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The diagnostic value of polymerase chain reaction for ocular tuberculosis diagnosis in relation to antitubercular therapy response: a meta-analysis

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ABSTRACT

Background: Polymerase chain reaction (PCR) is currently considered the method of choice for diagnosing ocular tuberculosis. However, the sensitivity and specificity of PCR using ocular samples remain uncertain. Our meta-analysis aimed to review the diagnostic accuracy of PCR testing in confirming ocular tuberculosis, with responses to antitubercular therapy (ATT) as reference indices.

Methods: A systematic literature search of the PubMed, EBSCOHost, Scopus, and Google Scholar databases was performed using the standardized PRISMA guideline. Observational studies reporting both PCR MTb positivity and ATT response were included. Meta-analysis was performed to estimate the pooled positivity rate, sensitivity, specificity, positive and negative likelihood ratios, diagnostic odds ratios (DOR), and summary receiver operating curves (SROC).

Results: The pooled positivity rate for PCR MTb was 0.55 (95% CI 0.44–0.67). The overall sensitivity and specificity were 88% (95% CI 83–92) and 71% (95% CI 60–80), respectively. The pooled DOR was 12.15 (95% CI 5.55–26.62). The area under the SROC was 0.83.

Conclusions: The diagnostic accuracy of PCR Mtb is not sufficient for use as a benchmark for ocular TB diagnosis routinely based on ATT response. A negative result may help avoid prescribing unnecessary ATT in dilemmatic cases.

Background

Tuberculosis (TB) remains the leading cause of morbidity and mortality due to infection, with approximately a quarter of the population infected (Global tuberculosis report 2020). Around 90% of these have latent TB infection, yet clinical disease can develop anytime during their life (Dye et al., 1999; Shakarchi, 2015). As a multisystem disease, TB may also cause ocular problems. Ocular TB represents a complex clinical problem due to its wide spectrum. Ocular inflammation can be uni- or bilateral, and can present as anterior, intermediate, posterior, and panuveitis (Shakarchi, 2015). The true prevalence of ocular TB is hard to determine, and has been reported as being between 0.2% and 10.5% among all uveitis cases in referral hospitals (Agrawal et al., 2020). This uncertainty is the result of many patients being diagnosed with presumptive ocular TB, based on a wide spectrum of clinical appearances and the use of corroborative evidence to find indirect evidence of TB infection, or after the exclusion of other possible causes (Testi et al., 2020). However, according to our previous study, TB-related uveitis might account for up to 48% of uveitis cases if interferon-gamma release assay (IGRA)-positive patients are accounted for (La Distia Nora et al., 2018).

The diagnosis of ocular TB is difficult, and poses problems. Specimen biopsy and direct examination to find *Mycobacterium tuberculosis* (MTb) under a microscope are impractical in proving oc-

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ular infection in many cases because the ocular manifestation may represent a delayed hypersensitivity reaction rather than a direct infection. Moreover, ocular TB patients can have no clinical signs or symptoms associated with pulmonary or other systemic TB. Mostly, the diagnosis of ocular TB is presumptive (Agrawal et al., 2020; Shakarchi, 2015).

Based on the current diagnostic criteria, ocular TB can be categorized as confirmed, probable, or possible (Gupta et al., 2015; Teixeira-Lopes et al., 2018). The presence of Mtb from ocular samples is mandatory for the diagnosis of confirmed TB (Agrawal et al., 2020; Gupta et al., 2015; Teixeira-Lopes et al., 2018). Polymerase chain reaction (PCR) is considered a rapid and easy method for detecting the cause of uveitis due to infection, including Mtb (Mochizuki et al., 2017). In practice, the application of a gold-standard test to find Mtb from ocular fluid is difficult to achieve by culture or smear. Thus, experts suggest starting ATT based on several criteria, rather than depending solely on Mtb found in ocular samples (Agarwal et al., 2019a; Agrawal et al., 2020; Testi et al., 2020). The sensitivity and specificity of PCR from ocular samples have often been assessed using a clinical diagnosis of presumptive ocular TB (Barik et al., 2018). Moreover, a PCR result — considered easier and more reliable in a paucibacillary setting — does not relate to the management of ocular TB in the real setting (Agarwal et al., 2019b; Agrawal et al., 2020).

Our systematic review and meta-analysis aimed to evaluate the diagnostic accuracy of PCR using aqueous/vitreous samples. The response to antitubercular therapy (ATT) was chosen as the reference test, since benefits derived from ATT reflect definitive evidence of ocular TB (Dalvin and Smith, 2017).

Methods

Search strategy and selection criteria

For this systematic review and meta-analysis, PubMed, EBSCOHost, Scopus, and Google Scholar databases were searched for relevant studies published up to July 30, 2020. The following search strategy, with similar terms, was used: “polymerase chain reaction” AND “uveitis/ocular tuberculosis”. All studies published in English that included patients being investigated for ocular tuberculosis, and which reported PCR results and clinical responses to antitubercular therapy, were included.

Only observational studies were accepted. To be eligible, studies had to recruit adults based on ocular signs and symptoms suggestive of tuberculosis, PCR (both conventional and real-time) from any ocular fluids as the index test, and response to ATT as the reference test. Our study is registered with the International Prospective Register of Systematic Reviews (PROSPERO), registration number CRD42020199579. Our study protocol was prepared, the systematic review performed, and the report prepared according to recommendations of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2009).

