MYCOBACTERIUM *W* IMMUNOTHERAPY FOR TREATING PULMONARY TUBERCULOSIS – A SYSTEMATIC REVIEW

BY

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PNDSHA001

SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

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SUPERVISOR: PROFESSOR BM MAYOSI
UNIVERSITY OF CAPE TOWN, FACULTY OF HEALTH SCIENCES, DEPARTMENT OF MEDICINE.

DECLARATION

I, Shaheen Pandie, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, or is to submitted for another degree in this or any other university.

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SIGNATURE: Signed by candidate

DATE: 14 APRIL 2011

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PART A: PROTOCOL

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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MYCOBACTERIUM *W* ADJUVANT IMMUNOTHERAPY IN PULMONARY TUBERCULOSIS – PROTOCOL FOR A SYSTEMATIC REVIEW

SHAHEEN PANDIE, MARK ENGEL, ZITA KERBELKER, BONGANI MAYOSI

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ABSTRACT

BACKGROUND

Tuberculosis (TB) remains a major health problem in the developing world, accounting for approximately 2 million deaths per year. The search for appropriate and applicable therapies to reduce the burden of TB is essential. Mycobacterium *w* (M *w*) is a heat-killed immune-modulating vaccine designed to attenuate the effects of TB infection and reduce the time to sputum negativity, thereby improving cure rates and decreasing transmission rates.

HYPOTHESIS

The use of M *w* immunotherapy in patients with pulmonary TB (PTB) is associated with a reduction in time to sputum negativity (i.e. proxy for cure).

OBJECTIVES

To assess the effects of M *w* immunotherapy on sputum conversion in participants with PTB.

SEARCH METHODS

Two independent investigators will perform a comprehensive search for randomised and quasi-randomised controlled trials of M *w* use in PTB. This will include:

- An electronic search of the following trial registries: The Cochrane Infectious Diseases Group specialised trials register, the Cochrane Controlled Trials Register, the Pan African National Clinical Trials Registry (PACTR), the World Health Organisation (WHO) International Clinical Trials Registry Platform (ICTRP), and ClinicalTrials.gov
- An electronic search of the following databases: The Cochrane Library (February 2010 Issue), MEDLINE, OVID, and LILACS

- A handsearch of the reference lists of identified articles, abstracts, and conference proceedings including: The International Union Against Tuberculosis and Lung Disease World Congress (IUATLD), The American Thoracic Society International Congress (ATS) and The European Respiratory Society World Congress (ERS)
- Correspondence to authors, experts and organisations working in the field of TB immunotherapy requesting information about unpublished or work-in-progress data.

SELECTION CRITERIA

Studies selected for review will be randomised or quasi-randomised controlled trials of M *w* treatment in participants with PTB.

DATA COLLECTION AND ANALYSIS

Data will be extracted using a standardised data extraction form. Each article will have an assessment of risk of bias, which will include information on sequence generation, allocation concealment, blinding, incomplete outcome or missing data, and selective outcome reporting. Data will be analysed using Review Manager 5.0.15 (RevMan5), comparing M *w* to control for the outcomes of: (i) sputum conversion (cure), (ii) death and (iii) adverse events.

BACKGROUND

DESCRIPTION OF THE CONDITION

The 2009 Global Tuberculosis Control Update estimated that there were 9.4 million incident cases, 11.1 prevalent cases and 1.82 million deaths (0.52 million in HIV positive individuals) attributed to tuberculosis (TB). The major burden of disease is concentrated in the developing world (WHO 2009). The annual incidence continues to increase in Africa because of the human immunodeficiency virus (HIV) epidemic (Murray 2004). In South Africa, TB has been identified as a major public health challenge. In the World Health Organization's (WHO) 2008 Global high-burden TB rankings, South Africa ranked 4th out of 22 (WHO 2008). This epidemic has been compounded by the emergence of drug resistant TB. Multi-drug resistant (MDR) TB is defined as resistance to both isoniazid and rifampicin, the two drugs regarded as the most effective anti-tuberculous treatment. Extensively drug resistant (XDR) TB is defined as MDR plus resistance to a fluoroquinolone and a second-line injectable agent. On a global scale, MDR TB is reported to account for 10% of the 9 million new cases of TB that occur annually (Mitnick 2007). Current treatment strategies for TB, MDR TB and XDR TB include prolonged oral and injectable drug therapies. Treatment regimens are problematic because of high pill burdens and toxicity, which result in low cure and treatment completion rates. Therefore, the investigation of new, applicable anti-tuberculous strategies is essential.

The immunopathogenesis of TB involves a complex interplay between bacteriostatic and bactericidal immune pathways. Bacteriostatic processes result in a walled off granuloma, while the bactericidal processes of autophagy, apoptosis, and cytotoxic T-cell destruction, result in cell death (Churchyard 2009). A combination of the traditionally known T-Helper 1 response (key cytokines include INFgamma, TNF-ɣ, IL-15) and cytotoxic T-lymphocyte (CTL) killing is thought to provide the optimal immune protection against TB. The T-Helper 2 response (IL-4, TGF-ß, IL -10) directly reduces the immune response to TB by both delaying the maturation of Th1 cells, and inhibiting the production of cytokines that drive Th1 and CTL cells. Therefore, the ideal immunotherapeutic strategy would be to inhibit Th2 while enhancing the protective Th1 and CTL pathways.

DESCRIPTION OF THE INTERVENTION

Mycobacterium w **(M *w*)** is a non-pathogenic, saprophytic, rapidly growing atypical *Mycobacterium* species with immuno-potentiating properties. Recent polyphasic taxonomic analysis classified M *w* as a distinct species, *Mycobacterium indicus pranii*, placing it in the Runyon Group IV along with *M Vaccae, M fortuitum and M smegmatis* (Saini 2009). Its uniqueness stems from its ability to undergo antigen-driven blast leukocyte transformation. When administered as an intra-dermal heat killed vaccine, it

stimulates a Th1 cellular immune response against shared epitopes for both *Mycobacterium leprae* and *Mycobacterium tuberculosis*. An extensive body of data supports the safety and efficacy of M *w* in the prevention and treatment of leprosy (Nath 1998, Sharma 2005). Laboratory, animal and clinical work investigating M *w* use in TB suggests reduction in time to sputum conversion, a proxy for cure, and thus a potential reduction in spread of disease (Rieder 1996).

HOW THE INTERVENTION MIGHT WORK

The precise mechanism of action of M *w* is not understood. M *w* shares B- and T-cell antigen epitopes with *Mycobacterium leprae* and with *Mycobacterium tuberculosis* (Ganju 1990, Singh 1991). The initial work (animal trials and phase I - III human clinical trials) has resulted in the widespread use of M *w* as adjuvant treatment for leprosy. This outcome is testament to the hypothesis that the use of M *w* as an immune modulator for diseases with overlapping *Mycobacterium* antigens is both scientifically plausible and clinically relevant to investigate. In humans, injection of heat-killed M *w* was tested as an adjunct to standard antibiotic therapy in phase 3 clinical trials of lepromatous leprosy. Participants with leprosy received multidrug therapy (MDT) plus M *w*, while the control arm received MDT plus placebo injection. This study showed that bacteriological clearance was more rapid in the M *w* group ($P < 0.03$). There was also an associated decrease in the number of organisms (bacillary load), shorter duration of antibiotic therapy, and earlier discharge from care (Zaheer 1995, Sharma 2000, Kaur 2002). In healthy contacts of leprosy patients, M *w* was associated with protection from lepromatous infection (Sharma 2005). Sharma et al vaccinated a total of 24060 household contacts with M *w* or placebo in a double-blind, randomised trial. Participants were followed up for 8-10 years, at 3 yearly intervals. M *w* showed a protective efficacy of 68%, 59% and 39.3% at the respective follow-up intervals. The safety and efficacy of M *w* has thus been well established through widespread use as adjuvant therapy for the treatment and prevention of leprosy.

As regards TB, multiple laboratory studies have demonstrated that mice immunised with heat-killed M *w* had increased Th1 lymphocyte and macrophage activity, with a cytokine environment that was predominantly IL2 and INFgamma. In addition, immunised mice were protected from sub-lethal challenge with *M. tuberculosis* (Gupta 2009, Guleria 1993, Singh 1992). Human data of heat-killed M *w* use as immunotherapy for active TB are not definitive. Results from published and unpublished clinical trials suggest that M *w* administration is associated with a reduction in time to sputum negativity and improved cure rates (Patel 2002, Patel 2003, Luhadia 2004). In addition, Katoch reviewed the participants (healthy contacts) of the leprosy studies looking for evidence of new TB infection (Katoch 2008). The results suggest that M *w* significantly ($P < 0.01$) reduced the rate of new TB infection. In terms of safety, M *w* has been administered to thousands of participants in multiple trials and studies,

with no reports of serious adverse events. All published studies have concluded that M *w* is safe. Reported side effects are related to local injection site erythema, induration and pain.

WHY IT IS IMPORTANT TO DO THIS REVIEW

The aim of this systematic review is to summarise the evidence of the effectiveness of M *w* vaccination as adjunctive treatment for PTB. There is currently no consensus on the effectiveness of immune-modulating vaccines use in TB. This information will be of help to policy makers, healthcare practitioners and researchers in this area.

Our hypothesis is that in patients with PTB:

- M *w* immunotherapy is associated with a reduction in time to sputum conversion (i.e. proxy for cure)
- M *w* immunotherapy is associated with a decrease in mortality
- M *w* immunotherapy is safe in patients with PTB

OBJECTIVES

To review the available data as regards M *w* usage in PTB, focusing on the effects of M *w* immunotherapy on:

- Cure/ sputum conversion
- Mortality
- Adverse reactions.

CRITERIA FOR CONSIDERING STUDIES FOR THIS REVIEW

TYPES OF STUDIES

All randomised and quasi-randomised controlled trials of M *w* immunotherapy in participants diagnosed with PTB.

TYPES OF PARTICIPANTS

Participants diagnosed with PTB either by sputum smear microscopy, sputum culture or culture of material from a clinically affected anatomical site.

TYPES OF INTERVENTIONS

Intervention: Inoculation with at least one dose of heat-killed M *w*.

Control: Placebo injection or no control administered.

Chemotherapy for TB must be according to WHO guidelines for category I and category II TB.

TYPES OF OUTCOME MEASURES

PRIMARY OUTCOMES

To determine the effect of M *w* therapy on:

- Sputum conversion (sputum culture negativity) during the course of TB treatment (assessed at day 15, 30, 60, 120, 120+)
- Mortality

SECONDARY OUTCOMES

To determine the frequency of:

- Serious adverse reactions (fatal, life threatening or requiring hospitalisation)
- Other adverse events related to the immunotherapy

SEARCH METHODS FOR IDENTIFICATION OF STUDIES

We plan to do a detailed literature search for data pertaining to the use of M *w* in PTB. Selected articles will then be reviewed for standardised data extraction and presentation in the format of a meta-analysis.

The project team will consist of three main contributors:

- Dr. Shaheen Pandie (SP), who will function as the primary investigator and be responsible for all aspects of the project including final data analysis and publication(s) that may arise
- Mark Engel (ME), who will be responsible for independently completing the literature search and act as co-supervisor
- Dr. Zita Kerbelker (ZK), who will be responsible for independently completing the data extraction, thereby verifying the data collected and generated
- Professor Bongani Mayosi (BM) who will function as the project supervisor.

SP and ME will perform an exhaustive and comprehensive search to identify all relevant studies regardless of language or publication status (published, unpublished, *in press* and *in progress*).

ELECTRONIC SEARCHES

This process will include searching the following journal and trial databases:

- Cochrane Central Register of Controlled Trials (CENTRAL) latest issue
- MEDLINE 1966 to March 2011
- OVID 1980 to March 2011
- LILACS 1982 to March 2011 (La Literatura Latinoamericana y del Caribe de Information en Ciencias de la Salud) (www.bireme.br)
- Cochrane Infectious Diseases Group Specialised Trials Register
- Pan African National Clinical Trials Registry (PACTR)
- World Health Organisation (WHO) International Clinical Trials Registry Platform (ICTRP)
- ClinicalTrials.gov

The following search terms (including the use of MeSH terms) will be used:

- Tuberculosis AND ("*Mycobacterium w*" OR immunotherapy OR immunoadjuvant OR immunomodulator OR immu-vac OR *Mycobacterium indicus pranii*), cross referenced with an isolated search for "Mycobacterium *w*".

ADDITIONAL SEARCHES

We will perform a handsearch of the reference lists of identified articles and relevant review articles. We will also do a manual search of abstracts or proceedings of the following conferences (2000 to present):

- The International Union Against Tuberculosis and Lung Disease World Congress (IUATLD)
- The American Thoracic Society International Congress (ATS)
- The European Respiratory Society World Congress (ERS)

In addition, we will send correspondence to all the authors of the relevant articles for any updates on their research. Finally, individuals and organisations working in the field of TB immunotherapy will be consulted for information regarding unpublished data and work in progress.

DATA COLLECTION AND ANALYSIS

SELECTION OF STUDIES

SP and ZK will review all relevant material identified from the above search. After reading the titles and abstracts of the identified articles, we will acquire the full text articles of all citations deemed to meet the inclusion criteria. These articles will be independently inspected to verify that they meet the pre-specified inclusion criteria.

DATA EXTRACTION AND MANAGEMENT

We will then extract the data using a standardised data extraction form (Appendix 1). Any discrepancies will be resolved through discussion of the original articles with ME and BM. The following characteristics will be extracted from each included study:

- Administrative details
    - trial identification number; title; author(s); published or unpublished; year of publication; number of studies included in paper; year in which the study was conducted; and details of other relevant papers cited
- Verification assessment
    - assessment to ensure that the study met the inclusion criteria for the systematic review
- Details of study
    - study design; duration and completeness of follow-up; country and location of study; informed consent; and ethics approval
- Details of participants
    - setting; number; relevant baseline characteristics; and category of PTB (I or II)
- Details of intervention
    - M *w* dosage; duration; and mode of administration
- Details of control
    - placebo or no vaccine control; and completeness of treatment
- Details of outcomes
    - sputum conversion at various stages of therapy; mortality; and adverse events

- Notes
    - general comments

ASSESSMENT OF RISK OF BIAS IN INCLUDED STUDIES

Each included study will also be assessed for risk of bias. The assessment will include information on sequence generation, allocation concealment, blinding, incomplete outcome data or missing data, selective outcome reporting and other sources of bias. Each methodological component will be assessed as being adequate, inadequate or unclear as per the Cochrane Handbook of Systematic Reviews of Interventions (Higgins 2008).

MEASURES OF TREATMENT EFFECT

Data will be analysed using Review Manager Version 5.0.15 (RevMan5). Outcomes (sputum conversion, death, adverse events) will be considered as dichotomous variables. Outcome measures will be calculated using risk ratios and 95% confidence intervals.

DEALING WITH MISSING DATA

Every effort will be made to contact the original authors or investigators of the selected articles to help resolve the issues of missing or incomplete data.

ASSESSMENT OF HETEROGENEITY

Heterogeneity between trials will be assessed using the chi-squared test set at a 10% level of significance. The impact of any statistical heterogeneity will be quantified using the I^2 statistic (Appendix 2). If there is an acceptable degree of heterogeneity and it is appropriate to pool the data, the Mantel-Haenszel statistical method and Random Effects Analysis Model will be used; and the results will be presented in the form of a meta-analysis. If we are unable to combine the studies, the data will be presented in a narrative form.

SUBGROUP ANALYSIS AND INVESTIGATION OF HETEROGENEITY

In addition to evaluating all PTB participants, we also plan to analyse the subgroups of Category I (new infections) and Category II (retreatment or relapse) PTB. The same statistical analyses will be used as described above.

ACKNOWLEDGEMENTS

The research team would like to acknowledge:

- Peter Nyasulu for assisting with the development of this protocol
- Dr. Olefemi Ajayi (research assistant) for assisting with the project.

