Molecular evidence for the role of mycobacteria in sarcoidosis: a meta-analysis

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ABSTRACT: The aetiology of sarcoidosis is currently unknown. Due to the clinical and histological similarities between sarcoidosis and tuberculosis, the role of mycobacteria has been repeatedly investigated as an aetiological agent for sarcoidosis. The current meta-analysis aimed to evaluate the available molecular evidence on the possible role of mycobacteria in the development of sarcoidosis.

The MEDLINE, EMBASE, CINAHL, DARE and CENTRAL databases were searched for relevant studies published from 1980 to 2006, and studies evaluating the presence of mycobacteria using molecular techniques in biological samples of patients with sarcoidosis were included in the current analysis. The 95% confidence intervals (CI) were calculated for the expected proportion (of individual studies); the data was then pooled to obtain a summary success rate with 95% CI. The odds ratio (95% CI) was also calculated in order to assess the presence of mycobacteria in samples of patients with sarcoidosis *versus* those from nonsarcoidosis control samples.

The database search yielded 31 studies. All studies used polymerase chain reaction for nucleic acid amplification followed by identification of nucleic acid sequences specific for different types of mycobacteria. Overall, 231 out of the 874 patients were positive for mycobacteria with a positive signal rate of 26.4 (23.6–29.5%), and the odds of finding mycobacteria in samples of patients with sarcoidosis *versus* controls were 9.67 (4.56–20.5%) using the random effects model and 19.49 (11.21–35.54%) using the exact method. There was methodological and statistical heterogeneity and evidence of publication bias.

The results of the current study illustrate a demonstrable mycobacterial presence in sarcoidosis lesions suggesting an association between mycobacteria and some cases of sarcoidosis. To avoid methodological diversity, larger multicentre trials with a central laboratory for sample testing should be designed.

KEYWORDS: Mycobacteria, polymerase chain reaction, sarcoidosis, tuberculosis

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Sarcoidosis is a granulomatous disease of unknown aetiology characterised by non-caseating granulomas involving the lung, lymph nodes and other organs [1]. It is believed that granulomatous diseases may be the result of continued presentation of a poorly degradable antigen [2]. In the case of sarcoidosis, this agent remains unclear. Numerous aetiological agents have been incriminated, both infective and non-infective [3]. Noninfective agents have been implicated because of their epidemiological association [4], but these implications have not stood the test of time [5]. Currently, the focus is on infective agents, and the two strongest contenders are Propionibacterium and Mycobacterium [6]. A recent study of culture and PCR analysis of Propionibacterium species from lung tissues and lymph nodes from subjects with and without sarcoidosis, showed *Propionibacterium acnes* to be a common commensal bacteria in peripheral lung tissues and mediastinal lymph nodes, suggesting that it is less likely to be the trigger for sarcoidosis [7].

Since sarcoidosis was first described, there has always been a belief that the disease is in some way related to tuberculosis [8]. However, the inability to identify mycobacteria by histological staining or culture from pathological tissues continues to be one of the strongest arguments against a potential role for mycobacteria. Of late, molecular analysis (such as polymerase chain reaction (PCR) techniques) for nucleic acids of putative agents serves as an alternative method for isolating fastidious organisms. This method

has been used successfully in identifying *Tropheryma whippelii* as a causative agent for Whipple's disease [9] and coronaviruses [10] as an agent for severe acute respiratory syndrome.

Several studies have been carried out using molecular techniques to study the presence of mycobacteria in sarcoidosis patients [5]. The aim of the current study was to systematically analyse the available literature on this issue.

MATERIAL AND METHODS

Search strategy and selection criteria

To identify the studies for inclusion in this review, all authors independently searched the National Library of Medicine's MEDLINE, EMBASE, CINAHL, DARE and CENTRAL databases for relevant studies published from 1980–2006 using the terms: sarcoidosis AND mycobacteria; sarcoidosis AND mycobacterium; sarcoidosis AND *Mycobacterium tuberculosis*; and sarcoidosis AND tuberculosis. Bibliographies of all selected articles and review articles that included information on the relationship between sarcoidosis and mycobacteria were reviewed. In addition, the current authors' studies were also reviewed. All the studies irrespective of language were identified.