Data extraction

Two reviewers (DFK and IS) independently screened the titles and abstracts of the articles identified through the electronic searches against the eligibility criteria. DFK, IS, and IP independently assessed the full texts of the included papers, documented non-inclusion reasons, and identified additional articles from reference lists. Any disagreements were resolved by consensus. Three authors extracted data from the eligible articles into an Excel database.

The following data were extracted from eligible papers: first author, year of publication, country of data collection, ocular fluids being examined, PCR and its gene target, ATT employed and the clinical response, method of assessing response to ATT, and the numbers of patients who underwent PCR (ocular fluid samples) and who achieved a good clinical response to ATT. Articles were defined as eligible for meta-analysis estimation of sensitivity and specificity if they provided data on numbers of patients who were true positives, false positives, false negatives, and true negatives. The determination of these values was based on the positivity of PCR and the clinical resolution of inflammation after ATT. True positive referred to the number of suspected ocular TB cases with positive PCR results who responded to ATT, whereas true negative referred to the number of patients with negative PCR results and who did not respond to ATT or achieved inflammation resolution with treatment other than ATT. For studies with missing or incomplete information for the meta-analysis, data were requested from the authors. In cases where data were unavailable, as much information as the study could provide was included in the narrative synthesis.

Assessment of study bias

The risk of bias at the study level was assessed using QUADAS-2 (DFK and IS), the recommended tool for evaluating primary studies for inclusion in systematic reviews involving the assessment of diagnostic accuracy (Whiting et al., 2011). The risk of bias concerns were assessed using four domains: patient selection, index test, reference standard, and patient flow/timing of tests. The level of risk or concern was reported as either high, some concerns, or low. For the patient selection domain, a low risk of bias meant that the study included all available probable or possible ocular TB patients. A study that only analyzed PCR-positive patients receiving ATT was considered to be at a high risk of bias. The threshold effect was not applicable in the index test domain as PCR TB was only defined as a positive or negative result. In the reference standard test, the study was graded as low risk of bias if the ATT and its response were interpreted regardless of the PCR result.

Statistical analysis

The meta-analysis included all studies that allowed us to calculate PCR sensitivity and specificity from any type of ocular fluid. It was performed using MetaDisc, as previously described (Zamora et al., 2006). Point estimates were ascertained using the DerSimonian-Laird random-effects model and 95% CIs for sensitivity and specificity for each study and pooled data. The pooled positivity rate for PCR was calculated using the MetaXL (www.epigear.com) add-in for Microsoft Excel, with 95% CI, using the random-effects model.

To provide an inference of diagnostic quality, a summary receiver operating characteristic curve was plotted, in which the diagnostic accuracy of the PCR was estimated according to the area under the curve and the summary operating point. Heterogeneity was assessed across studies using the I^2 statistic. If a contingency 2×2 table had a cell with no events, diagnostic quantitative analysis with 95% CIs was calculated by adding 0.5 to all cells. To explore potential sources of heterogeneity, the Spearman correlation test and meta-regression analysis were performed; these analyzed factors such as study design, recruiting patients with posterior inflammation only, using the MPB64 primer, and giving oral steroids to all patients.

Results

The study selection process is described in Figure 1. The quality assessment of included studies is provided in Figure 2 and Supplementary Table 1. In the final analysis, 13 studies were included as suitable for this review. The 13 eligible articles were

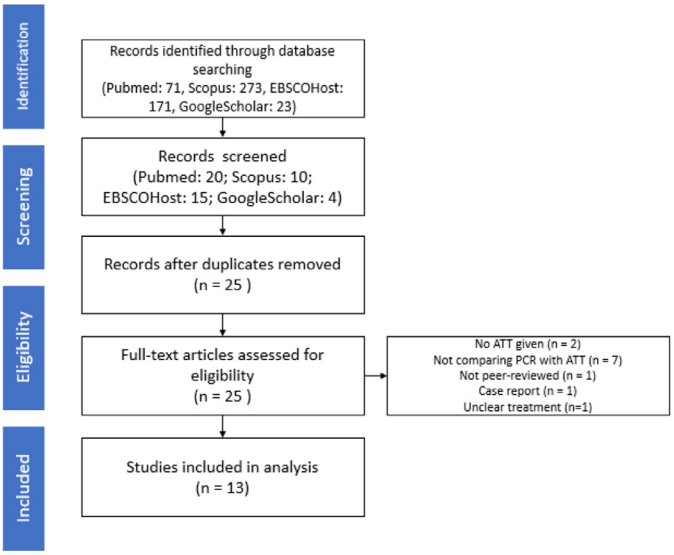

Figure 1. Flowchart of the article selection process

published between 1998 and 2017. Most of the studies recruited patients from India. In total, 347 ocular TB patients with PCR testing results from ocular fluid samples, and who received ATT, were analyzed.

Spectrum of presumed ocular tuberculosis

The diagnostic criteria for presumed ocular TB being tested for PCR MTb were variable between studies (Table 1). Several studies specifically included ocular TB patients with multifocal serpiginous choroiditis (MSC), subretinal abscess, or retinal vascular involvement (e.g. Eales disease). The numbers of patients being tested for PCR and treated with ATT are shown in Table 2. Most ocular samples were taken from aqueous or a combination of aqueous and vitreous fluid. The pooled positivity rate for PCR MTb from the included studies was 0.55 (95% CI 0.44–0.67; Figure 3). IS6110 and MPB64 primers were mostly used.

