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Molecular Evidence for the Role of Mycobacteria in Sarcoidosis: A Meta-Analysis

Dr Dheeraj Gupta[1]
Dr Ritesh Agarwal[2]
Dr Ashutosh N Aggarwal[3]
Dr Surinder K Jindal[4]
[2]Assistant Professor, [3]Associate Professor, [1]Additional Professor and [4]Professor
Department of Pulmonary Medicine,
Postgraduate Institute of Medical Education and Research,
Chandigarh, India.

Corresponding Author:
Dr. Dheeraj Gupta
Additional Professor
Department of Pulmonary Medicine,
Postgraduate Institute of Medical Education and Research,
Sector-12,
Chandigarh -160012.
India.
Phone: 91-172-2756823
Fax: 91-172- 2748215
E-mail: dheeraj@indiachest.org

ABSTRACT

Background and Aims: The etiology of sarcoidosis remains unknown. Because of the clinical and histological similarities with tuberculosis, the role of mycobacteria has been repeatedly investigated as etiologic agent for sarcoidosis. This meta-analysis is aimed at evaluating the available molecular evidence on the possible role of mycobacteria in causation of sarcoidosis.

Methods: We searched the MEDLINE, EMBASE, CINAHL, DARE, CENTRAL databases for relevant studies published from 1980 to 2006, and included studies that have evaluated the presence of mycobacteria using molecular techniques in biological samples of patients with sarcoidosis. We then calculated the 95% confidence intervals (CI) for the expected proportion (of individual studies) and pooled the data to get a summary success rate with 95% CI. We also calculated the odds ratio (OR) and 95% CI to assess the presence of mycobacteria in samples of patients with sarcoidosis versus those from non-sarcoidosis control samples.

Results: Our search yielded 31 studies. All studies had used polymerase chain reaction (PCR) 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% (95% CI, 23.6-29.5), and the odds of finding mycobacteria in samples of patients with sarcoidosis versus controls were 9.67 (95% CI, 4.56-20.5) using the random effects model and 19.49 (95% CI, 11.21-35.54) using the exact method. There was methodological and statistical heterogeneity (Cochran Q statistic 30.31, P = 0.034; I^2 40.6%; 95% CI, 8.2% to 73.1%; chi-square statistic 35.02, P <0.0001) and evidence of publication bias.

Conclusions: The results of this study show that there is a demonstrable mycobacterial presence in sarcoidosis lesions suggesting an association between mycobacteria and some cases of sarcoidosis. To avoid methodological diversity, larger multicenter trials with a central laboratory for testing of the samples should be designed.

Key words: sarcoidosis, mycobacteria, tuberculosis, polymerase chain reaction

INTRODUCTION

Sarcoidosis is a granulomatous disease of unknown etiology characterized 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 etiologic agents have been incriminated, both infective and non-infective.[3] Non-infective agents have been implicated because of their epidemiologic association,[4] but have not stood the test of time.[5] Currently, infective agents are in focus and the two strongest contenders are the *Propionibacterium* and the *Mycobacterium*.[6] A recent study of culture and PCR analysis for *Propionibacterium* species from lung tissues and lymph nodes from subjects with and without sarcoidosis showed that *P. acnes* is a common commensal in peripheral lung tissues and mediastinal lymph nodes, suggesting that it is less likely to be the trigger for sarcoidosis.[7]

From the time sarcoidosis has been 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 histologic staining or culture from pathologic 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 the putative agents serves as an alternative method for isolating fastidious organisms. This method has been used successfully in identifying *Tropheryma whippelli* 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 this study is to systematically analyze the available literature on the issue.

MATERIAL AND METHODS

Search strategy and selection criteria

To identify the studies for inclusion in this review, all the authors independently searched the National Library of Medicine's MEDLINE, EMBASE, CINAHL, DARE, CENTRAL databases for relevant studies published from 1980 to 2006 using free text terms : sarcoidosis AND mycobacteria, sarcoidosis AND mycobacterium, sarcoidosis AND mycobacterium tuberculosis, 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, we reviewed our personal files. All the studies irrespective of language were identified.

