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Role of endoscopic ultrasound-guided tissue acquisition for the diagnosis of gastric wall thickening: a retrospective study with meta-analysis

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Abstract

Background Tissue acquisition from a thickened gastric wall using biopsy forceps may not always lead to diagnosis, given the submucosal location of the pathology. Endoscopic ultrasound (EUS)-guided tissue acquisition (TA) may serve as a minimally invasive diagnostic tool in such cases. Hence, we aimed to assess the diagnostic outcome and safety of EUS-TA from thickened gastric walls.

Methods Data from patients with gastric wall thickening undergoing EUS-TA at 5 tertiary care centers from August 2020 to August 2022 were retrospectively analyzed. These data were pooled with studies obtained from a comprehensive search of Medline, Embase and Scopus from January 2000 to November 2022 and a meta-analysis was performed. Pooled event rates were calculated using an inverse variance model.

Results The search strategy yielded 13 studies that were combined with data from 30 patients from our centers; a total of 399 patients were included in the analysis. The pooled rate of sample adequacy was 94.1% (95% confidence interval [CI] 90.0-98.2), while the pooled rate of diagnostic accuracy was 91.3% (95%CI 87.0-95.5). The pooled sensitivity and specificity for diagnosing malignant lesions with EUS-TA from gastric wall thickening were 94.8% (95%CI 91.3-97.2) and 100% (95%CI 93.6-100), respectively. There were no reported adverse events in any of the studies.

Conclusions EUS-TA offers a safe and accurate diagnostic modality for the etiological diagnosis of thickened gastric walls. Further research is required to identify the needle type and optimal technique for improving outcomes.

Keywords Endoscopic ultrasonography, fine-needle aspiration and biopsy, gastric lymphoma, linitis plastica

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Conflict of Interest: None

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Introduction

Abnormal gastric wall thickening poses a diagnostic challenge for the endoscopist. When the pathology is located in the subepithelial layers, with accompanying reactive fibrosis in the mucosal layer, endoscopic mucosal biopsies are often inadequate for diagnosis [1]. Ji *et al* showed a low diagnostic yield of 28% when deep mucosal biopsies using the bite-on-bite technique were taken for subepithelial gastric lesions [2]. Abnormal gastric wall thickening can be caused by a wide range of benign and malignant conditions, and expedient diagnosis is required to commence the appropriate treatment [3,4].

Various techniques have been described to obtain histology from the deeper layers of the stomach. Bite-on-bite mucosal

biopsies are not highly targeted and may not sample deep enough to reach the abnormal area of interest [2]. Endoscopic submucosal dissection allows for deeper sampling, but the abnormal area may not be clearly visible on an endoscopic view, increasing the risk of unsuccessful sampling. Moreover, endoscopic submucosal dissection (ESD) is associated with adverse events (AE) such as bleeding and gastric wall perforation [5,6].

Endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) or biopsy (FNB) is a promising solution to this diagnostic challenge. The lesion can be localized and targeted via EUS. Certain EUS features, such as a thickened *muscularis propria* layer, non-preserved gastric wall layers, and the presence of ascites and lymphadenopathy, are highly suggestive of malignancy [7,8]. EUS-guided tissue acquisition (TA) is also a reliable method of obtaining histology required for subsequent resection or systemic chemotherapy. There is a paucity of data on the diagnostic utility of EUS-TA for abnormal gastric wall thickening, with wide variation in the reported data on the outcomes.

Therefore, we conducted a retrospective analysis to determine the diagnostic outcomes of EUS-TA for abnormal gastric wall thickening. We also aimed to summarize the current evidence on the diagnostic outcomes of EUS-TA for abnormal gastric wall thickening by performing a systematic review and meta-analysis.

Patients and methods

Present study

We retrospectively analyzed the data from the endoscopic databases of 5 tertiary care centers in India from October 2020 to October 2022. The data on patients undergoing EUS-FNA/FNB from gastric thickening were collected and analyzed. The present study was performed in accordance with the Declaration of Helsinki and reported as per the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines for observational studies (Supplementary Table 1) [9].

Technique

After informed consent, endosonographers with experience of at least 300 independent EUS procedures carried out the procedures. Guided by a linear echoendoscope (Olympus GF-UCT 180, Tokyo, Japan), procedures were carried out under intravenous conscious or deep sedation (a combination of pentazocine and midazolam or propofol), using a 22-G EUS-FNA/FNB needle (EchoTip® Ultra FNA needle or Acquire needle, Boston Scientific Ltd, USA) with a slow stylet pull and fanning method. A minimum of 2 passes with at least 10 actuations were performed in all cases. Where feasible, rapid on-site evaluation (ROSE) was carried out. After each pass, the materials were put on a glass slide for macroscopic observation. Short tissue pieces and drop-like components were spread between two glass slides, while lengthy tissue fragments were transferred to a 10% formalin fixative for histopathological analysis [10]. Half of the slides were air-dried, half fixed with absolute alcohol, and sent for cytological examination. Gastrointestinal pathologists with more than 5 years of experience analyzed the pathological specimen for sample adequacy and final diagnosis.

Outcomes

The primary outcomes of the present study were sample adequacy and diagnostic accuracy. Sample adequacy was defined as samples adequate for histopathologic examination and immunohistochemical analyses. Diagnostic accuracy was defined as the proportion of true positives + true negatives in the total number of patients. Histopathological examination of the surgically resected specimen or clinical follow up for a minimum of 6 months (in patients not undergoing surgery) was considered the gold standard for diagnosis. Secondary outcomes included AE related to the procedures, reported as per the standard Lexicon of the American Society for Gastrointestinal Endoscopy [11].

Systematic review and meta-analysis

A meta-analysis was conducted in accordance with the updated Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Supplementary Table 2) [12].

Database search

The keywords used for the search strategy were: (EUS OR 'Endoscopic ultrasound') AND (FNA OR FNB) AND (Gastric OR Stomach OR Linitis OR Lymphoma). Using the above keywords, electronic databases of MEDLINE, Embase, and Scopus were searched from January 2000 to November 2022 (SG, SA). The bibliographies of the included studies were also searched for any relevant studies. A third reviewer resolved any disagreement (SS).

Study inclusion

All cohort studies (prospective or retrospective), randomized controlled trials, and cross-sectional studies were screened for the following inclusion criteria: (a) *Study population* – patients with a thickened gastric wall; (b) *Intervention* – EUS-guided

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FNA or FNB within/of the gastric wall; and (c) *Outcomes* – sample adequacy, diagnostic accuracy, and safety. Two reviewers independently assessed each study's title and abstract in line with the aforementioned selection criteria (SG, CCHW). A third reviewer (SB) resolved any differences. Studies with participants under 18 years of age, case series with fewer than 5 patients, and those with insufficient or irrelevant clinical data were also excluded.