Most of the patients received oral steroids. The duration of treatment monitoring was variable (Table 2). There was no consistent definition of the response to treatment in the included studies. The approaches for measuring the response to treatment were largely subjective in all studies, based on clinical findings of inflammation resolution.

Overall diagnostic accuracy of PCR MTb from ocular samples in diagnosing ocular TB

Since heterogeneity was evident in this study, the random-effects model was used. Compared with the response to ATT therapy, the pooled sensitivity of the PCR test was 88% (95% CI 83–92; I^2 = 74.1%), and the pooled specificity was 71% (95% CI 60–80; I^2 = 54.9%). Figure 4 displays the forest plots of pooled sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio (details are provided in Supplementary Table 2). The pooled diagnostic odds ratio (DOR) was 12.15 (95% CI 5.55–26.62; Figure 5). The area under the summary receiver operating characteristic curve (SROC) was 0.83 (standard error (SE) = 0.05; Figure 6).

There was a significant correlation (r = 0.652, p = 0.016) between sensitivity and 1-specificity, which indicated the potential influence of patient spectrum or selection in each study in determining sensitivity and specificity results. For instance, the pooled analysis that included studies with only a low risk of bias in the patient selection (see Supplementary Tables) yielded higher sensitivity (96%; 95% CI 90–99) but lower specificity (39%; 95% CI 16–66). Additional analysis to explore the potential sources of heterogeneity was performed using meta-regression analysis (Table 3), with no factors found to have contributed to the heterogeneity.

Studies by Agarwal et al. (Agarwal et al., 2019b) and Bhagya et al. (Sudheer et al., 2018) were among those with higher DOR. Agarwal et al. (Agarwal et al., 2019b) reported that samples were obtained from patients with a diagnostic dilemma, and had already performed a thorough examination to exclude other potential causes. Failure to respond to treatment was noted from the persistence or recurrence of inflammation after 6 months of completing treatment. This was by far the most extensive study, forming part of the the Collaborative Ocular Tuberculosis Study (COTS) report. The study by Bhagya et al. (Sudheer et al., 2018) yielded a high DOR as they also performed PCR in selective cases with suspicion of being caused by TB in clinical appearance, or those unresponsive to steroid treatment.

Discussion

The accurate and timely diagnosis of ocular TB is crucial in reducing morbidity (Basu et al., 2014). However, ocular TB diagnosis usually needs to exclude other possible causes and conclude based on thorough corroborative evidence. Blood tests, chest X-rays, and either tuberculin skin tests or interferon-gamma release essays are usually performed before a diagnosis of presumptive ocular TB is decided (Agrawal et al., 2020; Shakarchi, 2015). Determining the diagnosis of ocular TB by confirming the presence of MTb in ocular tissue/fluid, including PCR, is not usually performed in all patients (Table 2). Moreover, our study found a dis-

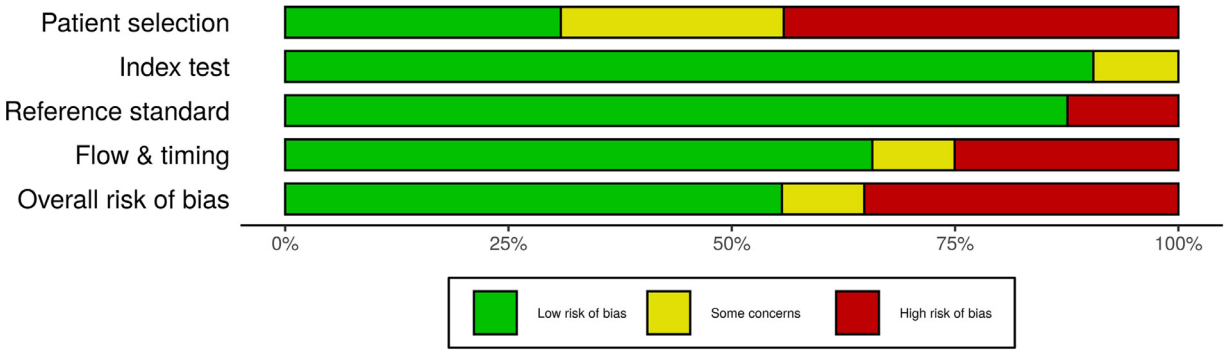

Figure 2. Risk of bias (QUADAS-2) concerns as percentages across the included studies, using ATT response as the reference standard for determining ocular TB

Table 1
Clinical spectrum of presumed ocular tuberculosis for the included studies