Data abstraction

The abstracts of the studies were independently reviewed by two authors (RA and DG), without blinding to study the details. Any disagreement was resolved by discussion between the authors. We included studies that have 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:

- Publication details (title; author(s); other citation details)
- Number of patients with sarcoidosis and the number and type (tissue or cellular samples) of samples used for analysis
- Details of the molecular test used for evaluating the presence of mycobacteria in samples; the type of mycobacteria (tuberculous vs. non-tuberculous mycobacteria)

- Percentage of the samples positive for mycobacteria in patients with sarcoidosis versus the samples positive in control samples

Determination of the pooled treatment effect:

To calculate the percentage positivity of mycobacteria in sarcoid samples, we used binomial proportions in which the numerator was success in isolation of mycobacteria and denominator the total study sample. The expected proportion was the success rate of each study included. We then calculated the 95% confidence intervals (CI) for the expected proportion using the Newcombe-Wilson method.[11, 12] The data from individual studies was then pooled, and a summary success rate with 95% CI was calculated by summing up the positivity rate as numerator and the total samples evaluated as the denominator.[13] The statistical package StatsDirect (StatsDirect version 2.5.7 for MS-Windows, England, StatsDirect Ltd, 2005. http://www.statsdirect.com), Review Manager (RevMan, Version 4.2.8 for MS-Windows, Copenhagen, The Nordic Cochrane Centre, The Cochrane Collaboration, 2003) and EXACTMA (downloaded at http://www.sph.emory.edu/~haustin/exactma.html) were used to perform the statistical analysis.

We also calculated the odds ratio (OR) and 95% confidence intervals (CI) to assess the presence of mycobacteria in samples of patients with sarcoidosis versus the samples from 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 with 95% confidence intervals described by Martin et al [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 (p value) level at which heterogeneity should be diagnosed is unclear, given that the Q statistic has low power, and Fleiss et al. has recommended a value of at least 0.1.[16]

The impact of heterogeneity upon the pooled estimates of the individual outcomes of the meta-analysis was assessed using the chi-square test and/or the I^2 tests (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 more than 40% indicates significant heterogeneity. As the chi-square test has a low sensitivity for detecting heterogeneity, a P value of less than 0.1 was considered significant for the presence of statistical heterogeneity.[17]

Assessment of Publication bias:

We examined the presence of a small study effect 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 open circle represents each study in the meta-analysis. The line in the centre indicates the summary OR and the other two lines indicate 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.

The institutional review board clearance was not required for this manuscript as this was a meta-analysis of published studies.

RESULTS

Our initial data search yielded a total of 1718 citations (Figure 1). We excluded 1679 articles by reviewing both the title and abstract as they did not meet our inclusion criteria. Forty studies were identified that have used nucleic acid amplification techniques to identify mycobacterial nucleic acid sequences; nine studies were further excluded- seven were single patient case reports [19-25], one study had analyzed mycobacterial DNA in non-sarcoid granulomatous disease [26] and one study had re-analyzed the *M tuberculosis* positive samples of sarcoid patients using a different technique to further clarify the type of *M tuberculosis* [27]. We finally selected 31 articles for our analysis [28-58] (Table 1).

All the studies have provided detailed methodology and have been published from around the globe (Table 1). In all, the studies involved 882 samples (711 tissue samples and 71 fluid samples from which cells were isolated and further analyzed) from 874 patients with proven sarcoidosis and 611 samples from 600 controls (lung cancer, bronchiectasis and other lung disorders). All but six studies [36, 38, 48, 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 studies have used positive control samples of patients with proven mycobacterial infections [28, 30, 32, 54, 55]. The details regarding the sites of biopsy were not provided by all the studies but from the available data the lymph node (n=356) samples have been used in most studies followed by lung (n=146) and others (bronchial fluid- 70, skin-56, others (lacrimal gland, pituitary and others) - 12, cerebrospinal fluid- one). All studies have used polymerase chain reaction (PCR) for nucleic acid amplification followed by identification

of nucleic acid sequences specific for different types of mycobacteria (Table 1). Most studies had used primers to detect the presence of *M tuberculosis* complex (MTC) and non-tuberculous mycobacteria (NTM); however, some studies detected only *M tuberculosis* [30, 41-44, 46, 47, 54, 55, 57, 58] and some atypical mycobacterial species alone.[33, 38] As there were more samples than patients which can lead to both over- and under-estimation bias we excluded extra samples from patients and included only 874 patients (8 samples excluded- six negative and two positive) with 874 samples; similarly 600 controls had 611 samples evaluated and 11 samples were excluded (two positive and nine negative).

