCUSSION

## 9.1.Summary of findings, international comparisons (including all studies)

In this work, we investigated sample processing techniques and sampling equipment used in EUS-guided TA from solid pancreatic and abdominal masses. Specifically, we compared CEH-EUS to conventional EUS, investigating sample adequacy and diagnostic parameters, and two types of cytology (CS, LBC) and their combination. As primary results we found no benefit from use of CEH-EUS over conventional EUS, but a significant benefit both in terms of diagnostic sensitivity and sample adequacy for the combination of LBC and CS over each individual method alone. Furthermore, we found a potentially lower inadequacy rate for CS, when investigating the two cytology methods alone.

These results mostly align with the individual papers included, with a few notable exceptions. One of the randomized trials included in the comparison of CEH-EUS to conventional EUS found a significant benefit when using CEH-EUS compared to conventional, particularly on the first pass(34). While this study had a small sample size which in itself could weaken the importance of that result, investigating the reason for this discrepancy is still interesting. However, in this particular case, the first pass specifically was performed by inexperienced endoscopists, while from the second pass onwards, experienced endoscopists took over the investigation. From that point on the benefit of CEH-EUS visibly decreased. This raises the question if CEH-EUS could serve some benefit in training situations, or if experience is lacking.

Further, in the subgroup of non-randomized studies there was a significant benefit following CEH-EUS use, and all studies indicated this benefit. This was the case even though several studies were well-designed. This finding points towards undetected confounders at play, some of which might help narrow down instances in which CEH-EUS could be beneficial. Importantly, this factor might also explain why our results differ from those in a previously published meta-analysis(17), which pooled randomized and non-randomized studies.

One included study investigated factors which may affect the performance of the two EUS methods, investigating portal hypertension, biliary stents, tumor necrosis, tumor site and chronic pancreatitis. While no significant differences were found, some tendencies were visible and the authors highlighted particularly chronic pancreatitis as a setting in which CEH-EUS may provide added benefit for targeting the lesion.

When comparing LBC to CS, we found that the combination of the two performed best both for diagnostic sensitivity and sample adequacy. While this was a large, clinically important and statistically significant difference, it is important to note that for some studies, the result of the combination of methods was based on a single pass split into two samples, and for others it was based on two separate samples. As sensitivity increases with the number of needle passes performed, this might have introduced confounding where they were compared to single samples for the other methods. However, the two studies(52, 58) that did combine several passes for the combination did not rank best in sensitivity for the combined methods, with one ranking fifth for improvement for the combination. Ideally, this should have been subgrouped or separately analyzed, unfortunately there was not enough data available.

We were, however, able to subgroup based on whether a single sample was used for LBC and CS, or whether one sample was split into two for processing. In this subgrouping, CS performed significantly worse with split samples than with individual samples, while LBC was not affected similarly.

Our results are partially aligned, partially in contradiction with previous meta-analyses. One previous meta-analysis(64) also found a benefit to combining the two methods, but found that LBC was somewhat better than CS. This paper included only data on pancreatic masses, and only 8 papers. Another meta-analysis(65) focusing on comparing only single methods approached it by separating LBC by LBC technique and found that precipitation-based LBC was superior to CS, while filtration-based LBC was less effective than CS – this meta-analysis did not investigate the combined method. A third meta-analysis(66) investigated smears in the presence of ROSE, and found that if ROSE was available, CS outperformed LBC. This may have been a confounding factor in our analysis.

In contrast, we were able to evaluate both LBC, CS and their combination, the effects on the rate of inadequate samples, and investigated it both in pancreatic masses alone, and all abdominal samples.

An important finding during our investigation was that all studies were affected by different types of confounding, and the significant clinical heterogeneity among studies. Particularly needle size and brand differed among the studies, but also the sampling approach, presence of rapid on-site evaluation (ROSE), and clinical definitions (like adequacy). We were able to subgroup based on needle size, finding that primarily CS was impacted by a change in needle size. While this is a weak analysis, it does suggest that further investigations should consider this factor.

### 9.2.Strengths (including all studies)

Both studies followed the most up-to-date methodology as recommended by the Cochrane collaboration, had a pre-registered protocol and few deviations. Particularly Study I had studies primarily of low risk of bias and an up to moderate level of evidence, while study II was able to include a great number of patients for a question of its nature, even while including only studies directly comparing the two methods.

### 9.3.Limitations (including all studies)

Both studies were somewhat limited by heterogeneity between the studies, including both clinical heterogeneity (different needle types, staining and techniques for processing and sampling itself). Also the methodology of included studies differed, with one study including both non-randomized and randomized studies, and the other a combination of prospective studies, with and without crossover, and retrospective case review.

Particularly study II was also impacted by temporal bias, including studies across two decades, during which particularly LBC drastically changed and likely improved in technology. It was also impacted by incorporation bias, as the outcome of cytology itself was frequently included in the reference standard, inflating specificity and leading to zero false positives.

## 10. CONCLUSIONS

Based on our findings, CEH-EUS does not improve sample adequacy or diagnostic performance for tissue acquisition from solid pancreatic masses. Combining liquid-based cytology and conventional smear for the sample assessment, however, did significantly improve both adequacy and sensitivity for malignance.

## 11. IMPLICATIONS FOR PRACTICE

Based on the findings in these two reviews and meta-analyses, CEH-EUS is not currently indicated for use in tissue acquisition from solid pancreatic masses. There is potential for benefit in training situations, however, this remains to be investigated.

Once sampling has been performed, performing a combination of CS and LBC provides the highest sensitivity and lowest rate of inadequate samples. If only one method is to be performed, conventional smear may cause slightly fewer inadequate samples. CS is however affected by needle size and sample processing, and if possible a split sample should be avoided.

These suggestions should be incorporated into current clinical guidelines.

# 12. IMPLICATIONS FOR RESEARCH

## 12.1. Methodology and Study design

Further studies conducted should take care to transparently report needle size used, details for sample processing and definitions of inadequacy. Future studies should ensure that their reference standard does not include the outcome of the sampling itself, and a consistent reference standard applied to all patients. In future meta-analyses, accounting for temporal bias (e.g. by trial sequential analysis) and clinical heterogeneity should be attempted. Subgrouping based on staining, LBC platform, fixation and sample type would be ideal, as well as based on needle size.

## 12.2. New Areas

The potential use of CEH-EUS for training purposes should be investigated further, as should the question of use of CEH-EUS for chronic pancreatitis. The impact of splitting the sample and needle size should also be further investigated.

## 13. IMPLICATIONS FOR POLICY MAKERS

The results of this thesis should be considered for inclusion in current endoscopy guidelines: there is evidence for no benefit of CEH-EUS, and a recommendation of combining cytology methods where possible. Additionally, CEH-EUS can be considered for inclusion in endoscopy training set-ups, after cost-benefit analysis.

## 14. FUTURE PERSPECTIVES

To continue this work, I plan to finish an ongoing network meta-analysis comparing different needle designs and sizes to improve the diagnostic adequacy of FNA/FNB after EUS-guided TA.

Speaking more broadly, I will take my lessons from this journey into my future medical and academic life. The scientific thinking, critical assessment of published literature and interpretation and applications of scientific results will not only enable me to further conduct research in the field I will choose, but also to apply evidence-based medicine for my future patients, treating them according to the most up-to-date scientific outputs.

Ultimately, I am looking forward to combining a scientific and medical life, educating future scientists and doctors, researching and treating patients.

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## 16. BIBLIOGRAPHY

### 16.1. Publications related to the thesis

**Engh, M.A**.; Teutsch, B.; Schulze, Wenning A.; Hadani, Y.; Almog, O.; Veres, D.S.; Hegyi, P.; Erőss, B.

Contrast-enhanced endoscopic ultrasound likely does not improve diagnostic adequacy during endoscopic ultrasound guided tissue acquisition: A systematic review and meta-analysis

PANCREATOLOGY 24: 4 pp. 649-660., 12 p. (2024)

*Citing papers: 2 | Independent citation: 2 | Self citation: 0 | Unknown citation: 0 | Number of citations in WoS: 2 | Number of citations in Scopus: 2 | WoS/Scopus assigned: 2 | Number of citations with DOI: 2*
*Journal subject: Scopus - Gastroenterology Rank: Q1*
*Journal subject: Scopus - Endocrinology Rank: Q2*
*Journal subject: Scopus - Endocrinology, Diabetes and Metabolism Rank: Q2*
*Journal subject: Scopus - Hepatology Rank: Q2*
*IF: 2.7*

**Engh, M.A**.; Teutsch, B.; Schulze, Wenning A.; Koi T.; Hegyi, P.; Erőss, B.

Combined liquid-based cytology and conventional smear provides better sensitivity and adequacy rates after endoscopic ultrasound-guided tissue acquisition of abdominal masses: A systematic review and meta-analysis

JOURNAL OF CLINICAL MEDICINE (2025)

*Journal subject: Scopus - Medicine (miscellaneous) Rank: Q1*
*IF: 2.9*

### 16.2. Publications not related to the thesis

Lugosi Katalin, **Engh Marie A**, Kói Tamás, Molnár Zsolt, Csukly Gábor, Horváth Klaudia, Hargitai Emma, Hegyi Péter, Mezei Zsolt

Cognitive Impairment in Multiple Sclerosis: The Role of Clinical and Sociodemographic Factors - A Systematic Review and Meta-Analysis

ANNALS OF CLINICAL AND TRANSLATIONAL NEUROLOGY 2025 Paper:

DOI:10.1002/acn3.70172, 13 p. (2025)

*Scopus - Neurology (clinical) Rank: Q1*
*Scopus - Neuroscience (miscellaneous) Rank: Q1*
*IF: 3,9*

Zhubi, Esra; Bissenov, Azamat; **Engh, Marie Anne**; Tóth, Réka; Horváth, András Attila; Hegyi, Peter; Gunda, Bence

Bridging therapy versus direct endovascular thrombectomy in basilar artery occlusion stroke: a systematic review and meta-analysis

GEROSCIENCE: OFFICIAL JOURNAL OF THE AMERICAN AGING ASSOCIATION (AGE) (2025)

*Journal subject: Scopus - Complementary and Alternative Medicine Rank: D1*
*Journal subject: Scopus - Veterinary (miscellaneous) Rank: D1*
*Journal subject: Scopus - Aging Rank: Q1*
*Journal subject: Scopus - Cardiology and Cardiovascular Medicine Rank: Q1*
*Journal subject: Scopus - Geriatrics and Gerontology Rank: Q1*
*IF:5.4*

Creangă-Murariu, Ioana; Rezuș, Ioana-Irina; Karami, Roshanak; Rancz, Anett; Zolcsák, Ádám; **Engh, Marie Anne**; Obeidat, Mahmoud; Tamba, Bogdan-Ionel**; Hegyi, Péter** ; Bunduc, Stefania

Indications of Cannabinoids for the Palliation of Cancer-Associated Symptoms: A Systematic Review and Meta-Analysis

CURRENT ONCOLOGY REPORTS (2025)

*Journal subject: Scopus - Oncology Rank: Q1*
*IF: 5*

Gresits, Orsolya Zsuzsanna; Vezér, Mátyás; **Engh, Marie Anne**; Szabó, László; Molnár, Zsolt; Hegyi, Péter; Terebessy, Tamás ✉

Limited Evidence of Functional Benefit After Upper Limb Botulinum Toxin Treatment in Children With Cerebral Palsy: Systematic Review and Meta-analysis

AMERICAN JOURNAL OF PHYSICAL MEDICINE & REHABILITATION 104: 2 pp. 108-116., 9 p. (2025)

*Journal subject: Scopus - Rehabilitation Rank: Q1*
*Journal subject: Scopus - Physical Therapy, Sports Therapy and Rehabilitation Rank: Q2*
*Journal subject: Scopus - Sports Science Rank: Q3*
*IF: 2.4*

Kancsev, Alexander; Virág-Tulassay, Eszter Éva; **Engh, Marie Anne**; Kiss-Dala, Szilvia; Horváth, András Attila; Hegyi, Péter; Kéri, Szabolcs

Glucose homeostasis and cognitive functions in schizophrenia: a systematic review and meta-analysis

SCIENTIFIC REPORTS 15: 1 Paper: 22898, 14 p. (2025)

*Journal subject: Scopus - Multidisciplinary Rank: Q1*
*IF: 3.9*

Lányi, Orsolya; Zahemszky, Daniel; Wenning, Alexander Schulze; **Engh, Marie Anne**; Molnár, Zsolt; Horváth, András Attila; Hegyi, Péter; Csukly, Gábor ✉

Cerebello-Thalamo-Cortical Dysconnectivity in Schizophrenia Spectrum Disorders: A Resting-State fMRI Meta-Analysis

BIOLOGICAL PSYCHIATRY: COGNITIVE NEUROSCIENCE AND NEUROIMAGING (2025)

*Journal subject: Scopus - Biological Psychiatry Rank: D1*
*Journal subject: Scopus - Cognitive Neuroscience Rank: D1*
*Journal subject: Scopus - Neurology (clinical) Rank: D1*
*Journal subject: Scopus - Radiology, Nuclear Medicine and Imaging Rank: D1*
*IF: 4.8*

Rakovics, Márton; Meznerics, Fanni Adél; Fehérvári, Péter; Kói, Tamás; Csupor, Dezső; Bánvölgyi, András; Rapszky, Gabriella Anna; **Engh, Marie Anne**; Hegyi, Péter; Harnos, Andrea

Deep neural networks excel in COVID-19 disease severity prediction - a meta-regression analysis

SCIENTIFIC REPORTS 15: 1 Paper: 10350 , 12 p. (2025)

*Journal subject: Scopus - Multidisciplinary Rank: Q1*
*IF:3.9*

Sebestyén, Anna Réka; Turan, Caner*; Szemere, Ambrus; Virág, Marcell; Ocskay, Klementina; Dembrovszky, Fanni; Szabó, László; Hegyi, Péter; **Engh, Marie Anne****; Molnár, Zsolt

**Joint last authorship

Preoperative carbohydrate loading reduces length of stay after major elective, non-cardiac surgery when compared to fasting: a systematic review and meta-analysis

SCIENTIFIC REPORTS 15: 1 Paper: 19119, 11 p. (2025)

*Journal subject: Scopus - Multidisciplinary Rank: Q1*
*IF: 3.9*

Szemere, Ambrus; Fazekas, Alíz; Sebestyén, Anna Réka; Ezzeddine, Rani; Upor, Veronika; **Engh, Marie Anne**; Hegyi, Péter; Molnár, Zsolt; Horváth, Klára

Protocolized sedation may reduce ventilation and sedation requirements in the pediatric intensive care unit: a systematic review and meta-analysis

CLINICAL AND EXPERIMENTAL PEDIATRICS 68: 6 pp. 406-416. , 11 p. (2025)

*Journal subject: Scopus - Pediatrics Rank: D1*
*Journal subject: Scopus - Pediatrics, Perinatology and Child Health Rank: Q1*
*IF: 3.9*

Veres, Boglarka; Kiss, Boldizsar; Fehervari, Peter; **Engh, Marie Anne**; Hegyi, Peter; Zima, Endre; Merkely, Bela**; Kosztin, Annamaria

Enhancing Heart Failure Management: A Systematic Review and Meta-Analysis of Continuous Remote Telemedical Management vs. In-Person Visit in Patients with Cardiac Implantable Electronic Devices

JOURNAL OF CLINICAL MEDICINE 14: 12 Paper: 4278, 18 p. (2025)

*Journal subject: Scopus - Medicine (miscellaneous) Rank: Q1*
*IF: 2.9*

Bakó, Eszter; Böszörményi, Andrea; Vargáné Szabó, Bettina; **Engh, Marie Anne**; Hegyi, Péter; Ványolós, Attila; Csupor, Dezső

Chemometric analysis of monoterpenes and sesquiterpenes of conifers

FRONTIERS IN PLANT SCIENCE 15 Paper: 1392539, 17 p. (2024)

*Citing papers: 1 | Independent citation: 1 | Self citation: 0 | Unknown citation: 0 | Number of citations in WoS: 1 | Number of citations in Scopus: 1 | WoS/Scopus assigned: 1 | Number of citations with DOI: 1*
*Journal subject: Scopus - Plant Science Rank: D1*
*IF: 4.8*

Ehrenberger, Réka; Masszi, Richárd; Zsigmond, Előd-János; Nguyen Do To, Uyen; Turan, Caner; Walter, Anna; Hegyi, Péter; **Engh, Marie Anne**; Duray, Gábor Zoltán; Molnár, Zsolt et al.

Modified Balloon Use After Rotational Atherectomy Reduces Major Adverse Cardiovascular Event Rates in Severely Calcified Coronary Lesions: A Systematic Review and Meta-Analysis

JOURNAL OF CLINICAL MEDICINE 13: 22 Paper: 6853, 12 p. (2024)

*Journal subject: Scopus - Medicine (miscellaneous) Rank: Q1*
*IF: 2.9*

Gulyás, E.; Horváth, I.L.; **Engh, M.A.**; Bunduc, S.; Dembrovszky, F.; Fehérvári, P.; Bánvölgyi, A.; Csupor, D.; Hegyi, P.; Karvaly, G.B.