No.	Author (year)	Presumed ocular TB criteria in each study	Presumed ocular TB (*n*)	Patients' criteria prior to ocular samples being tested for MTb	Type of uveitis (*n*, %)[a]					PCR (+)/underwent PCR (*n*)
					Anterior	Intermediate	Posterior	Panuveitis	Others[b]	
1	Agarwal et al. (2017) (Agarwal et al., 2019b)	Clinical signs suggestive of uveitis TB and others where the specific cause had been excluded; corroborative evidence suggestive of uveitis TB	962	Selective cases on discretionary basis among dilemmatic cases or among those with diagnostic vitrectomy	3/59 (5.1%)	2/59 (3.4%)	33/59 (55.9%)	21/59 (35.6%)	0	33/59
2	Bhagya et al. (2017) (Sudheer et al., 2018)	Based on clinical features: hypopyon, granulomatous keratic percipitate, iris, choroid, or disc granulomas, active vasculitis, choroiditis, and healed chorioretinal scars along blood vessels; minimum 6 months of follow-up; no response to oral steroids	85	Suspected to have ocular TB or showing no response to oral steroids alone/other treatment	10/76 (13.2%)	9/76 (11.8%)	17/76 (22.4%)	36/76 (47.4%)	4/76 (5.3%)	24/85
3	Bansal et al. (2015) (Bansal et al., 2015)	Multifocal serpiginous choroiditis (MSC), vitreous cells, positive IGRA or TST; other possible causes excluded	13	MSC presumed to be caused by TB leading to pars plana vitrectomy	0	0	11/11 (100%)	0	0	10/11
4	Balne et al. (2014) (Balne et al., 2014)	One or more of the following: granulomatous anterior uveitis, intermediate uveitis, retinal vasculitis, serpiginous-like choroiditis, focal or multifocal choroiditis, and panuveitis; exclusion of other uveitic entities	114	Aqueous samples in patients with anterior chamber inflammation; vitreous samples in selected cases who underwent therapeutic vitrectomy	18/114 (15.8%)	15/114 (13.2%)	52/114 (45.6%)	25/114 (21.9%)	4/114 (3.5%)	80/114
5	Biswas et al. (2016) (Biswas et al., 2016)	MSC or choroiditis suspected for TB	40	All patients were included	0	0	40/40 (100%)	0	0	21/40
6	Sharma et al. (2013) (Sharma et al., 2013)	Clinical signs suggestive of uveitis TB with other specific causes excluded; corroborative evidence suggestive of uveitis TB	9	All patients were included	2/9 (22.2%)	1/9 (11.1%)	5/9 (55.6%)	1/9 (11.1%)	0	7/9
7	Gupta et al. (2003) (Gupta et al., 2003)	Serpiginous choroiditis	7	Selected patients with anterior chamber inflammation and additional vitreous samples	0	0	5/5 (100%)	0	0	5/5
8	Arora et al. (1999) (Arora et al., 1999)	Presumed uveitis TB with anterior chamber inflammation, with at least one of the following: (a) vasculitis, (b) anterior vitreous cells, (c) snowball, (d) snowbanking, or (e) retinochoroiditis	53	All patients were included	22/53 (42.5%)	0	28/53 (52.8%)	3/53 (5.7%)	0	20/53
9	Gupta et al. (1998) (Gupta et al., 1998)	Presumed uveitis TB with (a) vasculitis, (b) anterior vitreous cells, (c) snowball, (d) snowbanking, or (e) retinochoroiditis	17	All patients were included	4/17 (23.5%)	0	12/17 (70.6%)	1/17 (5.9%)	0	13/30
10	Murugan et al. (2016) (Murugan et al., 2016)	Presumed ocular TB based on examination	22	Cases who underwent both uniplex and nested PCR	n/a	n/a	n/a	n/a	n/a	5/22
11	Majumder et al. (2016) (Dutta Majumder et al., 2018)	Subretinal abscess	12	Patients with doubtful diagnosis and based on affordability for the patients	0	0	12/12 (100%)	0	0	8/12
12	Mohan et al. (2014) (Mohan et al., 2014)	MSC or choroiditis suspected for TB	13	Eyes with inflammation in the anterior chamber	0	0	13/13 (100%)	0	0	7/13
13	Singh et al. (2012) (Singh et al., 2012)	Eales disease	28	All patients were included	0	0	28/28 (100%)	0	0	16/28

[a] Proportions of uveitis type might be different from total numbers of ocular TB patients or patients undergoing PCR due to reporting variability.
[b] Including scleritisn/a: data not available

Table 2
Characteristics of included studies

No.	Author (year)	Country	Underwent PCR and given ATT (*n*)	PCR test Samples	Gene target	Method	Oral steroid	ATT	Treatment duration	Response to treatment criteria
1	Agarwal et al. (2017) (Agarwal et al., 2019b)	Multiple countries (most samples from India)	49	Aqueous and/or vitreous	IS6110, MPB64, and protein b	In-house PCR (across testing centers)	All	Variable (depending on individual institutional protocol)	Variable	Failure: (a) persistence or recurrence of inflammation within 6 months of completing ATT; (b) inability to taper oral steroid to < 10 mg/day or topical steroid drops < 2 drops/day; or (c) recalcitrant inflammation necessitating immunosuppresive therapy
2	Bhagya et al. (2017) (Sudheer et al., 2018)	India	56	Aqueous and/or vitreous	MPB64	Conventional (electrophoresis)	All	a*	6 months	Improvement in visual acuity and two-step decrease in inflammation
3	Bansal et al. (2015) (Bansal et al., 2015)	India	9	Vitreous	IS6110, MPB64, and protein b	Conventional (electrophoresis)	All	b* or MDR treatment	≥ 6 months	Clinical improvement
4	Balne et al. (2014) (Balne et al., 2014)	India	77	Aqueous (mostly)/ vitreous	IS6110, MPB64, and protein b	Conventional (electrophoresis)	All	a*	6 months	Clinical improvement (two-step decrease in AC cells, visual acuity improvement, disapperance of MSC lesion)
5	Biswas et al. (2016) (Biswas et al., 2016)	India	21	Aqueous	IS6110, MPB64	Real-time nested PCR	All	Unexplained	9 months	Choroiditis resolved
6	Sharma et al. (2013) (Sharma et al., 2013)	India	9	Aqueous or vitreous	IS6110, MPB64, and protein b	Conventional (electrophoresis)	All	b* or MDR treatment	Unclear	Clinical improvement
7	Gupta et al. (2003) (Gupta et al., 2003)	India	5	Aqueous or vitreous	IS6110	Conventional (electrophoresis)	All	b* or MDR treatment	≥ 12 months	Clinical improvement
8	Arora et al. (1999) (Arora et al., 1999)	India	53	Aqueous	H_{37}RA DNA (150 bp fragment)	Conventional (electrophoresis)	Unclear	Unexplained	12 months	Clinical improvement (two-step decrease in AC cells, decrease in leak of FFA for vasculitis, and ≥ 2 lines visual acuity improvement)
9	Gupta et al. (1998) (Gupta et al., 1998)	India	10	Aqueous	150 bp fragment	Conventional (electrophoresis)	All	b* or MDR treatment	18 months	Resolution of inflammation
10	Murugan et al. (2016) (Murugan et al., 2016)	India	22	Aqueous or vitreous	MPB64	–	All	b*	≥ 3 months	Reduction in inflammatory cells/flare or vitreous haze with ≥ 2 increments
11	Majumder et al. (2016) (Dutta Majumder et al., 2018)	India	12	Aqueous and vitreous	Unexplained	–	7/12	c*	Variable	Healed (resolving size of abscess and AC cells)
12	Mohan et al. (2014) (Mohan et al., 2014)	India	13	Aqueous	IS6110, MPB64, and protein b	Conventional (electrophoresis)	All	a*	6 months	Healed
13	Singh et al. (2012) (Singh et al., 2012)	India	11	Vitreous	MPB64	Real-time PCR	Unclear	Unexplained	12 months (median)	No recurrence

