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

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4 1 Meta-analysis of Anti- *Saccharomyces Cerevisiae* Antibodies as diagnostic markers of
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10 3 **Linlin Cheng, MD,^a Liubing Li, MD,^a Chenxi Liu, MD,^a Songxin Yan, MS,^a Yongzhe**
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26 8 **Email address: yongzhelipumch@126.com**
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33 10 **Abstract**
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37 11 **Objective:** To assess the diagnostic value of anti-*saccharomyces cerevisiae* antibodies
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39 12 (ASCA) in Behçet's disease (BD) patients and explore their relationship with other
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41 13 autoimmune diseases(AID).
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46 14 **Methods:** Relevant studies investigating ASCA levels in BD patients were retrieved from
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48 15 PubMed, EMBASE, Web of Science, SOCPUS, and the Cochrane Library. Review
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50 16 Manager 5.3, Meta-DiSc 1.4 and Stata/SE 12.0 were used to perform quality assessment,
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52 17 meta-analysis, and sensitivity analysis. Subgroup analysis were performed disaggregated
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4 18 by isotypes of ASCA. We also summarized the diagnostic performance of ASCA in AID
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7 19 based on a comprehensive database search.
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10 20 **Results:** Nine studies were included in the meta-analysis. All four types of ASCA were
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12
13 21 useful to distinguish between gastrointestinal BD (GIBD) and healthy controls (HC).
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16 22 ASCA-IgG was useful to differentiate between GIBD and HC [odds ratio (OR) 5.74 (95%
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18 23 confidence interval (CI), 2.83–11.65); sensitivity 0.34 (95% CI, 0.27–0.41); specificity
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21 24 0.93 (95% CI, 0.87–0.96)]; based on summary receiver operating characteristic curve, the
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24 25 positive rate in Crohn's disease (CD) was higher than that in BD/GIBD, while patients with
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27 26 negative results were more likely to be diagnosed as CD. However, it was difficult to
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30 27 distinguish GIBD from intestinal tuberculosis (iTb) and ulcerative colitis (UC), and to
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32 28 distinguish BD from UC and HC, with the area under the curve approximately 0.5 for all.
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35 29 **Conclusion:** ASCA may not be a useful serologic marker of BD/GIBD. It does not play a
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38 30 significant role in the differential diagnosis from intestinal diseases. The prevalence of
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41 31 ASCA in AID suggests a common pathogenetic role in AID.
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44 32 Key words: Behçet's disease; Anti-saccharomyces cerevisiae antibodies; autoimmune
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47 33 diseases; meta-analysis; autoantibodies
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4 **36 Strengths and limitations of this study**

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8 37 Behcet's disease is a non-marker autoimmune/inflammatory disease in which
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10 38 autoantibodies play an important role.

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14 39 We perform group analysis according to antibody subtypes of ASCA.

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17 40 The control groups include healthy controls and differential diagnosis disease
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20 41 (inflammatory bowel disease)

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24 42 Comprehensive summary of ASCA antibodies in autoimmune diseases is included.

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27 43 Too much subgrouping may lead to potential heterogeneity
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34 **45 Introduction**

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38 46 Behçet's disease (BD) is a chronic systemic vascular inflammatory disease with a high
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41 47 propensity for recurrence. The etiopathogenesis of BD is yet to be elucidated. The
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44 48 condition is characterized by recurrent oral ulcers, genital ulcers, ophthalmitis, and skin
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47 49 lesions. It can also involve blood vessels, nervous system, digestive tract, joints, and other
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50 50 organs in the body. BD not only impairs the quality of life of patients but can also cause
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53 51 serious consequences and even death. Involvement of eyes, the central nervous system, and
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56 52 large blood vessels may lead to serious complications. The onset of BD typically occurs in

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4 53 the third or fourth decade of life; the condition is rarely seen in children or in individuals
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7 54 older than 50 years [1]. BD has a typical geographical distribution consistent with the
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10 55 historical "Silk Road"; therefore, the condition is also referred to as the "Silk Road Disease".
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12 56 Its prevalence has considerably increased in the Mediterranean region, the Middle East and
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15 57 the Far East; Turkey has the highest prevalence rate of BD (420/100,000) [2]. This peculiar
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18 58 geographical distribution suggests a role of genetic factors in the pathogenesis of BD. An
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21 59 increasing number of studies have shown a strong correlation between human leukocyte
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24 60 antigen HLA-B51 and BD [3, 4]. However, a significant number of patients with BD test
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27 61 negative for HLA-B51; in addition, the HLA-B51 positivity rate is lower in patients with
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30 62 bone disease, especially in non-endemic areas; this indicates that other factors may play a
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33 63 role in the pathogenesis of BD [5]. Due to the lack of specific laboratory tests, the diagnosis
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36 64 of BD is typically challenging and is mainly based on clinical manifestations. The 1990
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39 65 International Study Group (ISG) criteria [6] were earlier used for the clinical diagnosis of
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42 66 BD; however, owing to its low sensitivity, the criteria were updated in 2013 and are now
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45 67 referred to as the International Criteria for Behçet's Disease (ICBD) [7]. However, these
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48 68 criteria do not include any laboratory tests, and are based on the clinician's judgment, which
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51 69 is relatively subjective and may lead to misdiagnosis. Due to the non-specific clinical
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54 70 characteristics, those with prominent involvement of a particular organ system are easily
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57 71 misdiagnosed. Patients with gastrointestinal involvement as the main manifestation are

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4 72 easily misdiagnosed as Crohn's disease (CD), ulcerative colitis (UC), or intestinal
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7 73 tuberculosis (iTB); joint symptoms are misdiagnosed as rheumatoid arthritis or ankylosing
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10 74 spondylitis; skin mucosal damage is misdiagnosed as polymorphic erythema, nodular
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12 75 erythema, syphilis, or systemic lupus erythematosus. Likewise, nervous system damage is
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15 76 misdiagnosed as infectious or allergic meningitis, cerebrospinal tumor, multiple sclerosis,
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18 77 or psychosis. In addition, diagnosis is frequently delayed until the development of clinical
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20 78 manifestations to qualify the criteria. Studies have shown that BD is often diagnosed after
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23 79 a gap of several years from the first appearance of symptoms. For example, patients with
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26 80 oral ulcers were diagnosed with BD after a mean delay of 3.77 ± 4.43 years after the
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29 81 appearance of oral ulcers [8]. The delay in diagnosis may have deleterious effects on the
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31 82 patients.

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35 83 Several recent studies have shown that anti-Saccharomyces Cerevisiae antibody (ASCA),
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38 84 directed against the phosphopeptidomannan part of the cell wall of the yeast, is an
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41 85 important serological marker of BD, especially in patients with gastrointestinal
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44 86 manifestations. However, patients with inflammatory bowel disease such as Crohn's
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47 87 disease (CD) also have a high prevalence rate of ASCA [9, 10, 11, 12, 13, 14]. Thus,
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50 88 comprehensive quantitative analysis to assess the relevance of ASCA as a routine
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53 89 laboratory test for diagnosis for BD is a key imperative. We performed a meta-analysis of
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56 90 relevant studies to assess the diagnostic relevance of ASCA as a marker of BD.

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8 92 **Methods**9
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14 94 A comprehensive literature search was performed in 5 biomedical databases, i.e., PubMed,
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17 95 EMBASE, Web of Science, SOCPUS, and the Cochrane Library. The key words used were
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20 96 Behçet's disease and anti-Saccharomyces Cerevisiae antibody. Search range was "all fields"
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23 97 or "all text". No restrictions were imposed with respect to time of publication, region, or
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26 98 ethnicity of the study population. All documents were updated to January 2019.

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32 100 Eligibility and exclusion criteria

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36 101 The inclusion criteria were: (1) studies that evaluated the diagnostic accuracy of ASCA in
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39 102 BD; (2) availability of adequate data pertaining to the prevalence rate or serum levels of
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42 103 ASCA in patients with BD; (3) studies with healthy population and/or disease controls; (5)
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45 104 meeting abstracts or letters to the editor were also included.

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48 105 The exclusion criteria were: (1) studies with incomplete data; (2) review articles; (3) non-
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51 106 English articles; (4) in case of studies with overlapping study population, studies with
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54 107 smaller sample size were excluded. Two investigators independently performed the

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4 108 literature search, screened the titles and abstracts, followed by full-text review of eligible
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7 109 studies.
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14 111 Data extraction and quality assessment

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17 112 Two independent investigators reviewed the full-text articles, extracted the data, and
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20 113 assessed the study quality using the Quality Assessment of Diagnostic Accuracy Studies
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23 114 (QUADAS-2) through Revman 5.3; the included items were evaluated as yes, no, or
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26 115 uncertain. Inter-researcher disagreements were resolved by consensus, or by a third
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29 116 investigator. Data pertaining to the following variables were extracted: publication year,
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32 117 article type, first author's name, country, isotypes of ASCA detected, age and sex, research
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35 118 design, sample size, experimental method, trade names of experimental materials, cut-off
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38 119 values, diagnostic criteria, and serum titers and/or prevalence rate of ASCA in BD,
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41 120 gastrointestinal BD (GIBD), healthy controls (HC), patients with Crohn's disease (CD),
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44 121 ulcerative colitis (UC), and intestinal tuberculosis (iTb). The data were either obtained
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47 122 directly from the article, calculated, or requested from the author via e-mail.

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51 124 Statistical analysis

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4 125 We used Stata/SE 12.0, Review Manager 5.3, and Meta-DiSc 1.4 for data analysis.
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7 126 Subgroup analysis was performed disaggregated by the isotypes of ASCA and different
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10 127 disease controls. Heterogeneity among the included studies was evaluated using the
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12 128 Cochran's Q-statistic as well as the I²-statistic. P value > 0.10 for the Q-statistic, or I² < 50%
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15 129 was considered indicative of lack of significant heterogeneity and the fixed effects model
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18 130 (FEM) was used for the analysis; in case of significant heterogeneity, the random effects
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21 131 models (REM) was used for analysis. We analyzed the pooled diagnostic odds ratio (DOR),
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24 132 sensitivity, specificity, positive likelihood ratio (LR+), and negative likelihood ratio (LR-),
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27 133 and their corresponding 95% confidence intervals (CI). The area under the summary
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30 134 receiver operating characteristic (SROC) curve was used to assess the overall diagnostic
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33 135 performance of ASCA. Sensitivity analysis was performed using Stata/SE 12.0 to evaluate
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36 136 stability of the results after sequential exclusion of one study at a time.
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39 137 Studies for which adequate data was not available or for whom adequate control group
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42 138 information was not available (especially meeting abstracts and letters to the editors) were
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45 139 also reviewed but not included in the meta-analysis.
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50 141 Patient and public involvement

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4 142 The present study is a meta-analysis and systematic review based on published data, patient
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7 143 and public are not involved in the study design, conduct, data analysis and result
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10 144 dissemination

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146 **Results**

147 Literature search and characteristics of studies

148 A total of 599 documents were retrieved on database and manual search. Forty-eight
149 duplicate publications were excluded using the document management software. A total of
150 126 records were retained after screening of titles and/or abstracts; the excluded records
151 included review articles, animal model studies, therapeutic or drug research, genetic
152 research, book chapters, duplicate publications not recognized by software, and other
153 irrelevant records. After full-text review for eligibility, 21 records were selected. Finally,
154 we identified 14 [9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23] studies; of these,
155 adequate data was available only for 9 studies and were included in the meta-analysis
156 (Figure 1). Two studies were included after obtaining the relevant data by contacting the
157 respective authors [12, 13]. In addition, we also verified 2 studies [18, 19] with overlapping
158 study population; of these, only one study was included in the meta-analysis. Three studies
159 [9, 11, 21] were presented as meeting abstracts without adequate data to allow the

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4 160 construction of a 2×2 table. One [10] article was a letter to the editor and only reported the
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7 161 prevalence rate of ASCA antibody in patients with BD, without information about the
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10 162 control group. One study [20] had employed a unique calculation method and could not be
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12 163 included in the meta-analysis. Among the included studies, there were 326 cases of BD,
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15 164 294 cases of GIBD, 520 cases of CD, 598 cases of UC, 112 cases of iTB, and 428 HCs
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18 165 (Table 1 and Table S1).

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22 23 24 25 167 Quality assessment

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28 168 There were 8 prognostic studies and 1 retrospective study [12]. The results of quality
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31 169 assessment including the risk of bias and applicability concerns pertaining to each domain
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34 170 are shown in Fig S1. The results indicated that the included studies were of high quality in
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37 171 general; however, 8 studies showed a high risk of bias with respect to patient selection
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39 172 (avoidance of inappropriate exclusion). Overall, none of the 9 included studies showed any
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42 173 major methodological bias or flaws, which indicates robustness of our meta-analysis.

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4 176 We conducted a meta-analysis of 9 studies that involved detection of ASCA in patients
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7 177 with BD and controls. Subsequently, we performed subgroup analysis based on different
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10 178 controls and isotypes of ASCA.

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15 180 1. Meta-analysis of prevalence rate of ASCA in various groups of patients using Meta-
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18 181 DiSc 1.4

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21 182 We analyzed the diagnostic accuracy of ASCA in BD (without gastrointestinal
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24 183 involvement)/GIBD/HC/UC/CD/iTB using the Revman 5.3 diagnostic test accuracy
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27 184 review model and Meta-DiSc 1.4. The results obtained with Meta-DiSc 1.4 are summarized
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30 185 in Table 2; some of the results are presented separately (Figure S2A-F). In addition, AUC
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33 186 are also showed separately by Revman 5.3 (Figure 2A-G).

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36 187 When we compared BD and HC, we found limited overall diagnostic value, with the
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39 188 exception of slightly higher ORs for IgA and IgG/IgA [IgA, 2.26 (95% CI 0.56–9.12);
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42 189 IgG/IgA, 2.85 (95% CI 0.57–14.29)] and LR+ [IgA, 2.03 (95% CI 0.58–7.17); IgG/IgA,
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45 190 2.08 (95% CI 0.67–6.41)]. However, both showed a high specificity (> 90%) and low
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48 191 sensitivity (< 20%) (Table 2).

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51 192 Overall, ASCA showed the highest diagnostic value in the GIBD vs. HC sub-group
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54 193 analysis, with high OR and LR+. ASCA-IgG had the highest OR [5.46 (95% CI 2.58–

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4 194 11.55)] and highest sensitivity [0.34 (95% CI 0.27–0.41)] in all groups (Figure S2A, B). In
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7 195 addition, the diagnostic value of ASCA was apparently higher than that in BD vs. HC sub-
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10 196 group analysis (Table 2).

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13 197 When we compared GIBD and CD, both the OR and the LR+ were less than 1 (Table 2,
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16 198 Figure S2C-F), which suggests that ASCA negative results are more likely to be diagnosed
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19 199 as BD at the time of differential diagnosis.

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22 200 The diagnostic value of ASCA was also limited when comparing GIBD and UC. Both the
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25 201 OR and LR+ were approximately 2. Both IgG and IgA positivity increased the value of
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28 202 LR+ [2.02 (95% CI 1.04–3.95)] (Table 2).

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31 203 When comparing GIBD and iTB, although IgG/IgA increased the sensitivity [0.32 (95%
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34 204 CI (0.24–0.41)], the OR and LR+ were both approximately 1, which suggests that either
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37 205 IgG or IgA positivity may also increase the detection rate of ASCA in iTB, not just GIBD
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40 206 (Table 2).

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43 207 On ROC curve analysis for distinguishing between BD and HC, the AUC for most antibody
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46 208 subtypes was slightly higher than 0.5 (or even lower than 0.5); of these, ASCA-IgG/IgA
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49 209 had the highest diagnostic efficacy (Figure 2A). In the BD vs. CD analysis, the AUC of
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52 210 ASCA was less than 0.5 (Figure 2B). The AUCs for BD vs. UC (Figure 2C), GIBD vs. CD
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55 211 (Figure 2E), and GIBD vs. iTB analyses (Figure 2G) were approximately 0.5. The AUC

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4 212 for GIBD vs. UC analysis was at a general level (Figure 2F). The AUC was highest for the
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7 213 GIBD vs. HC analysis, especially for ASCA-IgG/IgA/IgM, although only one study was
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10 214 included (Figure 2D)

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16 216 2. Meta-analysis of serum levels of ASCA in groups by Stata/SE 12.0

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20 217 In order to increase the robustness of the meta-analysis, we also extracted the data
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23 218 pertaining to serum levels of ASCA from five studies and performed meta-analysis using
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26 219 the Continuous data module of Stata/SE 12.0. The FEM was used for the analysis and
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29 220 weighted mean difference (WMD) was used as the effect measure. We used FEM for five
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31 221 subgroups with $I^2 < 50\%$ and $p > 0.1$ (Figure 3A). WMD was used because the same unit was
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34 222 used in these studies and there were only minor differences (less than three times) with
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37 223 respect to the serum levels of ASCA (Figure 3A). We found that ASCA-IgA in GIBD was
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39 224 significantly greater than that in HC and UC, in contrast to ASCA-IgG. On the contrary,
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42 225 levels of ASCA-IgG in CD were apparently higher than that in BD. For subgroups with
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45 226 $I^2 > 50\%$ and $p < 0.1$, we chose the REM for analysis using the WMD. We found no
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48 227 significant difference between GIBD and CD with respect to the serum levels of ASCA-
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50 228 IgA (Figure 3B).

