The Diagnostic Value of Polymerase Chain Reaction for *Mycobacterium tuberculosis* to Distinguish Intestinal Tuberculosis from Crohn's Disease: A Meta-analysis

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ABSTRACT

Background/Aim: Intestinal tuberculosis (ITB) and Crohn's disease (CD) are important differential diagnoses that can be difficult to distinguish. Polymerase chain reaction (PCR) for *Mycobacterium tuberculosis* (MTB) is an efficient and promising tool. This meta-analysis was performed to systematically and objectively assess the potential diagnostic accuracy and clinical value of PCR for MTB in distinguishing ITB from CD. **Materials and Methods:** We searched PubMed, Embase, Web of Science, Science Direct, and the Cochrane Library for eligible studies, and nine articles with 12 groups of data were identified. The included studies were subjected to quality assessment using the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. **Results:** The summary estimates were as follows: sensitivity 0.47 (95% CI: 0.42–0.51); specificity 0.95 (95% CI: 0.93–0.97); the positive likelihood ratio (PLR) 10.68 (95% CI: 6.98–16.35); the negative likelihood ratio (NLR) 0.49 (95% CI: 0.33–0.71); and diagnostic odds ratio (DOR) 21.92 (95% CI: 13.17–36.48). The area under the curve (AUC) was 0.9311, with a Q^* value of 0.8664. Heterogeneity was found in the NLR. The heterogeneity of the studies was evaluated by meta-regression analysis and subgroup analysis. **Conclusions:** The current evidence suggests that PCR for MTB is a promising and highly specific diagnostic method to distinguish ITB from CD. However, physicians should also keep in mind that negative results cannot exclude ITB for its low sensitivity. Additional prospective studies are needed to further evaluate the diagnostic accuracy of PCR.

Key Words: Crohn's disease, intestinal tuberculosis, meta-analysis, *Mycobacterium tuberculosis*, polymerase chain reaction

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Intestinal tuberculosis (ITB) and Crohn's disease (CD) are both chronic granulomatous inflammatory disorders.[1] Clinical, radiological, endoscopic, and histologic findings are similar between the two disorders, and these disorders have common underlying pathologies.[2,3] The overall resurgence of TB,[4] which was probably caused by the pandemic of human immunodeficiency virus (HIV) infection and the large wave of population migration,[5] has led to an increase in ITB. As the sixth most common presentation of extrapulmonary TB, ITB is observed in 11% of extrapulmonary TB patients.[6,7] CD, consisting of chronic relapsing mucosal inflammation that can affect any part of the gastrointestinal tract, results from a dysfunctional innate immune response.[8] The clinical misdiagnosis rate between CD and ITB ranges from 50 to 70%.[3,9-11] When ITB is misdiagnosed, unnecessary anti-TB therapy poses a risk of toxicity; in fact, a delayed diagnosis of ITB has been reported to carry a risk of intestinal stenosis, perforation, and septic shock.[7,12] In addition, severe deterioration or even death may occur if ITB is treated with immunosuppressive therapy.[13] Hence, identifying an

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accurate and rapid method of distinguishing CD from ITB is necessary and urgent.

Finding evidence of *Mycobacterium tuberculosis* (MTB) in intestinal tissues is the most credible way of distinguishing CD from ITB. At present, there are various methods that help in confirming the clinical diagnosis of ITB, such as the tuberculin skin test (TST), tuberculin acid-fast bacilli (AFB) staining, MTB culture, and interferon-gamma release assays (IGRAs). Unfortunately, the current routine methods for diagnosing TB are inefficient. For instance, AFB staining is not very sensitive and biopsy culture for MTB is time consuming (requiring 4–8 weeks).[14] In addition, the results of biopsy culture are frequently inaccurate (with accuracy ranging from 25 to 35%).[15] The IGRA is a new method based on MTB-specific antigens identified through genomic research.[16] However, the inability of T-cell-based IGRAs to distinguish between latent and active TB infection[17] partly limits the assays' utility in disease diagnosis.

