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Meta-analysis of Anti- Saccharomyces Cerevisiae Antibodies as diagnostic markers of Behçet's disease

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Abstract

Objective: To assess the diagnostic value of anti-saccharomyces cerevisiae antibodies (ASCA) in Behçet's disease (BD) patients and explore their relationship with other autoimmune diseases(AID).

Methods: Relevant studies investigating ASCA levels in BD patients were retrieved from PubMed, EMBASE, Web of Science, SOCPUS, and the Cochrane Library. Review Manager 5.3, Meta-DiSc 1.4 and Stata/SE 12.0 were used to perform quality assessment, meta-analysis, and sensitivity analysis. Subgroup analysis were performed disaggregated

by isotypes of ASCA. We also summarized the diagnostic performance of ASCA in AID based on a comprehensive database search.

Results: Nine studies were included in the meta-analysis. All four types of ASCA were useful to distinguish between gastrointestinal BD (GIBD) and healthy controls (HC). ASCA-IgG was useful to differentiate between GIBD and HC [odds ratio (OR) 5.74 (95% confidence interval (CI), 2.83–11.65); sensitivity 0.34 (95% CI, 0.27–0.41); specificity 0.93 (95% CI, 0.87–0.96)]; based on summary receiver operating characteristic curve, the positive rate in Crohn's disease (CD) was higher than that in BD/GIBD, while patients with negative results were more likely to be diagnosed as CD. However, it was difficult to distinguish GIBD from intestinal tuberculosis (iTB) and ulcerative colitis (UC), and to distinguish BD from UC and HC, with the area under the curve approximately 0.5 for all.

Conclusion: ASCA may not be a useful serologic marker of BD/GIBD. It does not play a significant role in the differential diagnosis from intestinal diseases. The prevalence of ASCA in AID suggests a common pathogenetic role in AID.

Key words: Behçet's disease; Anti-saccharomyces cerevisiae antibodies; autoimmune diseases; meta-analysis; autoantibodies

Strengths and limitations of this study

- Behcet's disease is a non-marker autoimmune/inflammatory disease in which autoantibodies play an important role.
- We perform group analysis according to antibody subtypes of ASCA.
- The control groups include healthy controls and differential diagnosis disease (inflammatory bowel disease)
- Comprehensive summary of ASCA antibodies in autoimmune diseases is included.
- Too much subgrouping may lead to potential heterogeneity

Introduction

Behçet's disease (BD) is a chronic systemic vascular inflammatory disease with a high propensity for recurrence. The etiopathogenesis of BD is yet to be elucidated. The condition is characterized by recurrent oral ulcers, genital ulcers, ophthalmitis, and skin lesions. It can also involve blood vessels, nervous system, digestive tract, joints, and other organs in the body. BD not only impairs the quality of life of patients but can also cause serious consequences and even death. Involvement of eyes, the central nervous system, and large blood vessels may lead to serious complications. The onset of BD typically occurs in

the third or fourth decade of life; the condition is rarely seen in children or in individuals older than 50 years [1]. BD has a typical geographical distribution consistent with the historical "Silk Road"; therefore, the condition is also referred to as the "Silk Road Disease". Its prevalence has considerably increased in the Mediterranean region, the Middle East and the Far East; Turkey has the highest prevalence rate of BD (420/100,000) [2]. This peculiar geographical distribution suggests a role of genetic factors in the pathogenesis of BD. An increasing number of studies have shown a strong correlation between human leukocyte antigen HLA-B51 and BD [3, 4]. However, a significant number of patients with BD test negative for HLA-B51; in addition, the HLA-B51 positivity rate is lower in patients with bone disease, especially in non-endemic areas; this indicates that other factors may play a role in the pathogenesis of BD [5]. Due to the lack of specific laboratory tests, the diagnosis of BD is typically challenging and is mainly based on clinical manifestations. The 1990 International Study Group (ISG) criteria [6] were earlier used for the clinical diagnosis of BD; however, owing to its low sensitivity, the criteria were updated in 2013 and are now referred to as the International Criteria for Behçet's Disease (ICBD) [7]. However, these criteria do not include any laboratory tests, and are based on the clinician's judgment, which is relatively subjective and may lead to misdiagnosis. Due to the non-specific clinical characteristics, those with prominent involvement of a particular organ system are easily misdiagnosed. Patients with gastrointestinal involvement as the main manifestation are

easily misdiagnosed as Crohn's disease (CD), ulcerative colitis (UC), or intestinal tuberculosis (iTB); joint symptoms are misdiagnosed as rheumatoid arthritis or ankylosing spondylitis; skin mucosal damage is misdiagnosed as polymorphic erythema, nodular erythema, syphilis, or systemic lupus erythematosus. Likewise, nervous system damage is misdiagnosed as infectious or allergic meningitis, cerebrospinal tumor, multiple sclerosis, or psychosis. In addition, diagnosis is frequently delayed until the development of clinical manifestations to qualify the criteria. Studies have shown that BD is often diagnosed after a gap of several years from the first appearance of symptoms. For example, patients with oral ulcers were diagnosed with BD after a mean delay of 3.77±4.43 years after the appearance of oral ulcers [8]. The delay in diagnosis may have deleterious effects on the patients.

Several recent studies have shown that anti-Saccharomyces Cerevisiae antibody (ASCA), directed against the phosphopeptidomannan part of the cell wall of the yeast, is an important serological marker of BD, especially in patients with gastrointestinal manifestations. However, patients with inflammatory bowel disease such as Crohn's disease (CD) also have a high prevalence rate of ASCA [9, 10, 11, 12, 13, 14]. Thus, comprehensive quantitative analysis to assess the relevance of ASCA as a routine laboratory test for diagnosis for BD is a key imperative. We performed a meta-analysis of relevant studies to assess the diagnostic relevance of ASCA as a marker of BD.

Methods

Literature search

A comprehensive literature search was performed in 5 biomedical databases, i.e., PubMed, EMBASE, Web of Science, SOCPUS, and the Cochrane Library. The key words used were Behçet's disease and anti-Saccharomyces Cerevisiae antibody. Search range was "all fields" or "all text". No restrictions were imposed with respect to time of publication, region, or ethnicity of the study population. All documents were updated to January 2019.

Eligibility and exclusion criteria

The inclusion criteria were: (1) studies that evaluated the diagnostic accuracy of ASCA in BD; (2) availability of adequate data pertaining to the prevalence rate or serum levels of ASCA in patients with BD; (3) studies with healthy population and/or disease controls; (5) meeting abstracts or letters to the editor were also included.

The exclusion criteria were: (1) studies with incomplete data; (2) review articles; (3) non-English articles; (4) in case of studies with overlapping study population, studies with smaller sample size were excluded. Two investigators independently performed the

literature search, screened the titles and abstracts, followed by full-text review of eligible studies.

Data extraction and quality assessment

Two independent investigators reviewed the full-text articles, extracted the data, and assessed the study quality using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) through Revman 5.3; the included items were evaluated as yes, no, or uncertain. Inter-researcher disagreements were resolved by consensus, or by a third investigator. Data pertaining to the following variables were extracted: publication year, article type, first author's name, country, isotypes of ASCA detected, age and sex, research design, sample size, experimental method, trade names of experimental materials, cut-off values, diagnostic critera, and serum titers and/or prevalence rate of ASCA in BD, gastrointestinal BD (GIBD), healthy controls (HC), patients with Crohn's disease (CD), ulcerative colitis (UC), and intestinal tuberculosis (iTB). The data were either obtained directly from the article, calculated, or requested from the author via e-mail.

Statistical analysis

We used Stata/SE 12.0, Review Manager 5.3, and Meta-DiSc 1.4 for data analysis. Subgroup analysis was performed disaggregated by the isotypes of ASCA and different disease controls. Heterogeneity among the included studies was evaluated using the Cochran's Q-statistic as well as the I^2-statistic. P value > 0.10 for the Q-statistic, or $I^2 < 50\%$ was considered indicative of lack of significant heterogeneity and the fixed effects model (FEM) was used for the analysis; in case of significant heterogeneity, the random effects models (REM) was used for analysis. We analyzed the pooled diagnostic odds ratio (DOR), sensitivity, specificity, positive likelihood ratio (LR+), and negative likelihood ratio (LR-), and their corresponding 95% confidence intervals (CI). The area under the summary receiver operating characteristic (SROC) curve was used to assess the overall diagnostic performance of ASCA. Sensitivity analysis was performed using Stata/SE 12.0 to evaluate stability of the results after sequential exclusion of one study at a time.

Studies for which adequate data was not available or for whom adequate control group information was not available (especially meeting abstracts and letters to the editors) were also reviewed but not included in the meta-analysis.

Patient and public involvement

The present study is a meta-analysis and systematic review based on published data, patient and public are not involved in the study design, conduct, data analysis and result dissemination

Results

Literature search and characteristics of studies

A total of 599 documents were retrieved on database and manual search. Forty-eight duplicate publications were excluded using the document management software. A total of 126 records were retained after screening of titles and/or abstracts; the excluded records included review articles, animal model studies, therapeutic or drug research, genetic research, book chapters, duplicate publications not recognized by software, and other irrelevant records. After full-text review for eligibility, 21 records were selected. Finally, we identified 14 [9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23] studies; of these, adequate data was available only for 9 studies and were included in the meta-analysis (Figure 1). Two studies were included after obtaining the relevant data by contacting the respective authors [12, 13]. In addition, we also verified 2 studies [18, 19] with overlapping study population; of these, only one study was included in the meta-analysis. Three studies [9, 11, 21] were presented as meeting abstracts without adequate data to allow the

construction of a 2×2 table. One [10] article was a letter to the editor and only reported the prevalence rate of ASCA antibody in patients with BD, without information about the control group. One study [20] had employed a unique calculation method and could not be included in the meta-analysis. Among the included studies, there were 326 cases of BD, 294 cases of GIBD, 520 cases of CD, 598 cases of UC, 112 cases of iTB, and 428 HCs (Table 1 and Table S1).

Quality assessment

There were 8 prognostic studies and 1 retrospective study [12]. The results of quality assessment including the risk of bias and applicability concerns pertaining to each domain are shown in Fig S1. The results indicated that the included studies were of high quality in general; however, 8 studies showed a high risk of bias with respect to patient selection (avoidance of inappropriate exclusion). Overall, none of the 9 included studies showed any major methodological bias or flaws, which indicates robustness of our meta-analysis.

Meta-analysis

We conducted a meta-analysis of 9 studies that involved detection of ASCA in patients with BD and controls. Subsequently, we performed subgroup analysis based on different controls and isotypes of ASCA.

1. Meta-analysis of prevalence rate of ASCA in various groups of patients using Meta-DiSc 1.4

We analyzed the diagnostic accuracy of ASCA in BD (without gastrointestinal involvement)/GIBD/HC/UC/CD/iTB using the Revman 5.3 diagnostic test accuracy review model and Meta-DiSc 1.4. The results obtained with Meta-DiSc 1.4 are summarized in Table 2; some of the results are presented separately (Figure S2A-F). In addition, AUC are also showed separately by Revman 5.3 (Figure 2A–G).

When we compared BD and HC, we found limited overall diagnostic value, with the exception of slightly higher ORs for IgA and IgG/IgA [IgA, 2.26 (95% CI 0.56–9.12); IgG/IgA, 2.85 (95% CI 0.57–14.29)] and LR+ [IgA, 2.03 (95% CI 0.58–7.17); IgG/IgA, 2.08 (95% CI 0.67–6.41)]. However, both showed a high specificity (> 90%) and low sensitivity (< 20%) (Table 2).

Overall, ASCA showed the highest diagnostic value in the GIBD vs. HC sub-group analysis, with high OR and LR+. ASCA-IgG had the highest OR [5.46 (95% CI 2.58–

11.55)] and highest sensitivity [0.34 (95% CI 0.27–0.41)] in all groups (Figure S2A, B). In addition, the diagnostic value of ASCA was apparently higher than that in BD vs. HC sub-group analysis (Table 2).

When we compared GIBD and CD, both the OR and the LR+ were less than 1 (Table 2, Figure S2C-F), which suggests that ASCA negative results are more likely to be diagnosed as BD at the time of differential diagnosis.

The diagnostic value of ASCA was also limited when comparing GIBD and UC. Both the OR and LR+ were approximately 2. Both IgG and IgA positivity increased the value of LR+ [2.02 (95% CI 1.04–3.95)] (Table 2).

When comparing GIBD and iTB, although IgG/IgA increased the sensitivity [0.32 (95% CI (0.24–0.41)], the OR and LR+ were both approximately 1, which suggests that either IgG or IgA positivity may also increase the detection rate of ASCA in iTB, not just GIBD (Table 2).

On ROC curve analysis for distinguishing between BD and HC, the AUC for most antibody subtypes was slightly higher than 0.5 (or even lower than 0.5); of these, ASCA-IgG/IgA had the highest diagnostic efficacy (Figure 2A). In the BD vs. CD analysis, the AUC of ASCA was less than 0.5 (Figure 2B). The AUCs for BD vs. UC (Figure 2C), GIBD vs. CD (Figure 2E), and GIBD vs. iTB analyses (Figure 2G) were approximately 0.5. The AUC

for GIBD vs. UC analysis was at a general level (Figure 2F). The AUC was highest for the GIBD vs. HC analysis, especially for ASCA-IgG/IgA/IgM, although only one study was included (Figure 2D)

2.Meta-analysis of serum levels of ASCA in groups by Stata/SE 12.0

In order to increase the robustness of the meta-analysis, we also extracted the data pertaining to serum levels of ASCA from five studies and performed meta-analysis using the Continuous data module of Stata/SE 12.0. The FEM was used for the analysis and weighted mean difference (WMD) was used as the effect measure. We used FEM for five subgroups with $I^2<50\%$ and $p>0.1$ (Figure 3A). WMD was used because the same unit was used in these studies and there were only minor differences (less than three times) with respect to the serum levels of ASCA (Figure 3A). We found that ASCA-IgA in GIBD was significantly greater than that in HC and UC, in contrast to ASCA-IgG. On the contrary, levels of ASCA-IgG in CD were apparently higher than that in BD. For subgroups with $I^2>50\%$ and $p<0.1$, we chose the REM for analysis using the WMD. We found no significant difference between GIBD and CD with respect to the serum levels of ASCA-IgA (Figure 3B).

Heterogeneity and sensitivity analysis

We performed sensitivity analysis to assess the stability of the results. The results showed that the studies by Krause et al (2002), Zhang et al (2018), Kocazeybe et al (2010), and Fresko et al (2005) were the key contributors to the heterogeneity (Figure S3). Thus, the results of related subgroup analysis are considered to be less stable. We further applied REM to analyze these seven subgroups; the forest plot is shown in Figure S4.

Summary of the relationship of ASCA with autoimmune disease

We searched the database for the association between ASCA and AID. The sensitivity, specificity, LR+, and LR- are summarized in Table 3.

Discussion

The diagnosis of BD is typically challenging prior to the appearance of clinical symptoms to qualify the diagnostic criteria. Currently, there are no specific laboratory biomarkers of BD; however, some specific autoantibodies in the context of BD have been reported. Therefore, identification of non-invasive specific diagnostic and prognostic biomarkers of BD is of much clinical relevance and a key focus area of research.

Several recent studies have assessed the relation between ASCA and BD. Saccharomyces cerevisiae, also known as the baker's or brewer's yeast, has long been utilized to ferment the sugars in rice, wheat, barley, and corn to produce alcoholic beverages; it is also used in the baking industry to raise dough. As a consequence, we are now commonly exposed to yeast [24]. IgG and IgA antibodies against the phosphopeptidomannan of the *S. cerevisiae* cell wall have been discovered as autoantibodies in the sera of patients with BD, especially those with gastrointestinal involvement. This suggests a role of environmental stimuli in the pathogenesis of BD. However, patients with gastrointestinal involvement, especially those with Crohn's disease, also have a high prevalence rate of ASCA , which is a controversial issue [25]. Moreover, a growing number of studies have assessed ASCA in several systemic and organ-specific AID, which led to postulation of molecular mimicry as a possible link between ASCA and AID, such as scleroderma, systemic lupus erythematosus, primary Sjögren's syndrome, rheumatoid arthritis, autoimmune liver disease, and spondyloarthritis (Table 3). Their correlation with elevated IgA suggests that ASCA may be an indirect sign of enhanced mucosal immunity [26]. Therefore, detection of ASCA may be a useful serologic marker of BD and other AID, especially those with gastrointestinal involvement.

To the best of our knowledge, this is the second meta-analysis of evidence pertaining to autoantibodies in patients with BD after anticardiolipin antibodies [27]. ASCA have been

widely researched in BD, Crohn's disease and other autoimmune disease; in order to investigate the diagnostic value and possible pathogenetic role of ASCA in BD, we included 9 studies to assess the diagnostic accuracy of ASCA in BD. Among these, some studies included BD patients with various clinical manifestations. Some studies included BD patients with systemic involvement symptoms including or excluding gastrointestinal involvement, while others included only BD patients with gastrointestinal involvement. Therefore, in order to reduce the impact of differences with respect to frequency distribution of gastrointestinal symptoms in each study, we disaggregated patients with BD into those with gastrointestinal involvement only and those without gastrointestinal manifestations. The isotype antibodies of ASCA tested and the results presented by the studies are also different. Studies had investigated IgG, IgA, either IgG or IgA, both IgG and IgA, IgG, IgA and IgM, and even IgG subtypes IgG1–IgG4. Several studies have shown a higher prevalence of ASCA among patients with BD/GIBD/CD; however, the results of meta-analysis showed that ASCA is not a useful biomarker for the differential diagnosis between GIBD and CD; however, ASCA negative results may be more likely to be diagnosed as GIBD/BD when compared with CD. In addition, ASCA showed the highest diagnostic value in the GIBD vs. HC sub-group analysis; all four types of ASCA were found to help distinguish between GIBD and HC. However, we found limited diagnostic value of ASCA in BD vs. HC and GIBD vs. UC sub-group analyses. Moreover,

it failed to distinguish between GIBD and iTB. Overall, use of ASCA-IgG in combination with IgA helped improve the specificity of the diagnosis of BD in all groups. Moreover, we used three different software (Meta-DiSc 1.4, Revman5.3, and Stata/SE 12.0) to perform multiple analyses, which helped increase the credibility of our results. For example, we used both data pertaining to prevalence rate and serum levels of ASCA. Combined with the results of QUADAS-2, we found that the heterogeneity in this meta-analysis was largely attributable to the following reasons (see Table 1, Figure S1). The first reason was the different diagnostic criteria used in the included studies. Different criteria may have different thresholds for diagnosis or place more weight on some symptoms than others. Specifically, the 1990 ISG criteria requires the presence of oral ulceration plus any two of the following: genital ulceration, typical eye lesions, typical skin lesions, or positive pathergy test for diagnosis of BD [6]. In contrast, the 1987 Japan criteria require all four characteristics for diagnosis of BD, i.e., oral ulceration, typical eye lesions, typical skin lesions, and genital ulceration [28]. The ISG criteria and the Japanese criteria often fail to classify some patients with BD; in addition, the Japanese criteria may also cause misclassified diagnosis. This may have caused the different diagnostic sensitivity and specificity for BD [29]. The second contributor to heterogeneity was differences with respect to the characteristics of the study population. The clinical features and laboratory findings tend to exhibit wide variability in different populations and clinical settings [30,

31]. However, we could not perform subgroup analysis disaggregated by the type of population, owing to the small sample size of various population groups after classification according to isotypes of ASCA. Third, different investigation methods and cut-off values also contributed to the heterogeneity. The two main methods used in the included studies were ELISA and indirect immunofluorescence assay. Notably, although the kits were manufactured by the same company, different cut-off values were used. Different values for ELISA were used for Inova Diagnostic [13, 16, 18, 19, 22] and Euroimmune Leubeck kits [9, 12]. Some studies performed testing through self-coated plates by purchasing ELISA plates and antibodies; thus, the effect of human error and inadequate repeatability cannot be ignored [17, 20].

In order to fully evaluate the value of ASCA for differential diagnosis of BD, we included patients with CD, UC, and iTB as the comparison objects in our meta-analysis. However, there are still some limitations of this meta-analysis. (1) Gray literature database, paper database, and other language databases were not used for the literature search. (2) Our primary goal was to assess the diagnostic efficacy of ASCA in BD, and therefore we did not include all studies pertaining to ASCA in inflammatory bowel disease and iTB. (3) Restricted by the number of included studies and the isotypes of ASCA, we could not perform subgroup analysis disaggregated by different populations and diagnostic criteria. (4) Some studies with incomplete data were excluded after lack of response from the author.

Our meta-analysis results, together with the review of ASCA in AID strongly suggest that ASCA (especially its certain isotypes) may be helpful biomarkers for GIBD, especially with respect to their possible predictive/pathogenic/diagnostic role in clinical settings [32]. Furthermore, ASCA may be detectable years before the diagnosis of some AID as they were retrospectively found in the preserved blood samples of soldiers who were affected by Crohn's disease years later [33]. However, due to its presence in several other AID, ASCA may have a limited value for clinical diagnosis.

Compliance with Ethical Standards:

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Figure 1 Schematic illustration of the literature search and study-selection criteria for the meta-analysis

Figure 2 AUC of diagnostic accuracy of ASCA when comparing BD and HC (A), BD and CD (B), BD and UC (C), GIBD and HC (D), GIBD and CD (E), GIBD and UC (F), and GIBD and iTB (G)

Figure 3 A. Forest plot of serum levels of ASCA in GIBD/CD/UC/HC using FEM; B. Forest plot of serum levels of ASCA in GIBD/BD/HC/CD using REM

Fig S1 Results of quality assessment of the included studies based on the QUADAS-1 tool

Figure S2 Forest plot of A. pooled diagnostic OR of ASCA-IgG when comparing GIBD and HC; B. pooled sensitivity of ASCA-IgG when comparing GIBD and HC; C. pooled diagnostic OR of ASCA-IgG when comparing GIBD and CD; D. pooled diagnostic OR of ASCA-IgA when comparing GIBD and CD; E. pooled diagnostic OR of ASCA-

IgG+IgA when comparing GIBD and CD; F. pooled diagnostic OR of ASCA-IgG/IgA when comparing GIBD and CD

Figure S3 Results of sensitivity analysis showing sources of heterogeneity

Figure S4 Forest plot of diagnostic OR of ASCA in 6 subgroups using the random effect model after sensitivity analysis.