Of the 874 samples, 187 were found positive for *M tuberculosis* complex and 43 samples were positive for non-tuberculous mycobacteria; one sample was found to have nucleic acid sequence for both the types of mycobacteria (Table 1). Overall, 231 samples from 874 patients were positive for mycobacteria with a positive signal rate of 26.4% (95% CI, 23.6-29.5) [Figure 2]. Seven studies found a positivity rate more than 50%, eight studies between 20-50%, nine studies found positive signal in less than 20% and seven studies did not find any evidence of mycobacteria in their analysis (Figure 2).

Of the 31 studies, six studies had no control arm and were excluded for the calculation of odds ratio 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 (95% CI, 2.74-140.64) and 30.72 (8.32-177.18) by the random effects model and exact method respectively (Figure 3). The odds of finding both MTC and NTM mycobacteria (680 cases and 600 controls) in samples of patients with sarcoidosis versus controls were 9.67

(95% CI, 4.56-20.5) using the random effects model (Figure 3) and 19.49 (95% CI, 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%; 95% CI, 8.2% to 73.1%; chi-square statistic 35.02, P <0.0001). The funnel plot also showed evidence of publication bias (Figure 4).

DISCUSSION

Meta-analysis is a statistical procedure that integrates the results of several relevant independent studies, and allows one to arrive at a common conclusion from an entire body of research. It provides a more precise estimate of a treatment effect, and may explain heterogeneity between the results of individual studies. The results of this meta-analysis indicate that almost 30% of patients with sarcoidosis have the presence of mycobacterial nucleic acids in the lesions and there is significant odds of finding mycobacteria in samples of patients with sarcoidosis (OR=9.67 by random effects model and 19.49 by the exact method), thereby suggesting an association between mycobacteria and sarcoidosis. Apart from the conventional technique of random-effects meta-analysis, we also used the partial polynomial multiplication algorithm in this study;[15] because the method is exact, sparseness of individual studies, which was seen in this analysis, is not an issue. Although the odds ratio doubled by the exact technique, the confidence intervals also widened, suggesting a weaker but definite association.

There are numerous factors that favor mycobacteria being a trigger for sarcoidosis. These include histopathological appearances of the granulomas [2], reports of mycobacterial disease either existing coincidentally, succeeding or antedating 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 a potential of mycobacteria in sarcoidosis.[68] It has also been suggested that the organism might exist in a cell wall deficient L-form and may be difficult to

isolate.[69] However, there is no clear evidence to say whether the tubercle bacillus, is really the cause of sarcoidosis.

The presence of mycobacteria can be considered incidental but it may also suggest that sarcoidosis lesion 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 the Koch's postulates but this is not always true and many pathogens for example the *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 it may be difficult to culture those using existing microbiologic 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] This meta-analysis does provide evidence to suggest an association in at least 30% cases. Future research should probably focus on establishing the causality.

Interestingly, the presence of mycobacteria also seem to influence outcome, with mycobacterial-nucleic acid negative patients showing better outcomes.[58] In one study it was reported that all the patients with the 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.[48] Also, patients

with mycobacterial-nucleic acid positivity have been found to show tendency for a chronic course compared to the 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 but the results of these trials have been discouraging. These trials were generally small studies and limited by time-bias and the fact that they have used older regimens based on isoniazid, amino-salicylic acid and streptomycin.[71-76] Our experience (in a high endemic zone for tuberculosis) with a large (n=500) number of patients with sarcoidosis show that a substantial number (on an average 40%- unpublished data) of patients with sarcoidosis get treated for tuberculosis and anti-tuberculous therapy probably does not influence outcome, at least 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 randomized controlled trial.

The major limitation of this systematic review is reflected in the highly variable results with positivity rates ranging from 0-100% which precludes any definite causal association. The reasons may be manifold and includes issues of technique (including the type of mycobacteria detected) and thus the sensitivity and specificity of the PCR [77]; the organ specificity of the biopsy specimens may also play some role. Some variation may also be observed because of the difference in local environmental exposures and the fact that some sarcoid patients may have a disorder initiated by organisms or triggers other than mycobacteria.

In conclusion, the results of this study suggest an association between mycobacteria (both MTC and NTM) and sarcoidosis. These mycobacteria might be

members of slow-growing types with low pathogenic potential but still capable of eliciting a type IV immune response. To avoid methodological diversity, larger multicenter trials with a central laboratory for testing of the samples should be designed.