Data abstraction

The abstracts of the studies were independently reviewed by two authors (R. Agarwal and D. Gupta), who were not blind to study details. Any disagreement was resolved by discussion between the authors. Included studies were those that evaluated the presence of mycobacteria using molecular techniques in samples (both histological and cellular) of patients with sarcoidosis. Data was recorded on a standard data extraction form. The following items were extracted. 1) Publication details including title, author(s) and other citation details. 2) The number of patients with sarcoidosis and the number and type (tissue or cellular samples) of samples used for analysis. 3) Details of the molecular test used to evaluate the presence of mycobacteria in samples. 4) The type of mycobacteria (tuberculous *versus* nontuberculous). 5) Percentage of the samples positive for mycobacteria in patients with sarcoidosis *versus* the percentage of positive control samples.

Determination of the pooled treatment effect

To calculate the percentage of mycobacteria-positive sarcoid samples, binomial proportions were used in which the numerator was success in isolation of mycobacteria and the denominator was the total study sample. The expected proportion was the success rate of each study included and 95% confidence intervals (CIs) were calculated for the expected proportion using the Newcombe–Wilson method [11, 12]. Data from individual studies was then pooled and a summary success rate with 95% CI was calculated by summing up the rate of mycobacteria-positive samples as the numerator and the total number of samples evaluated as the denominator [13].

The odds ratio (OR) and 95% CI were also calculated to assess the presence of mycobacteria in sarcoisosis samples *versus* controls. The results from individual studies were pooled using the random effects model of DERSIMONIAN and LAIRD [14] and the exact method with conditional maximal likelihood as described by MARTIN and AUSTIN [15].

Assessment of heterogeneity

The extent of heterogeneity was assessed by the Cochran Q-statistic (weighted sum of squared differences between individual study effects and the pooled effect across studies, with the weights being those used in the pooling method). The level at which heterogeneity should be diagnosed is unclear, given that the Q-statistic has low power, and FLEISS [16] recommended a p-value of >0.1.

The impact of heterogeneity upon the pooled estimates of the individual outcomes of the meta-analysis was assessed using the Chi-squared test and/or the I^2 tests (this measures the extent of inconsistency among the studies' results and is interpreted as approximately the proportion of total variation in study estimates that is due to heterogeneity rather than sampling error). An I^2 value >40% indicates significant heterogeneity. As the Chi-squared test has a low sensitivity for detecting heterogeneity, a p-value <0.1 was considered significant for the presence of statistical heterogeneity [17].

Assessment of publication bias

The presence of a small study effect was examined by means of funnel plots [18]. The funnel plot is a measure of the log of the OR (in the X-axis; a measure of diagnostic accuracy) against the standard error of the log of the OR (in the Y-axis; an indicator of sample size). Each point represents each study in the meta-analysis. The central line indicates the summary OR with other lines indicating the 95% CI. In the absence of publication bias, the OR estimates from smaller studies are expected to be scattered above and below the summary estimate, producing a triangular or funnel shape.

Institutional review board clearance was not required for this study as this was a meta-analysis of published studies.

RESULTS

The initial data search yielded a total of 1,718 citations (fig. 1). On review of the title and abstract, 1,679 articles were excluded as they did not meet the inclusion criteria. A total of 40 studies were identified that used nucleic acid amplification techniques to identify mycobacterial nucleic acid sequences. Of these 40 studies, nine were further excluded; seven were single patient case reports [19–25], one study analysed mycobacterial DNA in nonsarcoid granulomatous disease [26] and one study had re-analysed the *Mycobacterium tuberculosis*-positive samples of sarcoid patients using a different technique to further clarify the type of *M. tuberculosis* [27]. Therefore, 31 articles were selected for analysis (table 1) [28–58].