Assessment of the practical impact of adjusting beta-lactam dosages based on therapeutic drug monitoring in critically ill adult patients: a systematic review and meta-analysis of randomized clinical trials and observational studies

SCIENTIFIC REPORTS 14: 1 Paper: 7793, 13 p. (2024)

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*Journal subject: Scopus - Multidisciplinary Rank: Q1*
*IF: 3.9*

Huszár, Zsolt; **Engh, Marie Anne**; Pavlekovics, Márk; Sato, Tomoya; Steenkamp, Yalea ; Hanseeuw, Bernard; Terebessy, Tamás; Molnár, Zsolt; Hegyi, Péter; Csukly, Gábor ✉

Risk of conversion to mild cognitive impairment or dementia among subjects with amyloid and tau pathology: a systematic review and meta-analysis

ALZHEIMERS RESEARCH & THERAPY 16: 1 Paper: 81, 19 p. (2024)

*Citing papers: 8 | Independent citation: 7 | Self citation: 1 | Unknown citation: 0 | Number of citations in WoS: 6 | Number of citations in Scopus: 7 | WoS/Scopus assigned: 8 | Number of citations with DOI: 8*

*Journal subject: Scopus - Cognitive Neuroscience Rank: D1*
*Journal subject: Scopus - Neurology (clinical) Rank: D1*
*Journal subject: Scopus - Neurology Rank: D1*
*IF: 7.6*

Huszár, Zsolt; Solomon, Alina; **Engh, Marie Anne**; Koszovácz, Vanda; Terebessy, Tamás; Molnár, Zsolt; Hegyi, Péter; Horváth, András; Mangialasche, Francesca; Kivipelto, Miia et al.

Association of modifiable risk factors with progression to dementia in relation to amyloid and tau pathology.

ALZHEIMERS RESEARCH & THERAPY 16: 1 Paper: 238, 15 p. (2024)

*Citing papers: 4 | Independent citation: 4 | Self citation: 0 | Unknown citation: 0 | Number of citations in WoS: 3 | Number of citations in Scopus: 3 | WoS/Scopus assigned: 4 | Number of citations with DOI: 4*
*Journal subject: Scopus - Cognitive Neuroscience Rank: D1*
*Journal subject: Scopus - Neurology (clinical) Rank: D1*
*Journal subject: Scopus - Neurology Rank: D1*
*IF: 7.6*

Lányi, Orsolya; Koleszár, Boróka; Schulze, Wenning Alexander; Balogh, David; **Engh, Marie Anne**; Horváth, András Attila; Fehérvari, Péter; Hegyi, Péter; Molnár, Zsolt; Unoka, Zsolt et al.

Excitation/inhibition imbalance in schizophrenia: a meta-analysis of inhibitory and excitatory TMS-EMG paradigms

SCHIZOPHRENIA 10: 1 Paper: 56, 12 p. (2024)

*Citing papers: 6 | Independent citation: 5 | Self citation: 1 | Unknown citation: 0 | Number of citations in WoS: 6 | Number of citations in Scopus: 6 | WoS/Scopus assigned: 6 | Number of citations with DOI: 6*
*IF: 4.1*

Lugosi, Katalin; **Engh, Marie A**; Huszár, Zsolt; Hegyi, Péter; Mátrai, Péter; Csukly, Gábor; Molnár, Zsolt; Horváth, Klaudia; Mátis, Dóra; Mezei, Zsolt ✉

Domain-specific cognitive impairment in multiple sclerosis: A systematic review and meta-analysis

ANNALS OF CLINICAL AND TRANSLATIONAL NEUROLOGY 11: 3 pp. 564-576. , 13 p. (2024)

*Citing papers: 10 | Independent citation: 10 | Self citation: 0 | Unknown citation: 0 | Number of citations in WoS: 9 | Number of citations in Scopus: 9 | WoS/Scopus assigned: 10 | Number of citations with DOI: 10*
*Journal subject: Scopus - Neurology (clinical) Rank: Q1*
*Journal subject: Scopus - Neuroscience (miscellaneous) Rank: Q1*
*IF: 3.9*

Olah, Csilla; Kubik, András; Mátrai, Péter; **Engh, Marie Anne**; Barna, Viktória; Hegyi, Péter; Reis, Henning; Nyirády, Péter; Szarvas, Tibor ✉

Estimation of the incidence of urachal cancer: A systematic review and meta-analysis of registry-based studies

UROLOGIC ONCOLOGY: SEMINARS AND ORIGINAL INVESTIGATIONS 42 : 7 pp. 221.e1-221.e7. (2024)

*Citing papers: 5 | Independent citation: 3 | Self citation: 2 | Unknown citation: 0 | Number of citations in WoS: 5 | Number of citations in Scopus: 4 | WoS/Scopus assigned: 5 | Number of citations with DOI: 5*
*Journal subject: Scopus - Urology Rank: Q1*
*Journal subject: Scopus - Oncology Rank: Q2*
*IF: 2.3*

Szabó, Gergő Vilmos; Szigetváry, Csenge; Turan, Caner; **Engh, Marie Anne**; Terebessy, Tamás; Fazekas, Alíz; Farkas, Nelli; Hegyi, Péter; Molnár, Zsolt ✉

Fluid resuscitation with balanced electrolyte solutions results in faster resolution of diabetic ketoacidosis than with 0.9% saline in adults - A systematic review and meta-analysis.

DIABETES-METABOLISM RESEARCH AND REVIEWS 40: 5 Paper: e3831, 13 p. (2024)

*Citing papers: 1 | Independent citation: 1 | Self citation: 0 | Unknown citation: 0 | Number of citations in WoS: 1 | Number of citations in Scopus: 1 | WoS/Scopus assigned: 1 | Number of citations with DOI: 1*
*Journal subject: Scopus - Endocrinology Rank: Q1*
*Journal subject: Scopus - Endocrinology, Diabetes and Metabolism Rank: Q1*
*Journal subject: Scopus - Internal Medicine Rank: Q1*
*IF: 6*

Szigetváry, Csenge; Szabó, Gergő V.; Dembrovszky, Fanni; Ocskay, Klementina; **Engh, Marie A.**; Turan, Caner; Szabó, László; Walter, Anna; Kobeissi, Fadl; Terebessy, Tamás et al.

Individualised Positive End-Expiratory Pressure Settings Reduce the Incidence of Postoperative Pulmonary Complications: A Systematic Review and Meta-Analysis

JOURNAL OF CLINICAL MEDICINE 13: 22 Paper: 6776, 16 p. (2024)

*Citing papers: 1 | Independent citation: 1 | Self citation: 0 | Unknown citation: 0 | Number of citations in WoS: 1 | Number of citations in Scopus: 1 | WoS/Scopus assigned: 1 | Number of citations with DOI: 1*
*Journal subject: Scopus - Medicine (miscellaneous) Rank: Q1*
*IF: 2.9*

Szirmai, D.; Zabihi, A.; Kói, T.; Hegyi, P.; Wenning, A.S.; **Engh, M.A.**; Molnár, Z.; Csukly, G.; Horváth, A.A.

EEG connectivity and network analyses predict outcome in patients with disorders of consciousness – A systematic review and meta-analysis

HELIYON 10: 10 Paper: e31277, 12 p. (2024)

*Journal subject: Scopus - Multidisciplinary Rank: Q1*
*IF: 3.6*

Turan, Caner; Szigetváry, Csenge E.; Kói, Tamás; **Engh, Marie A.;** Atakan, Işıl; Zubek, László; Terebessy, Tamás; Hegyi, Péter; Molnár, Zsolt ✉

Hemoadsorption Therapy for Critically Ill Patients with Acute Liver Dysfunction: A Meta-Analysis and Systematic Review

BIOMEDICINES 12: 1 Paper: 67, 16 p. (2024)

*Citing papers: 6 | Independent citation: 6 | Self citation: 0 | Unknown citation: 0 | Number of citations in WoS: 6 | Number of citations in Scopus: 6 | WoS/Scopus assigned: 6 | Number of citations with DOI: 6*
*Journal subject: Scopus - Biochemistry, Genetics and Molecular Biology (miscellaneous) Rank: Q1*
*Journal subject: Scopus - Medicine (miscellaneous) Rank: Q1*
*IF: 3.9*

Varga, P.; Obeidat, M.; Máté, V.; Kói, T.; Kiss-Dala, S.; Major, G.S.; Tímár, Á.E.; Li, X.; Szilágyi, Á.; Csáki, Z. **et al**.

From simple factors to artificial intelligence: evolution of prognosis prediction in childhood cancer: a systematic review and meta-analysis

ECLINICALMEDICINE 78 Paper: 102902, 15 p. (2024)

*Citing papers: 2 | Independent citation: 2 | Self citation: 0 | Unknown citation: 0 | Number of citations in WoS: 1 | Number of citations in Scopus: 1 | WoS/Scopus assigned: 2 | Number of citations with DOI: 2*
*Journal subject: Scopus - Medicine (miscellaneous) Rank: D1*
*IF: 10*

Vezér, M.; Gresits, O.; **Engh, M.A.**; Szabó, B.; Molnár, Z.; Hegyi, P.; Terebessy, T.

Effectiveness of Video-Game-Based Therapy to Improve Hand Function in Children with Cerebral Palsy: A Systematic Review and Meta-Analysis

JOURNAL OF CLINICAL MEDICINE 13: 24 Paper: 7524, 16 p. (2024)

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*Journal subject: Scopus - Medicine (miscellaneous) Rank: Q1*
*IF: 2.9*

Kulyassa, Péter; **Engh, Marie Anne**; Vámosi, Péter; Fehérvári, Péter; Hegyi, Péter; Merkely, Béla; Édes, István Ferenc

Drug-coated balloon therapy is more effective in treating late drug-eluting stent in-stent restenosis than the early occurring one-a systematic review and meta-analysis

FRONTIERS IN CARDIOVASCULAR MEDICINE 10 Paper: 1062130, 10 p. (2023)

*Citing papers: 2 | Independent citation: 2 | Self citation: 0 | Unknown citation: 0 | Number of citations in WoS: 2 | Number of citations in Scopus: 2 | WoS/Scopus assigned: 2 | Number of citations with DOI: 2*
*Journal subject: Scopus - Cardiology and Cardiovascular Medicine Rank: Q2*
*IF: 2.9*

Pálinkás, Dániel; Teutsch, Brigitta; Gagyi, Endre Botond; **Engh, Marie Anne**; Kalló, Patrícia; Veres, Dániel S; Földvári-Nagy, László; Hosszúfalusi, Nóra; Hegyi, Péter; Erőss, Bálint

No Association between Gastrointestinal Rebleeding and DOAC Therapy Resumption: A Systematic Review and Meta-Analysis

BIOMEDICINES 11: 2 Paper: 554, 14 p. (2023)

*Citing papers: 1 | Independent citation: 1 | Self citation: 0 | Unknown citation: 0 | Number of citations in Scopus: 1 | WoS/Scopus assigned: 1 | Number of citations with DOI: 1*
*Journal subject: Scopus - Biochemistry, Genetics and Molecular Biology (miscellaneous) Rank: Q1*
*Journal subject: Scopus - Medicine (miscellaneous) Rank: Q1*
*IF: 3.9*

Pavlekovics, Mark; **Engh, Marie Anne**; Lugosi, Katalin; Szabo, Laszlo; Hegyi, Peter; Terebessy, Tamas; Csukly, Gabor; Molnar, Zsolt; Illes, Zsolt; Lovas, Gabor ✉

Plasma Exchange versus Intravenous Immunoglobulin in Worsening Myasthenia Gravis: A Systematic Review and Meta-Analysis with Special Attention to Faster Relapse Control

BIOMEDICINES 11: 12 Paper: 3180, 15 p. (2023)

*Citing papers: 11 | Independent citation: 11 | Self citation: 0 | Unknown citation: 0 | Number of citations in WoS: 8 | Number of citations in Scopus: 10 | WoS/Scopus assigned: 11 | Number of citations with DOI: 11*
*Journal subject: Scopus - Biochemistry, Genetics and Molecular Biology (miscellaneous) Rank: Q1*
*Journal subject: Scopus - Medicine (miscellaneous) Rank: Q1*
*IF: 3.9*

Rancz, Anett; Teutsch, Brigitta; **Engh, Marie Anne**; Veres, Daniel Sandor; Foldvari-Nagy, Laszlo; Eross, Balint; Hosszufalusi, Nora; Juhasz, Mark Felix; Hegyi, Peter; Mihaly, Emese

Microscopic colitis is a risk factor for lowbone density: a systematic review andmeta-analysis

THERAPEUTIC ADVANCES IN GASTROENTEROLOGY 16 pp. 1-13., 13 p. (2023)

*Citing papers: 1 | Independent citation: 1 | Self citation: 0 | Unknown citation: 0 | Number of citations in WoS: 1 | Number of citations in Scopus: 1 | WoS/Scopus assigned: 1 | Number of citations with DOI: 1*
*Journal subject: Scopus - Gastroenterology Rank: Q1*
*IF: 3.9*

Szabados, Márton; Kolumbán, Erika; Agócs, Gergely; Kiss-Dala, Szilvia; **Engh, Marie Anne**; Hernádfői, Márk; Takács, Kata; Tuboly, Eszter; Párniczky, Andrea; Hegyi, Péter et al.

Association of tumor location with anxiety and depression in childhood brain cancer survivors: a systematic review and meta-analysis

CHILD AND ADOLESCENT PSYCHIATRY AND MENTAL HEALTH 17: 1 Paper: 124 , 11 p. (2023)

*Citing papers: 5 | Independent citation: 5 | Self citation: 0 | Unknown citation: 0 | Number of citations in WoS: 3 | Number of citations in Scopus: 5 | WoS/Scopus assigned: 5 | Number of citations with DOI: 5*
*Journal subject: Scopus - Pediatrics, Perinatology and Child Health Rank: D1*
*Journal subject: Scopus - Psychiatry and Mental Health Rank: Q1*
*IF: 4.6*

Szigetváry, Csenge Erzsébet; Turan, Caner; Kovács, Emőke Henrietta; Kói, Tamás; **Engh, Marie Anne**; Hegyi, Péter; Csukly, Gábor; Ruszkai, Zoltán; Molnár, Zsolt

Hemoadsorption as Adjuvant Therapy in Acute Respiratory Distress Syndrome (ARDS): A Systematic Review and Meta-Analysis

BIOMEDICINES 11: 11 Paper: 3068, 18 p. (2023)

*Citing papers: 2 | Independent citation: 1 | Self citation: 1 | Unknown citation: 0 | Number of citations in WoS: 2 | Number of citations in Scopus: 1 | WoS/Scopus assigned: 2 | Number of citations with DOI: 2*
*Journal subject: Scopus - Biochemistry, Genetics and Molecular Biology (miscellaneous) Rank: Q1*
*Journal subject: Scopus - Medicine (miscellaneous) Rank: Q1*
*IF: 3.9*

Veres, Boglárka; Fehérvari, Péter; **Engh, Marie Anne**; Hegyi, Péter; Gharehdaghi, Sara; Zima, Endre; Duray, Gábor; Merkely, Béla**; Kosztin, Annamária

Time-trend treatment effect of Cardiac Resynchronization Therapy with or without Defibrillator on Mortality -A Systematic Review and Meta-Analysis.

EUROPACE 25: 10 Paper: euad289, 13 p. (2023)

*Citing papers: 9 | Independent citation: 6 | Self citation: 3 | Unknown citation: 0 | Number of citations in WoS: 7 | Number of citations in Scopus: 9 | WoS/Scopus assigned: 9 | Number of citations with DOI: 9*
*Journal subject: Scopus - Cardiology and Cardiovascular Medicine Rank: D1*

*Journal subject: Scopus - Physiology (medical) Rank: D1*
*IF: 7.9*

Vezér, Mátyás; Gresits, Orsolya; **Engh, Marie Anne**; Szabó, Laszlo; Molnar, Zsolt; Hegyi, Peter; Terebessy, Tamás

Evidence for gait improvement with robotic-assisted gait training of children with cerebral palsy remains uncertain.

GAIT & POSTURE 107 pp. 8-16., 9 p. (2023)

*Citing papers: 6 | Independent citation: 6 | Self citation: 0 | Unknown citation: 0 | Number of citations in WoS: 4 | Number of citations in Scopus: 6 | WoS/Scopus assigned: 6 | Number of citations with DOI: 6*
*Journal subject: Scopus - Rehabilitation Rank: Q1*
*Journal subject: Scopus - Biophysics Rank: Q2*
*Journal subject: Scopus - Orthopedics and Sports Medicine Rank: Q2*
*Journal subject: Scopus - Sports Science Rank: Q3*
*IF: 2.2*

Kulyassa, Péter; Németh, Balázs T.; Ehrenberger, Réka; Ruzsa, Zoltán; Szük, Tibor; Fehérvári, Péter; **Engh, Marie Anne**; Becker, Dávid; Merkely, Béla; Édes, István F.