Polymerase chain reaction (PCR) is an interesting and promising approach for differentiating ITB from CD, which is also a quick etiological diagnostic method. The procedure of TB-PCR is mainly divided into the following three parts: DNA extraction, DNA amplification, and DNA detection. The target sequence for PCR amplification is IS6110, which is a specific gene segment from *M. tuberculosis* that has not been detected in other mycobacteria or organisms.[18] Currently, MT-PCR is primarily applied in the clinical samples of fecal and biopsy. Many studies have explored the diagnostic value of PCR, with mixed results. To assess whether PCR can be used as an efficient diagnostic tool, we conducted a meta-analysis of published studies to derive a more precise and comprehensive assessment of the ability of PCR to differentiate ITB from CD.

MATERIALS AND METHODS

Data sources and search strategy

We performed a systematic search of PubMed, Embase, Web of Science, Science Direct, and the Cochrane Library for studies published through February 2015. The search terms were as follows: ("tuberculo*" or "TB") and ("polymerase chain reaction" or "PCR") and ("Crohn's disease" or "Crohn disease" or "CD"). Additional studies were identified via a manual review of the references to avoid missing potentially relevant studies.

Inclusion and exclusion criteria

The inclusion criteria used in this analysis were as follows: (1) The aim of the study was to explore the value of PCR in distinguishing ITB from CD, (2) the study used recognized criteria to diagnose ITB and CD, (3) the target gene sequence for PCR was IS6110 and the samples were intestinal tissue or feces, (4) the reported primary data were sufficient for separately calculating both the sensitivity and the specificity of PCR in diagnosing ITB or CD samples, and (5) the article was written in English. The exclusion criteria were as follows: (1) Duplicate articles that contained all or a selection of previously published data, (2) reviews, case reports, letters, conference proceedings, and comments, (3) studies without diagnostic criteria information, and (4) articles in a language other than English.

Data extraction and quality assessment

Information on author, year, and country of publication, mean patient age, total number of patients; study type, specimen source, technique, number of true positives, number of false positives, number of false negatives, and number of true negatives was obtained.

The quality of the eight included articles was assessed using the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool,[19] which was recommended by the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy.[20] The risk of bias was assessed for each domain, and the applicability of the first three domains was evaluated. Almost none of the articles reported whether the investigators were blinded, so we changed the signal question "Were the index test results interpreted without knowledge of the results of the reference standard?" to "Were the index test results interpreted without the investigators being affected by the results of the reference standard?"

The data extraction and quality assessment were performed independently by two authors (Ting Jin And Yu Zhang). Any discrepancies were discussed with a third author (Baoying Fei) until a consensus was reached.

Data analysis

We used Meta-Disc 1.4 software (XI Cochrane Colloquium, Barcelona, Spain) and Stata 12.0 software (Stata Corporation, College Station, TX, USA) for the data analysis. We calculated Spearman's correlation coefficient, Cochran's Q, and the inconsistency (I2) of the diagnostic odds ratio (DOR) to evaluate the existence of heterogeneity caused by a threshold or non-threshold effect. A $P < 0.05$ or I2 >50% indicated the existence of heterogeneity. We combined estimates of the sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and DOR. We also generated a symmetric receiver operator characteristic (SROC) curve to calculate the area under curve (AUC). If no threshold effect was detected but significant heterogeneity existed, regression meta-analysis and subgroup analysis were performed. Publication bias was additionally evaluated using Stata statistical software (version 12.0).

RESULTS

Selection of studies

We identified 538 studies in our initial search of the databases. We excluded 129 duplicate studies using EndNote software (Thomson Reuters, USA) and 378 irrelevant studies after screening the titles and abstracts; a total of 31 full-text articles remained for full-text screening. Of these publications, six articles contained repeated data and were therefore excluded. Another six articles were excluded because they were case reports, reviews, letters, or conference proceedings, and an additional six articles were excluded because they were unrelated to the purpose of the meta-analysis. Two excluded articles only had an abstract and no full text. Another study was written in the Korean language, and one study did not provide sufficient data to calculate the sensitivity and specificity. Therefore, a total of only nine articles were eligible for further analysis.[4,14,15,21-26] The study selection process is shown in Figure 1, and the basic characteristics of the selected studies are presented in Table 1.

Evaluation of the study quality

Figure 2 illustrates the quality assessment of the studies. Five of the nine articles were retrospective studies; therefore, the patient selection criterion was considered unsatisfactory.