Table 1 Characteristics of studies included in the meta-analysis of Anti-Saccharomyces Cerevisiae antibodies in Behçet's disease, its main differential diagnoses, and healthy controls

/:no sample; IIF: indirect immunofluorescence assay; NR: not reported; SD: standard deviation; *: all without gastrointestinal manifestations; #: lack of corresponding data; 1990 ISG criteria: the 1990 criteria of Behçet's Disease International Study Group; 1987 Japan criteria: the 1987 criteria by the Behçet's Disease Research Committee of Japan; BD: Behçet's disease; GIBD: gastrointestinal Behçet's disease; CD: Crohn's disease; UC: ulcerative colitis; iTB: intestinal tuberculosis; HC: healthy control

Year and author	Countries	Type	Type of article	Design	Sample size						Methods	Brands of experimental materials	Cut-off	Diagnostic criteria
					BD	GIBD	CD	UC	iTB	HC				
2018 Shulan Zhang [13]	China	IgG; IgA; IgG/IgA; IgG+IgA	Original article	case-control	/	71	171	208	57	70	ELISA	Inova Diagnostic	25	NR

2017 Shulan Zhang [12]	China	IgG; IgA; IgG/IgA; IgG+IgA	Original article	Retrospective study	/	34	128	140	31	/	ELISA	Euroimmun, Luebeck	20	NR
2011 George Vaiopoulos [23]	Greece	IgG; IgA	Original article	case-control	58	4#	/	/	/	56	ELISA	Inova Diagnostic	NR	1990 ISG criteria
2010 B. Kocazeybek. [11]	Turkey	IgG/IgA	conference Abstract	case-control	/	13	63	102	10	165	IIF	Euroimmun, Luebeck	NR	NR
2006 Chang Hwan Choi [15]	Korea	IgG	Original article	case-control	30*	106	/	/	/	45	IIF	Euroimmun, Luebeck	1:1000	1987 Japan criteria
2005 I. Fresko [16]	Turkey	IgG; IgA; IgG/IgA; IgG+IgA	Original article	case-control	85	8	24	25	/	21	ELISA	Inova Diagnostic	28 for IgG; 25 for IgA	1990 ISG criteria
2005 Seung-Ho Rhee [22]	Korea	IgG	Original article	case-control	/	16	/	/	/	4	ELISA	Inova Diagnostic	25	1987 Japan criteria
2002 I. Krause [18]	Israel	IgG; IgA; IgG/IgA; IgG+IgA	Original article	case-control	27*	/	/	/	/	10	ELISA	Inova Diagnostic	25	1990 ISG criteria
2002 Byeong Gwan Kim [17]	Korea	IgG+IgA+IgM	Original article	case-control	/	36	85	77	14	20	ELISA	plate: Sigma Chemical antibody: Biosoft	ROC curve	1987 Japan criteria

Table 2. Summary of subgroup meta-analysis of ASCA by Meta-DiSc 1.4

/ indicates that the number of included studies was less than three and the ROC curve could not be drawn by Meta-DiSc 1.4. BD: Behçet's disease; GIBD: gastrointestinal Behçet's disease; CD: Crohn's disease; UC: ulcerative colitis; iTB: intestinal tuberculosis; HC: healthy control

Subgroup	Antibody	Number of studies	Diagnostic OR (95% CI)	I^2(%)	Pooled sensitivity (95% CI)	I^2(%)	Pooled specificity (95% CI)	I^2(%)	LR+(95% CI)	I^2(%)	LR-(95% CI)	I^2(%)
BD vs. HC	ASCA-IgG	3	0.91(0.15-5.64)	56.10	0.11(0.06-0.18)	91.40	0.91(0.82-0.96)	0	0.86(0.18-4.12)	50.10	0.98(0.81-1.18)	70.60
	ASCA-IgA	2	2.26(0.56-9.12)	0	0.16(0.10-0.25)	0	0.94(0.79-0.99)	38.40	2.03(0.58-7.17)	0	0.90 (0.79-1.03)	0
	ASCA-IgG+IgA	2	1.13(0.19-6.64)	0	0.03(0.01-0.08)	55.60	0.97(0.83-1.00)	0	0.66(0.09-4.91)	0.40	1.02(0.93-1.11)	0
	ASCA-IgG/IgA	2	2.85(0.57-14.29)	40.90	0.27(0.19-0.37)	87.20	0.88(0.71-0.96)	0	2.08(0.67-6.41)	17.60	0.76(0.47-1.22)	76.80
GIBD vs. HC	ASCA-IgG	3	5.46(2.58-11.55)	0	0.34(0.27-0.41)	83.70	0.93(0.87-0.96)	0	4.17(2.13-8.17)	0	0.75(0.58-0.97)	76.10
	ASCA-IgA	2	2.62(1.24-5.51)	0	0.27(0.17-0.38)	0	0.88(0.79-0.94)	0	2.19(1.13-4.23)	0	0.83(0.71-0.98)	0
	ASCA-IgG+IgA	2	5.27(1.43-19.33)	0	0.14(0.07-0.24)	0	0.97(0.91-0.99)	0	4.61(1.30-16.31)	0	0.88(0.80-0.98)	0
	ASCA-IgG/IgA	3	2.82(1.50-5.33)	0	0.30(0.21-0.41)	0	0.92(0.88-0.95)	78.30	2.25(1.31-3.86)	0	0.81(0.70-0.94)	0
GIBD vs. CD	ASCA-IgG	3	0.48(0.28-0.83)	0	0.18(0.11-0.26)	0	0.71(0.66-0.76)	88.60	0.58(0.38-0.89)	0	1.18(1.06-1.32)	35.40
	ASCA-IgA	3	0.91(0.56-1.46)	0	0.28(0.20-0.38)	0	0.69(0.64-0.74)	76.60	0.93(0.67-1.30)	0	1.03(0.90-1.18)	0
	ASCA-IgG+IgA	3	0.58(0.30-1.11)	0	0.12(0.06-0.19)	19.10	0.83(0.78-0.87)	85.20	0.63(0.36-1.11)	0	1.08(0.99-1.170	0
	ASCA-IgG/IgA	4	0.57(0.28-1.15)	50.20	0.33(0.24-0.41)	0	0.56(0.51-0.61)	72.30	0.74(0.56-0.97)	39.90	1.31(0.97-1.75)	66.40
GIBD vs. UC	ASCA-IgG	3	1.77(1.07-2.92)	0	0.18(0.11-0.26)	0	0.89(0.86-0.92)	0	1.63(0.99-2.68)	0	0.92(0.84-1.01)	0
	ASCA-IgA	3	2.12(1.38-3.26)	0	0.28(0.20-0.38)	0	0.84(0.80-0.88)	0	1.80(1.24-2.62)	0	0.85(0.75-0.96)	0
	ASCA-IgG+IgA	3	2.15(1.16-4.00)	0	0.12(0.06-0.19)	19.10	0.94(0.92-0.96)	0	2.02(1.04-3.95)	0	0.94(0.88-1.01)	20.10
	ASCA-IgG/IgA	4	2.02(1.38-2.98)	0	0.33(0.24-0.41)	0	0.82(0.78-0.85)	44.90	1.68(1.23-2.31)	0	0.84(0.74-0.95)	0
GIBD vs. iTB	ASCA-IgG	2	1.08(0.53-2.16)	0	0.17(0.10-0.26)	7.50	0.84(0.75-0.91)	32.70	1.06(0.56-2.00)	0	0.99(0.87-1.12)	0

ASCA-IgA	2	1.51(0.75-3.04)	0	0.21(0.14-0.30)	63.90	0.85(0.76-0.92)	4.30	1.39(0.75-2.59)	0	0.93(0.82-1.06)	0
ASCA-IgG+IgA	2	1.01(0.43-2.39)	0	0.10(0.05-0.18)	19.30	0.90(0.81-0.95)	0	1.01(0.44-2.34)	0	1.00(0.91-1.10)	0
ASCA-IgG/IgA	3	1.04(0.62-1.74)	0	0.32(0.24-0.41)	19.10	0.68(0.58-0.77)	10.40	1.03(0.70-1.52)	0	0.99(0.82-1.8)	0

508

509 Table 3 Summary of the diagnostic performance of ASCA in AID.

510 SEN: sensitivity; SPE: specificity; LR+: positive likelihood; LR-: negative likelihood

Reference	Autoimmune disease	Type	SEN (%)	SPE (%)	LR+	LR-	Supplementary information
[31]	Scleroderma	IgG	43.24	98.25	24.65	0.58	African descendants showed higher positivity rates for ASCA-IgG. ASCA-IgA was less frequently detected in patients with severe disease
		IgA	16.22	94.74	3.08	0.88	
[34, 35, 36, 37]	Ankylosing spondylitis	IgG	0–11.63	89.74–98.72	1.13–3.00	0.97–0.98	ASCA IgA levels were significantly increased in patients with HLA-B27-associated SpA, particularly in AS and uSpA
		IgA	1.28–23.26	91.03–100.00	2.59–3.71	0.84–0.99	ASCA positivity may be associated with peripheral involvement and uveitis.
[38]	Antiphospholipid syndrome	IgG/IgA	20.00	95.00	/	/	
[39]	Juvenile Idiopathic Arthritis	IgA	0–50.00	94.74	9.50	0.53	
[26, 40, 41]	Autoimmune hepatitis	IgG	16.42–27.53	100.00	/	0.84	
		IgA	11.94	94.74	2.27	0.93	
		IgG/IgA	18.52	84.00	1.16	0.97	
[26, 41, 42]	Primary biliary cirrhosis	IgG	10.57–18.95	97.50–100.00	7.58	0.83–0.89	
		IgA	11.58–18.70	94.74–98.75	3.55–9.26	0.86–0.90	
		IgG/IgA	20.26–24.21	84.00–96.25	1.27–6.46	0.79–0.95	
[26, 41]	Primary sclerosing cholangitis	IgG	28.00	100.00	/	0.72	
		IgA	32.00	94.74	6.08	0.72	
		IgG/IgA	30.51	84.00	1.91	0.83	
[14, 25, 26, 34, 43]	Crohn's disease	IgG	13.75–69.57	97.96–100	6.74	0.30–0.88	Patients with more complicated disease course showed a trend for greater seroreactivity towards ASCA.
		IgA	19.30–71.43	94.74–100.00	9.91–29.40	0.50–0.71	ASCA was detected in 25% of first-degree relatives of patien ts with Crohn's disease

[43]	Cryoglobulinemia	IgG	7.10	99.50	/	/	
[43, 44, 45]	Graves' disease	IgG	5.70–12.50	94.17–99.50	2.15–3.76	0.91–0.93	ASCA was elevated in Graves' disease but not in Hashimoto's thyroiditis
		IgA	8.40–16.67	94.17–96.88	2.69–2.86	0.88–0.95	
[35, 46]	Rheumatoid arthritis	IgG	10.13–20.00	89.74–91.45	0.99–2.34	0.87–1.00	ASCA IgA levels strongly correlated with C-reactive protein l evels and erythrocyte sedimentation rate
		IgA	17.72–40.00	91.03–94.74	1.97–7.60	0.63–0.90	
		IgM	13.33	94.74	2.53	0.91	
[43, 47, 48]	Systemic lupus erythematosus	IgG	4.50–57.50	91.45–99.50	6.72–9.38	0.46–0.73	ASCA IgG levels in SLE patients during remission were relat ively lower, indicating a possible correlation with disease acti vity
		IgA	7.50–12.07	94.74–99.38	1.43–19.31	0.88–0.98	
		IgG/IgA	31.90	96.25	8.51	0.71	
[49]	Type 1 diabetes	IgG	20.98	98.09	10.98	0.81	
		IgA	9.82	98.73	7.71	0.91	
		IgG/IgA	24.55	97.45	9.64	0.77	
[50]	Primary Sjögren's syndrome	IgG/IgA	4.81	100.00	/	0.95	ASCA positivity was associated with pSS specific clinical and serological features
[43]	Vasculitides	IgG	6.50	99.50	/	/	

511

512 Table S1 Demographic characteristics of patients and healthy controls included in the

513 meta-analysis

514 BD: Behçet's disease; GIBD: gastrointestinal Behçet's disease; CD: Crohn's disease; UC: ulcerative colitis; iTB:

515 intestinal tuberculosis; HC: healthy control; /: No such group included; Blank: no such information in the article

	BD	GIBD	CD	UC	iTB	HC
Female, n (%)						
2018 Shulan Zhang	/					/
2017 Shulan Zhang			37 (28.9)	69 (49.3)		
2011 George Vaiopoulos	28 (48.3)	/	/	/	/	
2010 B. Kocazeybek	/	5(38.5)	39 (61.9)	51 (50.0)	3 (30.0)	99 (60.0)
2006 Chang Hwan Choi	16 (86.6)	47(44.3)	/	/	/	24 (53.3)
2005 I. Fresko	20 (23.5)	2(25.0)	10 (41.7)	10 (40.0)	/	9 (42.9)
2005 Seung-Ho Rhee	/	28(63.6)	/	/	/	
2002 I. Krause	20 (74.1)					
2002 Byeong Gwan Kim	/	20	28	36	7	
Median age at study (max, min)						
2018 Shulan Zhang	/					
2017 Shulan Zhang	/		33 (69,13)	42 (76,13)		/
2011 George Vaiopoulos	38.5 (17,70)	/	/	/	/	
2010 B. Kocazeybek	/	32.11 (23.22,41)	37.56 (24.91,50.21)	40.72 (27.28,54.16)	SD 9.96	35.07 (24.58,45.56)
2006 Chang Hwan Choi	38 (18,65)	37	/	/	/	39 (16,69)

2005 I. Fresko	34.3 (32.0,36.7)	26.9 (24.1,29.6)	38.9 (34.6,43.2)	35.6 (33.0,38.3)	/	33.7 (30.4,37.0)
2005 Seung-Ho Rhee	/	37.6	/	/	/	
2002 I. Krause	41.6 (36.9,46.2)					
2002 Byeong Gwan Kim	/	43.2	40.9	30.6	33.6	

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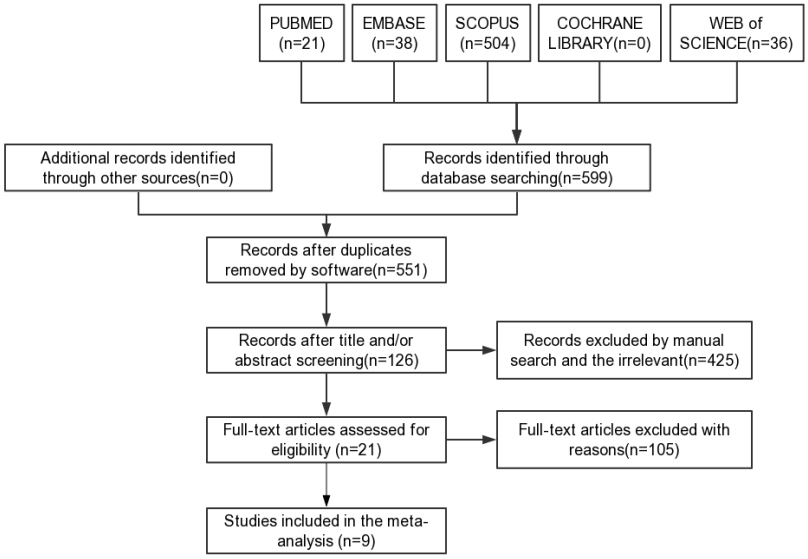

Figure 1 Schematic illustration of the literature search and study-selection criteria for the meta-analysis

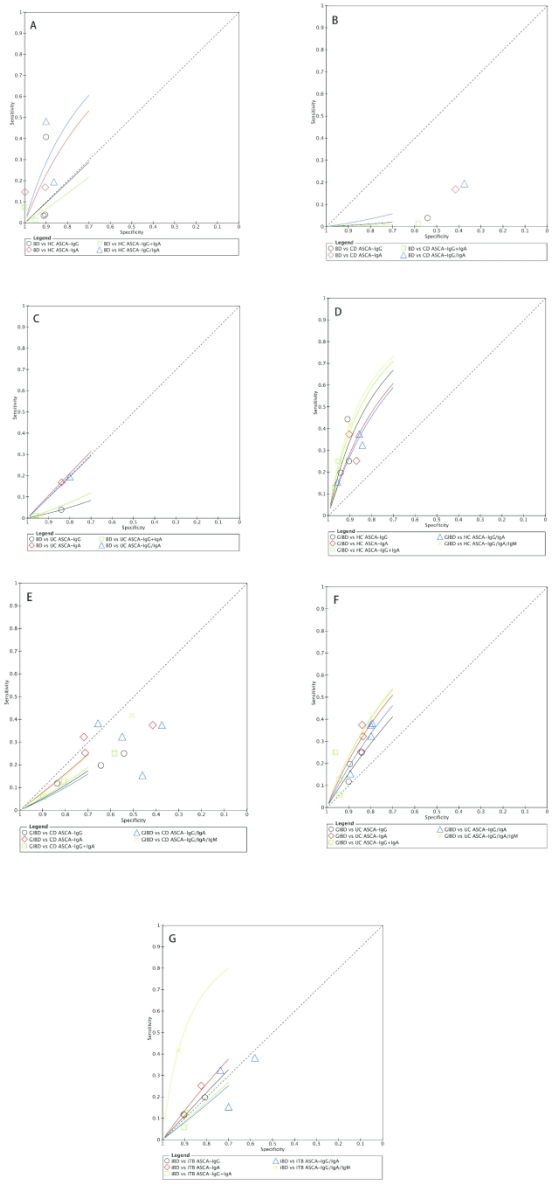

Figure 2 AUC of diagnostic accuracy of ASCA when comparing BD and HC (A), BD and CD (B), BD and UC (C), GIBD and HC (D), GIBD and CD (E), GIBD and UC (F), and GIBD and iTB (G)

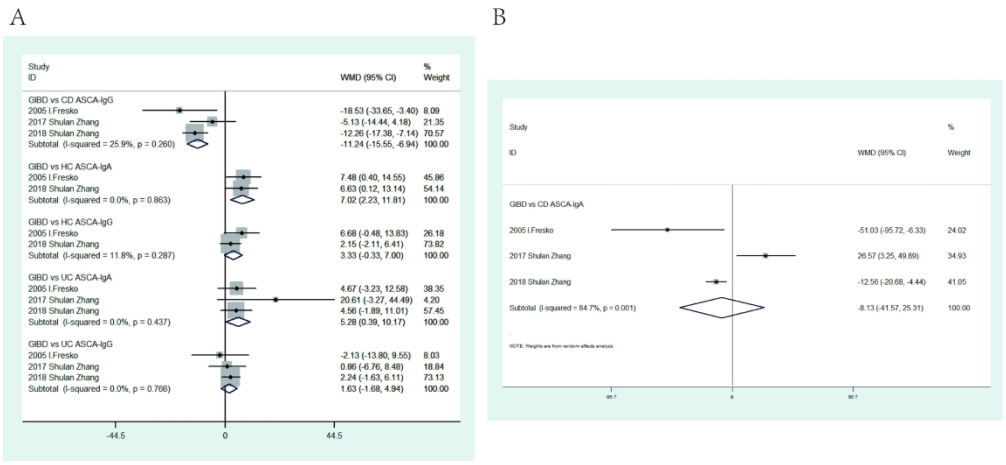

Figure 3 A. Forest plot of serum levels of ASCA in GIBD/CD/UC/HC using FEM; B. Forest plot of serum levels of ASCA in GIBD/BD/HC/CD using REM

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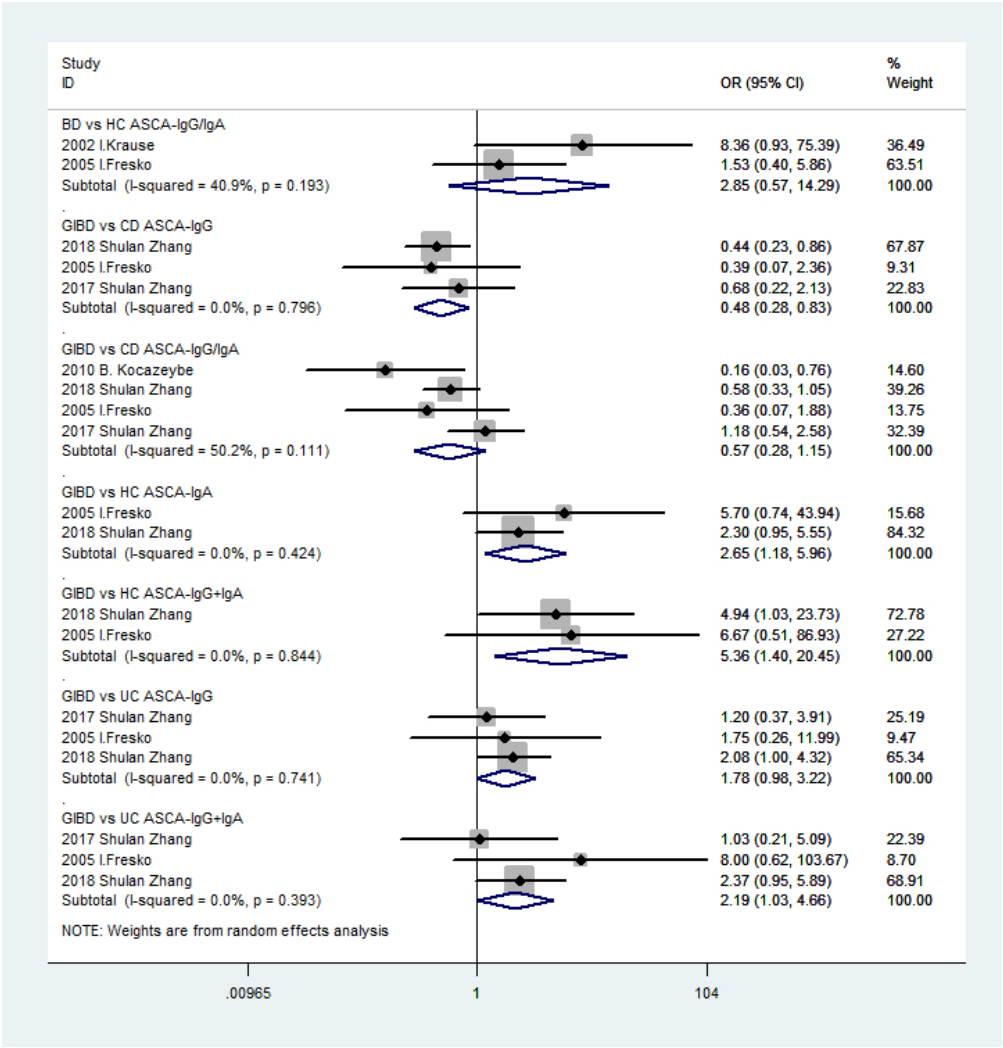

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Reporting checklist for meta-analysis of observational studies.

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	Reporting Item	Page Number
Title		
#1	Identify the study as a meta-analysis of observational research	1
Abstract		
#2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number (From PRISMA checklist)	1-2
Background		
#3a	Problem definition	3
#3b	Hypothesis statement	4-5
#3c	Description of study outcomes	n/a

	#3d	Type of exposure or intervention used	5
	#3e	Type of study designs used	5
	#3f	Study population	5
Methods			
Search strategy	#4a	Qualifications of searchers (eg, librarians and investigators)	6-7
Search strategy	#4b	Search strategy, including time period included in the synthesis and keywords	6
Search strategy	#4c	Effort to include all available studies, including contact with authors	7
Search strategy	#4d	Databases and registries searched	6
Search strategy	#4e	Search software used, name and version, including special features used (eg, explosion)	n/a
Search strategy	#4f	Use of hand searching (eg, reference lists of obtained articles)	n/a
Search strategy	#4g	List of citations located and those excluded, including justification	6
Search strategy	#4h	Method of addressing articles published in languages other than English	6
Search strategy	#4i	Method of handling abstracts and unpublished studies	6
Search strategy	#4j	Description of any contact with authors	7
	#5a	Description of relevance or appropriateness of studies gathered for assessing the hypothesis to be tested	7
	#5b	Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	n/a
	#5c	Documentation of how data were classified and coded (eg, multiple raters, blinding, and interrater reliability)	n/a

	#5d	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	8
	#5e	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	7
	#5f	Assessment of heterogeneity	8
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Meta-analysis of anti- Saccharomyces cerevisiae antibodies as diagnostic markers of Behçet's disease with gastrointestinal involvement

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Meta-analysis of anti- *Saccharomyces cerevisiae* antibodies as diagnostic markers of Behçet's disease with gastrointestinal involvement

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Meta-analysis of anti- *Saccharomyces cerevisiae* antibodies as diagnostic markers of Behçet's disease with gastrointestinal involvement

Abstract

Objective: Due to common exposure to yeast in the alcoholic and baking industry, positive rate of anti-*Saccharomyces cerevisiae* antibodies (ASCA) is reportedly high in patients with Behçet's disease (BD) who have gastrointestinal symptoms (gastrointestinal BD [GIBD]). We performed a meta-analysis to assess the diagnostic value of ASCA in differentiating patients with BD from those with other chronic inflammatory bowel diseases.

Methods: The meta-analysis is compliant with the PRISMA and MOOSE checklist. Relevant studies that investigated ASCA levels in BD patients were retrieved from PubMed, EMBASE, Web of Science, SCOPUS, and the Cochrane Library on July 12, 2019; the search was rerun on February 12, 2020. Stata/SE 12.0 and Meta-DiSc 1.4 were used to perform the meta-analysis and sensitivity analysis, disaggregated by isotypes of ASCA.

Results: Nine studies were included in the meta-analysis. The results revealed a strong association between ASCA and GIBD, especially ASCA-IgG [odds ratio (OR)=5.50 (95%

CI 2.58–11.55, p=0.000) and ASCA-IgG+IgA [OR=5.36 (95% CI 1.40–20.45), p=0.014]. The positivity rate of ASCA in GIBD was significantly higher than that in ulcerative colitis: IgA [OR=2.13 (95% CI 1.30–3.50), p=0.003]; IgG+IgA [OR=2.19 (95% CI 1.03–4.66), p=0.042]; IgG/IgA [OR=2.03 (95% CI 1.30–3.17), p=0.002]. However, the frequency of ASCA-IgG was significantly higher in patients with Crohn's disease than GIBD [OR=5.36 (95% CI 1.40–20.45), p=0.009]. There was no significant difference in ASCA positivity between BD without gastrointestinal involvement and healthy controls and between GIBD and intestinal tuberculosis (p>0.05).

Conclusion: ASCA may play a role in the pathogenesis of gastrointestinal involvement. Negative result of IgG favors the diagnosis of GIBD/BD when differentiated from Crohn's disease. ASCA-IgA showed moderate diagnostic performance in distinguishing GIBD and ulcerative colitis and the diagnostic performance was better in combination with IgG. However, ASCA may not be a useful serologic marker distinguishing GIBD and intestinal tuberculosis.

Key words: Behçet's disease; Anti-*Saccharomyces* cerevisiae antibodies; autoimmune diseases; meta-analysis; autoantibodies

Strengths and limitations of this study

- In addition to the healthy controls, we included patients with other gastrointestinal diseases that are considered in the differential diagnosis of gastrointestinal Behcet's disease in clinical settings (such as ulcerative colitis, Crohn's disease, and intestinal tuberculosis), in order to improve the clinical awareness of ASCA.
- Inclusion of both categorical data (positivity rate) and continuous data (serum concentration) pertaining to anti-*Saccharomyces cerevisiae* antibodies (ASCA) increases the reliability of the results of meta-analysis.
- We separately performed meta-analysis of IgG, IgA, and IgG+IgA, which provides insights into their ability to differentiate BD from other gastrointestinal diseases.
- Comprehensive summary of evidence linking ASCA and autoimmune diseases provides preliminary insights into the pathogenicity of *Saccharomyces cerevisiae.*
- Analysis of too many subgroups contributed to potential heterogeneity due to the small number of studies included in each subgroup.

Introduction

Behçet's disease (BD) is a chronic systemic vascular autoimmune/inflammatory disease with a high propensity for recurrence; the pathogenetic mechanisms of this disease are not well elucidated [1]. Virtually no specific histological or laboratory features of BD have been identified. Therefore, the diagnosis of BD is typically challenging as it is mainly based on clinical features [2, 3]. The diagnosis is frequently delayed until the development of clinical manifestations that qualify the diagnostic criteria. The estimated duration between the onset of symptoms and the fulfilment of diagnostic criteria is approximately 4 years [4].