All studies provided detailed methodology and the meta-analysis included studies from around the world (table 1). In total, the studies involved 882 samples (711 tissue samples and 71 fluid samples from which cells were isolated and further analysed) from 874 patients with proven sarcoidosis and 611 samples from 600 controls (lung cancer, bronchiectasis and other lung disorders). All but six studies [36, 37, 46, 49, 54, 55] were case–control studies where the cases and controls have been randomly sampled; all cases who have clinically confirmed sarcoidosis have been included. All but five [28, 30, 32, 54, 55] studies used positive control samples of patients with proven mycobacterial infections. The details regarding the sites of biopsy were not provided by all studies but from

TABLE 1 Studies evaluating the role of mycobacteria in sarcoidosis using molecular techniques

First author [Ref.]	Site	Molecular technique	Patients				Controls			
			n	Tissue/fluid	n/N	Mycobacteria	n	Diagnosis	n/N	Mycobacteria
BOCART [28]	France	PCR of 65 kDa mycobacterial antigen and IS6110	22	16/6	2/22	MTB	22	Benign or malignant tumours, nongranulomatous diseases	0/22	
GERDES [29]	Germany	PCR of 16S rDNA	14	NA	0/14		10	Lung cancer, nonmycobacterial lung diseases	0/10	
MITCHELL [30]	UK	Mycobacterial rRNA detection by liquid phase hybridisation	5	5/0	5/5	MTB	5	Nonmycobacterial lung diseases	0/5	
SABOOR [31]	UK	PCR of IS986/IS6110 and groEL	20	0/20	14/20	10 MTB, 4 NTM	22	Lung cancer, haemoptysis, ILD	5/22	3 MTB, 2 NTM
THAKKER [32]	UK	PCR of groEL and staining with AvaII	14	14/0	1/14	MTB	11	Nonspecific reactive lymph nodes	1/11	MTB
FIDLER [33]	UK	PCR of 65 kDa mycobacterial antigen and IS6110	16	16/0	7/16	MTB	16	Squamous cell cancer, Hodgkin's disease	1/16	MTB
LISBY [34]	Denmark	Nested PCR for IS900 of M. paratuberculosis	18	18/0	0/18		18	Normal donors	0/18	
GHOSSEIN [35]	USA	PCR of 65 kDa mycobacterial antigen	10	10/0	0/10	MTB	10	Normal tissue, benign or malignant neoplasms	0/10	
POPPER [36]	Austria	PCR of 65 kDa mycobacterial antigen and IS6110	15	15/0	2/15	MTB	0	None	0/0	
EL-ZAATARI [37]	USA	PCR of IS902/IS900	9	8/1	8/9	NTM	0	None	0/0	
RICHTER [38]	Germany	PCR of mycobacterial 16S rDNA	24	10/14	1/24	MTB	57	Cancer, fibrosis, pneumonia and Mycoplasma infection	3/57	MTB
CANNONE [39]	Italy	PCR10 of IS6110	30	30/0	2/30	MTB	10	Nontuberculous, nonsarcoid lung disease	0/10	
OZCELIK [40]	Turkey	PCR of IS6110	5	11/0	5/11	MTB	15	Nontuberculous, nonsarcoid lung disease	2/15	MTB
POPPER [41]	Austria	PCR of 65 kDa mycobacterial antigen, IS1110 and IS6110	35	35/0	11/35	NTM	39	Cancer, bronchiectasis, BOOP, pneumococcal and viral pneumonia	0/39	
VOKURKA [42]	France	PCR of IS6110 and DR region	15	15/0	0/15	MTB	27	Lung cancer, WG, BO, PLCH	0/27	
VAGO [43]	Italy	PCR of IS6110	30	30/0	2/30	MTB	17	Nontuberculous, nonsarcoid lung disease	0/17	
WILSHER [44]	NZ	PCR of IS6110, nested PCR to further amplify 85 bp sequence within the 123 bp product	31	31/0	0/31		10	Lung cancer, lymphoma, seminoma, bronchiectasis, reactive lymph nodes	0/10	
GROSSER [45]	Germany	PCR of IS986/IS6110	65	50/15	35/65	MTB	34	Lung tissue from still birth, iliac lymph nodes	1/34	MTB
IKONOMOPOULOS [46]	Greece	PCR of MPB 64, IS1110 and 383 bp region of 65 kDa mycobacterial antigen gene	25	25/0	9/25	MTB	0	None	0/0	
ISHIGE [47]	Japan	PCR of IS6110	15	15/0	3/15	MTB	15	Stomach cancer	1/15	MTB
LI [48]	USA	PCR of 65 kDa mycobacterial antigen and RFLP analysis	20	20/0	16/20	2 MTB, 14 NTM	20	Foreign body granuloma, normal skin, dermatitis	0/20	
IKONOMOPOULOS [49]	Greece	PCR of MPB 64, outward primer PCR of IS1110 and amplification of IS1110 and 16S rRNA	25	25/0	6/25	MTB	0	None	0/0	
KLEMEN [50]	Austria	PCR of IS6110 and mycobacterial chaperonin	4	4/0	3/4	NTM	39	Cancer, bronchiectasis, BOOP, pneumococcal and viral pneumonia	0/39	
DRAKE [51]	USA	PCR of 16S rRNA, rpoB, and IS6110	25	25/0	15/25	11 MTB, 3 NTM, 1 both	25	Cancer, lymphoma	0/25	
EISHI [52]	Japan	PCR of IS6110/IS900	108	108/0	5/108	5 MTB	86	Cancer lung, nonspecific lymphadenitis	2/86	
GAZOULI [53]	Greece	PCR of IS6110, MPB64 and mtp40	46	46/0	33/46	33 MTB	20	Lung cancer	0/20	
MILMAN [54]	Denmark	Cobas Amplicor®	15	0/15	0/15		0	None	0/0	
GROSSER [55]	Germany	PCR of 123 bp fragment of IS6110/IS986	105	NA	36/105	MTB	0	None	0/0	
MARCOVAL [56]	Spain	NAAT for rRNA of MTB	35	35/0	0/35		39	Not mentioned	0/39	
DUBANIEWICZ [57]	Poland	BD ProbeTec IS6110 amplification	50	50/0	3/50	MTB	10	Ca lung, reactive lymph nodes	0/10	
FITE [58]	Spain	PCR of IS6110 followed by Southern blot hybridisation	23	23/0	9/23	MTB	23	Lung neoplasm	1/23	MTB