Data extraction and quality assessment

The data extraction was carried out by 2 reviewers (SG, SA), and a third reviewer (SS) settled any disputes. Data were collected under the following headings: study author and year, country of study, study design, number of patients, age and sex distribution, details of the lesion, details of the procedure, diagnostic outcomes, and AE. Two independent reviewers (SG, CCHW) assessed the quality of the included studies using a Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [13]. A third independent individual (SB) was consulted in case of any discrepancy.

Statistical analysis

Using a random-effects inverse-variance model, the pooled proportions were calculated by combining data from previous studies and the present study. When the incidence of an outcome in a study was zero, a continuity adjustment of 0.5 was applied before statistical analysis. The heterogeneity of the studies was assessed using Cochran's Q test and I^2 statistics. Significant results were defined as either an I^2 value >75% or a Q test P-value <0.1. Visual inspection of funnel plots was used for publication bias assessment. In order to examine each study's impact on the total effect-size estimate and identify influential studies, a sensitivity analysis was carried out, in the form of a leave-one-out meta-analysis, where one study is eliminated at each iteration. STATA software (version 17, StataCorp., College Station, TX) was used for the statistical analysis.

Results

Present study

The analysis included 30 patients (18 male; median age 46, range 23-76 years). All patients had prior inconclusive endoscopic biopsies (median 2, range 1-4). Table 1 shows the baseline characteristics of each patient, along with the details of the lesion and procedure. Fig. 1 shows the details of one of the cases included in this study.

The median thickness of the gastric wall, measured by EUS, was 16 (range 10-28) mm. The most common site of involvement was the gastric body, with or without the involvement of the antrum. An FNB needle was used in 93.3% of cases. The median number of passes was 2 (range 1-5). Additional samples were taken from involved perigastric lymph nodes in 14/30 (46.7%) patients. The perigastric lymph nodes were mostly hypoechoic, heterogeneous, oval or round-shaped, with sizes ranging from 12-30 mm. ROSE was available

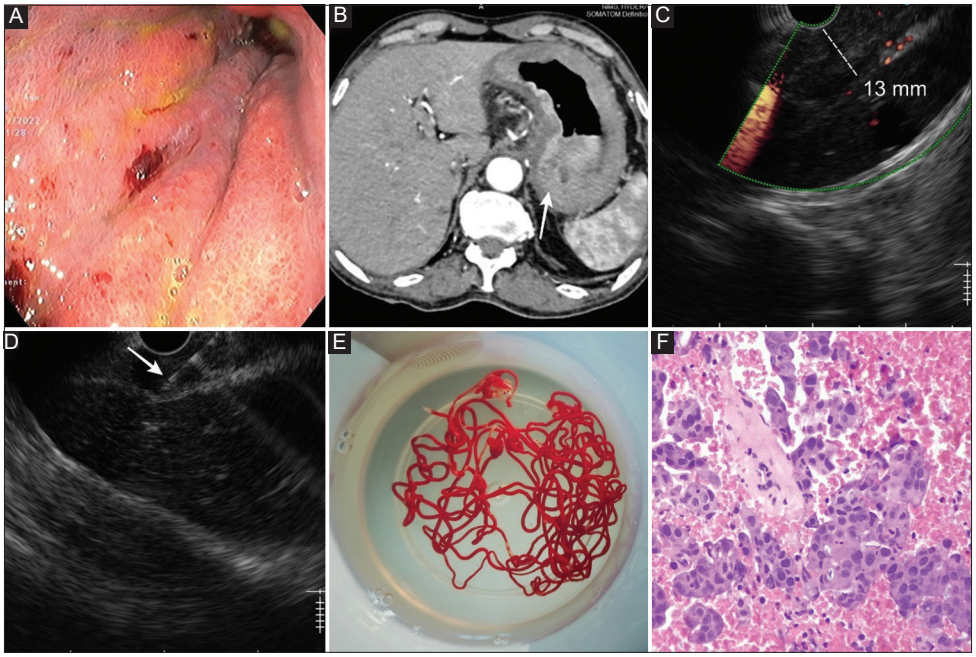

Figure 1 (A) Endoscopy showing thickened gastric wall along the greater curvature. (B) Computed tomography showing thickened gastric wall (arrow) with loss of stratification. (C) Endoscopic ultrasound (EUS) demonstrating hypoechoic thickened gastric wall of 13 mm with loss of normal layered pattern. (D) Fine-needle aspiration under EUS guidance with 22-G needle (arrow). (E) Aspiration specimen in formalin. (F) Histopathology suggestive of poorly differentiated adenocarcinoma

Table 1 Characteristics, procedural details, and outcomes of patients in the present study

Case	Age	Sex	Location	Inconclusive endoscopic biopsies	Needle type	Needle size	No. of passes	ROSE	Thickness, in mm	Lymph nodes	Final diagnosis
Case 1	35	F	Fundus, body, antrum	2	FNB	22G	2	N	16	Y	Adenocarcinoma
Case 2	28	M	Body, antrum	1	FNB	22G	2	N	18	N	Signet cell carcinoma
Case 3	63	F	Fundus, body	2	FNB	22G	3	N	19	Y	Signet cell carcinoma
Case 4	54	M	Body, antrum	2	FNB	22G	5	N	13	Y	Adenocarcinoma
Case 5	51	M	Fundus, body	3	FNB	22G	4	N	14	N	Signet cell carcinoma
Case 6	73	M	Body	2	FNB	22G	2	N	12	N	Metastatic
Case 7	50	F	Body, antrum	1	FNB	22G	2	Y	22	N	Inconclusive
Case 8	36	M	Body, antrum	2	FNB	22G	2	N	25	N	Adenocarcinoma
Case 9	31	F	Body	2	FNB	22G	2	N	10	N	Signet cell carcinoma
Case 10	45	F	Body	3	FNB	22G	1	Y	18	N	Lymphoma
Case 11	36	M	Body, antrum	2	FNB	22G	2	N	20	Y	Tuberculosis
Case 12	36	F	Body, antrum	4	FNB	22G	2	N	10	N	Adenocarcinoma
Case 13	36	M	Body, antrum	1	FNB	22G	2	N	28	Y	Lymphoma
Case 14	23	F	Body	2	FNB	22G	2	N	10	N	Adenocarcinoma
Case 15	36	M	Body	2	FNB	22G	2	N	12	N	Inconclusive
Case 16	36	F	Body, antrum	2	FNB	22G	2	N	10	Y	Adenocarcinoma
Case 17	35	F	Body	3	FNB	22G	1	Y	20	N	Inconclusive
Case 18	23	M	Body	1	FNB	22G	2	N	23	Y	Adenocarcinoma
Case 19	34	F	Body	2	FNB	22G	2	N	10	Y	Lymphoma
Case 20	51	M	Antrum	2	FNB	22G	2	Y	18	Y	Adenocarcinoma
Case 21	44	M	Antrum	3	FNB	22G	4	Y	19	Y	Tuberculosis
Case 22	72	M	Body, antrum	1	FNB	22G	2	N	25	N	Adenocarcinoma
Case 23	53	M	Body, antrum	2	FNB	22G	4	Y	20	Y	Lymphoma
Case 24	47	F	Fundus, body	3	FNB	22G	2	Y	13	Y	Adenocarcinoma
Case 25	75	M	Antrum	2	FNB	22G	3	N	13	Y	Lymphoma
Case 26	73	M	Fundus, body	4	FNB	22G	2	N	28	N	Metastatic
Case 27	64	M	Body, antrum	2	FNB	22G	2	N	13	N	Adenocarcinoma
Case 28	54	F	Body	2	FNB	22G	3	N	13	Y	Lymphoma
Case 29	76	M	Body, antrum	3	FNA	22G	3	N	16	N	Adenocarcinoma
Case 30	65	M	Body, antrum	1	FNA	22G	3	N	15	N	Adenocarcinoma