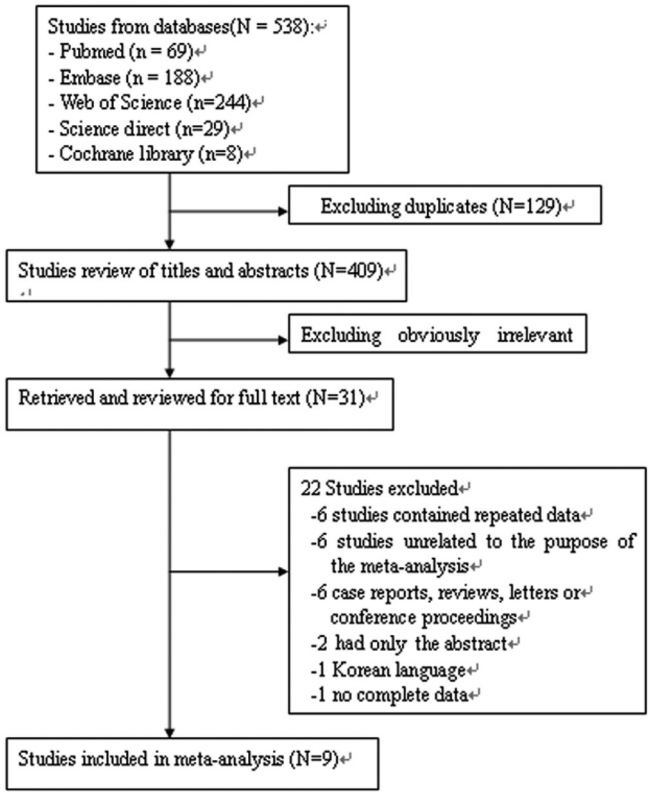

Figure 1: Flowchart of study identification and the inclusion and exclusion criteria

Data synthesis and meta-analysis

Spearman's correlation coefficient for PCR was 0.442 (P = 0.151). Cochran's Q and I2 for the DOR were 10.59 (P = 0.4782) and 0.0%, respectively. These values indicate an absence of heterogeneity caused by a threshold or non-threshold effect. Thus, a fixed-effects model was used. The overall diagnostic sensitivity, specificity, PLR, and NLR were 0.47 (95% CI: 0.42–0.51), 0.95 (95% CI: 0.93–0.97), 10.68 (95% CI: 6.98–16.35), and 0.53% (95% CI: 0.48–0.58), respectively [Figure 3a-d]. The AUC for PCR was 0.9311 [Figure 3e]. Thus, PCR for *M. tuberculosis* was reasonably accurate in differentiating ITB from CD. Patients with ITB had an approximately 10-fold higher chance of being PCR positive than patients with CD did, and there was no heterogeneity associated with the PLR (heterogeneity Chi-squared = 6.58 (P = 0.8320) and I2 = 0.0%). The heterogeneity Chi-squared for the NLR was 300.87 (P = 0.0000), and the I2 was 96.3%, indicating that there was significant heterogeneity for all of the included studies. We, therefore, used a random-effects model instead of a fixed-effects model. The recalculated pooled NLR was 0.49 (95% CI: 0.33–0.71) [Figure 3f].

Possible sources of heterogeneity

The pooled data showed that there was considerable heterogeneity among the studies. However, Spearman's correlation coefficient indicated that the heterogeneity was not caused by a threshold effect. A meta-regression analysis was used to estimate whether the heterogeneity was caused by differences in the study design and/or the specimen source and/or the assay method of the eligible studies, but the data suggest that these factors were not the source of heterogeneity. The results of the subgroup analysis are shown in Table 2. Deeks' funnel plot asymmetry test indicated that there was no publication bias (P = 0.81) [Figure 4].[27]

DISCUSSION

ITB and CD are difficult to distinguish, and misdiagnosis of either disorder can have serious repercussions. Thus, an efficient and accurate diagnostic tool that can distinguish between these disorders is a critical need. PCR is a simple and time-saving diagnostic method for detecting ITB. The present study is the first meta-analysis to comprehensively analyze the predictive power of PCR in this context using previously published studies.