PROFILES OF CONTRIBUTORS

PRIMARY INVESTIGATOR (PI)

Dr. Shaheen Pandie MBChB DipPEC FCP (SA)

On completion of my physician's fellowship exam in May 2009, I was awarded the Discovery Foundation Academic Fellowship, and appointed as a research fellow in the Department of Medicine, Division of Cardiology, at UCT. While working fulltime as a sub-investigator on the IMPI Project, I gained invaluable insight and understanding into both randomised controlled clinical trials and TB pericarditis. Under the mentorship of Professor Bongani Mayosi and Dr. Mpiko Nstekhe (both experts in TB pericarditis) at the UCT Project Coordinating Office, I gained experience in the management, coordination and basic structures of conducting clinical research. In addition, I had the opportunity to participate in the following clinical trial seminars and short courses as part of my research training: Good Clinical Practice (GCP) Training (University of Stellenbosch, Bioethics Department), Short Course in Clinical Trials (University of WITS), Short Course in Statistics in Clinical Trials (University of WITS), the International Research Methodology Course (MRC Hosted), and Cochrane Review protocol development and RevMan training course. My role as the PI on this project would include protocol development and implementation; data acquisition, management, and data processing; and completing any publications that arise from the data.

I currently have no published work.

CO-INVESTIGATOR

Dr. Zita Kerbelker MBChB

Dr Zita Kerbelker attained her MBChB from the University of Cape Town (UCT) in 2008; having been awarded the joint medal for Primary Health Care in her fourth year of study. Her interests include clinically-relevant research and she has assisted in numerous clinical trials during her two year

internship (2009/2010) at Groote Schuur Hospital. She is currently completing her community service and hopes to further her studies in internal medicine.

As yet, she has no published work.

SUPERVISOR

Professor Bongani Mayosi DPhil FCP (SA) FACC FESC MASSAf OMS

Professor Mayosi trained in internal medicine and cardiology at the University of Cape Town before taking up the Nuffield Medical Fellowship at the University of Oxford (1998), and going on to complete his doctorate in genetics in 2001. In 2006 he was appointed as Chair of Medicine and Head of the Department of Medicine at the University of Cape Town. His research interests and activities include genetic epidemiology of heart muscle disease, TB pericarditis and rheumatic fever. He has published over 50 papers in these various fields, including numerous systematic reviews. In addition, he has extensive experience in the mentorship of M.Med, MPhil, MD and PhD fellows. Relevant publications include:

1. Mayosi BM, Wiysonge CS, Ntsekhe M, Gumedze F, Volmink JA, Maartens G, et al. Mortality in patients treated for tuberculous pericarditis in sub-Saharan Africa. *S Afr Med J* 2008; 98:36-40.
2. Mayosi BM, Wiysonge CS, Ntsekhe M, Volmink JA, Gumedze F, Maartens G, et al. Clinical characteristics and initial management of patients with tuberculous pericarditis in the HIV era: the Investigation of the Management of Pericarditis in Africa (IMPI Africa) registry. *BMC Infect Dis* 2006; 6:2.
3. Mayosi BM, Burgess LJ, Doubell AF. Tuberculous pericarditis. *Circulation* 2005; 112:3608-3616.
4. Mayosi BM, Ntsekhe M, Volmink JA, Commerford PJ. Interventions for treating tuberculous pericarditis. *Cochrane Database Syst Rev* 2002; (4):CD000526.
5. Ntsekhe M, Wiysonge C, Volmink JA, Commerford PJ, Mayosi BM. Adjuvant corticosteroids for tuberculous pericarditis: promising, but not proven. *QJM* 2003; 96:593-599.

CO-SUPERVISOR

Mark Engel BSc(MED) Hons, MPH

Mark Engel initially trained as a Medical Laboratory Scientist at the Cape Peninsula University of Technology. He was awarded a British Council award to undertake further studies in molecular biology techniques in the UK, after which he went on to complete an honours degree in Human Genetics in the Faculty of Health Sciences at the University of Cape Town. Following a scholarship to Harvard

University's School of Public Health in 2001/2002, Engel was appointed as a Research Fellow at the South African Cochrane Centre where he gained extensive experience in teaching and conducting systematic reviews. After completing a Master in Public Health degree in Epidemiology and Biostatistics, also at the University of Cape Town, Engel joined the Department of Medicine at the University of Cape Town as a Project Manager of the ASAP Programme in Rheumatic Fever and Rheumatic Heart Disease. Currently, he is reading for a PhD in the area of Rheumatic Fever and Rheumatic Heart Disease; his research interest and activity focuses on genetic epidemiology of rheumatic fever. He has published NN papers in various fields, including systematic reviews. In addition, he has experience in teaching and the mentorship of MPH fellows. Relevant work includes:

Dave JA, Engel ME, , Freercks R, Peter J, May W, Badri M, Van Niekerk L, Levitt NS. Abnormal glucose metabolism in non-diabetic patients presenting with an acute stroke: prospective study and systematic review. Q J Med, doi:10.1093/qjmed/hcq062

Ford N, Engel ME, Jean B Nachega JB, Mills EJ. Directly Observed Antiretroviral Therapy: A Systematic Review And Meta-Analysis Of Randomized Clinical Trials. Lancet. 2009; 374: 2064–71

Engel ME, Matchaba P, Volmink J. Corticosteroids for tuberculous pleurisy. Cochrane Database Syst Rev. 2007: (4):CD001876.

Munro SA, Lewin SA, Smith HJ, Engel ME, Fretheim A, Volmink J. Patient adherence to tuberculosis treatment: a systematic review of qualitative research. PLoS Med 2007;4:1230-45.

Atkins SA, Lewin SA, Smith HJ, Engel M, Fretheim A, Volmink J. Conducting a meta-ethnography of qualitative literature: lessons learnt. BMC Medical Research Methodology 2008, 8:21.

SOURCES OF SUPPORT

- University of Cape Town Research Committee
- University of Cape Town, Department of Medicine: Cardiology Research Unit

POTENTIAL STUDY SHORTCOMINGS / LIMITATIONS

The background literature search highlighted that there is an extensive body of data as regards M *w* usage in leprosy, but only limited published randomised controlled trials on M *w* usage for PTB. This issue will be addressed by:

- being as comprehensive in our literature search as possible
- by making direct correspondence with all the investigators of the selected articles.

ETHICAL CONSIDERATIONS

Due to the nature of this review, no ethical approval is required. Authors of the selected articles will receive correspondence as regards the results of this review. They will be given the opportunity to respond to the final discussion before any data is published.

DECLARATIONS OF INTEREST

The authors of this review are currently conducting a trial of *Mycobacterium w* immunotherapy in tuberculous pericarditis (Appendix 3).

REFERENCES

Churchyard 2009

Churchyard GJ, Kaplan G, Fallows D, Wallis RS, Onyebujoh P, Rook GA. Advances in immunotherapy for tuberculosis treatment. Clinics in chest medicine 2009;30(4):769-82, ix.

Ganju 1990

Ganju L, Mukherjee R, Batra HV, Talwar G. Immuno-blot analysis of antigens of Mycobacterium w: a candidate anti-leprosy vaccine using monoclonal antibodies and patient sera. Zentralblatt fur Bakteriologie : international journal of medical microbiology 1990;273(3):378-85.

Guleria 1993

Guleria I, Mukherjee R, Kaufmann SH. In vivo depletion of CD4 and CD8 T lymphocytes impairs Mycobacterium w vaccine-induced protection against M. tuberculosis in mice. Medical microbiology and immunology 1993;182(3):129-35.

Gupta 2009

Gupta A, Geetha N, Mani J, Upadhyay P, Katoch VM, Natrajan M, et al. Immunogenicity and protective efficacy of "Mycobacterium w" against Mycobacterium tuberculosis in mice immunized with live versus heat-killed M. w by the aerosol or parenteral route. Infection and immunity 2009;77(1):223-31. [PubMed: 18981249]

Higgins 2008

Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions. Wiley-Blackwell, 2008.

Katoch 2008

Katoch K, Singh P, Adhikari T, Benara SK, Singh HB, Chauhan DS, et al. Potential of Mw as a prophylactic vaccine against pulmonary tuberculosis. Vaccine 2008;26(9):1228-34. [PubMed: 18243430]

Kaur 2002

Kaur I, Dogra S, Kumar B, Radotra BD. Combined 12-month WHO/MDT MB regimen and Mycobacterium w. vaccine in multi-bacillary leprosy: a follow-up of 136 patients. International journal of leprosy and other mycobacterial diseases : official organ of the International Leprosy Association 2002;70(3):174-81.

Luhadia 2004

Luhadia S, Saugat R, Joshi V. Mycobacterium w as an adjuvant to chemotherapy in management of pulmonary tuberculosis. In: National Conference on Pulmonary Diseases (NAPCON). 2004.

Mitnick 2007

Mitnick C.D., Castro K.G., Harrington M., Sacks L.V., and Burman W. Randomized trials to optimise treatment of multi drug-resistant tuberculosis: The time for action is now. Plos. Medicine 2007;4(11):e292.

Murray 2004

Murray JF. A century of tuberculosis. American journal of respiratory and critical care medicine 2004;169(11):1181-6. [PubMed: 15161611]

Nath 1998

Nath I. A vaccine for leprosy. Nature medicine 1998;4(5):548-50. [PubMed: 9585220]

Patel 2002

Patel N, Deshpande MM, Shah M. Effect of an immunomodulator containing Mycobacterium w on sputum conversion in pulmonary tuberculosis. Journal of the Indian Medical Association 2002;100(3):191-3. [PubMed: 12408283]

Patel 2003

Patel N, Trapathi SB. Improved cure rates in pulmonary tuberculosis category II (retreatment) with mycobacterium w. Journal of the Indian Medical Association 2003;101(11):680, 682. [PubMed: 15198421]

Rieder 1996

Rieder HL. Sputum smear conversion during directly observed treatment for tuberculosis. Tubercle and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease 1996;77(2):124-9. [PubMed: 8762846]

Saini 2009

Saini V, Raghuvanshi S, Talwar GP, Ahmed N, Khurana JP, Hasnain SE, et al. Polyphasic taxonomic analysis establishes Mycobacterium indicus pranii as a distinct species. PloS one 2009;4(7):e6263.

Sharma 2000

Sharma P, Misra RS, Kar HK, Mukherjee A, Poricha D, Kaur H, et al. Mycobacterium w vaccine, a useful adjuvant to multidrug therapy in multi-bacillary leprosy: a report on hospital-based immunotherapeutic clinical trials with a follow-up of 1-7 years after treatment. Leprosy review 2000;71(2):179-92.

Sharma 2005

Sharma P, Mukherjee R, Talwar G.P, Sarathchandra K.G, Walia R, Parida S K, et al. Immunoprophylactic effects of the anti-leprosy M *w* vaccine in household contacts of leprosy patients: clinical field trials with a follow up of 8 to 10 years. Leprosy review June 2005;Vol. 76(Issue 2):Pg. 127-43.

Singh 1991

Singh IG, Mukherjee R, Talwar GP. Resistance to intravenous inoculation of Mycobacterium tuberculosis H37Rv in mice of different inbred strains following immunization with a leprosy vaccine based on Mycobacterium w. Vaccine 1991;9(1):10-4.

Singh 1992

Singh IG, Mukherjee R, Talwar GP, Kaufmann SH. In vitro characterization of T cells from Mycobacterium w-vaccinated mice. Infection and immunity 1992;60(1):257-63.

WHO 2008

World Health Organisation (WHO). Anti-tuberculosis Drug Resistance in the World Report 2008. Fourth Global Report 2008.

WHO 2009

World Health Organisation (WHO). GlobalTuberculosisControl: A short update to the 2009 report. WHO Library Cataloguing-in-Publication Data 2009;(ISBN 978 92 4 159886 6).

Zaheer 1995

Zaheer SA, Beena KR, Kar HK, Sharma AK, Misra RS, Mukherjee A, et al. Addition of immunotherapy with Mycobacterium w vaccine to multi-drug therapy benefits multi-bacillary leprosy patients. Vaccine 1995;13(12):1102-10.

APPENDICES

APPENDIX 1: DATA EXTRACTION SHEET

Date:

Reviewer ID:

Administrative details	
Study ID	
Trial Number	
Author(s)	
Publication details	
Year of Publication	
Number of studies in this paper	
Year in which study was concluded	
Other relevant papers cited	

Study Details	
Study Verification	
Study Design	
Type, duration and completeness of follow-up	
Country/ location of study	
Informed consent	
Ethics	

Participant details	
Setting / diagnosis	
Number	
Baseline characteristics	

Interventions (I) / Controls (C)	
I dosage / regimen	
Control	

Background treatment	

Risk of bias	Judgement	Description
Adequate sequence generation		
Allocation concealment		
Blinding		
Incomplete outcome data addressed		
Free of selective reporting		
Free of other bias		

Primary Outcomes	Overall PTB	Category I PTB	Category II PTB
Sputum culture conversion **15 days**			
30 days			
60 days			
120 days			
120+ days			
Mortality			

Secondary outcomes	
Serious adverse reactions	
Adverse events related to the immunotherapy	
Additional notes	

APPENDIX 2: HIGGENS I^2

Higgins I^2 = Q-df / Q x 100%

Where

Q = χ^2 statistic

df = degrees of freedom

I^2 calculates the proportion of variation in the effect estimates that is due to heterogeneity rather than chance

Thresholds for interpreting I^2:

0 – 40 % = might not be important

30 – 60 % = moderate

50 – 90 % = substantial

75 – 100% = considerable

*From **"Analysing Data and Undertaking Meta-analyses":** Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions. Pages 277 – 8.Wiley-Blackwell; 2008.*

APPENDIX 3: IMPI TRIAL OVERVIEW

Title	**The IMPI (*I*nvestigation of the *M*anagement of *P*eri*c*ard*itis*) Trial** A Pilot Trial of Adjunctive Prednisolone and *Mycobacterium w* Immunotherapy in Tuberculous Pericarditis ClinicalTrials.gov Identifier: NCT00810849
Study Size	1400 participants from Africa and India.
Study Design	International multi-centre randomised controlled, double-blind, 2x2 factorial design trial of: a) Prednisolone vs. placebo and/or b) *Mycobacterium w* vs. placebo
Primary Objective	To assess the effects of Prednisolone and/or *Mycobacterium w* immunotherapy on mortality and pericardial complications (i.e. constriction and tamponade).
Inclusion Criteria	Confirmed pericardial effusion on echocardiography. Evidence of definite or probable tuberculous pericarditis, signified by at least one of the following: Tubercle bacilli found in stained smear or culture of pericardial fluid, or Tubercle bacilli or caseating granulomata found on histological examination of pericardium, or Tubercle bacilli found in stained smear or culture of sputum, gastric or lymph node aspirate, or Lymphocytic pericardial exudates with elevated ADA activity Tygerberg Index Score > 6 Within 1 week of starting anti-tuberculous treatment. Availability of fixed address and contactable relatives. Willingness to participate for the full duration of the trial (24 months).
Exclusion Criteria	Presence of an alternative cause of pericardial disease, e.g. penetrating chest trauma in the preceding 12 months; and malignancy. Use of corticosteroids within the previous month. History of hypersensitivity or allergy to the *Mycobacterium w* vaccine. Pregnancy. Age < 18 years.
Treatment Regimen	Prednisolone or matching placebo; tapering dose daily for 6 weeks given

	orally and/or *Mycobacterium w* or matching placebo given intradermally on day 0 (enrolment), week 2, week 4, week 6 and at 3 months.
Project Coordinating Office (PCO)	Mrs. Veronica Francis, Project Manager Department of Medicine, H47 Old Main Building, Groote Schuur Hospital University of Cape Town, Observatory 7925 Telephone: +27-21-4472777; Fax: +27-21-4472765 Randomisation Tel. No.: +27-72-9011126 Email Address: veronica.francis@uct.ac.za
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MYCOBACTERIUM *W* IMMUNOTHERAPY FOR TREATING PULMONARY TUBERCULOSIS – A SYSTEMATIC REVIEW

BY

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PART B: LITERATURE REVIEW

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INTRODUCTION

Tuberculosis (TB) remains a global health catastrophe. The 2009 Global TB Control Update reported 9.4 million incident cases and 11.1 prevalent cases of TB; with 1.82 million TB associated deaths (0.52 million in human immunodeficiency virus [HIV] positive individuals[1]). The major burden of disease is concentrated in the developing world. In Africa, the annual incidence continues to increase because of the HIV epidemic[2] . In South Africa, TB has been identified as a major public health challenge. The epidemic has been compounded by the emergence of drug resistant TB. Multi-drug resistant (MDR) TB is defined as resistance to both isoniazid and rifampicin, the two drugs regarded as the most effective anti-tuberculous treatment. On a global scale, MDR TB is reported to account for 10% of the 9.4 million new cases of TB that occur annually[3]. Extensively drug resistant (XDR) TB is defined as MDR plus resistance to a fluoroquinolone and a second-line injectable drug. Current treatment strategies for drug-sensitive, MDR and XDR TB include prolonged oral and injectable drug therapies. Treatment regimens are problematic because of high pill burden and drug toxicity, which result in low cure and treatment completion rates. Therefore, the investigation of new anti-tuberculous treatment strategies is essential. To date, there has been extensive research into the immunopathogenesis of TB, and possible immuno-therapies that could supplement current anti-tuberculous therapies[4].