*a: 2 months of isoniazid, pyrazinamid, rifampicin, and ethambutol, then 4 months of rifampicin + isoniazid; b: Four-drug ATT — isoniazid (5 mg/kg daily), rifampicin (450 mg daily if body weight < 50 kg, otherwise 600 mg daily), ethambutol (15 mg/kg daily), and pyrazinamide (25–30 mg/kg daily) for 2–3 months, then continue rifampicin + isoniazid for ± 9 months; c: Four-drug regimen (isoniazid, rifampicin, pyrazinamid, and ethambutol) for a minimum of 6 months.

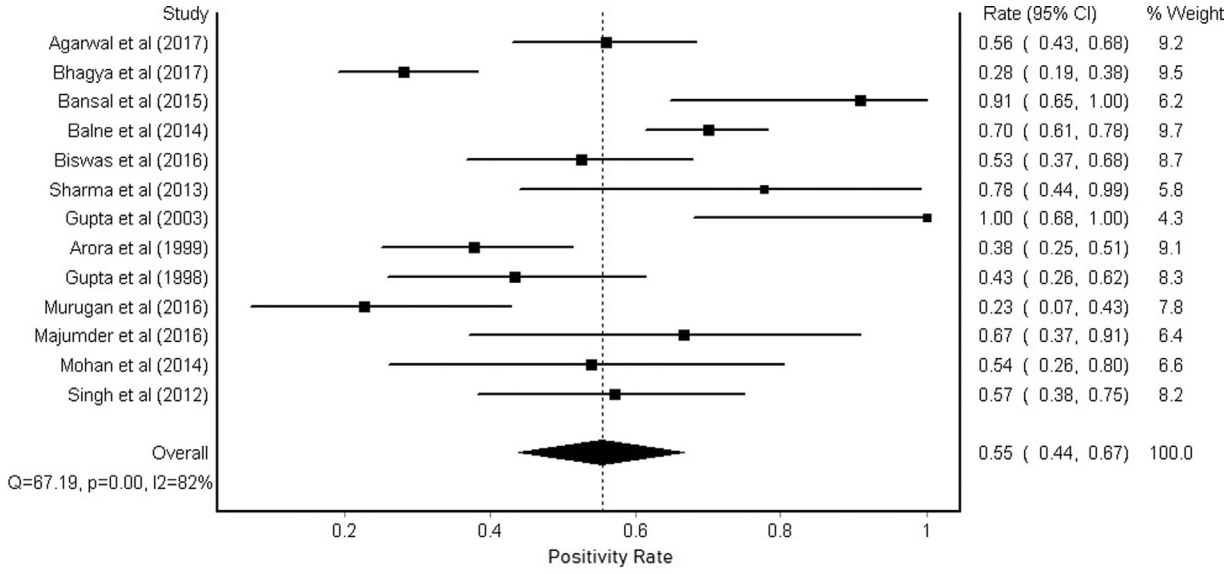

Figure 3. Pooled positivity rate of PCR test for MTb among presumed ocular TB patients