FIGURE LEGENDS

Figure 1: Flow diagram showing the trial selection process for this meta-analysis

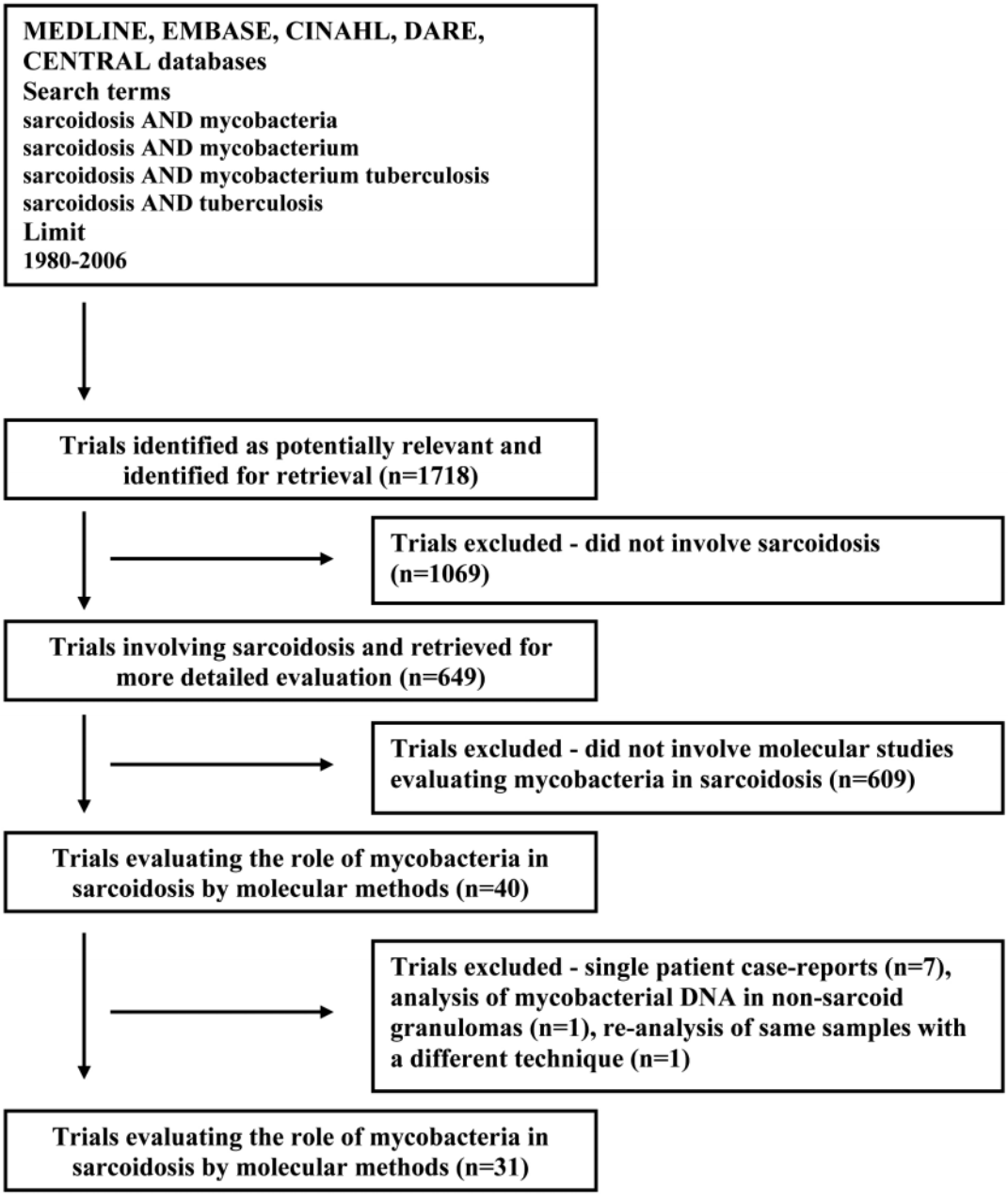

Figure 2: Forest plot showing the prevalence rate (with 95% confidence intervals) of mycobacteria in samples of patients with sarcoidosis