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4 230 Heterogeneity and sensitivity analysis
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7 231 We performed sensitivity analysis to assess the stability of the results. The results showed
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10 232 that the studies by Krause et al (2002), Zhang et al (2018), Kocazeybe et al (2010), and
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13 233 Fresko et al (2005) were the key contributors to the heterogeneity (Figure S3). Thus, the
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16 234 results of related subgroup analysis are considered to be less stable. We further applied
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19 235 REM to analyze these seven subgroups; the forest plot is shown in Figure S4.
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22 236 Summary of the relationship of ASCA with autoimmune disease
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25 237 We searched the database for the association between ASCA and AID. The sensitivity,
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28 238 specificity, LR+, and LR- are summarized in Table 3.
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35 240 **Discussion**

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39 241 The diagnosis of BD is typically challenging prior to the appearance of clinical symptoms
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42 242 to qualify the diagnostic criteria. Currently, there are no specific laboratory biomarkers of
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45 243 BD; however, some specific autoantibodies in the context of BD have been reported.
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48 244 Therefore, identification of non-invasive specific diagnostic and prognostic biomarkers of
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51 245 BD is of much clinical relevance and a key focus area of research.
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4 246 Several recent studies have assessed the relation between ASCA and BD. *Saccharomyces*
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7 247 *cerevisiae*, also known as the baker's or brewer's yeast, has long been utilized to ferment
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10 248 the sugars in rice, wheat, barley, and corn to produce alcoholic beverages; it is also used in
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12 249 the baking industry to raise dough. As a consequence, we are now commonly exposed to
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15 250 yeast [24]. IgG and IgA antibodies against the phosphopeptidomannan of the *S. cerevisiae*
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18 251 cell wall have been discovered as autoantibodies in the sera of patients with BD, especially
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21 252 those with gastrointestinal involvement. This suggests a role of environmental stimuli in
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23 253 the pathogenesis of BD. However, patients with gastrointestinal involvement, especially
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26 254 those with Crohn's disease, also have a high prevalence rate of ASCA , which is a
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29 255 controversial issue [25]. Moreover, a growing number of studies have assessed ASCA in
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32 256 several systemic and organ-specific AID, which led to postulation of molecular mimicry
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35 257 as a possible link between ASCA and AID, such as scleroderma, systemic lupus
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38 258 erythematosus, primary Sjögren's syndrome, rheumatoid arthritis, autoimmune liver
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41 259 disease, and spondyloarthritis (Table 3). Their correlation with elevated IgA suggests that
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44 260 ASCA may be an indirect sign of enhanced mucosal immunity [26]. Therefore, detection
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47 261 of ASCA may be a useful serologic marker of BD and other AID, especially those with
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50 262 gastrointestinal involvement.

51 263 To the best of our knowledge, this is the second meta-analysis of evidence pertaining to
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54 264 autoantibodies in patients with BD after anticardiolipin antibodies [27]. ASCA have been

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4 265 widely researched in BD, Crohn's disease and other autoimmune disease; in order to
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7 266 investigate the diagnostic value and possible pathogenetic role of ASCA in BD, we
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10 267 included 9 studies to assess the diagnostic accuracy of ASCA in BD. Among these, some
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12 268 studies included BD patients with various clinical manifestations. Some studies included
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15 269 BD patients with systemic involvement symptoms including or excluding gastrointestinal
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18 270 involvement, while others included only BD patients with gastrointestinal involvement.
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21 271 Therefore, in order to reduce the impact of differences with respect to frequency
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23 272 distribution of gastrointestinal symptoms in each study, we disaggregated patients with BD
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26 273 into those with gastrointestinal involvement only and those without gastrointestinal
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29 274 manifestations. The isotype antibodies of ASCA tested and the results presented by the
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32 275 studies are also different. Studies had investigated IgG, IgA, either IgG or IgA, both IgG
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35 276 and IgA, IgG, IgA and IgM, and even IgG subtypes IgG1–IgG4. Several studies have
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38 277 shown a higher prevalence of ASCA among patients with BD/GIBD/CD; however, the
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41 278 results of meta-analysis showed that ASCA is not a useful biomarker for the differential
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44 279 diagnosis between GIBD and CD; however, ASCA negative results may be more likely to
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47 280 be diagnosed as GIBD/BD when compared with CD. In addition, ASCA showed the
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50 281 highest diagnostic value in the GIBD vs. HC sub-group analysis; all four types of ASCA
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53 282 were found to help distinguish between GIBD and HC. However, we found limited
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56 283 diagnostic value of ASCA in BD vs. HC and GIBD vs. UC sub-group analyses. Moreover,

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4 284 it failed to distinguish between GIBD and iTB. Overall, use of ASCA-IgG in combination
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7 285 with IgA helped improve the specificity of the diagnosis of BD in all groups. Moreover,
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10 286 we used three different software (Meta-DiSc 1.4, Revman5.3, and Stata/SE 12.0) to
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12 287 perform multiple analyses, which helped increase the credibility of our results. For example,
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15 288 we used both data pertaining to prevalence rate and serum levels of ASCA. Combined with
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18 289 the results of QUADAS-2, we found that the heterogeneity in this meta-analysis was
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21 290 largely attributable to the following reasons (see Table 1, Figure S1). The first reason was
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24 291 the different diagnostic criteria used in the included studies. Different criteria may have
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27 292 different thresholds for diagnosis or place more weight on some symptoms than others.
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30 293 Specifically, the 1990 ISG criteria requires the presence of oral ulceration plus any two of
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33 294 the following: genital ulceration, typical eye lesions, typical skin lesions, or positive
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36 295 pathergy test for diagnosis of BD [6]. In contrast, the 1987 Japan criteria require all four
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39 296 characteristics for diagnosis of BD, i.e., oral ulceration, typical eye lesions, typical skin
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42 297 lesions, and genital ulceration [28]. The ISG criteria and the Japanese criteria often fail to
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45 298 classify some patients with BD; in addition, the Japanese criteria may also cause
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48 299 misclassified diagnosis. This may have caused the different diagnostic sensitivity and
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51 300 specificity for BD [29]. The second contributor to heterogeneity was differences with
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54 301 respect to the characteristics of the study population. The clinical features and laboratory
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57 302 findings tend to exhibit wide variability in different populations and clinical settings [30,
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4 303 31]. However, we could not perform subgroup analysis disaggregated by the type of
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7 304 population, owing to the small sample size of various population groups after classification
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10 305 according to isotypes of ASCA. Third, different investigation methods and cut-off values
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12 306 also contributed to the heterogeneity. The two main methods used in the included studies
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15 307 were ELISA and indirect immunofluorescence assay. Notably, although the kits were
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18 308 manufactured by the same company, different cut-off values were used. Different values
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20 309 for ELISA were used for Inova Diagnostic [13, 16, 18, 19, 22] and Euroimmune Leubeck
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23 310 kits [9, 12]. Some studies performed testing through self-coated plates by purchasing
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26 311 ELISA plates and antibodies; thus, the effect of human error and inadequate repeatability
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29 312 cannot be ignored [17, 20].

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32 313 In order to fully evaluate the value of ASCA for differential diagnosis of BD, we included
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35 314 patients with CD, UC, and iTB as the comparison objects in our meta-analysis. However,
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38 315 there are still some limitations of this meta-analysis. (1) Gray literature database, paper
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41 316 database, and other language databases were not used for the literature search. (2) Our
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44 317 primary goal was to assess the diagnostic efficacy of ASCA in BD, and therefore we did
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47 318 not include all studies pertaining to ASCA in inflammatory bowel disease and iTB. (3)
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50 319 Restricted by the number of included studies and the isotypes of ASCA, we could not
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53 320 perform subgroup analysis disaggregated by different populations and diagnostic criteria.
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56 321 (4) Some studies with incomplete data were excluded after lack of response from the author.

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4 322 Our meta-analysis results, together with the review of ASCA in AID strongly suggest that
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7 323 ASCA (especially its certain isotypes) may be helpful biomarkers for GIBD, especially
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10 324 with respect to their possible predictive/pathogenic/diagnostic role in clinical settings [32].
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12 325 Furthermore, ASCA may be detectable years before the diagnosis of some AID as they
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15 326 were retrospectively found in the preserved blood samples of soldiers who were affected
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18 327 by Crohn's disease years later [33]. However, due to its presence in several other AID,
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21 328 ASCA may have a limited value for clinical diagnosis.
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3 330 **Compliance with Ethical Standards:**
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9 334 B&R-01)

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13 335 **Ethical approval:** This article does not contain any studies with human participants
14 336 performed by any of the authors.

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17 337 **Author Statement:** Study concept and design: Linlin Cheng, Yongzhe Li. Acquisition of
18 338 data: Linlin Cheng, Liubing Li, Chenxi Liu, Songxin Yan. Statistical analysis and
19 339 interpretation of data: Linlin Cheng, Liubing Li, Chenxi Liu. Drafting of the manuscript:
20 340 Linlin Cheng. Revision of manuscript: Yongzhe Li, Linlin Cheng, Liubing Li.
21 341 Supervision of work: Yongzhe Li. All authors read and approved the final manuscript.

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26 342 **competing interests:** None.
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31 344 **References**
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4 470 Figure 1 Schematic illustration of the literature search and study-selection criteria for the
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7 471 meta-analysis
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14 473 Figure 2 AUC of diagnostic accuracy of ASCA when comparing BD and HC (A), BD
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16 474 and CD (B), BD and UC (C), GIBD and HC (D), GIBD and CD (E), GIBD and UC (F),
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19 475 and GIBD and iTB (G)
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26 477 Figure 3 A. Forest plot of serum levels of ASCA in GIBD/CD/UC/HC using FEM; B.
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29 478 Forest plot of serum levels of ASCA in GIBD/BD/HC/CD using REM
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36 480 Fig S1 Results of quality assessment of the included studies based on the QUADAS-1
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39 481 tool
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46 483 Figure S2 Forest plot of A. pooled diagnostic OR of ASCA-IgG when comparing GIBD
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48 484 and HC; B. pooled sensitivity of ASCA-IgG when comparing GIBD and HC; C. pooled
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51 485 diagnostic OR of ASCA-IgG when comparing GIBD and CD; D. pooled diagnostic OR
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54 486 of ASCA-IgA when comparing GIBD and CD; E. pooled diagnostic OR of ASCA-

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4 487 IgG+IgA when comparing GIBD and CD; F. pooled diagnostic OR of ASCA-IgG/IgA
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7 488 when comparing GIBD and CD
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14 490 Figure S3 Results of sensitivity analysis showing sources of heterogeneity

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17 491 Figure S4 Forest plot of diagnostic OR of ASCA in 6 subgroups using the random effect
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20 492 model after sensitivity analysis.
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30 495 Table 1 Characteristics of studies included in the meta-analysis of Anti-Saccharomyces
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32 496 Cerevisiae antibodies in Behçet’s disease, its main differential diagnoses, and healthy
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36 497 controls
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39 498 /:no sample; IIF: indirect immunofluorescence assay; NR: not reported; SD: standard deviation; *: all without
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41 499 gastrointestinal manifestations; #: lack of corresponding data; 1990 ISG criteria: the 1990 criteria of Behçet’s Disease
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43 500 International Study Group; 1987 Japan criteria: the 1987 criteria by the Behçet’s Disease Research Committee of
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45 501 Japan; BD: Behçet’s disease; GIBD: gastrointestinal Behçet’s disease; CD: Crohn's disease; UC: ulcerative colitis; iTB:
46 502 intestinal tuberculosis; HC: healthy control

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48 **Sample size**

Year and author	Count ries	Type	Type of article	Design	Sample size						Methods	Brands of experimental materials	Cut-off	Diagnostic criteria
					BD	GIB D	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

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3	2017	Shulan Zhang [12]	China	IgG; IgA; IgG/IgA; IgG+IgA	Original article	Retrospective study	/	34	128	140	31	/	ELISA	Euroimmun, Luebeck	20		NR	
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5																		
6	2011	George Vaiopoulos [23]	Greece	IgG; IgA	Original article	case-control	58	4*	/	/	/	56	ELISA	Inova Diagnostic	NR		1990 ISG criteria	
7																		
8	2010	B. Kocazeybek. [11]	Turkey	IgG/IgA	conference Abstract	case-control	/	13	63	102	10	165	IIF	Euroimmun, Luebeck	NR		NR	
9																		
10	2006	Chang Hwan Choi [15]	Korea	IgG	Original article	case-control	30*	106	/	/	/	45	IIF	Euroimmun, Luebeck	1:1000		1987 Japan criteria	
11																		
12	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	
13																		
14	2005	Seung-Ho Rhee [22]	Korea	IgG	Original article	case-control	/	16	/	/	/	4	ELISA	Inova Diagnostic	25		1987 Japan criteria	
15																		
16	2002	I. Krause [18]	Israel	IgG; IgA; IgG/IgA; IgG+IgA	Original article	case-control	27*	/	/	/	/	10	ELISA	Inova Diagnostic	25		1990 ISG criteria	
17																		
18	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	
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Table 2. Summary of subgroup meta-analysis of ASCA by Meta-DiSc 1.4

505 / indicates that the number of included studies was less than three and the ROC curve could not be drawn by Meta-DiSc

506 1.4. BD: Behçet's disease; GIBD: gastrointestinal Behçet's disease; CD: Crohn's disease; UC: ulcerative colitis; iTB:

507 intestinal tuberculosis; HC: healthy control

Subgroup	Antibody	Number of studies	Diagnostic OR (95% CI)	<i>P</i> (%)	Pooled sensitivity (95% CI)	<i>P</i> (%)	Pooled specificity (95% CI)	<i>P</i> (%)	LR+(95% CI)	<i>P</i> (%)	LR-(95% CI)	<i>P</i> (%)
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(0.99-1.17)	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

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

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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	
		IgG	16.42–27.53	100.00	/	0.84	
[26, 40, 41]	Autoimmune hepatitis	IgA	11.94	94.74	2.27	0.93	
		IgG/IgA	18.52	84.00	1.16	0.97	
		IgG	10.57–18.95	97.50–100.00	7.58	0.83–0.89	
[26, 41, 42]	Primary biliary cirrhosis	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	
		IgG	28.00	100.00	/	0.72	
[26, 41]	Primary sclerosing cholangitis	IgA	32.00	94.74	6.08	0.72	
		IgG/IgA	30.51	84.00	1.91	0.83	
		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.
[14, 25, 26, 34, 31, 43]	Crohn's disease	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 patients with Crohn's disease

3	[43]	Cryoglobulinemia	IgG	7.10	99.50	/	/	
5	[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
6			IgA	8.40–16.67	94.17–96.88	2.69–2.86	0.88–0.95	
8			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
9	[35, 46]	Rheumatoid arthritis	IgA	17.72–40.00	91.03–94.74	1.97–7.60	0.63–0.90	
10			IgM	13.33	94.74	2.53	0.91	
13			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
14	[43, 47, 48]	Systemic lupus erythematosus	IgA	7.50–12.07	94.74–99.38	1.43–19.31	0.88–0.98	
16			IgG/IgA	31.90	96.25	8.51	0.71	
18			IgG	20.98	98.09	10.98	0.81	
19	[49]	Type 1 diabetes	IgA	9.82	98.73	7.71	0.91	
20			IgG/IgA	24.55	97.45	9.64	0.77	
23	[50]	Primary Sjögren's syndrome	IgG/IgA	4.81	100.00	/	0.95	ASCA positivity was associated with pSS specific clinical and serological features
25	[43]	Vasculitides	IgG	6.50	99.50	/	/	

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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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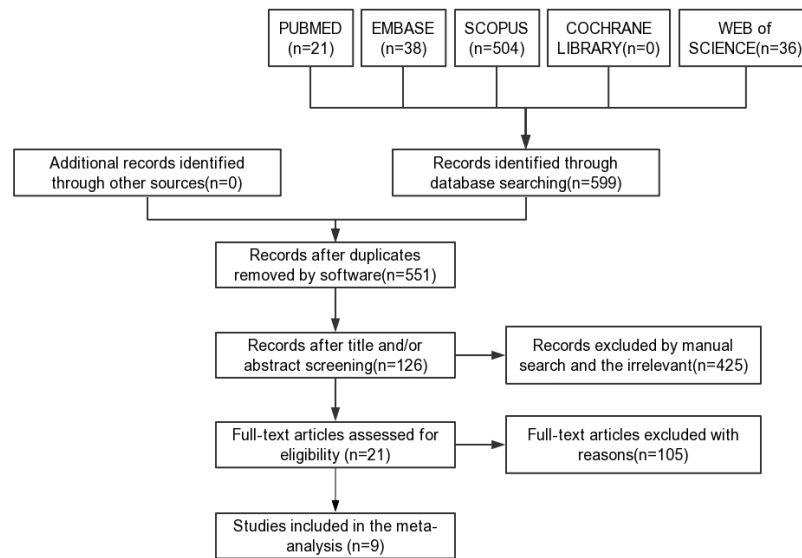


Figure 1 Schematic illustration of the literature search and study-selection criteria for the meta-analysis