In the current study, nine eligible studies, including 369 patients with ITB and 340 patients with CD, were identified. In all of the studies, the patients were diagnosed with their respective diseases using a previously established gold-standard method. We chose to use the QUADAS-2 tool to assess the quality of the studies. Although nearly none of the articles reported whether the investigators were

Table 1: Main characteristics and results of the nine eligible studies

Author, year, reference	Country	Mean age	Patients (*n*) (male:female)	Study design	Specimen source	Assay method	True positive	False positive	False negative	True negative
B.Y. Fei (2014)	China	ITB: 30.3±11.6 CD: 31.7±12.5	ITB: 29 (14:15) CD: 36 (22:14)	Prospective	Fecal samples, biopsy specimens	FQ-PCR	Fecal: 24 Biopsy: 16	3 2	5 13	33 34
B. Ramadass (2010)	India	ITB: 26 (15-57) CD: 28 (10-63)	ITB: 24 (14:10) CD: 44 (30:14)	Prospective	Fecal samples	C -PCR	19	5	5	39
Deepak N. Amarapurkar (2008)	India	ITB: 37.2±8.6 CD: 36.6±8.6	ITB: 26 (15:11) CD: 26 (16:10)	Prospective	Biopsy specimens	C -PCR	17	0	9	26
Xian Ji Jin (2010)	South Korea	ITB: 39 CD: 27	ITB: 55 (?) CD: 42 (?)	Prospective	Biopsy specimens	Nest-PCR C- PCR	Nest-PCR: 20 C- PCR: 3	0 0	35 52	42 42
Anna B. Pulimood (2008)	India, England	?	ITB: 20 (?) CD: 20 (?)	Retrospective	Biopsy specimens	In situ PCR C-PCR	*In situ* PCR: 6 C-PCR: 5	1 1	14 15	19 19
D.N. Amarapurkar (2004)	India	ITB: 32.2±5.6 CD: 34.2±7.3	ITB: 60 (3:2) CD: 20 (3:1)	Retrospective	Biopsy specimens	C-PCR	13	1	47	19
Hua Tian Gan (2002)	China	?	ITB: 39 (?) CD: 30 (?)	Retrospective	Biopsy specimens	C-PCR	25	0	14	30
Hua Tian Gan (1995)	China	?	ITB: 36 (?) CD: 26 (?)	Retrospective	Biopsy specimens	C-PCR	27	0	9	26
Yuan Lei (2013)	China	ITB: 37.0±15.7 CD: 36.2±14.1	ITB: 80 (?) CD: 96 (?)	Retrospective	Biopsy specimens	C-PCR	46	7	34	89

Note: PCR: Polymerase chain reaction, C-PCR: Conventional PCR, FQ-PCR: Fluorescent quantitative PCR, TP: True positive, FP: False positive, FN: False negative, TN: True negative

Table 2: Variables affecting the DOR

Subgroup	Number of studies	Se (95% CI)	Sp (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC	SE (AUC)
Design	Randomized controlled trial (*n*=6)	0.45 (0.39-0.52)	0.95 (0.92-0.98)	9.17 (5.21-16.17)	0.42 (0.19-0.94)	32.00 (14.92-68.62)	0.9342	0.0270
	Retrospective study (*n*=6)	0.48 (0.42-0.54)	0.95 (0.91-0.98)	8.16 (4.51-14.78)	0.54 (0.37-0.80)	16.21 (6.69-39.29)	0.9404	0.0541
Assay method	Conventional PCR (*n*=8)	0.46 (0.40-0.51)	0.95 (0.92-0.97)	8.16 (5.02-13.27)	0.48 (0.28-0.83)	20.47 (9.98-41.96)	0.9255	0.0325
	Unconventional PCR (*n*=4)	0.50 (0.41-0.58)	0.96 (0.91-0.98)	10.09 (4.71-21.59)	0.51 (0.34-0.78)	27.51 (10.81-70.05)	0.9411	0.0393
Specimen source	Biopsy specimens (*n*=10)	0.42 (0.38-0.47)	0.97 (0.94-0.98)	9.13 (5.44-15.32)	0.56 (0.39-0.79)	18.15 (10.07-32.73)	0.9203	0.0467
	Fecal samples (*n*=2)	0.81 (0.68-0.91)	0.90 (0.81-0.96)	7.96 (4.07-15.58)	0.21 (0.12-0.37)	38.24 (13.89-105.33)	/	/