The objectives of this literature review are:

1. To review the recent advances in TB immunotherapy
2. To provide background information on the immune modulator Mycobacterium *w* (M *w*)
3. To review the use of M *w* therapy in pulmonary TB (PTB)

LITERATURE SEARCH STRATEGY

For general background information, electronic sources such as MEDLINE, Google Scholar, and EMBASE were searched using key words such as: tuberculosis, immunotherapy, vaccines, immunology, and Mycobacterium *w*.

For the formal systematic review of "*Mycobacterium w immunotherapy in treating pulmonary tuberculosis*", the search criteria were clearly defined in the protocol (Appendix 1).

IMMUNOTHERAPY

As a result of advancements in the understanding of the immunopathogenesis of TB, there has been an increasing interest in immunotherapies as adjunctive treatments to standard TB drug regimens. There are three major categories of immunotherapeutic agents:

1. Immune modulators: alter the nature of the immune response
2. Immunosuppressives: suppress the immune response
3. Supplement effector cytokines: assist anti-microbicidal activity

IMMUNE MODULATORS

MYCOBACTERIUM VACCAE (M. VACCAE)

Murine models have shown that *M. vaccae* induces the perfect cytokine and immune milieu for protection against TB. *M. vaccae* induces T-regulatory cells responsible for inhibiting the T-helper 2 (Th2) response, whilst stimulating T-helper 1 (Th1) and cytotoxic T-lymphocyte (CTL) responses[5] . A systematic review of *M. vaccae* treatment for TB analysed 8 trials involving a total of 2140 adult participants. The meta-analysis suggested no significant effect on mortality (RR 1.09, 95% CI 0.83 to 1.42; 1741 participants, 4 trials), and only a small effect on participants who were sputum negative or culture negative at 2 months. The conclusion drawn was that *M. vaccae* was of no benefit and further trials were not warranted. A major criticism of the meta-analysed trials was under-dosing, with 7 out of the 8 trials reviewed only giving a single dose of *M. Vaccae* [6]. The focus has now turned to multi-dose schedules, and the results of a meta-analysis performed in China reviewing 11 trials focusing on multi-dose *M. vaccae* concluded that it is an effective adjuvant. As a result, *M. vaccae* has been approved for MDR-TB treatment in China[7].

MYCOBACTERIUM W (*M W*)

Discussed in detail later.

HIGH-DOSE INTRAVENOUS IMMUNOGLOBULIN

Intravenous immunoglobulin (IVIg) is used for a variety of inflammatory disorders (e.g. idiopathic thrombocytopenic purpura, Kawasaki disease, Guillain Barre syndrome). It has been shown to reduce the bacterial load and the severity of TB infection in mice[8]. The proposed

mechanism is due to a subset of anti-inflammatory immunoglobulins with fully sialylated oligosaccharides[9]. No human data are available yet.

HE2000 (16A-BROMOEPIANDROSTERONE)

HE2000 is a modified form of the steroid dehydroepiandrosterone (DHEA). In mouse models it was shown to be therapeutic and to accelerate bacillary clearance in TB[10]. The precise mechanism is unclear. In a small study comprising 25 HIV positive participants, HE2000 reduced the incidence of TB co-infection[10, 11].

NEUTRALISING ANTIBODIES TO INTERLEUKIN-4 (IL-4)

TGF-β (a polypeptide of the transforming growth factor beta superfamily of cytokines) is known to be detrimental in TB. Neutralising IL-4 antibody decreases TGF-β and this beneficial therapeutic effect has been proved in mouse model[12, 13]. A humanised antibody to IL-4 (pascolizumab) has been developed, but has not yet undergone clinical trials.

DNA VACCINE ENCODING A MYCOBACTERIAL PROTEIN (HEAT SHOCK PROTEIN 65)

The immune response targets proteins such as Mycobacterial heat shock protein (HSP) 65. DNA vaccines encoding proteins that modulate the immune response are novel, and are currently in Phase I human clinical trials[14].

PLANT EXTRACTS (E.G. DZHERELO)

Dzherelo is an over-the-counter phytoconcentrate containing multiple plant extracts. A small, open-labelled study assessing the adjunctive use of Dzherelo in TB patients shows promising results[15]. No randomised controlled trials have been done as yet.

IMMUNOSUPPRESSIVE THERAPY

THALIDOMIDE AND THALIDOMIDE ANALOGUES

TNF-α is essential for granuloma formation[16]. Thalidomide partially inhibits TNF-α, thereby preventing granuloma formation, and thus rendering bacilli in an actively growing state, which are then more susceptible to antimicrobials. There have been phase I studies demonstrating safety, reduction in TNF-α, and increased weight gain in adults with PTB[17].

However, a study of high dose thalidomide in children with TB meningitis was stopped early because of excess adverse events[18]. Thalidomide analogues have been produced to reduce toxic side effects, but these agents are still in the early phases of testing.

ETANERCEPT

Etanercept is a soluble TNF-α receptor. It was evaluated in a small, case-controlled study of 16 HIV positive participants with TB. It was considered safe and suggested a significant reduction in the time to sputum conversion[19]. Further studies are waranted.

HIGH-DOSE PREDNISONE/PREDNISOLONE

Clinical trials using adjunctive prednisolone as anti-TB treatment in participants with PTB have shown great promise; particularly as regards the time to sputum conversion and reduction of constitutional symptoms[20, 21]. There are, however, concerns regarding detrimental side-effects such as glucose intolerance, hypertension and fluid retention. In HIV positive patients, there was a transient increase in the plasma HIV RNA viral loads, which receded when treatment was completed. There was no significant increase in opportunistic infections. In order to reduce these side-effects, future studies may have to look at reducing steroid dosages, or at introding novel routes of administration (e.g. inhaled, nasal).

SUPPLEMENT EFFECTOR CYTOKINES

RECOMBINANT HUMAN INTERFERRON GAMMA

Interferron gamma is a crucial cytokine for defence against bacterial infections. Recombinant human (rh) INFgamma has been trialled in aerosol, subcutaneous and intramuscular forms. Initial studies in MDR participents showed minimal, non-sustained benefit[21,22]. In 2009 Dawson *et al* published a study that concluded that the use of adjuvant recombinant interferon in participants with cavitary PTB can reduce inflammatory cytokines, improve constitutional symptoms, and reduce time to sputum conversion[23].

OTHER CYTOKINES

Other cytokine effectors such as rh-IL-2 and rh-Granulocyte-Macrophage Colony-Stimulating Factor (rh GM-CSF) have been investigated. IL-2 showed promise as adjuvant treatment to drug-resistant TB, but showed no benefit in a study of drug-sensitive TB patients [24,25, 26]. In one small trial including HIV-uninfected participants with PTB, rh-GM-CSF was shown to have a trend to

faster sputum conversion[27]. In addition, it has been shown to have an adjuvant role in boosting the protection afforded by bacillus Calmette-Guerin (BCG)-derived vaccines[28].

The common theme illustrated in this synopsis of immunotherapies, is the need for further investigation. An appropriate starting point would therefore be to critically review the available data, and then make recommendations on future research.

MYCOBACTERIUM *w*

INTRODUCTION

Mycobacterium w (M *w*) is a non-pathogenic, saprophytic, rapidly growing atypical *Mycobacterium* species with immuno-potentiating properties. Recent polyphasic taxonomic analysis classified M *w* as a distinct species, *Mycobacterium indicus pranii*, placing it in the Runyon Group IV along with *M vaccae, M fortuitum and M smegmatis*[29]. Its uniqueness stems from its ability to undergo antigen-driven blast leukocyte transformation. Recent scientific experiments have shown that M *w* exerts its influence on the innate immune system at the level of the Toll-like-receptor (TLR) and TLR-ligands. It has the ability to inhibit TLRs (especially TLR 3, 4, 5, 6, 7, 8, 9) and antagonise TLR ligands[30]. This finding has spurred new interest into the potential benefits of M *w* usage, particularly in conditions that are associated with up-regulation or over-expression of TLRs, such as sepsis and chronic airway diseases. Interestingly, PTB is also associated with selective up-regulation of TLRs[31].

RECOGNISED USES OF M *w*

An extensive body of data supports the safety and efficacy of M *w* in the prevention and treatment of leprosy[32,33]. There are two different clinical manifestations of leprosy i.e. tuberculoid and lepromatous. In the milder tuberculoid form, mycobacterium proliferation and destruction are relatively contained by a cell-mediated immune response. Mycobacteria are engulfed by macrophages, but initially survive because the phagosomes and lysosomes do not fuse[34]. Macrophages require activation by Th1 lymphocytes for phagosome-lysosome fusion. The complete process of mycobacterial destruction involves: (i) Th1-macrophage activation, followed by (ii) phagosome-lysosome fusion, and (iii) mycobacterial destruction by proteases resulting in (iv) mycobacterial peptide fragments binding to MHC class II molecules and finally, (v) presentation at the cell surface to CD4 T cells[35].

Th1 or Th2 cell differentiation is determined by the cytokine milieu produced after the undifferentiated CD4 helper T cell recognises the antigen presented on the MHC class II molecules of infected macrophages. IL-12 and INFgamma promote differentiation of Th1 subtypes, leading to a cell-mediated immune response and the less severe tuberculoid manifestation of the disease. Th1 cells specific for *M. leprae* antigens are capable of activating macrophages and inducing destruction. Lepromatous leprosy, on the other hand, results from the failure to produce an effective cell-mediated immune response, and predominance of

humeral immune responses. Secretion of IL-4 and IL-6 lead to the T cell differentiation into the Th2 subtypes. B cells are activated to make neutralising antibodies, which are not able to reach intracellular bacteria and therefore constitute an ineffective immune response. *M. leprae* are able to grow in macrophages, resulting in tissue destruction[35].

When administered as an intra-dermal heat killed vaccine, M *w* stimulates a Th1 cellular immune response against shared epitopes for both *Mycobacterium leprae* and *Mycobacterium tuberculosis*[36-38]. As explained above, the stimulation of the Th1 response leads to an improved cell-mediated immune response, and therefore less severe tuberculoid disease.

M *w* was tested as an adjunct to standard antibiotic therapy in phase 3 clinical trials of lepromatous leprosy. Participants with leprosy received multidrug therapy (MDT) plus M *w*; while the control arm received MDT plus placebo injections. These studies showed that bacteriological clearance was more rapid in the M *w* group ($p<0.03$)[39]. There was also an associated decrease in the number of organisms (bacillary load), shorter duration of antibiotic therapy was required; and earlier discharge from care was possible[39-41].

In healthy contacts of leprosy patients, M *w* was associated with protection from leprosy infection. Sharma et al vaccinated a total of 24,060 household contacts with M *w* or placebo in a double-blind randomised trial. Participants were followed at 3 yearly intervals for 8 to 10 years. M *w* showed a protective efficacy of 68%, 59% and 39.3% at the respective follow-up intervals[33]. The safety and efficacy of M *w* has thus been well established through widespread use as adjuvant therapy for leprosy treatment and prevention.

M *w* AND TB

The immunopathogenesis of TB is similar to that of leprosy. The initial immune response to TB infection results in either active disease or protection against disease. This immune response involves a complex interplay between bacteriostatic and bactericidal immune pathways. Bacteriostatic processes results in a walled off granuloma, while the bactericidal processes of autophagy, apoptosis, and cytotoxic T-cell destruction, result in cell death[42]. A combination of a Th1 response (key cytokines include INFgamma, TNFα, IL-15) and the CTL killing is thought to provide the optimal immune protection against TB. A Th2 response (IL-4, TGFβ, IL -10) directly reduces the immune response to TB by both inhibiting the maturation of Th1 cells, and inhibiting the production of cytokines that drive Th1 and CTL cells. Therefore, the ideal immunotherapeutic strategy would be to inhibit Th2 while enhancing the protective Th1 and CTL pathways.

Multiple laboratory studies have demonstrated that mice immunised with heat-killed M *w* had increased Th1 lymphocyte and macrophage activity, with a cytokine environment that was predominantly IL2 and INFgamma[43]. In addition, immunised mice were protected from sub-lethal challenge with *M. tuberculosis*[44, 45].

Human data of heat-killed M *w* use as immunotherapy for active TB are not definitive. Results from published and unpublished clinical trials investigating M *w* administration in patients with PTB suggest a reduction in time to sputum negativity and improved cure rates[46-48]. In addition, Katoch reviewed the healthy contacts of the leprosy studies' participants looking for evidence of new TB infection. The results suggest that M *w* significantly ($p<0.01$) reduced the rate of new TB infection[49]. There is, however, concern as regards the quality of the available data, particularly in terms of methodological flaws.

The aim of the proposed systematic review is to evaluate the evidence of the effectiveness of M *w* vaccination as adjunctive treatment for PTB. This critical evaluation of the available data will include assessment of: (i) risk of bias, (ii) heterogeneity, and (iii) comparison of available data sets in the form of a meta-analysis.

M *w* USAGE IN OTHER CONDITIONS

M *w* AND HIV

It has become increasingly difficult to separate the entities of TB and HIV. As mentioned previously, out of the 1.82 million TB associated deaths that were reported in 2009, 0.52 million were in HIV positive individuals[1]. For this reason, it would be important to know if there was any impact of M *w* usage in HIV infected individuals.

There is limited data of *M w* use in the HIV population. The only relevant conclusions that can be drawn from work done by Kharkar, is that *M w* was safely used in a cohort of 55 HIV positive participants without any major adverse events, and no reports of immune reconstitution inflammatory syndrome (IRIS)[50]. Further evidence of the safety of heat-killed atypical mycobacterium immune-modulating vaccines can be drawn from the work done with M *vaccae* vaccine, which is similar in structure and immune mechanism to *M w*. The literature suggests that the use of heat-killed TB vaccines is safe in the HIV population and at least as effective as in non-HIV individuals[6, 51]. Proper randomised controlled trials are both necessary and essential, especially in areas of high HIV and TB prevalence.

M *w* AND CANCER

Immunotherapy with bacillus Calmette-Guérin (BCG) instillation is well recognised as adjuvant therapy for high-risk non-muscle invasive bladder cancer[52]. In view of this, M *w* has been trialled for use as an adjuvant immunotherapy in various types of cancers including invasive bladder cancer, non-small cell lung cancer, and head and neck cancers.

Chaudhuri et al investigated the use of *Mycobacterium w* adjunctive therapy with radiation therapy in the management of invasive bladder cancer. Even though it was a small trial (only 5 patients), at the two year follow up period, all the patients were disease free[53].

Sur's controlled trial showed that the use of M *w* immunotherapy as adjuvant to combination of chemotherapy and radiotherapy in non-small lung cancer showed an improvement in quality of life (measured by Karnofsky performance scores), a significant regression of tumour size in the intervention group, and an overall improvement in lung functions. This was also, however, a very small study, with only 10 participants in each arm[54].

In a non-randomised trial of M *w* adjuvant treatment as palliative care for advanced head and neck cancers, M *w* was considered safe and beneficial for improving the quality of life[55].

MISCELLANEOUS USES OF M *w*

M *w* has also been trialled in a variety of miscellaneous conditions, including psoriasis, asthma, and genital warts[30, 56-58]. These were mostly pilot or proof of concept studies, with small sample sizes and results that could not be generalised.

SAFETY OF M *w*

In terms of safety, M *w* has been administered to thousands of patients in multiple trials and studies, with no reports of serious adverse events. The accepted chronology of the "normal" local skin reaction to the vaccine is:

1. A small pustule between days four to five
2. Mild ulceration between days seven and ten
3. Formation of a scab by 1 month after administration

Any skin changes that are not in keeping with this sequence of events are considered an accelerated or exaggerated reaction i.e. adverse reaction[47]. Even so, all published studies have concluded that M *w* is safe, with the reported side effects invariably being self-limiting.