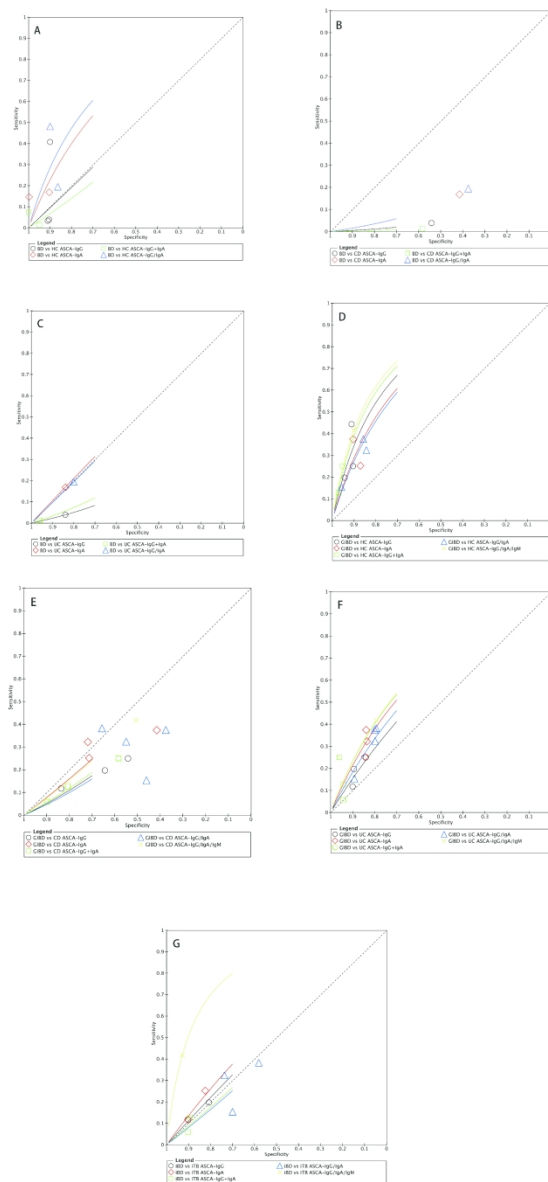


Figure 2 AUC of diagnostic accuracy of ASCA when comparing BD and HC (A), BD and CD (B), BD and UC (C), GIBD and HC (D), GIBD and CD (E), GIBD and UC (F), and GIBD and iTB (G)

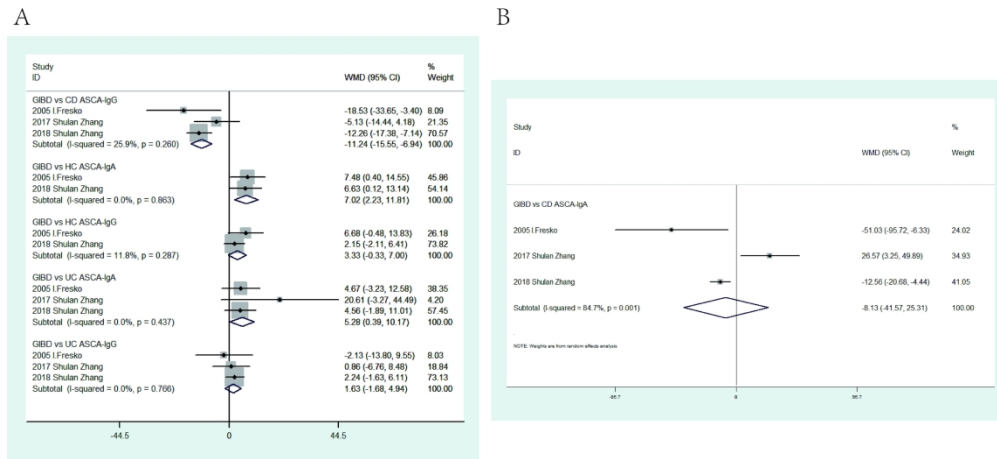
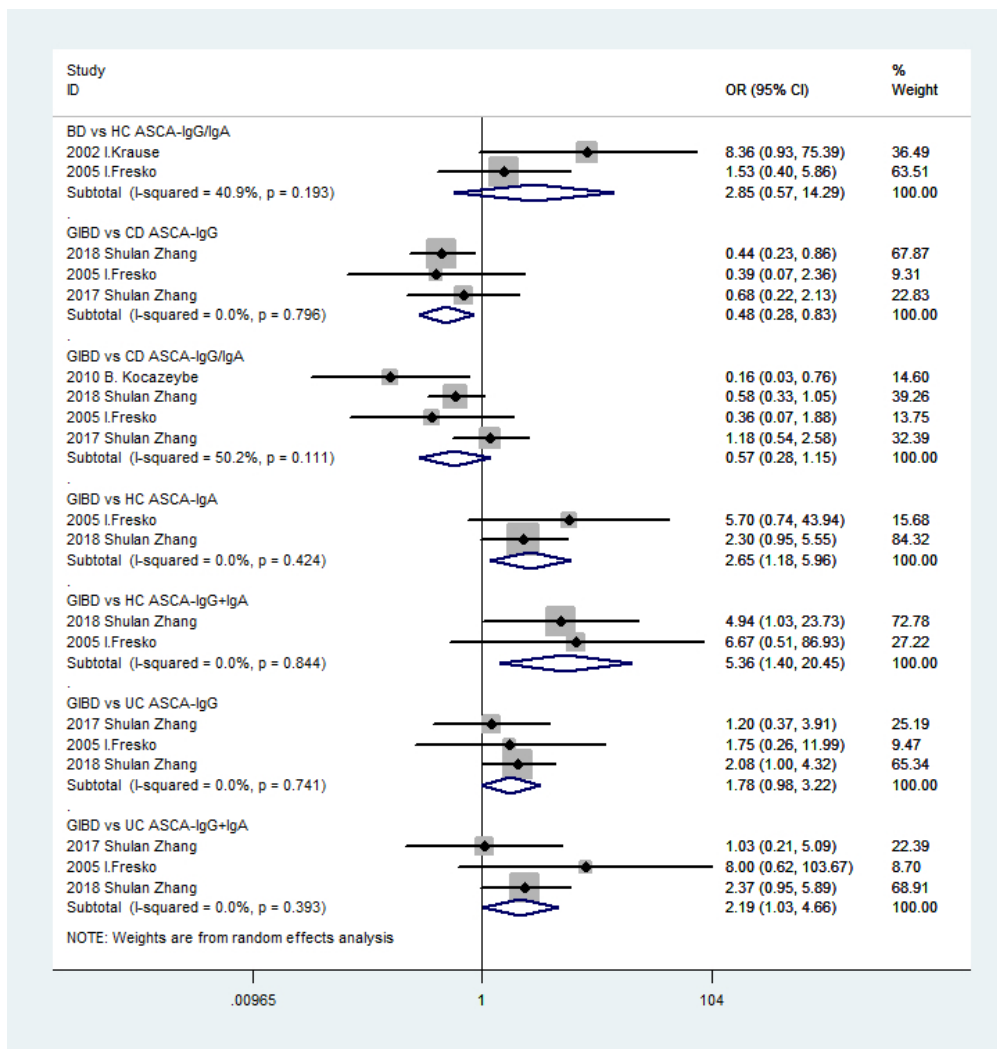


Figure 3 A. Forest plot of serum levels of ASCA in GIBD/CD/UC/HC using FEM; B. Forest plot of serum levels of ASCA in GIBD/BD/HC/CD using REM

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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 MOOSE reporting 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	3
	#3b Hypothesis statement	4-5
	#3c Description of study outcomes	n/a

1	#3d	Type of exposure or intervention used	5
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3	#3e	Type of study designs used	5
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5	#3f	Study population	5
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8	Methods		
9			
10	Search	#4a Qualifications of searchers (eg, librarians and investigators)	6-7
11	strategy		
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14	Search	#4b Search strategy, including time period included in the synthesis and keywords	6
15	strategy		
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18	Search	#4c Effort to include all available studies, including contact with authors	7
19	strategy		
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22	Search	#4d Databases and registries searched	6
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26	Search	#4e Search software used, name and version, including special features used (eg,	n/a
27	strategy	explosion)	
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29	Search	#4f Use of hand searching (eg, reference lists of obtained articles)	n/a
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33	Search	#4g List of citations located and those excluded, including justification	6
34	strategy		
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37	Search	#4h Method of addressing articles published in languages other than English	6
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41	Search	#4i Method of handling abstracts and unpublished studies	6
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45	Search	#4j Description of any contact with authors	7
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49		#5a Description of relevance or appropriateness of studies gathered for assessing	7
50		the hypothesis to be tested	
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52		#5b Rationale for the selection and coding of data (eg, sound clinical principles or	n/a
53		convenience)	
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56		#5c Documentation of how data were classified and coded (eg, multiple raters,	n/a
57		blinding, and interrater reliability)	
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1	#5d	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	8
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5	#5e	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	7
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11	#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
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18	#5h	Provision of appropriate tables and graphics	n/a
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20	Results		
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22	#6a	Graphic summarizing individual study estimates and overall estimate	26
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24	#6b	Table giving descriptive information for each study included	25
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27	#6c	Results of sensitivity testing (eg, subgroup analysis)	27
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29	#6d	Indication of statistical uncertainty of findings	n/a
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32	Discussion		
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34	#7a	Quantitative assessment of bias (eg, publication bias)	16
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36	#7b	Justification for exclusion (eg, exclusion of non-English-language citations)	n/a
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39	#7c	Assessment of quality of included studies	16
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41	Conclusion		
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43	#8a	Consideration of alternative explanations for observed results	n/a
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45	#8b	Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)	19
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49	#8c	Guidelines for future research	19
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51	#8d	Disclosure of funding source	19
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BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-033880.R1
Article Type:	Original research
Date Submitted by the Author:	24-Feb-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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4 1 **Meta-analysis of anti- *Saccharomyces cerevisiae* antibodies as diagnostic**
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7 2 **markers of Behçet's disease with gastrointestinal involvement**
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14 4 **Linlin Cheng, MD,^a Liubing Li, MD,^a Chenxi Liu, MD,^a Songxin Yan, MS,^a Yongzhe**
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4 17 **Meta-analysis of anti- *Saccharomyces cerevisiae* antibodies as diagnostic**
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14 20 **Abstract**
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17 21 **Objective:** Due to common exposure to yeast in the alcoholic and baking industry, positive
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20 22 rate of anti-*Saccharomyces cerevisiae* antibodies (ASCA) is reportedly high in patients
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23 23 with Behçet's disease (BD) who have gastrointestinal symptoms (gastrointestinal BD
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25 24 [GIBD]). We performed a meta-analysis to assess the diagnostic value of ASCA in
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28 25 differentiating patients with BD from those with other chronic inflammatory bowel
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31 26 diseases.
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34 27 **Methods:** The meta-analysis is compliant with the PRISMA and MOOSE checklist.
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37 28 Relevant studies that investigated ASCA levels in BD patients were retrieved from
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40 29 PubMed, EMBASE, Web of Science, SCOPUS, and the Cochrane Library on July 12,
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43 30 2019; the search was rerun on February 12, 2020. Stata/SE 12.0 and Meta-DiSc 1.4 were
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46 31 used to perform the meta-analysis and sensitivity analysis, disaggregated by isotypes of
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49 32 ASCA.
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52 33 **Results:** Nine studies were included in the meta-analysis. The results revealed a strong
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55 34 association between ASCA and GIBD, especially ASCA-IgG [odds ratio (OR)=5.50 (95%
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4 35 CI 2.58–11.55, p=0.000) and ASCA-IgG+IgA [OR=5.36 (95% CI 1.40–20.45), p=0.014].

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7 36 The positivity rate of ASCA in GIBD was significantly higher than that in ulcerative colitis:

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10 37 IgA [OR=2.13 (95% CI 1.30–3.50), p=0.003]; IgG+IgA [OR=2.19 (95% CI 1.03–4.66),

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13 38 p=0.042]; IgG/IgA [OR=2.03 (95% CI 1.30–3.17), p=0.002]. However, the frequency of

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16 39 ASCA-IgG was significantly higher in patients with Crohn's disease than GIBD [OR=5.36

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19 40 (95% CI 1.40–20.45), p=0.009]. There was no significant difference in ASCA positivity

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22 41 between BD without gastrointestinal involvement and healthy controls and between GIBD

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24 42 and intestinal tuberculosis (p>0.05).

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27 43 **Conclusion:** ASCA may play a role in the pathogenesis of gastrointestinal involvement.

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30 44 Negative result of IgG favors the diagnosis of GIBD/BD when differentiated from Crohn's

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33 45 disease. ASCA-IgA showed moderate diagnostic performance in distinguishing GIBD and

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36 46 ulcerative colitis and the diagnostic performance was better in combination with IgG.

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38 47 However, ASCA may not be a useful serologic marker distinguishing GIBD and intestinal

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40 48 tuberculosis.

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44 49 Key words: Behçet's disease; Anti-*Saccharomyces cerevisiae* antibodies; autoimmune

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47 50 diseases; meta-analysis; autoantibodies

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54 Strengths and limitations of this study

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

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78 71 **Introduction**
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11 72 Behçet's disease (BD) is a chronic systemic vascular autoimmune/inflammatory disease
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13 73 with a high propensity for recurrence; the pathogenetic mechanisms of this disease are not
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16 74 well elucidated [1]. Virtually no specific histological or laboratory features of BD have
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19 75 been identified. Therefore, the diagnosis of BD is typically challenging as it is mainly based
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22 76 on clinical features [2, 3]. The diagnosis is frequently delayed until the development of
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25 77 clinical manifestations that qualify the diagnostic criteria. The estimated duration between
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28 78 the onset of symptoms and the fulfilment of diagnostic criteria is approximately 4 years
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30 79 [4].

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34 80 Moreover, patients with prominent involvement of a particular organ system are easily
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37 81 misdiagnosed. For example, patients who have gastrointestinal symptoms as the main
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40 82 manifestation are liable to be misdiagnosed as having Crohn's disease (CD), ulcerative
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43 83 colitis (UC), or intestinal tuberculosis (iTb). These features make formulating disease
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45 84 criteria difficult, causing deleterious effects on the patients.

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48 85 Several recent studies (but not all) have reported the diagnostic value of anti-
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51 86 *Saccharomyces cerevisiae* antibody (ASCA) in BD. *Saccharomyces cerevisiae*, also
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54 87 known as the baker's or brewer's yeast, has long been utilized to ferment the sugars in
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4 88 cereals to produce alcoholic beverages; it is also used in the baking industry to raise dough.
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7 89 As a consequence, we are now commonly exposed to yeast [5]. IgG and IgA antibodies
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10 90 against the phosphopeptidomannan of the *S. cerevisiae* cell wall have been discovered as
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12 91 autoantibodies in the sera of patients with BD, especially those with gastrointestinal
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15 92 involvement. This suggests a role of environmental stimuli in the pathogenesis of BD.
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18 93 However, patients with inflammatory bowel disease such as Crohn's disease (CD) also
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21 94 have a high prevalence rate of ASCA due to their similarities [6, 7, 8, 9, 10, 11]. In this
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24 95 context, identification of ASCA as a diagnostic marker for BD is a key imperative. The
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27 96 objectives of this study were to summarize the findings pertaining to the relevance of
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30 97 ASCA in BD and other gastrointestinal diseases and to perform a meta-analysis to assess
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33 98 its diagnostic accuracy for BD.
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100 **Methods**

101 Study design

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

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4 106 predefined protocol was registered with PROSPERO (Registration No.
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7 107 CRD42020115245).
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14 109 Literature search

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17 110 A comprehensive literature search was performed to identify studies pertaining to ASCA
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20 111 as biomarkers for BD in 5 biomedical databases, i.e., PubMed, EMBASE, Web of Science,
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23 112 SCOPUS, and the Cochrane Library on July 12, 2019. The search terms for Behçet's
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26 113 disease were: Behçet, triple symptom complex, triple symptom complices, Adamantiades
27
28 114 Behçet and old silk route disease; the search terms for *Saccharomyces cerevisiae* were: *S.*
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31 115 *cerevisiae*, *Saccaromyces cerevisiae*, *Saccharomyces capensis*, *Saccharomyces*
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33
34 116 *diastaticus*, *Saccharomyces italicus*, *Saccharomyces oviformis*, *Saccharomyces uvarum*,
35
36
37 117 brewer yeast or baker yeast, mannan, manna, polymannan, glucomannan, yeast mannan,
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39 118 dicoman, humamil, ASCA. Combination of keywords using "AND" was used to retrieve
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41
42 119 studies in the range of "all fields" or "all text". The search was rerun on February 12, 2020
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44
45 120 to ensure inclusion of recent studies. No restrictions were imposed with respect to time of
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48 121 publication, region, or ethnicity of the study population. In addition, the reference list of
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51 122 obtained articles was also examined to identify possible relevant studies.
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4 124 Eligibility and exclusion criteria
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7 125 The inclusion criteria were: (1) studies that evaluated the diagnostic accuracy of ASCA in
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10 126 BD; (2) availability of adequate data pertaining to the prevalence rate or serum levels of
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13 127 ASCA in patients with BD; (3) studies with healthy population and/or disease controls; (4)
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16 128 meeting abstracts or letters to the editor were also included.
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19 129 The exclusion criteria were: (1) studies with incomplete data; (2) review articles; (3) non-
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22 130 English articles; (4) in case of studies with overlapping study population, studies with
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25 131 smaller sample size were excluded. Two investigators independently performed the
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28 132 literature search, screened the titles and abstracts, followed by full-text review of eligible
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31 133 studies.
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37 135 Data extraction and quality assessment
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41 136 Two independent investigators reviewed the full-text articles, extracted the data, and
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44 137 assessed the study quality using the modified version (nine-star scoring system) of the
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47 138 Newcastle-Ottawa Scale (NOS) for case-control studies and the Quality Assessment of
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50 139 Diagnostic Accuracy Studies (QUADAS-2) and; For NOS, studies with higher NOS scores
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53 140 (☆) were considered as higher quality (low risk of bias). For QUADAS-2, the included
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56 141 items were evaluated as yes, no, or uncertain. Inter-researcher disagreements were resolved
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4 142 by consensus, or by a third investigator. Data pertaining to the following variables were
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7 143 extracted: publication year, article type, first author's name, country, isotypes of ASCA
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10 144 detected, age and sex, research design, sample size, experimental method, trade names of
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13 145 experimental materials, cut-off values, diagnostic criteria, and serum titers and/or
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15 146 prevalence rate of ASCA in BD, gastrointestinal BD (GIBD), healthy controls (HC),
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18 147 patients with Crohn's disease (CD), ulcerative colitis (UC), and intestinal tuberculosis
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21 148 (iTB). The data were either obtained directly from the article, calculated, or requested from
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24 149 the author via e-mail.
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30 151 Statistical analysis

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34 152 Pooled odds ratios (OR) with 95% confidence intervals (CI) were calculated to evaluate
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37 153 the association between ASCA and BD (without gastrointestinal
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40 154 involvement)/GIBD/CD/UC/iTB using Stata/SE 12.0. Meta-DiSc 1.4 was used to calculate
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43 155 the sensitivity, specificity, AUC values, and the area under the summary receiver operating
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46 156 characteristic (SROC) curve to assess the overall diagnostic performance of ASCA.
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49 157 Heterogeneity among the included studies was evaluated using the Cochran's Q-statistic.
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52 158 P values > 0.10 were considered indicative of lack of significant heterogeneity. We chose
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55 159 the random effects models (REM) since REM tends to generalize findings beyond the
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58 160 included studies by assuming that the selected studies are random samples from a larger

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4 161 population [15]. Subgroup analysis was performed disaggregated by the isotypes of ASCA
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7 162 and different disease controls. The isotypes of ASCA were classified and defined as
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10 163 follows: IgG, IgA, IgG/IgA (positive results of either IgG or IgA), and IgG+IgA (positive
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12 164 results of both IgG and IgA).