Note: The random-effects model was used

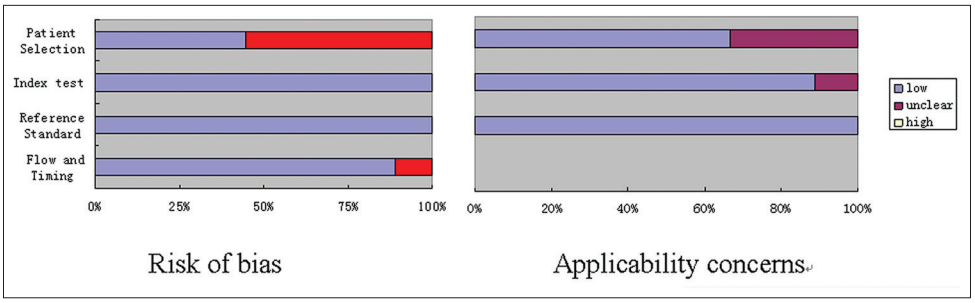

Figure 2: Quality assessment of the nine included studies using the modified QUADAS-2 tool

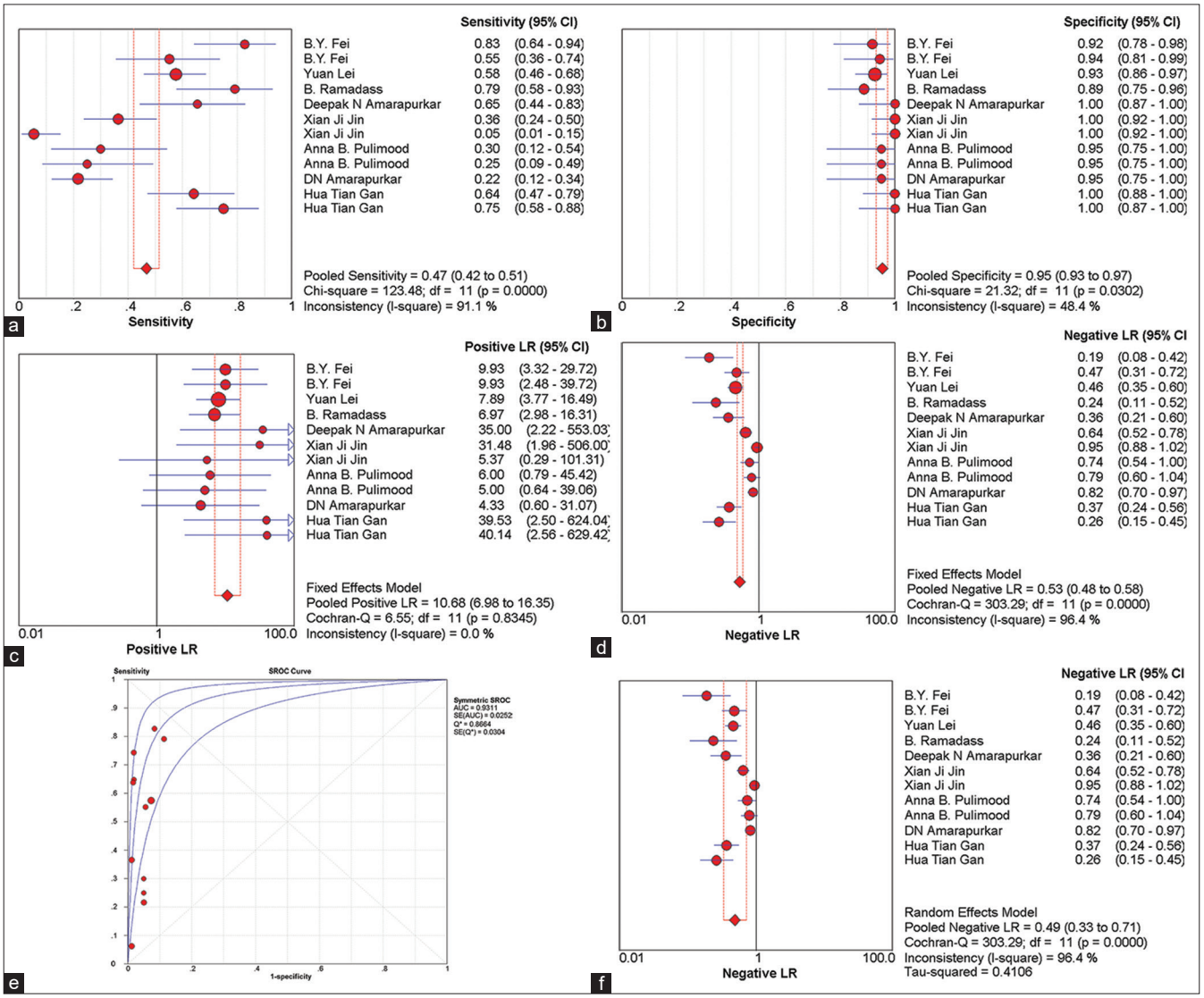

Figure 3: (a) Pooled sensitivity; (b) Pooled specificity; (c) PLR (fixed-effects model); (d) NLR (fixed-effects model); (e) SROC curve and AUC; (f) NLR (random-effects model)

blinded to the study, the cutoff of the PCR machine was pre-specified, and the result of the index test was therefore not judged subjectively. We, therefore, believe that the lack of blinding did not significantly affect the study results.

In our meta-analysis, we calculated Spearman's correlation coefficient and the DOR to estimate whether heterogeneity existed. Our results indicate that there was minimal heterogeneity. Three of the nine articles contained two sets of data due to the use of different PCR assays (conventional or unconventional) and different specimens (fecal samples or biopsy specimens). These studies were analyzed twice, which may have decreased the overall heterogeneity. For this reason, we excluded one dataset from each of the three articles to determine whether the heterogeneity was affected. The recalculated Spearman's correlation coefficient and DOR indicated a lack of heterogeneity caused by a threshold or non-threshold effect. In contrast, the pooled NLR showed significant heterogeneity ($P < 0.05$).