Moreover, patients with prominent involvement of a particular organ system are easily misdiagnosed. For example, patients who have gastrointestinal symptoms as the main manifestation are liable to be misdiagnosed as having Crohn's disease (CD), ulcerative colitis (UC), or intestinal tuberculosis (iTB). These features make formulating disease criteria difficult, causing deleterious effects on the patients.

Several recent studies (but not all) have reported the diagnostic value of anti-*Saccharomyces cerevisiae* antibody (ASCA) in BD. *Saccharomyces cerevisiae*, also known as the baker's or brewer's yeast, has long been utilized to ferment the sugars in

cereals to produce alcoholic beverages; it is also used in the baking industry to raise dough. As a consequence, we are now commonly exposed to yeast [5]. IgG and IgA antibodies against the phosphopeptidomannan of the S. cerevisiae cell wall have been discovered as autoantibodies in the sera of patients with BD, especially those with gastrointestinal involvement. This suggests a role of environmental stimuli in the pathogenesis of BD. However, patients with inflammatory bowel disease such as Crohn's disease (CD) also have a high prevalence rate of ASCA due to their similarities [6, 7, 8, 9, 10, 11]. In this context, identification of ASCA as a diagnostic marker for BD is a key imperative. The objectives of this study were to summarize the findings pertaining to the relevance of ASCA in BD and other gastrointestinal diseases and to perform a meta-analysis to assess its diagnostic accuracy for BD.

Methods

Study design

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis Diagnostic Test Accuracy (PRISMA-DTA) guidelines [12] (Table S1) and Meta-analysis of Observational Studies in Epidemiology (MOOSE) [13](Table S2) were followed throughout the literature search process to structure and design the framework for the review [14]. Besides, a

predefined protocol was registered with PROSPERO (Registration No. CRD42020115245).

Literature search

A comprehensive literature search was performed to identify studies pertaining to ASCA as biomarkers for BD in 5 biomedical databases, i.e., PubMed, EMBASE, Web of Science, SCOPUS, and the Cochrane Library on July 12, 2019. The search terms for Behçet's disease were: Behcet, triple symptom complex, triple symptom complices, Adamantiades Behcet and old silk route disease; the search terms for *Saccharomyces cerevisiae* were*: S. cerevisiae, Saccaromyces cerevisiae, Saccharomyces capensis, Saccharomyces diastaticus, Saccharomyces italicus, Saccharomyces oviformis, Saccharomyces uvarum,* brewer yeast or baker yeast, mannan, manna, polymannan, glucomannan, yeast mannan, dicoman, humamil, ASCA. Combination of keywords using "AND" was used to retrieve studies in the range of "all fields" or "all text". The search was rerun on February 12, 2020 to ensure inclusion of recent studies. No restrictions were imposed with respect to time of publication, region, or ethnicity of the study population. In addition, the reference list of obtained articles was also examined to identify possible relevant studies.

Eligibility and exclusion criteria

The inclusion criteria were: (1) studies that evaluated the diagnostic accuracy of ASCA in BD; (2) availability of adequate data pertaining to the prevalence rate or serum levels of ASCA in patients with BD; (3) studies with healthy population and/or disease controls; (4) meeting abstracts or letters to the editor were also included.

The exclusion criteria were: (1) studies with incomplete data; (2) review articles; (3) non-English articles; (4) in case of studies with overlapping study population, studies with smaller sample size were excluded. Two investigators independently performed the literature search, screened the titles and abstracts, followed by full-text review of eligible studies.

Data extraction and quality assessment

Two independent investigators reviewed the full-text articles, extracted the data, and assessed the study quality using the modified version (nine-star scoring system) of the Newcastle-Ottawa Scale (NOS) for case-control studies and the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) and; For NOS, studies with higher NOS scores (☆) were considered as higher quality (low risk of bias). For QUADAS-2, the included items were evaluated as yes, no, or uncertain. Inter-researcher disagreements were resolved

by consensus, or by a third investigator. Data pertaining to the following variables were extracted: publication year, article type, first author's name, country, isotypes of ASCA detected, age and sex, research design, sample size, experimental method, trade names of experimental materials, cut-off values, diagnostic criteria, and serum titers and/or prevalence rate of ASCA in BD, gastrointestinal BD (GIBD), healthy controls (HC), patients with Crohn's disease (CD), ulcerative colitis (UC), and intestinal tuberculosis (iTB). The data were either obtained directly from the article, calculated, or requested from the author via e-mail.

Statistical analysis

Pooled odds ratios (OR) with 95% confidence intervals (CI) were calculated to evaluate the association between ASCA and BD (without gastrointestinal involvement)/GIBD/CD/UC/iTB using Stata/SE 12.0. Meta-DiSc 1.4 was used to calculate the sensitivity, specificity, AUC values, and the area under the summary receiver operating characteristic (SROC) curve to assess the overall diagnostic performance of ASCA. Heterogeneity among the included studies was evaluated using the Cochran's Q-statistic. P values > 0.10 were considered indicative of lack of significant heterogeneity. We chose the random effects models (REM) since REM tends to generalize findings beyond the included studies by assuming that the selected studies are random samples from a larger

population [15]. Subgroup analysis was performed disaggregated by the isotypes of ASCA and different disease controls. The isotypes of ASCA were classified and defined as follows: IgG, IgA, IgG/IgA (positive results of either IgG or IgA), and IgG+IgA (positive results of both IgG and IgA).

In order to increase the robustness of the meta-analysis, we also extracted the data pertaining to serum levels of ASCA from five studies and performed meta-analysis using the Continuous data module of Stata/SE 12.0. The REM was used for the analysis and weighted mean difference (WMD) was used as the effect measure if the same unit was used in these studies and there were minor differences with respect to the serum levels of ASCA. Sensitivity analysis was performed using Stata/SE 12.0 to evaluate stability of the results after sequential exclusion of one study at a time.

Patient and public involvement

The present study was a meta-analysis and systematic review based on published data. Patients and public were not involved in the study design, conduct, data analysis, and result dissemination.

Relationship between ASCA and autoimmune disease

We searched the PubMed for studies pertaining to the relationship between ASCA and autoimmune diseases. The two search terms used were autoimmune disease and *Saccharomyces cerevisiae.* We performed an interval statistic of four indicators of ASCA– sensitivity, specificity, positive likelihood (LR+) and negative likelihood (LR-) based on the included studies sorted by diseases.

Results

Literature search and characteristics of studies

A total of 625 documents were retrieved on database and manual search. Fifty-one duplicate publications were excluded using the document management software. A total of 127 records were retained after screening of titles and/or abstracts; the excluded records included review articles, animal model studies, therapeutic or drug research, genetic research, book chapters, duplicate publications not recognized by software, and other irrelevant records. After full-text review for eligibility, 22 records were selected. Finally, we included 9 available studies with adequate data in the meta-analysis (Figure 1). Two studies were included after obtaining the relevant data by contacting the respective authors [9, 10]. In addition, we also verified 2 studies [16, 17] with overlapping study population; of these, only 1 study was included in the meta-analysis. Three studies [6, 8, 18] were

presented as meeting abstracts without adequate data to allow the construction of a 2×2 table. One article[7] was a letter to the editor and only reported the prevalence rate of ASCA antibody in patients with BD, without information about the control group. One study [19] had employed a unique calculation method and could not be included in the meta-analysis. Among the included studies, there were 326 cases of BD, 294 cases of GIBD, 520 cases of CD, 598 cases of UC, 112 cases of iTB, and 428 HCs (Table 1 and Table S3).

Quality assessment

There were 8 case-control studies and 1 retrospective study [9]. The results of quality assessment by NOS including the selection of the study groups, the comparability of the groups and the ascertainment of the exposure of interest for case-control studies are shown in Table 2, and by QUADAS-2 including the risk of bias and applicability concerns pertaining to each domain are shown in Figure S1. The results indicated that the included studies were of high quality in general. Overall, none of the 9 included studies showed any major methodological bias or flaws, which indicates robustness of our meta-analysis.

Meta-analysis

Association between ASCA and BD (without gastrointestinal involvement), GIBD and other intestinal diseases

Data pertaining to correlation between ASCA and BD (without gastrointestinal involvement)/GIBD/CD/UC/iTB are listed in Table 3. No substantial heterogeneity ($p>0.1$ for all) was observed by using REM to calculate the OR. The results revealed a strong association between all detection types of ASCA and GIBD, especially for ASCA-IgG [OR=5.50 (95% CI 2.58–11.55, p=0.000) and ASCA-IgG+IgA [OR=5.36 (95% CI 1.40–20.45), p=0.014]. When comparing GIBD and UC, of the positivity rate for ASCA in GIBD was significantly higher than that for UC: IgA [OR=2.13 (95% CI 1.30–3.50), p=0.003], IgG+IgA [OR=2.19 (95% CI 1.03–4.66), p=0.042], and IgG/IgA [OR=2.03 (95% CI 1.30–3.17), p=0.002]. Conversely, the frequency of only ASCA-IgG in patients with CD was significantly higher than that in the GIBD [OR=5.36 (95% CI 1.40–20.45), p=0.009]. Further, on stratified analysis according to detection method, ASCA-IgG was associated with GIBD using both the ELISA method (OR = 3.83, 95% CI 1.37–10.70, p = 0.010) and the immunoprecipitation method (IIF) (OR = 8.17, 95% CI 2.73–24.43, p = 0.000) (Figure 2). However, no significant difference was observed with respect to ASCA positivity between BD without gastrointestinal involvement and HC and between GIBD and iTB ($p>0.05$).

Diagnostic ability of ASCA for GIBD

The overall sensitivity for ASCA-IgG in patients with GIBD detected by IIF was 0.44, which is much higher than that of ELISA [0.20 (95%CI 0.12–0.31)] (Table 4). Combined detection of IgG and IgA by ELISA increased the sensitivity to 0.33 (95% CI 0.23–0.44). However, we observed a low level of sensitivity of ASCA-IgG/IgA by IIF, which may be attributable to the inclusion of only one study with few GIBD patients (n=13).

Difference in serum levels of ASCA in GIBD and other intestinal diseases

Serum levels of ASCA-IgA observed in GIBD were significantly greater than that in HC [WMD=7.02 (95% CI 2.23–11.81), p=0.004) and UC [WMD=5.28 (95% CI 0.39–10.17), p=0.034] in contrast to ASCA-IgG (p>0.05) (Figure 3). On the contrary, serum levels of ASCA-IgG in CD were significantly greater than that in GIBD [WMD=-11.04 (95% CI -16.74–-5.34), p=0.000] (Figure 3). However, we found no significant difference in serum levels of ASCA between BD without gastrointestinal symptoms and HC (p>0.05) (Figure 3).

Heterogeneity and sensitivity analysis

We performed sensitivity analysis to assess the stability of the results. The results showed that the studies by Krause et al (2002), Zhang et al (2018), Kocazeybe et al (2010), and Fresko et al (2005) were the key contributors to the heterogeneity (Figure S2). Thus, the

results of related subgroup analysis are considered to be less stable.

Summary of the relationship of ASCA with autoimmune disease

Sixteen studies reporting the relevance of ASCA and autoimmune diseases were included in the summary. The sensitivity, specificity, LR+, and LR- of ASCA for different autoimmune diseases are summarized in Table 5. Although the diagnostic results of ASCA reported by different studies vary, the summary revealed an overall association between ASCA and autoimmune diseases especially in patients with scleroderma, juvenile idiopathic arthritis, Crohn's disease, and systemic lupus erythematosus with high SEN (>40%), high SPE (>95), high LR+ (>5) (Table 5).

Discussion

Serological markers in BD. The diagnosis of BD is typically challenging prior to the appearance of clinical symptoms necessary to qualify the diagnostic criteria. Currently, there are no specific laboratory biomarkers of BD; however, some specific autoantibodies in the context of BD have been reported. Therefore, identification of non-invasive specific diagnostic and prognostic biomarkers of BD is of much clinical relevance and a key focus area of research.

ASCA in BD and autoimmune diseases. Several recent studies have investigated the relationship of ASCA with BD or other autoimmune diseases. *Saccharomyces cerevisiae* has long been utilized in alcoholic and baking industry, and for the production of vaccines owing to its antigenic component. However, during long-term and ubiquitous presence, even the commensal and classically non-pathogenic microbiota can trigger autoimmunity due to loss of immune tolerance towards the resident bacterial flora, like in gastrointestinal tract [20, 21]. The reported similarity of sequences involving the eukaryotic microorganism and self-antigens suggest a mechanism of molecular mimicry and also the plausibility of shared epitopes in different autoimmune diseases. The production of ASCA by the subsequent activation of the humoral immune response may lead to a direct pathogenic role through a costimulatory CD80/86-CD28-mediated effect [20]. Moreover, healthy family members but not spouses of BD patients were also found to have increased levels of ASCA, which indicated a role of genetic factors in addition to environmental stimuli [17, 21]. A large number of studies have assessed the role of ASCA in the context of several systemic and organ-specific autoimmune diseases, such as BD, scleroderma, systemic lupus erythematosus, primary Sjögren's syndrome, and rheumatoid arthritis (Table 5). The results suggest that the relation of ASCA with BD or other autoimmune diseases may represent a potential pathogenic mechanism between ASCA and autoimmunity; this underlines the importance of ASCA as a valuable serologic marker for autoimmune diseases including BD.

Results of the meta-analysis. To the best of our knowledge, this is the second meta-analysis of evidence pertaining to autoantibodies in patients with BD after anticardiolipin antibodies [22]. ASCA have been widely researched in BD, Crohn's disease and other autoimmune disease; in order to investigate the diagnostic value and possible pathogenetic role of ASCA in BD, we included 9 studies in this meta-analysis. Among these, some studies included BD patients with systemic involvement including or excluding gastrointestinal involvement, while others included only BD patients with gastrointestinal involvement. Therefore, in order to reduce the impact of differences with respect to frequency distribution of gastrointestinal symptoms in each study, we disaggregated patients with BD into those with gastrointestinal involvement only and those without gastrointestinal manifestations. The isotype antibodies of ASCA tested and the results presented by the studies are also different. The meta-analysis revealed a strong association of ASCA with GIBD and not with BD with no gastrointestinal involvement; this suggests the role of ASCA in the pathogenesis of gastrointestinal involvement. ASCA showed a moderate diagnostic performance as a biomarker for the differential diagnosis between GIBD and CD, and the negative result of ASCA-IgG may slightly favor the diagnosis of GIBD/BD when compared with CD, especially with concomitant positive HLA-B51 tests [23]. In addition, ASCA-IgA showed a moderate diagnostic value for distinguishing GIBD and UC and would perform better with concomitant detection of IgG. However, ASCA failed to distinguish between GIBD and iTB. Besides, the concomitant evaluation of both

continuous data (sensitivity and specificity) and discontinuous data (serum levels) helped increase the credibility of our results.

Heterogeneity. Combined with the results of QUADAS-2, we found that the heterogeneity in this meta-analysis was largely attributable to the following reasons (see Table 1, Figure S1). 1) The different diagnostic criteria used in the included studies. Different criteria may have different thresholds for diagnosis or place more weight on some symptoms than others. Specifically, the 1990 ISG criteria requires the presence of oral ulceration plus any two of the following: genital ulceration, typical eye lesions, typical skin lesions, or positive pathergy test for diagnosis of BD [2]. In contrast, the 1987 Japan criteria require all four characteristics for the diagnosis of BD, i.e., oral ulceration, typical eye lesions, typical skin lesions, and genital ulceration [24]. The ISG criteria and the Japanese criteria often fail to classify some patients with BD; in addition, the Japanese criteria may also cause misclassified diagnosis. This may have caused the different diagnostic sensitivity and specificity for BD [25]. 2) Differences in demographic characteristics of included studies. The clinical features and laboratory findings tend to exhibit wide variability in different populations and clinical settings [26, 27]. However, we failed to perform subgroup analysis disaggregated by ethnicity owing to the small sample size in each subgroup. 3) Different antibody assays and cut-off values. Commercial kits and in-house tests from different laboratories have variable performance, which may affect the diagnosis and management

of patients. We found that different methods and cut-off values were adopted by the studies included in this meta-analysis. Notably, there was significant association between ASCA-IgG and GIBD using both methods; however, it seems that IIF has a higher sensitivity than ELISA. Nonetheless, further investigations with larger study population are required to provide more definitive evidence. Although previous studies have shown that IIF has a better performance, ELISA provides the titer change of serum antibodies and could have an equal performance to IIF by changing cut-off values to optimize the overall diagnostic performance [28].

Limitations of the meta-analysis. In order to fully evaluate the value of ASCA for differential diagnosis of BD, we included patients with CD, UC, and iTB as the comparison objects in our meta-analysis. However, there are still some limitations of this meta-analysis. (1) Gray literature database, paper database, and other language databases were not used for the literature search. Quite a few non-English studies were excluded due to incomplete data or unavailability of full text. (2) Our primary goal was to assess the diagnostic efficacy of ASCA in BD, and therefore we did not include all studies pertaining to ASCA in inflammatory bowel disease and iTB. (3) Restricted by the number of included studies and the isotypes of ASCA, we could not perform subgroup analysis disaggregated by different populations and diagnostic criteria. (4) Some studies with incomplete data were excluded after lack of response from the author. (5) There are some inherent statistical shortcomings

using Meta-DiSc during the separate pooling of sensitivity and specificity, as the between-study variance is not included. More advanced methods are not implemented [29].

Conclusion. Our meta-analysis results, together with the review of ASCA in autoimmune diseases strongly suggest that ASCA (especially its certain isotypes) may be helpful biomarkers for GIBD, especially with respect to their possible predictive/pathogenic/diagnostic role in clinical settings [20]. Furthermore, ASCA may be detectable years before the diagnosis of some autoimmune diseases as they were retrospectively found in the preserved blood samples of soldiers who were affected by Crohn's disease years later [30]. However, due to its presence in several other autoimmune diseases, ASCA may have a limited value for clinical diagnosis.

Compliance with Ethical Standards:

Contributorship statement: Study concept and design: Linlin Cheng, Yongzhe Li. Acquisition of data: Linlin Cheng, Liubing Li, Chenxi Liu, Songxin Yan. Statistical analysis and interpretation of data: Linlin Cheng, Liubing Li, Chenxi Liu. Drafting of the manuscript: Linlin Cheng. Revision of manuscript: Yongzhe Li, Linlin Cheng, Liubing Li. Supervision of work: Yongzhe Li. All authors read and approved the final manuscript.

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Figure 1 PRISMA flow diagram illustrating the literature screening process and the criteria for inclusion of studies in the meta-analysis

Figure 2 Forest plot of the association between the presence of ASCA-IgG and GIBD stratified by detection methods

Figure 3 Forest plot comparing serum levels of ASCA between BD without gastrointestinal symptom/GIBD and HC/CD/UC

Table 1 Characteristics of studies included in the meta-analysis of Anti-*Saccharomyces cerevisiae* antibodies in Behçet's disease, its main differential diagnoses, and healthy controls.

/:no sample; ELISA: enzyme-linked immunosorbent assay; IIF: indirect immunofluorescence assay; NR: not reported; SD: standard deviation; *: all without gastrointestinal manifestations; #: lack of corresponding data; 1990 ISG criteria: the 1990 criteria of Behçet's Disease International Study Group; 1987 Japan criteria: the 1987 criteria by the Behçet's Disease Research Committee of Japan; BD: Behçet's disease; GIBD: gastrointestinal Behçet's disease; CD: Crohn's disease; UC: ulcerative colitis; iTB: intestinal tuberculosis; HC: healthy control

Year and author	Countries	Type	Type of article	Design	Sample size						Methods	Brands of experimental materials	Cut-off (U/ml)	Diagnostic criteria
					BD	GIBD	CD	UC	iTB	HC				
2018 Shulan Zhang [31]	China	IgG; IgA; IgG/IgA; IgG+IgA	Original article	case-control	/	71	171	208	57	70	ELISA	Inova Diagnostic	25	NR
2017 Shulan Zhang [32]	China	IgG; IgA; IgG/IgA; IgG+IgA	Original article	Retrospective study	/	34	128	140	31	/	ELISA	Euroimmun, Luebeck	20	NR
2011 George Vaiopoulos [33]	Greece	IgG; IgA	Original article	case-control	58	4#	/	/	/	56	ELISA	Inova Diagnostic	NR	1990 ISG criteria
2010 B. Kocazeybek. [34]	Turkey	IgG/IgA	conference Abstract	case-control	/	13	63	102	10	165	IIF	Euroimmun, Luebeck	NR	NR
2006 Chang Hwan Choi [35]	Korea	IgG	Original article	case-control	30*	106	/	/	/	45	IIF	Euroimmun, Luebeck	1:1000	1987 Japan criteria
2005 I. Fresko [36]	Turkey	IgG; IgA; IgG/IgA; IgG+IgA	Original article	case-control	85	8	24	25	/	21	ELISA	Inova Diagnostic	28 for IgG; 25 for IgA	1990 ISG criteria
2005 Seung-Ho Rhee [37]	Korea	IgG	Original article	case-control	/	16	/	/	/	4	ELISA	Inova Diagnostic	25	1987 Japan criteria
2002 I. Krause [16]	Israel	IgG; IgA; IgG/IgA; IgG+IgA	Original article	case-control	27*	/	/	/	/	10	ELISA	Inova Diagnostic	25	1990 ISG criteria
2002 Byeong Gwan Kim [38]	Korea	IgG+IgA+IgM	Original article	case-control	/	36	85	77	14	20	ELISA	plate: Sigma Chemical antibody: Biosoft	ROC curve	1987 Japan criteria

Table 2 Risk of bias assessment of the included studies according to the modified Newcastle-Ottawa Scale (NOS).

NOS item / Study ID	2018 Shulan Zhang	2017 Shulan Zhang	2011 George Vaiopoulos	2010 B. Kocazeybek	2006 Chang Hwan Choi	2005 I. Fresko	2005 Seung-Ho Rhee	2002 I. Krause	2002 Byeong Gwan Kim
Is the case definition adequate?	✸	✸	✸		✸	✸	✸	✸	✸
Representativeness of the cases	✸	✸				✸			
Selection of controls	✸		✸	✸	✸	✸	✸	✸	✸
Definition of controls	✸	✸	✸	✸	✸	✸	✸	✸	✸
Study controls for the most important factor (i.e., age)	✸		✸			✸			✸
Study controls for the second important factor (i.e., sex)	✸		✸			✸			✸
Was the measurement method of ASCA described?	✸	✸	✸	✸	✸	✸	✸	✸	✸
Same method of ascertainment for cases and controls	✸	✸	✸	✸	✸	✸	✸	✸	✸
Non-response rate	✸	✸	✸	✸	✸	✸	✸	✸	✸
Total Score	9	6	8	5	6	9	6	6	8

✸was awarded when the respective information was available.