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16 165 In order to increase the robustness of the meta-analysis, we also extracted the data
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18 166 pertaining to serum levels of ASCA from five studies and performed meta-analysis using
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21 167 the Continuous data module of Stata/SE 12.0. The REM was used for the analysis and
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24 168 weighted mean difference (WMD) was used as the effect measure if the same unit was
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27 169 used in these studies and there were minor differences with respect to the serum levels of
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29 170 ASCA. Sensitivity analysis was performed using Stata/SE 12.0 to evaluate stability of the
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32 171 results after sequential exclusion of one study at a time.

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42 174 The present study was a meta-analysis and systematic review based on published data.
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45 175 Patients and public were not involved in the study design, conduct, data analysis, and result
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48 176 dissemination.

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53 54 55 178 Relationship between ASCA and autoimmune disease

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4 179 We searched the PubMed for studies pertaining to the relationship between ASCA and
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7 180 autoimmune diseases. The two search terms used were autoimmune disease and
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10 181 *Saccharomyces cerevisiae*. We performed an interval statistic of four indicators of ASCA–
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12 182 sensitivity, specificity, positive likelihood (LR+) and negative likelihood (LR-) based on
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15 183 the included studies sorted by diseases.
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22 185 **Results**

26 186 Literature search and characteristics of studies

29 187 A total of 625 documents were retrieved on database and manual search. Fifty-one
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32 188 duplicate publications were excluded using the document management software. A total of
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35 189 127 records were retained after screening of titles and/or abstracts; the excluded records
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37 190 included review articles, animal model studies, therapeutic or drug research, genetic
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40 191 research, book chapters, duplicate publications not recognized by software, and other
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43 192 irrelevant records. After full-text review for eligibility, 22 records were selected. Finally,
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45 193 we included 9 available studies with adequate data in the meta-analysis (Figure 1). Two
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48 194 studies were included after obtaining the relevant data by contacting the respective authors
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51 195 [9, 10]. In addition, we also verified 2 studies [16, 17] with overlapping study population;
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54 196 of these, only 1 study was included in the meta-analysis. Three studies [6, 8, 18] were

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4 197 presented as meeting abstracts without adequate data to allow the construction of a 2×2
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7 198 table. One article[7] was a letter to the editor and only reported the prevalence rate of
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10 199 ASCA antibody in patients with BD, without information about the control group. One
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12 200 study [19] had employed a unique calculation method and could not be included in the
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15 201 meta-analysis. Among the included studies, there were 326 cases of BD, 294 cases of
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17 202 GIBD, 520 cases of CD, 598 cases of UC, 112 cases of iTB, and 428 HCs (Table 1 and
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20 203 Table S3).
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27 205 Quality assessment

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31 206 There were 8 case-control studies and 1 retrospective study [9]. The results of quality
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34 207 assessment by NOS including the selection of the study groups, the comparability of the
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36 208 groups and the ascertainment of the exposure of interest for case-control studies are shown
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39 209 in Table 2, and by QUADAS-2 including the risk of bias and applicability concerns
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42 210 pertaining to each domain are shown in Figure S1. The results indicated that the included
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45 211 studies were of high quality in general. Overall, none of the 9 included studies showed any
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47 212 major methodological bias or flaws, which indicates robustness of our meta-analysis.
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54 214 Meta-analysis

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4 215 Association between ASCA and BD (without gastrointestinal involvement), GIBD and
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7 216 other intestinal diseases
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10 217 Data pertaining to correlation between ASCA and BD (without gastrointestinal
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13 218 involvement)/GIBD/CD/UC/iTB are listed in Table 3. No substantial heterogeneity
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16 219 ($p>0.1$ for all) was observed by using REM to calculate the OR. The results revealed a
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19 220 strong association between all detection types of ASCA and GIBD, especially for ASCA-
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21 221 IgG [OR=5.50 (95% CI 2.58–11.55, $p=0.000$) and ASCA-IgG+IgA [OR=5.36 (95% CI
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23
24 222 1.40–20.45), $p=0.014$]. When comparing GIBD and UC, of the positivity rate for ASCA
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26
27 223 in GIBD was significantly higher than that for UC: IgA [OR=2.13 (95% CI 1.30–3.50),
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29 224 $p=0.003$], IgG+IgA [OR=2.19 (95% CI 1.03–4.66), $p=0.042$], and IgG/IgA [OR=2.03
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32 225 (95% CI 1.30–3.17), $p=0.002$]. Conversely, the frequency of only ASCA-IgG in patients
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35 226 with CD was significantly higher than that in the GIBD [OR=5.36 (95% CI 1.40–20.45),
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38 227 $p=0.009$]. Further, on stratified analysis according to detection method, ASCA-IgG was
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40 228 associated with GIBD using both the ELISA method (OR = 3.83, 95% CI 1.37–10.70, p
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43 229 = 0.010) and the immunoprecipitation method (IIF) (OR = 8.17, 95% CI 2.73–24.43, p =
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46 230 0.000) (Figure 2). However, no significant difference was observed with respect to
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49 231 ASCA positivity between BD without gastrointestinal involvement and HC and between
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51 232 GIBD and iTB ($p>0.05$).
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55 233 Diagnostic ability of ASCA for GIBD
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4 234 The overall sensitivity for ASCA-IgG in patients with GIBD detected by IIF was 0.44,
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7 235 which is much higher than that of ELISA [0.20 (95%CI 0.12–0.31)] (Table 4). Combined
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10 236 detection of IgG and IgA by ELISA increased the sensitivity to 0.33 (95% CI 0.23–0.44).
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12 237 However, we observed a low level of sensitivity of ASCA-IgG/IgA by IIF, which may be
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15 238 attributable to the inclusion of only one study with few GIBD patients (n=13).

18 239 Difference in serum levels of ASCA in GIBD and other intestinal diseases

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22 240 Serum levels of ASCA-IgA observed in GIBD were significantly greater than that in HC
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24 241 [WMD=7.02 (95% CI 2.23–11.81), p=0.004] and UC [WMD=5.28 (95% CI 0.39–10.17),
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27 242 p=0.034] in contrast to ASCA-IgG (p>0.05) (Figure 3). On the contrary, serum levels of
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30 243 ASCA-IgG in CD were significantly greater than that in GIBD [WMD=-11.04 (95% CI -
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32 244 16.74--5.34), p=0.000] (Figure 3). However, we found no significant difference in serum
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35 245 levels of ASCA between BD without gastrointestinal symptoms and HC (p>0.05) (Figure
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38 246 3).

45 248 Heterogeneity and sensitivity analysis

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49 249 We performed sensitivity analysis to assess the stability of the results. The results showed
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52 250 that the studies by Krause et al (2002), Zhang et al (2018), Kocazeybe et al (2010), and
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55 251 Fresko et al (2005) were the key contributors to the heterogeneity (Figure S2). Thus, the

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4 252 results of related subgroup analysis are considered to be less stable.
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11 254 Summary of the relationship of ASCA with autoimmune disease
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15 255 Sixteen studies reporting the relevance of ASCA and autoimmune diseases were included
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17 256 in the summary. The sensitivity, specificity, LR+, and LR- of ASCA for different
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19
20 257 autoimmune diseases are summarized in Table 5. Although the diagnostic results of ASCA
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23 258 reported by different studies vary, the summary revealed an overall association between
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26 259 ASCA and autoimmune diseases especially in patients with scleroderma, juvenile
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28 260 idiopathic arthritis, Crohn's disease, and systemic lupus erythematosus with high SEN
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31 261 (>40%), high SPE (>95), high LR+ (>5) (Table 5).
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38 263 **Discussion**
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41 264 **Serological markers in BD.** The diagnosis of BD is typically challenging prior to the
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44 265 appearance of clinical symptoms necessary to qualify the diagnostic criteria. Currently,
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47 266 there are no specific laboratory biomarkers of BD; however, some specific autoantibodies
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50 267 in the context of BD have been reported. Therefore, identification of non-invasive specific
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53 268 diagnostic and prognostic biomarkers of BD is of much clinical relevance and a key focus
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56 269 area of research.
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4 270 **ASCA in BD and autoimmune diseases.** Several recent studies have investigated the
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7 271 relationship of ASCA with BD or other autoimmune diseases. *Saccharomyces cerevisiae*
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10 272 has long been utilized in alcoholic and baking industry, and for the production of vaccines
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12 273 owing to its antigenic component. However, during long-term and ubiquitous presence,
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15 274 even the commensal and classically non-pathogenic microbiota can trigger autoimmunity
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17 275 due to loss of immune tolerance towards the resident bacterial flora, like in gastrointestinal
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20 276 tract [20, 21]. The reported similarity of sequences involving the eukaryotic microorganism
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23 277 and self-antigens suggest a mechanism of molecular mimicry and also the plausibility of
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26 278 shared epitopes in different autoimmune diseases. The production of ASCA by the
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28 279 subsequent activation of the humoral immune response may lead to a direct pathogenic role
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31 280 through a costimulatory CD80/86-CD28-mediated effect [20]. Moreover, healthy family
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34 281 members but not spouses of BD patients were also found to have increased levels of ASCA,
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37 282 which indicated a role of genetic factors in addition to environmental stimuli [17, 21]. A
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40 283 large number of studies have assessed the role of ASCA in the context of several systemic
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42 284 and organ-specific autoimmune diseases, such as BD, scleroderma, systemic lupus
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45 285 erythematosus, primary Sjögren's syndrome, and rheumatoid arthritis (Table 5). The
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48 286 results suggest that the relation of ASCA with BD or other autoimmune diseases may
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51 287 represent a potential pathogenic mechanism between ASCA and autoimmunity; this
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53 288 underlines the importance of ASCA as a valuable serologic marker for autoimmune
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56 289 diseases including BD.

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4 290 **Results of the meta-analysis.** To the best of our knowledge, this is the second meta-
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7 291 analysis of evidence pertaining to autoantibodies in patients with BD after anticardiolipin
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10 292 antibodies [22]. ASCA have been widely researched in BD, Crohn's disease and other
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12 293 autoimmune disease; in order to investigate the diagnostic value and possible pathogenetic
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15 294 role of ASCA in BD, we included 9 studies in this meta-analysis. Among these, some
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17 295 studies included BD patients with systemic involvement including or excluding
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20 296 gastrointestinal involvement, while others included only BD patients with gastrointestinal
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23 297 involvement. Therefore, in order to reduce the impact of differences with respect to
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26 298 frequency distribution of gastrointestinal symptoms in each study, we disaggregated
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29 299 patients with BD into those with gastrointestinal involvement only and those without
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31 300 gastrointestinal manifestations. The isotype antibodies of ASCA tested and the results
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34 301 presented by the studies are also different. The meta-analysis revealed a strong association
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37 302 of ASCA with GIBD and not with BD with no gastrointestinal involvement; this suggests
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40 303 the role of ASCA in the pathogenesis of gastrointestinal involvement. ASCA showed a
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42 304 moderate diagnostic performance as a biomarker for the differential diagnosis between
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45 305 GIBD and CD, and the negative result of ASCA-IgG may slightly favor the diagnosis of
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48 306 GIBD/BD when compared with CD, especially with concomitant positive HLA-B51 tests
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50 307 [23]. In addition, ASCA-IgA showed a moderate diagnostic value for distinguishing GIBD
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53 308 and UC and would perform better with concomitant detection of IgG. However, ASCA
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56 309 failed to distinguish between GIBD and iTB. Besides, the concomitant evaluation of both

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4 310 continuous data (sensitivity and specificity) and discontinuous data (serum levels) helped
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7 311 increase the credibility of our results.
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10 312 **Heterogeneity.** Combined with the results of QUADAS-2, we found that the heterogeneity
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12 313 in this meta-analysis was largely attributable to the following reasons (see Table 1, Figure
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15 314 S1). 1) The different diagnostic criteria used in the included studies. Different criteria may
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18 315 have different thresholds for diagnosis or place more weight on some symptoms than
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21 316 others. Specifically, the 1990 ISG criteria requires the presence of oral ulceration plus any
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24 317 two of the following: genital ulceration, typical eye lesions, typical skin lesions, or positive
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27 318 pathergy test for diagnosis of BD [2]. In contrast, the 1987 Japan criteria require all four
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30 319 characteristics for the diagnosis of BD, i.e., oral ulceration, typical eye lesions, typical skin
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33 320 lesions, and genital ulceration [24]. The ISG criteria and the Japanese criteria often fail to
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36 321 classify some patients with BD; in addition, the Japanese criteria may also cause
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39 322 misclassified diagnosis. This may have caused the different diagnostic sensitivity and
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42 323 specificity for BD [25]. 2) Differences in demographic characteristics of included studies.
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45 324 The clinical features and laboratory findings tend to exhibit wide variability in different
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48 325 populations and clinical settings [26, 27]. However, we failed to perform subgroup analysis
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51 326 disaggregated by ethnicity owing to the small sample size in each subgroup. 3) Different
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54 327 antibody assays and cut-off values. Commercial kits and in-house tests from different
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57 328 laboratories have variable performance, which may affect the diagnosis and management

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4 329 of patients. We found that different methods and cut-off values were adopted by the studies
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7 330 included in this meta-analysis. Notably, there was significant association between ASCA-
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10 331 IgG and GIBD using both methods; however, it seems that IIF has a higher sensitivity than
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12 332 ELISA. Nonetheless, further investigations with larger study population are required to
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15 333 provide more definitive evidence. Although previous studies have shown that IIF has a
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17 334 better performance, ELISA provides the titer change of serum antibodies and could have
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20 335 an equal performance to IIF by changing cut-off values to optimize the overall diagnostic
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23 336 performance [28].
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26 337 **Limitations of the meta-analysis.** In order to fully evaluate the value of ASCA for
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28 338 differential diagnosis of BD, we included patients with CD, UC, and iTB as the comparison
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31 339 objects in our meta-analysis. However, there are still some limitations of this meta-analysis.
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34 340 (1) Gray literature database, paper database, and other language databases were not used
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37 341 for the literature search. Quite a few non-English studies were excluded due to incomplete
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40 342 data or unavailability of full text. (2) Our primary goal was to assess the diagnostic efficacy
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43 343 of ASCA in BD, and therefore we did not include all studies pertaining to ASCA in
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46 344 inflammatory bowel disease and iTB. (3) Restricted by the number of included studies and
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49 345 the isotypes of ASCA, we could not perform subgroup analysis disaggregated by different
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52 346 populations and diagnostic criteria. (4) Some studies with incomplete data were excluded
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55 347 after lack of response from the author. (5) There are some inherent statistical shortcomings
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4 348 using Meta-DiSc during the separate pooling of sensitivity and specificity, as the between-
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7 349 study variance is not included. More advanced methods are not implemented [29].
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10 350 **Conclusion.** Our meta-analysis results, together with the review of ASCA in autoimmune
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13 351 diseases strongly suggest that ASCA (especially its certain isotypes) may be helpful
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16 352 biomarkers for GIBD, especially with respect to their possible
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19 353 predictive/pathogenic/diagnostic role in clinical settings [20]. Furthermore, ASCA may be
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22 354 detectable years before the diagnosis of some autoimmune diseases as they were
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25 355 retrospectively found in the preserved blood samples of soldiers who were affected by
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28 356 Crohn's disease years later [30]. However, due to its presence in several other autoimmune
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31 357 diseases, ASCA may have a limited value for clinical diagnosis.
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359 **Compliance with Ethical Standards:**

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

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369 **Ethical approval:** This article does not contain any studies with human participants
370 performed by any of the authors.

371 **Data availability statement:** All data relevant to the study are included in the article or
372 uploaded as supplementary information.

373

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4 579 Figure 1 PRISMA flow diagram illustrating the literature screening process and the
5 580 criteria for inclusion of studies in the meta-analysis
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10 582 Figure 2 Forest plot of the association between the presence of ASCA-IgG and GIBD
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13 583 stratified by detection methods
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20 585 Figure 3 Forest plot comparing serum levels of ASCA between BD without
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23 586 gastrointestinal symptom/GIBD and HC/CD/UC
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596 Table 1 Characteristics of studies included in the meta-analysis of Anti-*Saccharomyces*

597 *cerevisiae* antibodies in Behçet's disease, its main differential diagnoses, and healthy

598 controls.