SUMMARY

The HIV-TB epidemic is a health crisis, and current therapeutic options for TB are suboptimal. Investigating immunotherapeutic interventions is one of the options that may facilitate the fight against TB. Understanding the immunopathogenesis of TB is crucial in the development of appropriate anti-TB strategies[59]. Laboratory, animal and clinical work investigating M *w* use in TB suggests a reduction in time to sputum conversion (a proxy for cure), and thus the potential to reduce the spread of disease. The aim of this systematic review is to summarise the evidence of the effectiveness of M *w* vaccination as adjunctive treatment for PTB. This information will be helpful for policy makers, healthcare practitioners and researchers in the field of TB management and control.

FUTURE RESEARCH

There are currently several trials in progress evaluating M *w* as adjuvant therapy (Appendix 2). The trials that are specifically related to M *w* usage in patients with TB:

- The Efficacy and Safety of Immunomodulator as an Adjunct Therapy in New Pulmonary Tuberculosis (Category I) Patients (ClinicalTrials.gov Identifier: NCT00341328)
- The Efficacy and Safety Study of Immunomodulator as an Adjunct Therapy in Pulmonary Tuberculosis (TB) Retreatment Patients (ClinicalTrials.gov Identifier: NCT00265226)
- The Trial of Adjunctive Prednisolone and Mycobacterium *w* Immunotherapy in Tuberculous Pericarditis: IMPI (ClinicalTrials.gov Identifier: NCT00810849)

These three large, randomised controlled trials would hopefully provide more definitive answers as regards the effectiveness of M *w* usage.

REFERENCES

1. World Health Organisation (WHO). Global Tuberculosis Control: A short update to the 2009 report. WHO Library Cataloguing-in-Publication Data 2009; (ISBN 978 92 4 159886 6).

2. Murray JF. A Century of Tuberculosis. Am J Respir Crit Care Med 2004; 169(11): 1181-6.

3. Mitnick CD, Castro KG, Harrington M, Sacks LV, and Burman W. Randomized trials to optimize treatment of multi drug-resistant tuberculosis: The time for action is now. Plos.Medicine 2007; 4(11): e292.

4. Hoft DF. Tuberculosis vaccine development: goals, immunological design, and evaluation. Lancet 2008; 372(9633): 164-75.

5. Skinner MA, Yuan S, Prestidge R, Chuk D, Watson JD, Tan PL. Immunization with heat-killed Mycobacterium vaccae stimulates CD8+ cytotoxic T cells specific for macrophages infected with Mycobacterium tuberculosis. Infect Immun 1997; 65(11): 4525-30.

6. De Bruyn G, Garner P. Mycobacterium vaccae immunotherapy for tuberculosis. Cochrane Database of Systematic Reviews 2003.

7. Fan M, Chen X, Wang K. Adjuvant effect ofMycobacterium vaccae on treatment of recurrent treated pulmonary tuberculosis: a meta-analysis. Chinese Journal of Evidence-Based Medicine 2007; 7(6): 449–55.

8. Roy E, Stavropoulos E, Brennan J, Coade S, Grigorieva E, Walker B, et al. Therapeutic efficacy of high-dose intravenous immunoglobulin in Mycobacterium tuberculosis infection in mice. Infect Immun 2005; 73(9): 6101-9.

9. Anthony RM, Wermeling F, Karlsson MC, Ravetch JV. Identification of a receptor required for the anti-inflammatory activity of IVIG. Proc Natl Acad Sci U S A 2008; 105(50): 19571-8.

10. Hernandez-Pando R, Aguilar-Leon D, Orozco H, Serrano A, Ahlem C, Trauger R, et al. 16 alpha-Bromoepiandrosterone restores T helper cell type 1 activity and accelerates chemotherapy-induced bacterial clearance in a model of progressive pulmonary Tuberculosis. J Infect Dis 2005; 191(2): 299-306.

11. Stickney DR, Noveljic Z, Garsd A, Destiche DA, Frincke JM. Safety and activity of the immune modulator HE2000 on the incidence of tuberculosis and other opportunistic infections in AIDS patients. Antimicrob Agents Chemother 2007; 51(7): 2639-41.

12. Roy E, Lowrie DB, Jolles SR. Current strategies in TB immunotherapy. Curr Mol Med 2007; 7(4): 373-86.

13. Roy E, Brennan J, Jolles S, Lowrie DB. Beneficial effect of anti-interleukin-4 antibody when administered in a murine model of tuberculosis infection. Tuberculosis (Edinb) 2008; 88(3): 197-202.

14. Okada M, Kita Y, Nakajima T, Kanamaru N, Hashimoto S, Nagasawa T, et al. Novel prophylactic and therapeutic vaccine against tuberculosis. Vaccine 2009; 27(25-26): 3267-70.

15. Zaitzeva SI, Matveeva SL, Gerasimova TG, Pashkov YN, Butov DA, Pylypchuk VS, et al. Treatment of cavitary and infiltrating pulmonary tuberculosis with and without the immunomodulator Dzherelo. Clin Microbiol Infect 2009; 15(12): 1154-62.

16. Algood HM, Lin PL, Yankura D, Jones A, Chan J, Flynn JL. TNF influences chemokine expression of macrophages in vitro and that of CD11b+ cells in vivo during Mycobacterium tuberculosis infection. J Immunol 2004; 172(11): 6846-57.

17. Tramontana JM, Utaipat U, Molloy A, Akarasewi P, Burroughs M, Makonkawkeyoon S, et al. Thalidomide treatment reduces tumor necrosis factor alpha production and enhances weight gain in patients with pulmonary tuberculosis. Mol Med 1995; 1(4): 384-97.

18. Schoeman JF, Springer P, van Rensburg AJ, Swanevelder S, Hanekom WA, Haslett PA, et al. Adjunctive thalidomide therapy for childhood tuberculous meningitis: results of a randomized study. J Child Neurol 2004; 19(4): 250-7.

19. Wallis RS, Kyambadde P, Johnson JL, Horter L, Kittle R, Pohle M, et al. A study of the safety, immunology, virology, and microbiology of adjunctive etanercept in HIV-1-associated tuberculosis. AIDS 2004; 18(2): 257-64.

20. Mayanja-Kizza H, Jones-Lopez E, Okwera A, Wallis RS, Ellner JJ, Mugerwa RD, et al. Immunoadjuvant prednisolone therapy for HIV-associated tuberculosis: a phase 2 clinical trial in Uganda. J Infect Dis 2005; 191(6): 856-65.

21. Koh WJ, Kwon OJ, Suh GY, Chung MP, Kim H, Lee NY, et al. Six-month therapy with aerosolized interferon-gamma for refractory multidrug-resistant pulmonary tuberculosis. J Korean Med Sci 2004; 19(2): 167-71.

22. Suarez-Mendez R, Garcia-Garcia I, Fernandez-Olivera N, Valdes-Quintana M, Milanes-Virelles MT, Carbonell D, et al. Adjuvant interferon gamma in patients with drug - resistant pulmonary tuberculosis: a pilot study. BMC Infect Dis 2004; 4: 44.

23. Dawson R, Condos R, Tse D, Huie ML, Ress S, Tseng CH, et al. Immunomodulation with recombinant interferon-gamma1b in pulmonary tuberculosis. PLoS One 2009; 4(9): e6984.

24. Johnson B, Bekker LG, Ress S, Kaplan G. Recombinant interleukin 2 adjunctive therapy in multidrug-resistant tuberculosis. Novartis Found Symp 1998; 217: 99,106; discussion 106-11.

25. Johnson BJ, Bekker LG, Rickman R, Brown S, Lesser M, Ress S, et al. rhuIL-2 adjunctive therapy in multidrug resistant tuberculosis: a comparison of two treatment regimens and placebo. Tuber Lung Dis 1997; 78(3-4): 195-203.

26. Johnson JL, Ssekasanvu E, Okwera A, Mayanja H, Hirsch CS, Nakibali JG, et al. Randomized trial of adjunctive interleukin-2 in adults with pulmonary tuberculosis. Am J Respir Crit Care Med 2003; 168(2): 185-91.

27. Pedral-Sampaio DB, Netto EM, Brites C, Bandeira AC, Guerra C, Barberin MG, et al. Use of Rhu-GM-CSF in pulmonary tuberculosis patients: results of a randomized clinical trial. Braz J Infect Dis 2003; 7(4): 245-52.

28. Ryan AA, Wozniak TM, Shklovskaya E, O'Donnell MA, Fazekas de St Groth B, Britton WJ, et al. Improved protection against disseminated tuberculosis by Mycobacterium bovis bacillus Calmette-Guerin secreting murine GM-CSF is associated with expansion and activation of APCs. J Immunol 2007; 179(12): 8418-24.

29. Saini V, Raghuvanshi S, Talwar GP, Ahmed N, Khurana JP, Hasnain SE. Polyphasic taxonomic analysis establishes Mycobacterium indicus pranii as a distinct species. PloS one 2009; 4(7): e6263.

30. Khamar BM, Modi RI. Process for manufacturing pharmaceutical composition comprises of mycobacterium w in the treatment of asthma (obstructive lung disease). 2010(May / 18).

31. Chang JS, Huggett JF, Dheda K, Kim LU, Zumla A, Rook GA. Myobacterium tuberculosis induces selective up-regulation of TLRs in the mononuclear leukocytes of patients with active pulmonary tuberculosis. J Immunol 2006; 176(5): 3010-8.

32. Nath I. A vaccine for leprosy. Nat Med 1998; 4(5): 548-50.

33. SharmaP, MukherjeeR, TalwarGP, SarathchandraKG, WaliaR, ParidaSK, PandeyRM, RaniR, KarH, MukherjeeA, KatochK, BenaraSK, SinghT, SinghP. Immunoprophylactic effects of the anti-leprosyM wvaccine in household contacts of leprosypatients: clinical field trials with a follow up of 8-10 years. Lepr Rev 2005; Vol. 76(Issue 2): Pg. 127-43.

34. Kumar V, Abbas AK. Robbins & Cotran Pathologic Basis of Disease. 7th edition ed. Saunders; 2004.

35. Murphy KM, Travers P, Walport M. Janeway's Immunobiology Seventh Edition. Seventh ed. ; 2007.

36. Ganju L, Mukherjee R, Batra HV, Talwar GP. Immuno-blot analysis of antigens of Mycobacterium w: a candidate anti-leprosy vaccine using monoclonal antibodies and patient sera. Zentralblatt fur Bakteriologie : international journal of medical microbiology 1990; 273(3): 378-85.

37. Mustafa AS. Identification of T-cell-activating recombinant antigens shared among three candidate antileprosy vaccines, killed M. leprae, M. bovis BCG, and mycobacterium w. Int J Lepr Other Mycobact Dis 1988; 56(2): 265-73.

38. Singh IG, Mukherjee R, Talwar GP. Resistance to intravenous inoculation of Mycobacterium tuberculosis H37Rv in mice of different inbred strains following immunization with a leprosy vaccine based on Mycobacterium w. Vaccine 1991; 9(1): 10-4.

39. Zaheer SA, Beena KR, Kar HK, Sharma AK, Misra RS, Mukherjee A. Addition of immunotherapy with Mycobacterium w vaccine to multi-drug therapy benefits multibacillary leprosy patients. Vaccine 1995; 13(12): 1102-10.

40. Sharma P, Misra RS, Kar HK, Mukherjee A, Poricha D, Kaur H. Mycobacterium w vaccine, a useful adjuvant to multidrug therapy in multibacillary leprosy: a report on hospital based immunotherapeutic clinical trials with a follow-up of 1-7 years after treatment. Lepr Rev 2000; 71(2): 179-92.

41. Kaur I, Dogra S, Kumar B, Radotra BD. Combined 12-month WHO/MDT MB regimen and Mycobacterium w. vaccine in multibacillary leprosy: a follow-up of 136 patients. International journal of leprosy and other mycobacterial diseases : official organ of the International Leprosy Association 2002; 70(3): 174-81.

42. Churchyard GJ, Kaplan G, Fallows D, Wallis RS, Onyebujoh P, Rook GA. Advances in immunotherapy for tuberculosis treatment. Clin Chest Med 2009; 30(4): 769,82, ix.

43. Singh IG, Mukherjee R, Talwar GP, Kaufmann SH. In vitro characterization of T cells from Mycobacterium w-vaccinated mice. Infect Immun 1992; 60(1): 257-63.

44. Gupta A, Geetha N, Mani J, Upadhyay P, Katoch VM, Natrajan M. Immunogenicity and protective efficacy of "Mycobacterium w" against Mycobacterium tuberculosis in mice immunized with live versus heat-killed M. w by the aerosol or parenteral route. Infect Immun 2009; 77(1): 223-31.

45. Guleria I, Mukherjee R, Kaufmann SH. In vivo depletion of CD4 and CD8 T lymphocytes impairs Mycobacterium w vaccine-induced protection against M. tuberculosis in mice. Med Microbiol Immunol (Berl) 1993; 182(3): 129-35.

46. Patel N, Deshpande MM, Shah M. Effect of an immunomodulator containing Mycobacterium w on sputum conversion in pulmonary tuberculosis. J Indian Med Assoc 2002; 100(3): 191-3.

47. Dr. Luhadia S, Dr. Saugat R, Dr. Joshi V. Mycobacterium w as an adjuvant to chemotherapy in management of pulmonary tuberculosis. Personal correspondence.

48. Patel N, Trapathi SB. Improved cure rates in pulmonary tuberculosis category II (retreatment) with mycobacterium w. J Indian Med Assoc 2003; 101(11): 680, 682.

49. Katoch K, Singh P, Adhikari T, Benara SK, Singh HB, Chauhan DS. Potential of Mw as a prophylactic vaccine against pulmonary tuberculosis. Vaccine 2008; 26(9): 1228-34.

50. Kharkar R. Immune recovery in HIV with Mycobacterium W. J Indian Med Assoc 2002; 100(9): 578-9.

51. Mwinga A, Nunn A, Ngwira B, Chintu C, Warndorff D, Fine P, et al. Mycobacterium vaccae (SRL172) immunotherapy as an adjunct to standard antituberculosis treatment in HIV-infected adults with pulmonary tuberculosis: a randomised placebo-controlled trial. Lancet 2002; 360(9339): 1050-5.

52. Malmstrom PU, Loskog AS, Lindqvist CA, Mangsbo SM, Fransson M, Wanders A, et al. AdCD40L Immunogene Therapy for Bladder Carcinoma - The First Phase I/IIa Trial. Clin Cancer Res 2010; .

53. Chaudhuri P, Mukhopadhyay S. Bladder preserving approach for muscle invasive bladder cancer--role of mycobacterium w. J Indian Med Assoc 2003; 101(9): 559-60.

54. Sur PK, Dastidar AG. Role of Mycobacterium w as Adjuvant Treatment of Lung Cancer (Non-small Cell Lung Cancer). JIMA 2003; 101(02).

55. Dave RI, Tripathi UB, Parikh BJ, Patel KM, Shukla HK. Improvement in quality of life and symptom control with MYCOBACTERIUM W (CADI-05) in head and neck cancer. Journal of Clinical Oncology 2007; 25(18S).

56. Kumar B, Sandhu K, Kaur I. Role of Mycobacterium w vaccine in the management of psoriasis. Br J Dermatol 2005; 152(2): 380-2.

57. Rath N, Kar HK. Efficacy of intradermal heat-killed Mycobacterium w in psoriasis: a pilot study. Int J Dermatol 2003; 42(9): 756-7.

58. Gupta S, Malhotra AK, Verma KK, Sharma VK. Intralesional immunotherapy with killed Mycobacterium w vaccine for the treatment of ano-genital warts: an open label pilot study. J Eur Acad Dermatol Venereol 2008; 22(9): 1089-93.

59. Dheda K, Schwander SK, Zhu B, van Zyl-Smit RN, Zhang Y. The immunology of tuberculosis: from bench to bedside. Respirology 2010; 15(3): 433-50.

APPENDICES

APPENDIX 1: SEARCH STRATEGY (FROM FORMAL PROTOCOL)

INCLUSION CRITERIA

TYPES OF STUDIES

All randomised and quasi-randomised controlled trials of *Mycobacterium w* immunotherapy in participants diagnosed with PTB.

TYPES OF PARTICIPANTS

Patients diagnosed with PTB either by sputum smear microscopy, sputum culture or culture of tissue/samples from other parts of the body.

TYPES OF INTERVENTIONS

Intervention: Inoculation with at least one dose of heat killed M *w*.

Control: Placebo injection or no control administered.