Table 3. Association between the presence of ASCA and BD (without gastrointestinal symptom)/GIBD and other intestinal diseases

BD: Behçet's disease without gastrointestinal symptom; GIBD: gastrointestinal Behçet's disease; CD: Crohn's disease; UC: ulcerative colitis; iTB: intestinal tuberculosis; HC: healthy control

Subgroup	Antibody	Number of studies	Diagnostic OR (95% CI)	Significance test (p)
BD vs. HC	ASCA-IgG	4	1.00 (0.28–3.53)	0.997
	ASCA-IgA	2	2.50 (0.63–9.96)	0.194
	ASCA-IgG+IgA	2	1.06 (0.17–6.78)	0.954
	ASCA-IgG/IgA	2	2.88 (0.62–13.44)	0.179
GIBD vs. HC	ASCA-IgG	3	5.50 (2.58–11.55)	0.000
	ASCA-IgA	2	2.65 (1.18–5.96)	0.018
	ASCA-IgG+IgA	2	5.36 (1.40–20.45)	0.014
	ASCA-IgG/IgA	3	2.90 (1.47–5.74)	0.002
GIBD vs. CD	ASCA-IgG	3	0.48(0.28–0.83)	0.009
	ASCA-IgA	3	0.91(0.56–1.46)	0.685
	ASCA-IgG+IgA	3	0.58(0.30–1.11)	0.100
	ASCA-IgG/IgA	4	0.57 (0.28–1.15)	0.117
GIBD vs. UC	ASCA-IgG	3	1.78 (0.98–3.22)	0.057
	ASCA-IgA	3	2.13 (1.30–3.50)	0.003
	ASCA-IgG+IgA	3	2.19 (1.03–4.66)	0.042
	ASCA-IgG/IgA	4	2.03 (1.30–3.17)	0.002
GIBD vs. iTB	ASCA-IgG	2	1.08 (0.50–2.32)	0.854
	ASCA-IgA	2	1.51 (0.71–3.22)	0.290
	ASCA-IgG+IgA	2	1.02 (0.40–2.62)	0.972
	ASCA-IgG/IgA	3	1.05 (0.58–1.87)	0.883

Table 4 Pooled sensitivity and specificity of ASCA-IgG and IgG/IgA for diagnosis of GIBD assessed by ELISA and IIF

ELISA: enzyme-linked immunosorbent assay; IIF: indirect immunofluorescence assay

Methods	ELISA		IIF	
Diagnostic accuracy	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	Sensitivity	Specificity
ASCA-IgG	0.20 (0.12–0.31)	0.93 (0.86–0.98)	0.44	0.91
ASCA-IgG/IgA	0.33 (0.23–0.44)	0.85 (0.76–0.91)	0.15	0.96

639 Table 5 Summary of the diagnostic performance of ASCA in autoimmune disease

640 SEN: sensitivity; SPE: specificity; LR+: positive likelihood; LR-: negative likelihood

Reference	Autoimmune disease	Type	SEN (%)	SPE (%)	LR+	LR-	Supplementary information
[27]	Scleroderma	IgG	43.24	98.25	24.65	0.58	African descendants showed higher positivity rates for ASCA-IgG. ASCA-IgA was less frequently detected in patients with severe disease
		IgA	16.22	94.74	3.08	0.88	
[39, 40, 41, 42]	Ankylosing spondylitis	IgG	0–11.63	89.74–98.72	1.13–3.00	0.97–0.98	ASCA IgA levels were significantly increased in patients with HLA-B27-associated SpA, particularly in AS and uSpA
		IgA	1.28–23.26	91.03–100.00	2.59–3.71	0.84–0.99	
[43]	Antiphospholipid syndrome	IgG/IgA	20.00	95.00	/	/	
[44]	Juvenile Idiopathic Arthritis	IgA	0–50.00	94.74	9.50	0.53	
[45, 46, 47]	Autoimmune hepatitis	IgG	16.42–27.53	100.00	/	0.84	
		IgA	11.94	94.74	2.27	0.93	
		IgG/IgA	18.52	84.00	1.16	0.97	
[45, 47, 48]	Primary biliary cirrhosis	IgG	10.57–18.95	97.50–100.00	7.58	0.83–0.89	
		IgA	11.58–18.70	94.74–98.75	3.55–9.26	0.86–0.90	
		IgG/IgA	20.26–24.21	84.00–96.25	1.27–6.46	0.79–0.95	
[45, 47]	Primary sclerosing cholangitis	IgG	28.00	100.00	/	0.72	
		IgA	32.00	94.74	6.08	0.72	
		IgG/IgA	30.51	84.00	1.91	0.83	
[1, 39, 45, 49, 50]	Crohn's disease	IgG	13.75–69.57	97.96–100	6.74	0.30–0.88	Patients with more complicated disease course showed a trend for greater seroreactivity towards ASCA.
		IgA	19.30–71.43	94.74–100.00	9.91–29.40	0.50–0.71	
[50]	Cryoglobulinemia	IgG	7.10	99.50	/	/	
[50, 51, 52]	Graves' disease	IgG	5.70–12.50	94.17–99.50	2.15–3.76	0.91–0.93	ASCA was elevated in Graves' disease but not in Hashimoto's thyroiditis
		IgA	8.40–16.67	94.17–96.88	2.69–2.86	0.88–0.95	
[40, 53]	Rheumatoid arthritis	IgG	10.13–20.00	89.74–91.45	0.99–2.34	0.87–1.00	ASCA IgA levels strongly correlated with C-reactive protein levels and erythrocyte sedimentation rate
		IgA	17.72–40.00	91.03–94.74	1.97–7.60	0.63–0.90	
		IgM	13.33	94.74	2.53	0.91	
[39, 54, 55]	Systemic lupus erythematosus	IgG	4.50–57.50	91.45–99.50	6.72–9.38	0.46–0.73	ASCA IgG levels in SLE patients during remission were relatively lower, indicating a possible correlation with disease activity
		IgA	7.50–12.07	94.74–99.38	1.43–19.31	0.88–0.98	
		IgG/IgA	31.90	96.25	8.51	0.71	
[56]	Type 1 diabetes	IgG	20.98	98.09	10.98	0.81	
		IgA	9.82	98.73	7.71	0.91	
		IgG/IgA	24.55	97.45	9.64	0.77	
[57]	Primary Sjögren's syndrome	IgG/IgA	4.81	100.00	/	0.95	ASCA positivity was associated with pSS specific clinical and serological features
[50]	Vasculitides	IgG	6.50	99.50	/	/	

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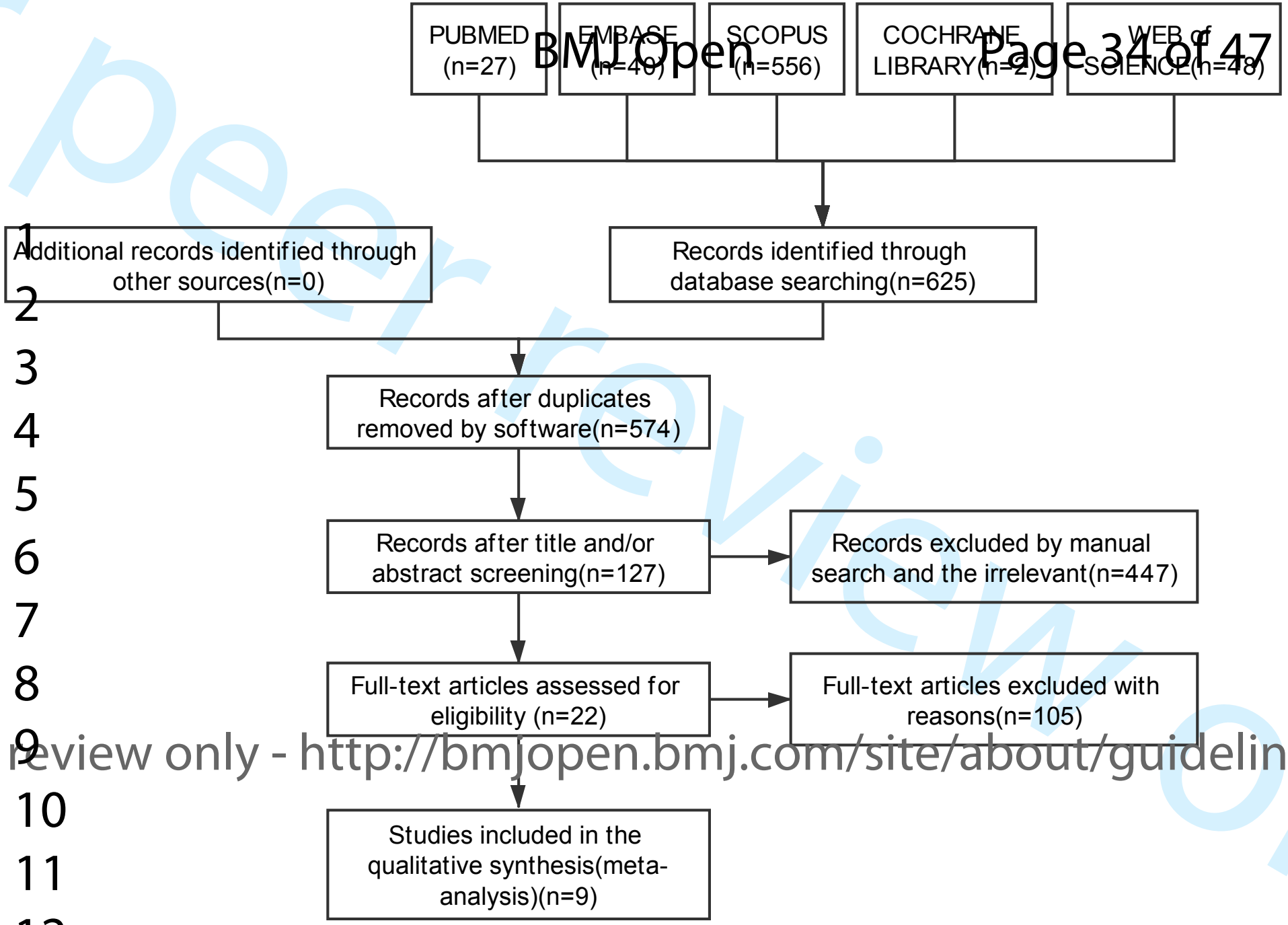
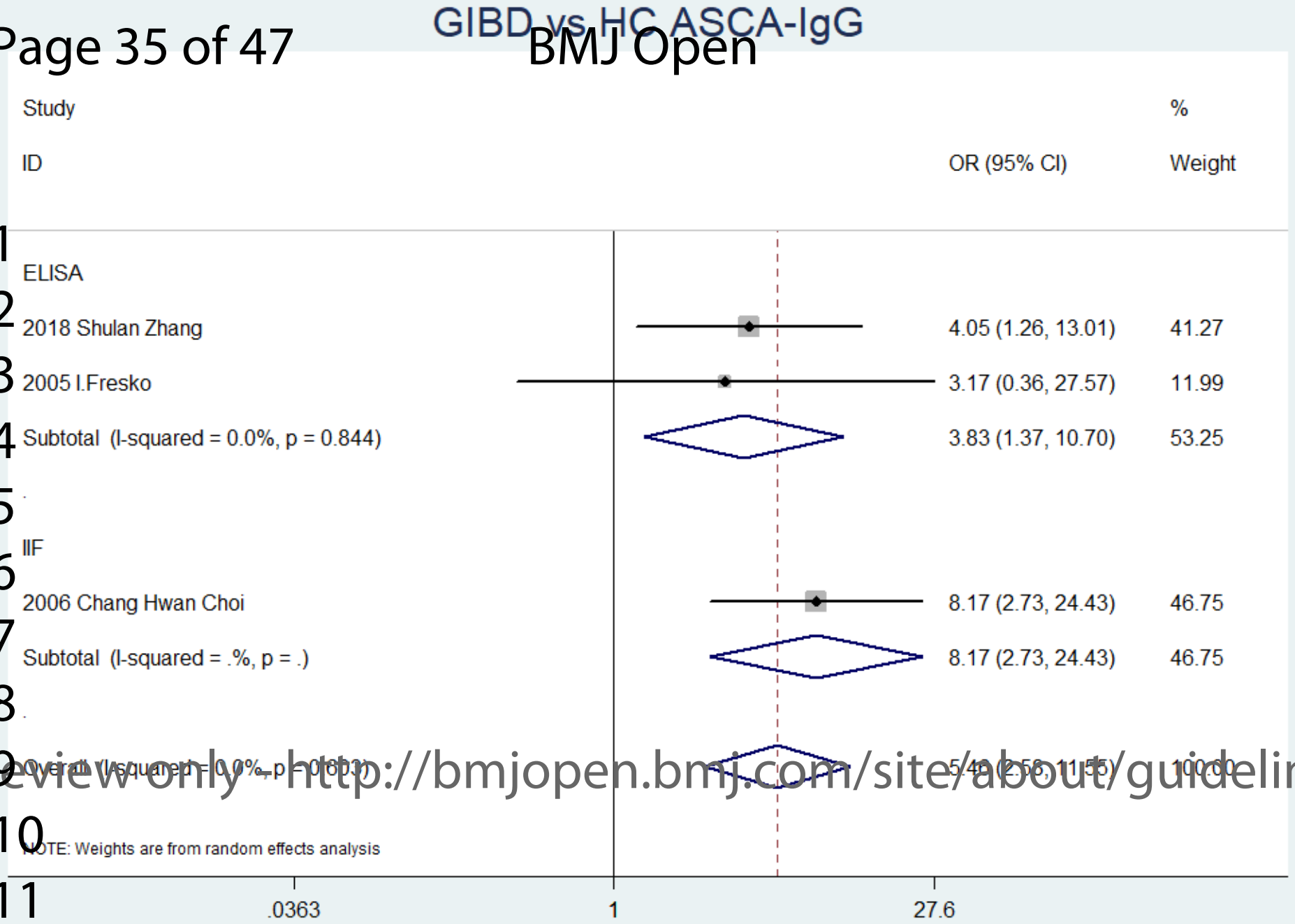




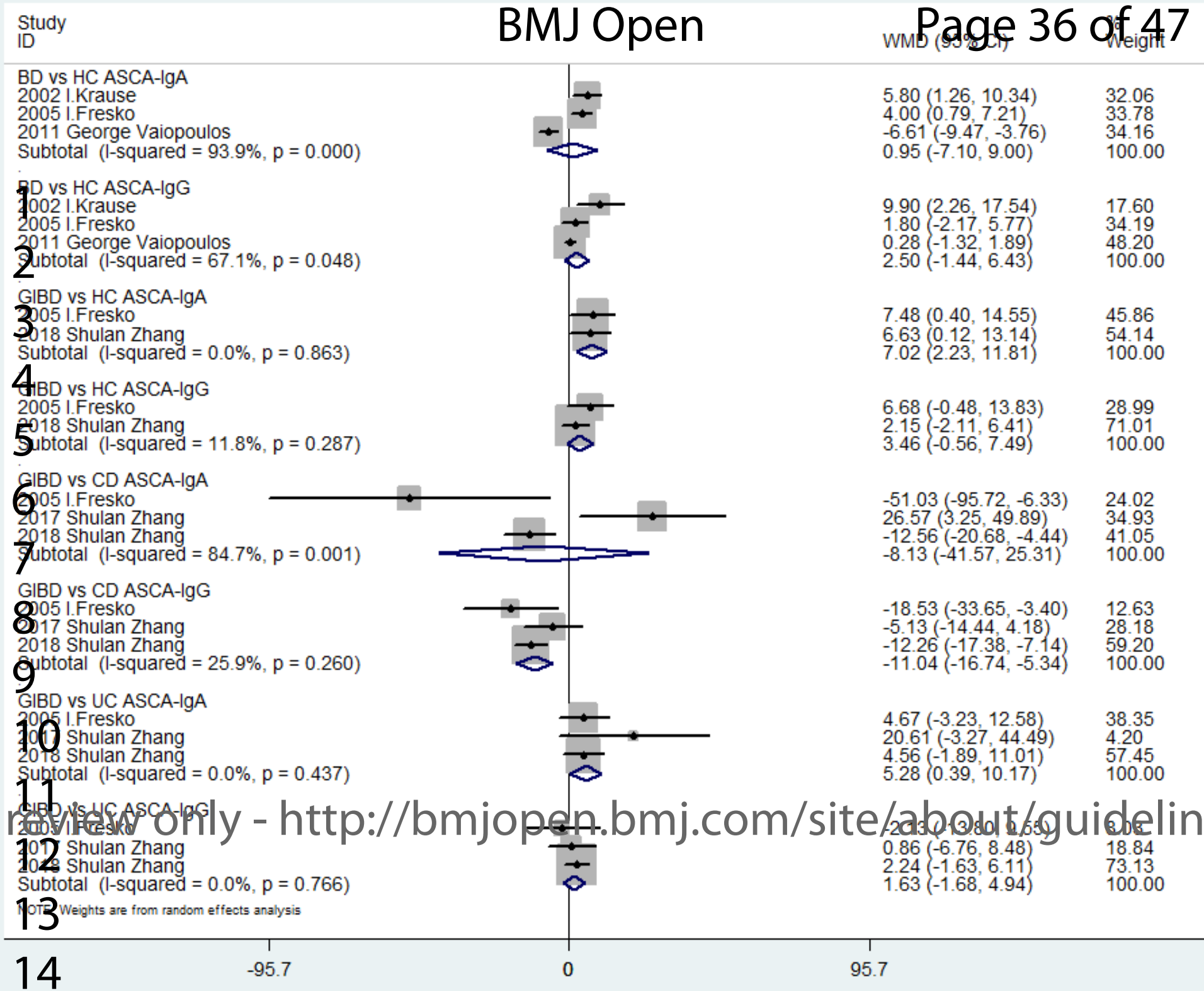
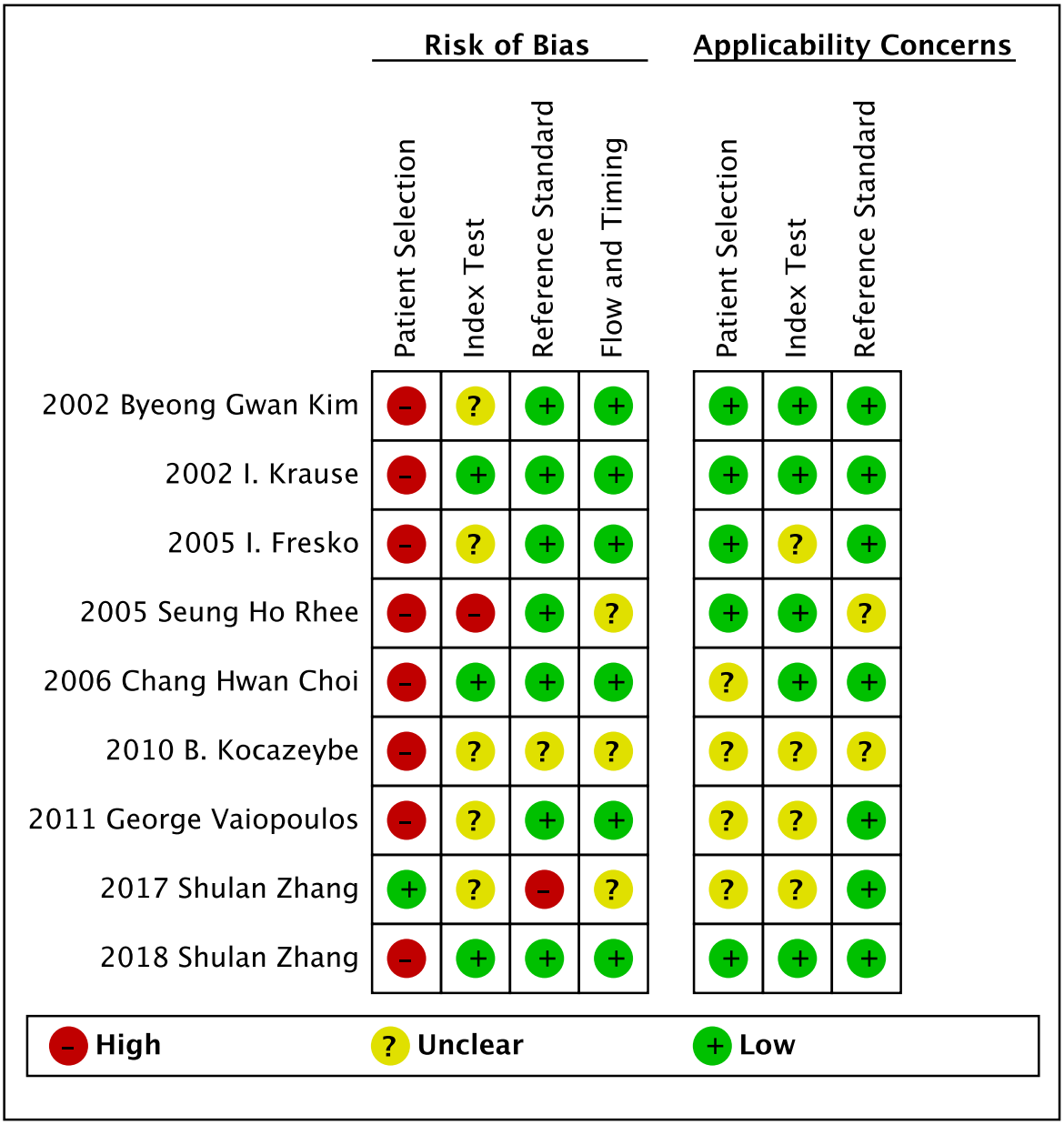
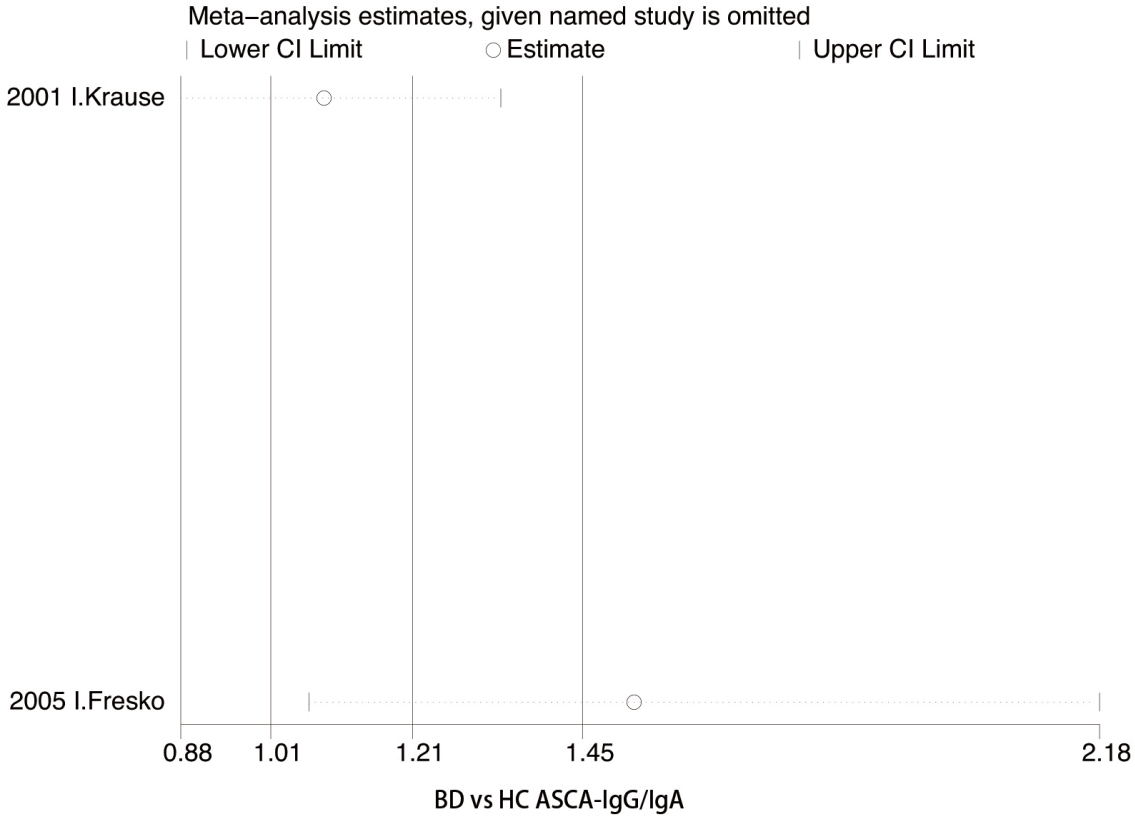


Fig S1 Results of quality assessment of the included studies based on the QUADAS-1 tool