n: Mycobacteria-positive samples; N: total samples; PCR: polymerase chain reaction; IS6110: insertion sequence to identify Mycobacterium tuberculosis (MTB) complex (MTB, Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium bovis and bacillus Calmette–Guerin); 16S rDNA: ribosomal DNA (rDNA) common to all mycobacteria; NA: not applicable; rRNA: ribosomal RNA; IS986: insertion sequence to identify MTB complex; groEL: gene encoding 65 kDa surface antigen common to all mycobacteria; NTM: nontuberculous mycobacteria; ILD: interstitial lung disease; AvaII: restriction endonuclease; IS900: insertion sequence to identify M. avium subspecies and Mycobacterium paratuberculosis (M. paratuberculosis); IS902: insertion sequence to identify M. avium subspecies silvaticum and M. paratuberculosis; IS1110: insertion sequence to identify M. avium; BOOP: bronchiolitis obliterans (BO) with organising pneumonia; DR: direct repeat; WG: Wegener's granulomatosis; PLCH: pulmonary Langerhans cell histiocytosis; NZ: New Zealand; bp: base pair; MPB: MTB complex-specific mycobacterial protein; RFLP: restriction fragment length polymorphism; rpoB: RNA polymerase β-subunit gene; mtp 40: MTB species-specific primers; NAAT: nucleic acid amplification test; BD: Beckton Dickinson (Franklin Lakes, NJ, USA).

the available data, lymph node samples (n=356) were used in most studies, followed by the lung (n=146) and others (bronchial fluid (n=70), skin (n=56), others (lacrimal gland, pituitary; n=12), cerebrospinal fluid (n=1)). All studies used PCR for nucleic acid amplification followed by identification of nucleic acid sequences specific for different types of myco-bacteria (table 1). Most studies used primers to detect the presence of *M. tuberculosis* complex (MTC) and nontuberculous mycobacteria (NTM); however, some studies detected only *M. tuberculosis* [30, 39, 40, 43–45, 47, 54, 55, 57, 58] and some atypical mycobacterial species alone [34, 37]. As there were more samples than patients, which can lead to both over- and under-estimation bias, further patient samples were excluded (eight samples excluded: six negative and two positive) and only 874 patients with 874 samples were included. Similarly, 600 controls had 611 samples evaluated, therefore 11 samples were excluded (two positive and nine negative).

Of the 874 samples, 187 were positive for MTC and 43 samples were positive for NTM; one sample was found to have a nucleic acid sequence for both types of mycobacteria (table 1). In total, 231 samples from 874 patients were positive for mycobacteria with a positive signal rate of 26.4 (23.6–29.5%; fig. 2). Seven studies found a positive signal rate >50%, eight studies had a rate between 20–50%, nine studies found a positive signal in <20% and seven studies did not find any evidence of mycobacteria in the analysis (fig. 2).