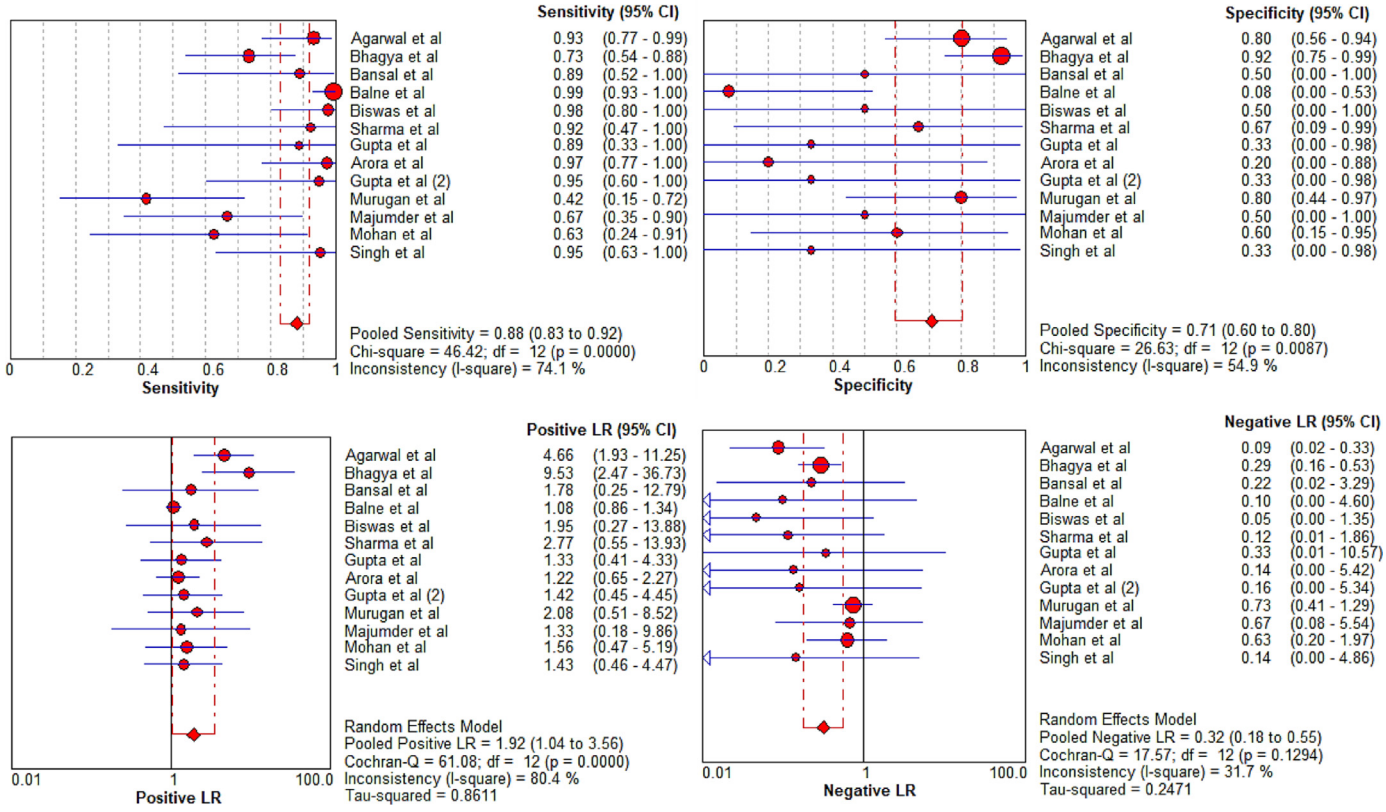

Figure 4. Diagnostic sensitivity (a), specificity (b), positive likelihood ratio (c), and negative likelihood ratio (d) for PCR MTb test from ocular samples versus response to ATT therapy

Table 3
Meta regression analysis of potential sources of heterogeneity

Factors	Coefficient	Standard error	*p*	RDOR	95% CI
Using the MPB64 primer	−0.153	1.536	0.477	0.32	0.01–11.92
Giving oral steroids to all patients	0.174	1.766	0.924	1.19	0.02–77.42
Design (retrospective or prospective)	0.941	1.593	0.573	2.56	0.06–110.81
Only recruiting patients with posterior segment inflammation	1.415	1.068	0.227	4.12	0.33–51.46