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Lower CI Limit
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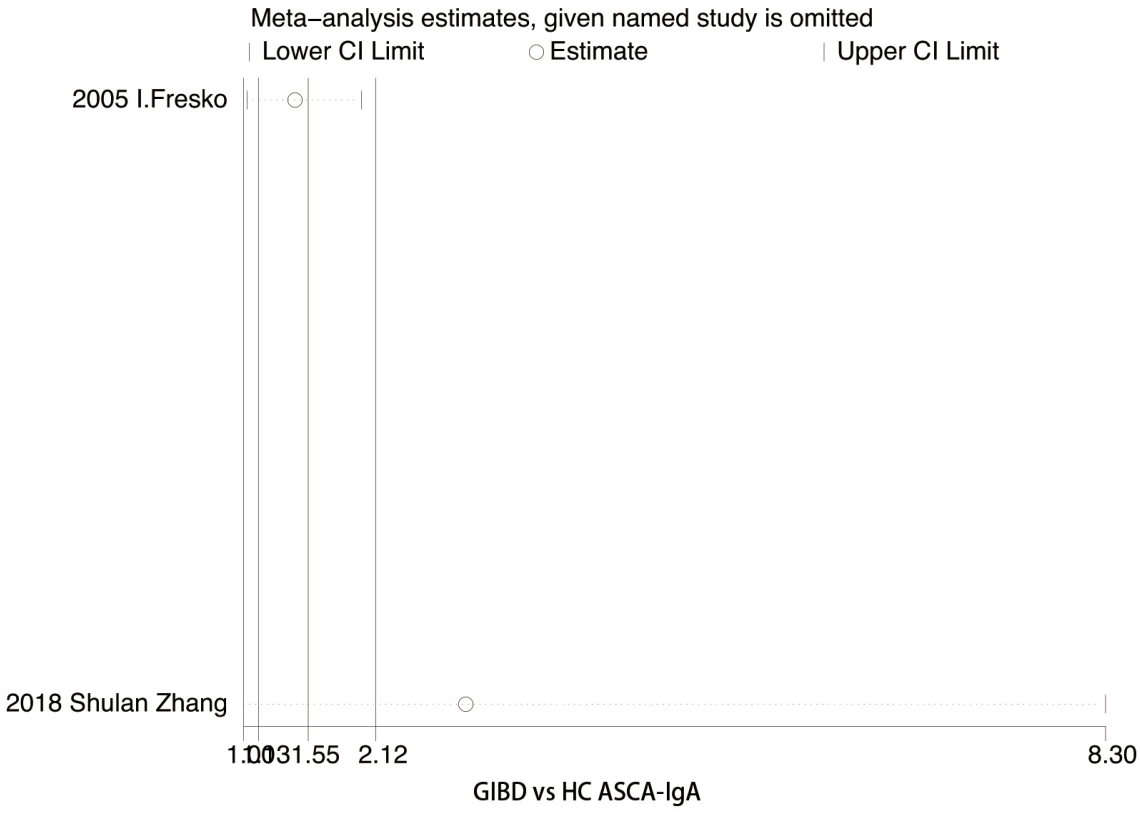
BD vs HC ASCA-IgG/IgA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Lower CI Limit
Estimate
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2005 I.Fresko
2018 Shulan Zhang
1.00
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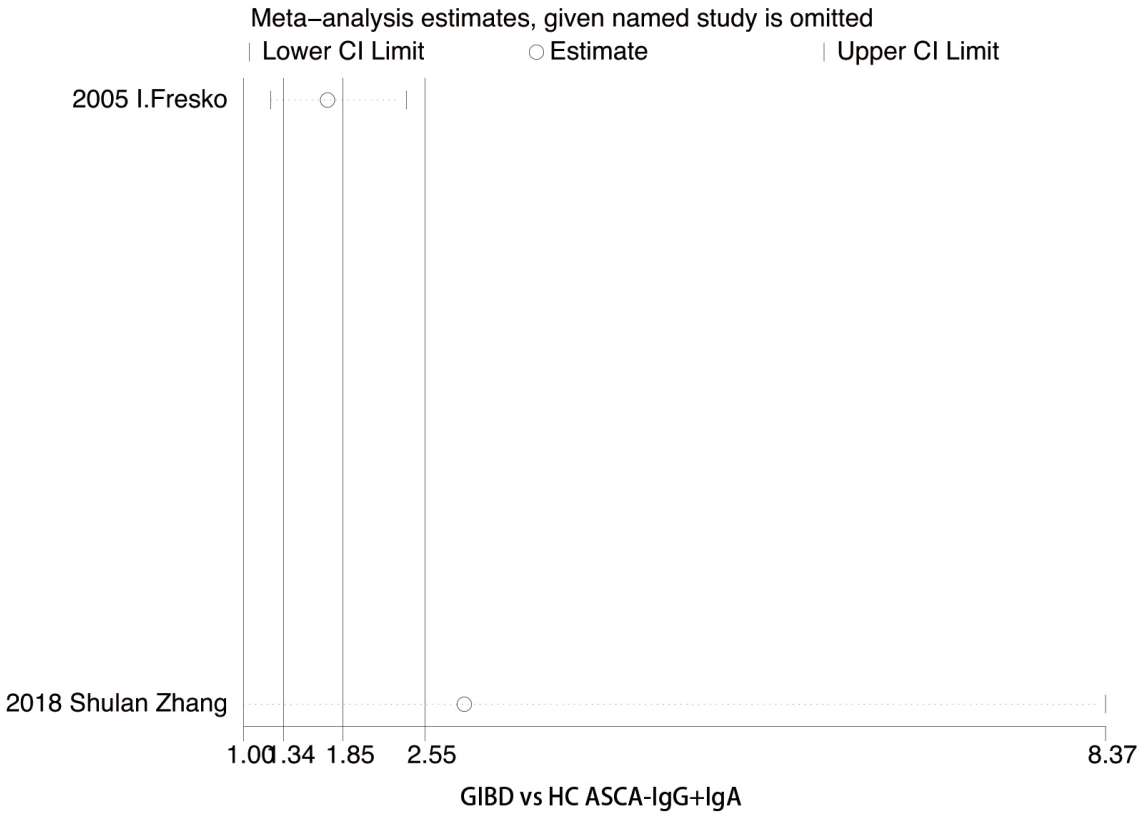
8.30
GIBD vs HC ASCA-IgA


Meta−analysis estimates, given named study is omitted
Lower CI Limit
Estimate
Upper CI Limit
2005 I.Fresko
2018 Shulan Zhang
1.00
1.34
1.85
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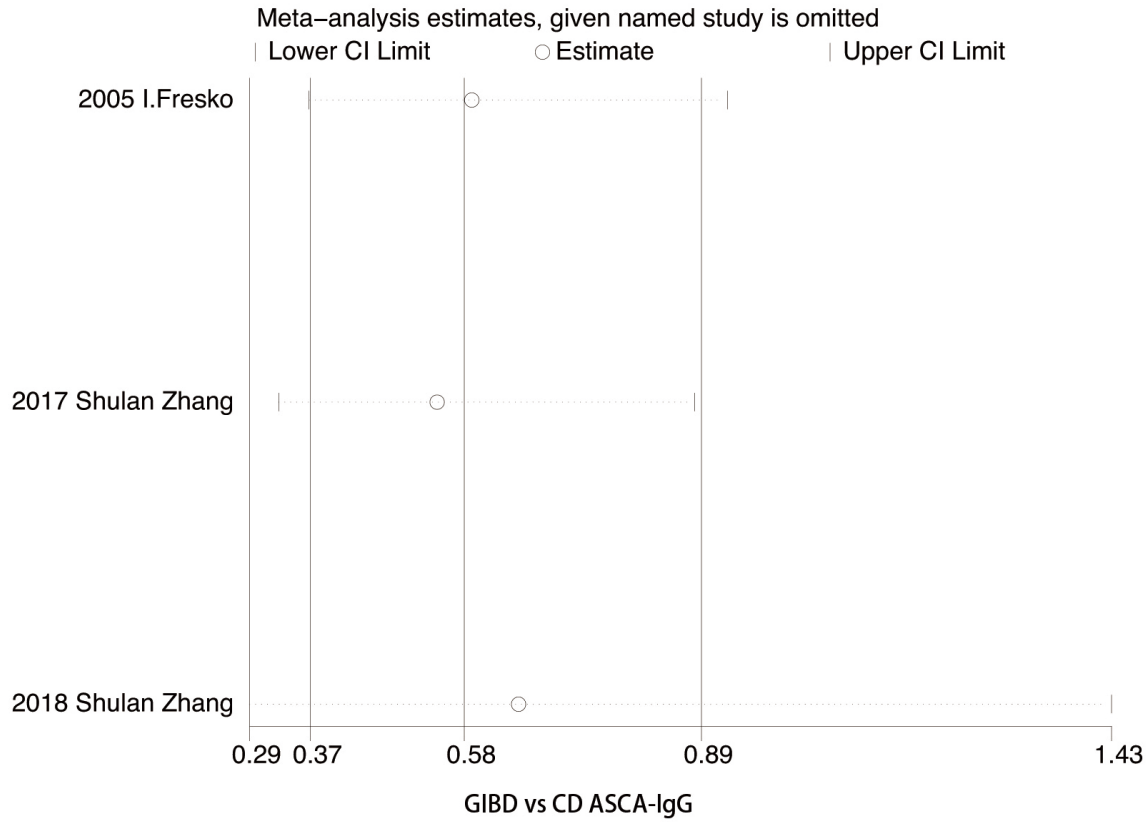
GIBD vs HC ASCA-IgG+IgA


Meta−analysis estimates, given named study is omitted
Lower CI Limit
Estimate
Upper CI Limit
2005 I.Fresko
2017 Shulan Zhang
2018 Shulan Zhang
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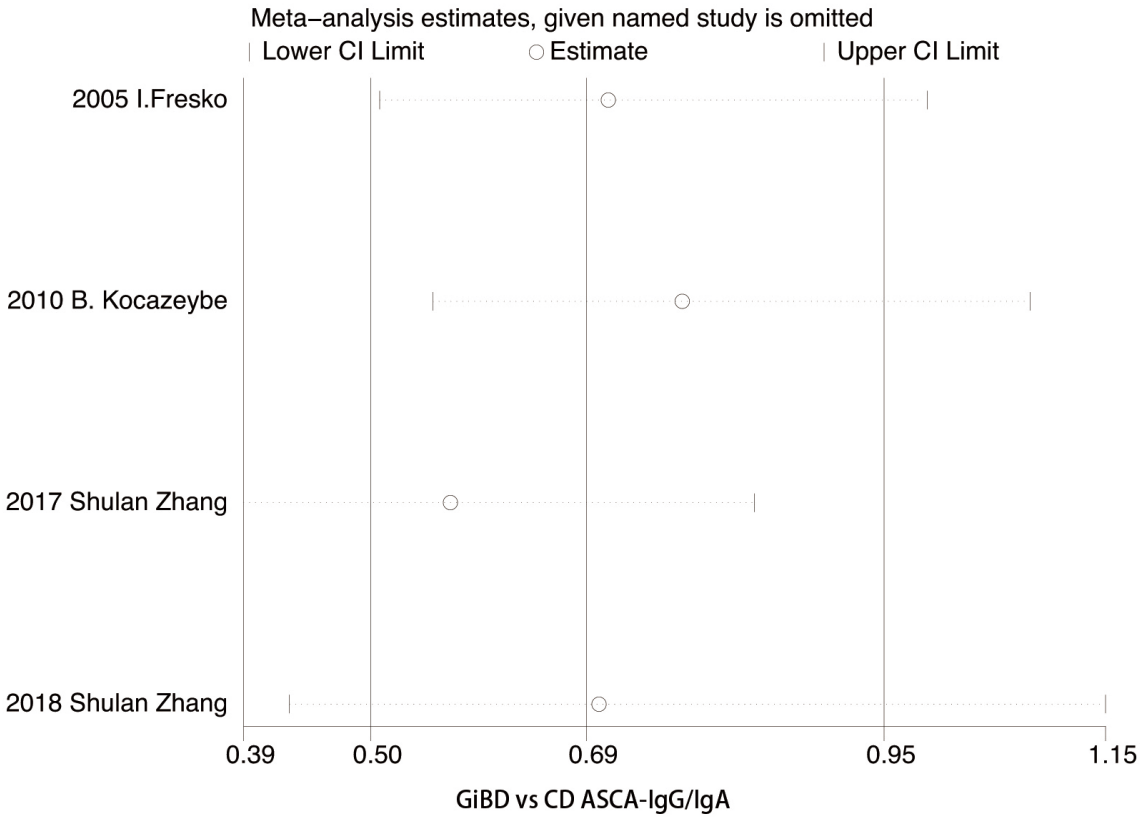
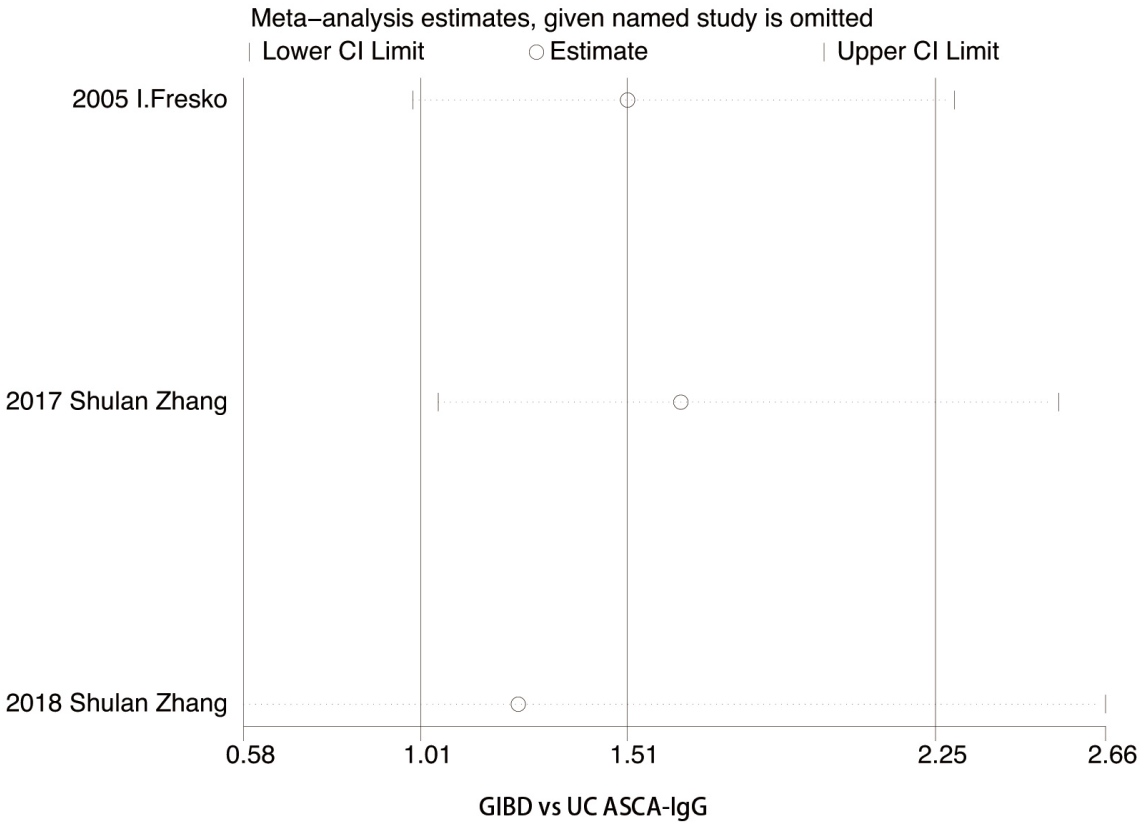
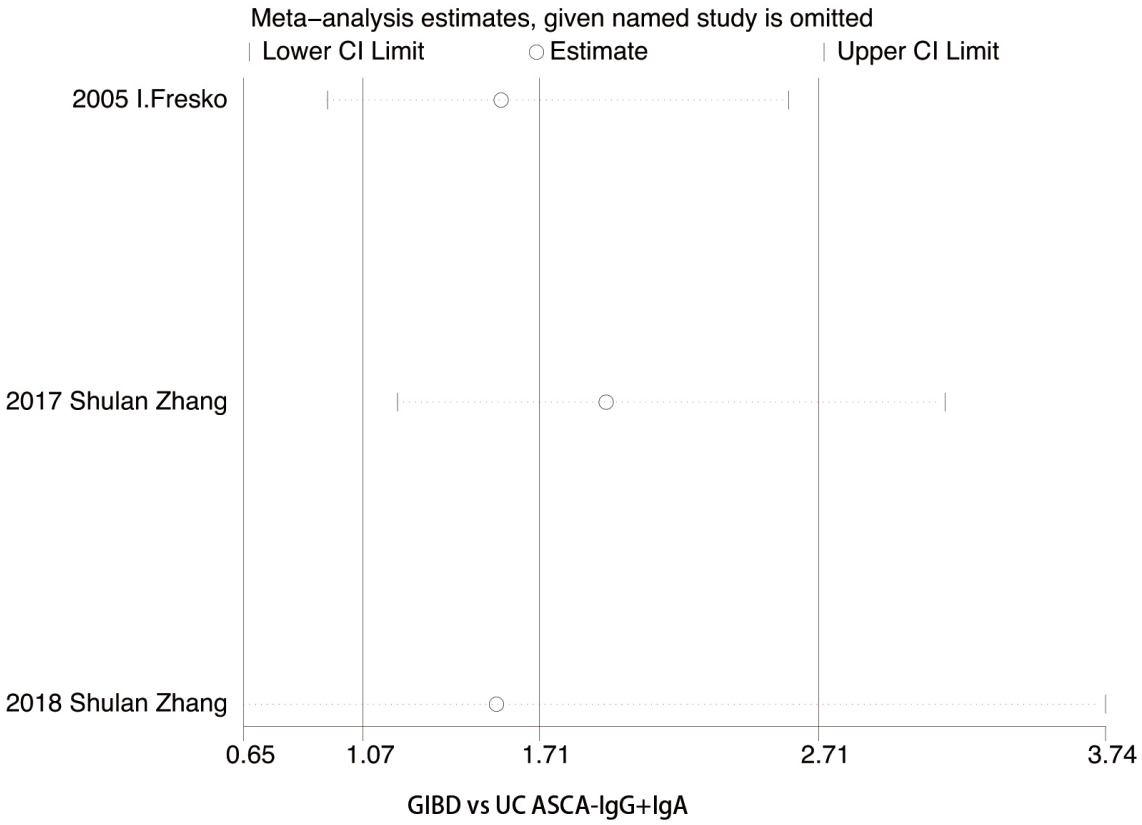
0.37
0.58
0.89
1.43
GIBD vs CD ASCA-IgG


Meta−analysis estimates, given named study is omitted
Lower CI Limit
Estimate
Upper CI Limit
2005 I.Fresko
2010 B. Kocazeybe
2017 Shulan Zhang
2018 Shulan Zhang
0.39
0.50
0.69
0.95
1.15
GiBD vs CD ASCA-IgG/IgA


Meta−analysis estimates, given named study is omitted
Lower CI Limit
Estimate
Upper CI Limit
2005 I.Fresko
2017 Shulan Zhang
2018 Shulan Zhang
0.58
1.01
1.51
2.25
2.66
GIBD vs UC ASCA-IgG



PRISMA-DTA Checklist

Section/topic	#	PRISMA-DTA Checklist Item	Reported on page #
TITLE / ABSTRACT			
Title	1	Identify the report as a systematic review (+/- meta-analysis) of diagnostic test accuracy (DTA) studies.	1
Abstract	2	Abstract: See PRISMA-DTA for abstracts.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Clinical role of index test	D1	State the scientific and clinical background, including the intended use and clinical role of the index test, and if applicable, the rationale for minimally acceptable test accuracy (or minimum difference in accuracy for comparative design).	4, 5
Objectives	4	Provide an explicit statement of question(s) being addressed in terms of participants, index test(s), and target condition(s).	4, 5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5 CRD42020115245
Eligibility criteria	6	Specify study characteristics (participants, setting, index test(s), reference standard(s), target condition(s), and study design) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6, 7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6, 7
Search	8	Present full search strategies for all electronic databases and other sources searched, including any limits used, such that they could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Definitions for data extraction	11	Provide definitions used in data extraction and classifications of target condition(s), index test(s), reference standard(s) and other characteristics (e.g. study design, clinical setting).	7
Risk of bias and applicability	12	Describe methods used for assessing risk of bias in individual studies and concerns regarding the applicability to the review question.	7
Diagnostic accuracy measures	13	State the principal diagnostic accuracy measure(s) reported (e.g. sensitivity, specificity) and state the unit of assessment (e.g. per-patient, per-lesion).	7, 8
Synthesis of results	14	Describe methods of handling data, combining results of studies and describing variability between studies. This could include, but is not limited to: a) handling of multiple definitions of target condition. b) handling of multiple thresholds of test positivity, c) handling multiple index test readers, d) handling of indeterminate test results, e)	7, 8

PRISMA-DTA Checklist

		grouping and comparing tests, f) handling of different reference standards	

Section/topic	#	PRISMA-DTA Checklist Item	Reported on page #
Meta-analysis	D2	Report the statistical methods used for meta-analyses, if performed.	7, 8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7-9
RESULTS			
Study selection	17	Provide numbers of studies screened, assessed for eligibility, included in the review (and included in meta-analysis, if applicable) with reasons for exclusions at each stage, ideally with a flow diagram.	9, 10
Study characteristics	18	For each included study provide citations and present key characteristics including: a) participant characteristics (presentation, prior testing), b) clinical setting, c) study design, d) target condition definition, e) index test, f) reference standard, g) sample size, h) funding sources	9, 10
Risk of bias and applicability	19	Present evaluation of risk of bias and concerns regarding applicability for each study.	10
Results of individual studies	20	For each analysis in each study (e.g. unique combination of index test, reference standard, and positivity threshold) report 2x2 data (TP, FP, FN, TN) with estimates of diagnostic accuracy and confidence intervals, ideally with a forest or receiver operator characteristic (ROC) plot.	10
Synthesis of results	21	Describe test accuracy, including variability; if meta-analysis was done, include results and confidence intervals.	10-12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression; analysis of index test: failure rates, proportion of inconclusive results, adverse events).	12
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence.	14
Limitations	25	Discuss limitations from included studies (e.g. risk of bias and concerns regarding applicability) and from the review process (e.g. incomplete retrieval of identified research).	16, 17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence. Discuss implications for future research and clinical practice (e.g. the intended use and clinical role of the index test).	17
FUNDING			
Funding	27	For the systematic review, describe the sources of funding and other support and the role of the funders.	18

Adapted From: McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PM, The PRISMA-DTA Group (2018). Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies: The PRISMA-DTA Statement. JAMA. 2018 Jan 23;319(4):388-396. doi: 10.1001/jama.2017.19163.

For more information, visit: www.prisma-statement.org.

Reporting checklist for meta-analysis of observational studies.

Based on the MOOSE guidelines.

		Reporting Item	Page Number
Title			
	#1	Identify the study as a meta-analysis of observational research	1
Abstract			
	#2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number (From PRISMA checklist)	1-2
Background			
	#3a	Problem definition	4
	#3b	Hypothesis statement	4-5
	#3c	Description of study outcomes	n/a
			The study outcomes were reported in the section of Results and Discussion.
	#3d	Type of exposure or intervention used	4
	#3e	Type of study designs used	4
	#3f	Study population	4, 5
Methods			
Search	#4a	Qualifications of searchers (eg, librarians and	6, 7

strategy		investigators)	
Search strategy	#4b	Search strategy, including time period included in the synthesis and keywords	5, 6
Search strategy	#4c	Effort to include all available studies, including contact with authors	7
Search strategy	#4d	Databases and registries searched	5, 6
Search strategy	#4e	Search software used, name and version, including special features used (eg, explosion)	n/a The search was performed on website of databases
Search strategy	#4f	Use of hand searching (eg, reference lists of obtained articles)	6
Search strategy	#4g	List of citations located and those excluded, including justification	6, 7
Search strategy	#4h	Method of addressing articles published in languages other than English	6
Search strategy	#4i	Method of handling abstracts and unpublished studies	6
Search strategy	#4j	Description of any contact with authors	7
	#5a	Description of relevance or appropriateness of studies gathered for assessing the hypothesis to be tested	7
	#5b	Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	7
	#5c	Documentation of how data were classified and coded (eg, multiple raters, blinding, and interrater reliability)	7
	#5d	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	7
	#5e	Assessment of study quality, including blinding of quality	7

		assessors; stratification or regression on possible predictors of study results	
	#5f	Assessment of heterogeneity	8
	#5g	Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	8
	#5h	Provision of appropriate tables and graphics	n/a The methods were described in text instead of tables and graphics.
Results			
	#6a	Graphic summarizing individual study estimates and overall estimate	10-12
	#6b	Table giving descriptive information for each study included	9, 10
	#6c	Results of sensitivity testing (eg, subgroup analysis)	12
	#6d	Indication of statistical uncertainty of findings	12
Discussion			
	#7a	Quantitative assessment of bias (eg. publication bias)	n/a Small number of studies in each subgroup prevented publication bias analysis
	#7b	Justification for exclusion (eg, exclusion of non–English-language citations)	16

	#7c	Assessment of quality of included studies	15, 16
Conclusion			
	#8a	Consideration of alternative explanations for observed results	17
	#8b	Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)	17
	#8c	Guidelines for future research	17
	#8d	Disclosure of funding source	18

Table S3 Demographic characteristics of patients and healthy controls included in the meta-analysis

BD: Behçet's disease; GIBD: gastrointestinal Behçet's disease; CD: Crohn's disease; UC: ulcerative colitis; iTB: intestinal tuberculosis; HC: healthy control; /: No such group included; Blank: no such information in the article

	BD	GIBD	CD	UC	iTB	HC
Female, n (%)						
2018 Shulan Zhang	/					/
2017 Shulan Zhang			37 (28.9)	69 (49.3)		
2011 George Vaiopoulos	28 (48.3)	/	/	/	/	
2010 B. Kocazeybek	/	5 (38.5)	39 (61.9)	51 (50.0)	3 (30.0)	99 (60.0)
2006 Chang Hwan Choi	16 (86.6)	47 (44.3)	/	/	/	24 (53.3)
2005 I. Fresko	20 (23.5)	2 (25.0)	10 (41.7)	10 (40.0)	/	9 (42.9)
2005 Seung-Ho Rhee	/	28 (63.6)	/	/	/	
2002 I. Krause	20 (74.1)					
2002 Byeong Gwan Kim	/	20	28	36	7	
Median age at study (max, min)						
2018 Shulan Zhang	/					
2017 Shulan Zhang	/		33 (69,13)	42 (76,13)		/
2011 George Vaiopoulos	38.5 (17,70)	/	/	/	/	
2010 B. Kocazeybek	/	32.11 (23.22,41)	37.56 (24.91,50.21)	40.72 (27.28,54.16)	SD 9.96	35.07 (24.58,45.56)
2006 Chang Hwan Choi	38 (18,65)	37	/	/	/	39 (16,69)
2005 I. Fresko	34.3 (32.0,36.7)	26.9 (24.1,29.6)	38.9 (34.6,43.2)	35.6 (33.0,38.3)	/	33.7 (30.4,37.0)
2005 Seung-Ho Rhee	/	37.6	/	/	/	
2002 I. Krause	41.6 (36.9,46.2)					
2002 Byeong Gwan Kim	/	43.2	40.9	30.6	33.6	

BMJ Open

Meta-analysis of anti- Saccharomyces cerevisiae antibodies as diagnostic markers of Behçet's disease with gastrointestinal involvement

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Manuscript ID	bmjopen-2019-033880.R2
Article Type:	Original research
Date Submitted by the Author:	15-May-2020
Complete List of Authors:	Cheng, Linlin; Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Department of Clinical Laboratory Li, Liubing; Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Department of Clinical Laboratory Liu, Chenxi; Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Department of Clinical Laboratory Yan, Songxin; Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Department of Clinical Laboratory Li, Yongzhe; Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Department of Clinical Laboratory
Primary Subject Heading:	Rheumatology
Secondary Subject Heading:	Diagnostics, Epidemiology, Gastroenterology and hepatology, Immunology (including allergy)
Keywords:	Behçet's disease, Anti-saccharomyces cerevisiae antibodies, autoimmune diseases, meta-analysis, autoantibodies

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Meta-analysis of anti- *Saccharomyces cerevisiae* antibodies as diagnostic markers of Behçet's disease with gastrointestinal involvement

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Meta-analysis of anti- *Saccharomyces cerevisiae* antibodies as diagnostic markers of Behçet's disease with gastrointestinal involvement

Abstract

Objective: Due to common exposure to yeast in the alcoholic and baking industry, positive rate of anti-*Saccharomyces cerevisiae* antibodies (ASCA) is reportedly high in patients with Behçet's disease (BD) who have gastrointestinal symptoms (gastrointestinal BD [GIBD]). We performed a meta-analysis to assess the diagnostic value of ASCA in differentiating patients with BD from those with other chronic inflammatory bowel diseases.