SEARCH METHODS FOR IDENTIFICATION OF STUDIES

SP and OA will perform an exhaustive and comprehensive search to identify all relevant studies regardless of language or publication status (published, unpublished, *in press* and *in progress*).

ELECTRONIC SEARCHES

This process will include searching the following journal and trial databases:

- Cochrane Central Register of Controlled Trials (CENTRAL) – latest issue
- MEDLINE 1966 to March 2011
- EMBASE 1980 to March 2011
- LILACS 1982 to March 2011 (La Literatura Latinoamericana y del Caribe de Information en Ciencias de la Salud) (www.bireme.br)
- Cochrane Infectious Diseases Group Specialised Trials Register
- Pan African National Clinical Trials Registry (PACTR)
- World Health Organisation (WHO) International Clinical Trials Registry Platform (ICTRP)
- ClinicalTrials.gov

The following search terms (including the use of MeSH terms) will be used:

- tuberculosis AND ("*Mycobacterium w*" OR immunotherapy OR immunoadjuvant OR immunomodulator OR immu-vac OR *Mycobacterium indicus pranii*) crossed referenced with an isolated search for "Mycobacterium *w*"

OTHER RESOURCES

We will perform a hand-search of the reference lists of identified articles and relevant review articles. We will also do a manual search of abstracts or proceedings of the following conferences (2000 to present):

- The International Union Against Tuberculosis and Lung Disease World Congress (IUATLD)
- The American Thoracic Society International Congress (ATS)
- The European Respiratory Society World Congress (ERS)

In addition, we will send correspondence to all the authors of the relevant articles for any updates on their research. Finally, individuals and organisations working in the field of TB immunotherapy will be consulted for information regarding unpublished data and work in progress.

APPENDIX 2: CURRENT TRIALS USING MYCOBACTERIUM W (TRIAL NUMBER AVAILABLE AT CLINICALTRIALS.GOV)

1. STUDY OF MYCOBACTERIUM W IN BCG REFRACTORY SUPERFICIAL TRANSITIONAL CELL CARCINOMA OF BLADDER

Condition: Superficial Transitional Cell Carcinoma

Intervention: Biological: Mycobacterium *w*

2. A STUDY OF MYCOBACTERIUM W PLUS DOCETAXEL FOR HORMONE REFRACTORY METASTATIC PROSTATE CANCER

Condition: Hormone Refractory Prostate Cancer

Interventions: Biological: Mycobacterium *w*

Drug: Docetaxel

3. A PILOT TRIAL OF ADJUNCTIVE PREDNISOLONE AND MYCOBACTERIUM WITH IMMUNOTHERAPY IN TUBERCULOUS PERICARDITIS

Condition: Tuberculous Pericarditis

Interventions: Drug: Prednisolone

Biological: Mycobacterium w immunotherapy

4. STUDY OF MYCOBACTERIUM W IN SUPERFICIAL TRANSITIONAL CELL CARCINOMA OF BLADDER

Condition: Superficial Transitional Cell Carcinoma of Bladder

Interventions: Biological: Mycobacterium *w*

Biological: BCG (bacillus Calmette-Guerin)

5. EFFICACY AND SAFETY OF IMMUNOMODULATOR AS AN ADJUNCT THERAPY IN NEW PULMONARY TUBERCULOSIS (CATEGORY I) PATIENTS

Condition: Tuberculosis

Intervention: Biological: Intradermal injection of Mycobacterium *w*

6. A STUDY OF MYCOBACTERIUM W IN COMBINATION WITH PACLITAXEL PLUS CISPLATIN IN ADVANCED NON SMALL CELL LUNG CANCER

Condition: Non Small Cell Lung Cancer

Interventions: Drug: Paclitaxel & Cisplatin

Biological: Mycobacterium *w*

7. EFFICACY AND SAFETY STUDY OF IMMUNOMODULATOR AS AN ADJUNCT THERAPY IN PULMONARY TUBERCULOSIS (TB) RETREATMENT PATIENTS

Condition: Tuberculosis

Intervention: Biological: Intra-dermal administration of M *w*

MYCOBACTERIUM *W* IMMUNOTHERAPY FOR TREATING PULMONARY TUBERCULOSIS – A SYSTEMATIC REVIEW

BY

SHAHEEN PANDIE

PNDSHA001

PART C: MANUSCRIPT

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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MYCOBACTERIUM *W* ADJUVANT IMMUNOTHERAPY IN PULMONARY TUBERCULOSIS – A SYSTEMATIC REVIEW

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ABSTRACT

BACKGROUND

Tuberculosis (TB) remains a global health catastrophe, with the major burden of disease concentrated in the developing world. Current TB chemotherapies include prolonged oral and injectable drugs. High pill burdens and drug toxicities result in low cure and treatment completion rates. The investigation of new, applicable anti-TB strategies is therefore essential. Immunotherapies that manipulate the immune-pathogenic pathways of TB provide attractive options as possible adjuncts to standard TB chemotherapies. *Mycobacterium w* is a heat-killed immune-modulating vaccine designed to attenuate the effects of TB, reduce time to sputum conversion, and thereby decrease transmission and improve cure rates.

OBJECTIVES

To evaluate *Mycobacterium w (M w)* immunotherapy as an adjunct to chemotherapy in participants with pulmonary TB (PTB).

SEARCH STRATEGY

In March2011, we searched the Cochrane Infectious Diseases Group Specialised Register, CENTRAL (*The Cochrane Library* 2010, issue 1), MEDLINE, OVID, LILACS, the Pan African National Clinical Trials Registry (PACTR), the World Health Organisation (WHO) International Clinical Trials Registry Platform (ICTRP) and ClinicalTrials.gov. In addition, we handsearched the reference lists of the relevant articles, and abstracts or proceedings from the following

conferences: the International Union Against Tuberculosis and Lung Disease World Congress (IUATLD), the American Thoracic Society International Congress (ATS) and the European Respiratory Society World Congress (ERS). Individuals and organisations working in the field of TB immunotherapy were also consulted for information regarding unpublished data and work in progress.

SELECTION CRITERIA

Randomised and quasi-randomised controlled trials of *M w* immunotherapy versus placebo (or no control) for participants with PTB.

DATA COLLECTION AND ANALYSIS

Two of the authors (SP and ZK) independently extracted data and assessed trial quality. Discrepancies were resolved through discussion with a third independent reviewer (ME). Dichotomous outcomes were analysed using risk ratios (RR) and 95% confidence intervals (CI).

MAIN RESULTS

Three trials (four papers) involving a total of 368 participants were included. All four papers had methodological flaws including inadequate or unclear sequence generation, allocation concealment, blinding, selective reporting, and other forms of bias. The interpretation of results must be viewed in this context.

Overall, 173 participants received multi-dose *M w* and 168 participants received either a placebo vaccine or no alternative. *M w* immunotherapy was effective at reducing the time to sputum conversion at days 15 (RR 2.31; 95% CI 1.75 to 3.06; P < 0.001) and 30 (RR 1.83; 95% CI 1.12 to 2.98; P = 0.02) for both category I (new) and category II (re-treatment) TB participants. After day 30, benefit was only demonstrated in the category II TB (Day 60: RR 1.50; 95% CI 1.10 to 2.03; P = 0.01, Day 120: RR 1.35; 95% CI 1.05 to 1.72; P = 0.02, and Day 120+: RR 1.34; 95% CI 1.11 to 1.62; P = 0.003). We were unable to perform a meta-analysis for the outcomes of death and adverse events because they were reported in only one out of the four papers.

AUTHORS' CONCLUSIONS

Even though the meta-analysis suggests benefit as regards the time to sputum conversion, the available data on *M w* immunotherapy for participants with PTB are methodologically flawed. It is therefore difficult to advocate for routine *M w* usage as an adjuvant to TB chemotherapy. The evidence is sufficient to advise that *M w* be investigated in a well-structured, large, randomised controlled trial.

PLAIN LANGUAGE SUMMARY

MYCOBACTERIUM W IMMUNOTHERAPY FOR PEOPLE WITH PULMONARY TUBERCULOSIS

Immunotherapies are injections or vaccines that alter the way the human body responds to an infection. These therapies are often derived from actual organisms, like the organisms that cause leprosy and tuberculosis (TB). In preparing the immunotherapy, the organisms are killed by exposure to heat, and the remaining particles are used to produce the injection or vaccine. As a result of this heat killing process, there is no risk of the injection causing an infection.

Mycobacterium w (M *w*) is an immunotherapy. Scientists and doctors have studied the response to *M w* in both mice and humans. The research suggests that it may be beneficial in people with TB, especially in that it reduces the time it takes for the TB organisms to clear from the lungs. The trials conducted thus far strongly suggest that the addition of M *w* to standard TB treatment produces better results. Our review analysed three trials that used M *w* as immunotherapy. Even though the results of this overview support the fact that M *w* improved the time it took for sputum to become TB-organism free (sputum conversion), the trials themselves were poorly designed, making it difficult to put much statistical weight behind the results.

Thus, given the lack of valid evidence, we recommend that a well-structured, well-designed trial needs to be conducted in order to answer the question about the effectiveness of M *w* as an immunotherapy for people with TB.

BACKGROUND

Tuberculosis (TB) remains a global health catastrophe. The 2009 Global TB Control Update reported 9.4 million incident cases and 11.1 prevalent cases of TB; with 1.82 million TB-associated deaths (0.52 million in HIV positive individuals). The major burden of disease is concentrated in the developing world (WHO 2009). In Africa, the annual incidence continues to increase because of the Human Immunodeficiency Virus (HIV) epidemic (Murray 2004). The TB-HIV epidemic has been compounded by the emergence of drug resistant TB. Multi-drug resistant (MDR) TB is defined as resistance to both isoniazid and rifampicin, the two drugs regarded as the most effective TB chemotherapy. On a global scale, MDR TB is reported to account for 10% of the 9 million new cases of TB that occur annually (Mitnick 2007). Extensively-drug resistant (XDR) TB is defined as MDR plus resistance to a fluoroquinolone and second-line injectable. Current treatment strategies for drug-sensitive, MDR and XDR TB include prolonged oral and injectable drug therapies. Treatment regimens are problematic because of high pill burdens and drug toxicities, which result in low cure and treatment completion rates. The investigation of novel, applicable anti-tuberculosis strategies is therefore essential.

The immunopathogenesis of TB involves a complex interplay between bacteriostatic and bactericidal immune pathways. Bacteriostatic processes result in a walled-off granuloma, while the bactericidal processes of autophagy, apoptosis, and cytotoxic T-cell destruction, result in cell death (Churchyard 2009). A combination of the traditionally known T-helper 1 (Th1) response (key cytokines include interferon (IFN) gamma, tumour necrosis factor (TNF) alpha, and interleukin (IL) 15) and cytotoxic T-lymphocyte (CTL) killing is thought to provide the optimal immune protection against TB. The T-helper 2 (Th2) response (IL-4, Transforming Growth Factor (TGF) beta, IL -10) directly reduces the immune response to TB by both delaying the maturation of Th1 cells, and by inhibiting the production of cytokines that drive Th1 and CTL cells. Therefore, the ideal immunotherapeutic strategy would be to inhibit Th2 while enhancing the protective Th1 and CTL pathways.

Mycobacterium w (*M w*) is a non-pathogenic, saprophytic, rapidly growing atypical *Mycobacterium* species with immuno-potentiating properties. Recent polyphasic taxonomic analysis classified *M w* as a distinct species, *Mycobacterium indicus pranii*, placing it in the Runyon group IV along with *M Vaccae, M fortuitum and M smegmatis* (Saini 2009). Its uniqueness stems from its ability to undergo antigen-driven blast leukocyte transformation. Recent scientific experiments have shown that *M w* exerts its influence on the innate immune system at the level of the Toll-like-receptor (TLR) and TLR ligands. It has the ability to inhibit

TLRs (especially TLR 3, 4, 5, 6, 7, 8, and 9) and antagonise TLR ligands (Khamar 2008). This finding has spurred new interest in the potential benefits of *M w* usage, particularly in conditions that are associated with up-regulation or over-expression of TLRs, such as sepsis and chronic airway diseases. Interestingly, PTB is also associated with selective up-regulation of TLRs (Chang 2006).

An extensive body of data supports the safety and efficacy of *M w* in the prevention and treatment of leprosy (Nath 1998, Sharma 2005). Laboratory, animal and clinical work investigating *M w* use in TB suggests reduction in time to sputum conversion (a proxy for cure), and thus a potential reduction in spread of disease.

M w shares B and T-cell antigen epitopes with *Mycobacterium leprae* and *Mycobacterium tuberculosis* (Ganju 1990, Singh 1991). The initial work (animal trials and phase I - III human clinical trials) has resulted in the use of *M w* as an adjuvant treatment for leprosy. This outcome is testament to the hypothesis that the use of *M w* as an immune modulator for diseases with overlapping *Mycobacterium* antigens, is both scientifically plausible and clinically relevant to investigate.

In humans, injection of heat-killed *M w* was tested as an adjunct to standard antibiotic therapy in phase three clinical trials of lepromatous leprosy. Participants with leprosy received multi-drug therapy (MDT) plus *M w*, while the control arm received MDT plus a placebo injection. This study showed that bacteriological clearance was more rapid in the *M w* group ($P < 0.03$) (Zaheer 1995). There was also an associated decrease in the number of organisms (bacillary load), shorter duration of antibiotic therapy, and earlier discharge from care (Zaheer 1995, Sharma 2000, Kaur 2002). In healthy contacts of leprosy patients, *M w* was associated with protection from leprosy infection (Sharma 2005). Sharma et al vaccinated a total of 24,060 household contacts with *M w* or placebo in a double-blind, randomised controlled trial. Participants were followed at 3 yearly intervals for 8-10 years. *M w* showed a protective efficacy of 68%, 59% and 39.3% at the respective follow-up intervals. The safety and efficacy of *M w* has thus been well established through widespread use as an adjuvant therapy for leprosy treatment and prevention.

As regards TB, multiple laboratory studies have demonstrated that mice immunised with heat-killed *M w* had increased Th1 lymphocyte and macrophage activity, with a cytokine environment that was predominantly IL-2 and IFN-gamma. In addition, immunised mice were protected from sub-lethal challenge with *M. tuberculosis* (Gupta 2009, Guleria 1993, Singh 1992). Human data of heat-killed *M w* use as immunotherapy for active TB are not definitive. Results from published and unpublished clinical trials suggest that *M w* administration is associated with a reduction in time to sputum negativity and improved cure rates (Patel 2002,

Patel 2003, Luhadia 2004). In addition, Katoch reviewed the participants (healthy contacts) of the leprosy studies looking for evidence of new TB infection (Katoch 2008). The results suggest that *M w* significantly ($P < 0.01$) reduced the rate of new TB infection.

In terms of safety, *M w* has been administered to thousands of participants in multiple trials and studies, with no reports of any serious adverse events. The accepted chronology of the "normal" local skin reaction to the vaccine is:

(1) a small pustule between days four to five;

(2) mild ulceration between days seven and ten; and

(3) formation of a scab by 1 month post administration.

Any skin changes that are not in keeping with this sequence of events are considered an accelerated or exaggerated reaction i.e. adverse reaction (Luhadia 2004). Even so, all published studies have concluded that *M w* is safe, with reported side effects invariably being self-limiting.

The TB-HIV epidemic is a health crisis, and current therapeutic options for TB are sub-optimal. Immunotherapeutic interventions may facilitate the fight against TB. Understanding the immunopathogenesis of TB is crucial in the development of appropriate anti-TB strategies (Dheda 2010). Laboratory, animal and clinical work investigating *M w* use in TB suggests a reduction in time to sputum conversion (a proxy for cure), and thus the potential to reduce the spread of disease. This systematic review aims to analyse the evidence of the effectiveness of *M w* vaccination as an adjunctive treatment for PTB. This information will be helpful for policy makers, healthcare practitioners and researchers in the field of TB management and control.

OBJECTIVES

To evaluate *M w* immunotherapy as an adjunct to chemotherapy in participants with PTB.

We reviewed the available data as regards *M w* usage in PTB, focusing on the effects of *M w* immunotherapy on:

(1) sputum conversion;

(2) mortality; and

(3) adverse reactions.

CRITERIA FOR CONSIDERING STUDIES FOR THIS REVIEW

TYPES OF STUDIES

Randomised and quasi-randomised controlled trials of *M w* immunotherapy in participants diagnosed with PTB.