FNA, fine-needle aspiration; FNB, fine-needle biopsy; ROSE, rapid on-site evaluation

in 7/30 (23.3%) of cases. There were no reported intra- or postprocedural adverse events following the EUS-TA.

An adequate sample was obtained in 29/30 (96.6%) cases of gastric wall thickening. The EUS-FNA from gastric thickening was diagnostic in 27/30 (90%) of patients. Gastric adenocarcinoma was the most common diagnosis (13/30, 43.3%), followed by gastric lymphoma (6/30, 20%), signet ring cell carcinoma (4/30, 13.3%), metastatic disease (2/30, 6.7%), and tuberculosis (2/30, 6.7%). Of the 3 cases with an inconclusive diagnosis from pathological examination of the EUS-FNA/B sample, 1 was diagnosed as adenocarcinoma with an FNB sample from a metastatic lymph node. Two other patients with gastric thickening did not develop any progression over a 6-month follow up. Fig. 1 and 2 show the details of 2 cases of gastric thickening with negative endoscopic biopsy, 1 of which was diagnosed as gastric adenocarcinoma and another as gastric lymphoma.

Systematic review and meta-analysis

Literature search and study characteristics

A total of 3215 records were retrieved using the above search strategy, of which 14 studies were included in the final analysis [14-26]. Fig. 3 shows the PRISMA flowchart for the study selection and inclusion process. The baseline characteristics of the included studies are summarized in Table 2. The majority of the studies were retrospective in nature, with numbers of patients ranging from 8-104. The thickness of the gastric wall varied from 5 mm to 30 mm. Two studies used FNA needles [16,18], 2 used trucut biopsy needles [14,15], 2 used FNB needles [21,25] and the rest used either FNA or FNB. The dry suction method was used in the majority of studies. The median number of passes was 2 in the majority of the studies. Supplementary Fig. 1 shows the study quality analysis using the QUADAS-2 tool. One study had a low risk of bias [22], 3 had an intermediate risk of bias [16,20,23], and the rest had a high risk of bias.

Sample adequacy

Data on sample adequacy were reported by 10 studies (n=195) [14,15,17,20-22,24-26]. The pooled rate of sample adequacy with EUS-TA from gastric wall thickenings was 94.1% (95% confidence interval [CI] 90.0-98.2; I^2=28.5%) (Supplementary Fig. 2). Considering only malignant lesions (n=157), the sample adequacy rate was 92.4% (95%CI 87.4-97.4; I^2=26.2%).

Sensitivity and specificity for the diagnosis of malignancy

Data from 9 studies were used to calculate the pooled sensitivity and specificity [14,15,18-20,22,23,25]. The pooled sensitivity and specificity for diagnosing malignant lesions with EUS-TA from gastric wall thickening were 94.8% (95%CI 91.3-97.2; I^2=26.4%) and 100% (95%CI 93.6-100; I^2=0.0%), respectively (Fig. 4). The Fagan nomogram showed that a positive result increased the pretest probability of malignancy from 50-99%, whereas a negative result decreased the pretest probability from 50% to 6% (Supplementary Fig. 3). The summary receiver operating characteristic curve showed an area under the curve of 1.00 (95%CI 0.99-1.00) (Supplementary Fig. 4).

Diagnostic accuracy

All 14 studies (n=399) reported diagnostic accuracy with EUS-TA from gastric wall thickening [11-23]. The pooled diagnostic accuracy was 91.3% (95%CI 87.0-95.5; I^2=63.0%), with moderate heterogeneity among the studies (Fig. 5). With respect only to malignant lesions, the data from 357 patients showed a pooled diagnostic accuracy of 89.5% (95%CI 84.5-94.5; I^2=63.6%).

AEs

None of the studies reported any AEs with EUS-TA from gastric wall thickening.

Publication bias and sensitivity analysis

Funnel-plot assessment showed the presence of publication bias for sample adequacy and accuracy (Supplementary Fig. 5). Deek's plot showed significant publication bias for pooled sensitivity and specificity (Supplementary Fig. 6). Egger's test showed the presence of a small-study effect for all the outcomes (Supplementary Table 3). There was no change in the overall effect with the leave-one-out analysis.