The Design and Feasibility of the: Radial Artery Puncture Hemostasis Evaluation – RAPHE Study, a Prospective, Randomized, Multicenter Clinical Trial

FRONTIERS IN CARDIOVASCULAR MEDICINE 9 Paper: 881266, 7 p. (2022)

*Citing papers: 1 | Independent citation: 0 | Self citation: 1 | Unknown citation: 0 | Number of citations in Scopus: 1 | WoS/Scopus assigned: 1 | Number of citations with DOI: 1*
*Journal subject: Scopus - Cardiology and Cardiovascular Medicine Rank: Q1*
*IF: 3.6*

Szakó, Lajos; Németh, Dávid; Farkas, Nelli; Kiss, Szabolcs; Dömötör, Réka Zsuzsa; **Engh, Marie Anne**; Hegyi, Péter; Eross, Balint; Papp, András

Network meta-analysis of randomized controlled trials on esophagectomies in esophageal cancer: The superiority of minimally invasive surgery

WORLD JOURNAL OF GASTROENTEROLOGY 28: 30 pp. 4201-4210. , 10 p. (2022)

*Citing papers: 20 | Independent citation: 13 | Self citation: 7 | Unknown citation: 0 | Number of citations in WoS: 14 | Number of citations in Scopus: 17 | WoS/Scopus assigned: 17 | Number of citations with DOI: 18*
*Journal subject: Scopus - Gastroenterology Rank: Q1*
*Journal subject: Scopus - Medicine (miscellaneous) Rank: Q1*
*IF:4.3*

Vörhendi, N.; Soós, A.; **Engh, MA**; Tinusz, B.; Szakács, Z.; Pécsi, D.; Mikó, A.; Sarlós, P.; Hegyi, P.; Erőss, B.

Accuracy of the Helicobacter pylori diagnostic tests in patients with peptic ulcer bleeding: a systematic review and network meta-analysis

THERAPEUTIC ADVANCES IN GASTROENTEROLOGY 13 Paper: 1756284820965324, 14 p. (2020)

*Citing papers: 20 | Independent citation: 19 | Self citation: 1 | Unknown citation: 0 | Number of citations in WoS: 16 | Number of citations in Scopus: 19 | WoS/Scopus assigned: 20 | Number of citations with DOI: 20*
*Journal subject: Scopus - Gastroenterology Rank: Q1*
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# Contrast-enhanced endoscopic ultrasound likely does not improve diagnostic adequacy during endoscopic ultrasound guided tissue acquisition: A systematic review and meta-analysis

Marie Anne Engh [a, b], Brigitta Teutsch [a, b], Alexander Schulze Wenning [a], Yael Hadani [a], Omer Almog [a], Dániel Sándor Veres [a, c], Péter Hegyi [a, b, d, e], Bálint Erőss [a, b, d, *]

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Pancreatic neoplasms
Endoscopic ultrasound-guided fine needle aspiration
Fine needle biopsy

ABSTRACT

*Background and aims:* Solid pancreatic masses are sampled through tissue acquisition by endoscopic ultrasound (EUS). Inadequate samples may significantly delay diagnosis, increasing costs and carrying risks to the patients. Aim: assess the diagnostic adequacy of tissue acquisition using contrast-enhanced harmonic endoscopic ultrasound (CEH-EUS) compared to conventional EUS.

*Methods:* Five databases (PubMed, Embase, CENTRAL, Scopus and Web of Science) were searched in November 2023. Studies comparing diagnostic adequacy, accuracy and safety using CEH-EUS versus conventional EUS for tissue acquisition of solid pancreatic masses were included. Risk of bias was assessed using the Risk of Bias tool for randomized controlled trials (RoB2) and the Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) tool for non-randomized studies, level of evidence using the GRADE approach, Odds Ratios (RR) with 95 % Confidence Intervals (CI) calculated and pooled using a random-effects model. $I^2$ quantified heterogeneity.

*Results:* The search identified 3858 records; nine studies (1160 patients) were included. OR for achieving an adequate sample was 1.467 (CI: 0.850–2.533), for randomized trials 0.902 (CI: 0.541–1.505), for non-randomized 2.396 (CI: 0.916–6.264), with significant subgroup difference. OR for diagnostic accuracy was 1.326 (CI: 0.890–1977), for randomized trials 0.997 (CI: 0.593–1.977) and for non-randomized studies 1.928 (CI: 1.096–3.393), significant subgroup difference ($p = 0.0467$). No differences were observed for technical failures or adverse events. Heterogeneity was low, risk of bias "low" to "some concerns" for most outcomes, mostly moderate for non-randomized studies.

*Conclusion:* Non-randomized studies indicated differences in favor of contrast-enhanced EUS, randomized studies showed no difference in diagnostic adequacy, accuracy or sensitivity when using CEH-EUS.


## 1. Introduction

Pancreatic cancer is a leading cause of cancer death worldwide [1,2], with cases usually detected late and treatment options being scarce. One commonly used diagnostic tool for solid pancreatic masses is endoscopic ultrasound (EUS) guided tissue acquisition – aspiration or biopsy [3–5]. The sampling is an invasive procedure that requires sedation, clinical resources, and risks side effects, including pancreatitis and bleeding. Fourteen percent of samples are not adequate for histology and eight percent not adequate for cytology after up to two needle passes [6], as pancreatic masses may be difficult to target and are often surrounded by scar tissue or necrotic areas, which may decrease diagnostic sensitivity [3,7,8]. Improving the efficacy of EUS-guided tissue acquisition (EUS-TA) is important for a few reasons. A higher number of needle passes in one session may prolong the procedure and the need for sedation.

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**List of abbreviations**

| | |
|---|---|
| **CEH-EUS** | Contrast enhanced harmonic endoscopic ultrasound |
| **CI** | Confidence Interval |
| **EUS** | Endoscopic Ultrasound |
| **FNA** | Fine needle aspiration |
| **FNB** | Fine needle biopsy |
| **GRADE** | Grading of Recommendations, Assessment, Development and Evaluations |
| **OR** | Odds Ratio |
| **PRISMA** | Preferred reporting items for systematic reviews and meta-analyses |
| **RR** | Risk Ratio |
| **TA** | Tissue acquisition |

It may also lead to higher costs due to greater equipment use [9].

Several strategies have been tested to decrease the rate of inadequate sampling and to protect patients from unnecessary re-intervention or re-puncture. Among the suggested strategies are the use of different needle tip designs, different types of suction, and variations of other technologies used.

Contrast-enhanced ultrasound allows better visualization of vessels in tissue, thereby allowing more precise discrimination of scar tissue from biologically active tissues [3,10]. This ability may allow better targeting of the mass for sampling, and studies have been carried out to determine whether this may increase diagnostic sensitivity and decrease the rate of inadequate samples. Contrast-enhanced EUS during the puncture has previously been shown to be cost-effective in a retrospective study that suggested that reducing the number of needles used off-set the cost of using contrast during the EUS [9]. CEH-EUS is discussed in the most recent European Society of Gastrointestinal Endoscopy (ESGE) guidelines for sampling of solid masses as a potential method to improve the sampling rate of solid pancreatic masses in patients with chronic pancreatitis. Here, guidelines highlight the inconclusive results in recent studies [5,11,12]. Recent ESGE guidelines on technical aspects of EUS-guided sampling specifically chose to not give recommendations due to the lack of evidence on the subject [13].

In this systematic review and meta-analysis, we aimed to assess the published evidence of the impact of contrast-enhanced ultrasound on sampling efficacy and safety during EUS-guided tissue acquisition of pancreatic solid masses.

## 2. Methods

### 2.1. Reporting and protocol

We report this systematic review and meta-analysis according to the recommendations of the PRISMA 2020 guideline (see Supplementary Table S1), and during the process, we followed the methodological guidance of the Cochrane Handbook [14]. The protocol of this review was registered on PROSPERO (CRD42022285023). The following protocol deviations occurred: Search and selection was expanded to include non-randomized studies during peer-review, diagnostic parameters from the included studies were pooled for added information on the clinical importance of any potential differences.

### 2.2. Inclusion criteria

Studies reporting on patients undergoing EUS-guided tissue acquisition (EUS-TA) for a solid pancreatic mass were included if they compared the use of contrast-enhanced EUS to that of conventional EUS and investigated the diagnostic adequacy, rate of adverse events and technical failures, number of needle passes or tissue yield. Randomized controlled trials, non-randomized interventional studies and prospective and retrospective cohort studies were eligible for inclusion. In cases where studies reported having assessed an outcome but did not publish results for that outcome, the corresponding author was contacted, and the relevant data were requested.

### 2.3. Outcomes

1.) Diagnostic adequacy

Diagnostic adequacy was chosen as the primary outcome, as EUS-TA is a sampling method, not a diagnostic method – and the diagnosis is made by a histopathologist following the sampling. Diagnostic adequacy was defined using the definition used in the papers, or where unavailable, as the inverse of inadequate or non-diagnostic samples.

2.) Diagnostic test parameters

The included studies and a previous meta-analysis [15] reported diagnostic test parameters. However, in most studies these comparisons were not reported in a way appropriate for diagnostic test meta-analysis, as diagnostic test meta-analysis requires sensitivity and specificity reported together, to analyze these as mutually dependent [16].

To assess diagnostic test parameters, the following outcome measures were used:

Sensitivity, Specificity and Accuracy for malignant versus benign cases were treated as regular dichotomous outcomes and used both for individual quantification of efficacy of both types of ultrasound (as proportions), and to compare the two using ratios (Risk or Odds Ratios). True negatives (TN), false positives (FP), true positives (TP) and false negatives (FN) were extracted or calculated from sensitivity or specificity and case numbers where available. Sensitivity was calculated as the proportion of TP to all malignant cases, specificity was calculated as the proportion of TN to all benign cases, and accuracy was calculated as the proportion of all correctly identified patients (benign or malignant) to all cases. Further, these numbers were used to conduct a regular, bivariate diagnostic meta-analysis as well.

3.) Adverse events

Adverse events were included as a safety outcome where available, using the definitions of the included papers.

4.) Needle passes needed

The number of needle passes needed to achieve an adequate sample were extracted from papers and pooled as a continuous outcome.

### 2.4. Eligibility for synthesis

As the papers differed in number of needle passes performed and this was considered an important confounding factor, this was considered when deciding which articles to pool. The data was

tabulated, and an analysis was performed for each needle pass for which basic requirements for analysis were satisfied (minimum of 3 eligible articles for the outcome). For studies which only gave a mean number of passes, this was the number considered. An additional analysis was performed including the final pass from each study.

### 2.5. Search and selection

The systematic search was performed on November 19th, 2023, in five major databases (Medline – via PubMed, Embase, CENTRAL, Scopus, and Web of Science). The search key consisted of domains representing pancreatic masses, tissue acquisition, and contrast-enhanced ultrasound (see supplementary material).

After automatic and subsequent manual duplicate removal, the selection was performed by two independent review authors (MAE, ASW) in two stages (by title and abstract, then by full text), with any disagreements resolved by a discussion. The degree of agreement was quantified using Cohen's kappa [17].

References of the included articles were systematically searched using an online tool [18].

### 2.6. Data extraction

Data extraction was performed by two reviewers independently (YH, OA) and compared by a third author (MAE). It was done in a pre-designed Excel sheet, and data were extracted on basic data of the study (author, year, location, number of centers), population data (age, sex, location of pancreatic mass), procedure data (details of sampling, the experience of the endoscopist and pathologist), outcomes (diagnostic adequacy, adverse events, technical failures, number of needle passes, tissue yield, rates of accurate diagnoses, rates of diagnostic sensitivity, diagnostic data (TP, FP, TN, FN) and their definitions.

### 2.7. Risk of bias and quality of evidence assessment

For randomized controlled trials (RCTs), the risk of bias was assessed using version 2 of the Cochrane Risk of Bias tool [19]. This assessment was performed by two independent reviewers (YH, OA), with disagreements resolved by a third reviewer (ME). For non-randomized studies of interventions, the Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) tool [20] was used and the assessments were performed by two independent reviewers (MAE, ASW) with disagreements resolved by discussion.

The strength of evidence was assessed with the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) approach [21] with the help of the GradePRO software [22].

### 2.8. Synthesis methods

Risk ratios (RRs) with a 95 % confidence interval (CI) were used for the effect size measure for the results from RCTs, as it is easier to interpret and RCTs have a higher level of evidence. When including non-randomized studies, odds ratios (ORs) were used instead. To calculate these ratios, the total number of patients and those with the event of interest (in each group separately) was extracted from each study. The results are reported as risk or odds of event of interest in the CEH-EUS group, versus the risk or odds of event of interest in the conventional group. For continuous outcomes, mean differences (MDs) with 95 % confidence interval (CI) were used and reported as the mean in the CEH-EUS group minus the mean in the conventional group. For diagnostic outcomes, different effect sizes were used: RRs or ORs for direct comparison, proportions for sensitivity, specificity and accuracy, As we anticipated considerable between-study heterogeneity, a random-effects model was used to pool effect sizes. Pooled RR and OR based on raw data was calculated by the Mantel-Haenszel method [23–25]. The exact Mantel-Haenszel method (without continuity correction) was used to handle zero cell counts. We used Hartung-Knapp adjustments for CIs. To estimate the heterogeneity variance measure $\tau 2$, the Paule-Mandel method [26] with the Q profile for confidence interval was applied [27]. Forest plots were used to graphically summarize the results. However, due to the small number of studies, assessing publication bias or performing outlier and influential analyses were not possible. All statistical analyses were made with R (R Core Team 2022, v4.2.1) using the meta (v5.5.0) package [28] and dmetar [29] for meta-analysis calculations. More detailed descriptions of analysis can be found in the supplementary material.

## 3. Results

### 3.1. Search and selection

Our search identified 7200 studies, of which 3852 remained after duplicate removal. Cohen's kappa of title abstract selection was 0.79 (substantial agreement), while that of full-text selection was 1.0 (perfect agreement). Nine studies [9,11,12,30–35] were included for synthesis, reporting on 1160 patients. Our search also identified two protocols of ongoing randomized trials [36,37]. The exact progression of selection is detailed in the PRISMA flowchart (Fig. 1).

The baseline characteristics of included studies are detailed in Table 1. Supplementary Table S2 lists the identified protocols of ongoing trials not already published and their details.

### 3.2. Diagnostic adequacy

#### 3.2.1. Final pass

Seven studies total reported adequacy, four randomized trials [11,12,32,33] (three [11,32,33] reported the outcome in the text, data for the fourth [12] was provided by the corresponding author at our request), and three non-randomized studies [9,31,35]. The pooled OR for achieving an adequate sample was 1.467 (CI: 0.850–2.533), with subgroup totals of 0.902 (CI: 0.541–1.505) for randomized trials and 2.396 (CI: 0.916–6.264) for observational studies(Fig. 2). The test for subgroup differences was significant ($p = 0.0045$). For the analysis of randomized trials only, the pooled RR for achieving an adequate sample was 1.002 (95 % CI: 0.81–1.39), $i^2$ was 0 % (Fig. S1).

#### 3.2.2. 1st pass

Four studies reported the diagnostic adequacy after the first pass, three RCTs [11,12,32] and one non-randomized study [35]. The pooled OR for adequacy was 2.263 (CI: 0.960–5.334) (Fig. S2). Two studies appeared to indicate no difference [12,32], while two studies [11,35] were significantly in favor of CEH-EUS. Pooling only randomized trials, RR was 1.171 (CI: 0.433–3.170), see Fig. S3.