TYPES OF PARTICIPANTS

Participants diagnosed with PTB either by sputum smear microscopy, sputum culture, or culture of material from a clinically affected anatomical site.

TYPES OF INTERVENTIONS

Intervention: inoculation with at least one dose of heat-killed *M w*.

Control: placebo injection or no control.

Other: chemotherapy for TB (according to World Health Organisation (WHO) guidelines for category I and category II TB).

TYPES OF OUTCOME MEASURES

PRIMARY OUTCOMES

To determine the effect of *M w* therapy on:

(1) sputum conversion (sputum culture negativity), assessed at days 15, 30, 60, 120, and beyond 120; and

(2) mortality.

SECONDARY OUTCOMES

To determine the frequency of:

(1) serious adverse reactions (fatal, life threatening or requiring hospitalisation); and

(2) other adverse events related to the immunotherapy.

SEARCH METHODS FOR IDENTIFICATION OF STUDIES

We performed a detailed literature search for reports pertaining to the use of *M w* in PTB. Selected articles were reviewed for standardised data extraction and analysis.

The project team consisted of three main contributors:

(1) Dr. Shaheen Pandie (SP), who functioned as the primary investigator and was responsible for all aspects of the project;
(2) Mr. Mark Engel (ME), who was responsible for independently completing the literature search and verifying the data that were collected, as well as functioning as co-supervisor on the project;
(3) Dr. Zita Kerbelker (ZK), who was responsible for independently extracting the data; and
(4) Professor Bongani Mayosi (BM) who functioned as the project supervisor.

We performed an exhaustive and comprehensive search to identify all relevant studies regardless of language or publication status (published, unpublished, *in press* and *in progress*).

ELECTRONIC SEARCHES

This process included searching the following journal and trial databases:

(1) the Cochrane Infectious Diseases Group Specialised Trials Register
(2) CENTRAL (*The Cochrane Library* 2010, issue 1);
(3) MEDLINE (January 1966 to March 2011);
(4) OVID (January 1980 to March2011);
(5) LILACS (La Literatura Latinoamericana y del Caribe de Information en Ciencias de la Salud)(January 1982 to March 2011);
(6) the Pan African National Clinical Trials Registry (PACTR);
(7) the World Health Organisation (WHO) International Clinical Trials Registry Platform (ICTRP); and
(8) ClinicalTrials.gov.

The following search terms (including the use of MeSH terms) were used: tuberculosis AND ("*Mycobacterium w*" OR immunotherapy OR immunoadjuvant OR immunomodulator OR Immu-vac® OR *Mycobacterium indicus pranii*) cross-referenced with an isolated search for "*Mycobacterium w* ".

SEARCHING OTHER RESOURCES

We performed a handsearch of the reference lists of identified articles and relevant review articles. We also did a manual search of abstracts or proceedings of the following conferences:

(1) the International Union Against Tuberculosis and Lung Disease World Congress (IUATLD) (January 2000 to May 2010);
(2) the American Thoracic Society International Congress (ATS) (January 2000 to May 2010); and
(3) the European Respiratory Society World Congress (ERS).

In addition, we sent correspondence to the authors of the relevant articles, enquiring about any updates on their research. Finally, we consulted individuals and organisations working in the field of TB immunotherapy for information regarding unpublished data and work in progress.

DATA COLLECTION AND ANALYSIS

SELECTION OF STUDIES

SP and ZK reviewed all relevant material identified from the above search. After reading the titles and abstracts of the identified articles, we acquired the full text articles of all citations deemed to meet the inclusion criteria. These articles were then independently inspected to verify that they met the inclusion criteria.

DATA EXTRACTION AND MANAGEMENT

We extracted the data using a standardised data extraction form (Appendix 1). Any discrepancies were resolved through discussion of the original articles with BM. The following characteristics were extracted from each included study:

(1) Administrative details: trial identification number, title, author (s), published or unpublished, year of publication, number of studies included in the paper, year in which the study was conducted, and details of other relevant papers cited;
(2) Verification assessment: assessment to ensure that the study met the inclusion criteria for the systematic review;
(3) Details of study: study design, duration and completeness of follow-up, country and location of study, informed consent, and ethics approval;
(4) Details of participants: setting, number and relevant baseline characteristics, category of PTB (I or II);
(5) Details of intervention: *M w* dosage, duration, and mode of administration;
(6) Details of control: placebo or no vaccine control, completeness of treatment;
(7) Details of outcomes: sputum conversion at various stages of therapy, mortality, adverse events; and
(8) Notes: general comments.

ASSESSMENT OF RISK OF BIAS IN INCLUDED STUDIES

We independently examined the components of each included trial for risk of bias. The assessment focused on information regarding sequence generation, allocation concealment, blinding, incomplete outcome or missing data, selective outcome reporting and other sources of bias. Each methodological component was assessed as being adequate, inadequate or unclear as

per the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2008). It was then recorded on the data collection sheet.

MEASURES OF TREATMENT EFFECT

Data were analysed using Review Manager 5.0. Outcomes (sputum conversion (cure), death, adverse events) were considered as dichotomous variables. Outcome measures were calculated using risks ratios (RR) with 95% confidence intervals (CI).

DEALING WITH MISSING DATA

We made every effort to contact the original authors or investigators of the selected articles to help address the issues of missing or incomplete data.

ASSESSMENT OF HETEROGENEITY

Heterogeneity between trials was assessed using the chi-squared test set at a 10% level of significance. The impact of statistical heterogeneity was quantified using the I^2 statistic (Appendix 2). Because there was an acceptable degree of lack of heterogeneity, it was appropriate to pool the data. Using the Mantel-Haenszel statistical method and random-effects model, the results were generated in the form of a meta-analysis. Data that could not be analysed as part of the meta-analysis are presented in a narrative form.

SUBGROUP ANALYSIS AND INVESTIGATION OF HETEROGENEITY

In addition to evaluating all PTB participants, we analysed the subgroups of category I (new infections) and category II (retreatment or relapse) PTB. The same statistical analysis was used as described above.

DESCRIPTION OF INCLUDED STUDIES

Luhadia (Luhadia 2004) presented the findings of his single-blind, placebo-controlled study of *M w* as immunotherapy for treating PTB at the National Conference on Pulmonary Diseases (NAPCON) in India in 2004. Two hundred sputum-positive participants were randomised to either *M w* or placebo, with background standard TB chemotherapy. There were 100 category I (new cases) and 100 category II (retreatment) TB cases. 0.1 ml *M w* or saline placebo was given intradermally on days zero, 15, 30, 60 and then two-monthly until completion of treatment. The diagnosis of TB was made on sputum examination. Sputum samples were collected at each follow-up visit (days 0, 15, 30, 60, and 120). Radiographs were performed on days zero, 60, 120, and at the end of treatment. For the category I group, 50 participants were randomised to *M w,* and 50 to the placebo arm. The same division occurred with the category II participants (50 *M w* versus 50 placebo). The groups were evenly matched for age (mean age 35.5) and weights (41kg in males and 32kg in females).

In the category I subgroup, sputum conversion was 97% at 15 days and 100% at 30 days in the *M w* group compared to 42% at 15 days and 75 % at 30 days in the placebo group. At the end of two months (60 days) the control group reach 93.5% sputum conversion rate. *M w* thus preponed sputum conversion by 45 days. There were no deaths or adverse reactions reported.

In the category II sub-group, 41% sputum conversion was achieved at the end of 15 days in the *M w* arm; while it took 60 days to achieve a 39% sputum conversion in the placebo arm. There were four deaths, two reported in each arm respectively. The *M w* arm also had two participants with an accelerated local skin reaction.

Patel (Patel 2002) completed a single-centre pilot study that randomised 134 consecutive PTB participants who were sputum positive. Both category I (58) and category II (76) cases were randomly assigned to TB chemotherapy plus *M w*, or TB chemotherapy alone. *M w* was administered on day one, then fortnightly for two months. Sixty-nine participants were randomised to the *M w* arm and 65 to the control arm. Sputa specimens were collected for laboratory examination before treatment, and then on days 15, 30, 45 and 60. The laboratory examiner was blinded to the type and duration of treatment. The *M w* treatment group had a significantly faster sputum conversion rate at days 15, 30, and 45 overall; and for both category I and category II subgroups. The sputum conversion rate achieved at day 60 in the control arm was achieved at day 30 in the *M w* group, i.e. *M w* preponed sputum conversion by at least 30 days. No major adverse events were reported, and local side effects like skin induration and ulceration were self-limiting.

Even though these results are very impressive, a major criticism of the trial is the flawed methodology. Even though the two arms seem to be evenly matched (69 versus 65 participants), there was inequality in the sex distribution (102 males and 32 females), and in the category I and II subgroups. It is not clear whether this reflects a lack of appropriate sequence generation, allocation or stratification of the randomisation. As a result, the *M w* arm had 20 category I and 49 category II participants, while the control arm had 38 and 27 respectively. In addition, it is not clear whether the category I and II subgroups were prespecified.

In 2003, Patel (Patel 2003) published a post-hoc analysis of the 2002 paper. It involved a records review of the category II TB participants that were originally enrolled in the trial. Sputa samples were analysed at three, five and eight months (for sputum negative participants); and at four, six and nine months (for sputum positive participants). Again, the laboratory technician was blinded to the treatment type and duration. The total number of category II cases reviewed was 76, of which 49 were in the *M w* arm and 27 in the control group. Participants that were randomly assigned to the *M w* arm received 0.2 ml *M w* (0.1 ml in each deltoid) at day one, then 0.1 ml fortnightly for two months. No placebo was given to the control arm. All the participants completed the respective treatments. The results showed that sputum clearance at follow up visits (three and four months, and 2 months post-intensive phase of TB treatment) was better in the *M w* arm. The *M w* arm also had lower treatment failure rates. No major adverse events, including all-cause and TB-related mortality, were reported. As mentioned previously, this is a post hoc analysis of a non-a priori specified subgroup with unequal numbers in the two comparative groups, which makes the value of the data questionable and non-generalisable.

Parikh's (Parikh 2006) study was a randomised, double-blind, placebo-controlled trial investigating the role of *M w* in the management of TB hydropneumothorax as an adjuvant to TB chemotherapy and intercostal tube drainage (ICTD). Thirty-four participants with TB-related pleural effusions (including category I and II TB participants, both sputum smear positive and negative) were randomised to *M w* or control arms. Eighteen participants were randomised to *M w* plus TB chemotherapy and ICTD (4 out of 18 smear positive, 14 out of 18 smear negative, 2 out of 18 category I, and 16 out of 18 category II). *M w* was administered intradermally (0.2 ml on day zero, followed by 0.1 ml on days 15, 30, 60, 120, 180 and till the end of TB treatment). Sixteen participants were randomised to the control arm which included TB chemotherapy and ICTD (3 out of 16 smear positive, 13 out of 16 smear negative, 2 out of 16 category I, and 14 out of 16 category II).

The *M w* arm had faster time to removal of ICTD with 72% removal within 22 days versus 37.5% in the control arm. The decision to remove the ICTD was determined by the attending physician, and was based on clinical and radiological resolution. Student's t-Test was used to

compare number of days taken for removal of ICTD between the two groups: the *M w* arm had a faster (15.1 ± 8.58 days) removal as compared to the control arm (43.9 ± 31.6 days); with a P value of < 0.001. Sputum conversion was faster in the *M w* arm (18.8 ± 7.5 days) compared to the control arm (96.7 ± 40.4 days), with a P value of < 0.012.

This was a very small study, with only 7 out of 34 participants being sputum positive. There was no comment made about adverse events related to *M w* usage.

RESULTS

RESULTS OF LITERATURE SEARCH

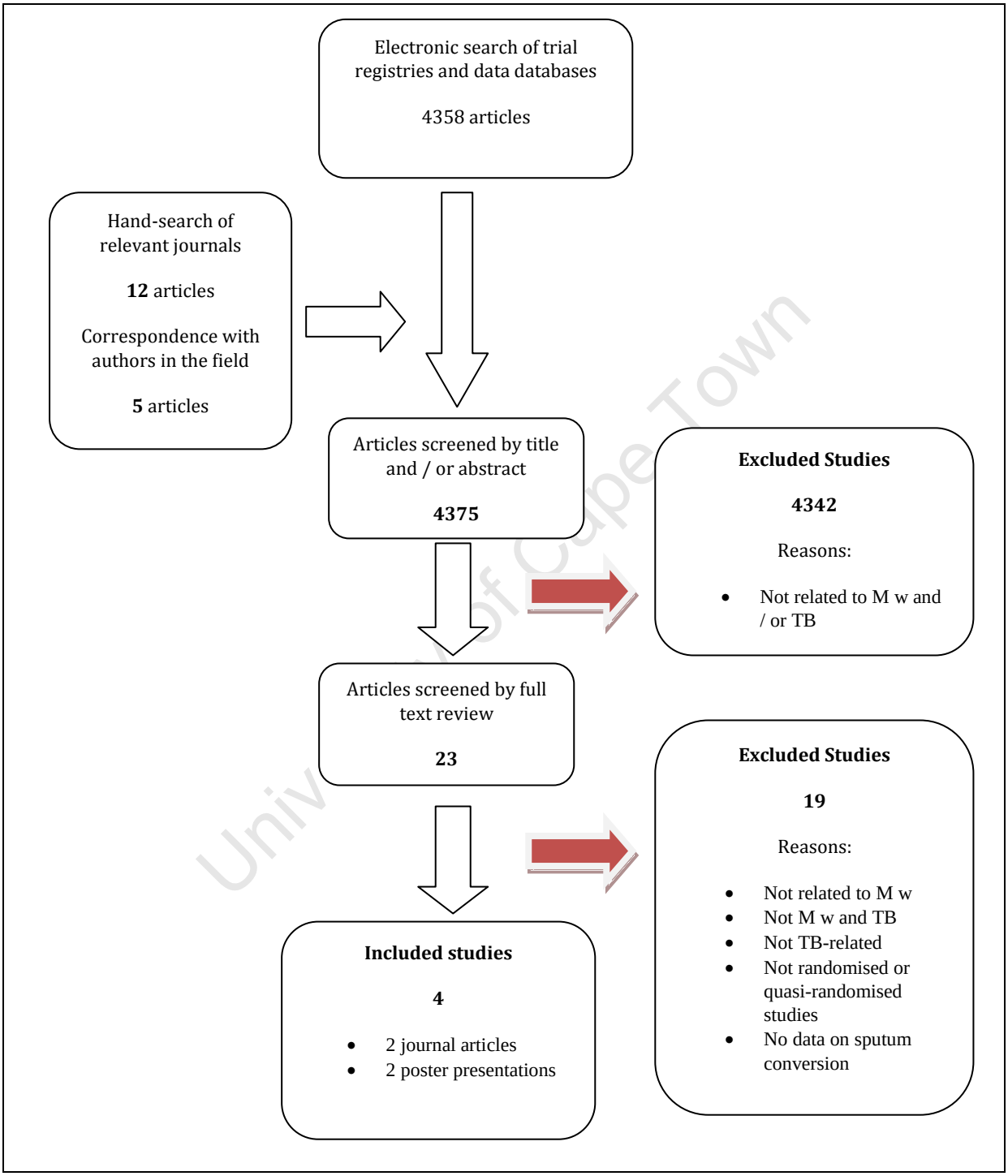


FIGURE 1: FLOW DIAGRAM OF SEARCH RESULTS

Figure 1 summarises the results of the literature search. There were three studies (four papers) that met the inclusion criteria. Two studies were randomised controlled trials using M w as an

immunotherapy in confirmed PTB (category I and II) (Luhadia 2004, Patel 2002), one study was a post-hoc analysis (with extended follow up) of M w usage in category II participants (Patel 2003), and one study included participants with TB pleural effusions (Parikh 2006). Two of the papers were published (Patel 2002, Patel 2003), while the remaining two were abstracts presented at conferences (Luhadia 2004, Parikh 2006).

METHODOLOGICAL QUALITY

Figures 2 and 3 are graphic representations of the assessment of risk of bias including the components of allocation, blinding, dealing with incomplete data, selective reporting and other potential sources of bias. All these components were assessed as being adequate, inadequate or unclear (Higgins 2008). Limited information was provided as regards the aspects of randomisation (i.e. sequence generation and allocation concealment). All three studies were regarded as either inadequate or unclear. For all three studies, blinding was considered adequate in terms of the primary outcome. For other outcomes (adverse events, clinical and radiological improvements), the blinding was inadequate. There was no missing data in any of the studies. Two out of the three studies (Patel 2002, Luhadia 2004, Parikh 2006) include reporting on all the expected outcomes, and were therefore scored as adequate. The Patel 2003 paper could be considered as selective reporting because there was no pre-specification of the category II TB subgroup. All three studies are at risk of other sources of bias, including selection, performance, and clinical bias.