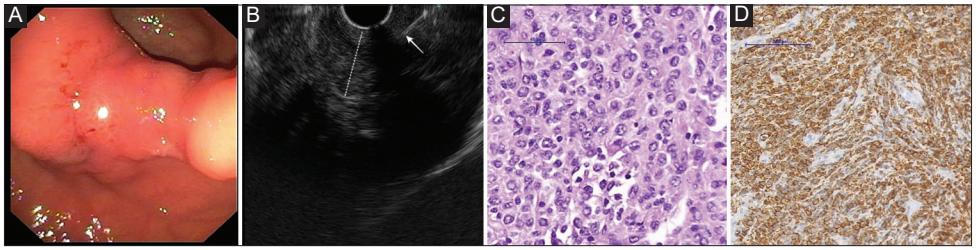

Figure 2 (A) Endoscopy showing thickening of the antropyloric region with (B) endoscopic ultrasound-guided fine-needle aspiration (arrow) from gastric wall thickening with a maximum thickness of 16 mm (dotted line); (C) high-power microscopy (400×) showing sheets of large atypical lymphoid cells; and (D) immunohistochemistry (400×) showing diffusely CD20 positivity suggestive of diffuse large B-cell lymphoma

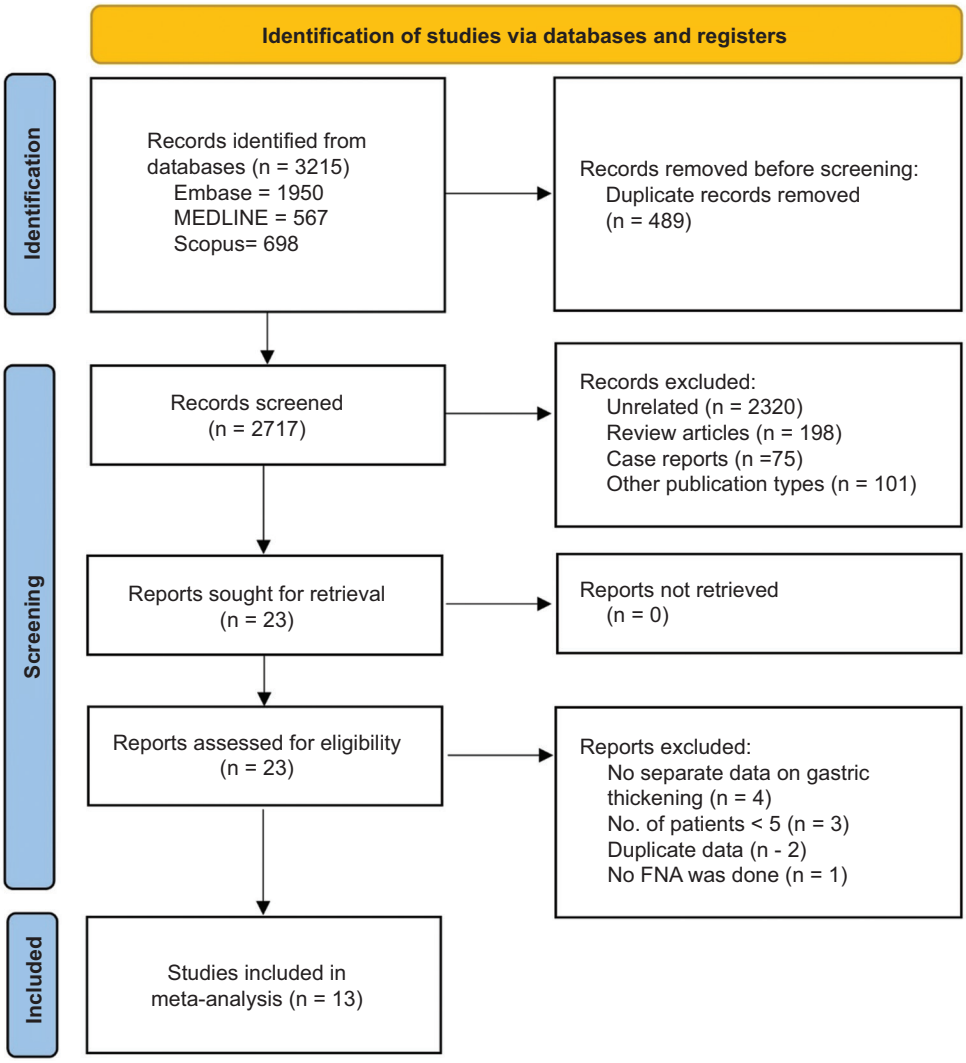

Figure 3 PRISMA flowchart for study identification, selection, and inclusion process
FNA, fine-needle aspiration

Discussion

Tissue diagnosis is essential for managing thickened gastric walls detected on endoscopy or cross-sectional imaging. EUS-TA is a valuable tool for the etiological diagnosis of thickened gastric walls in patients with prior negative endoscopic biopsies. The present multicenter study of 30 patients undergoing EUS-TA for evaluation of gastric wall thickening showed a sample adequacy of 96.6% and a diagnostic accuracy of 90%, without any procedure-related AE. The meta-analysis of data from 427 patients showed a pooled rate of sample adequacy and a diagnostic accuracy of 94.1% and 91.3%, respectively, for EUS-TA from gastric wall thickening. The pooled sensitivity and specificity for diagnosing malignant lesions with EUS-TA from gastric wall thickening were 94% and 99%, respectively.

Gastric wall thickening is due to a variety of conditions, both benign and malignant. Of concern are the malignant etiologies such as linitis plastica and lymphoma. EUS plays an important role in the management of early gastric cancer by determining the T stage and N stage, aiding in treatment by endoscopic therapy. In instances of diffuse wall thickening, a diagnostic EUS aids in identifying the layer of origin and the preservation of layers and extension through various layers [27]. As the standard biopsy forceps provide samples with only a mucosal layer, the yield is very low in patients with these malignancies, which usually present as wall thickening in the absence of mucosal abnormalities. Standard endoscopic biopsies have a low yield of 51.2% in diagnosing these malignancies of the infiltrative pattern [28]. This led to the European Society of Gastrointestinal Endoscopy's guideline recommending EUS-guided sampling after the failure of standard biopsy techniques in patients with diffuse gastric wall thickening [29].

Multiple features of EUS can give clues to an underlying malignant etiology. Ascites, loss of wall structure, impaired gastric distension, and presence of pathologic lymph nodes point towards malignancy [19]. Further, Thomas *et al* [5] reported a significant difference in the wall thickness between