599 /:no sample; ELISA: enzyme-linked immunosorbent assay; IIF: indirect immunofluorescence assay; NR: not reported;

600 SD: standard deviation; *: all without gastrointestinal manifestations; #: lack of corresponding data; 1990 ISG criteria:

601 the 1990 criteria of Behçet's Disease International Study Group; 1987 Japan criteria: the 1987 criteria by the Behçet's

602 Disease Research Committee of Japan; BD: Behçet's disease; GIBD: gastrointestinal Behçet's disease; CD: Crohn's

603 disease; UC: ulcerative colitis; iTB: intestinal tuberculosis; HC: healthy control

Year and author	Count ries	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
1995 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
2012 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

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609 Table 2 Risk of bias assessment of the included studies according to the modified
610 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

611 * was awarded when the respective information was available.

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

620 BD: Behçet's disease without gastrointestinal symptom; GIBD: gastrointestinal Behçet's disease; CD: Crohn's disease;
 621 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

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

629 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

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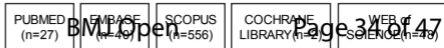
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639 Table 5 Summary of the diagnostic performance of ASCA in autoimmune disease

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

Reference	Autoimmune disease	Type	SEN (%)	SPE (%)	LR+	LR-	Supplementary information
10	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
11 [27]		IgA	16.22	94.74	3.08	0.88	
12	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
13		IgA	1.28–23.26	91.03–100.00	2.59–3.71	0.84–0.99	
14 [39, 40, 41, 42]	Antiphospholipid syndrome	IgG/IgA	20.00	95.00	/	/	
15 [43]	Juvenile Idiopathic Arthritis	IgA	0–50.00	94.74	9.50	0.53	
16	Autoimmune hepatitis	IgG	16.42–27.53	100.00	/	0.84	
17 [44]		IgA	11.94	94.74	2.27	0.93	
18 [45, 46, 47]	Primary biliary cirrhosis	IgG/IgA	18.52	84.00	1.16	0.97	
19		IgG	10.57–18.95	97.50–100.00	7.58	0.83–0.89	
20	Primary sclerosing cholangitis	IgA	11.58–18.70	94.74–98.75	3.55–9.26	0.86–0.90	
21 [45, 47, 48]		IgG/IgA	20.26–24.21	84.00–96.25	1.27–6.46	0.79–0.95	
22	Crohn's disease	IgG	28.00	100.00	/	0.72	
23 [45, 47]		IgA	32.00	94.74	6.08	0.72	
24	Cryoglobulinemia	IgG/IgA	30.51	84.00	1.91	0.83	
25 [11, 39, 45, 49, 50]		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.
26	IgA	19.30–71.43	94.74–100.00	9.91–29.40	0.50–0.71		
27 [50]	Graves' disease	IgG	7.10	99.50	/	/	
28 [50, 51, 52]	Rheumatoid arthritis	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
29		IgA	8.40–16.67	94.17–96.88	2.69–2.86	0.88–0.95	
30	Systemic lupus erythematosus	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
31 [40, 53]		IgA	17.72–40.00	91.03–94.74	1.97–7.60	0.63–0.90	
32	Type 1 diabetes	IgM	13.33	94.74	2.53	0.91	
33		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
34 [49, 54, 55]	IgA	7.50–12.07	94.74–99.38	1.43–19.31	0.88–0.98		
35	Primary Sjögren's syndrome	IgG/IgA	31.90	96.25	8.51	0.71	
36		IgG	20.98	98.09	10.98	0.81	
37 [56]	Vasculitides	IgA	9.82	98.73	7.71	0.91	
38		IgG/IgA	24.55	97.45	9.64	0.77	
39 [57]	Primary Sjögren's syndrome	IgG/IgA	4.81	100.00	/	0.95	ASCA positivity was associated with pSS specific clinical and serological features
40 [50]	Vasculitides	IgG	6.50	99.50	/	/	

641



1 Additional records identified through
2 other sources(n=0)

Records identified through
database searching(n=625)

3
4 Records after duplicates
removed by software(n=574)

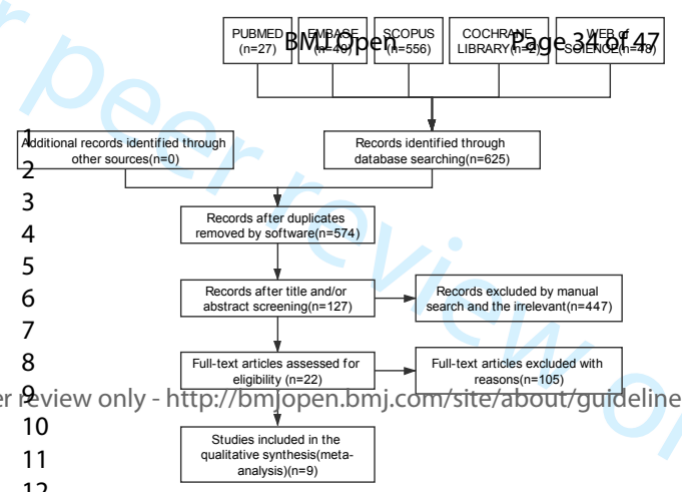
5
6 Records after title and/or
abstract screening(n=127)

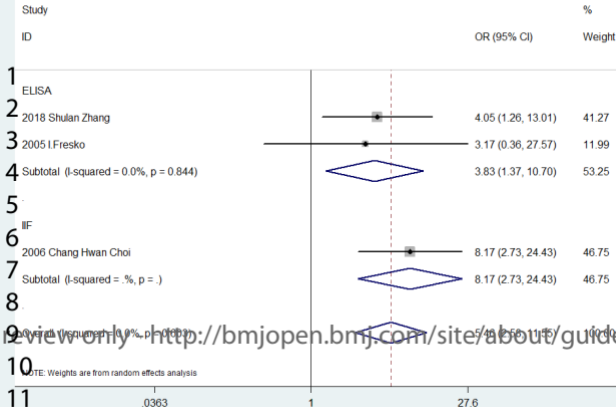
Records excluded by manual
search and the irrelevant(n=447)

7
8 Full-text articles assessed for
eligibility (n=22)

Full-text articles excluded with
reasons(n=105)

9
10
11 Studies included in the
qualitative synthesis(meta-
analysis)(n=9)
12





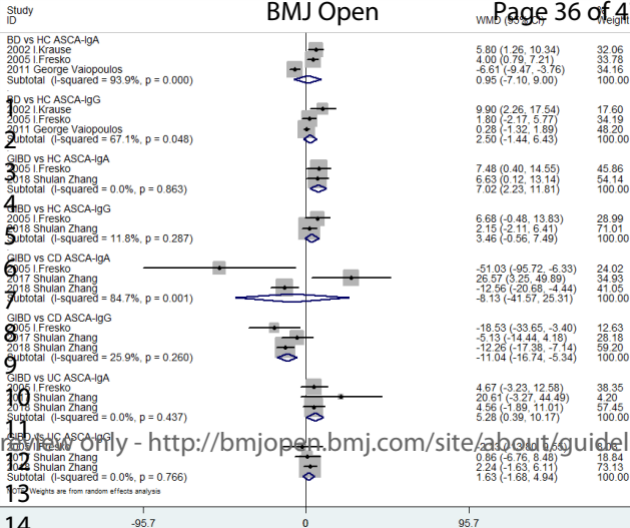
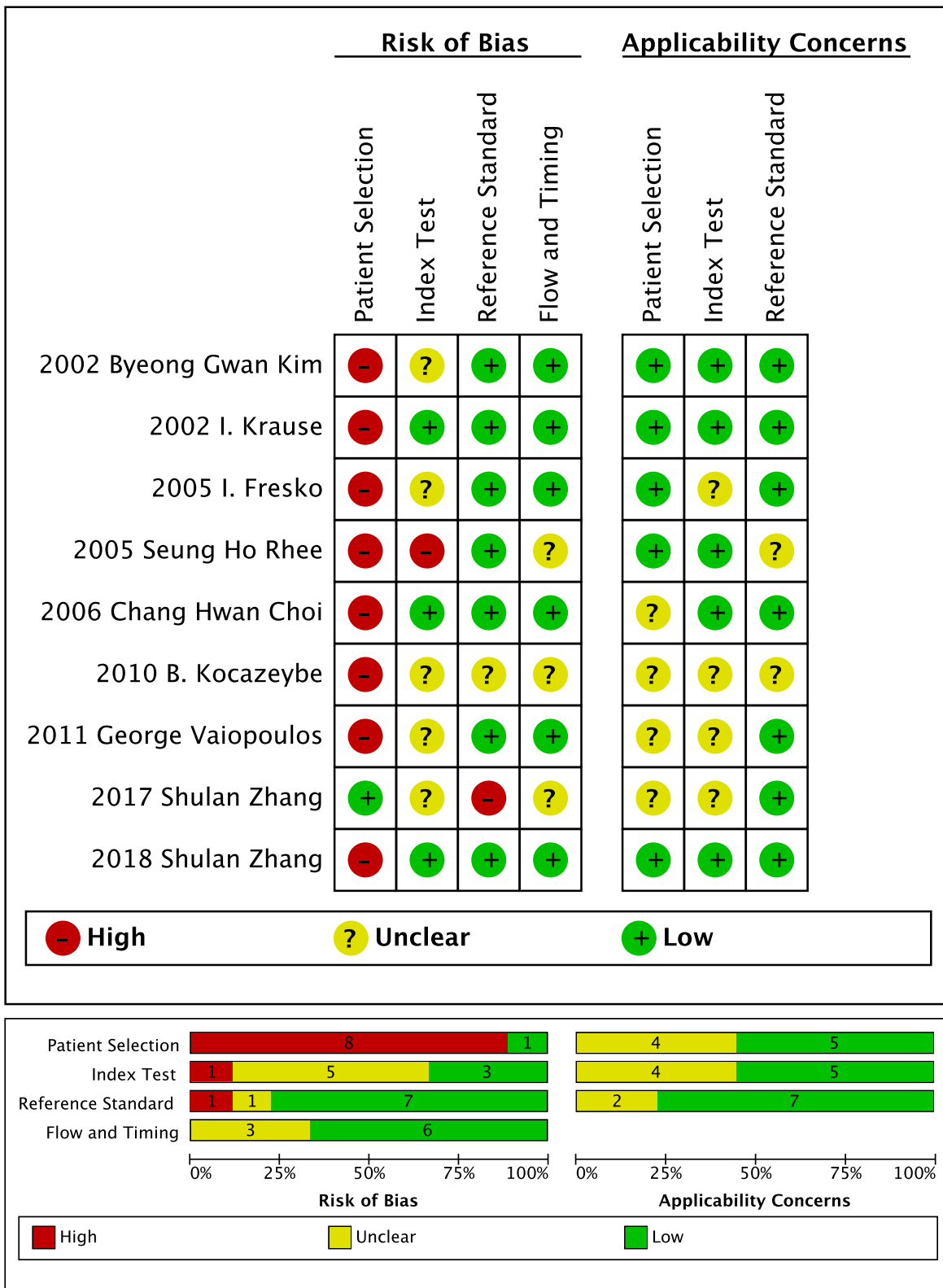
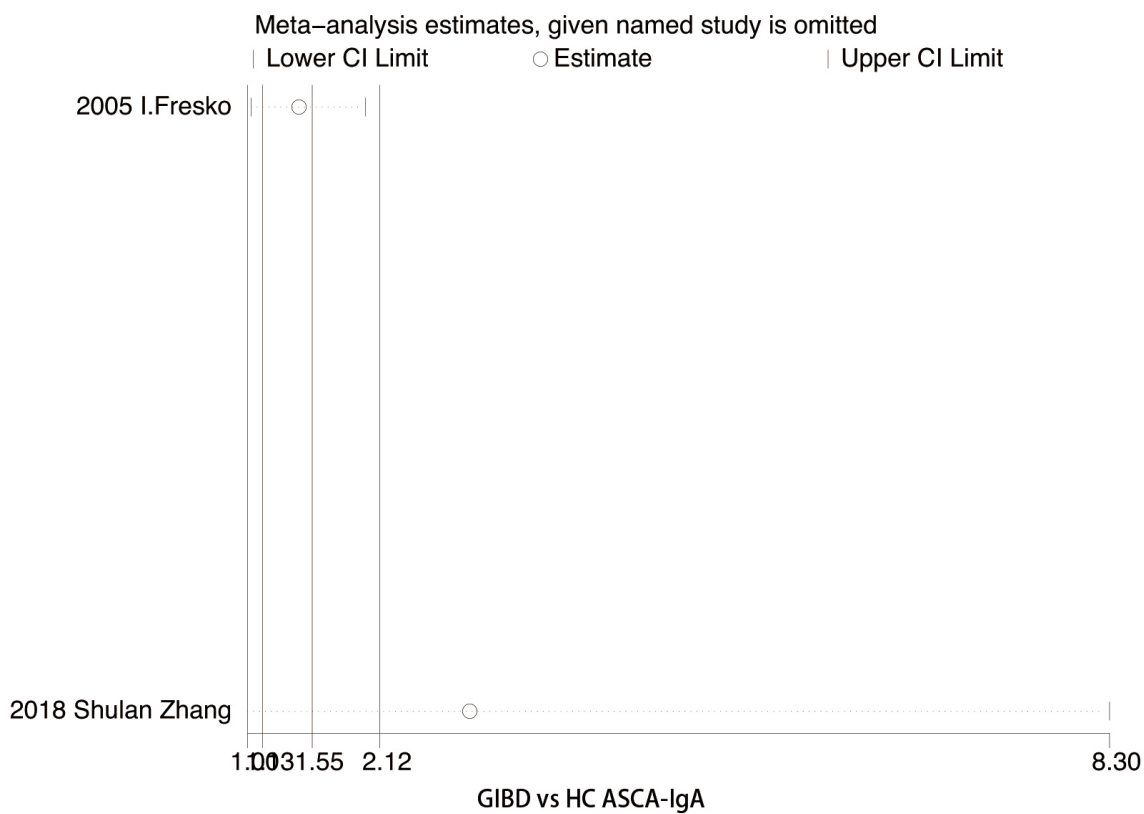
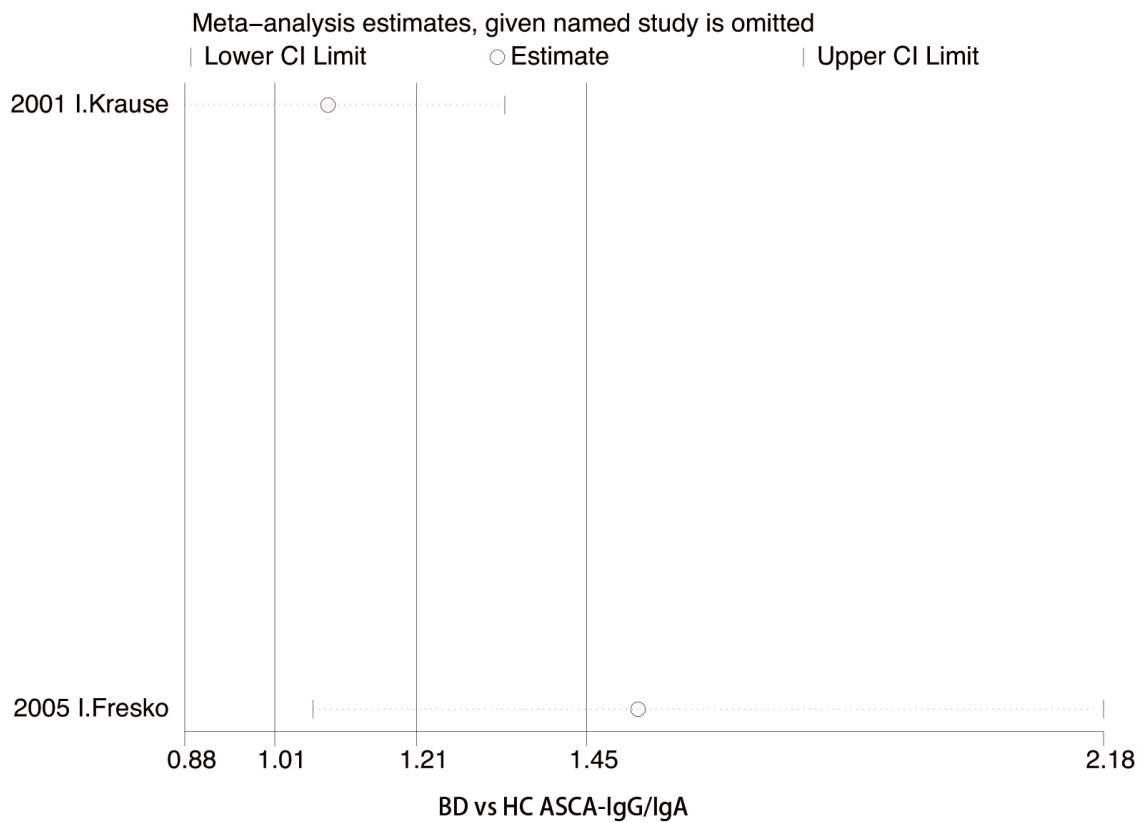
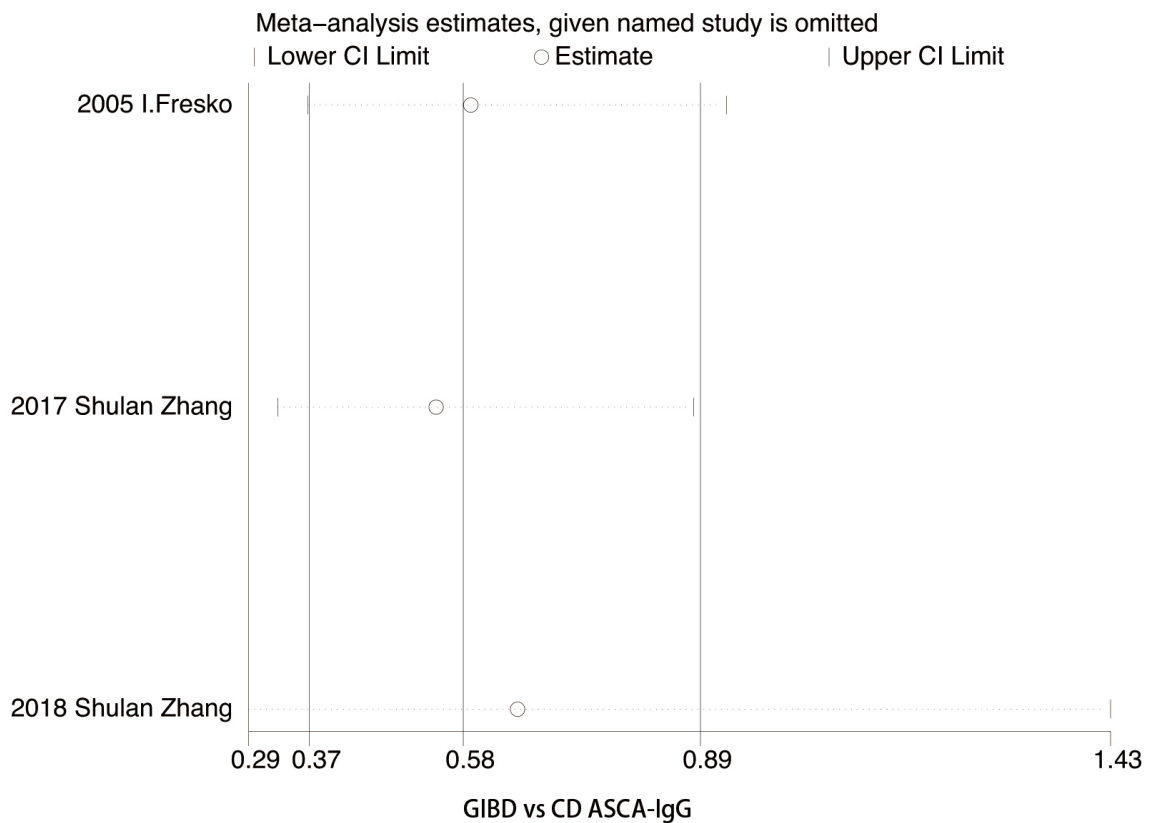
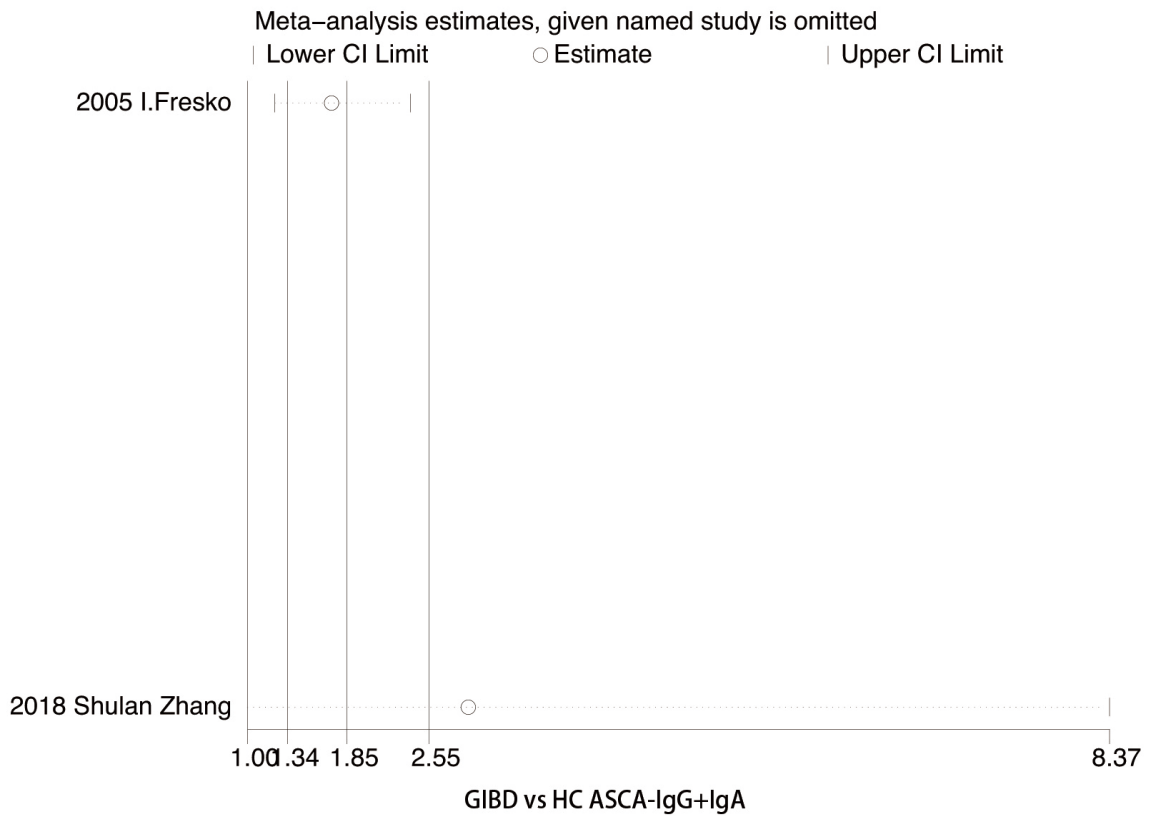
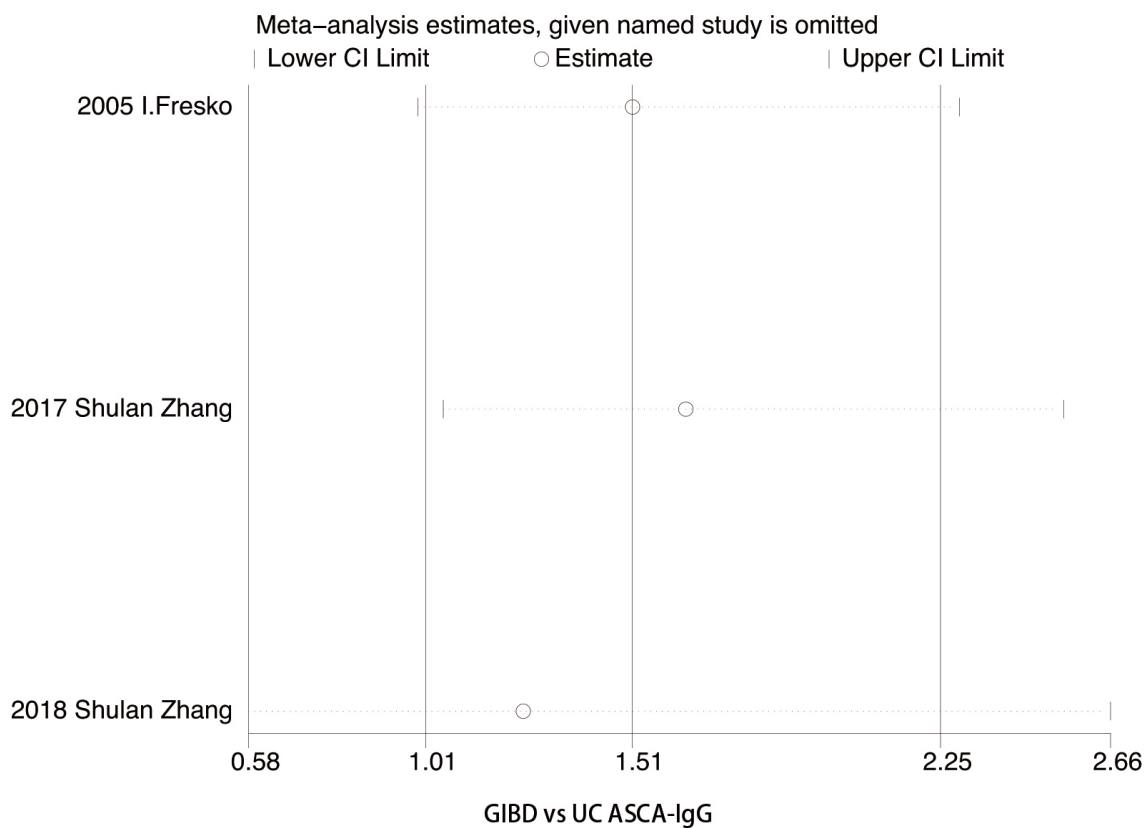
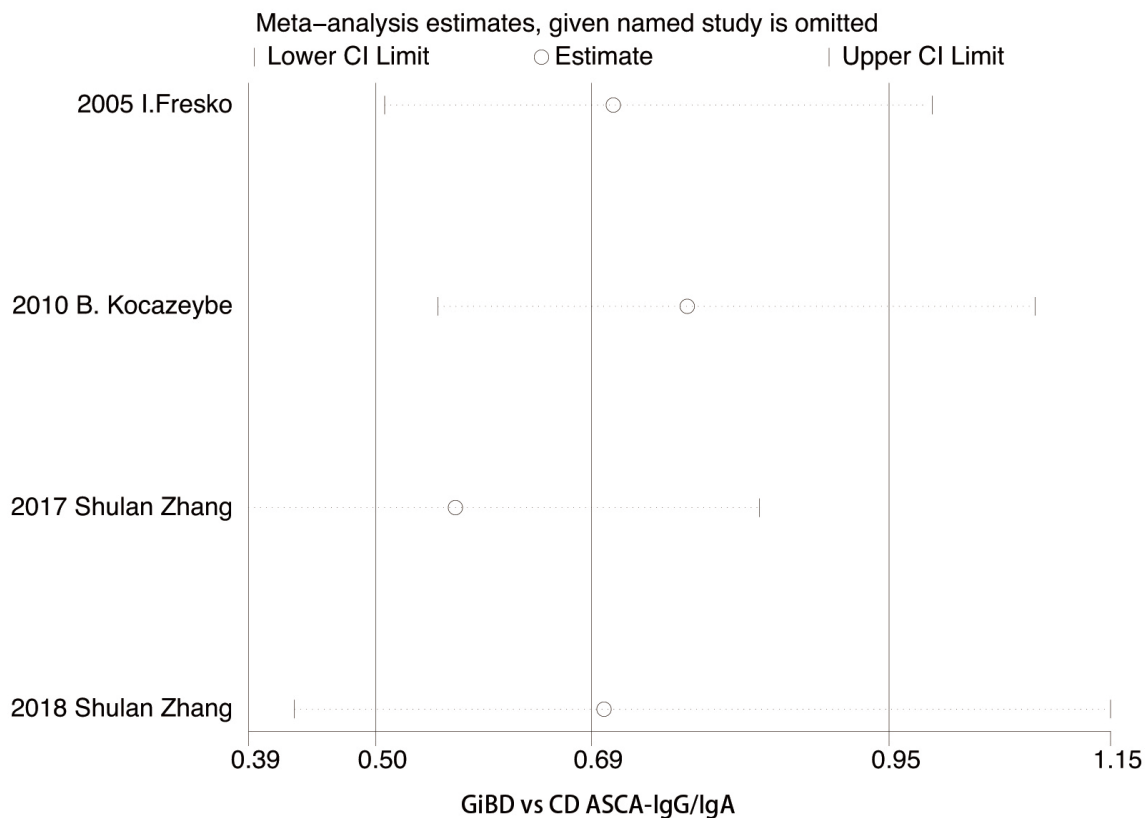