### 3.3. Diagnostic accuracy

#### 3.3.1. Final pass

Seven studies in total reported data necessary to calculate the accuracy, four randomized trials [11,12,32,33] and three non-randomized studies [9,30,31]. The pooled OR for diagnostic accuracy was 1.326 (CI: 0.890–1977), with subgroup totals of 0.997 (CI: 0.593–1.977) for randomized trials and 1.928 (CI: 1.096–3.393) for observational studies. The test for subgroup difference was significant ($p = 0.0467$), heterogeneity was low ($i^2 = 0$ %) (Fig. 3). The RR

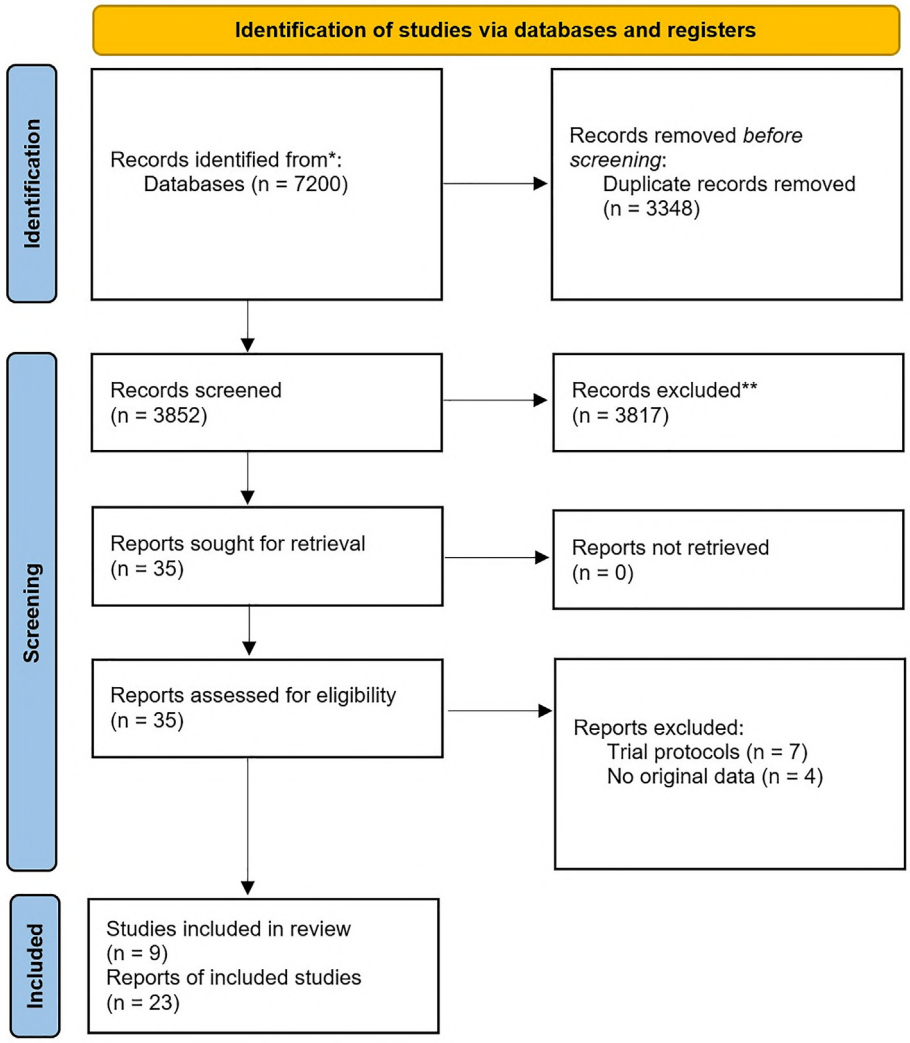


**Fig. 1.** PRISMA Flowchart detailing the selection process.

analysis including only randomized trials was 0.988 (CI: 0.959–1.017), heterogeneity was low ($i^2 = 0$ %) (Fig. S4).

*3.3.2. 1st pass*

Three studies [12,32,33] reported the accuracy after the first pass. The pooled OR for diagnostic accuracy was 1.182 (CI: 0.806–1.733). Heterogeneity was low ($i^2 = 0$ %). The forest plot of this analysis may be found in the supplementary material (Fig. S5).

*3.3.3. 2. pass*

Three studies [12,32,33] reported the accuracy after the second pass. The pooled OR for diagnostic accuracy after the second pass was 1.123 (CI: 0.340–3.706). The forest plot of this analysis may be found in the supplementary material (Fig. S6).

*3.4. Sensitivity and specificity*

1.) Ratios

Sensitivity: Nine studies reported data necessary to calculate sensitivity ratios, the pooled OR was 1.494 (CI: 1.052–2.121). In the subgroup for RCTs, the pooled OR was 0.968 (0.535–1.753), in that for non-randomized studies it was 1.950 (1.294–2.940). The test for subgroup differences was significant ($p = 0.0125$), heterogeneity was low ($i^2 = 0$ %). Including only RCTs, the pooled RR was 0.998 (CI: 0.965–1.033). Heterogeneity was low ($i^2 = 0$ %) (Fig. 4).

Specificity: Six studies [9,30–32,35] reported data necessary to calculate specificity ratios, however, due to a 100 % specificity rate in all but 1 study [32], pooling was not feasible.

2.) Proportions

Sensitivity: All studies reported data necessary to calculate sensitivity proportions. The pooled proportion in the case of CEH-EUS was 0.887 (CI: 0.826–0.928), with RCTS at 0.923 (0.694–0.985) and non-randomized studies at 0.858 (CI: 0.766–0.918). Heterogeneity was low ($i^2 = 7$ %), test for subgroup differences not significant ($p = 0.2281$) (Fig. S6). For conventional EUS the proportion was 0.854 (CI:0.740–0.924), in the subgroup of randomized trials 0.923 (CI: 0.696–0.985), in the subgroup of non-randomized studies 0.780 (CI: 0.620–0.885). Heterogeneity was substantial ($i^2 = 79$ %), the test for subgroup differences was significant ($p = 0.0384$) (Fig. S7).

Specificity: Six studies [9,30–32,35] reported data necessary to calculate specificity proportions, however, due to a 100 % specificity rate in all but 1 study [32], pooling was not feasible.

**Table 1**
Baseline characteristics of included studies. RCT: Randomized controlled trial, EUS-FNA: Endoscopic Ultrasound-guided Fine Needle Aspiration, CEH-EUS: contrast-enhanced harmonic endoscopic ultrasound, mm: millimeter.

| Author (Year) | Country | Enrollment Period | Number of patients (female %) | Age (years) | Size of lesion (mm) | Study design | Endoscopist experience | Sampling technique | Ultrasound technique | Needle | Reference for diagnostic test parameters | Malignant NET Benign |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cho (2021) | South Korea | March 2016 −September 2019 | 240 (47.1 %) | 67.3 (±11.85) EUS: 68.28 (±11.90), CEH-EUS: 66.31 (±11.78)[a] | 32.03 (±14.41) EUS: 33.09 (±16.39), CEH-EUS: 30.96 (±12.09)[a] | Parallel RCT | "Experienced endosonographers" | 10 mL negative pressure 20 to-and-fro movements | GF-UCT 260; Olympus. CEH-EUS: 2.4 mL SonoVue, 10 mL saline flush | 19-25G FNA or FNB | Pathology results of FNA/FNB sampling or the surgical specimen. If unavailable, imaging studies 6 months after the endoscopic procedure. Malignancy where lesion progression or metastasis was observed on follow-up imaging, benign disease with a stable lesion without an increasing size or metastasis. | 90.8 % 3.8 % 5.4 % |
| Facciorusso (2020) | Italy | January 2008 −December 2019 | 362 (40.6 %), 206 (45.1 %) after propensity score matching | Matched population: EUS: 66 ± 8 CEH-EUS ±6 | Matched population: EUS: 32 ± 1, CEH-EUS: 32 ± 1.1 | Propensity-score matched analysis, prospective | "Board certified gastroenterologist with 20 years' experience" | 10 mL negative pressure "more than 10 to-and-fro movements" | Pentax FG-36UA CEH-EUS: 4.8 mL SonoVue followed by 20 mL saline flush | 22G FNA (EchoTip Ultra, Cook Medical) | Surgical pathology or clinical course (progression or death, clinical changes indicative of diagnosis of benign disease) during follow-up of 12 months | 73.9 % 6.7 % 19.4 % |
| Hou (2015) | China | October 2010−July 2013 | 163, CEH-EUS: 59 (38 %), Conventional: 105 (40 %) | CEH-EUS: 55.1 (±11.7)[a] Conventional: 56.2 (±12.5)[a] | Ceh-EUS: 38 (±12)[a] Conventional: 39 (±8) [a] | Post-hoc analysis of prospectively collected data | "Experienced Endosonographer" | NA | GFUCT2000(Olympus) CEH-EUS: GFUC-30p (Olympus) 4.8 mL SonoVue, 20 mL saline flush | 22G needle (Wilson Cook Medical) | Surgical pathology, malignant cytology with clinical progression compatible with the diagnosis, or death from malignancy. In the absence of surgical confirmation, 12 month follow-up for disease progression or resolution of imaging or clinical changes. | 61.3 % 6.13 % 32.5 % |
| Itonaga (2020) | Japan | October 2016 −October 2017 | 93 (46.3 %) | 72.5 (34−89) [c] | 25.2 (12−56) [c] | Prospective cohort with crossover | >300 EUS-FNA procedures | Negative pressure with 20 mL syringe, 20 to-and-fro movements | GF-UCT260 (Olympus) CEH-EUS: No information regarding contrast agent. | 22G FNA (EZ shot 3, Olympus) | Surgical pathology result or 12 month follow-up with US, CT, MRI and/or EUS every 2−6 months or until death. | 90.3 % 4.3 % 5.4 % |
| Kuo (2023) | Taiwan | February 2019 −January 2021 | 118 (39 %) | 64.4 (±12.1) [a] | 37.5 (30−46) [b] | Parallel RCT | "Experienced Endosonographers" | No suction. Conventional: 4x4 to-and-fro movement, fanning technique. CEH-EUS: 16 to-and-fro movements | GF-UCT260, (Olympus) CEH-EUS: 0.015 mL/kg body weight Sonazoid, 10 mL saline flush | 22G FNB (Acquire, Boston Scientific) | Histopathological diagnosis surgical specimen, EUS-FNB with a compatible clinical course, or negative FNB diagnosis with no deterioration on imaging studies for follow-up time of 6 months | 89 % 2.5 % 3.4 % |
| Lai (2022) | Taiwan | January 2019 −March 2021 | 155 (53.5 %) | 63.64 (±12.58) | 31.8 (±16.0) CEH-EUS: 29.5 (±11.5) Conventional: 34.8 (±18.2) | Retrospective chart review, CEH-EUS patients volunteered to self-pay procedure | "Two endoscopists who achieved the FNA learning curve" | Fanning method from at least 4 areas, slow-pull or low-negative suction | GF-UCT260, (Olympus) CEH-EUS: 0.015 mg/kg Sonazoid | 22G FNB (Acquire, Boston Scientific) | Successful FNB diagnosis (suspicious or positive), surgical direct biopsy or transabdominal echo-guided metastatic lesion biopsy. In benign diagnosis: Imaging follow-up for at least 6 months. | 74.2 % 11.6 % 12.2 % |

(*continued on next page*)

**Table 1** (*continued*)

| Author (Year) | Country | Enrollment Period | Number of patients (female %) | Age (years) | Size of lesion (mm) | Study design | Endoscopist experience | Sampling technique | Ultrasound technique | Needle | Reference for diagnostic test parameters | Malignant<br>NET<br>Benign |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seicean (2015) | Romania | November 2012−March 2013 | 51 (41.2 %) | 54 (30−83) [b] | 35 | Prospective cohort with crossover | No information | No suction, fanning technique used where possible. | GF-UCT180-AL5 (Olympus).<br>CEH-EUS:<br>2.4 mL SonoVue followed by 5 mL saline flush- | 22G FNA (Olympus) | FNA results in 38 patients, FNA + Surgical pathology in 13, in case of negative FNA findings 12-month clinical follow up, transabdominal ultrasound at 3-month intervals, repeated spiral CT/EUS if needed. | 78.4 %<br>1.9 %<br>19.6 % |
| Seicean (2020) | Romania | January 2017 −October 2019 | 150 (43.2 %) | 64.5 (±11.3) [a] | 30 (20.8−35) [b] | Crossover RCT | >7000 EUS-FNA and >500 CEH-EUS | Slow-pull, 10 to-and-fro movements | GF-UCT 180 AL5 (Olympus)<br>CEH-EUS:<br>2.4 ml SonoVue, 5 mL saline flush | 22G FNA (Expect, Boston Scientific) | FNA results or post-surgical histopathological examination. Negative FNA findings: 12 month clinical follow up, CT at 3 months, subsequent transabdominal ultrasound at 3-month intervals. | 78.3 %<br>8.8 %<br>12.8 % |
| Sugimoto (2015) | Japan | September 2013−June 2014 | 40 (62.5 %) | CEH-EUS: 69.5 (±10.5) [a]<br>Conventional: 67.1 (±9.9) [a] | CEH-EUS: 25.0 (±8.0) [a]<br>Conventional: 26.5 (±9.2) [a] | Parallel RCT | 1st pass <100 EUS-FNA, 2nd pass >300 EUS-FNA | Negative pressure with 10 ml syringe, 20 to-and-fro movements | GF-UCT 260, GF-UCT24-AL5 (Olympus)<br>CEH-EUS:<br>0.015 ml/kg Sonazoid | CEH-EUS: 22G FNB (Expect, Boston Scientific),<br>Conventional: 22G or 25G Expect (Boston Scientific), 25G Echotip (Cook Medical), 22G EZ shot2 (Olympus Medical Systems) | Surgical specimens, if unresectable then based on EUS-FNA and imaging. Cytology class IV/V was deemed malignant | 100 %<br>0 %<br>0 % |

[a] Mean + SD.
[b] Median and range.
[c] Mean and range.

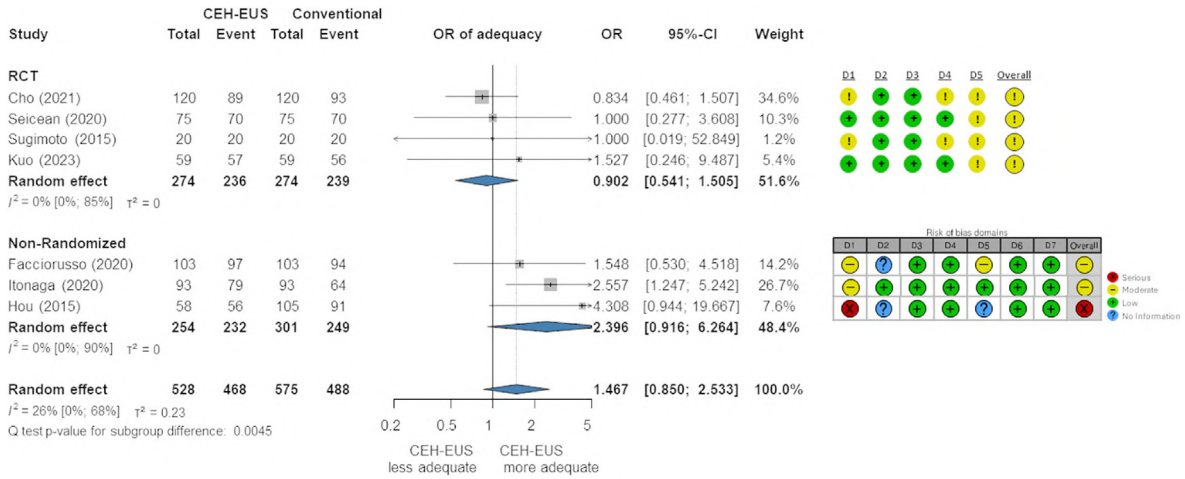


**Fig. 2.** Forest plot of pooled odds ratios for an adequate sample following tissue acquisition (FNA/FNB) using contrast-enhanced versus conventional ultrasound. Data for final needle pass used in each study. Results of the are summarized on the right by study, domain and overall. For Cochrane risk-of-bias tool v.2 (RoB2): Domains: D1: Risk arising from the randomization process, D2: Bias due to deviations from intended intervention, D3: Bias due to missing outcome data, D4: Bias in the measurement of the outcome, D5: Bias in the selection of the reported result. For Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) tool: D1: Bias due to confounding, D2: Bias due to selection of participants, D3: Bias in classification of interventions, D4: Bias due to deviations from intended interventions, D5: Bias due to missing data, D6: Bias in measurement of outcomes, D7: Bias in selection of the reported results
CEH-EUS: Contrast-enhanced harmonic endoscopic ultrasound, OR: Odds Ratio.

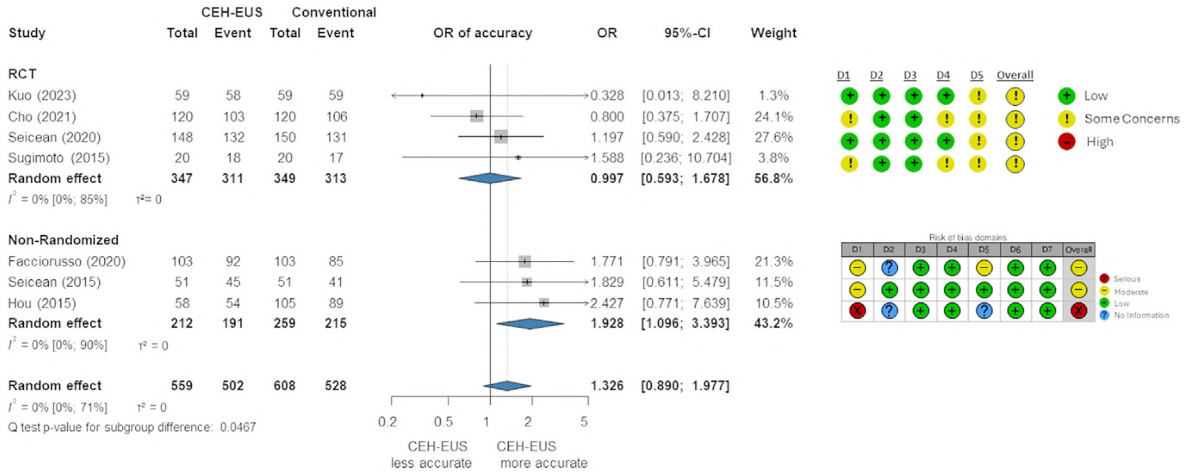


**Fig. 3.** Forest plot of pooled odds ratios for accurately diagnosing both negative and positive cases (diagnostic accuracy). Results of the Risk of Bias assessment are summarized on the right by study, domain and overall. For Cochrane risk-of-bias tool v.2 (RoB2): Domains: D1: Risk arising from the randomization process, D2: Bias due to deviations from intended intervention, D3: Bias due to missing outcome data, D4: Bias in the measurement of the outcome, D5: Bias in the selection of the reported result. For Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) tool: D1: Bias due to confounding, D2: Bias due to selection of participants, D3: Bias in classification of interventions, D4: Bias due to deviations from intended interventions, D5: Bias due to missing data, D6: Bias in measurement of outcomes, D7: Bias in selection of the reported results
CEH-EUS: Contrast-enhanced harmonic endoscopic ultrasound, OR: Odds Ratio.