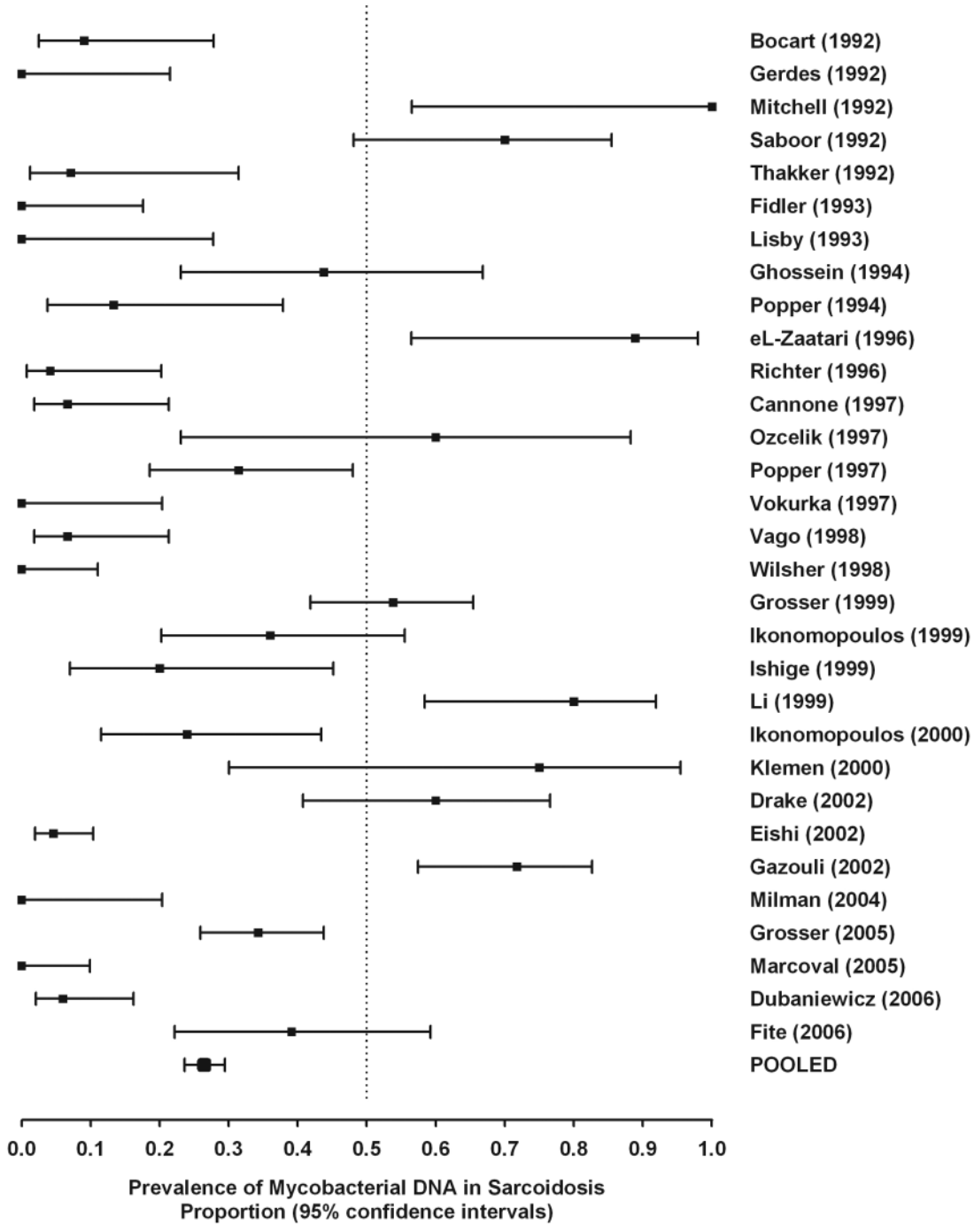

Figure 3: Forest plot showing the presence of mycobacteria in samples of patients with sarcoidosis versus control samples (odds ratio with 95% CI intervals, random effects model)