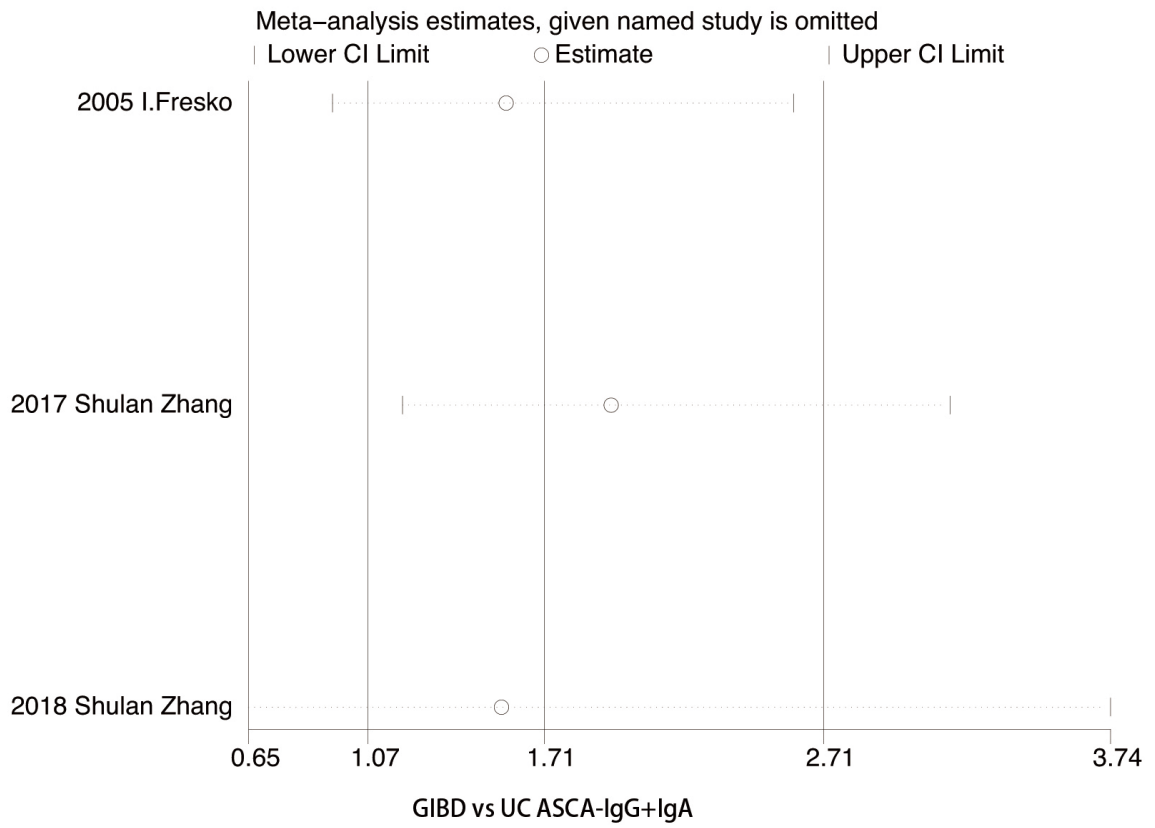
Fig S1 Results of quality assessment of the included studies based on the QUADAS-1 tool











Review only



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

Page 1 of 2

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, Thoms 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

1	strategy	investigators)	
2			
3	Search	#4b Search strategy, including time period included in the	5, 6
4	strategy	synthesis and keywords	
5			
6	Search	#4c Effort to include all available studies, including contact	7
7	strategy	with authors	
8			
9			
10	Search	#4d Databases and registries searched	5, 6
11	strategy		
12			
13			
14	Search	#4e Search software used, name and version, including	n/a
15	strategy	special features used (eg, explosion)	
16			The search was performed on website of
17			databases
18			
19			
20			
21			
22	Search	#4f Use of hand searching (eg, reference lists of obtained	6
23	strategy	articles)	
24			
25			
26	Search	#4g List of citations located and those excluded, including	6, 7
27	strategy	justification	
28			
29			
30	Search	#4h Method of addressing articles published in languages	6
31	strategy	other than English	
32			
33			
34	Search	#4i Method of handling abstracts and unpublished studies	6
35	strategy		
36			
37			
38	Search	#4j Description of any contact with authors	7
39	strategy		
40			
41			
42		#5a Description of relevance or appropriateness of studies	7
43		gathered for assessing the hypothesis to be tested	
44			
45			
46		#5b Rationale for the selection and coding of data (eg,	7
47		sound clinical principles or convenience)	
48			
49			
50		#5c Documentation of how data were classified and coded	7
51		(eg, multiple raters, blinding, and interrater reliability)	
52			
53			
54		#5d Assessment of confounding (eg, comparability of cases	7
55		and controls in studies where appropriate)	
56			
57			
58		#5e Assessment of study quality, including blinding of quality	7
59			
60			

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

1	#7c	Assessment of quality of included studies	15, 16
2			
3	Conclusion		
4			
5			
6	#8a	Consideration of alternative explanations for observed results	17
7			
8			
9			
10	#8b	Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)	17
11			
12			
13			
14			
15	#8c	Guidelines for future research	17
16			
17			
18	#8d	Disclosure of funding source	18
19			

20 Reproduced with permission from JAMA. 2000. 283(15):2008-2012. Copyright © 2000 American
21 Medical Association. All rights reserved. This checklist was completed on 26. August 2019 using
22 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
23 [Penelope.ai](#)
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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

Journal:	<i>BMJ Open</i>
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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4 1 **Meta-analysis of anti- *Saccharomyces cerevisiae* antibodies as diagnostic**
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7 2 **markers of Behçet's disease with gastrointestinal involvement**
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14 4 **Linlin Cheng, MD,^a Liubing Li, MD,^a Chenxi Liu, MD,^a Songxin Yan, MS,^a Yongzhe**
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16 5 **Li, PhD^{a*}.**

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7 18 **markers of Behçet's disease with gastrointestinal involvement**
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14 20 **Abstract**

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17 21 **Objective:** Due to common exposure to yeast in the alcoholic and baking industry, positive
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20 22 rate of anti-*Saccharomyces cerevisiae* antibodies (ASCA) is reportedly high in patients
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23 23 with Behçet's disease (BD) who have gastrointestinal symptoms (gastrointestinal BD
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26 24 [GIBD]). We performed a meta-analysis to assess the diagnostic value of ASCA in
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28 25 differentiating patients with BD from those with other chronic inflammatory bowel
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31 26 diseases.