3.) Bivariate Diagnostic Meta-Analysis

Conventional EUS: Six studies [9,12,30–32,35] reported data to calculate diagnostic test parameters in a bivariate basis. Bivariate analysis showed a sensitivity of 0.835 (CI: 0.673–0.926) for conventional EUS. Subgroup analysis for study type was not feasible. Heterogeneity was substantial ($i^2$ = 75 %). Pooled specificity with bivariate analysis was 1.000 (CI: 0.000–1.000), heterogeneity was low ($i^2$ = 0 %) (Fig. S8).

CEH-EUS: Six studies [9,12,30–32,35] reported data to calculate diagnostic test parameters in a bivariate basis. Bivariate analysis showed a sensitivity of 0.892 (CI: 0.807–0.942) for conventional EUS. Subgroup analysis for study type was not feasible. Heterogeneity was low ($i^2$ = 22 %). Pooled specificity with bivariate analysis was 0.998 (CI: 0.476–1.000), heterogeneity was low ($i^2$ = 0 %) (Fig. S8).

### 3.5. Adverse events

Adverse events were reported in all randomized trials, however two studies [11,12] reported zero event rates, while the other two [32,33] observed equal events in both arms. The RR for adverse events was 1.00 (95 % CI: 0.29–3.41), shown in Fig. 5.

### 3.6. Technical failures

No article reported the rate of technical failures, however Seicean et al. provided data on the rate of technical failures upon our request for their randomized trial. No technical failures were observed in either treatment arm.

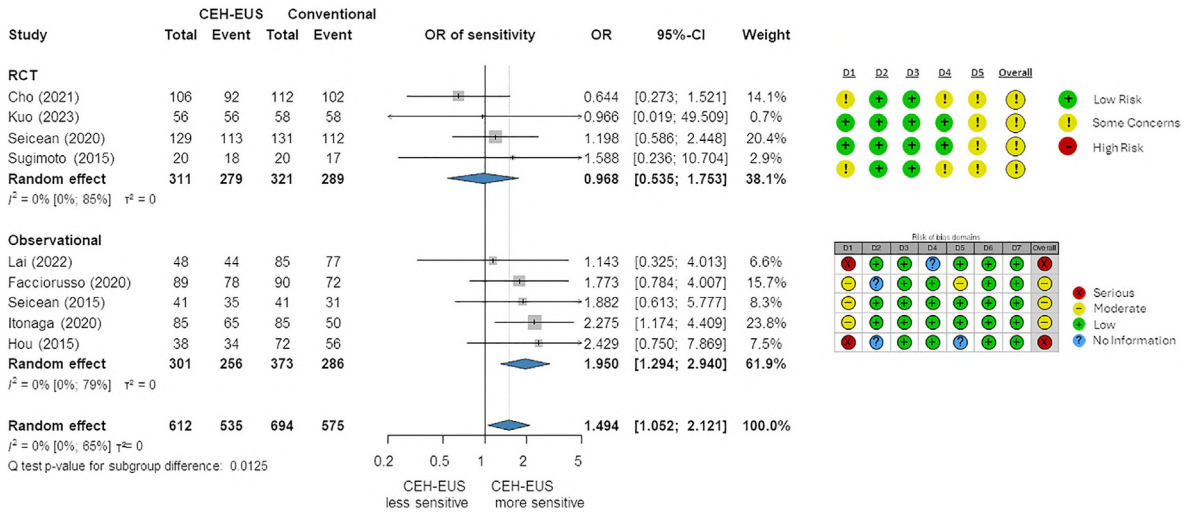


**Fig. 4.** Forest plot of pooled odds ratios for accurately identifying positive cases (diagnostic sensitivity). Results of the Risk of Bias assessment are summarized on the right by study, domain and overall. For Cochrane risk-of-bias tool v.2 (RoB2): Domains: D1: Risk arising from the randomization process, D2: Bias due to deviations from intended intervention, D3: Bias due to missing outcome data, D4: Bias in the measurement of the outcome, D5: Bias in the selection of the reported result. For Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) tool: D1: Bias due to confounding, D2: Bias due to selection of participants, D3: Bias in classification of interventions, D4: Bias due to deviations from intended interventions, D5: Bias due to missing data, D6: Bias in measurement of outcomes, D7: Bias in selection of the reported results
CEH-EUS: Contrast-enhanced harmonic endoscopic ultrasound, OR: Odds Ratio.

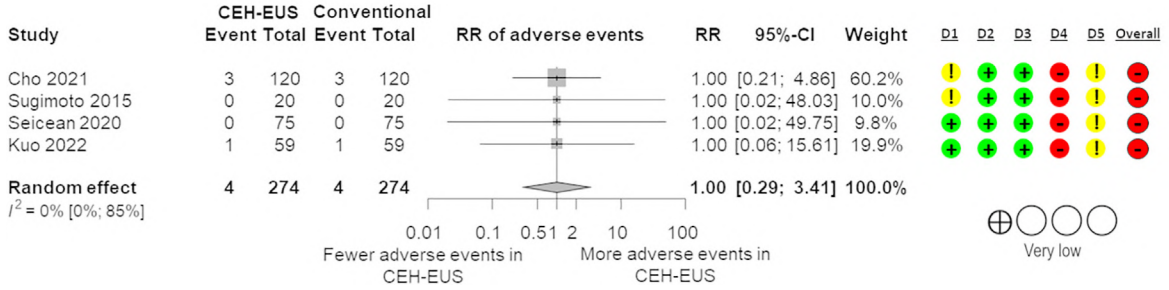


**Fig. 5.** Forest plot of pooled odds ratios for adverse events. Results of the Risk of Bias assessment are summarized on the right by study, domain and overall. For Cochrane risk-of-bias tool v.2 (RoB2): Domains: D1: Risk arising from the randomization process, D2: Bias due to deviations from intended intervention, D3: Bias due to missing outcome data, D4: Bias in the measurement of the outcome, D5: Bias in the selection of the reported result. For Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) tool: D1: Bias due to confounding, D2: Bias due to selection of participants, D3: Bias in classification of interventions, D4: Bias due to deviations from intended interventions, D5: Bias due to missing data, D6: Bias in measurement of outcomes, D7: Bias in selection of the reported results
CEH-EUS: Contrast-enhanced harmonic endoscopic ultrasound, OR: Odds Ratio.

### 3.7. Number of needle passes

Three studies [9,31,34] (all non-randomized) reported the mean number of needle passes to achieve an adequate sample. The mean difference was −0.54 (CI: 2.50-1.42), heterogeneity was substantial ($i^2 = 90$ %) (Fig. 6).

Kuo et al. reported the number of needle passes in terms of cumulative diagnostic accuracy after each needle pass, all given with 95 % Cis. They found that while the first needle pass yielded 76.3 % (CI: 63.4–86.4) accuracy in the CEH-EUS group and 72.9 % (CI: 59.7–83.6) accuracy in the conventional group (p-value: 0.833), this improved to 91.5 % (CI: 81.3–97.2) and 86.4 % (CI: 75.0–94.0) with the second pass (p-value: 0.558) and 93.2 % (CI: 88.3–99.6) and 94.9 % (CI: 85.9–98.9) with the third pass (p-value: 1). The fourth pass (CEH-EUS: 96.6 %, CI: 88.3–99.6 versus Conventional: 94.9 %, CI: 85.9–98.9), fifth pass (CEH-EUS: 96.6 %, CI: 88.3–99.6, Conventional: 96.6 %, CI: 88.3–99.6) and sixth pass (CEH-EUS: 98.3 %, CI: 90.9–100; Conventional: 100 %) also showed no difference (p-value: 1).

Sugimoto et al. reported adequacy after each needle passes up to 5 passes. They found that while the first needle pass yielded 60 % adequacy in the CEH-EUS group, and 25 % adequacy in the conventional group, this improved to 75 % and 65 % with the second pass and 90 % and 95 % with the third pass. In the CEH-EUS group, 100 % adequacy was achieved already on the fourth pass, while the conventional EUS group reached 95 % and finally 100 % on the fifth pass.

Cho et al. reported the number of needle passes in terms of diagnostic sensitivity after each needle pass, with 95 % Cis. They found that while the first needle pass yielded 70.0 % (CI: 61.2–77.5) sensitivity in the CEH-EUS group and 66.7 % (CI: 57.8–74.5) sensitivity in the conventional group, this improved to 80.0 % (CI: 71.9–86.2) and 83.3 % (CI: 75.6–89.0) with the second pass and 85.0 % (CI: 77.4–90.3) and 88.3 % (CI: 81.3–93.0) with the third pass. Further passes yielded limited improvement, at 85.8 % (CI: 78.4–91.0) and 88.3 % (CI: 81.3–93.0) for both the fourth and fifth needle passes.

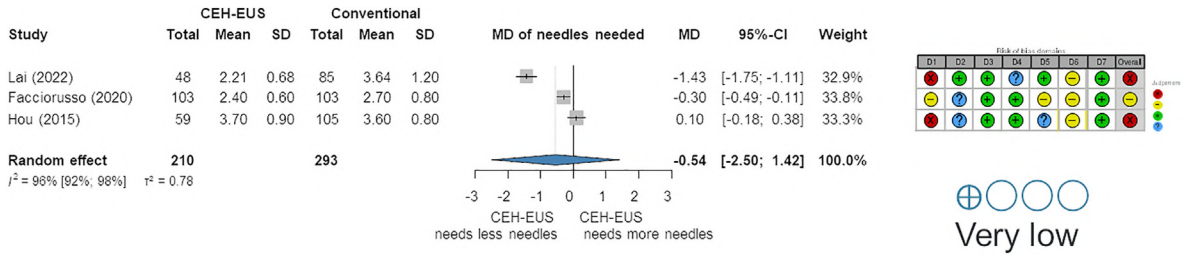


**Fig. 6.** Forest plot of mean differences of number of needle passes until adequacy. Results of the Risk of Bias assessment are summarized on the right by study, domain and overall. For Cochrane risk-of-bias tool v.2 (RoB2): Domains: D1: Risk arising from the randomization process, D2: Bias due to deviations from intended intervention, D3: Bias due to missing outcome data, D4: Bias in the measurement of the outcome, D5: Bias in the selection of the reported result. For Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) tool: D1: Bias due to confounding, D2: Bias due to selection of participants, D3: Bias in classification of interventions, D4: Bias due to deviations from intended interventions, D5: Bias due to missing data, D6: Bias in measurement of outcomes, D7: Bias in selection of the reported results
CEH-EUS: Contrast-enhanced harmonic endoscopic ultrasound.

### 3.8. Tissue yield

Only Kuo et al. reported sample size based on modality. In the contrast-enhanced group the median macroscopic visible core was 18 mm (IQR: 10–26), while the conventional/fanning group had a median macroscopic visible core of 18 mm (IQR: 11–30). There was no difference (p-value: 0.598).

### 3.9. Risk of bias assessment

Results of the Risk of Bias assessment are detailed on forest plots (Figs. 2–6). Some concerns were noted in Cho and Sugimoto regarding the randomization process, as there was no information about allocation concealment leading up to enrollment, as well as regarding the measurement of adequacy (no information/no blinding). The assessment of the selection of reported results also caused some concerns for the outcome of adequacy in all studies (lack of information in the pre-registered study plan), and for diagnostic test parameters in Kuo et al. and Sugimoto et al. (lack of information in the pre-registered study plan). Finally, for the outcome of adverse events, the risk of bias was high in all studies due to the measurement of outcome (no information regarding blinding, the definitions or measurement in any paper).

In the non-randomized studies, most domains were low or moderate, with not enough information for an assessment for domain 2 in two studies [9,31], for domain 4 in one [34] and for domain 5 in one study [9]. Most studies managed to mitigate the intrinsic bias of non-randomized studies through matching or crossover and received a rating of moderate for confounding, except for two: In one, patients who received the intervention were those who were willing to pay for it [34], in another [9], the reason for giving each intervention was not elaborated on and only patient charts were retrospectively reviewed. Both these papers received a rating of serious risk for domain 1 (confounding), and an overall rating of serious. All other studies received an overall rating of moderate.

### 3.10. Strength of evidence

Where results for randomized trials were pooled, GRADE assessment was performed only on that sub-analysis, as level of evidence is higher. For diagnostic adequacy, level of evidence was moderate. For diagnostic accuracy and sensitivity, level of evidence was low. For adverse events, number of needle passes and the not pooled evidence for technical failures the level of evidence was rated as very low. For details of reasons for downgrading and the complete table of the GRADE assessment, see Supplementary Table S3.

## 4. Discussion

While this review found a significant difference for sensitivity, favoring CEH-EUS, and strong trends for the outcomes of adequacy, accuracy and specificity, this difference was driven solely by differences in non-randomized studies, and the test for subgroup differences between randomized and non-randomized studies was significant in all cases. Individual analyses including only randomized controlled trials were all indicative of no difference in efficacy or safety between the two.

The difference in results between subgroups based on study type is strongly indicative of some baseline factors that may be at play, potentially contributing to better outcomes when using contrast-enhanced EUS: In the study by Facciorusso et al., patients were matched using propensity score matching, this was the observational study most closely aligned to the results of the randomized controlled trials. Both Seicean et al. and Itonaga et al. performed a crossover to match the patients, however always performing conventional EUS first. It stands to reason that, when performing these two passes consecutively in one intervention, the experience of performing the first pass may disproportionately benefit the success of the second. The study by Hou was a retrospective study and the criteria for receiving the different interventions were not clear. In the study by Lai et al., patients receiving CEH-EUS were those who were willing to pay for it out of pocket – however, this study was the exception among non-randomized studies, and showed no difference between conventional or contrast-enhanced EUS.

Interestingly, when proportions were pooled for diagnostic test parameters, significant subgroup differences were found between study types in the case of conventional EUS, but not in the case of CEH-EUS. This could potentially indicate that somehow, conventional EUS is performing worse in non-randomized studies than in the randomized trials.

All randomized trials reported adverse events/complications; however, two reported zero events, while the other two reported an equal but low (2.5 %, 1.7 %) rate of adverse events. While the meta-analysis of this outcome is weak, the individual results of studies still suggest that complications are rare and do not differ depending on the type of EUS used. Studies on EUS-guided tissue acquisition are commonly underpowered when assessing adverse events due to their rare nature [13].

The analysis of mean number of needle passes until adequacy showed a tendency towards fewer needle passes needed when using CEH-EUS, albeit an insignificant one. The point estimate showed a mean difference of half a needle pass less with the use of CEH-EUS, which – if a genuine difference – might mean that half the patients would need one fewer needle pass. While half a needle

pass may not be a clinically significant difference for an individual patient, one pass fewer for every second patient may still represent a beneficial effect on a population level. This difference however was largely driven by Lai et al., a retrospective study that was judged at serious risk of bias due to the way the treatments were assigned. The more well-designed, lower risk of bias paper by Facciorusso et al., which applied propensity score matching, showed a much smaller – albeit still statistically significant difference in favor of CEH-EUS. All these results should be considered with the caveat that they are based on non-randomized studies.

An additional three randomized trials [11,32,33] which could not be pooled for mean number of passes due to having a predetermined number of passes performed, reported data for separate needle passes. In these studies, CEH-EUS performed better for the first needle pass, albeit for diagnostic sensitivity or accuracy instead of adequacy in two studies, and for inexperienced endoscopists in the third. This difference quickly disappeared with repeated needle passes, even reversing non-significantly by the third needle pass in all three studies. The difference was only statistically significant in the study by Sugimoto et al., which will be further addressed.

On the topic of needle passes, another interesting trend was seen. In both the conventional and the CEH-EUS group, there was a clear tendency that the additional benefit after further needle passes plateaued after 3 needle passes. Although this does not answer the question of whether CEH-EUS may give an added benefit during tissue acquisition, it may be valuable knowledge for clinicians performing endoscopy procedures.

Attempts to improve the successful sampling rate from solid pancreatic masses have been ongoing for the past years, and multiple variables have been implicated as influencing factors. Among the factors discussed, the type and size of needles are most frequently highlighted, alongside suction techniques and differences between FNA and FNB [13]. Particularly needle size and design have been potential confounding factors in this review, as the choice of the needle was left up to the endoscopist in two of the four included randomized trials. Cho et al. [33], however, listed this as a potential factor in their baseline characteristics table and found that the groups did not differ significantly for either needle size or type (FNA or FNB). Sugimoto [11] used only 22G FNA needles in the CEH-FNA group and 22G and 25G needles in the conventional FNA group, unfortunately introducing an imbalance between the two groups. It has previously been suggested that 25G needles may be more effective for sampling than other sizes of needles [38], which may have disproportionately skewed the results in the direction of conventional EUS, especially due to their application for 5/20 patients in this study arm. Seicean et al. [12] [[,30], Hou et al. [9], Facciorusso et al. [31] and Itonaga et al. [35] performed all procedures using 22G FNA needles. Kuo et al. [32] and Lai et al. [34] performed all procedures using 22G Franseen type FNB needles.