Table 2 Baseline characteristics of studies included in the present analysis

Author, Year [ref.]	Country	Study design	Type of publication	No. of patients	Male/ female	Age, in years	Thickness, in mm	Size & type of needle	Type of suction	No. of passes
Aithal 2005 [14]	UK	Retrospective	Full-text	10	6/4	40-87	5-20	19-G TCB	-	2.5 (1-5)
Thomas 2009 [15]	UK	Retrospective	Full-text	31	20/11	67 (60-74)	18	19-G TCB	-	3 (1-5)
Yu 2016 [16]	China	Retrospective	Full-text	39	14/25	49.3±14.8	-	FNA	DS	2-4
Liu 2018 [17]	China	Retrospective	Full-text	26	9/17	54 (29 - 70)	8.3-22.7	19-, 22- or 25-G FNA/B	DS	1 to 3
Ye 2018 [18]	China	Retrospective	Full-text	46	20/26	47±10.3	15.7±5.8	19-G FNA	DS	4-8
Coronel 2019 [19]	USA	Retrospective	Abstract	12	7/5	63	-	22- or 25-G FNA/B	-	2 (2-3)
Téllez-Ávila 2019 [20]	Mexico	Retrospective	Full-text	22	10/12	57.8±14.7	15 (6-50)	19- or 22-FNA/B	DS	2 (1-10)
Karagyozov 2021 [21]	Bulgaria	Retrospective	Abstract	8	4/4	-	-	22-G FNB	-	-
Chavarria 2021 [22]	Spain	Retrospective	Full-text	15	-	68 (56-77)	10.9 (5-30)	19- or 22-FNA/B	DS	4 (3-5)
Fan 2021 [23]	China	Retrospective	Full-text	104	-	-	14±7	-	-	-
Takada 2021 [24]	Japan	Retrospective	Full-text	13	9/4	40-84	20 (15-25)	19- or 22-FNA/B	DS	3
Assaf 2022 [25]	France	Retrospective	Full-text	34	20/14	65 (36-89)	-	20- or 22-FNB	SSP	2-3
Liu 2022 [26]	China	Retrospective	Full-text	12	-	-	-	19-, 22- or 25-G FNA/B	-	-
Present study	India	Retrospective	Full-text	30	18/12	46 (23-76)	16 (10-28)	22-FNA/B	SSP	2 (1-5)

FNA, fine-needle aspiration; FNB, fine-needle biopsy; TCB, trucut biopsy; DS, dry suction; SSP, slow stylet pull

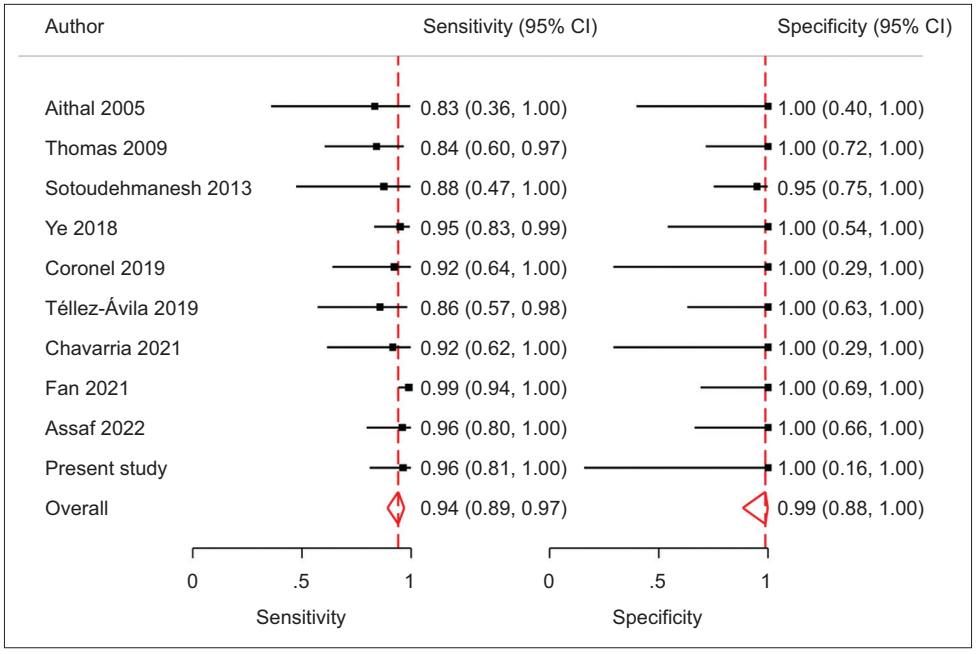

Figure 4 Forest plot for pooled sensitivity and specificity with endoscopic ultrasound-guided transesophageal tissue acquisition from gastric wall thickening
CI, confidence interval

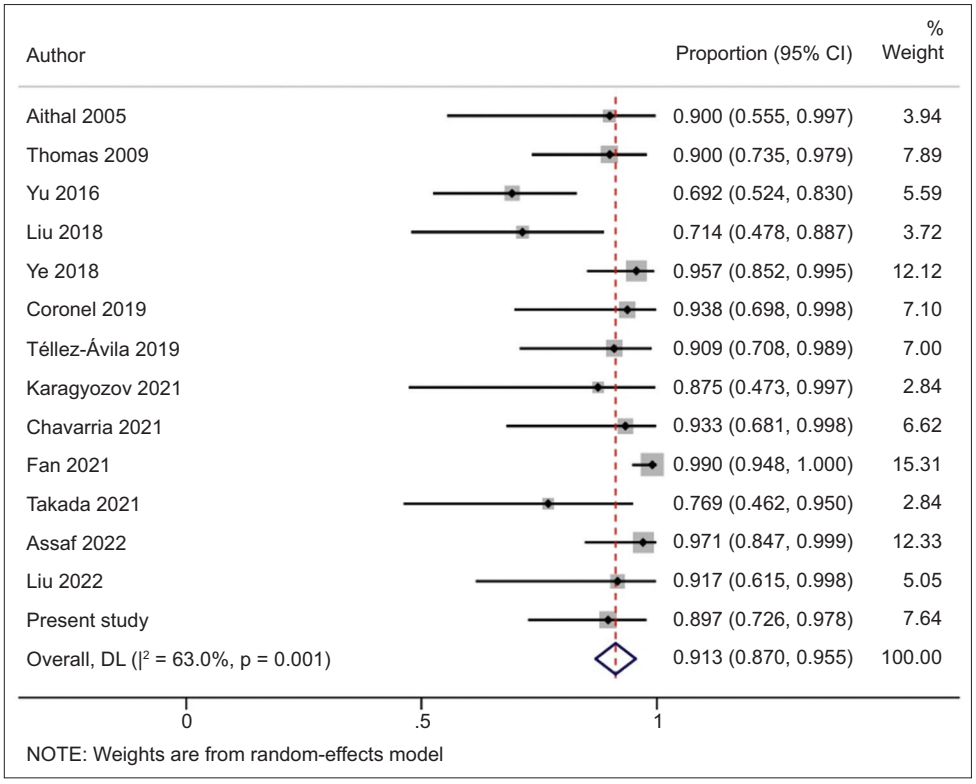

Figure 5 Forest plot for pooled diagnostic accuracy with endoscopic ultrasound-guided transesophageal tissue acquisition from gastric wall thickening
CI, confidence interval

benign disease and malignant disease (10 mm vs. 15 mm; P=0.037). In a study by Liu *et al* [17], certain features such as enlarged or effaced rugal folds, loss of distensibility despite air insufflation, circumferentially infiltrating lesions associated with hyperemic mucosal change, and small erosions on white light endoscopy (WLE) were used to determine the presence

and extent of gastric linitis plastica. Their study showed a higher positive rate with EUS-FNA (71.43%) than with WLE (47.37%). However, the difference did not reach statistical significance (P=0.12), perhaps because of the small number of patients.