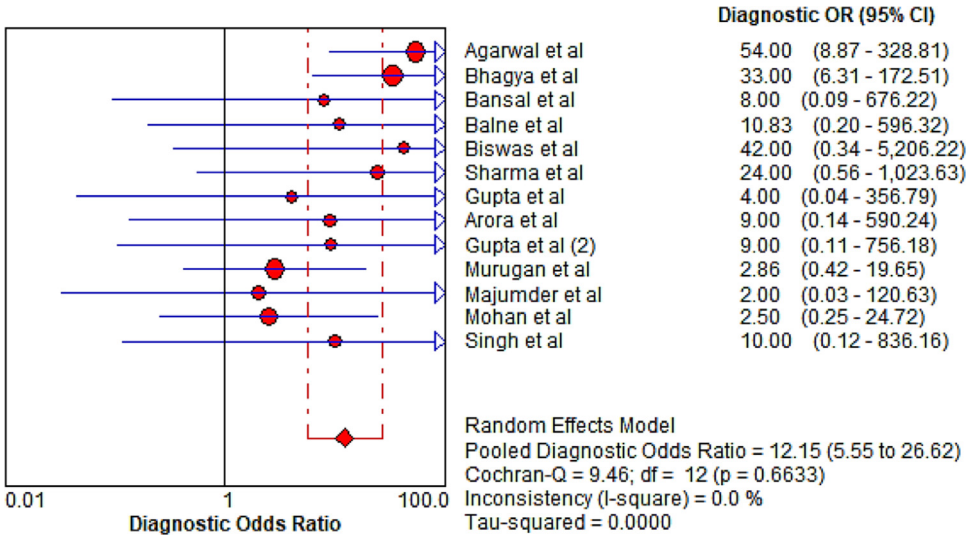

Figure 5. Diagnostic odds ratios of included studies

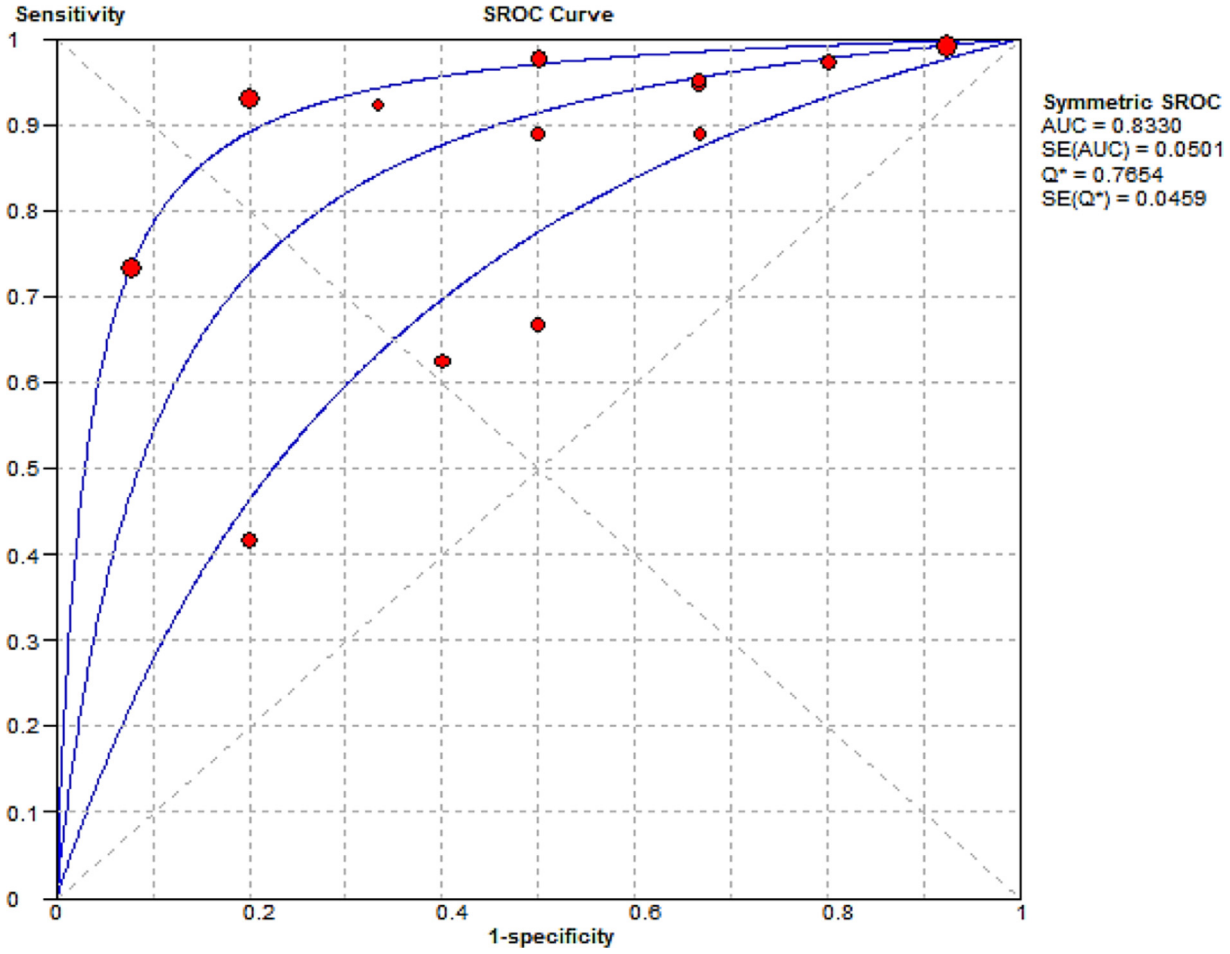

Figure 6. SROC meta-analysis of diagnostic performance of PCR MTb from ocular samples against response to ATT therapy

crepancy between the numbers of patients with positive PCR results and those receiving ATT. Based on our analysis, the diagnostic performance of PCR against response to ATT yielded acceptable sensitivity (pooled sensitivity, 88%) but relatively low specificity (pooled specificity, 71%). However, these results should be interpreted carefully in terms of possible false-positive and false-negative outcomes (Trevethan, 2017). The pooled positive likelihood ratio for PCR was very low (1.92), indicating that many patients with PCR-positive results did not show adequate response to ATT.

False-positive patients may be problematic in ocular TB. The presence of MTb is not exclusively correlated with treatment response — ATT response may be inadequate for some reasons. In a study by Bansal et al. (Bansal et al., 2015), the possibility of

MDR TB was further examined. The study found that among 10 eyes showing PCR-positive results, *rpoB* mutations were found in three samples, implicating the possibility of rifampicin resistance in patients with ocular TB. Second, there was variable treatment duration among the included studies, with a trend for higher success of ATT therapy among those with a treatment duration of 9 months or more, as previously described (Agrawal et al., 2015; Ang et al., 2012). Moreover, inflammation in ocular TB might be progressive, including paradoxical worsening, with an escalating dose of steroids perhaps influencing treatment outcomes (Basu et al., 2013). A study by Gupta et al. (Arora et al., 1999) also found ocular TB patients who showed suboptimal results with oral steroids, but the inflammation was significantly resolved after intravenous methylprednisolone. Moreover, possible coexistence with viral infection might be encountered, with inflammation subsiding after antiviral therapy (Mohan et al., 2014; Sudheer et al., 2018). Thus, the presence of MTb might not be directly linked with the clinical manifestation, because an inflammatory load of aqueous/vitreous samples increases the probability of so-called 'bystander' MTb DNA, especially in an endemic setting (Barik et al., 2018).