Analyzing confounding factors that may affect the difference between the two modalities, Seicean [12] performed an association analysis between the relative risk of successful sampling and different features of pancreatic disease. The factors assessed were portal hypertension, biliary stent, tumor necrosis, tumor site, and chronic pancreatitis. Although some trends were visible, none were statistically significant. The authors particularly highlighted the importance of chronic pancreatitis, a disorder characterized by a high level of fibrosis of the pancreas. They found that although CEH-EUS performed better for diagnostic sensitivity than conventional EUS in the context of chronic pancreatitis, this difference was not significant (82.8 % vs. 75.8 %, $p = 0.47$). The authors suggested that this may have been due to the relatively small number of patients. Other factors which were assessed as potentially influencing the results across the papers included the mass location, presence of necrosis, mass size, size of core histology and presence of portal hypertension or biliary stents. None of these factors were found to significantly affect results, although Seicean et al. [12] also found a slight trend favoring CEH-EUS in the presence of biliary stents. None of the papers factored in the final pathology and whether the two methodologies differed in the context of adenocarcinoma from that of neuroendocrine tumors, and we were also unable to subgroup based on pathology. However, the relative distribution of pathology is included in the baseline characteristics table for context.

As has already been established, RCTs showed no difference overall between the two EUS methods in the meta-analysis. In the analysis of diagnostic adequacy after the first needle pass a slight, statistically non-significant tendency favoring CEH-EUS (RR 1.171, 95%CI: 0.433–3.170) was visible. This tendency was largely driven by the trial by Sugimoto et al. [11], which included only 20 patients in each arm and was the first of the randomized trials to complete patient enrollment (2014 vs 2019). This study found a 2.4 times higher risk for inadequate samples in the conventional EUS group, with 75 % of samples taken using conventional EUS inadequate for analysis on the first pass, compared to only 40 % in the CEH-EUS group. Interestingly, in this study, the first pass was performed by endoscopists with an experience of <100 performed EUS-FNAs, albeit in the presence of an expert, while any subsequent pass was performed in the same session by an expert endoscopist (>300 performed EUS-FNA), and authors themselves suggested repeating the research with experienced endosonographers. In comparison, the endoscopists in the study performed by Seicean et al. [12] had a minimum of 7000 EUS-FNA, including 500 CEH-EUS-FNA, performed, and this study found no difference between the groups (RR 1.00, 95 % CI: 0.92–1.09). Cho et al. [28] did not specify the experience beyond that “experienced endoscopists” performed the intervention and found no difference (RR 0.96, 95 % CI: 0.83–1.10).

As Sugimoto et al. [11] individually found a large, statistically and clinically significant difference in diagnostic adequacy favoring CEH-EUS when inexperienced endosonographers performed one single pass, it could potentially suggest that CEH-EUS may benefit less experienced endoscopists, allowing them to achieve a higher rate of adequate samples. Unfortunately, this suggestion is weakened by the potential bias introduced by different needle designs in the two arms. All other results of this review and meta-analysis indicate no or little difference between the diagnostic adequacies and sensitivities of tissue acquisition performed using contrast-enhanced or conventional EUS.

### 4.1. Strengths and limitation

This systematic review and meta-analysis summarized all available studies in five major databases on this topic and thus presented the highest level of evidence on the topic to date. Level of evidence for several outcomes was moderate, and the risk of bias for most outcomes was Low to Some concerns. We strictly followed the most up-to-date methodology as suggested by the Cochrane Collaboration, including pre-registering a protocol and reporting all deviations from the protocol.

However, the study is limited by the slightly different definitions of adequacy across studies and clinical and methodological heterogeneity among the different studies. Furthermore, only four published RCTs were eligible for inclusion.

### 4.2. Implication for practice and research

Translating scientific results to community benefits and implementing them into the patient care are of major importance [39,40]. Based on the results of our analysis, we suggest that CEH-

EUS likely shows no benefit over conventional EUS for tissue acquisition of solid pancreatic masses, and further research is only warranted to assess its applicability in a setting of chronic pancreatitis, alongside potential benefits for inexperienced endoscopists – e.g. in a training or educational setting. Any further trials should be carefully designed to avoid the obvious confounding factors highlighted in our study, and trials investigating the benefit in chronic pancreatitis should ensure using appropriate inclusion criteria. Unknown factors appear to affect the outcome of conventional EUS in non-randomized settings, detecting their nature may help better select patients that could benefit from CEH-EUS.

## 5. Conclusion

The use of CEH-EUS likely does not improve diagnostic adequacy during sampling from solid pancreatic masses. However, it may show a benefit for inexperienced endoscopists, and its use in a setting of chronic pancreatitis remains to be explored.

## Data availability statement

All data in this study is publicly available in previously published studies.

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The sponsors had no role in the design, data collection, analysis, interpretation, and manuscript preparation.

## Ethical approval

No ethical approval was required for this systematic review with meta-analysis, as all data were already published in peer-reviewed journals. No patients were involved in the design, conduct, or interpretation of our study.

The datasets used in this study can be found in the full-text articles included in the systematic review and meta-analysis.

## CRediT author contribution

MAE: conceptualization, project administration, methodology, formal analysis, writing – original draft; YH: conceptualization, investigation, data curation, writing - review & editing; OA: conceptualization, investigation, data curation, writing - review & editing; ASW: validation, investigation, writing – review & editing, BT: conceptualization, methodology, writing - review & editing; DSV: conceptualization, formal analysis, visualization, writing – review & editing; PH: conceptualization, funding acquisition, writing - review & editing; BE: conceptualization; supervision; writing – original draft.

All authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

## Declaration of competing interest

None to declare.

## Acknowledgment

None to declare.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pan.2024.04.007.

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# Combined Liquid-Based Cytology and Conventional Smear Provides Better Sensitivity and Adequacy Rates After Endoscopic Ultrasound-Guided Tissue Acquisition of Abdominal Masses: A Systematic Review and Meta-Analysis

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## Abstract

**Background and Aims:** Endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) or biopsy (FNB) is the standard method for diagnosing abdominal masses, but sample inadequacy and diagnostic accuracy remain challenges. Conventional smear (CS) and liquid-based cytology (LBC) are standard processing methods, yet their comparative effectiveness and potential combined benefit remain unclear. We performed a systematic review and meta-analysis to evaluate and compare the diagnostic performance and adequacy of CS, LBC, and their combination. **Methods:** A systematic search was conducted in Medline, Embase, and CENTRAL on 17 November 2024. Studies comparing CS, LBC, or their combination following EUS-FNA/FNB for abdominal masses were included. Diagnostic parameters, including sensitivity, specificity, accuracy, and inadequacy rates, were extracted and analyzed. Methodological quality was assessed using QUADAS-2. **Results**: 16 studies (2128 patients) were included. Sensitivity for pancreatic masses was 71.4% (CI: 62.9–78.7) for CS, 74.7% (CI: 64.3–82.8) for LBC, and 86.2% (CI: 82.4–89.3) for combined methods ($p = 0.001$). For all abdominal masses, sensitivity was 76.3% (CI: 67.9–83.0) for CS, 73.6% (CI: 65.6–80.2) for LBC, and 88.0% (CI: 84.0–91.2) for combined methods ($p \leq 0.006$). Specificity was nearly 100%. Inadequacy rates were lowest for combined methods (1.5%, CI: 0–36.2), when compared to LBC (7.7%, CI: 2.7–20.4) and CS (4.4%, CI: 2.4–7.9). Moderate bias risk was noted, primarily due to incorporation bias. Domain 3 (reference standard) of QUADAS was uniformly moderate-risk across studies. **Conclusions**: Combining CS and LBC methods improves diagnostic sensitivity and reduces sample inadequacy after EUS-guided tissue acquisition for abdominal masses, particularly pancreatic lesions. Clinical guidelines should consider recommending the combined approach to enhance diagnostic yield and clinical outcomes.

**Keywords:** cytology; EUS; pancreatic masses; abdominal masses



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## 1. Introduction

Cancerous lesions in and around the gastrointestinal (GI) tract represent a major global health burden, accounting for 26% of global cancer incidence burden and 35% of all cancer-related deaths [1]. Notably, pancreatic cancer has some of the lowest survival rates, with pancreatic cancer five-year survival ranging from 10% to 18% depending on the country [2]. In the case of pancreatic cancer, this is largely due to late-stage diagnosis, as lesions are often detected after the disease has progressed, leading to a poor prognosis.

With advancements in minimally invasive techniques, endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) or biopsy (FNB) has become the preferred method for obtaining pathological samples from peri-GI lesions [3]. Despite being less invasive, these procedures still carry risks [4]. Moreover, sample adequacy is not guaranteed, with reported success rates ranging from 60% to 90%, depending on factors such as rapid on-site examination, needle type used, and other factors. This problem has prompted ongoing efforts to improve sample adequacy and diagnostic accuracy through innovations in equipment, technique, and processing methods [5,6].

One such area of focus is the optimal processing of cytology specimens. The Conventional Smear (CS) technique and Liquid-Based Cytology (LBC)—the latter of which is the gold standard in fields such as gynecology—are two primary approaches. Several studies, including meta-analyses, have compared these methods, though results have varied due to methodological differences and inconsistent findings [7–9]. Conflicting results across earlier reviews reflect methodological heterogeneity: outcome measures (diagnostic parameters vs. inadequacy), comparators (single method vs. CS + LBC), and study designs. In addition, ROSE availability, the LBC technique (filtration vs. precipitation), and paired vs. unpaired comparisons varied between evidence bases.

Current international guidelines reflect this uncertainty. The European Society for Gastrointestinal Endoscopy (ESGE) [10] recommends a combination of CS and LBC for pancreatic EUS samples, though this is based on low-quality evidence. In contrast, the Korean Society for Gastrointestinal Endoscopy (KSGE) [11] acknowledges the importance of cytology method selection but does not issue a specific recommendation.

In this study, we aim to systematically review and meta-analyze existing data by comparing CS, LBC, and their combination in terms of diagnostic yield and sample adequacy to provide a more substantial evidence base for future guidelines.

## 2. Methods

This systematic review and meta-analysis has been conducted according to the guidelines of the Cochrane Collaboration [12] and is reported following the PRISMA 2020 guideline [13]. The PRISMA checklist can be found in the Supplementary Material. The protocol of this study was fully adhered to and was registered on PROSPERO (registration number: CRD42024612112). An additional analysis was performed to assess the inadequacy rate of the different methods.

### 2.1. Eligibility Criteria

Studies including patients undergoing EUS-guided FNA or FNB for an abdominal lesion (pancreatic, gastrointestinal, or other) were eligible for inclusion. Only studies that reported diagnostic parameters of both liquid-based cytology and conventional smear were included to minimize confounders in the comparator. Due to a limited number of studies predicted, studies reporting on the combined diagnostic value of liquid-based cytology and conventional smear were eligible for inclusion regardless of whether the two cytology methods were also separately reported. Attempts were made to deduce the combined

diagnostic value from papers that did not report it through their degree of agreement, assuming that one positive test would mean a positive by combination.

Both indexed journal articles of any study design and conference abstracts were eligible for inclusion, provided they contained the information necessary for analysis.

### 2.2. Information Sources and Search Strategy

The systematic search was conducted on 17 November 2024, in Medline (via PubMed), Embase, and CENTRAL. The search strategy included a domain for the abdomen/GI/pancreas, a domain for tissue acquisition, and a domain for the type of tissue preparation. The exact search key can be found in the Supplementary Material (Supplementary Text S1).

References of the included studies were screened for further eligible studies, and papers citing the included studies were searched on 17 November 2024, using the citationchaser [14] tool.

### 2.3. Selection Process

The selection was performed by two independent review authors (MAE and ASW) after the duplicates were removed. Records were first screened by title and abstract, then by full text. Disagreements were resolved by discussion. Cohen's kappa was used to quantify the degree of interrater agreement. Citing papers and references were handled as two separate pools of records, and selection was performed in the same two stages.

### 2.4. Data Collection Process

Two independent reviewers (MAE, ASW) extracted data in a prospectively designed Excel sheet. The primary investigator (MAE) compared the data and any points of contention resolved by a consensus. Data were sought on the study design, years of patient enrollment, study population (type of lesions, age, and gender), sampling procedure (needle type), reference, number of benign and malignant cases, and outcomes. The outcome variables sought were true positives, false negatives, true negatives, and false positives. Where available, we also extracted accuracy, sensitivity, specificity, and positive and negative predictive values with confidence intervals (CIs).

The case numbers were reverse engineered from the available information if only sensitivity, specificity, and accuracy were given without CIs, but the total number of benign and malignant cases were known.

If diagnoses were given as a table with the different pathological definitions according to the Papanicolau Society of Cytopathology [15], malignant and suspicious were considered malignant, and all others were considered benign in the extraction.

In the primary analysis, to increase homogeneity, studies were only included if the definition of malignancy was either "malignant and suspicious", or case numbers were extractable in accordance with the definition.

Two analyses were done: First, studies on solid pancreatic masses were included, and second, all types of abdominal masses were included.

### 2.5. Study Risk of Bias Assessment

The risk of bias was assessed using the QUADAS-2 tool [16] by two independent reviewers (MAE, ASW) and discussed internally to resolve conflicts. Results were visualized using the robvis tool [17].

### 2.6. Synthesis Methods

Statistical analyses were performed using R statistical software (version 4.1.2) and the R script of the online tool described by Freeman [18]. Random-effect meta-analysis was

applied for each outcome. A *p*-value of less than 0.05 was considered significant for all statistical analyses.

Two by two contingency tables were directly extracted or calculated from the studies containing true positive, false positive, false negative, and true negative values. Several diagnostic measures can be calculated from these numbers. As usual in diagnostic meta-analysis, we only meta-analyzed the sensitivity and specificity. They are the preferred outcomes since they do not depend on the proportion of the malignant cases. To provide better insight, using the pooled sensitivity and specificity, we calculated accuracy, PPV and NPV, assuming several different malignancy prevalence assumptions. In the case of sensitivity and specificity, only the corresponding random effects can be correlated; the within-study correlation between them is zero. In contrast, when a study reports results corresponding to more than one diagnostic tool evaluated on the same patients, there is also within-study correlation in the data. The Clubsandwich R package (version 4.1.2) provides a robust tool for handling within-study correlation but only works with linear meta-analysis models. For this reason, instead of the usual mixed-effect logistic regression-based bivariate approach of Reitsma and Chu [19,20], we used a linear model on the logit transforms of sensitivity and specificity, and we had to analyse them separately. The fact that almost all specificities were precisely one further justifies the separate analyses.

The performances of the conventional smear, LBC, and combined methods were frequently evaluated in the same population within the studies. For this reason, for the logit transformed sensitivity, we constructed a three-dimensional model using the rma.mv() function of the metafor R package. To circumvent the problem caused by the unknown correlations, we supplemented the method with the robust approach of Pustejovsky [21], implemented in the coef_test() function of the clubSandwhich R package. Moreover, we repeated the approach under several within-study correlation assumptions. All sensitivity runs provided similar *p*-values. For the specificity, this approach was not feasible. Hence, we calculated pooled specificity using the generalized mixed-effect approach of Stijnen et al. [22]. We used a mixed approach to meta-analyze the inadequate sample ratio. Namely, due to the presence of studies with no inadequate sample, to calculate pooled results within the subgroup, we used the methodology of Stijnen et al., while after adding 0.1 to the zero frequencies (continuity correction), we followed the robust approach described above to generate *p*-values.

We visualized the results on forest plots. Heterogeneity was assessed by calculating the $I^2$ measure and its confidence interval. In the case of the robust multivariate sensitivity analysis, the $I^2$ statistics were calculated for each method separately using univariate methodology.

For the reasons mentioned above, we performed publication bias analyses only for sensitivity. In the case of prevalence, the effect size and the standard error are dependent. For this reason, following the suggestion of Hunter et al. [23], we created a modified funnel plot to access the publications bias visually: on the y-axis, we plotted the study size instead of the standard error. Moreover, instead of Egger's test, we used Peters' test [24] to test whether publication bias is present. We assessed publication bias separately in each subgroup with at least 10 studies.

## 3. Results

### *3.1. Search and Selection*

Our search strategy identified 134 records, of which 22 reports [25–46] (13 studies) were eligible for inclusion. Three further studies [47–49] were identified from the reference and citation search. All the studies included focused exclusively on FNA. The entire selection process is detailed in the PRISMA flowchart in Figure 1. One paper [50] was first

deemed eligible, then excluded, as the type of cytology was part of a compound change in sample processing, which would likely represent significant confounding while following a different definition of malignancy than that employed in this review.