In the present study, associated perigastric lymph nodes were seen in 46.7% of the cases, and EUS-FNA/B was carried out at these nodes as well as the gastric thickening. Of the 3 cases with an inconclusive diagnosis on the EUS-FNA/B sample, 1 was diagnosed as adenocarcinoma with an FNB sample from a metastatic lymph node. Hence, in the presence of associated perigastric lymphadenopathy, samples should be obtained from the gastric wall as well as from nodes, as this would improve the diagnostic accuracy.

In patients with suspected gastrointestinal lymphoma, auxiliary methods such as flow cytometry (FCM) and gene rearrangement provide additional information for diagnosis and management. Yu *et al* utilized a gastric thickening sample obtained by EUS-FNA for FCM and analysis of gene rearrangement [16]. Adequate material for FCM was obtained in 84.6% of cases (33/39), while only 50% (14/28) of the cases had adequate material for analyzing monoclonal gene rearrangement. An FNA needle was used for TA in this study. Whether an FNB needle can improve the adequacy of the sample for conducting auxiliary tests remains a topic for future research. FNB samples may also provide a further advantage in maintaining the tissue architecture for additional histopathological assessment. Recent studies have shown that genomic profiling of an EUS-FNB sample can identifying clinically applicable druggable mutations in various cancers [30]. Therefore, further studies on the role of EUS-FNB are required for patients with gastric thickening.

In the included studies, the median number of negative endoscopic biopsies prior to EUS-TA varied from 2 to 4. Though guidelines endorse EUS after the failure of sampling by standard techniques, the number of failed endoscopic biopsies [29] after which EUS-TA should be sought is not specified in the guidelines, and this delays the diagnosis. Furthermore, the high false negative rates with standard sampling methods in gastric wall thickening indicate the impending need to adopt EUS-guided tissue acquisition as the standard of care for these patients. Hence, the threshold for EUS-TA should be lower, and patients with gastric wall thickening should be scheduled for EUS-TA as the first-line modality for tissue acquisition or immediately after one report of a negative biopsy with forceps.

Multiple other techniques have been studied to improve tissue acquisition and diagnostic yield from gastric thickening. A new technique called after-EUS judgment (AEJ) biopsy was used by Liu *et al* [26]: after the target lesion in the gastric wall was identified using EUS, biopsies were performed using biopsy forceps under EUS guidance. For diffuse infiltrative lesions, EUS-FNA was performed. The positive rate of biopsy by WLE was 77.93%, whereas that of AEJ biopsy was 89.38%. Notably, for diffuse thickening of the gastric wall, the positive rate of EUS was 91.67%, while it was 0% for WLE [26]. Shan *et al* reported a technique similar to mucosal incision-assisted biopsy for subepithelial lesions [31], called via mucosa incision EUS-guided sampling [32]. After identification of the submucosal lesion by EUS, a small incision is made, through which biopsy forceps are inserted, and a biopsy is taken from the target lesion under EUS guidance. The authors reported no perforation or massive bleeding. However, an incision is always associated with a risk of bleeding, which may make this technique less useful. Further studies are required to study the role of these techniques in the diagnosis of gastric thickening.

To the best of our knowledge, this is the first meta-analysis on the diagnostic efficacy of EUS-TA from a thickened gastric wall. The lack of significant heterogeneity for the majority of outcomes adds to the strength of the study. Nevertheless, the present meta-analysis had multiple limitations. As most studies included in the meta-analyses were retrospective, it may be associated with selection bias. No data were available for comparison of the diagnostic outcomes of EUS-FNA and FNB. Further studies with large sample sizes are needed to determine the choice of needle and suction and also compare EUS-TA with other modalities, such as standard biopsy, mucosal incision-assisted biopsy and endoscopic submucosal sampling. Moreover, 3 studies with low diagnostic accuracy [16,17,24] did not mention the sensitivity, which led to a high pooled sensitivity in the analysis. Lastly, reference standards were not mentioned in the majority of studies.

To conclude, EUS-FNA/B from gastric wall thickening provides a safe and effective diagnostic modality after indeterminate endoscopic biopsy. EUS-TA also allows assessment of the depth of invasion and simultaneous sampling of perigastric lymph nodes, which may be helpful for staging and increasing the diagnostic accuracy. Further prospective studies are required to ascertain the optimal technique of EUS-TA to improve diagnostic accuracy, and whether EUS-TA should be advised early for gastric wall thickening rather than waiting for multiple negative endoscopic biopsies.

Summary Box

What is already known:

- Endoscopic biopsy from the thickened gastric wall may not always give a diagnosis, because of the submucosal location of the pathology
- Endoscopic ultrasound (EUS)-guided tissue acquisition (TA) may serve as a minimally invasive diagnostic tool in such cases
- There is a wide variation in the reported data on the outcomes of EUS-TA for abnormal gastric wall thickening

What the new findings are:

- The pooled sample adequacy and diagnostic accuracy rates were >90% with EUS-TA from abnormal gastric wall thickening
- The pooled sensitivity and specificity with EUS-TA from abnormal gastric wall thickening were more than 95%
- EUS-TA from gastric thickening is a safe technique with no reported adverse events