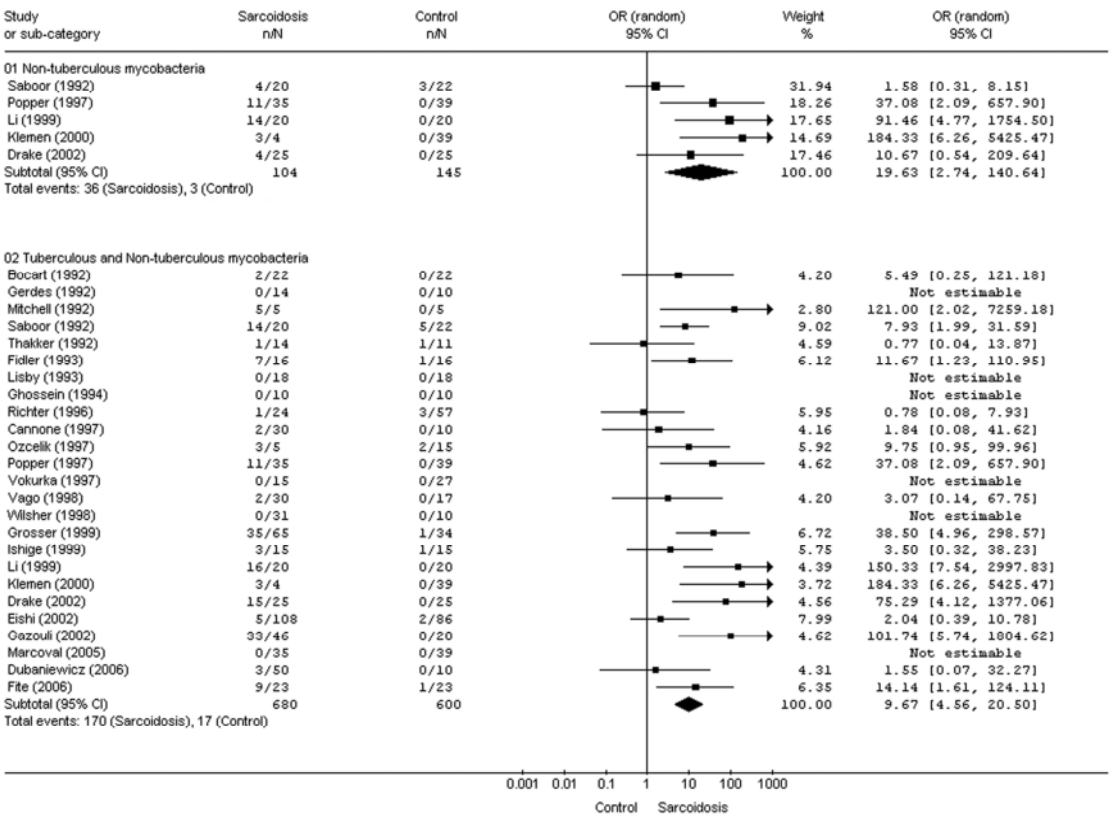

Figure 4: Funnel plot comparing log odds ratio (OR) versus the standard error of log OR. Open circles represent trials included in the meta-analysis. The line in the center indicates the summary log OR. The other lines represent the 95% confidence intervals. Asymmetry about the pooled OR line is consistent with the presence of publication bias.

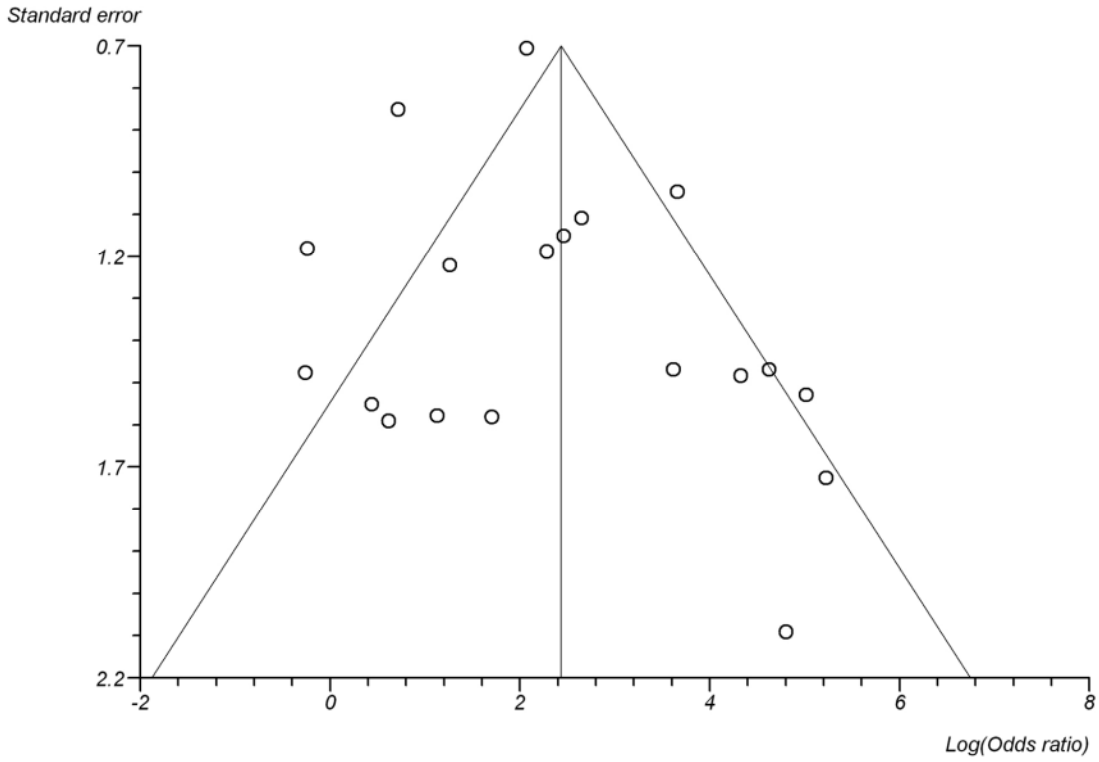
Standard error
0.7
1.2
1.7
2.2
-2
0
2
4
6
8
Log(Odds ratio)