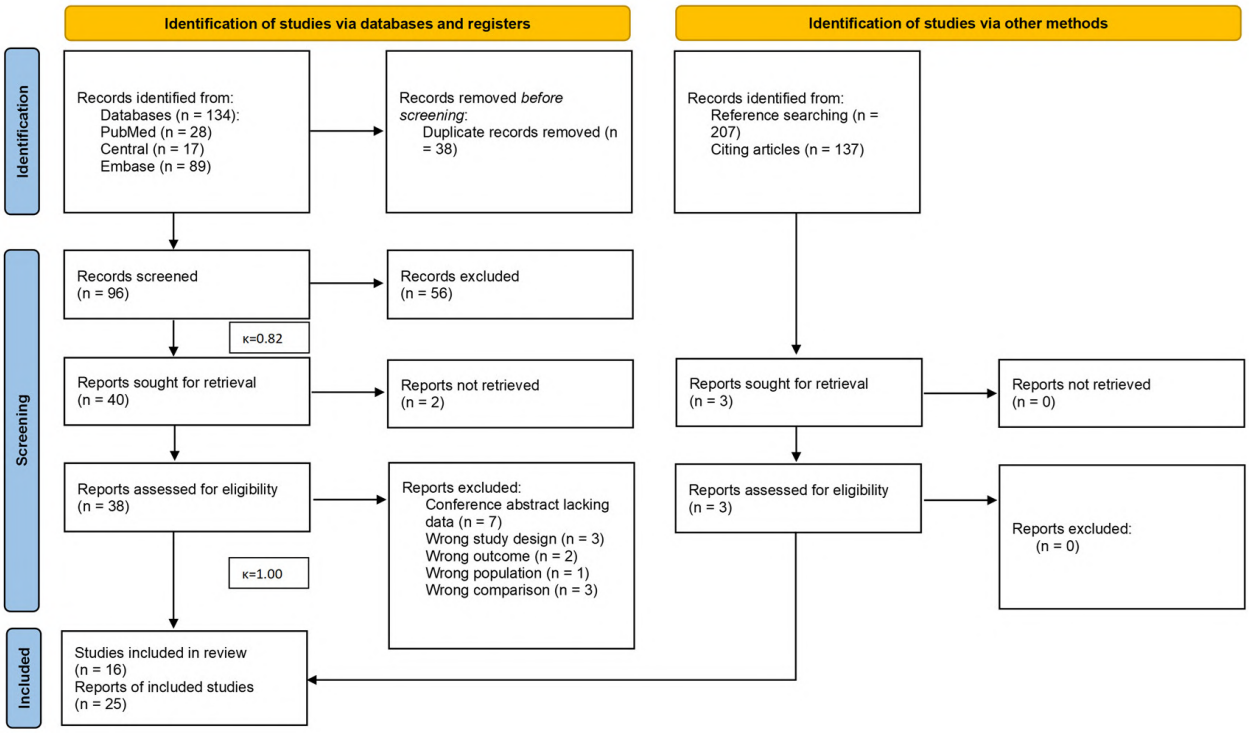


**Figure 1.** PRISMA 2020 flowchart representing the study selection process.

### 3.2. Basic Characteristics of Included Studies

The baseline characteristics, including patient characteristics, methods used for cytology, needle designs used, and the type of mass, are detailed in Table 1.

### 3.3. Sensitivity and Specificity

Eleven studies [25,27,32,33,38,41,42,45–49] were included for sensitivity when investigating only pancreatic masses, of which six [33,44–46,48,49] were used for the combined methods, seen in Supplementary Figure S1. For conventional smear, sensitivity was 0.714 (CI: 0.629–0.787, $I^2$: 82%); for LBC, it was 0.747 (CI: 0.643–0.828, $I^2$: 84.1%); and for the combination, sensitivity was 0.862 (CI: 0.824–0.893, $I^2$: 52.7%). The difference between conventional smear and LBC was not significant ($p$ = 0.5942). The difference between conventional smear/LBC and the combination was significant ($p$ = 0.001).

Thirteen studies [25,27,32,33,36,38,41,42,45–49] were included for sensitivity when investigating all abdominal masses, of which six [33,44–46,48,49] were used for combined methods, seen in Figure 2. For conventional smear, sensitivity was 0.763 (CI: 0.679–0.830, $I^2$: 86.5%); for LBC, it was 0.736 (CI: 0.656–0.802, $I^2$: 81.8%); and for the combination, sensitivity was 0.880 (CI: 0.840–0.912, $I^2$: 69.1%). The difference between conventional smear and LBC was not significant ($p$ = 0.611). The difference between conventional smear/LBC and the combination was significant ($p$ = 0.001/$p$ = 0.006).

**Table 1.** Basic characteristics of included studies. N: Number of patients, CS: Conventional Smear, LBC: Liquid-based cytology, EUS: Endoscopic ultrasound, FNA: fine needle aspiration, IQR: Interquartile range, NA: Not available, HE: Hematoxylin/Eosin, PSM: Propensity score matched. * Conference abstracts.

| Study | Study Design | Study Period | Country | Population | Mass | Needle Type | Reference | Description of Assessments | Staining | ROSE | Sample |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chun 2020 [27] | Randomized, crossover | April 2018–March 2019 | Republic of Korea | N: 170, Female: 44.1%, age: 64.8 ± 10.6 (37–88) | Pancreatic | 19/22G EZ Shot 3 Plus (Olympus Medical Systems, Tokyo, Japan) | LBC, CS; EUS-FNA core biopsy or surgical specimen, 6-month clinical/radiological follow-up | CS: alcohol, LBC: CytoRich Red, SurePath | Papanicolaou | Unclear/No | Individual |
| de Luna 2004 [47] | Retrospective, crossover | August 2000 to February 2002 | USA | N: 67, Female: 32.8%, age: 64 (39–87) | Pancreatic | NA | Histologic and clinical follow-up | CS: alcohol/air, LBC: PreservCyt, ThinPrep | Modified Giemsa for air-dried smears, Papanicolaou for alcohol-fixed and LBC samples | Unclear | Split |
| Hashimoto 2017 [32] | PSM | January 2009–August 2014 | Japan | N: 126, Female: 49.2%, age: CS: 65 (35–93), LBC: 66 (33–85) | Pancreatic | 19G/22G/25G | Surgical resection, additional EUS-FNA procedure, 6 months clinical/imaging follow-up | CS: alcohol, LBC: SurePath | HE for smears, Papanicolaou for LBC samples | No | Individual |
| Itonaga 2019 [33] | PSM | December 2011 to October 2017 | Japan | N: 311, Female: 42.8%, age: NA | Pancreatic | 19G/22G/25G Expect (Boston Scientific) or EZ Shot2 (Olympus Medical) | Surgical resection, 12-month clinical follow-up | LBC: ThinPrep, CS: Air/alcohol | Air-dried: Diff-quick, alcohol-fixed and LBC Papanicolaou | Yes | Split |
| Jun 2023 [36] | Randomized crossover | January 2019 to August 2022 | Republic of Korea | N: 60, Female: 53.3%, age: 60.7 ± 12.8 (24–85) | Abdominal | 19G/22G EZ Shot 3 Plus (Olympus) | Core biopsy, LBC; CS and surgical specimens, plus 6-month follow-up | LBC: CytoRich, SurePath vials, PrepStain processor, CS: alcohol | NA | No | Individual |
| LeBlanc 2010 [48] | Prospective crossover | April 2005 through April 2007 | USA | N: 50, Female: 36% (whole population), age: mean 63 | Subepithelial masses | 22G EUSN-3 or echo-1-22 | Final cytological diagnosis, surgical pathology, or follow-up | CS: air/alcohol, LBC: ThinPrep | Air-dried: Diff-quick, alcohol-fixed and LBC Papanicolaou | Yes | Individual |
| van Riet 2010 [43] | Prospective crossover | April 2016–2017 | The Netherlands | N: 71, Female: NA, age: NA | Solid pancreatic lesions | 19G, 22G, 25G Echotip/Expect | Surgical resection, 12-month clinical follow-up | CS: no information, LBC: Thinprep | Different per center: Hemocolor, no stain, Diff quick, or Giemsa | No | Split |

**Table 1.** *Cont.*

| Study | Study Design | Study Period | Country | Population | Mass | Needle Type | Reference | Description of Assessments | Staining | ROSE | Sample |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Yan 2023 [44] | Retrospective crossover | January 2014 to February 2022 | China | N: 251, Female: 41%, age: Median 60 (IQR: 13) | Pancreatic | 20G FNA | Needle biopsy/surgery sample, FNA sample, 6-month clinical/imaging follow-up | CS: alcohol, LBC: ThinPrep | NA | Not all | Individual |
| Yeon 2018 [45] | Prospective crossover | June 2012–October 2013 | Republic of Korea | N: 43, Female: 32.6%, age: 65.5 ± 12.5 | Pancreatic | 22G Echotip | Biopsy, surgery, or 6-month clinical follow-up | CS: 95% alcohol fixation, LBC: CellPrepPlus | NA | Unclear | Individual |
| Zhou 2020 [46] | Retrospective crossover | January 2015 to January 2019 | China | N: 514, Female: 37.2%, age: Median 60 (IQR 50–67) | Pancreatic | 22G/25G | FNA, surgical pathology, 6-month clinical follow-up | CS: alcohol, LBC: CytoRich | CS: HE, LBC: Papanicolaou | Not all | Split |
| Qin 2014 [41] | Prospective crossover | January 2011 to January 2014 | China | N: 72, Female: 19.4%, age: 54.6 (24–70) | Pancreatic | 22G FNA | Surgery, 9-month clinical follow-up | CS: alcohol/air, LBC: ThinPrep | Modified Diff-Quick, Papanicolaou | No | Individual |
| Cheng 2020 [25] * | NA | December 2016 to January 2018 | China | N: 52, Female: NA, age: NA | Abdominal | NA | Pathology, follow-up | NA | NA | NA | NA |
| Schmidt 2015 [42] * | NA | 2008 to 2011 | Germany | N: 172, Female: 39%, age: 64.8 (±12.4) | Pancreatic | NA | Surgical histology, 12-month follow-up | NA | NA | NA | NA |
| Min 2013 [38] * | prospective crossover | November 2010 to February 2013 | China | N: 32, Female: NA, age: NA | Pancreatic | NA | NA | NA | NA | NA | NA |
| Lee 2016 [49] | Retrospective crossover | July 2010 to June 2015 | Republic of Korea | N: 48, Female: 50%, age: Median 67, range 39–84 | Pancreatic | 22G FNA | Clinical and imaging follow-up of 12 months, CS, LBC, and pathology from metastatic sites | CS: alcohol, LBC: ThinPrep | Papanicolaou | No | Individual |

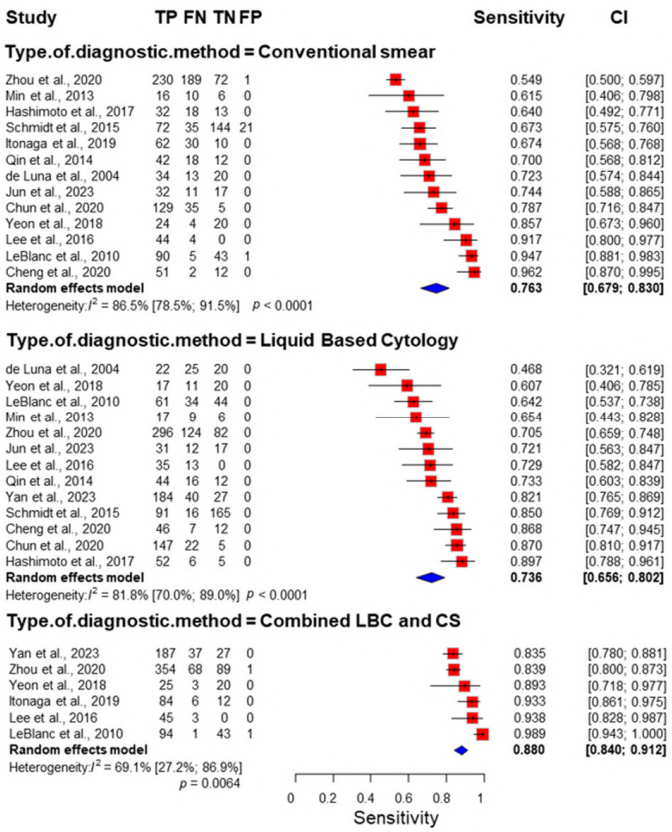


**Figure 2.** Forest plot representing the sensitivity of different cytology methods [25,27,32,33,36,38,41,42,45–49]. The difference between conventional smear and LBC was not significant ($p = 0.611$). The difference between conventional smear/LBC and the combination was significant ($p = 0.001$/$p = 0.006$). TP: True positives, FN: false negatives, TN: True negatives, FP: False positives. CI: Confidence interval, LBC: liquid-based cytology, CS: conventional smear.

The same thirteen studies also reported data for calculating specificity for all abdominal masses. Nearly all studies showed 100% specificity with no cases of false positives. Notable exceptions were Schmidt [42] with 21 false positives, LeBlanc [48] with 1, and Zhou with 1 [46]. We present in Figure 3 visualizations of the specificities.

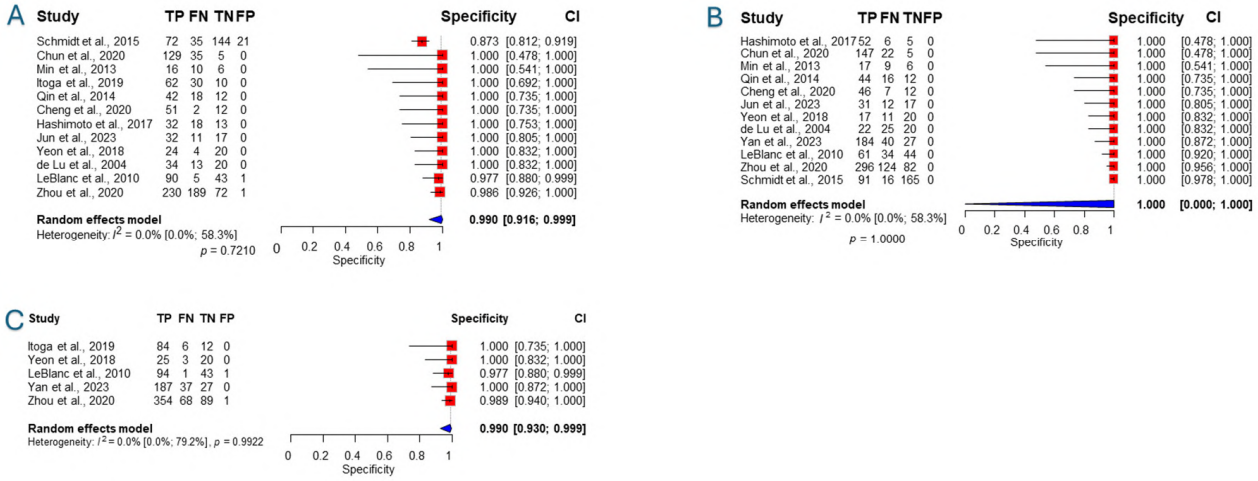


**Figure 3.** Forest plot representing the specificity of different cytology methods for all abdominal masses [25,27,32,33,36,38,41,42,45–49]. (**A**): Conventional Smear, (**B**): Liquid-based cytology, (**C**): Combination. TP: True positives, FN: false negatives, TN: True negatives, FP: False positives. CI: Confidence interval.

Three of the included studies were conference abstracts. To mitigate potential bias, the analysis was run omitting these three studies (Supplementary Figure S2). For conventional smear, sensitivity was 0.761 (CI: 0.665–0.836, $I^2$: 88.1%); for LBC, it was 0.719 (CI: 0.618–0.803, $I^2$: 83.3%); and for the combination, sensitivity was 0.881 (CI: 0.839–0.913, $I^2$: 69.1%).

### 3.4. Accuracy

The pooled sensitivity and specificity results were used to calculate accuracy. The accuracy of CS was 93.12% (actual w = 0.723), that of LBC was 93.45% (actual w = 0.739), and that of the combination was 97.44% (actual w = 0.805). The accuracy for w = 0.2, w = 0.4, w = 0.6, w = 0.8 and w = 1.0 is shown in Supplementary Table S1.

One study [43] was included in this review, which was not included in sensitivity and specificity analysis but reported accuracy. It reported that the accuracy for malignancy was 66.20% (47/71) for conventional smear and 81.70% (58/71) for liquid-based cytology.

### 3.5. Inadequacy Rate

Nine studies [27,32,33,44–49] were included for the rate of inadequate samples for pancreatic masses alone, of which four were included for the combination of methods, seen in Figure 4. The inadequacy rate of LBC was 7.7% (CI: 2.7–20.4, $I^2$: 93.7%), that of conventional smear was 4.4% (CI:2.4–7.9, $I^2$: 39.1%), and that of the combination was 1.5% (CI: 0–36.2%, $I^2$: 33.6%).