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34 27 **Methods:** The meta-analysis is compliant with the PRISMA and MOOSE checklist.

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37 28 Relevant studies that investigated ASCA levels in BD patients were retrieved from
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40 29 PubMed, EMBASE, Web of Science, SCOPUS, and the Cochrane Library on July 12,
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43 30 2019; the search was rerun on February 12, 2020. Stata/SE 12.0 and Meta-DiSc 1.4 were
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46 31 used to perform the meta-analysis and sensitivity analysis, disaggregated by isotypes of
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49 32 ASCA.

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52 33 **Results:** Nine studies were included in the meta-analysis. The results revealed a strong
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55 34 association between ASCA and GIBD, especially ASCA-IgG [odds ratio (OR)=5.50 (95%

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4 35 CI 2.58–11.55, p=0.000) and ASCA-IgG+IgA [OR=5.36 (95% CI 1.40–20.45), p=0.014].
5
6

7 36 The positivity rate of ASCA in GIBD was significantly higher than that in ulcerative colitis:
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9 37 IgA [OR=2.13 (95% CI 1.30–3.50), p=0.003]; IgG+IgA [OR=2.19 (95% CI 1.03–4.66),
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11 38 p=0.042]; IgG/IgA [OR=2.03 (95% CI 1.30–3.17), p=0.002]. However, the frequency of
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13 39 ASCA-IgG was significantly higher in patients with Crohn's disease than GIBD [OR=5.36
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15 40 (95% CI 1.40–20.45), p=0.009]. There was no significant difference in ASCA positivity
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17 41 between BD without gastrointestinal involvement and healthy controls and between GIBD
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19 42 and intestinal tuberculosis (p>0.05).
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26 43 **Conclusion:** ASCA may play a role in the pathogenesis of gastrointestinal involvement.
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28 44 Negative result of IgG favors the diagnosis of GIBD/BD when differentiated from Crohn's
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30 45 disease. ASCA-IgA showed moderate diagnostic performance in distinguishing GIBD and
31

32 46 ulcerative colitis and the diagnostic performance was better in combination with IgG.
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34 47 However, ASCA may not be a useful serologic marker distinguishing GIBD and intestinal
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36 48 tuberculosis.
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44 49 Key words: Behçet's disease; Anti-*Saccharomyces cerevisiae* antibodies; autoimmune
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46 50 diseases; meta-analysis; autoantibodies
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54 Strengths and limitations of this study

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

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78 71 **Introduction**
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11 72 Behçet's disease (BD) is a chronic systemic vascular autoimmune/inflammatory disease
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13 73 with a high propensity for recurrence; the pathogenetic mechanisms of this disease are not
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16 74 well elucidated [1]. Virtually no specific histological or laboratory features of BD have
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18
19 75 been identified. Therefore, the diagnosis of BD is typically challenging as it is mainly based
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22 76 on clinical features [2, 3]. The diagnosis is frequently delayed until the development of
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25 77 clinical manifestations that qualify the diagnostic criteria. The estimated duration between
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28 78 the onset of symptoms and the fulfilment of diagnostic criteria is approximately 4 years
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30 79 [4].

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34 80 Moreover, patients with prominent involvement of a particular organ system are easily
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37 81 misdiagnosed. For example, patients who have gastrointestinal symptoms as the main
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40 82 manifestation are liable to be misdiagnosed as having Crohn's disease (CD), ulcerative
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43 83 colitis (UC), or intestinal tuberculosis (iTb). These features make formulating disease
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45 84 criteria difficult, causing deleterious effects on the patients.

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48 85 Several recent studies (but not all) have reported the diagnostic value of anti-
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51 86 *Saccharomyces cerevisiae* antibody (ASCA) in BD. *Saccharomyces cerevisiae*, also
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54 87 known as the baker's or brewer's yeast, has long been utilized to ferment the sugars in
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4 88 cereals to produce alcoholic beverages; it is also used in the baking industry to raise dough.
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7 89 As a consequence, we are now commonly exposed to yeast [5]. IgG and IgA antibodies
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10 90 against the phosphopeptidomannan of the *S. cerevisiae* cell wall have been discovered as
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12 91 autoantibodies in the sera of patients with BD, especially those with gastrointestinal
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15 92 involvement. This suggests a role of environmental stimuli in the pathogenesis of BD.
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18 93 However, patients with inflammatory bowel disease such as Crohn's disease (CD) also
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21 94 have a high prevalence rate of ASCA due to their similarities [6, 7, 8, 9, 10, 11]. In this
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24 95 context, identification of ASCA as a diagnostic marker for BD is a key imperative. The
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27 96 objectives of this study were to summarize the findings pertaining to the relevance of
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30 97 ASCA in BD and other gastrointestinal diseases and to perform a meta-analysis to assess
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33 98 its diagnostic accuracy for BD.
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100 **Methods**

101 Study design

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

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4 106 the review [14]. Besides, a predefined protocol was registered with PROSPERO
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7 107 (Registration No. CRD42020115245).
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109 Literature search

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

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8 125 Eligibility and exclusion criteria
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11 126 The inclusion criteria were: (1) studies that evaluated the diagnostic accuracy of ASCA in
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14 127 BD; (2) availability of adequate data pertaining to the prevalence rate or serum levels of
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17 128 ASCA in patients with BD; (3) studies with healthy population and/or disease controls; (4)
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19 129 meeting abstracts or letters to the editor were also included.
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23 130 The exclusion criteria were: (1) studies with incomplete data; (2) review articles; (3) non-
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26 131 English articles; (4) in case of studies with overlapping study population, studies with
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29 132 smaller sample size were excluded. Two investigators independently performed the
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32 133 literature search, screened the titles and abstracts, followed by full-text review of eligible
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34 134 studies.
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41 136 Data extraction and quality assessment
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44 137 Two independent investigators reviewed the full-text articles, extracted the data, and
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47 138 assessed the study quality using the Quality Assessment of Diagnostic Accuracy Studies
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50 139 (QUADAS-2); The included items were evaluated as yes, no, or uncertain. Inter-researcher
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53 140 disagreements were resolved by consensus, or by a third investigator. Data pertaining to
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56 141 the following variables were extracted: publication year, article type, first author's name,
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4 142 country, isotypes of ASCA detected, age and sex, research design, sample size,
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7 143 experimental method, trade names of experimental materials, cut-off values, diagnostic
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10 144 criteria, and serum titers and/or prevalence rate of ASCA in BD, gastrointestinal BD
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12 145 (GIBD), healthy controls (HC), patients with Crohn's disease (CD), ulcerative colitis (UC),
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15 146 and intestinal tuberculosis (iTb). The data were either obtained directly from the article,
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18 147 calculated, or requested from the author via e-mail.
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24 149 Statistical analysis

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28 150 Pooled odds ratios (OR) with 95% confidence intervals (CI) were calculated to evaluate
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31 151 the association between ASCA and BD (without gastrointestinal
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34 152 involvement)/GIBD/CD/UC/iTB using Stata/SE 12.0. Meta-DiSc 1.4 was used to calculate
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37 153 the sensitivity, specificity, AUC values, and the area under the summary receiver operating
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40 154 characteristic (SROC) curve to assess the overall diagnostic performance of ASCA.
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42 155 Heterogeneity among the included studies was evaluated using the Cochran's Q-statistic.
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45 156 P values > 0.10 were considered indicative of lack of significant heterogeneity. We chose
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48 157 the random effects models (REM) since REM tends to generalize findings beyond the
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51 158 included studies by assuming that the selected studies are random samples from a larger
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54 159 population [15]. Subgroup analysis was performed disaggregated by the isotypes of ASCA
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57 160 and different disease controls. The isotypes of ASCA were classified and defined as

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4 161 follows: IgG, IgA, IgG/IgA (positive results of either IgG or IgA), and IgG+IgA (positive
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7 162 results of both IgG and IgA).
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10 163 In order to increase the robustness of the meta-analysis, we also extracted the data
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13 164 pertaining to serum levels of ASCA from five studies and performed meta-analysis using
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16 165 the Continuous data module of Stata/SE 12.0. The REM was used for the analysis and
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19 166 weighted mean difference (WMD) was used as the effect measure if the same unit was
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22 167 used in these studies and there were minor differences with respect to the serum levels of
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24 168 ASCA. Sensitivity analysis was performed using Stata/SE 12.0 to evaluate stability of the
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27 169 results after sequential exclusion of one study at a time.
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33 171 Patient and public involvement 34 35 36

37 172 The present study was a meta-analysis and systematic review based on published data.
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40 173 Patients and public were not involved in the study design, conduct, data analysis, and result
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43 174 dissemination.
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49 176 Relationship between ASCA and autoimmune disease 50 51 52

53 177 We searched the PubMed for studies pertaining to the relationship between ASCA and
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56 178 autoimmune diseases. The two search terms used were autoimmune disease and
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4 179 *Saccharomyces cerevisiae*. We performed an interval statistic of four indicators of ASCA–
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7 180 sensitivity, specificity, positive likelihood (LR+) and negative likelihood (LR-) based on
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10 181 the included studies sorted by diseases.

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14 15 16 183 **Results**

17 18 19 20 184 Literature search and characteristics of studies

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23 185 A total of 625 documents were retrieved on database and manual search. Fifty-one
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26 186 duplicate publications were excluded using the document management software. A total of
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29 187 127 records were retained after screening of titles and/or abstracts; the excluded records
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32 188 included review articles, animal model studies, therapeutic or drug research, genetic
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35 189 research, book chapters, duplicate publications not recognized by software, and other
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38 190 irrelevant records. After full-text review for eligibility, 22 records were selected. Finally,
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41 191 we included 9 available studies with adequate data in the meta-analysis (Figure 1). Two
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44 192 studies were included after obtaining the relevant data by contacting the respective authors
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47 193 [9, 10]. In addition, we also verified 2 studies [16, 17] with overlapping study population;
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50 194 of these, only 1 study was included in the meta-analysis. Three studies [6, 8, 18] were
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53 195 presented as meeting abstracts without adequate data to allow the construction of a 2×2
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56 196 table. One article[7] was a letter to the editor and only reported the prevalence rate of

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4 197 ASCA antibody in patients with BD, without information about the control group. One
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7 198 study [19] had employed a unique calculation method and could not be included in the
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10 199 meta-analysis. Among the included studies, there were 326 cases of BD, 294 cases of
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12 200 GIBD, 520 cases of CD, 598 cases of UC, 112 cases of iTB, and 428 HCs (Table 1 and
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15 201 supplementary file 4).

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21 203 Quality assessment

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25 204 There were 8 case-control studies and 1 retrospective study [9]. The results of quality
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28 205 assessment by QUADAS-2 including the risk of bias and applicability concerns pertaining
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31 206 to each domain [20] are shown in supplementary file 5. The results indicated that the
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34 207 included studies were of high quality in general. Overall, none of the 9 included studies
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37 208 showed any major methodological bias or flaws, which indicates robustness of our meta-
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39 209 analysis.

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44 45 46 211 Meta-analysis

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50 212 Association between ASCA and BD (without gastrointestinal involvement), GIBD and
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52 213 other intestinal diseases

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56 214 Data pertaining to correlation between ASCA and BD (without gastrointestinal
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4 215 involvement)/GIBD/CD/UC/iTB are listed in Table 2. No substantial heterogeneity
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7 216 ($p>0.1$ for all) was observed by using REM to calculate the OR. The results revealed a
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10 217 strong association between all detection types of ASCA and GIBD, especially for ASCA-
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12 218 IgG [OR=5.50 (95% CI 2.58–11.55, $p=0.000$) and ASCA-IgG+IgA [OR=5.36 (95% CI
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15 219 1.40–20.45), $p=0.014$]. When comparing GIBD and UC, of the positivity rate for ASCA
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18 220 in GIBD was significantly higher than that for UC: IgA [OR=2.13 (95% CI 1.30–3.50),
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20 221 $p=0.003$], IgG+IgA [OR=2.19 (95% CI 1.03–4.66), $p=0.042$], and IgG/IgA [OR=2.03
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22 222 (95% CI 1.30–3.17), $p=0.002$]. Conversely, the frequency of only ASCA-IgG in patients
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25 223 with CD was significantly higher than that in the GIBD [OR=5.36 (95% CI 1.40–20.45),
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28 224 $p=0.009$]. Further, on stratified analysis according to detection method, ASCA-IgG was
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31 225 associated with GIBD using both the ELISA method (OR = 3.83, 95% CI 1.37–10.70, p
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33 226 = 0.010) and the immunoprecipitation method (IIF) (OR = 8.17, 95% CI 2.73–24.43, p =
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36 227 0.000) (Figure 2). However, no significant difference was observed with respect to
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39 228 ASCA positivity between BD without gastrointestinal involvement and HC and between
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42 229 GIBD and iTB ($p>0.05$).

230 Diagnostic ability of ASCA for GIBD

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49 231 The overall sensitivity for ASCA-IgG in patients with GIBD detected by IIF was 0.44,
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52 232 which is much higher than that of ELISA [0.20 (95%CI 0.12–0.31)] (Table 3). Combined
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55 233 detection of IgG and IgA by ELISA increased the sensitivity to 0.33 (95% CI 0.23–0.44).

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4 234 However, we observed a low level of sensitivity of ASCA-IgG/IgA by IIF, which may be
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7 235 attributable to the inclusion of only one study with few GIBD patients (n=13).
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10 236 Difference in serum levels of ASCA in GIBD and other intestinal diseases
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14 237 Serum levels of ASCA-IgA observed in GIBD were significantly greater than that in HC
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16 238 [WMD=7.02 (95% CI 2.23–11.81), p=0.004] and UC [WMD=5.28 (95% CI 0.39–10.17),
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19 239 p=0.034] in contrast to ASCA-IgG (p>0.05) (Figure 3). On the contrary, serum levels of
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22 240 ASCA-IgG in CD were significantly greater than that in GIBD [WMD=-11.04 (95% CI -
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25 241 16.74--5.34), p=0.000] (Figure 3). However, we found no significant difference in serum
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28 242 levels of ASCA between BD without gastrointestinal symptoms and HC (p>0.05) (Figure
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37 245 Heterogeneity and sensitivity analysis
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41 246 We performed sensitivity analysis to assess the stability of the results. The results showed
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44 247 that the studies by Krause et al (2002), Zhang et al (2018), Kocazeybe et al (2010), and
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46 248 Fresko et al (2005) were the key contributors to the heterogeneity (supplementary file 6).
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49 249 Thus, the results of related subgroup analysis are considered to be less stable.
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56 251 Summary of the relationship of ASCA with autoimmune disease
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4 252 Sixteen studies reporting the relevance of ASCA and autoimmune diseases were included
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7 253 in the summary. The sensitivity, specificity, LR+, and LR- of ASCA for different
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10 254 autoimmune diseases are summarized in Table 4. Although the diagnostic results of ASCA
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12 255 reported by different studies vary, the summary revealed an overall association between
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15 256 ASCA and autoimmune diseases especially in patients with scleroderma, juvenile
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17 257 idiopathic arthritis, Crohn's disease, and systemic lupus erythematosus with high SEN
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20 258 (>40%), high SPE (>95), high LR+ (>5) (Table 4).
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27 260 **Discussion**

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31 261 **Serological markers in BD.** The diagnosis of BD is typically challenging prior to the
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33 262 appearance of clinical symptoms necessary to qualify the diagnostic criteria. Currently,
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35 263 there are no specific laboratory biomarkers of BD; however, some specific autoantibodies
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37 264 in the context of BD have been reported. Therefore, identification of non-invasive specific
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39 265 diagnostic and prognostic biomarkers of BD is of much clinical relevance and a key focus
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42 266 area of research.
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48 267 **ASCA in BD and autoimmune diseases.** Several recent studies have investigated the
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50 268 relationship of ASCA with BD or other autoimmune diseases. *Saccharomyces cerevisiae*
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52 269 has long been utilized in alcoholic and baking industry, and for the production of vaccines
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4 270 owing to its antigenic component. However, during long-term and ubiquitous presence,
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7 271 even the commensal and classically non-pathogenic microbiota can trigger autoimmunity
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10 272 due to loss of immune tolerance towards the resident bacterial flora, like in gastrointestinal
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12 273 tract [21, 22]. The reported similarity of sequences involving the eukaryotic microorganism
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15 274 and self-antigens suggest a mechanism of molecular mimicry and also the plausibility of
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18 275 shared epitopes in different autoimmune diseases. The production of ASCA by the
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21 276 subsequent activation of the humoral immune response may lead to a direct pathogenic role
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24 277 through a costimulatory CD80/86-CD28-mediated effect [21]. Moreover, healthy family
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27 278 members but not spouses of BD patients were also found to have increased levels of ASCA,
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30 279 which indicated a role of genetic factors in addition to environmental stimuli [17, 22]. A
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33 280 large number of studies have assessed the role of ASCA in the context of several systemic
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36 281 and organ-specific autoimmune diseases, such as BD, scleroderma, systemic lupus
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39 282 erythematosus, primary Sjögren's syndrome, and rheumatoid arthritis (Table 4). The
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42 283 results suggest that the relation of ASCA with BD or other autoimmune diseases may
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45 284 represent a potential pathogenic mechanism between ASCA and autoimmunity; this
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48 285 underlines the importance of ASCA as a valuable serologic marker for autoimmune
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51 286 diseases including BD.