Table 1: Studies that have evaluated the role of mycobacteria in sarcoidosis using molecular techniques

Author (year)	Site	Molecular technique used	Patients				Controls			
			No.	Tissue /fluid	n/N	Type of Myco-bacteria	No.	Diagnosis	n/N	Type of Myco-bacteria
Bocart (1992)	France	PCR amplification- of gene fragment encoding for 65 kDa mycobacterial antigen and *IS6110* sequence	22	16/6	2/22	MTB	22	Benign or malignant tumors, non-granulomatous diseases	0/22	-
Gerdes (1992)	Germany	PCR amplification- of mycobacterial gene fragment 16S rDNA	14	NA	0/14	-	10	Lung cancer, non-mycobacterial lung diseases	0/10	-
Mitchell (1992)	UK	Mycobacterial rRNA detection by liquid phase hybridization	5	5/0	5/5	MTB	5	Non-mycobacterial lung diseases	0/5	-
Saboor (1992)	UK	PCR amplification- of *IS986*/*IS6110* sequence and gene fragment encoding for mycobacterial *groEL* gene	20	0/20	14/20	MTB (10), NTM (4)	22	Lung cancer, hemoptysis, ILD	5/22	MTB (3), NTM (2)
Thakker (1992)	UK	PCR amplification- of gene fragment encoding for mycobacterial groEL gene followed by staining using *AvaII*	14	14/0	1/14	MTB	11	Non-specific reactive lymph nodes	1/11	MTB
Fidler (1993)	UK	PCR amplification- of gene fragment encoding for 65 kDa mycobacterial antigen and *IS6110* sequence	16	16/0	7/16	MTB	16	Squamous cell cancer, Hodgkin's disease	1/16	MTB
Lisby (1993)	Denmark	Nested PCR specific for *IS900* sequence of M paratuberculosis	18	18/0	0/18	-	18	Normal donors	0/18	-
Ghossein (1994)	US	PCR amplification- of gene fragment encoding for 65 kDa mycobacterial antigen	10	10/0	0/10	MTB	10	Normal tissue, benign or malignant neoplasms	0/10	-
Popper (1994)	Austria	PCR amplification- of gene fragment encoding for 65 kDa mycobacterial antigen and *IS6110* sequence	15	15/0	2/15	MTB	0	None	0/0	-
el-Zaatari (1996)	US	PCR amplification- of *IS902*/*IS900* sequence	9	8/1	8/9	NTM	0	None	0/0	-