A study by Agarwal et al. (Agarwal et al., 2019b) yielded the highest DOR, followed by that by Bhagya et al. (Sudheer et al., 2018) study. In these two studies, PCR was performed only selectively — when the diagnosis was problematic or the patient was unresponsive to initial treatment other than ATT.

COTS-1 had published diagnostic criteria for uveitis TB. Performing MTb PCR from ocular samples was not mandatory if MTb from other organ had been documented, or corroborative evidence (Mantoux, IGRA, or chest X-ray) strongly suggested TB, and other possible entities had been ruled out (Agrawal et al., 2017). Looking further at the numbers of patients with positive IGRA, tuberculin, and chest X-ray results suggestive of TB, patients with positive or negative PCR did not show any differences in these proportions (Agarwal et al., 2019b). This is relevant to the pooled sensitivity result, which implicates a good 'rule-out' for ocular TB based on fairly good sensitivity and NPV (Trevethan, 2017). After excluding other potential causes, when PCR results are negative, the likelihood that patients will benefit from ATT administration is quite low. Routine PCR analysis in uveitis cases with probable infectious etiology would lead to suboptimal utility. Scheepers et al. (Scheepers et al., 2013) found a general positivity rate of only 2% (1/43), and a specific positivity rate for presumptive ocular TB of 14% (1/7).

Our meta-analysis showed substantial heterogeneity, consistent with the non-standardized nature of included patients, ATT regimen, sample size, and the definition of response to treatment. Other factors potentially leading to heterogeneity included site-specific specimen positivity (aqueous vs vitreous), the timing of PCR being conducted in relation to disease course, and variability of PCR methods in each study site (see Supplementary Table 3). A significant correlation between sensitivity and specificity was found in our study. Since the threshold effect was not considered to be the cause of this correlation, different spectra or samples tested for PCR might be the reason for this finding (Zamora et al., 2006). The selection of patients or samples that increase sensitivity can decrease specificity, and vice versa, as seen in a sub-analysis of studies with low risk of bias in patient selection.

In addition to the risk of bias in patient selection, visual inspection of the plots (Figure 2) from studies by Balne et al. (Balne et al., 2014), Singh et al. (Singh et al., 2012), Arora et al. (Arora et al., 1999), and Gupta et al. (Gupta et al., 1998) showed lower specificity despite having good sensitivity. Although this finding could not be analyzed in depth, the PCR results might have been influenced by the samples obtained. Our study indicated that the PCR results may have been site-specific, depending on the main anatomical location of the inflammation. In those studies, samples for PCR were taken mostly from aqueous fluid, except the Singh et al. study (Singh et al., 2012). In comparison, Agarwal et al. (Agarwal et al., 2019b) and Bhagya et al. (Sudheer et al., 2018) took relatively more samples from vitreous taps/biopsies, as most patients in these studies presented with posterior uveitis. The paucibacillary nature and preferential localization of MTb in retinal pigment epithelium previously described by Rao et al. (Rao et al., 2006) could have influenced the positivity rate, depending on site-specific samples obtained for PCR, as described in quantitative analyses (Sharma et al., 2010). In an everyday setting, obtaining vitreous samples is not an easy task, with aqueous fluid often considered the sample of choice even in posterior uveitis (Figueira et al., 2017; Dos et al., 2020). Aside from the potential influence of site-specific samples, Bhagya et al. (Sudheer et al., 2018) found more positive results from patients with acute uveitis. However, further analysis of the relationship between the timing of samples obtained in terms of the disease course and the site-specific influence of fluid samples could not be analyzed due to the limited individual data available in this meta-analysis.

Our review encountered other limitations. Most of the studies reported a selective patient sub-group without clearly defined criteria for timing and conditions of PCR examination. Several studies only reported treatment responses for those who were PCR positive. Thus, false-positive cases would have been overestimated, whereas false-negative and true-negative cases might have been overlooked. Our analysis was not able to demonstrate which PCR method was superior to the others. However, using the MBP64 primer had been reported to increase the accuracy of the PCR test (Kataria et al., 2015). Also, reports on PCR positivity and treatment responses from countries other than India are scarce, thus limiting the extrapolation of these results to other settings with different prevalences of TB. There is still no randomized trial demonstrating the benefit of ATT following protocols for extrapulmonary TB. Moreover, patients' compliance with ATT treatment was not taken into account in this analysis. Lastly, treatment response criteria and assessments varied between included studies. Further studies that compare the diagnostic results of PCR from different ocular sample sites (vitreous and aqueous) and the responses to ATT are needed. Biomarker studies evaluating indirect inflammation related to TB in the eyes would also be beneficial.

Conclusion

The pooled estimate of diagnostic accuracy parameters for PCR in detecting MTb from ocular fluid samples, with ATT response as a reference standard, provided an unremarkable result, which therefore cannot be used as a benchmark for routine ocular TB diagnosis. The benefit of PCR for TB would be more useful in dilemmatic cases, in which other possible causes have already been excluded. A negative result may help to rule out any potential benefits from ATT. More data are needed to guide and standardize PCR testing in cases of presumptive ocular TB. In addition, more biomarkers that are easy to obtain, inexpensive, and less invasive to screen in establishing TB as the cause of uveitis are needed.

Declaration of Competing Interest

The authors declare no competing interests.

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Ethical approval

Not required

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2021.07.075.

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