FIGURE 2: RISK OF BIAS GRAPH: REVIEW AUTHORS' JUDGMENTS ABOUT EACH RISK OF BIAS ITEM PRESENTED AS PERCENTAGES ACROSS ALL INCLUDED STUDIES

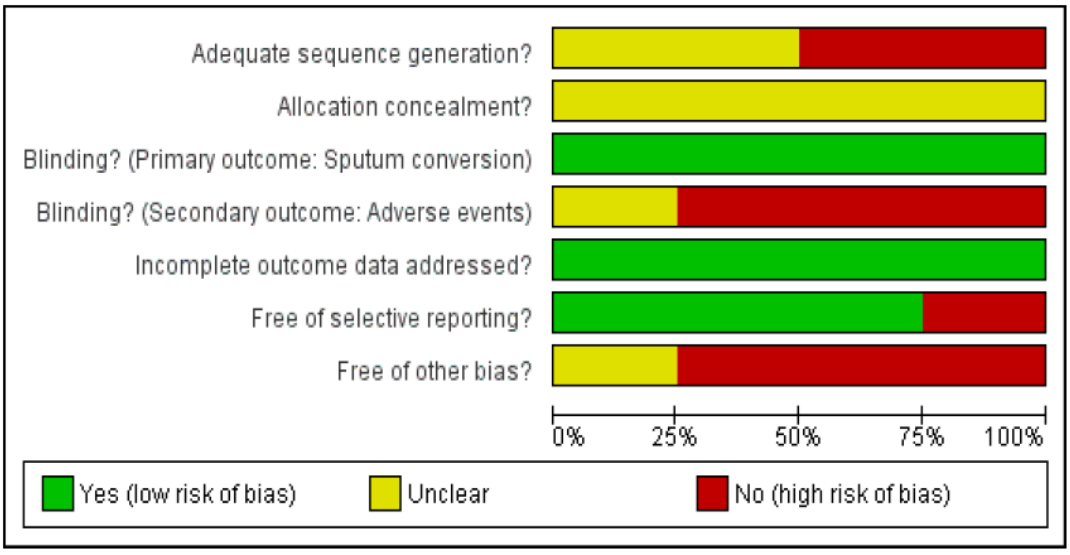
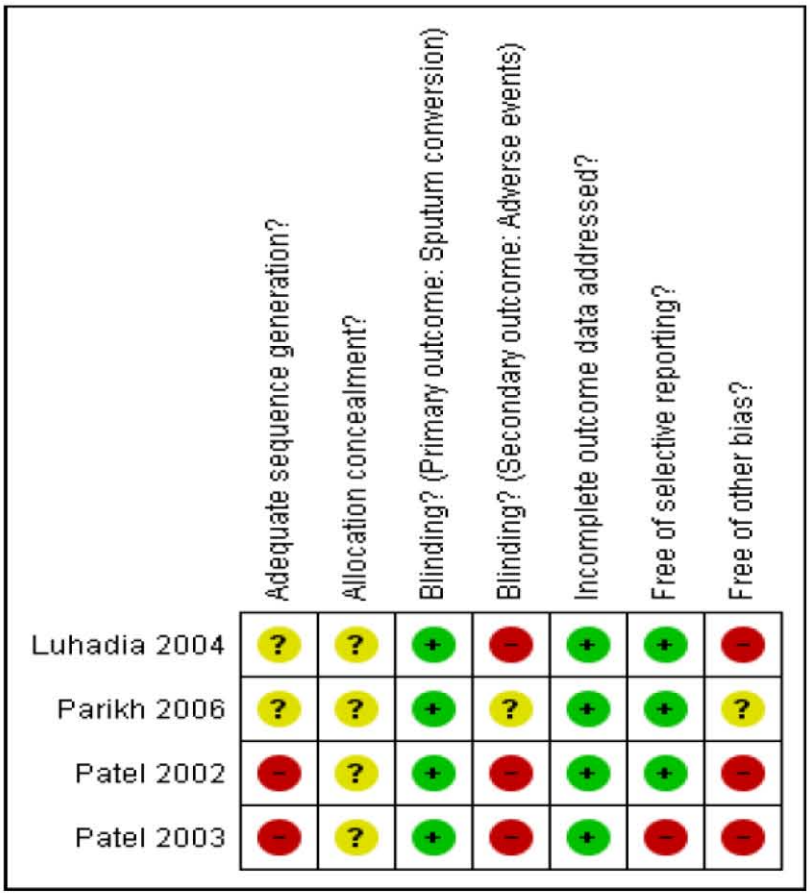


FIGURE 3: RISK OF BIAS SUMMARY: REVIEW AUTHORS' JUDGMENTS ABOUT EACH RISK OF BIAS ITEM FOR EACH INCLUDED STUDY



SUMMARY OF THE MAIN RESULTS

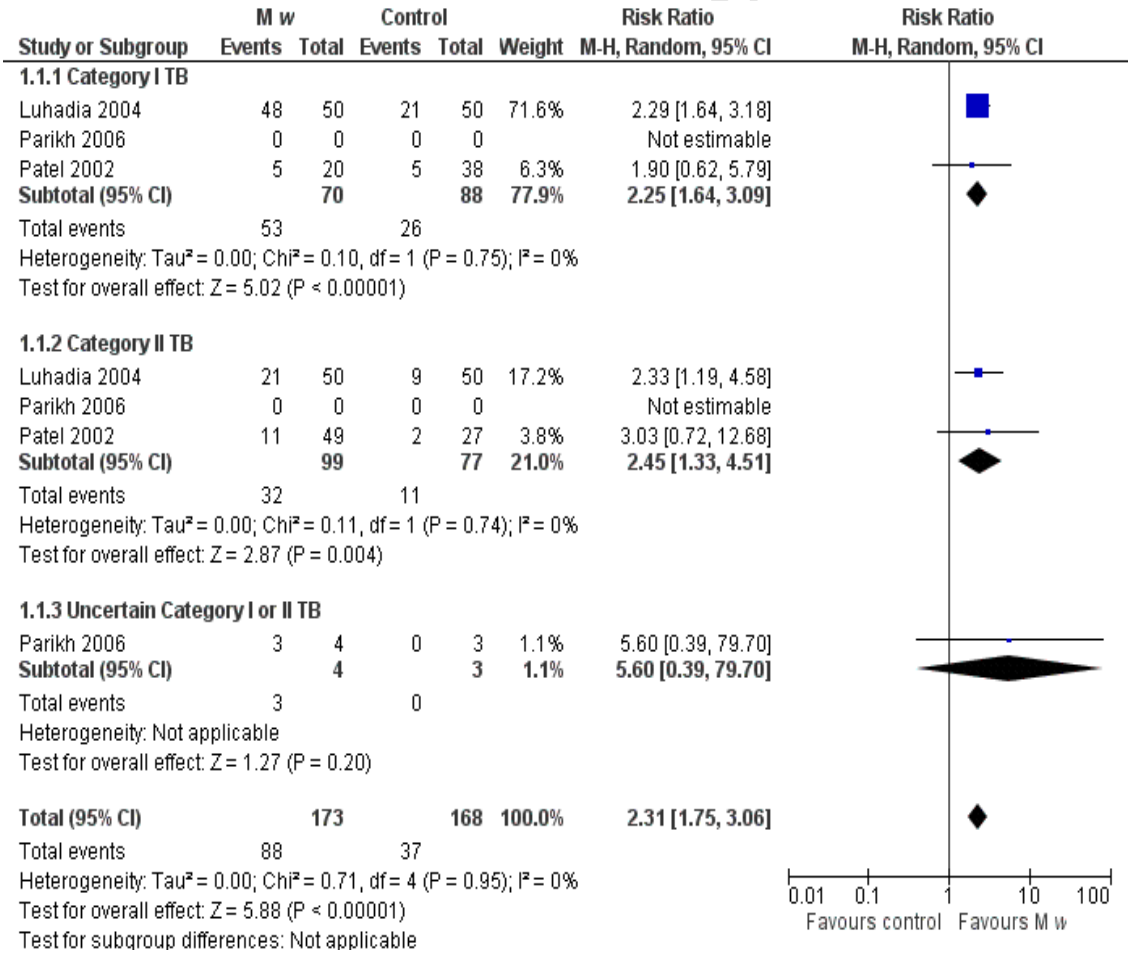
Three trials (four papers) involving 368 participants were included. There was a total of 341 confirmed, smear positive PTB participants. 158 Were regarded as category I TB, 176 as category II TB, and 7 were unclassified. Overall, 173 participants (70 category I, 99 category II, and 4 category uncertain TB) received multi-dose *M w* immunotherapy therapy, and 168 participants (88 category I, 77 category II, and 3 category uncertain TB) received either placebo vaccinations or no alternative. All participants had standard TB chemotherapy as per WHO guidelines. The meta-analysis was performed for the following outcomes of sputum conversion at days 15, 30, 60, 120 and beyond. Participants were divided into the subgroups of category I TB, category II TB and uncertain category.

SPUTUM CONVERSION AT DAY 15

See Analysis 1.1, Figure 4

M w usage has a significant effect on early sputum conversion from positive to negative at day 15 (P < 0.001). The meta-analysis included 341 participants. Overall, the *M w* group had 88 sputum negative participants out of 173, compared to 37 out of 168 in the control arm; with a risk ratio (RR) of 2.31 and a 95% confidence interval (CI) of 1.75 to 3.06. These results were reflected throughout the subgroups of category I (RR 2.25; 95% CI 1.64 to 3.09; P < 0.001) and category II (RR 2.25; 95% CI 1.33 to 4.51; P < 0.001). The uncertain category had 3 participants in the M w group and nil in the control group (RR 5.6 and a wide 95% CI 0.39 to 79.7). There was lack of heterogeneity (chi squared = 0.71; I^2 = 0%) making it appropriate to combine the studies and the subgroup.

FIGURE 4: FOREST PLOT OF COMPARISON: 1 SPUTUM CONVERSION, OUTCOME: 1.1 SPUTUM NEGATIVE AT DAY 15.

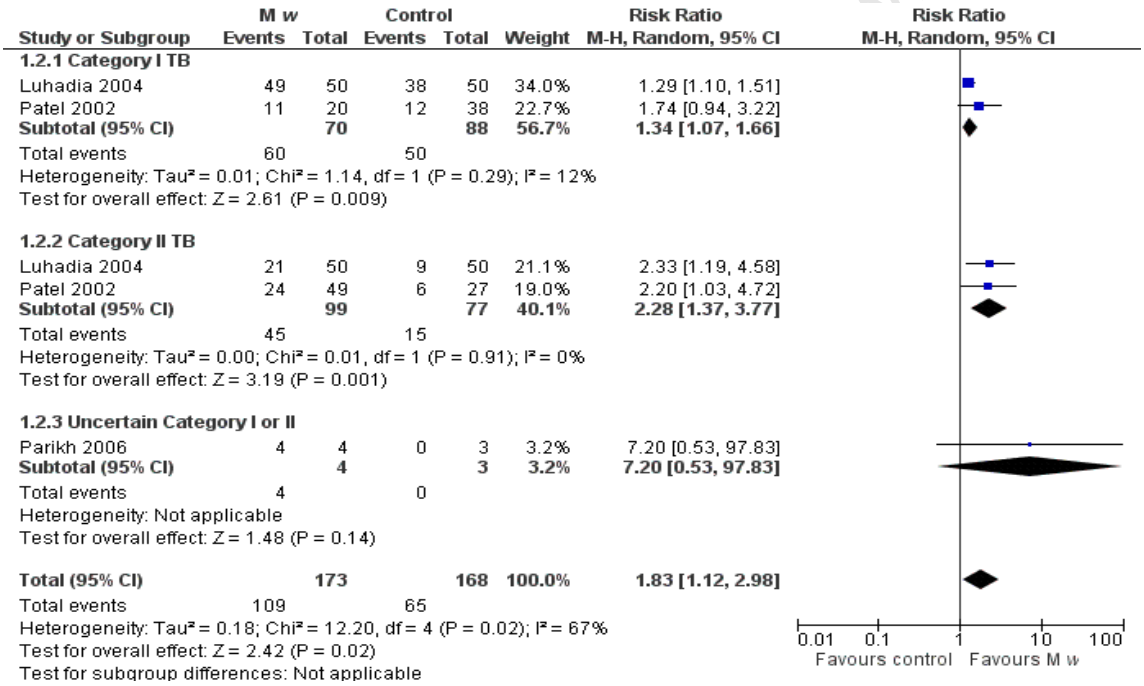


SPUTUM CONVERSION AT DAY 30

See Analysis 1.2, Figure 5

M w usage has a significant effect on sputum conversion from positive to negative at day 30 (P = 0.02). Of the 173 participants in the *M w* group, 109 were sputum negative, compared to 65 out of 168 in the control group (RR 1.83; 95% CI 1.12 to 2.98). Results for the subgroups were similar: category I TB subgroup had a RR of 1.34 with a 95% CI 1.07 to 1.66; and category II TB subgroup had a RR 2.28 and a 95% CI 1.37 to 3.77. There was heterogeneity within the subgroups (chi squared = 12.20; $I^2 = 67\%$).

FIGURE 5: FOREST PLOT OF COMPARISON: 1 SPUTUM CONVERSION, OUTCOME: 1.2 SPUTUM NEGATIVE AT DAY 30.

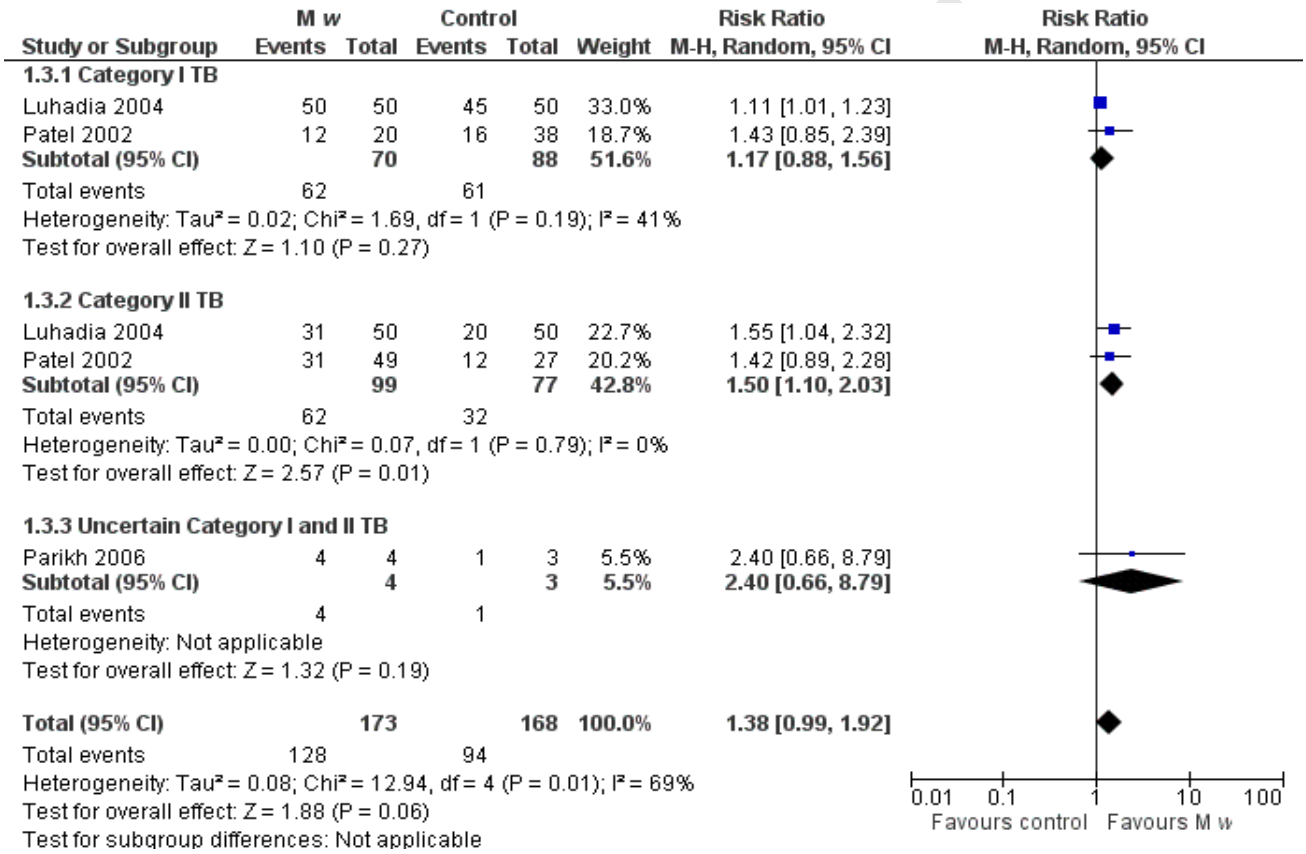


SPUTUM CONVERSION AT DAY 60

See Analysis 1.3, Figure 6

At day 60, the effect of *M w* on sputum conversion is no longer statistically significant for category I TB (RR 1.17; 95% CI 0.88 to 1.56; P = 0.19), and overall (RR 1.38; 95% CI 0.99 to 1.92; P = 0.06). There was still statistically significant benefit for the category II subgroup (RR 1.50 and 95% CI 1.10 to 2.03; P = 0.001). Again, the groups seem to be heterogenous (Chi^2 = 12.94 and I^2= 69%).