Of the 31 studies, six had no control arm and were excluded for the calculation of OR and 95% CI. Five studies specifically reported on the detection of NTM (104 cases and 145 controls), and the odds of finding NTM was 19.63 (2.74–140.64) and 30.72 (8.32–177.18) by the random effects model and exact method, respectively (fig. 3, table 2). The odds of finding both MTC and NTM (680 cases and 600 controls) in samples of patients with sarcoidosis *versus* controls were 9.67 (4.56–20.5) using the random effects model (fig. 3, table 2) and 19.49 (10.86–37.1) using the exact method.

There was methodological (table 1) and statistical heterogeneity (Cochran Q-statistic 30.31, p=0.034; I^2 40.6 (8.2–73.1%); Chi-squared 35.02, p<0.0001). The funnel plot also showed evidence of publication bias (fig. 4).

DISCUSSION
Meta-analysis is a statistical procedure that integrates the results of several relevant independent studies and allows a common conclusion to be drawn from an entire body of
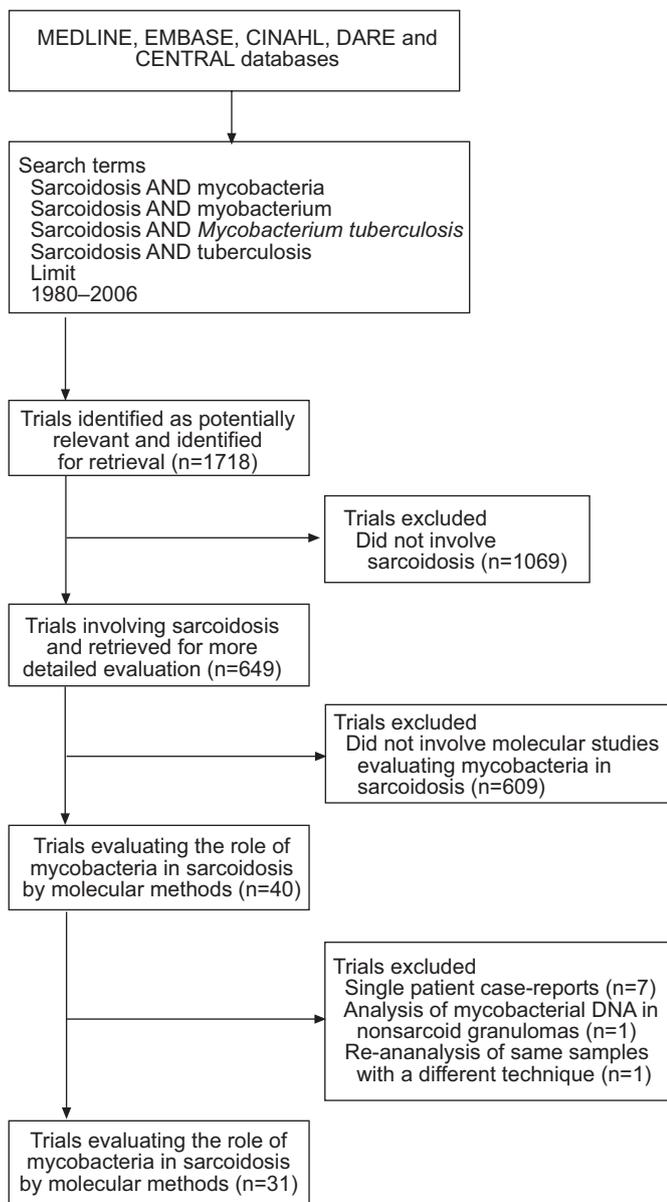
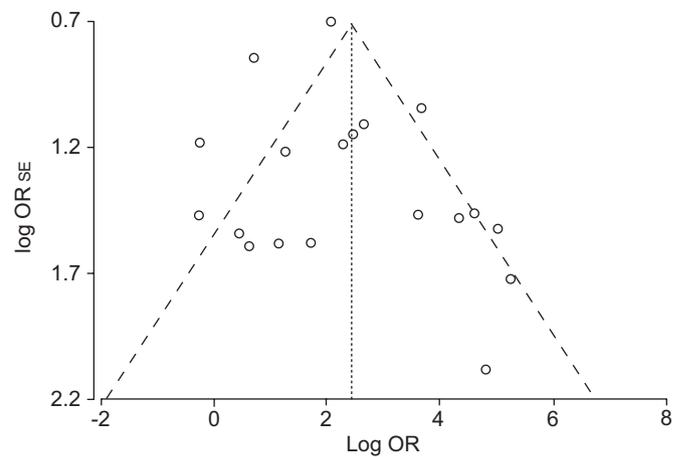**FIGURE 1.** Flow diagram of the current trial selection.
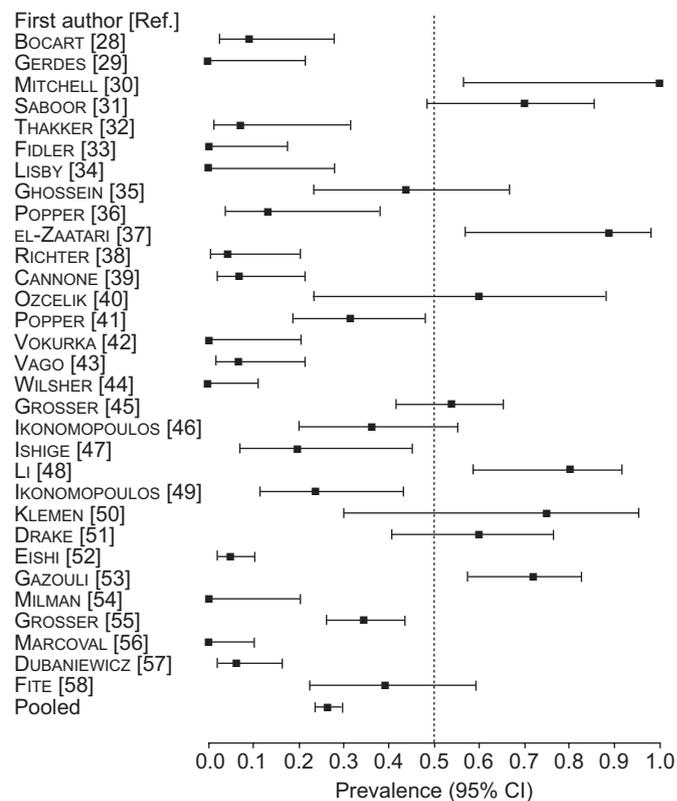
FIGURE 2. Forest plot of the mycobacterial DNA prevalence rate in samples of patients with sarcoidosis. CI: confidence interval.
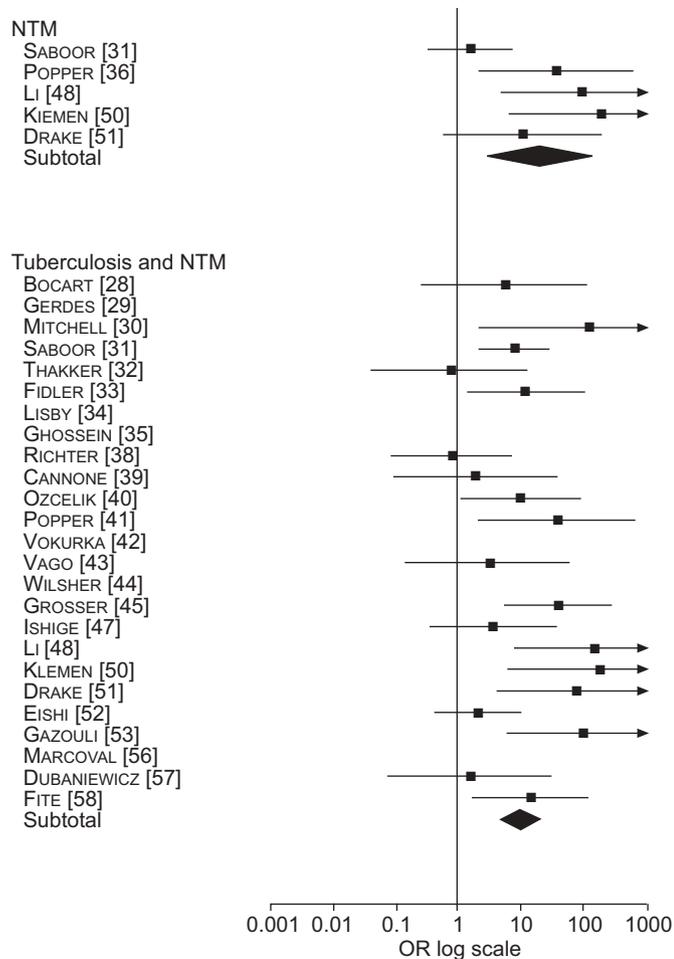
FIGURE 3. Forest plot showing the presence of mycobacteria in samples from sarcoidosis patients *versus* control samples. ■: odds ratio (OR); ——: 95% confidence interval, random effects model. NTM: nontuberculous mycobacterium.