Next, we further investigated the source of the heterogeneity. Five of the nine articles were retrospective studies, and others were prospective studies, which may affect the heterogeneity. The type of PCR for *M. tuberculosis* was also different in each study; certain studies used conventional PCR, whereas others used fluorescence quantitative PCR (FQ-PCR), *in situ* PCR or nested PCR. These varying methods could have increased the heterogeneity. The specimen sources in the articles also differed, including intestinal biopsies, fecal samples, freshly prepared samples, and paraffin-embedded tissue specimens stained with hematoxylin and eosin. These specimen sources may have further added to the heterogeneity. Therefore, we

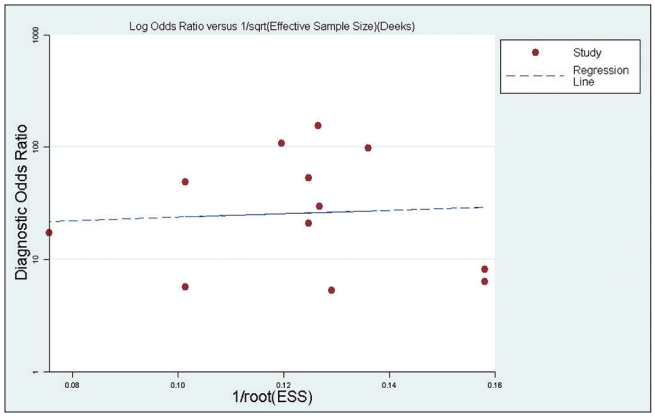

Figure 4: Funnel plot for the assessment of potential bias in PCR assays

conducted a meta-regression analysis to identify the source of heterogeneity. Unfortunately, our results indicated that the heterogeneity was not caused by the study design, the specimen source, or the assay method. The number of studies analyzed in this meta-analysis was small, which may limit the quality of our analysis. In addition, we speculate that the different test procedures, participant groups, and sample collection times in the studies may have affected the heterogeneity.