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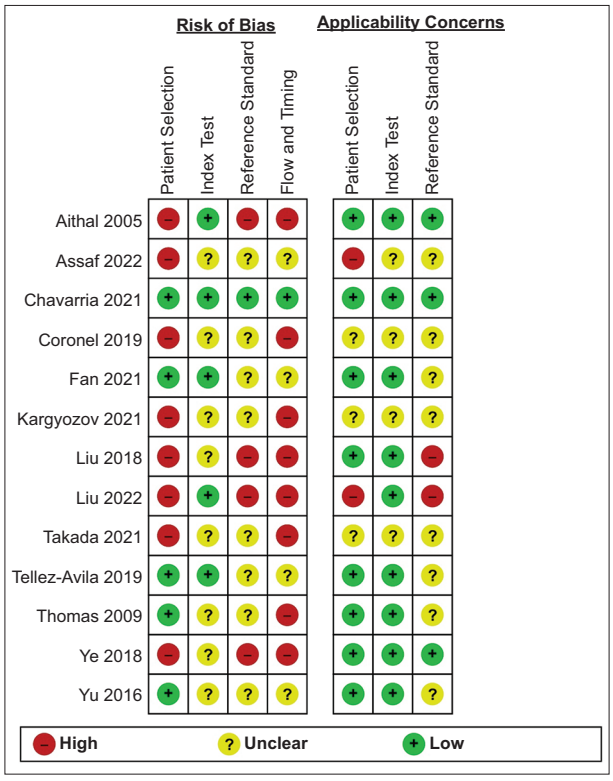
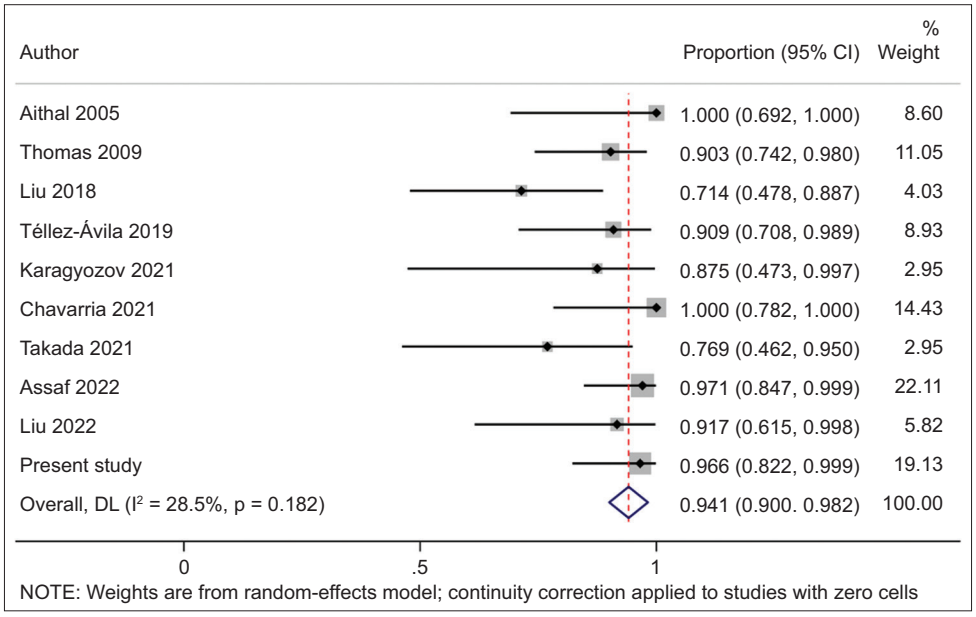
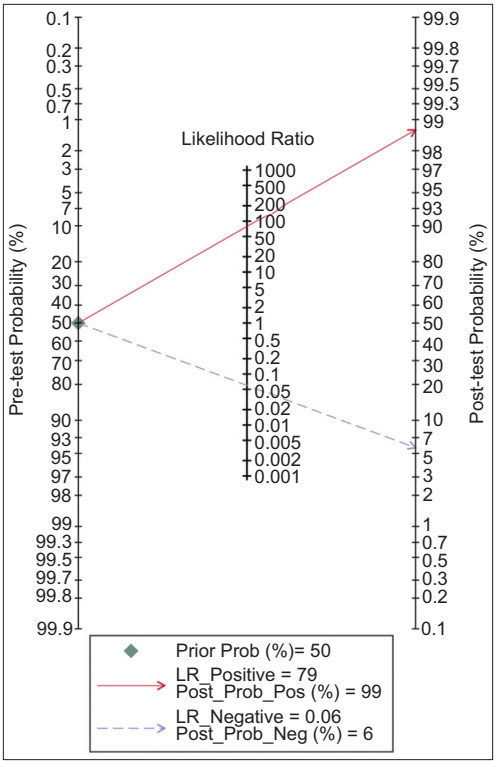
Supplementary material



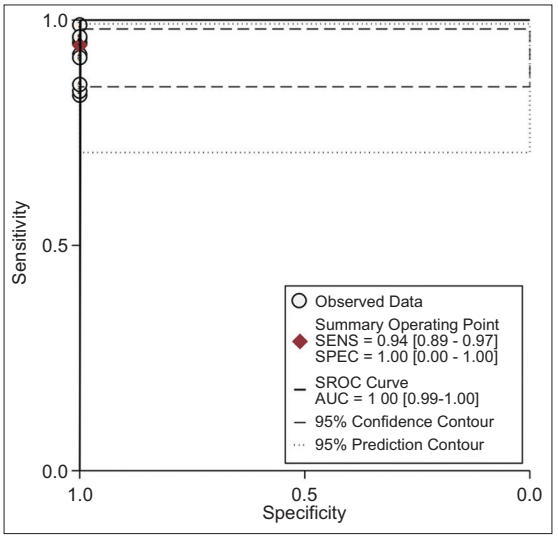
Supplementary Figure 1 Study quality assessment using the QUADAS-2 tool