Richter (1996)	Germany	PCR amplification- of mycobacterial gene fragment 16S rDNA	24	10/14	1/24	MTB	57	Cancer, Fibrosis, Pneumonia and infection with Mycoplsma	3/57	MTB
Cannone (1997)	Italy	PCR10 amplification- of *IS6110* sequence	30	30/0	2/30	MTB	10	Non-tuberculous, non-sarcoid lung disease	0/10	-
Ozcelik (1997)	Turkey	PCR ampl0ification- of *IS6110* sequence	5	11/0	5/11	MTB	15	Non-tuberculous, non-sarcoid lung disease	2/15	MTB
Popper (1997)	Austria	PCR amplification- of gene39 fragment encoding for 65 kDa mycobacterial antigen, *IS1110* and *IS6110* sequence	35	35/0	11/35	NTM	39	Cancer, bronchiectasis, BOOP, Pneumococcal and viral pneumonia	0/39	-
Vokurka (1997)	France	PCR amplification- of *IS6110* sequence and DR region	15	15/0	0/15	MTB	27	Cancer lung, WG, BO, PLCH	0/27	-
Vago (1998)	Italy	PCR amplification- of *IS6110* sequence	30	30/0	2/30	MTB	17	Non-tuberculous, non-sarcoid lung disease	0/17	-
Wilsher (1998)	NZealand	PCR amplification- of *IS6110* sequence and nested PCR to further amplify an 85 bp sequence within the 123 bp product	31	31/0	0/31	-	10	Cancer lung, Lymphoma, seminoma, bronchiectasis, reactive lymph nodes	0/10	-
Grosser (1999)	Germany	PCR amplification- of *IS986/IS6110* sequence	65	50/15	35/65	MTB	34	Lung tissue from still birth, iliac lymph nodes	1/34	MTB
Ikonomo-poulos (1999)	Greece	PCR amplification- of gene encoding the immunogenic protein MPB 64; of *IS1110* sequence and 383 bp region of the 65 kDa mycobacterial antigen gene	25	25/0	9/25	MTB	0	None	0/0	-
Ishige (1999)	Japan	PCR amplification- of *IS6110* sequence	15	15/0	3/15	MTB	15	Cancer stomach	1/15	MTB
Li (1999)	US	PCR amplification- of gene fragment encoding for 65 kDa mycobacterial antigen followed by restriction enzyme pattern analysis	20	20/0	16/20	MTB (2), NTM (14)	20	Foreign body granuloma, normal skin, dermatitis	0/20	-

Ikonomo-poulos (2000)	Greece	PCR amplification- of gene encoding the immunogenic protein MPB 64; outward primer PCR of *IS1110* sequence and amplification of *IS1110* and 16S rRNA sequences	25	25/0	6/25	MTB	0	None	0/0	-
Klemen (2000)	Austria	PCR amplification- of *IS6110* sequence and gene fragment encoding for mycobacterial chaperonin	4	4/0	3/4	NTM	39	Cancer, bronchiectasis, BOOP, Pneumococcal and viral pneumonia	0/39	-
Drake (2002)	US	PCR amplification- for Mycobacterium species 16S rRNA, rpoB, and *IS6110* sequences	25	25/0	15/25	MTB (11), NTM (3), both (1)	25	Cancer, Lymphoma	0/25	-
Eishi (2002)	Japan	PCR amplification- of *IS6110*/*IS900* sequence	108	108/0	5/108	MTB (5)	86	Cancer lung, non-specific lymphadenitis	2/86	-
Gazouli (2002)	Greece	PCR amplification- *IS6110*, MBP64 and mtp40 sequences	46	46/0	33/46	MTB (33)	20	Cancer lung	0/20	-
Milman (2004)	Denmark	Cobas Amplicor ®	15	0/15	0/15	-	0	None	0/0	-
Grosser (2005)	Germany	PCR amplification- of 123 bp fragment of *IS6110*/*IS 986* sequence	105	NA	36/105	MTB	0	None	0/0	-
Marcoval (2005)	Spain	NAAT for rRNA of M tuberculosis	35	35/0	0/35	-	39	Not mentioned	0/39	-
Dubanie-wicz (2006)	Poland	BD ProbeTec *IS6110* amplification	50	50/0	3/50	MTB	10	Ca lung, reactive lymph nodes	0/10	-
Fite (2006)	Spain	PCR amplification- of *IS6110* sequence followed by Southern blot hybridization	23	23/0	9/23	MTB	23	Lung neoplasm	1/23	MTB

n positive samples; N total samples; PCR- polymerase chain reaction; MTB- *M tuberculosis*; NTM- non tuberculous mycobacteria
IS6110- insertion sequence to identify MTB complex (MTB, *M africanum*, *M bovis*, bacillus Calmette-Guerin); IS1110- insertion sequence to *M avium*; IS900- insertion sequence to identify *M avium* subspecies and *M paratuberculosis*; 65 kDa surface antigen and 16S rRNA common to all mycobacteria; MBP 64- MTB complex-specific protein; mtp 40- MTB species-specific primers; groEL gene- codes for 65 kDa surface antigen common to all mycobacteria
ILD- interstitial lung disease, BO- bronchiolitis obliterans, BOOP- BO with organizing pneumonia, WG- Wegener's granulomatosis, PLCH- pulmonary Langerhans cell histiocytosis

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