Methods: The meta-analysis is compliant with the PRISMA and MOOSE checklist. Relevant studies that investigated ASCA levels in BD patients were retrieved from PubMed, EMBASE, Web of Science, SCOPUS, and the Cochrane Library on July 12, 2019; the search was rerun on February 12, 2020. Stata/SE 12.0 and Meta-DiSc 1.4 were used to perform the meta-analysis and sensitivity analysis, disaggregated by isotypes of ASCA.

Results: Nine studies were included in the meta-analysis. The results revealed a strong association between ASCA and GIBD, especially ASCA-IgG [odds ratio (OR)=5.50 (95%

CI 2.58–11.55, p=0.000) and ASCA-IgG+IgA [OR=5.36 (95% CI 1.40–20.45), p=0.014]. The positivity rate of ASCA in GIBD was significantly higher than that in ulcerative colitis: IgA [OR=2.13 (95% CI 1.30–3.50), p=0.003]; IgG+IgA [OR=2.19 (95% CI 1.03–4.66), p=0.042]; IgG/IgA [OR=2.03 (95% CI 1.30–3.17), p=0.002]. However, the frequency of ASCA-IgG was significantly higher in patients with Crohn's disease than GIBD [OR=5.36 (95% CI 1.40–20.45), p=0.009]. There was no significant difference in ASCA positivity between BD without gastrointestinal involvement and healthy controls and between GIBD and intestinal tuberculosis (p>0.05).

Conclusion: ASCA may play a role in the pathogenesis of gastrointestinal involvement. Negative result of IgG favors the diagnosis of GIBD/BD when differentiated from Crohn's disease. ASCA-IgA showed moderate diagnostic performance in distinguishing GIBD and ulcerative colitis and the diagnostic performance was better in combination with IgG. However, ASCA may not be a useful serologic marker distinguishing GIBD and intestinal tuberculosis.

Key words: Behçet's disease; Anti-*Saccharomyces* cerevisiae antibodies; autoimmune diseases; meta-analysis; autoantibodies

Strengths and limitations of this study

- In addition to the healthy controls, we included patients with other gastrointestinal diseases that are considered in the differential diagnosis of gastrointestinal Behcet's disease in clinical settings (such as ulcerative colitis, Crohn's disease, and intestinal tuberculosis), in order to improve the clinical awareness of ASCA.
- Inclusion of both categorical data (positivity rate) and continuous data (serum concentration) pertaining to anti-*Saccharomyces cerevisiae* antibodies (ASCA) increases the reliability of the results of meta-analysis.
- We separately performed meta-analysis of IgG, IgA, and IgG+IgA, which provides insights into their ability to differentiate BD from other gastrointestinal diseases.
- Comprehensive summary of evidence linking ASCA and autoimmune diseases provides preliminary insights into the pathogenicity of *Saccharomyces cerevisiae.*
- Analysis of too many subgroups contributed to potential heterogeneity due to the small number of studies included in each subgroup.

Introduction

Behçet's disease (BD) is a chronic systemic vascular autoimmune/inflammatory disease with a high propensity for recurrence; the pathogenetic mechanisms of this disease are not well elucidated [1]. Virtually no specific histological or laboratory features of BD have been identified. Therefore, the diagnosis of BD is typically challenging as it is mainly based on clinical features [2, 3]. The diagnosis is frequently delayed until the development of clinical manifestations that qualify the diagnostic criteria. The estimated duration between the onset of symptoms and the fulfilment of diagnostic criteria is approximately 4 years [4].

Moreover, patients with prominent involvement of a particular organ system are easily misdiagnosed. For example, patients who have gastrointestinal symptoms as the main manifestation are liable to be misdiagnosed as having Crohn's disease (CD), ulcerative colitis (UC), or intestinal tuberculosis (iTB). These features make formulating disease criteria difficult, causing deleterious effects on the patients.

Several recent studies (but not all) have reported the diagnostic value of anti-*Saccharomyces cerevisiae* antibody (ASCA) in BD. *Saccharomyces cerevisiae*, also known as the baker's or brewer's yeast, has long been utilized to ferment the sugars in

cereals to produce alcoholic beverages; it is also used in the baking industry to raise dough. As a consequence, we are now commonly exposed to yeast [5]. IgG and IgA antibodies against the phosphopeptidomannan of the S. cerevisiae cell wall have been discovered as autoantibodies in the sera of patients with BD, especially those with gastrointestinal involvement. This suggests a role of environmental stimuli in the pathogenesis of BD. However, patients with inflammatory bowel disease such as Crohn's disease (CD) also have a high prevalence rate of ASCA due to their similarities [6, 7, 8, 9, 10, 11]. In this context, identification of ASCA as a diagnostic marker for BD is a key imperative. The objectives of this study were to summarize the findings pertaining to the relevance of ASCA in BD and other gastrointestinal diseases and to perform a meta-analysis to assess its diagnostic accuracy for BD.

Methods

Study design

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis Diagnostic Test Accuracy (PRISMA-DTA) guidelines [12] (supplementary file 1) and Meta-analysis of Observational Studies in Epidemiology (MOOSE) [13] (supplementary file 2) were followed throughout the literature search process to structure and design the framework for

the review [14]. Besides, a predefined protocol was registered with PROSPERO (Registration No. CRD42020115245).

Literature search

A comprehensive literature search was performed to identify studies pertaining to ASCA as biomarkers for BD in 5 biomedical databases, i.e., PubMed, EMBASE, Web of Science, SCOPUS, and the Cochrane Library on July 12, 2019. The search terms for Behçet's disease were: Behcet, triple symptom complex, triple symptom complices, Adamantiades Behcet and old silk route disease; the search terms for *Saccharomyces cerevisiae* were*: S. cerevisiae, Saccaromyces cerevisiae, Saccharomyces capensis, Saccharomyces diastaticus, Saccharomyces italicus, Saccharomyces oviformis, Saccharomyces uvarum,* brewer yeast or baker yeast, mannan, manna, polymannan, glucomannan, yeast mannan, dicoman, humamil, ASCA. Combination of keywords using "AND" was used to retrieve studies in the range of "all fields" or "all text". The search was rerun on February 12, 2020 to ensure inclusion of recent studies. No restrictions were imposed with respect to time of publication, region, or ethnicity of the study population. In addition, the reference list of obtained articles was also examined to identify possible relevant studies. The full search strategy for EMBASE is shown in supplementary file 3.

Eligibility and exclusion criteria

The inclusion criteria were: (1) studies that evaluated the diagnostic accuracy of ASCA in BD; (2) availability of adequate data pertaining to the prevalence rate or serum levels of ASCA in patients with BD; (3) studies with healthy population and/or disease controls; (4) meeting abstracts or letters to the editor were also included.

The exclusion criteria were: (1) studies with incomplete data; (2) review articles; (3) non-English articles; (4) in case of studies with overlapping study population, studies with smaller sample size were excluded. Two investigators independently performed the literature search, screened the titles and abstracts, followed by full-text review of eligible studies.

Data extraction and quality assessment

Two independent investigators reviewed the full-text articles, extracted the data, and assessed the study quality using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2); The included items were evaluated as yes, no, or uncertain. Inter-researcher disagreements were resolved by consensus, or by a third investigator. Data pertaining to the following variables were extracted: publication year, article type, first author's name,

country, isotypes of ASCA detected, age and sex, research design, sample size, experimental method, trade names of experimental materials, cut-off values, diagnostic criteria, and serum titers and/or prevalence rate of ASCA in BD, gastrointestinal BD (GIBD), healthy controls (HC), patients with Crohn's disease (CD), ulcerative colitis (UC), and intestinal tuberculosis (iTB). The data were either obtained directly from the article, calculated, or requested from the author via e-mail.

Statistical analysis

Pooled odds ratios (OR) with 95% confidence intervals (CI) were calculated to evaluate the association between ASCA and BD (without gastrointestinal involvement)/GIBD/CD/UC/iTB using Stata/SE 12.0. Meta-DiSc 1.4 was used to calculate the sensitivity, specificity, AUC values, and the area under the summary receiver operating characteristic (SROC) curve to assess the overall diagnostic performance of ASCA. Heterogeneity among the included studies was evaluated using the Cochran's Q-statistic. P values > 0.10 were considered indicative of lack of significant heterogeneity. We chose the random effects models (REM) since REM tends to generalize findings beyond the included studies by assuming that the selected studies are random samples from a larger population [15]. Subgroup analysis was performed disaggregated by the isotypes of ASCA and different disease controls. The isotypes of ASCA were classified and defined as

follows: IgG, IgA, IgG/IgA (positive results of either IgG or IgA), and IgG+IgA (positive results of both IgG and IgA).

In order to increase the robustness of the meta-analysis, we also extracted the data pertaining to serum levels of ASCA from five studies and performed meta-analysis using the Continuous data module of Stata/SE 12.0. The REM was used for the analysis and weighted mean difference (WMD) was used as the effect measure if the same unit was used in these studies and there were minor differences with respect to the serum levels of ASCA. Sensitivity analysis was performed using Stata/SE 12.0 to evaluate stability of the results after sequential exclusion of one study at a time.

Patient and public involvement

The present study was a meta-analysis and systematic review based on published data. Patients and public were not involved in the study design, conduct, data analysis, and result dissemination.

Relationship between ASCA and autoimmune disease

We searched the PubMed for studies pertaining to the relationship between ASCA and autoimmune diseases. The two search terms used were autoimmune disease and

Saccharomyces cerevisiae. We performed an interval statistic of four indicators of ASCA– sensitivity, specificity, positive likelihood (LR+) and negative likelihood (LR-) based on the included studies sorted by diseases.

Results

Literature search and characteristics of studies

A total of 625 documents were retrieved on database and manual search. Fifty-one duplicate publications were excluded using the document management software. A total of 127 records were retained after screening of titles and/or abstracts; the excluded records included review articles, animal model studies, therapeutic or drug research, genetic research, book chapters, duplicate publications not recognized by software, and other irrelevant records. After full-text review for eligibility, 22 records were selected. Finally, we included 9 available studies with adequate data in the meta-analysis (Figure 1). Two studies were included after obtaining the relevant data by contacting the respective authors [9, 10]. In addition, we also verified 2 studies [16, 17] with overlapping study population; of these, only 1 study was included in the meta-analysis. Three studies [6, 8, 18] were presented as meeting abstracts without adequate data to allow the construction of a 2×2 table. One article[7] was a letter to the editor and only reported the prevalence rate of

ASCA antibody in patients with BD, without information about the control group. One study [19] had employed a unique calculation method and could not be included in the meta-analysis. Among the included studies, there were 326 cases of BD, 294 cases of GIBD, 520 cases of CD, 598 cases of UC, 112 cases of iTB, and 428 HCs (Table 1 and supplementary file 4).

Quality assessment

There were 8 case-control studies and 1 retrospective study [9]. The results of quality assessment by QUADAS-2 including the risk of bias and applicability concerns pertaining to each domain [20] are shown in supplementary file 5. The results indicated that the included studies were of high quality in general. Overall, none of the 9 included studies showed any major methodological bias or flaws, which indicates robustness of our meta-analysis.

Meta-analysis

Association between ASCA and BD (without gastrointestinal involvement), GIBD and other intestinal diseases

Data pertaining to correlation between ASCA and BD (without gastrointestinal

involvement)/GIBD/CD/UC/iTB are listed in Table 2. No substantial heterogeneity (p>0.1 for all) was observed by using REM to calculate the OR. The results revealed a strong association between all detection types of ASCA and GIBD, especially for ASCA-IgG [OR=5.50 (95% CI 2.58–11.55, p=0.000) and ASCA-IgG+IgA [OR=5.36 (95% CI 1.40–20.45), p=0.014]. When comparing GIBD and UC, of the positivity rate for ASCA in GIBD was significantly higher than that for UC: IgA [OR=2.13 (95% CI 1.30–3.50), p=0.003], IgG+IgA [OR=2.19 (95% CI 1.03–4.66), p=0.042], and IgG/IgA [OR=2.03 (95% CI 1.30–3.17), p=0.002]. Conversely, the frequency of only ASCA-IgG in patients with CD was significantly higher than that in the GIBD [OR=5.36 (95% CI 1.40–20.45), p=0.009]. Further, on stratified analysis according to detection method, ASCA-IgG was associated with GIBD using both the ELISA method (OR = 3.83, 95% CI 1.37–10.70, *p* = 0.010) and the immunoprecipitation method (IIF) (OR = 8.17, 95% CI 2.73–24.43, *p* = 0.000) (Figure 2). However, no significant difference was observed with respect to ASCA positivity between BD without gastrointestinal involvement and HC and between GIBD and iTB (p>0.05).

Diagnostic ability of ASCA for GIBD

The overall sensitivity for ASCA-IgG in patients with GIBD detected by IIF was 0.44, which is much higher than that of ELISA [0.20 (95%CI 0.12–0.31)] (Table 3). Combined detection of IgG and IgA by ELISA increased the sensitivity to 0.33 (95% CI 0.23–0.44).

However, we observed a low level of sensitivity of ASCA-IgG/IgA by IIF, which may be attributable to the inclusion of only one study with few GIBD patients (n=13).

Difference in serum levels of ASCA in GIBD and other intestinal diseases

Serum levels of ASCA-IgA observed in GIBD were significantly greater than that in HC [WMD=7.02 (95% CI 2.23–11.81), p=0.004) and UC [WMD=5.28 (95% CI 0.39–10.17), p=0.034] in contrast to ASCA-IgG (p>0.05) (Figure 3). On the contrary, serum levels of ASCA-IgG in CD were significantly greater than that in GIBD [WMD=-11.04 (95% CI -16.74–-5.34), p=0.000] (Figure 3). However, we found no significant difference in serum levels of ASCA between BD without gastrointestinal symptoms and HC (p>0.05) (Figure 3).

Heterogeneity and sensitivity analysis

We performed sensitivity analysis to assess the stability of the results. The results showed that the studies by Krause et al (2002), Zhang et al (2018), Kocazeybe et al (2010), and Fresko et al (2005) were the key contributors to the heterogeneity (supplementary file 6). Thus, the results of related subgroup analysis are considered to be less stable.

Summary of the relationship of ASCA with autoimmune disease

Sixteen studies reporting the relevance of ASCA and autoimmune diseases were included in the summary. The sensitivity, specificity, LR+, and LR- of ASCA for different autoimmune diseases are summarized in Table 4. Although the diagnostic results of ASCA reported by different studies vary, the summary revealed an overall association between ASCA and autoimmune diseases especially in patients with scleroderma, juvenile idiopathic arthritis, Crohn's disease, and systemic lupus erythematosus with high SEN (>40%), high SPE (>95), high LR+ (>5) (Table 4).

Discussion

Serological markers in BD. The diagnosis of BD is typically challenging prior to the appearance of clinical symptoms necessary to qualify the diagnostic criteria. Currently, there are no specific laboratory biomarkers of BD; however, some specific autoantibodies in the context of BD have been reported. Therefore, identification of non-invasive specific diagnostic and prognostic biomarkers of BD is of much clinical relevance and a key focus area of research.

ASCA in BD and autoimmune diseases. Several recent studies have investigated the relationship of ASCA with BD or other autoimmune diseases. *Saccharomyces cerevisiae* has long been utilized in alcoholic and baking industry, and for the production of vaccines

owing to its antigenic component. However, during long-term and ubiquitous presence, even the commensal and classically non-pathogenic microbiota can trigger autoimmunity due to loss of immune tolerance towards the resident bacterial flora, like in gastrointestinal tract [21, 22]. The reported similarity of sequences involving the eukaryotic microorganism and self-antigens suggest a mechanism of molecular mimicry and also the plausibility of shared epitopes in different autoimmune diseases. The production of ASCA by the subsequent activation of the humoral immune response may lead to a direct pathogenic role through a costimulatory CD80/86-CD28-mediated effect [21]. Moreover, healthy family members but not spouses of BD patients were also found to have increased levels of ASCA, which indicated a role of genetic factors in addition to environmental stimuli [17, 22]. A large number of studies have assessed the role of ASCA in the context of several systemic and organ-specific autoimmune diseases, such as BD, scleroderma, systemic lupus erythematosus, primary Sjögren's syndrome, and rheumatoid arthritis (Table 4). The results suggest that the relation of ASCA with BD or other autoimmune diseases may represent a potential pathogenic mechanism between ASCA and autoimmunity; this underlines the importance of ASCA as a valuable serologic marker for autoimmune diseases including BD.

Results of the meta-analysis. To the best of our knowledge, this is the second meta-analysis of evidence pertaining to autoantibodies in patients with BD after anticardiolipin

antibodies [23]. ASCA have been widely researched in BD, Crohn's disease and other autoimmune disease; in order to investigate the diagnostic value and possible pathogenetic role of ASCA in BD, we included 9 studies in this meta-analysis. Among these, some studies included BD patients with systemic involvement including or excluding gastrointestinal involvement, while others included only BD patients with gastrointestinal involvement. Therefore, in order to reduce the impact of differences with respect to frequency distribution of gastrointestinal symptoms in each study, we disaggregated patients with BD into those with gastrointestinal involvement only and those without gastrointestinal manifestations. The isotype antibodies of ASCA tested and the results presented by the studies are also different. The meta-analysis revealed a strong association of ASCA with GIBD and not with BD with no gastrointestinal involvement; this suggests the role of ASCA in the pathogenesis of gastrointestinal involvement. ASCA showed a moderate diagnostic performance as a biomarker for the differential diagnosis between GIBD and CD, and the negative result of ASCA-IgG may slightly favor the diagnosis of GIBD/BD when compared with CD, especially with concomitant positive HLA-B51 tests [24]. In addition, ASCA-IgA showed a moderate diagnostic value for distinguishing GIBD and UC and would perform better with concomitant detection of IgG. However, ASCA failed to distinguish between GIBD and iTB. Besides, the concomitant evaluation of both continuous data (sensitivity and specificity) and discontinuous data (serum levels) helped increase the credibility of our results.

Heterogeneity. Combined with the results of QUADAS-2, we found that the heterogeneity in this meta-analysis was largely attributable to the following reasons (see Table 1, supplementary file 5). 1) The different diagnostic criteria used in the included studies. Different criteria may have different thresholds for diagnosis or place more weight on some symptoms than others. Specifically, the 1990 ISG criteria requires the presence of oral ulceration plus any two of the following: genital ulceration, typical eye lesions, typical skin lesions, or positive pathergy test for diagnosis of BD [2]. In contrast, the 1987 Japan criteria require all four characteristics for the diagnosis of BD, i.e., oral ulceration, typical eye lesions, typical skin lesions, and genital ulceration [25]. The ISG criteria and the Japanese criteria often fail to classify some patients with BD; in addition, the Japanese criteria may also cause misclassified diagnosis. This may have caused the different diagnostic sensitivity and specificity for BD [26]. 2) Differences in demographic characteristics of included studies. The clinical features and laboratory findings tend to exhibit wide variability in different populations and clinical settings [27, 28]. However, we failed to perform subgroup analysis disaggregated by ethnicity owing to the small sample size in each subgroup. 3) Different antibody assays and cut-off values. Commercial kits and in-house tests from different laboratories have variable performance, which may affect the diagnosis and management of patients. We found that different methods and cut-off values were adopted by the studies included in this meta-analysis. Notably, there was significant association between ASCA-IgG and GIBD using both methods; however, it seems that IIF

has a higher sensitivity than ELISA. Nonetheless, further investigations with larger study population are required to provide more definitive evidence. Although previous studies have shown that IIF has a better performance, ELISA provides the titer change of serum antibodies and could have an equal performance to IIF by changing cut-off values to optimize the overall diagnostic performance [29]. 4) According to the QUADAS-2, there are certain concerns that most studies have risk of bias (internal validity) in patient selection, which, to some extent, would cause the the distorted estimation in diagnostic accuracy [20].

Limitations of the meta-analysis. In order to fully evaluate the value of ASCA for differential diagnosis of BD, we included patients with CD, UC, and iTB as the comparison objects in our meta-analysis. However, there are some limitations of this meta-analysis. (1) Gray literature database, paper database, and other language databases were not used for the literature search. Quite a few non-English studies were excluded due to incomplete data or unavailability of full text. (2) Our primary goal was to assess the diagnostic efficacy of ASCA in BD, and therefore we did not include all studies pertaining to ASCA in inflammatory bowel disease and iTB. (3) Restricted by the number of included studies and the isotypes of ASCA, we could not perform subgroup analysis disaggregated by different populations and diagnostic criteria. (4) Some studies with incomplete data were excluded after lack of response from the author. (5) There are some inherent statistical shortcomings

using Meta-DiSc during the separate pooling of sensitivity and specificity, as the between-study variance is not included. More advanced methods are not implemented [30].

Conclusion. Our study demonstrated the relationship between ASCA/*Saccharomyces cerevisiae* and gastrointestinal involvement in BD. Furthermore, ASCA may be detectable years before the diagnosis of some autoimmune diseases as they were retrospectively found in the preserved blood samples of soldiers who were affected by Crohn's disease years later [31]. However, detection of only ASCA may have a limited value for clinical diagnosis due to its moderate sensitivity and the presence in several other autoimmune diseases. In the future, further studies are needed to explore the role of ASCA and *Saccharomyces cerevisiae* in BD.

Compliance with Ethical Standards:

Contributorship statement: Study concept and design: Linlin Cheng, Yongzhe Li. Acquisition of data: Linlin Cheng, Liubing Li, Chenxi Liu, Songxin Yan. Statistical analysis and interpretation of data: Linlin Cheng, Liubing Li, Chenxi Liu. Drafting of the manuscript: Linlin Cheng. Revision of manuscript: Yongzhe Li, Linlin Cheng, Liubing Li. Supervision of work: Yongzhe Li. All authors read and approved the final manuscript.

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Figure 1 PRISMA flow diagram illustrating the literature screening process and the criteria for inclusion of studies in the meta-analysis

Figure 2 Forest plot of the association between the presence of ASCA-IgG and GIBD stratified by detection methods

Figure 3 Forest plot comparing serum levels of ASCA between BD without gastrointestinal symptom/GIBD and HC/CD/UC

Table 1 Characteristics of studies included in the meta-analysis of Anti-*Saccharomyces cerevisiae* antibodies in Behçet's disease, its main differential diagnoses, and healthy controls.