FIGURE 4. Funnel plot comparing log odds ratio (OR) *versus* the standard error (SE) of log OR. ○: trials included in the meta-analysis; ·····: summary log OR; – – –: 95% confidence intervals. Asymmetry about the pooled OR line is consistent with the presence of publication bias.

research. It provides a more precise estimate of a treatment effect, and may explain heterogeneity between the results of individual studies. The results of the present meta-analysis indicate that almost 30% of patients with sarcoidosis have the presence of mycobacterial nucleic acids within the lesions and there are significant odds of finding mycobacteria in samples from sarcoidosis patients (OR=9.67 and 19.49 by the random effects and exact methods, respectively), thereby suggesting an association between mycobacteria and sarcoidosis. In addition to the conventional technique of random effects meta-analysis, the partial polynomial multiplication algorithm [15] was also used in the present study; because the method is exact the sparseness of individual studies in the current analysis was not an issue. Although the OR doubled with the exact technique, the CIs also widened, suggesting a weaker but definite association.

There are numerous factors that favour mycobacteria as a trigger for sarcoidosis. These include: histopathological appearances of the granulomas [2]; reports of mycobacterial disease either existing coincidentally, succeeding or pre-dating sarcoidosis [59, 60]; and the finding of mycobacteria in occasional granulomas of sarcoidosis [61–63]. Passage experiments have

also suggested that mycobacteria with characteristics of *M. tuberculosis* may be the incriminating agent [64–67]. Recent studies on humoral immunity to mycobacterial antigens from sarcoidosis patients have renewed interest in the potential of mycobacteria in sarcoidosis [68]. It has also been suggested that the organism might exist in a cell-wall deficient bacteria (L-form) and may be difficult to isolate [69]. However, there is no clear evidence to determine whether the tubercle bacillus is really the cause of sarcoidosis.

The presence of mycobacteria can be considered incidental but it may also suggest that sarcoid lesions result as a continued reaction to mycobacteria. The isolation of genetic material from mycobacteria in granulomas of sarcoidosis, at least, suggests that the granuloma was initiated by mycobacteria but whether the organism is currently viable is a different question.

Do mycobacteria have a role to play in causing sarcoidosis? The case of sarcoidosis does not meet Koch's postulates but this is not always true and many pathogens, for example *Pneumocystis jiroveii*, cannot be grown outside the host but do clearly cause pulmonary disease. It is also possible that these mycobacteria may be different from the conventional mycobacteria in that they may be difficult to culture using existing microbiological techniques. In fact, the organism causing Whipple's disease (*T. whippelii*) was grown in culture using a technique that added the cytokine interleukin-4, which causes deactivation of macrophages [70]. The present meta-analysis does provide evidence to suggest an association in ≥30% of cases, but future research is necessary to establish the cause of this association.