PCR for *M. tuberculosis* was found to be highly specific for ITB but not very sensitive. MTB is not spread evenly or superficially throughout the diseased intestinal tissue, and hence the restricted depth and size of the obtained endoscopic mucosal biopsy specimens and the limited amount of extracted MTB DNA may have been responsible for the low sensitivity. In addition, the use of paraffin-embedded biopsy specimens may have degraded the DNA of the organism, decreasing the yield of the PCR.[28] Furthermore, certain MTB strains do not contain the IS6110 sequence,[29] which may have yielded false negatives. For the false positives, the high prevalence of *M. tuberculosis* worldwide and incidental infection of CD patients by contaminated water or food consumption may have led to the presence of MTB in CD patients. Moreover, CD patients may co-exist with latent TB infection, which can be activated by immunosuppressive therapy.[30,31] Another possible problem is contamination of the samples during collection or during handling for the extraction and amplification of MTB DNA.

The pooled sensitivity and specificity do not behave independently when they are pooled from various primary studies to generate separate averages.[32] In the current study, the AUC and DOR were calculated to evaluate the potential diagnostic value of PCR. We found that the AUC for PCR was 0.9311, indicating that PCR for *M. tuberculosis* is reasonably able to differentiate ITB from CD. The DOR is a single indicator of test accuracy;[33] the pooled DOR in the present meta-analysis was 21.92, further confirming that PCR is useful in distinguishing ITB from CD. Our subgroup analyses indicated that fecal PCR for MTB may have higher diagnostic value, as the DOR of biopsy specimens was 18.15 and that of the fecal samples was 38.24. However, in the literature, there are limited data on the use of fecal samples for detecting MTB DNA to distinguish ITB from CD. Two eligible articles using fecal samples [4,21] reported sensitivities of 0.83 and 0.79 and respective specificities of 0.92 and 0.89. In addition, Balamurugan *et al*.[18] showed that the sensitivity, specificity, PPV, and NPV were 88.8, 100, 100, and 93.7%, respectively. Theoretically, fecal PCR can detect MTB DNA anywhere along the gastrointestinal tract, which makes it less affected by sampling errors. Furthermore, fecal specimens can be obtained non-invasively and conveniently, so patients bear a decreased economic burden and are subjected to less physical discomfort. Thus, fecal PCR is an attractive and promising test that may be used as a complementary approach to identify ITB and CD. Because current research is limited, additional studies evaluating the use of PCR to identify ITB using fecal samples are needed.

Several studies have reported that certain *M. tuberculosis* strains in India contain either a single copy or no copy of IS6110, especially in the southern part of the country.[34-36] One study[22] showed that PCR had better sensitivity in detecting both the MPB64 and the IS6110 genes than in detecting the IS6110 gene sequence only. For IS6110-negative strains, more gene segments specific to *M. tuberculosis* need to be determined for future PCR assays.

We believe that PCR for MTB will be an important tool for the diagnosis of ITB. Future research should focus on the following tasks: (1) Using fresh samples from cases before treatment to improve sensitivity, (2) improving the use of fecal samples for PCR and including additional gene segments, and (3) standardizing the detection method and cutoff values. These tasks will improve the sensitivity and specificity of the test, which will facilitate a more accurate meta-analysis of the diagnostic value of PCR.

This meta-analysis has certain limitations. First, the conference abstracts, letters, studies without sufficient data, and non-English language studies were excluded, which may lead to publication bias; an inflation of accuracy estimates due to the preferential acceptance of papers reporting favorable results. Second, we included studies which used discrepant PCR methods and different samples (feces and tissue), which may affect the accuracy estimates, even though we have discussed this limitation by subgroup analysis. Third, we detected significant heterogeneity across the included studies, but were unable to determine the source by meta-regression. Finally, the number of studies

included was limited, and further studies are still needed to evaluate the diagnostic accuracy of PCR.

CONCLUSIONS

Evidence suggests that PCR for *M. tuberculosis* has potential diagnostic value. In consideration of the low sensitivity, physicians should also consider that negative results cannot exclude ITB and that additional diagnostic tests may be needed. Currently, there is no ideal diagnostic approach for distinguishing ITB from CD. PCR can be viewed as an important complement to conventional tests to help delete confirm the diagnosis, however, additional studies are needed to further evaluate the diagnostic accuracy of PCR.

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Conflicts of interest
There are no conflicts of interest.

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