Year and author	Countries	Type	Type of article	Design	Sample size						Methods	Brands of experimental materials	Cut-off (U/ml)	Diagnostic criteria
					BD	GIBD	CD	UC	iTB	HC				
2018 Shulan Zhang [32]	China	IgG; IgA; IgG/IgA; IgG+IgA	Original article	case-control	/	71	171	208	57	70	ELISA	Inova Diagnostic	25	NR
2017 Shulan Zhang [33]	China	IgG; IgA; IgG/IgA; IgG+IgA	Original article	Retrospective study	/	34	128	140	31	/	ELISA	Euroimmun, Luebeck	20	NR
2011 George Vaiopoulos [34]	Greece	IgG; IgA	Original article	case-control	58	4#	/	/	/	56	ELISA	Inova Diagnostic	NR	1990 ISG criteria
2010 B. Kocazeybek. [35]	Turkey	IgG/IgA	conference Abstract	case-control	/	13	63	102	10	165	IIF	Euroimmun, Luebeck	NR	NR
2006 Chang Hwan Choi [36]	Korea	IgG	Original article	case-control	30*	106	/	/	/	45	IIF	Euroimmun, Luebeck	1:1000	1987 Japan criteria
2005 I. Fresko [37]	Turkey	IgG; IgA; IgG/IgA; IgG+IgA	Original article	case-control	85	8	24	25	/	21	ELISA	Inova Diagnostic	28 for IgG; 25 for IgA	1990 ISG criteria
2005 Seung-Ho Rhee [38]	Korea	IgG	Original article	case-control	/	16	/	/	/	4	ELISA	Inova Diagnostic	25	1987 Japan criteria
2002 I. Krause [16]	Israel	IgG; IgA; IgG/IgA; IgG+IgA	Original article	case-control	27*	/	/	/	/	10	ELISA	Inova Diagnostic	25	1990 ISG criteria
2002 Byeong Gwan Kim [39]	Korea	IgG+IgA+IgM	Original article	case-control	/	36	85	77	14	20	ELISA	plate: Sigma Chemical antibody: Biosoft	ROC curve	1987 Japan criteria

/:no sample; ELISA: enzyme-linked immunosorbent assay; IIF: indirect immunofluorescence assay; NR: not reported; SD: standard deviation; *: all without gastrointestinal manifestations; #: lack of corresponding data; 1990 ISG criteria: the 1990 criteria of Behçet's Disease International Study Group; 1987 Japan criteria: the 1987 criteria by the Behçet's Disease Research Committee of Japan; BD: Behçet's disease; GIBD: gastrointestinal Behçet's disease; CD: Crohn's disease; UC: ulcerative colitis; iTB: intestinal tuberculosis; HC: healthy control

Table 2. Association between the presence of ASCA and BD (without gastrointestinal symptom)/GIBD and other intestinal diseases

Subgroup	Antibody	Number of studies	Diagnostic OR (95% CI)	Significance test (p)
BD vs. HC	ASCA-IgG	4	1.00 (0.28–3.53)	0.997
	ASCA-IgA	2	2.50 (0.63–9.96)	0.194
	ASCA-IgG+IgA	2	1.06 (0.17–6.78)	0.954
	ASCA-IgG/IgA	2	2.88 (0.62–13.44)	0.179
GIBD vs. HC	ASCA-IgG	3	5.50 (2.58–11.55)	0.000
	ASCA-IgA	2	2.65 (1.18–5.96)	0.018
	ASCA-IgG+IgA	2	5.36 (1.40–20.45)	0.014
	ASCA-IgG/IgA	3	2.90 (1.47–5.74)	0.002
GIBD vs. CD	ASCA-IgG	3	0.48(0.28–0.83)	0.009
	ASCA-IgA	3	0.91(0.56–1.46)	0.685
	ASCA-IgG+IgA	3	0.58(0.30–1.11)	0.100
	ASCA-IgG/IgA	4	0.57 (0.28–1.15)	0.117
GIBD vs. UC	ASCA-IgG	3	1.78 (0.98–3.22)	0.057
	ASCA-IgA	3	2.13 (1.30–3.50)	0.003
	ASCA-IgG+IgA	3	2.19 (1.03–4.66)	0.042
	ASCA-IgG/IgA	4	2.03 (1.30–3.17)	0.002
GIBD vs. iTB	ASCA-IgG	2	1.08 (0.50–2.32)	0.854
	ASCA-IgA	2	1.51 (0.71–3.22)	0.290
	ASCA-IgG+IgA	2	1.02 (0.40–2.62)	0.972
	ASCA-IgG/IgA	3	1.05 (0.58–1.87)	0.883

BD: Behçet's disease without gastrointestinal symptom; GIBD: gastrointestinal Behçet's disease; CD: Crohn's disease; UC: ulcerative colitis; iTB: intestinal tuberculosis; HC: healthy control

Table 3 Pooled sensitivity and specificity of ASCA-IgG and IgG/IgA for diagnosis of GIBD assessed by ELISA and IIF

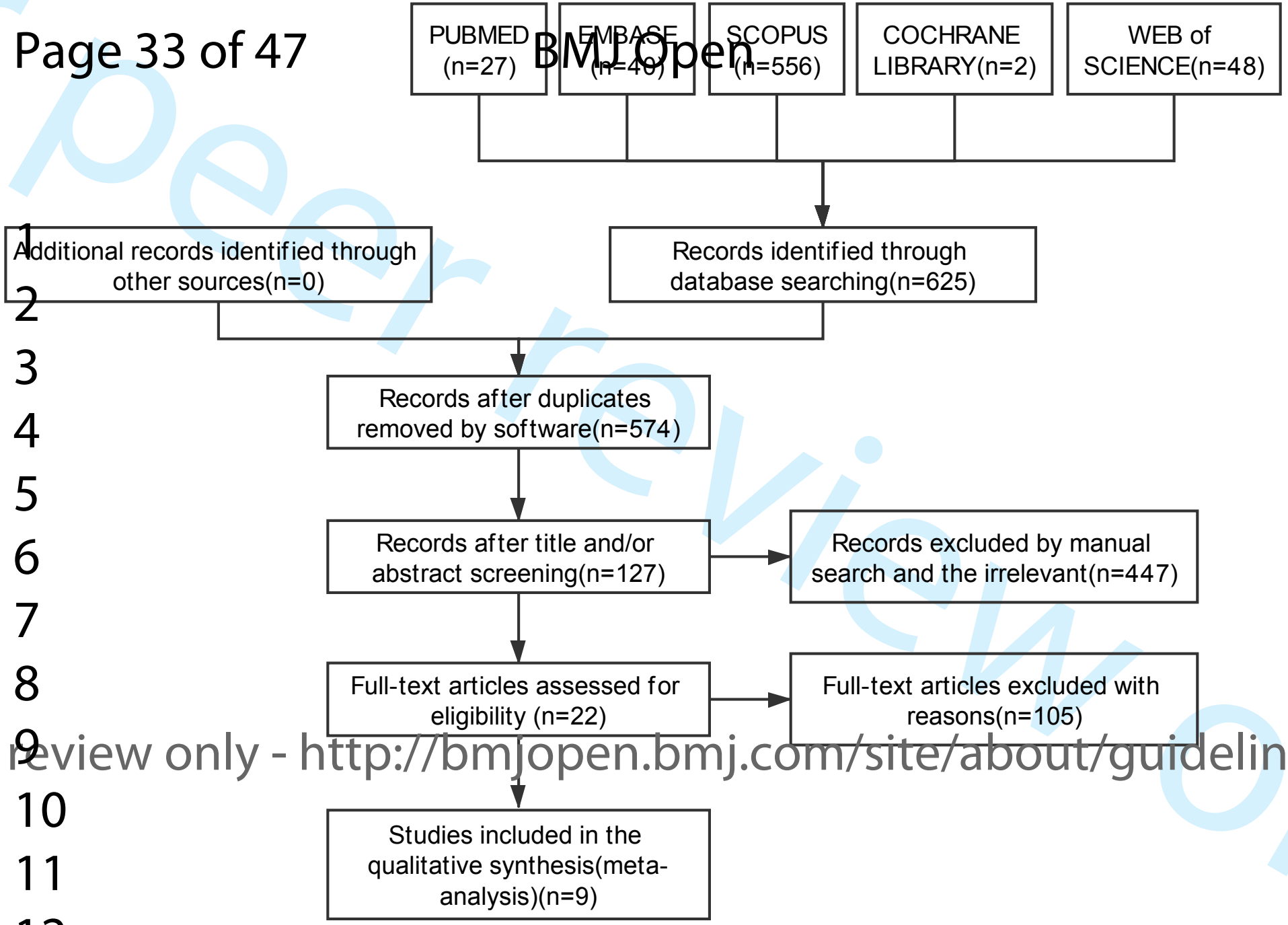
Methods	ELISA		IIF	
Diagnostic accuracy	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	Sensitivity	Specificity
ASCA-IgG	0.20 (0.12–0.31)	0.93 (0.86–0.98)	0.44	0.91
ASCA-IgG/IgA	0.33 (0.23–0.44)	0.85 (0.76–0.91)	0.15	0.96

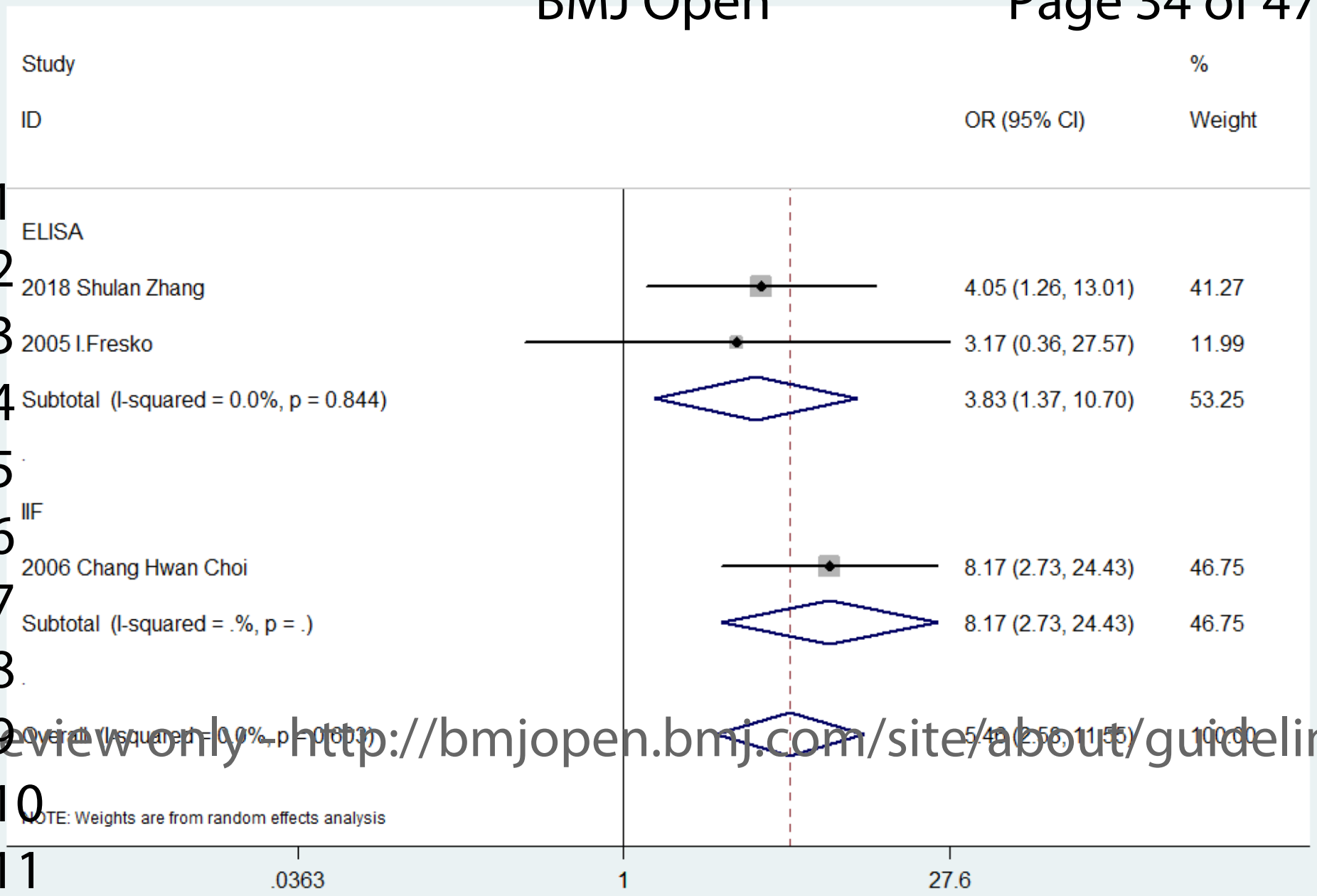
ELISA: enzyme-linked immunosorbent assay; IIF: indirect immunofluorescence assay

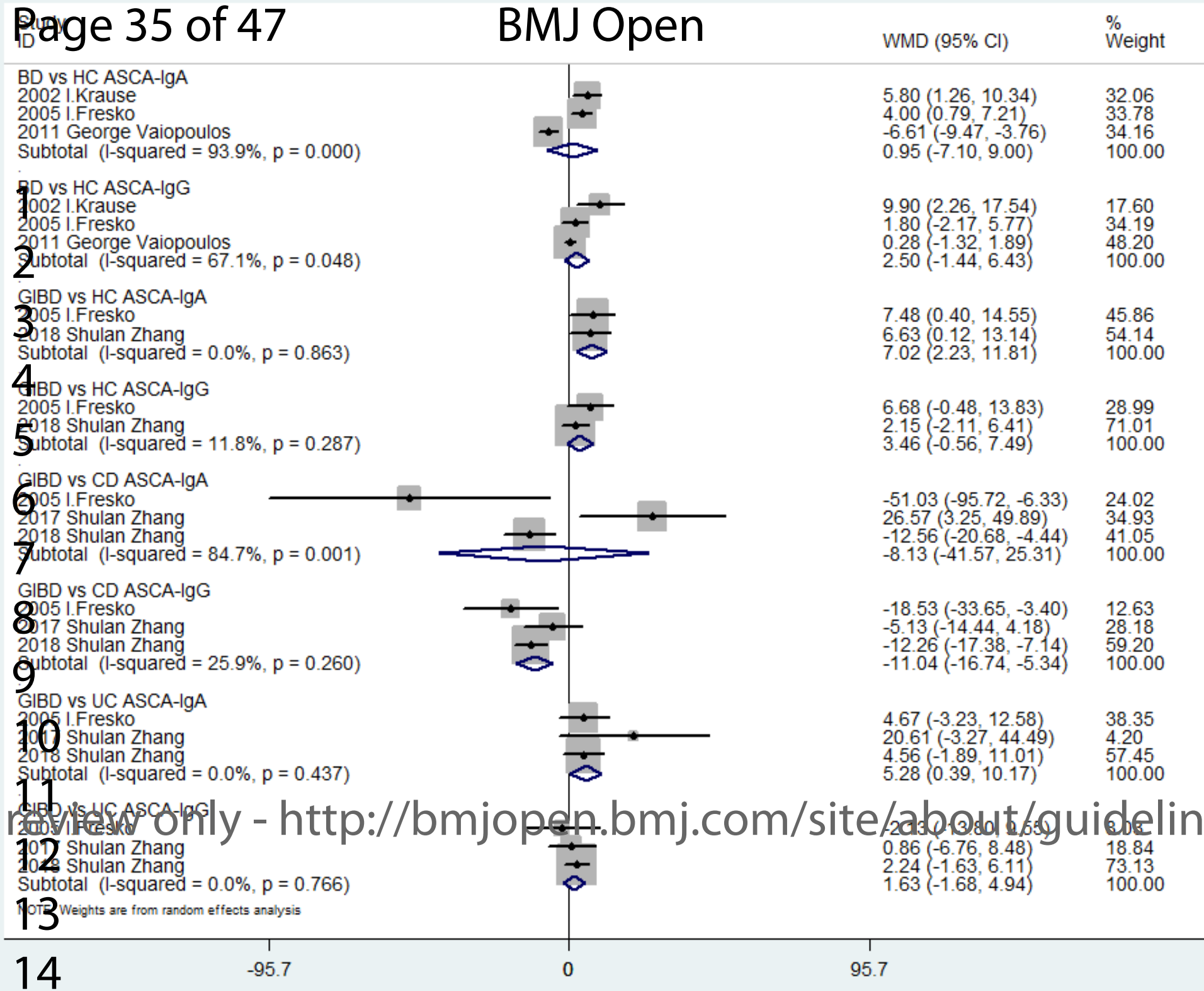
635 Table 4 Summary of the diagnostic performance of ASCA in autoimmune disease

Reference	Autoimmune disease	Type	SEN (%)	SPE (%)	LR+	LR-	Supplementary information
[28]	Scleroderma	IgG	43.24	98.25	24.65	0.58	African descendants showed higher positivity rates for ASCA-IgG. ASCA-IgA was less frequently detected in patients with severe disease
		IgA	16.22	94.74	3.08	0.88	
[40, 41, 42, 43]	Ankylosing spondylitis	IgG	0–11.63	89.74–98.72	1.13–3.00	0.97–0.98	ASCA IgA levels were significantly increased in patients with HLA-B27-associated SpA, particularly in AS and uSpA
		IgA	1.28–23.26	91.03–100.00	2.59–3.71	0.84–0.99	
[44]	Antiphospholipid syndrome	IgG/IgA	20.00	95.00	/	/	
[45]	Juvenile Idiopathic Arthritis	IgA	0–50.00	94.74	9.50	0.53	
[46, 47, 48]	Autoimmune hepatitis	IgG	16.42–27.53	100.00	/	0.84	
		IgA	11.94	94.74	2.27	0.93	
		IgG/IgA	18.52	84.00	1.16	0.97	
[46, 48, 49]	Primary biliary cirrhosis	IgG	10.57–18.95	97.50–100.00	7.58	0.83–0.89	
		IgA	11.58–18.70	94.74–98.75	3.55–9.26	0.86–0.90	
		IgG/IgA	20.26–24.21	84.00–96.25	1.27–6.46	0.79–0.95	
[46, 48]	Primary sclerosing cholangitis	IgG	28.00	100.00	/	0.72	
		IgA	32.00	94.74	6.08	0.72	
		IgG/IgA	30.51	84.00	1.91	0.83	
[38, 40, 46, 50, 51]	Crohn's disease	IgG	13.75–69.57	97.96–100	6.74	0.30–0.88	Patients with more complicated disease course showed a trend for greater seroreactivity towards ASCA.
		IgA	19.30–71.43	94.74–100.00	9.91–29.40	0.50–0.71	
[51]	Cryoglobulinemia	IgG	7.10	99.50	/	/	
[54, 52, 53]	Graves' disease	IgG	5.70–12.50	94.17–99.50	2.15–3.76	0.91–0.93	ASCA was elevated in Graves' disease but not in Hashimoto's thyroiditis
		IgA	8.40–16.67	94.17–96.88	2.69–2.86	0.88–0.95	
[41, 54]	Rheumatoid arthritis	IgG	10.13–20.00	89.74–91.45	0.99–2.34	0.87–1.00	ASCA IgA levels strongly correlated with C-reactive protein levels and erythrocyte sedimentation rate
		IgA	17.72–40.00	91.03–94.74	1.97–7.60	0.63–0.90	
		IgM	13.33	94.74	2.53	0.91	
[51, 55, 56]	Systemic lupus erythematosus	IgG	4.50–57.50	91.45–99.50	6.72–9.38	0.46–0.73	ASCA IgG levels in SLE patients during remission were relatively lower, indicating a possible correlation with disease activity
		IgA	7.50–12.07	94.74–99.38	1.43–19.31	0.88–0.98	
		IgG/IgA	31.90	96.25	8.51	0.71	
[57]	Type 1 diabetes	IgG	20.98	98.09	10.98	0.81	
		IgA	9.82	98.73	7.71	0.91	
		IgG/IgA	24.55	97.45	9.64	0.77	
[58]	Primary Sjögren's syndrome	IgG/IgA	4.81	100.00	/	0.95	ASCA positivity was associated with pSS specific clinical and serological features
[51]	Vasculitides	IgG	6.50	99.50	/	/	

636 SEN: sensitivity; SPE: specificity; LR+: positive likelihood; LR-: negative likelihood







PRISMA-DTA Checklist

Section/topic	#	PRISMA-DTA Checklist Item	Reported on page #
TITLE / ABSTRACT			
Title	1	Identify the report as a systematic review (+/- meta-analysis) of diagnostic test accuracy (DTA) studies.	1
Abstract	2	Abstract: See PRISMA-DTA for abstracts.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Clinical role of index test	D1	State the scientific and clinical background, including the intended use and clinical role of the index test, and if applicable, the rationale for minimally acceptable test accuracy (or minimum difference in accuracy for comparative design).	4, 5
Objectives	4	Provide an explicit statement of question(s) being addressed in terms of participants, index test(s), and target condition(s).	4, 5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5 CRD42020115245
Eligibility criteria	6	Specify study characteristics (participants, setting, index test(s), reference standard(s), target condition(s), and study design) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6, 7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6, 7
Search	8	Present full search strategies for all electronic databases and other sources searched, including any limits used, such that they could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Definitions for data extraction	11	Provide definitions used in data extraction and classifications of target condition(s), index test(s), reference standard(s) and other characteristics (e.g. study design, clinical setting).	7
Risk of bias and applicability	12	Describe methods used for assessing risk of bias in individual studies and concerns regarding the applicability to the review question.	7
Diagnostic accuracy measures	13	State the principal diagnostic accuracy measure(s) reported (e.g. sensitivity, specificity) and state the unit of assessment (e.g. per-patient, per-lesion).	7, 8
Synthesis of results	14	Describe methods of handling data, combining results of studies and describing variability between studies. This could include, but is not limited to: a) handling of multiple definitions of target condition. b) handling of multiple thresholds of test positivity, c) handling multiple index test readers, d) handling of indeterminate test results, e)	7, 8

PRISMA-DTA Checklist

		grouping and comparing tests, f) handling of different reference standards	

Section/topic	#	PRISMA-DTA Checklist Item	Reported on page #
Meta-analysis	D2	Report the statistical methods used for meta-analyses, if performed.	7, 8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7-9
RESULTS			
Study selection	17	Provide numbers of studies screened, assessed for eligibility, included in the review (and included in meta-analysis, if applicable) with reasons for exclusions at each stage, ideally with a flow diagram.	9, 10
Study characteristics	18	For each included study provide citations and present key characteristics including: a) participant characteristics (presentation, prior testing), b) clinical setting, c) study design, d) target condition definition, e) index test, f) reference standard, g) sample size, h) funding sources	9, 10
Risk of bias and applicability	19	Present evaluation of risk of bias and concerns regarding applicability for each study.	10
Results of individual studies	20	For each analysis in each study (e.g. unique combination of index test, reference standard, and positivity threshold) report 2x2 data (TP, FP, FN, TN) with estimates of diagnostic accuracy and confidence intervals, ideally with a forest or receiver operator characteristic (ROC) plot.	10
Synthesis of results	21	Describe test accuracy, including variability; if meta-analysis was done, include results and confidence intervals.	10-12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression; analysis of index test: failure rates, proportion of inconclusive results, adverse events).	12
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence.	14
Limitations	25	Discuss limitations from included studies (e.g. risk of bias and concerns regarding applicability) and from the review process (e.g. incomplete retrieval of identified research).	16, 17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence. Discuss implications for future research and clinical practice (e.g. the intended use and clinical role of the index test).	17
FUNDING			
Funding	27	For the systematic review, describe the sources of funding and other support and the role of the funders.	18

Adapted From: McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PM, The PRISMA-DTA Group (2018). Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies: The PRISMA-DTA Statement. JAMA. 2018 Jan 23;319(4):388-396. doi: 10.1001/jama.2017.19163.

For more information, visit: **www.prisma-statement.org**.

Reporting checklist for meta-analysis of observational studies.

Based on the MOOSE guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the MOOSEreporting guidelines, and cite them as:

Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA. 2000; 283(15):2008-2012.