51 287 **Results of the meta-analysis.** To the best of our knowledge, this is the second meta-
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54 288 analysis of evidence pertaining to autoantibodies in patients with BD after anticardiolipin
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4 289 antibodies [23]. ASCA have been widely researched in BD, Crohn's disease and other
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7 290 autoimmune disease; in order to investigate the diagnostic value and possible pathogenetic
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10 291 role of ASCA in BD, we included 9 studies in this meta-analysis. Among these, some
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12 292 studies included BD patients with systemic involvement including or excluding
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15 293 gastrointestinal involvement, while others included only BD patients with gastrointestinal
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18 294 involvement. Therefore, in order to reduce the impact of differences with respect to
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21 295 frequency distribution of gastrointestinal symptoms in each study, we disaggregated
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24 296 patients with BD into those with gastrointestinal involvement only and those without
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27 297 gastrointestinal manifestations. The isotype antibodies of ASCA tested and the results
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30 298 presented by the studies are also different. The meta-analysis revealed a strong association
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33 299 of ASCA with GIBD and not with BD with no gastrointestinal involvement; this suggests
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36 300 the role of ASCA in the pathogenesis of gastrointestinal involvement. ASCA showed a
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39 301 moderate diagnostic performance as a biomarker for the differential diagnosis between
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42 302 GIBD and CD, and the negative result of ASCA-IgG may slightly favor the diagnosis of
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45 303 GIBD/BD when compared with CD, especially with concomitant positive HLA-B51 tests
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48 304 [24]. In addition, ASCA-IgA showed a moderate diagnostic value for distinguishing GIBD
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51 305 and UC and would perform better with concomitant detection of IgG. However, ASCA
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54 306 failed to distinguish between GIBD and iTB. Besides, the concomitant evaluation of both
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57 307 continuous data (sensitivity and specificity) and discontinuous data (serum levels) helped
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60 308 increase the credibility of our results.

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4 309 **Heterogeneity.** Combined with the results of QUADAS-2, we found that the heterogeneity
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7 310 in this meta-analysis was largely attributable to the following reasons (see Table 1,
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10 311 supplementary file 5). 1) The different diagnostic criteria used in the included studies.
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12 312 Different criteria may have different thresholds for diagnosis or place more weight on some
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15 313 symptoms than others. Specifically, the 1990 ISG criteria requires the presence of oral
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18 314 ulceration plus any two of the following: genital ulceration, typical eye lesions, typical skin
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21 315 lesions, or positive pathergy test for diagnosis of BD [2]. In contrast, the 1987 Japan criteria
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24 316 require all four characteristics for the diagnosis of BD, i.e., oral ulceration, typical eye
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27 317 lesions, typical skin lesions, and genital ulceration [25]. The ISG criteria and the Japanese
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30 318 criteria often fail to classify some patients with BD; in addition, the Japanese criteria may
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33 319 also cause misclassified diagnosis. This may have caused the different diagnostic
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36 320 sensitivity and specificity for BD [26]. 2) Differences in demographic characteristics of
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39 321 included studies. The clinical features and laboratory findings tend to exhibit wide
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42 322 variability in different populations and clinical settings [27, 28]. However, we failed to
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45 323 perform subgroup analysis disaggregated by ethnicity owing to the small sample size in
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48 324 each subgroup. 3) Different antibody assays and cut-off values. Commercial kits and in-
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51 325 house tests from different laboratories have variable performance, which may affect the
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54 326 diagnosis and management of patients. We found that different methods and cut-off values
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57 327 were adopted by the studies included in this meta-analysis. Notably, there was significant
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60 328 association between ASCA-IgG and GIBD using both methods; however, it seems that IIF

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4 329 has a higher sensitivity than ELISA. Nonetheless, further investigations with larger study
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7 330 population are required to provide more definitive evidence. Although previous studies
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10 331 have shown that IIF has a better performance, ELISA provides the titer change of serum
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12 332 antibodies and could have an equal performance to IIF by changing cut-off values to
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15 333 optimize the overall diagnostic performance [29]. 4) According to the QUADAS-2, there
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18 334 are certain concerns that most studies have risk of bias (internal validity) in patient
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21 335 selection, which, to some extent, would cause the the distorted estimation in diagnostic
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24 336 accuracy [20].

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26 337 **Limitations of the meta-analysis.** In order to fully evaluate the value of ASCA for
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29 338 differential diagnosis of BD, we included patients with CD, UC, and iTB as the comparison
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32 339 objects in our meta-analysis. However, there are some limitations of this meta-analysis. (1)
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35 340 Gray literature database, paper database, and other language databases were not used for
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38 341 the literature search. Quite a few non-English studies were excluded due to incomplete data
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41 342 or unavailability of full text. (2) Our primary goal was to assess the diagnostic efficacy of
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44 343 ASCA in BD, and therefore we did not include all studies pertaining to ASCA in
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47 344 inflammatory bowel disease and iTB. (3) Restricted by the number of included studies and
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50 345 the isotypes of ASCA, we could not perform subgroup analysis disaggregated by different
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53 346 populations and diagnostic criteria. (4) Some studies with incomplete data were excluded
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56 347 after lack of response from the author. (5) There are some inherent statistical shortcomings

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4 348 using Meta-DiSc during the separate pooling of sensitivity and specificity, as the between-
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7 349 study variance is not included. More advanced methods are not implemented [30].
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10 350 **Conclusion.** Our study demonstrated the relationship between *ASCA/Saccharomyces*
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13 351 *cerevisiae* and gastrointestinal involvement in BD. Furthermore, ASCA may be detectable
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16 352 years before the diagnosis of some autoimmune diseases as they were retrospectively found
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19 353 in the preserved blood samples of soldiers who were affected by Crohn's disease years
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22 354 later [31]. However, detection of only ASCA may have a limited value for clinical
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25 355 diagnosis due to its moderate sensitivity and the presence in several other autoimmune
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28 356 diseases. In the future, further studies are needed to explore the role of ASCA and
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30 357 *Saccharomyces cerevisiae* in BD.
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359 **Compliance with Ethical Standards:**

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

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369 **Ethical approval:** This article does not contain any studies with human participants
370 performed by any of the authors.

371 **Data availability statement:** All data relevant to the study are included in the article or
372 uploaded as supplementary information.

373

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3 584 Figure 1 PRISMA flow diagram illustrating the literature screening process and the
4 585 criteria for inclusion of studies in the meta-analysis
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8 586 Figure 2 Forest plot of the association between the presence of ASCA-IgG and GIBD
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10 587 stratified by detection methods
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14 588 Figure 3 Forest plot comparing serum levels of ASCA between BD without
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16 589 gastrointestinal symptom/GIBD and HC/CD/UC
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4 601 Table 1 Characteristics of studies included in the meta-analysis of Anti-*Saccharomyces*
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6 602 *cerevisiae* antibodies in Behçet's disease, its main differential diagnoses, and healthy
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9 603 controls.
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Year and author	Count ries	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

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

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614 Table 2. Association between the presence of ASCA and BD (without gastrointestinal
615 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

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

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

Methods	ELISA		IIF	
	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

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

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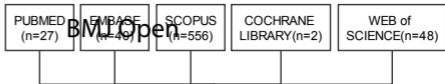
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635 Table 4 Summary of the diagnostic performance of ASCA in autoimmune disease

Reference	Autoimmune disease	Type	SEN (%)	SPE (%)	LR+	LR-	Supplementary information
7	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
8 [28]		IgA	16.22	94.74	3.08	0.88	
10, 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
12		IgA	1.28–23.26	91.03–100.00	2.59–3.71	0.84–0.99	
13	Antiphospholipid syndrome	IgG/IgA	20.00	95.00	/	/	
14 [44]	Juvenile Idiopathic Arthritis	IgA	0–50.00	94.74	9.50	0.53	
16	Autoimmune hepatitis	IgG	16.42–27.53	100.00	/	0.84	
17, 46, 47, 48		IgA	11.94	94.74	2.27	0.93	
18		IgG/IgA	18.52	84.00	1.16	0.97	
19	Primary biliary cirrhosis	IgG	10.57–18.95	97.50–100.00	7.58	0.83–0.89	
20, 48, 49		IgA	11.58–18.70	94.74–98.75	3.55–9.26	0.86–0.90	
21		IgG/IgA	20.26–24.21	84.00–96.25	1.27–6.46	0.79–0.95	
22	Primary sclerosing cholangitis	IgG	28.00	100.00	/	0.72	
23, 46, 48		IgA	32.00	94.74	6.08	0.72	
24		IgG/IgA	30.51	84.00	1.91	0.83	
25	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.
26, 40, 46, 50, 51		IgA	19.30–71.43	94.74–100.00	9.91–29.40	0.50–0.71	
27	Cryoglobulinemia	IgG	7.10	99.50	/	/	
28, 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
29		IgA	8.40–16.67	94.17–96.88	2.69–2.86	0.88–0.95	
30	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
31, 54		IgA	17.72–40.00	91.03–94.74	1.97–7.60	0.63–0.90	
32		IgM	13.33	94.74	2.53	0.91	
33	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
34, 55, 56		IgA	7.50–12.07	94.74–99.38	1.43–19.31	0.88–0.98	
35		IgG/IgA	31.90	96.25	8.51	0.71	
36	Type 1 diabetes	IgG	20.98	98.09	10.98	0.81	
37, 57		IgA	9.82	98.73	7.71	0.91	
38		IgG/IgA	24.55	97.45	9.64	0.77	
39	Primary Sjögren's syndrome	IgG/IgA	4.81	100.00	/	0.95	ASCA positivity was associated with pSS specific clinical and serological features
40	Vasculitides	IgG	6.50	99.50	/	/	

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



1 Additional records identified through
2 other sources(n=0)

Records identified through
database searching(n=625)

3
4 Records after duplicates
removed by software(n=574)

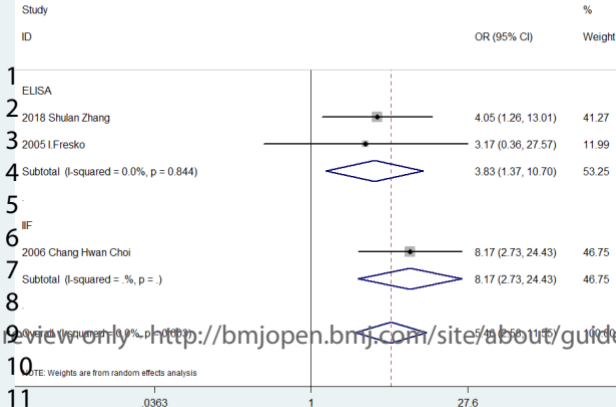
5
6 Records after title and/or
abstract screening(n=127)

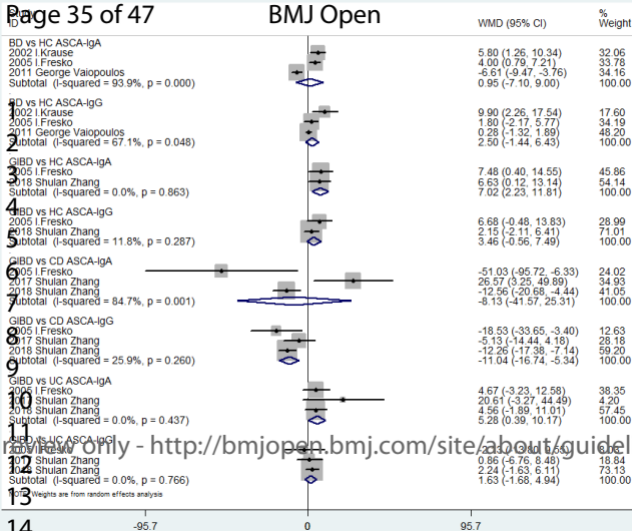
Records excluded by manual
search and the irrelevant(n=447)

7
8 Full-text articles assessed for
eligibility (n=22)

Full-text articles excluded with
reasons(n=105)

9
10
11 Studies included in the
qualitative synthesis(meta-
analysis)(n=9)
12







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

Page 1 of 2

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, Thoms 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 MOOSE reporting 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

1		#3b	Hypothesis statement	4-5
2				
3		#3c	Description of study outcomes	n/a
4				
5				
6				The study outcomes
7				were reported in the
8				section of Results an
9				d Discussion.
10				
11		#3d	Type of exposure or intervention used	4
12				
13		#3e	Type of study designs used	4
14				
15		#3f	Study population	4, 5
16				
17				
18				
19	Methods			
20				
21				
22	Search	#4a	Qualifications of searchers (eg, librarians and	6, 7
23	strategy		investigators)	
24				
25				
26	Search	#4b	Search strategy, including time period included in the	5, 6
27	strategy		synthesis and keywords	
28				
29				
30	Search	#4c	Effort to include all available studies, including contact	7
31	strategy		with authors	
32				
33				
34	Search	#4d	Databases and registries searched	5, 6
35	strategy			
36				
37	Search	#4e	Search software used, name and version, including	n/a
38	strategy		special features used (eg, explosion)	
39				The search was perf
40				ormed on website of
41				databases
42				
43				
44				
45	Search	#4f	Use of hand searching (eg, reference lists of obtained	6
46	strategy		articles)	
47				
48				
49	Search	#4g	List of citations located and those excluded, including	6, 7
50	strategy		justification	
51				
52				
53	Search	#4h	Method of addressing articles published in languages	6
54	strategy		other than English	
55				
56	Search	#4i	Method of handling abstracts and unpublished studies	6
57	strategy			
58				
59				
60				

1	Search	#4j	Description of any contact with authors	7
2	strategy			
3				
4				
5		#5a	Description of relevance or appropriateness of studies	7
6			gathered for assessing the hypothesis to be tested	
7				
8				
9		#5b	Rationale for the selection and coding of data (eg,	7
10			sound clinical principles or convenience)	
11				
12		#5c	Documentation of how data were classified and coded	7
13			(eg, multiple raters, blinding, and interrater reliability)	
14				
15				
16		#5d	Assessment of confounding (eg, comparability of cases	7
17			and controls in studies where appropriate)	
18				
19				
20		#5e	Assessment of study quality, including blinding of quality	7
21			assessors; stratification or regression on possible	
22			predictors of study results	
23				
24				
25		#5f	Assessment of heterogeneity	8
26				
27				
28		#5g	Description of statistical methods (eg, complete	8
29			description of fixed or random effects models,	
30			justification of whether the chosen models account for	
31			predictors of study results, dose-response models, or	
32			cumulative meta-analysis) in sufficient detail to be	
33			replicated	
34				
35				
36				
37		#5h	Provision of appropriate tables and graphics	n/a
38				
39				
40				
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46				
47	Results			
48				
49		#6a	Graphic summarizing individual study estimates and	10-12
50			overall estimate	
51				
52				
53		#6b	Table giving descriptive information for each study	9, 10
54			included	
55				
56				
57		#6c	Results of sensitivity testing (eg, subgroup analysis)	12
58				
59				
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The methods were described in text instead of tables and graphics.

1	#6d	Indication of statistical uncertainty of findings	12
2			
3	Discussion		
4			
5			
6	#7a	Quantitative assessment of bias (eg. publication bias)	n/a
7			
8			Small number of
9			studies in each
10			subgroup prevented
11			publication bias
12			analysis
13			
14			
15			
16	#7b	Justification for exclusion (eg, exclusion of non–English-	16
17		language citations)	
18			
19			
20	#7c	Assessment of quality of included studies	15, 16
21			
22	Conclusion		
23			
24			
25	#8a	Consideration of alternative explanations for observed	17
26		results	
27			
28			
29	#8b	Generalization of the conclusions (ie, appropriate for the	17
30		data presented and within the domain of the literature	
31		review)	
32			
33			
34	#8c	Guidelines for future research	17
35			
36	#8d	Disclosure of funding source	18
37			

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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 (saccharomyces 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

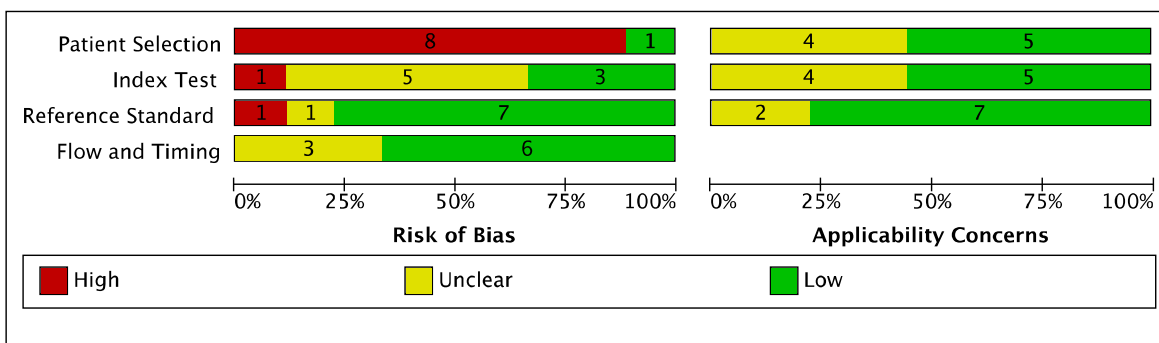
BD: Behçet's disease; GIBD: gastrointestinal Behçet's disease; CD: Crohn's disease; UC: ulcerative colitis; iTB: intestinal tuberculosis; HC: healthy control; /: No such group included; Blank: no such information in the article

	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

	<u>Risk of Bias</u>				<u>Applicability Concerns</u>		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
2002 Byeong Gwan Kim	-	?	+	+	+	+	+
2002 I. Krause	-	+	+	+	+	+	+
2005 I. Fresko	-	?	+	+	+	?	+
2005 Seung Ho Rhee	-	-	+	?	+	+	?
2006 Chang Hwan Choi	-	+	+	+	?	+	+
2010 B. Kocazeybe	-	?	?	?	?	?	?
2011 George Vaiopoulos	-	?	+	+	?	?	+
2017 Shulan Zhang	+	?	-	?	?	?	+
2018 Shulan Zhang	-	+	+	+	+	+	+

- High
 ? Unclear
 + Low



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