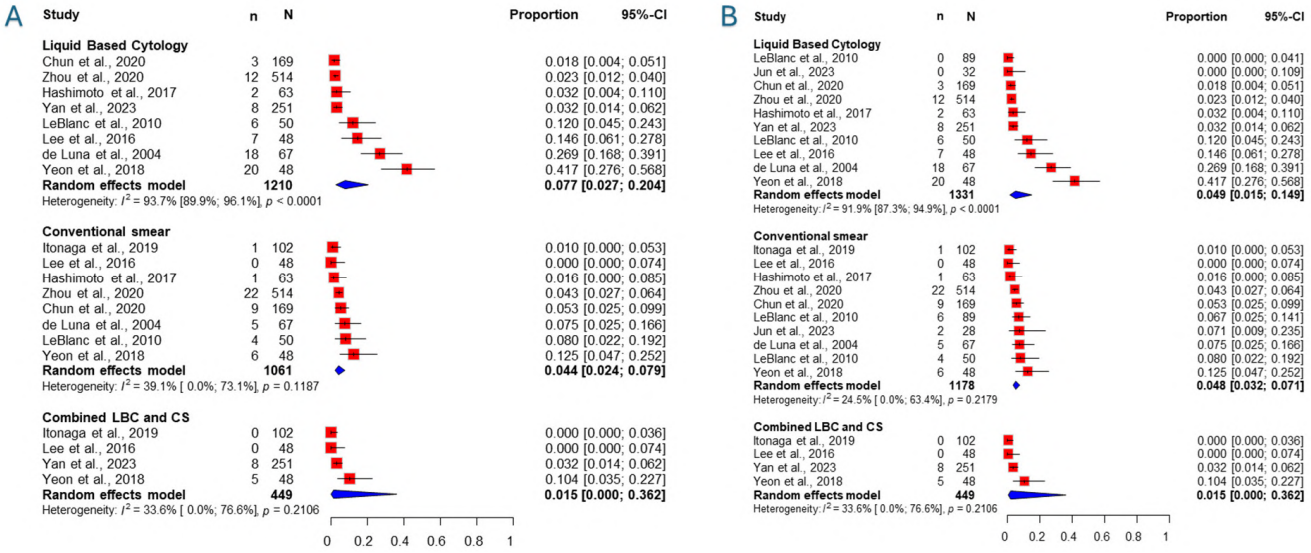


**Figure 4.** Forest plot representing the rate of inadequate samples of different cytology methods [27,32,33,36,44–49]. (**A**): Pancreatic masses, (**B**): All abdominal masses. N: Number of samples, n: number of inadequate samples, CI: confidence interval, LBC: liquid-based cytology, CS: conventional smear.

Eleven studies [27,32,33,36,44–49] were included for the rate of inadequate samples for all types of abdominal masses, of which four were included for the combination of methods, seen in Figure 4. The inadequacy rate of LBC was 4.9% (CI: 1.5–14.9, $I^2$: 91.9%), that of conventional smear was 4.8% (CI: 3.2–7.1, $I^2$: 24.5%), and that of the combination was 1.5% (CI: 0–36.2, $I^2$: 33.6%). *p*-values of the comparison of CS to the combination, LBC to the combination, and CS to LBC were 0.063, 0.15, and 0.66, respectively.

Importantly, confidence intervals were very wide, and differences between interventions should be considered hypothesis-generating.

### 3.6. Risk of Bias Assessment

The results of the risk of bias assessment are found in Figure 5, and a summary can be found in Figure 6. Most studies were found to have a moderate risk of bias overall, primarily due to an impaired domain 3 (reference domain). Due to the inclusion of the cytology result in the reference test, there is a significant risk of incorporation bias in most studies, with a moderate risk of impacting results. Three studies were classified mostly as "No information" due to being conference abstracts. To truly understand the results in context of the risk of bias, the analysis excluding conference abstracts could be referred to.

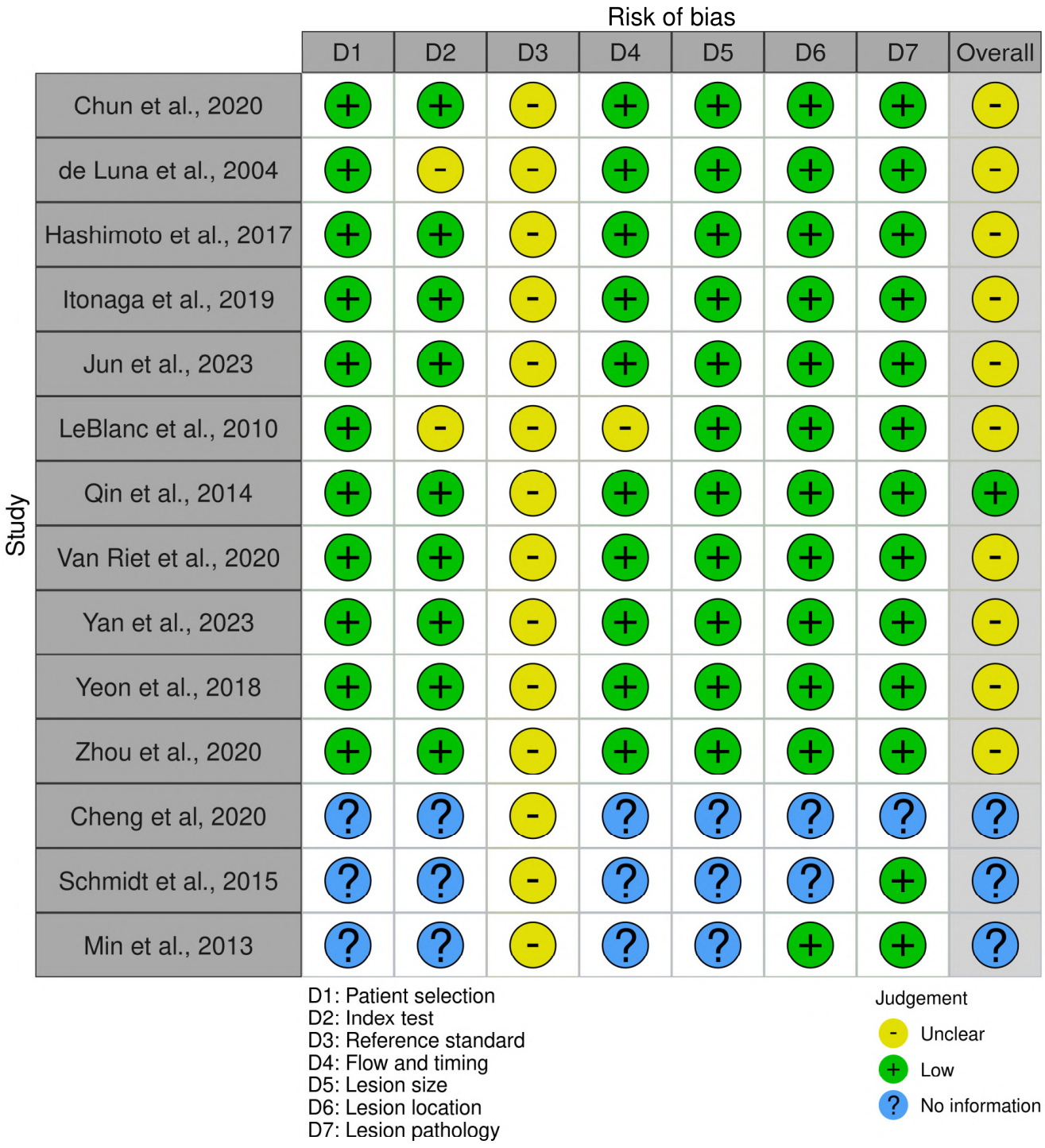


**Figure 5.** Result of the risk of bias assessment [25,26,32,33,36,38,41–48].

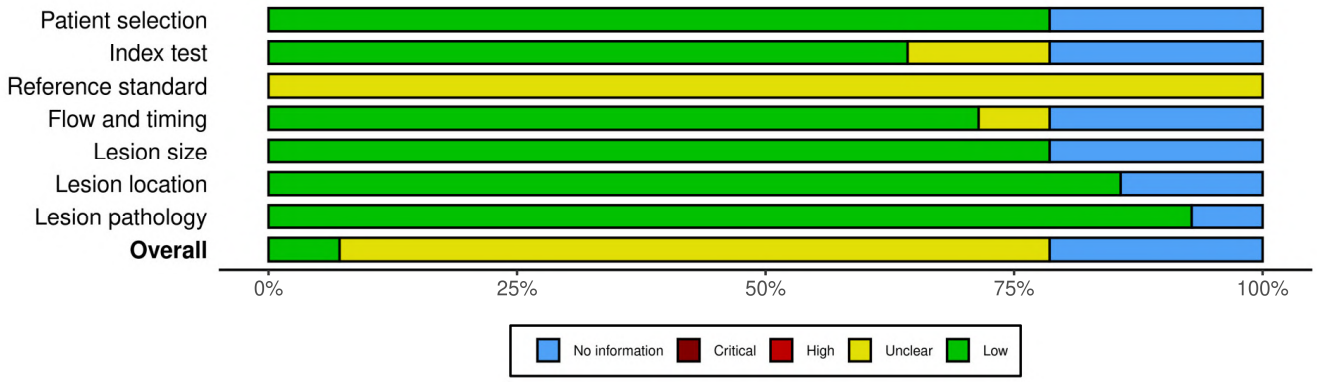


**Figure 6.** Overall result of the risk of bias assessment.

### *3.7. Publication Bias and Heterogeneity*

Peters' test for publication bias was not statistically significant; details can be found in the Supplementary Material (Supplementary Figures S3 and S4).

## 4. Discussion

In this systematic review and meta-analysis, we evaluated all available studies that directly compared LBC and CS for EUS-guided tissue acquisition from abdominal and pancreatic masses, as well as studies assessing their combined use. While LBC and CS performed similarly overall, their combination significantly improved sensitivity. Specificity was uniformly high across all methods, without notable differences observed. Furthermore, CS demonstrated a slightly lower inadequacy rate than LBC in pancreatic masses, while combining the two methods substantially reduced the rate of inadequate samples.

It is well known that performing multiple passes during EUS-guided tissue acquisition can increase sensitivity. Some of the included studies that assessed the combined use of CS and LBC obtained one pass per method, meaning that the combination group had a total of several passes while the single-method groups had only one—a potential confounder. However, among these studies, those using this approach (Yan and Lee) did not show the highest sensitivity, with one ranking last and the other second among the studies included. Another study (Yeon) performed five passes per method but ranked only fourth in sensitivity. The above observations suggest that the diagnostic benefit of combining CS and LBC is likely independent of the number of passes.

Our analysis confirmed that LBC and CS yield comparable results, but their combination improves diagnosis. As expected, combining methods can only enhance sensitivity, yet the observed difference was both statistically significant and clinically important—yielding nearly a 10% increase in sensitivity without any reduction in specificity. This result demonstrates that a nondiagnostic result from one method does not predict a nondiagnostic result from the other, as they may be able to diagnose different subsets of cases.

Specificity was uniformly high in all studies, with few or no false positives. However, this may be partly due to study design. Many included studies used a composite reference standard that incorporated surgical pathology, biopsy results, clinical follow-up, and, in some cases, cytology itself. While combining multiple confirmation methods and including follow-up strengthens diagnostic accuracy, including the index test in the reference standard introduces incorporation bias. This issue was reflected in our risk of bias assessment, where most studies were rated as having moderate risk due to reference standard limitations. However, the main impact of this incorporation bias would be on false positives and specificity, which we chose to only review in this paper.

We also analyzed the inadequacy rates for each method. When pooling data from all abdominal masses, CS and LBC performed similarly, with inadequacy rates of 4.8%

and 4.9%, respectively. In pancreatic masses, however, LBC showed a slightly higher inadequacy rate (7.7%) than CS (4.4%). Although confidence intervals were wide and statistical significance was not reached, four studies (de Luna, Lee, LeBlanc, and Yeon) reported higher inadequacy rates with LBC. These studies used various LBC platforms (PreservCyt, ThinPrep, CellPrepPlus), which were also used in other studies with better results. Notably, three of the four were among the earliest studies included (published in 2004, 2010, and 2016), and while Yeon was published more recently, patient enrollment ended in 2013. LBC as a method for non-gynecological cases was first introduced by a single publication in 1996 [51], followed by one further in 2002 [52]. The method has since been under development. It is possible that early user inexperience may have contributed to higher inadequacy rates for LBC in these studies, which resolved with time.

We also assessed the inadequacy rate of the combined CS and LBC method, which was markedly lower at 1.5%. Although this difference was not statistically significant, it is clearly of clinical relevance—representing a threefold reduction compared to either method alone. As with sensitivity, the potential confounding effect of multiple passes was considered. Nevertheless, studies using multiple passes for the combined method (Yan, Lee) did not show the best performance, while the study with five passes per method had the highest inadequacy rate (10.4%). This finding further supports the interpretation that the diagnostic advantage of combining CS and LBC is genuine and not solely due to the sampling technique. A sample deemed inadequate by one method may still be diagnostic when processed by the other.

We identified three previous meta-analyses on the topic. Zhang et al. [9] assessed the use of LBC and CS (with ROSE) in pancreatic masses and concluded that CS was the superior method in terms of sensitivity (78%, CI: 67–87%) over LBC (75%, CI: 67–81%), while finding, similarly to our investigation, a specificity of 100%. Chandan et al. [7] compared CS to precipitation-based (SurePath) and filtration-based (ThinPrep) LBC for pancreatic masses and concluded that precipitation-based LBC was the superior method in terms of sensitivity (79.2%, CI: 70.7–85.7%; 83.6%, CI: 70.7–91.5%; and 68.3%, CI: 55.3–79%, respectively). Pan et al. was able to compare these two methods to a combined approach based on 8 studies, finding a higher sensitivity for LBC than for CS (76%, CI: 72–79% vs. 68%, CI: 64–71%), and a superior sensitivity still for the combination (87%, CI: 84–90%). Overall, our results are similar for the combination at 89%; however, the 74% and 75% sensitivities for CS and LBC demonstrate the relative comparability of the two methods.

There was some clinical heterogeneity among the included studies. For one, the included studies used different needle sizes, with some limiting themselves to 22G or 20G needles, and others using a mix of other available needle sizes. Needle size has been investigated previously as impacting sensitivity of tissue acquisition, although a meta-analysis investigating different needle sizes for FNA [53] did not find a difference in sensitivity or adequacy between 22G and 25G needles. Regardless, there is a potential that needle size may impact the specific performance of one cytology method over the other.

Prior reviews indicate that ROSE materially modifies cytology performance and helps explain why meta-analyses have disagreed on the “preferred” method. Zhang et al. concluded that smear cytology with ROSE yields the highest diagnostic performance, recommending LBC primarily when ROSE is unavailable, whereas Chandan et al. emphasized that in no-ROSE settings, certain LBC platforms (e.g., precipitation-based) can outperform smears; Pan et al. did not stratify by ROSE but showed a clear incremental gain when combining LBC and smears [7–9]. In our dataset, ROSE use was inconsistently reported, precluding a formal moderator analysis; nevertheless, the consistently superior sensitivity of the combined CS+LBC strategy and its lowest inadequacy rate suggest that using both preparations likely mitigates ROSE-related variability—enhancing yield when ROSE is

present (by capturing discordant positives) and partially compensating when ROSE is absent (by reducing nondiagnostic samples).

Current ESGE guidelines [10] recommend preparing EUS-guided tissue acquisition samples using either LBC alone or a combination of CS and LBC, depending on local expertise. This recommendation is weak and based on limited evidence. Our findings support the combined use of CS and LBC, further suggesting that if only one method is selected, CS might be the preferred option to minimize the risk of inadequate samples.

### 4.1. Strengths and Limitations

In the performance of this study, we aimed to include only studies that allowed us to compare cytology methods directly, and we were able to include a great number of patients.

This study is limited somewhat by clinical heterogeneity among the studies, particularly in needle types, staining, and processing—all of which are known to impact performance. Additionally, articles were published across two decades (2004–2023), during which time cytology technology improvements may have introduced temporal bias. This was also reflected in substantial heterogeneity. For inadequacy rates, CIs were wide, particularly for inadequate samples, limiting interpretation.

### 4.2. Implication for Practice and Research

To translate these findings into clinical benefit [54,55], we recommend that both CS and LBC be used following EUS-guided tissue acquisition to improve diagnostic sensitivity and reduce the rate of inadequate samples. Current guidelines should be updated accordingly. Further studies are needed to confirm the observed reduction in inadequacy rates.

## 5. Conclusions

A combination of CS and LBC significantly increases sensitivity after EUS-guided tissue acquisition for abdominal masses, with a clinically important difference.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm14186685/s1, Supplemental Text, Figure S1: Forest plot representing the sensitivity of different cytology methods in pancreatic masses; Figure S2: Forest plot representing the sensitivity of different cytology methods in all abdominal masses, excluding conference abstracts; Figure S3: Funnel plot for publication bias of liquid based cytology; Figure S4: Funnel plot for publication bias of smear cytology; Table S1: Estimated accuracy for different values of w (disease prevalence).

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**Informed Consent Statement:** Patient consent was waived as no ethical approval was required for this systematic review with meta-analysis, which did not involve patient enrollment.

**Data Availability Statement:** The datasets used in this study can be found in the full-text articles included in the systematic review and meta-analysis. If further information is needed, it will be provided upon reasonable request to the corresponding author.

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