	Reporting Item	Page Number
Title		
#1	Identify the study as a meta-analysis of observational research	1
Abstract		
#2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number (From PRISMA checklist)	1-2
Background		
#3a	Problem definition	4

	#3b	Hypothesis statement	4-5
	#3c	Description of study outcomes	n/a The study outcomes were reported in the section of Results and Discussion.
	#3d	Type of exposure or intervention used	4
	#3e	Type of study designs used	4
	#3f	Study population	4, 5
Methods			
Search strategy	#4a	Qualifications of searchers (eg, librarians and investigators)	6, 7
Search strategy	#4b	Search strategy, including time period included in the synthesis and keywords	5, 6
Search strategy	#4c	Effort to include all available studies, including contact with authors	7
Search strategy	#4d	Databases and registries searched	5, 6
Search strategy	#4e	Search software used, name and version, including special features used (eg, explosion)	n/a The search was performed on website of databases
Search strategy	#4f	Use of hand searching (eg, reference lists of obtained articles)	6
Search strategy	#4g	List of citations located and those excluded, including justification	6, 7
Search strategy	#4h	Method of addressing articles published in languages other than English	6
Search strategy	#4i	Method of handling abstracts and unpublished studies	6

Search strategy	#4j	Description of any contact with authors	7
	#5a	Description of relevance or appropriateness of studies gathered for assessing the hypothesis to be tested	7
	#5b	Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	7
	#5c	Documentation of how data were classified and coded (eg, multiple raters, blinding, and interrater reliability)	7
	#5d	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	7
	#5e	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	7
	#5f	Assessment of heterogeneity	8
	#5g	Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	8
	#5h	Provision of appropriate tables and graphics	n/a The methods were described in text instead of tables and graphics.
Results			
	#6a	Graphic summarizing individual study estimates and overall estimate	10-12
	#6b	Table giving descriptive information for each study included	9, 10
	#6c	Results of sensitivity testing (eg, subgroup analysis)	12

	#6d	Indication of statistical uncertainty of findings	12
Discussion			
	#7a	Quantitative assessment of bias (eg. publication bias)	n/a
			Small number of studies in each subgroup prevented publication bias analysis
	#7b	Justification for exclusion (eg, exclusion of non–English-language citations)	16
	#7c	Assessment of quality of included studies	15, 16
Conclusion			
	#8a	Consideration of alternative explanations for observed results	17
	#8b	Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)	17
	#8c	Guidelines for future research	17
	#8d	Disclosure of funding source	18

Supplementary file 3 The full search strategy for EMBASE

#1 yeast?, AND baker? OR (baker? AND yeast?) OR (yeast?, AND brewer?) OR (brewer? AND yeast?) OR (s AND cerevisiae) OR (s. AND cerevisiae) OR (saccaromyces AND cerevisiae) OR (saccharomyces AND capensis) OR (saccharomyces AND cerevisia) OR (saccharomyces AND cerevisiae) OR (saccharomyces AND cerevisial) OR (saccharomyces AND cervisiae) OR (saccharomyces AND diastaticus) OR (saccharomyces AND italicus) OR (saccharomyces AND oviformis) OR (saccharomyces AND uvarum AND var. AND melibiosus) OR asca

#2 behcet? OR ('triple symptom' AND complex) OR (triple AND symptom AND complex) OR (complex, AND triple AND symptom) OR (complices, AND triple AND symptom) OR (symptom AND complex, AND triple) OR (symptom AND complices, AND triple) OR (triple AND symptom AND complices) OR 'adamantiades behcet' OR (old AND silk AND route AND disease) OR behçet

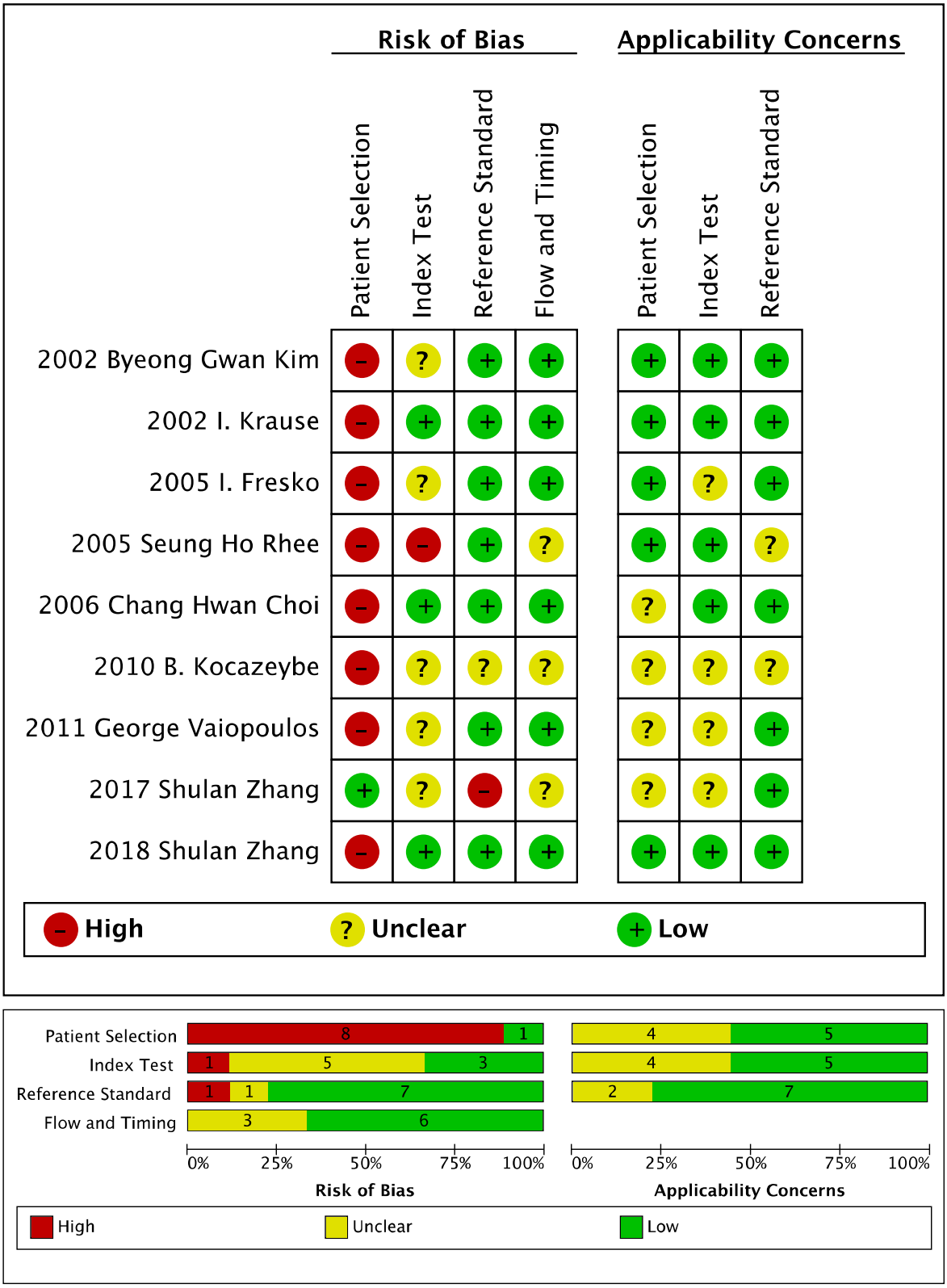
#3 #1 AND #2

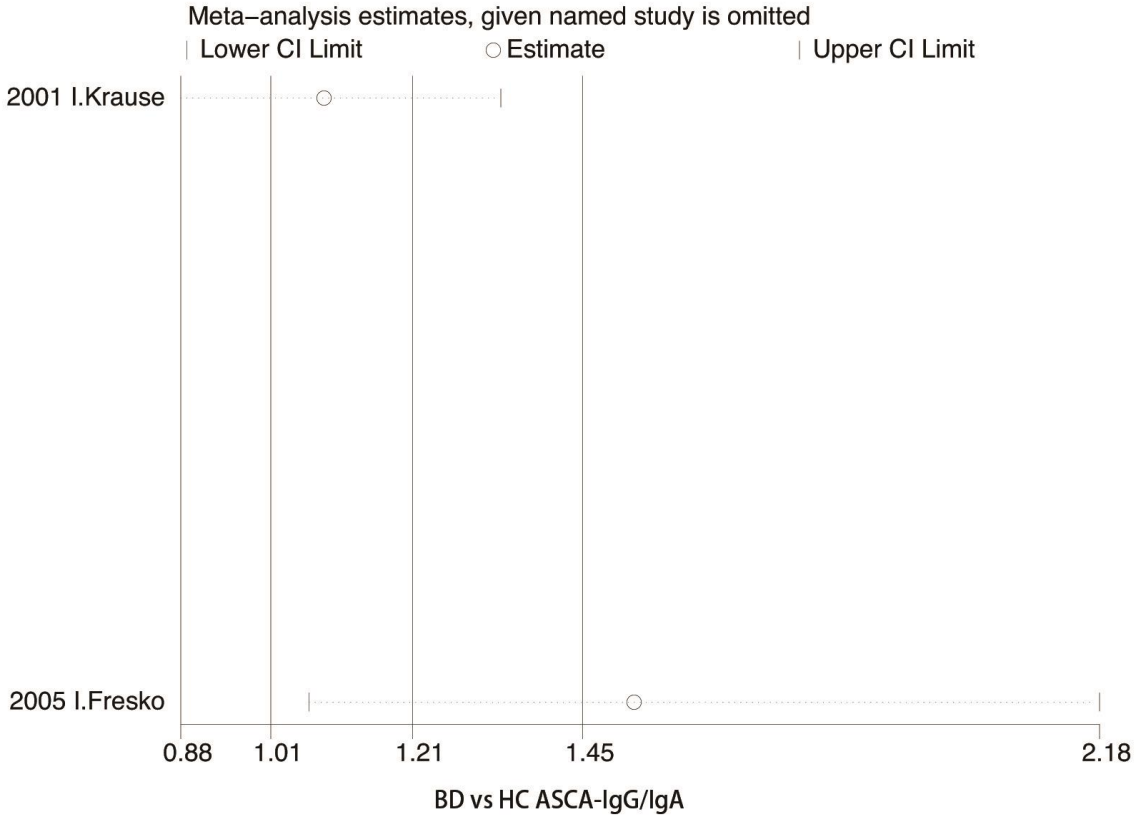
Supplementary file 4 Demographic characteristics of patients and healthy controls included in the meta-analysis

BD: Behçet's disease; GIBD: gastrointestinal Behçet's disease; CD: Crohn's disease; UC: ulcerative colitis; iTB: intestinal tuberculosis; HC: healthy control; /: No such group included; Blank: no such information in the article

	BD	GIBD	CD	UC	iTB	HC
Female, n (%)						
2018 Shulan Zhang	/					/
2017 Shulan Zhang			37 (28.9)	69 (49.3)		
2011 George Vaiopoulos	28 (48.3)	/	/	/	/	
2010 B. Kocazeybek	/	5 (38.5)	39 (61.9)	51 (50.0)	3 (30.0)	99 (60.0)
2006 Chang Hwan Choi	16 (86.6)	47 (44.3)	/	/	/	24 (53.3)
2005 I. Fresko	20 (23.5)	2 (25.0)	10 (41.7)	10 (40.0)	/	9 (42.9)
2005 Seung-Ho Rhee	/	28 (63.6)	/	/	/	
2002 I. Krause	20 (74.1)					
2002 Byeong Gwan Kim	/	20	28	36	7	
Median age at study (max, min)						
2018 Shulan Zhang	/					
2017 Shulan Zhang	/		33 (69,13)	42 (76,13)		/
2011 George Vaiopoulos	38.5 (17,70)	/	/	/	/	
2010 B. Kocazeybek	/	32.11 (23.22,41)	37.56 (24.91,50.21)	40.72 (27.28,54.16)	SD 9.96	35.07 (24.58,45.56)
2006 Chang Hwan Choi	38 (18,65)	37	/	/	/	39 (16,69)
2005 I. Fresko	34.3 (32.0,36.7)	26.9 (24.1,29.6)	38.9 (34.6,43.2)	35.6 (33.0,38.3)	/	33.7 (30.4,37.0)
2005 Seung-Ho Rhee	/	37.6	/	/	/	
2002 I. Krause	41.6 (36.9,46.2)					
2002 Byeong Gwan Kim	/	43.2	40.9	30.6	33.6	

Supplementary file 5 Results of quality assessment of the included studies based on the QUADAS-1 tool




Meta−analysis estimates, given named study is omitted
Lower CI Limit
Estimate
Upper CI Limit
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2005 I.Fresko
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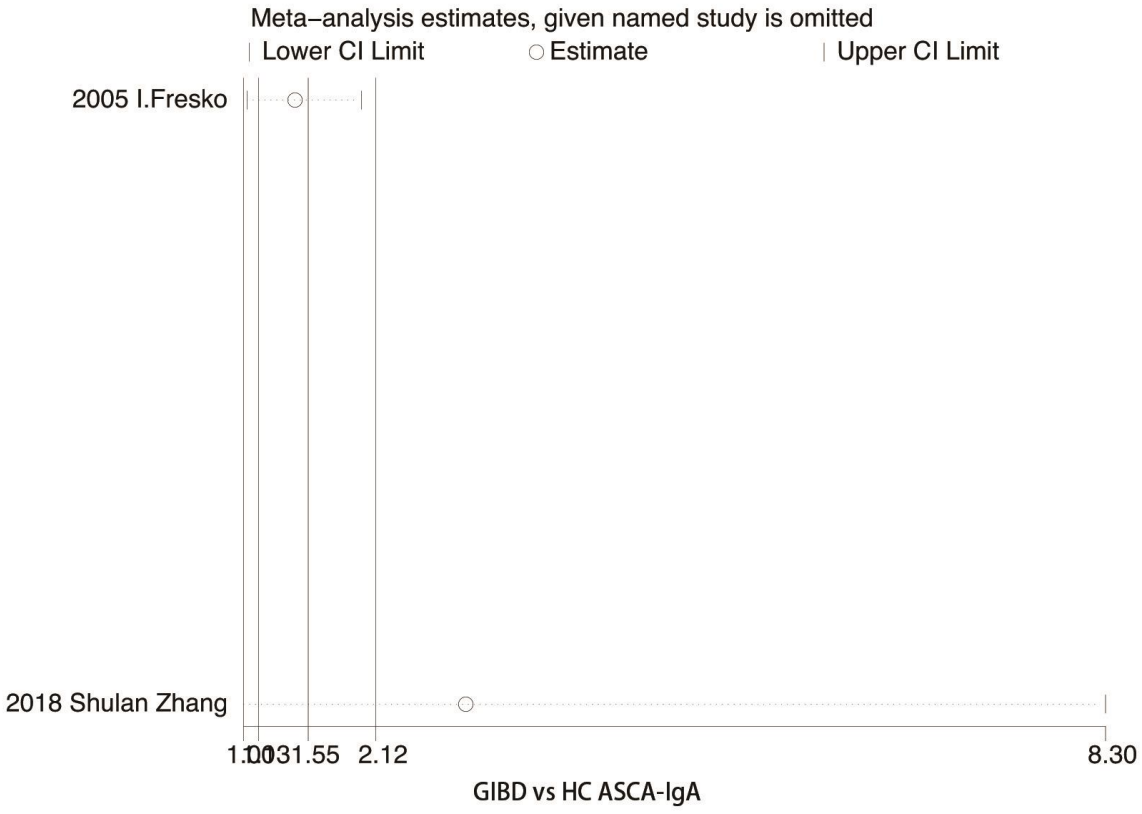
1.01
1.21
1.45
2.18
BD vs HC ASCA-IgG/IgA


Meta−analysis estimates, given named study is omitted
Lower CI Limit
Estimate
Upper CI Limit
2005 I.Fresko
2018 Shulan Zhang
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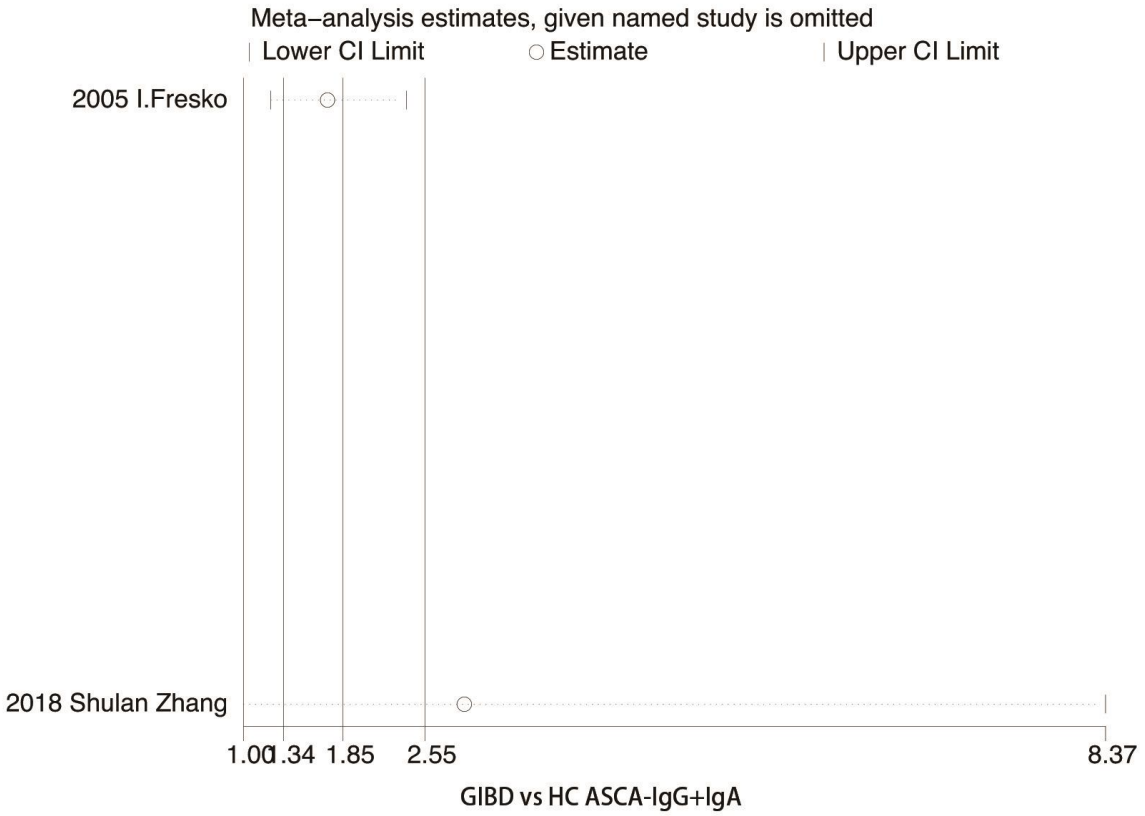
1.03
1.55
2.12
8.30
GIBD vs HC ASCA-IgA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Estimate
Upper CI Limit
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2018 Shulan Zhang
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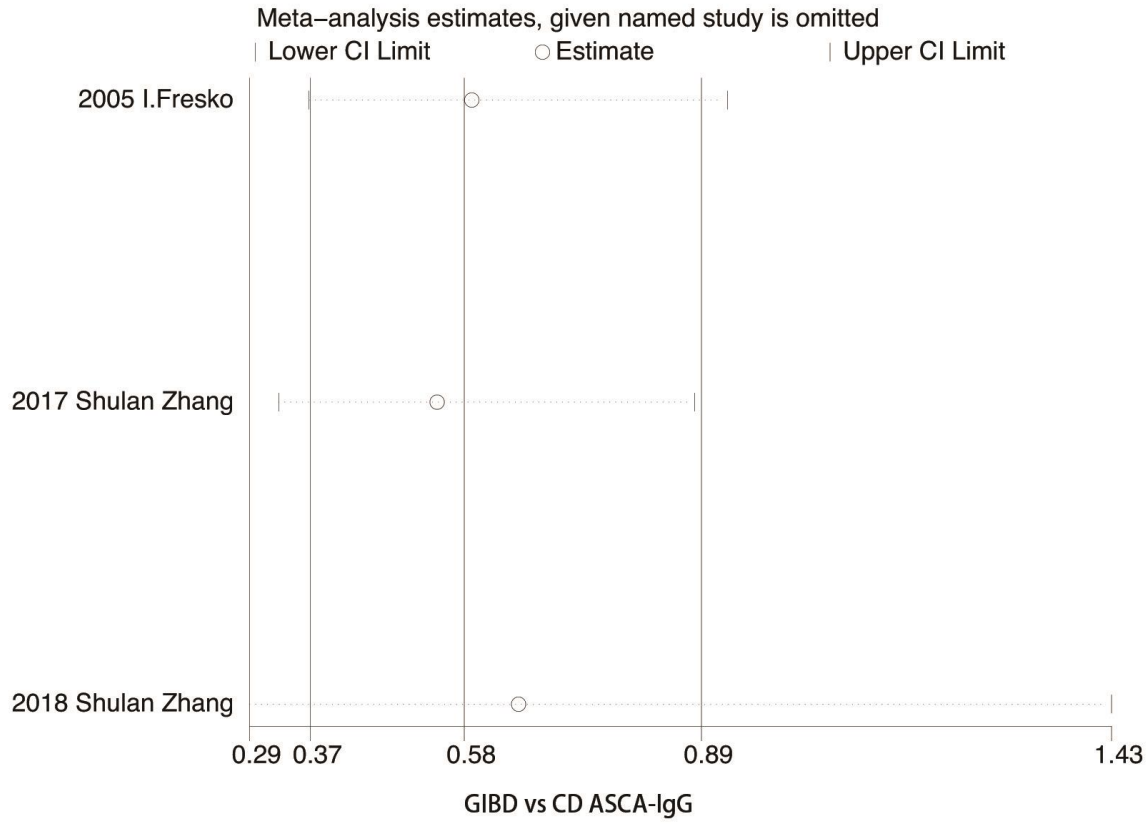
1.34
1.85
2.55
8.37
GIBD vs HC ASCA-IgG+IgA


Meta−analysis estimates, given named study is omitted
Lower CI Limit
Estimate
Upper CI Limit
2005 I.Fresko
2017 Shulan Zhang
2018 Shulan Zhang
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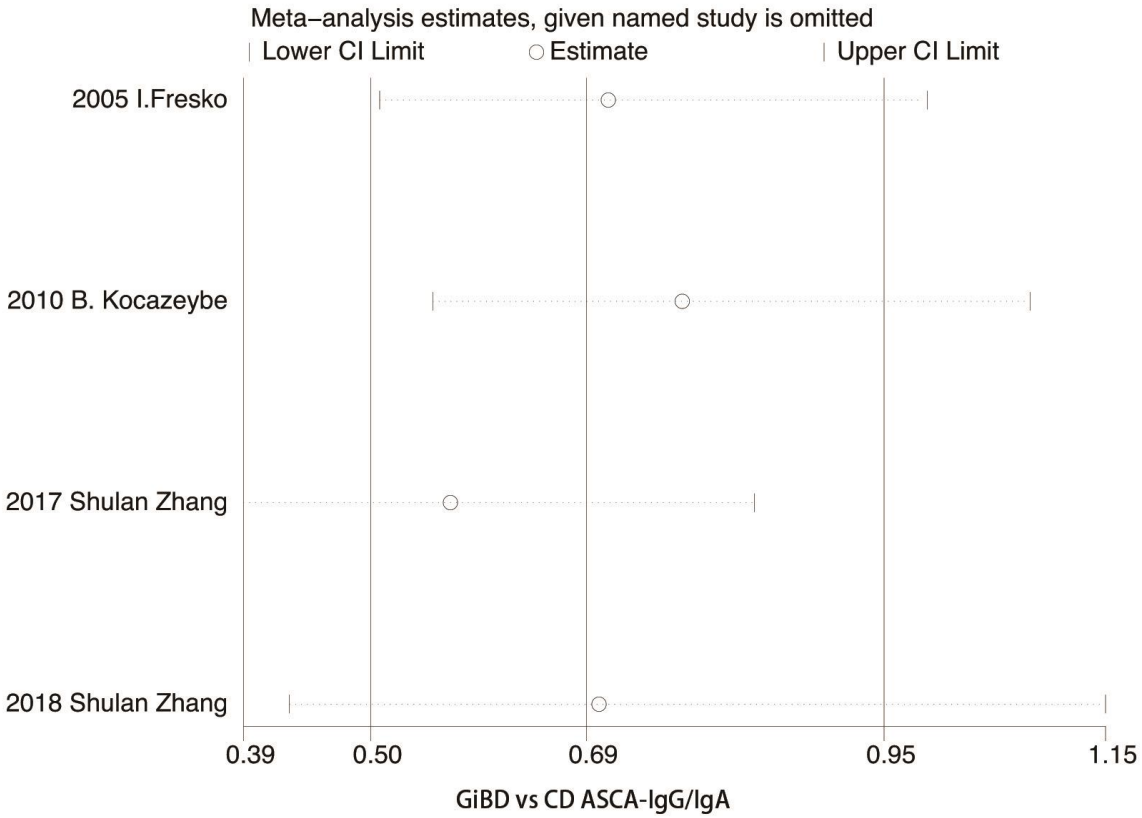
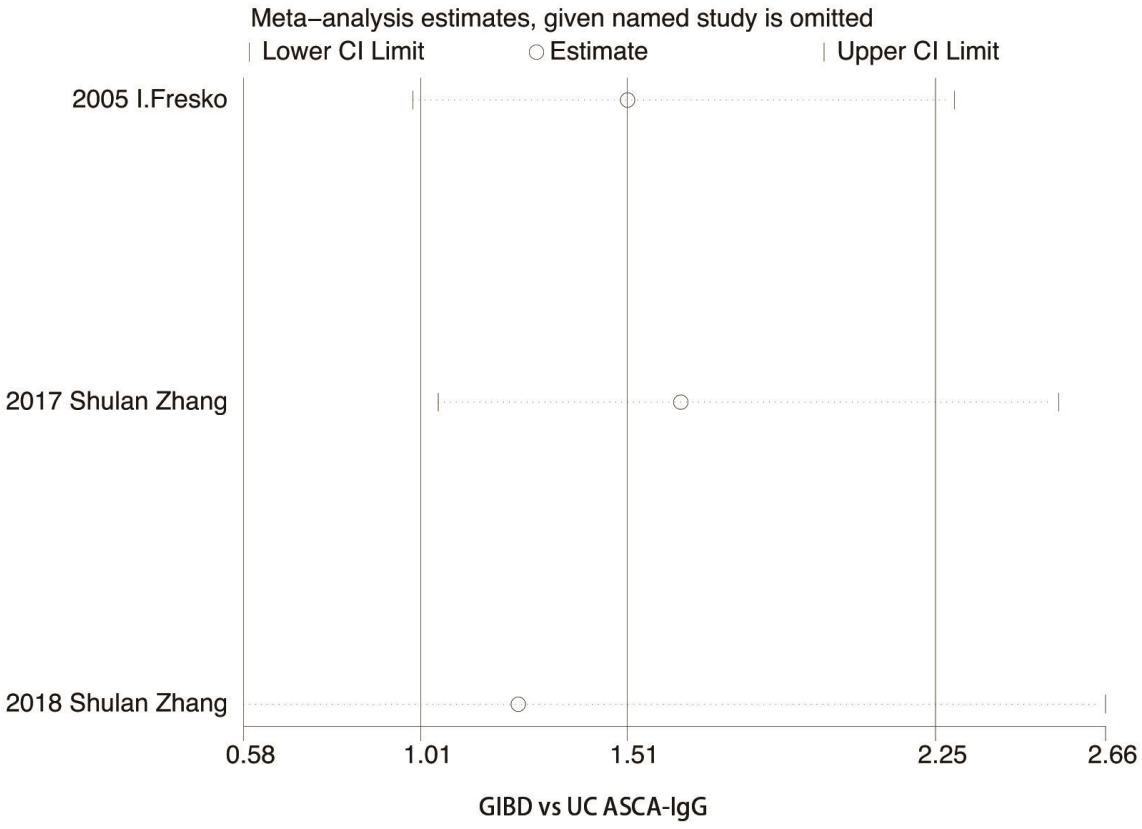
0.37
0.58
0.89
1.43
GIBD vs CD ASCA-IgG


Meta−analysis estimates, given named study is omitted
Lower CI Limit
Estimate
Upper CI Limit
2005 I.Fresko
2010 B. Kocazeybe
2017 Shulan Zhang
2018 Shulan Zhang
0.39
0.50
0.69
0.95
1.15
GiBD vs CD ASCA-IgG/IgA


Meta−analysis estimates, given named study is omitted
Lower CI Limit
Estimate
Upper CI Limit
2005 I.Fresko
2017 Shulan Zhang
2018 Shulan Zhang
0.58
1.01
1.51
2.25
2.66
GIBD vs UC ASCA-IgG