Interestingly, the presence of mycobacteria also seems to influence disease outcome, with mycobacterial nucleic acid-negative patients showing better outcomes [58]. In one study it was reported that all patients with mycobacteria-positive samples did not respond to the typical immunosuppressive treatment for sarcoidosis but the remaining mycobacteria-negative patients reacted positively to the same therapy [46]. Also, patients with positive mycobacterial nucleic acid samples

| TABLE 2 | Forest plot data of the presence of mycobacteria in samples from sarcoidosis patients *versus* control samples |

Study	Sarcoidosis n/N	Control n/N	Study weight	Random effects
NTM				
Saboor [31]	4/20	3/22	31.94	1.58 (0.31–8.15)
Popper [36]	11/35	0/39	18.26	37.08 (2.09–657.9)
Li [48]	14/20	0/20	17.65	91.46 (4.77–1754.5)
Klemen [50]	3/4	0/39	14.69	184.33 (6.26–5425.47)
Drake [51]	4/25	0/25	17.46	10.67 (0.54–209.64)
Subtotal	104	145	100	19.63 (2.74–140.64)
Tuberculous and NTM				
Bocart [28]	2/22	0/22	4.20	5.49 (0.25–121.18)
Gerdes [29]	0/14	0/10		NE
Mitchell [30]	5/5	0/5	2.80	121 (2.02–7259.18)
Saboor [31]	14/20	5/22	9.02	7.93 (1.99–31.59)
Thakker [32]	1/14	1/11	4.59	0.77 (0.04–13.87)
Fidler [33]	7/16	1/16	6.12	11.67 (1.23–110.95)
Lisby [34]	0/18	0/18		NE
Ghossein [35]	0/10	0/10		NE
Richter [38]	1/24	3/57	5.95	0.78 (0.08–7.93)
Cannone [39]	2/30	0/10	4.16	1.84 (0.08–41.62)
Ozcelik [40]	3/5	2/15	5.92	9.75 (0.95–99.96)
Popper [41]	11/35	0/39	4.62	37.08 (2.09–657.90)
Vokurka [42]	0/15	0/27		NE
Vago [43]	2/30	0/17	4.20	3.07 (0.14–67.75)
Wilsher [44]	0/31	0/10		NE
Grosser [45]	35/65	1/34	6.72	38.50 (4.96–298.57)
Ishige [47]	3/15	1/15	5.75	3.50 (0.32–38.23)
Li [48]	16/20	0/20	4.39	150.33 (7.54–2997.83)
Klemen [50]	3/4	0/39	3.72	184.33 (6.26–5425.47)
Drake [51]	15/25	0/25	4.56	75.29 (4.12–1377.06)
Eishi [52]	5/108	2/86	7.99	2.04 (0.39–10.78)
Gazouli [53]	33/46	0/20	4.62	101.74 (5.74–1804.62)
Marcoval [56]	0/35	0/39		NE
Dubaniewicz [57]	3/50	0/10	4.31	1.55 (0.07–32.27)
Fite [58]	9/23	1/23	6.35	14.14 (1.61–124.11)
Subtotal	680	600	100	9.67 (4.56–20.50)

Data are presented as % or odds ratio (95% confidence interval), unless otherwise stated. NTM: nontuberculous mycobacteria; NE: not estimable.

have been found to show a tendency for a chronic disease course compared with mycobacteria-negative patients [55].

Can treatment with anti-tuberculous drugs alter the natural history and course of sarcoidosis? Very few trials have been conducted in the past and the results of these trials have been discouraging. These trials were generally small studies and were limited by time-bias and the use of older regimens based on isoniazid, amino-salicylic acid and streptomycin [71–76]. The current authors' experience (in a high tuberculosis-endemic zone) with a large (n=500) number of patients with sarcoidosis show that a substantial number (on an average 40%, unpublished data) of patients are treated for tuberculosis

and anti-tuberculous therapy probably does not influence outcome in these patients. However, this data reflects only the patients who have been referred to the tertiary care institute and this observation needs to be confirmed by a randomised controlled trial.

The major limitation of the present systematic review is reflected in the highly variable results with rates of mycobacteria-positive samples ranging 0–100%, which precludes any definite causal association. The reasons may be manifold and include issues of technique (including the type of mycobacteria detected) and thus the sensitivity and specificity of PCR [77]; the organ specificity of the biopsy specimens may

also have some role to play. Some variation may also be observed because of the difference in local environmental exposures and the fact that some sarcoidosis patients may have a disorder initiated by organisms or triggers other than mycobacteria.

In conclusion, the results of the present study suggest an association between mycobacteria (both *Mycobacteria tuberculosis* complex and nontuberculous) and sarcoidosis. These mycobacteria might be members of slow-growing types with low pathogenic potential but the capability of eliciting a type IV immune response. To avoid methodological diversity, larger multicentre trials with a central laboratory for sample testing should be designed.

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