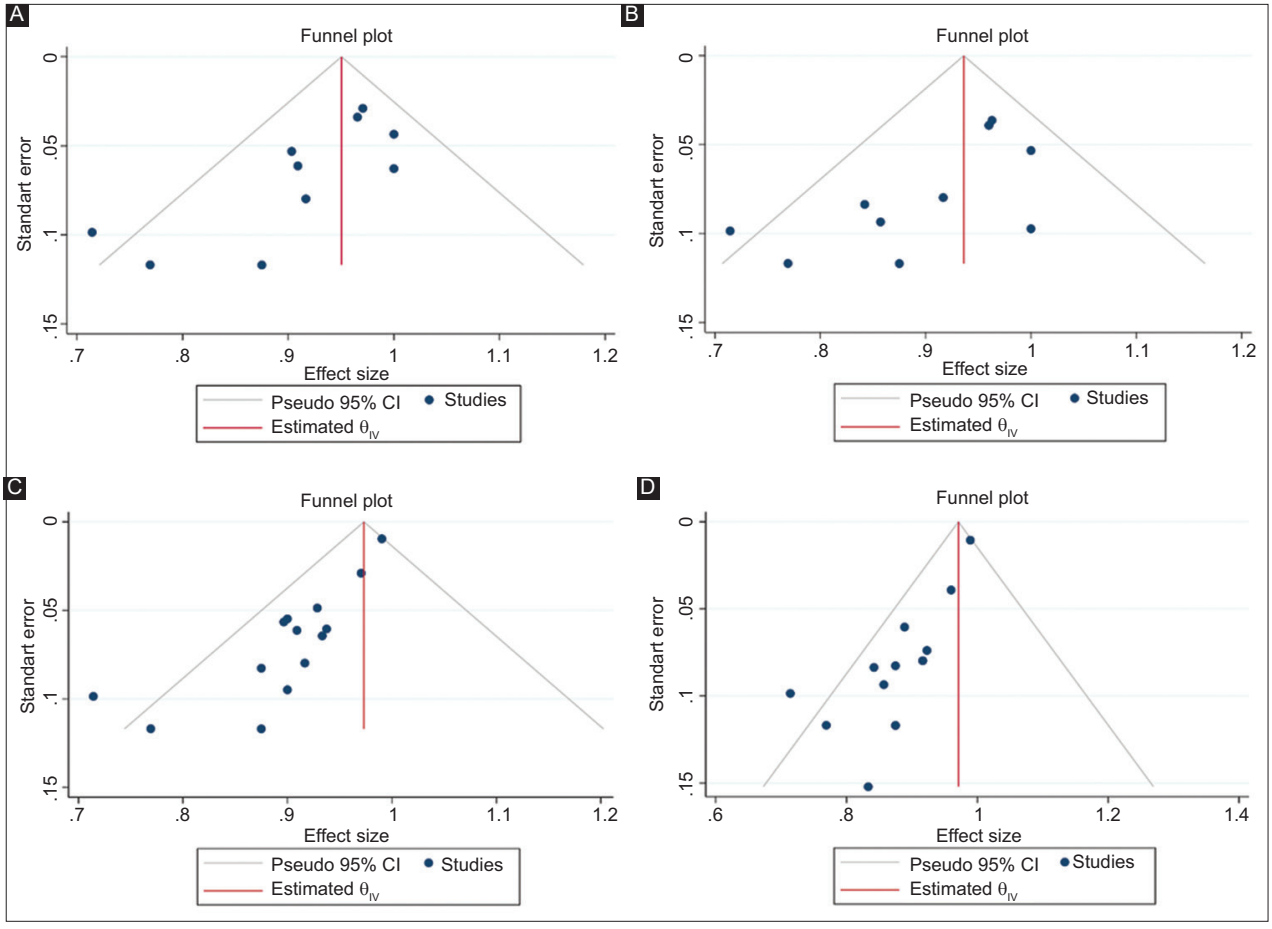
Supplementary Figure 2 Forest plot for pooled sample adequacy with endoscopic ultrasound-guided tissue acquisition from gastric thickening
CI, confidence interval



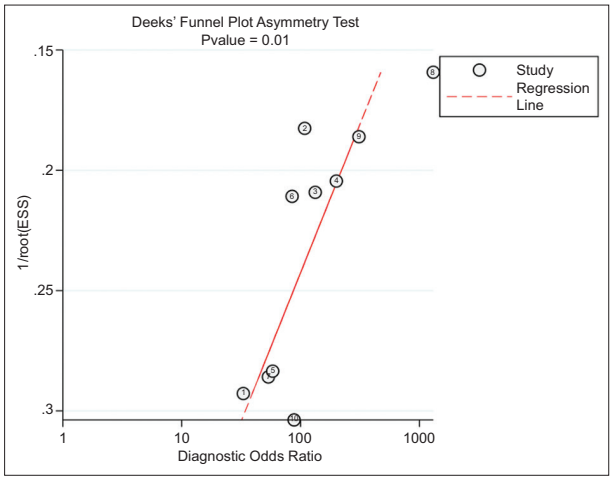
Supplementary Figure 3 Fagan nomogram for prediction of post-test probability of pathology based on the likelihood ratio



Supplementary Figure 4 Summary receiver operating characteristic curve



Supplementary Figure 5 Funnel plot for publication bias concerning (A) sample adequacy, (B) sample adequacy in malignant lesions, (C) diagnostic accuracy, and (D) diagnostic accuracy in malignant lesions



Supplementary Figure 6 Deek's plot for publication bias for sensitivity and specificity

Supplementary Table 1 STROBE statement—checklist of items that should be included in reports of observational studies

Checklist	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
		Introduction	
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
		Methods	
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than 1 group	3
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	N/A
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	N/A
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	N/A
		(*e*) Describe any sensitivity analyses	N/A
		Results	
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	6
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	N/A
		Cross-sectional study—Report numbers of outcome events or summary measures	N/A

(*Contd...*)

Supplementary Table 1 (*Continued*)

Checklist	Item No	Recommendation	Page No
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6
		(*b*) Report category boundaries when continuous variables were categorized	6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
		Discussion	
Key results	18	Summarise key results with reference to study objectives	7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
		Other information	
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org

Supplementary Table 2 PRISMA checklist for systematic review and meta-analysis

Section and Topic	Item #	Checklist item	Location where item is reported
		TITLE	
Title	1	Identify the report as a systematic review.	1
		ABSTRACT	
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
		INTRODUCTION	
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	3
		METHODS	
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	4
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	4
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	4
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	4

(*Contd...*)

Supplementary Table 2 (*Continued*)

Section and Topic	Item #	Checklist item	Location where item is reported
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	5
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g., for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	5
	10b	List and define all other variables for which data were sought (e.g., participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	5
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	5
Effect measures	12	Specify for each outcome the effect measure(s) (e.g., risk ratio, mean difference) used in the synthesis or presentation of results.	5
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g., tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	5
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	5
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	5
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	5
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g., subgroup analysis, meta-regression).	5
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	5
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	5
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	5
		RESULTS	
Study selection	16	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	5
Study characteristics	17	Cite each included study and present its characteristics.	Table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Supplementary Fig. 1
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g., confidence/credible interval), ideally using structured tables or plots.	6,7
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	6,7
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g., confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	6,7
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	7
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	7

(*Contd...*)

Supplementary Table 2 (*Continued*)

Section and Topic	Item #	Checklist item	Location where item is reported
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	7
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	7
		DISCUSSION	
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	7,8,9
	23b	Discuss any limitations of the evidence included in the review.	9
	23c	Discuss any limitations of the review processes used.	9
	23d	Discuss implications of the results for practice, policy, and future research.	9
		OTHER INFORMATION	
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	N/A
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	N/A
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	1
Competing interests	26	Declare any competing interests of review authors.	1
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	N/A

Supplementary Table 3 Egger's test for assessment of small-study effect

Parameter	β1	SE of β1	z	Prob > |z|
Sample adequacy	-1.91	0.740	-2.59	0.0097
Sample adequacy (malignant)	-1.84	0.757	-2.43	0.0150
Diagnostic accuracy	-1.59	0.363	-4.37	0.0000
Diagnostic accuracy (malignant)	-1.54	0.348	-4.43	0.0000