FIGURE 6: FOREST PLOT OF COMPARISON: 1 SPUTUM CONVERSION, OUTCOME: 1.3 SPUTUM NEGATIVE AT DAY 60.



SPUTUM CONVERSION AT DAY 120 AND BEYOND

See Analysis 1.4, Analysis 1.5, Figure 7 and Figure 8

Only participants in the category II TB and uncertain category subgroups were reported on at day 120. In the *M w* group, 77 participants (out of 103) had converted to sputum negative, compared to 41 out of 80 in the control group (RR 1.35; 95% CI 1.05 to 1.72; P = 0.02).

Beyond day 120, only data on the category II TB subgroup is reported. The benefit of *M w* usage for sputum conversion extends beyond 4 months in participants with category II TB (RR 1.34; 95% CI 1.11 to 1.62; P = 0.003).

FIGURE 7: FOREST PLOT OF COMPARISON: 1 SPUTUM CONVERSION, OUTCOME: 1.4 SPUTUM NEGATIVE AT DAY 120.

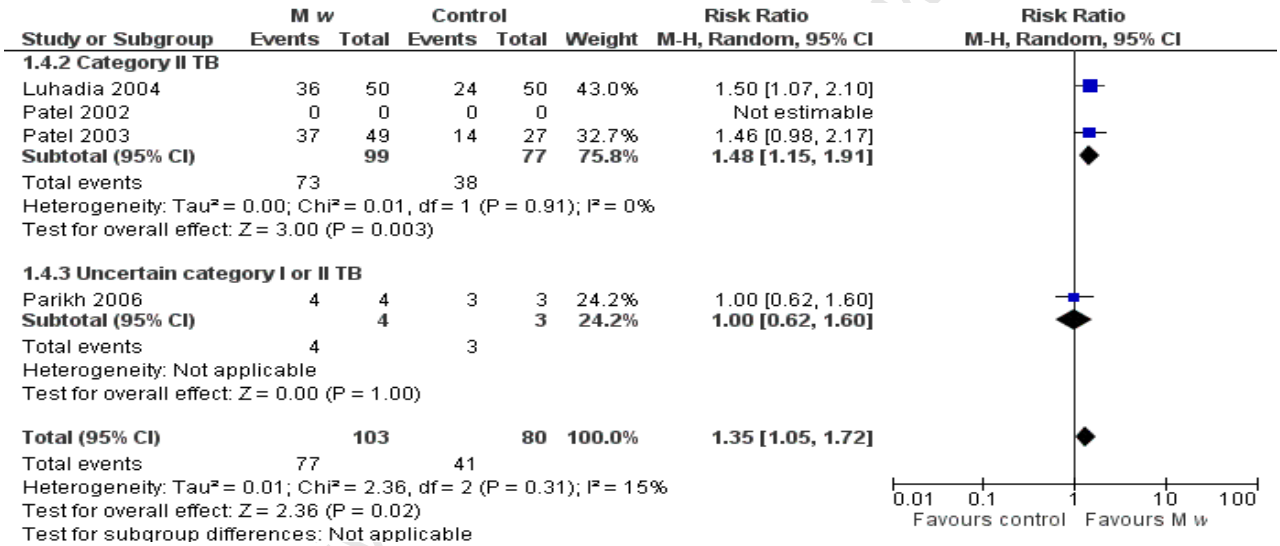
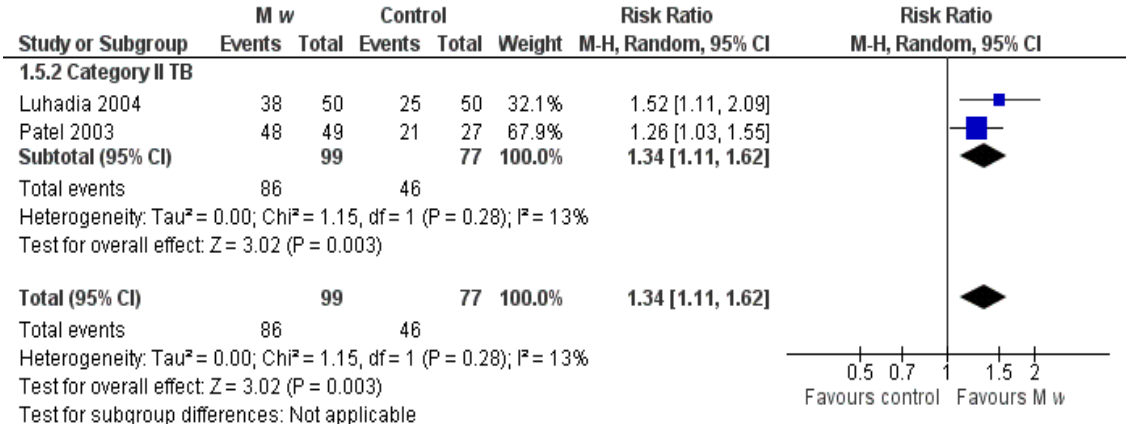


FIGURE 8: FOREST PLOT OF COMPARISON: 1 SPUTUM CONVERSION, OUTCOME: 1.5 SPUTUM NEGATIVE AFTER DAY 120.



MORBIDITY AND MORTALITY

Luhadia 2004 reported 2 deaths in the M w group and 2 deaths in the control group, as well as 2 adverse events in each group. No deaths or adverse events were reported in the other papers.

DISCUSSION

METHODOLOGICAL QUALITY

A major criticism of all the included studies is methodological flaws. These include published or presented data that do not meet the criteria set out in the CONSORT statement (Schulz 2010); making it difficult to provide any meaningful commentary on issues of randomisation, allocation concealment, and sources of bias.

ALLOCATION

As regards the aspects of randomisation (i.e. sequence generation and allocation concealment), all three studies provided limited information, and were therefore regarded as either inadequate or unclear. The lack of adequate randomisation seriously influences the way in which the results of all three studies are interpreted.

BLINDING

In terms of the primary outcome of sputum conversion, ensuring that the laboratory technician is blinded to both type and duration of therapy is the most pivotal step in ensuring the blinding process. For all three studies, blinding of assessors was considered adequate in terms of the primary outcome. For secondary outcomes (adverse events; clinical and radiological improvements), the blinding was inadequate. In general, the blinding process for vaccines that cause "normal" or "accepted" skin reactions, is difficult. Even with placebo vaccines (saline), less of a skin reaction is expected, making it difficult to blind a clinical assessor. We therefore considered that there is sufficient evidence to only evaluate our primary outcome.

INCOMPLETE OUTCOME DATA

No loss to follow up was reported, nor were there any missing data in the studies.

SELECTIVE REPORTING

For all three studies, there was no protocol available for review even after correspondence with the authors. Despite this, it is clear that two out of the three studies include reporting on all the expected outcomes, and were therefore scored as adequate. We considered the Patel 2003 paper as selective reporting due to there being no pre-specification of the category II TB subgroup.

OTHER POTENTIAL SOURCES OF BIAS

Due to the possible errors in randomisation, all three studies are at risk of other sources of bias including selection, performance, and detection bias.

The lack of placebo control in Luhadia's 2004 study may have led to bias in the evaluation of clinical outcomes such as adverse reactions, clinical improvement, and radiological resolution; as the assessor was not blinded to the intervention.

There is strong selection or referral bias evident in Patel 2002, Patel 2003 and Parikh 2006. These manifested as discrepant category I and II TB participants, and uneven numbers of males to females being enrolled. In Parikh 2006 there was also assessment bias in that the timing of intercostal drain removal was clinician determined.

EFFICACY OF *M w*

The meta-analysis of the included trials suggest that *M w* has a significant effect on sputum conversion for both category I and II TB at days 15 ($P < 0.001$) and 30 ($P = 0.02$). From day 60 onwards, the benefit persists for those participants with category II TB (day 60 $P = 0.001$; day 120 $P = 0.02$; and day 120+ $P = 0.003$). However, results were not available for all the outcomes we wished to evaluate. Out of the three included studies, only Luhadia 2004 reported deaths and adverse events.

In the comparison of the *M w* group to the control arm, *M w* early in the course of TB treatment was favoured in terms of reducing the time to sputum conversion. These results were reflected throughout the subgroups of category I and category II at days 15 and 30. These results suggest that *M w* usage has a significant effect on early sputum conversion from positive to negative.

By day 60, the initial effect of *M w* seems to taper for category I TB, and categories I and II combined. This may reflect the efficacy of the intensive phase of the WHO recommended anti-tuberculous treatment (WHO 2009). By the end of 2 months, standard therapy usually results in bacteriological clearance in approximately 75% (range 61.7 to 90.9%) of drug-sensitive smear positive cases (Rieder 1996). For the category II subgroup, the benefit of *M w* seems to persist at day 60. This is consistent with the understanding that category II TB patients require an extended intensive phase of anti-tuberculous treatment (Rieder 1996).

Only participants in the category II TB and uncertain category TB subgroups were reported at day 120. In the *M w* group, 77 out of 103 participants had converted to sputum negative, compared to 41 out of 80 in the control group, confirming that the benefit of *M w* usage for sputum conversion extends beyond 4 months in participants with category II TB. It therefore

implies that category II TB participants benefit from *M w* administration even after being on anti-tuberculous treatment for more than 4 months.

Only Luhadia 2004 reported on mortality and adverse reactions. Because so few events were reported, it is impossible to make any meaningful statistical assumptions, though it seems unlikely that the deaths were attributable to *M w.*

Even though the other papers eluded to self-limiting skin reactions, there was no formal reporting of adverse skin reactions. There appears to be an "accepted" degree of local reaction to the *M w* injection. Only reactions that occur too rapidly or that progress beyond mild ulceration are regarded as adverse. The lack of reporting on adverse events (including death) made it impossible to report these outcomes in the format of a meta-analysis.

QUALITY OF THE EVIDENCE

The above meta-analysis provides support for the efficacy of *M w* usage in participants with PTB, clearly demonstrating a significant reduction in the time to sputum conversion. Unfortunately, these results are overshadowed by the methodological flaws identified in the assessment of risk of bias. Inadequate randomisation, sequence generation, allocation concealment, and various forms of bias (selection, clinical, or performance) make the results of the included studies unreliable. For some of the measured outcomes, there appeared to be a moderate degree of heterogeneity. This is most likely attributable the combination of above mentioned methodological flaws, and the small numbers of participants in some of the included studies.

AUTHORS' CONCLUSIONS

IMPLICATIONS FOR CLINICAL PRACTICE

The use of *M w* immunotherapy for reducing the time for sputum conversion in participants with PTB appears promising, but the available data are methodologically flawed, with multiple sources of bias. There is insufficient evidence to make recommendations for clinical practice.

IMPLICATIONS FOR RESEARCH

This review has demonstrated the need for well-structured, randomised controlled trials assessing the role of *M w* adjuvant therapy. There are three such trials currently recruiting participants (NCT00265226; NCT00341328; NTC00810849).

ACKNOWLEDGEMENTS

The authors would like to acknowledge:

(1) Peter Nyasulu for his assistance in constructing the protocol for the project; and
(2) the staff of the South African Cochrane Centre for advice and support.

DECLARATIONS OF INTEREST

Shaheen Pandie and Bongani Mayosi are currently working on the IMPI trial (NTC00810849), a randomised clinical trial comparing M *w* and / or prednisolone with placebo in participants with tuberculous pericarditis.

REFERENCES

REFERENCES TO STUDIES INCLUDED IN THIS REVIEW

Luhadia 2004

Luhadia S, Saugat R, Joshi V. Mycobacterium w as an adjuvant to chemotherapy in management of pulmonary tuberculosis. In: National Conference on Pulmonary Diseases (NAPCON). 2004.

Parikh 2006

Parikh H, Shah N, Tewari T, Parswani JP, Maseeh A. Role of Mw Vaccine in management of Hydropneumothorax as an adjuvant to ATT (Anti Tuberculosis Therapy) with ICTD (Intercostal Tube Drainage). In: TB Vaccines for the World, Vienna, Austria. 2006.

Patel 2002

Patel N, Deshpande MM, Shah M. Effect of an immunomodulator containing Mycobacterium w on sputum conversion in pulmonary tuberculosis. Journal of the Indian Medical Association 2002; 100(3):191-3. [PubMed: 12408283]

Patel 2003

Patel N, Trapathi SB. Improved cure rates in pulmonary tuberculosis category II (retreatment) with mycobacterium w. Journal of the Indian Medical Association 2003; 101(11):680, 682. [PubMed: 15198421]

REFERENCES TO STUDIES EXCLUDED FROM THIS REVIEW

Chadda 2002

Chadda A, Shah NJ, Shah CM. Role of Mycobacterium *w* in management of tuberculous pleural effusion. medical@cadilapharma.co.in 2002.

Katiyar

Katiyar SK, Agrawal A, Singh L, Shahabuddin S. Salvage therapy of MDR failure using M *w* vaccine. medical@cadilapharma.co.in.

Katoch 2008

Katoch K, Singh P, Adhikari T, Benara SK, Singh HB, Chauhan DS, et al. Potential of Mw as a prophylactic vaccine against pulmonary tuberculosis. Vaccine 2008;26(9):1228-34. [PubMed: 18243430]

Marthur 2006

Marthur ML. Potential utility of Mycobacterium *w* vaccine in control of tuberculosis. Current Respiratory Medicine Reviews May 2006;2(2):183-88.

Zhou 2002

Zhou X, Lan J, Zhang T. A study on the use of mycobacterium vaccine in the treatment of tuberculous pleurisy. Zhonghua jie he he hu xi za zhi = Zhonghua jiehe he huxi zazhi = Chinese journal of tuberculosis and respiratory diseases 2002;25(10):595-7. [PubMed: 12490125]

REFERENCES TO ONGOING STUDIES

NCT00265226

Unpublished data only

Efficacy and safety study of immunomodulator (Mycobacterium w) as an adjunct therapy in category-II pulmonary tuberculosis along with assessment of immunological parameters.

NCT00341328

Unpublished data only

Efficacy and safety of immunomodulator (Mycobacterium w) as an adjunct therapy in category I pulmonary tuberculosis and along with assessment of immunological parameters.

NTC00810849

Unpublished data only

A pilot trial of adjunctive prednisolone and Mycobacterium w immunotherapy in tuberculous pericarditis.

ADDITIONAL REFERENCES

Chang 2006

Chang JS, Huggett JF, Dheda K, Kim LU, Zumla A, Rook GA. Myobacterium tuberculosis induces selective up-regulation of TLRs in the mononuclear leukocytes of patients with active pulmonary tuberculosis. Journal of immunology (Baltimore, Md. : 1950) 2006;176(5):3010-8. [PubMed: 16493059]

Chaudhuri 2003

Chaudhuri P, Mukhopadhyay S. Bladder preserving approach for muscle invasive bladder cancer: role of mycobacterium w. Journal of the Indian Medical Association 2003;101(9):559-60. [PubMed: 15168998]

Churchyard 2009

Churchyard GJ, Kaplan G, Fallows D, Wallis RS, Onyebujoh P, Rook GA. Advances in immunotherapy for tuberculosis treatment. Clinics in chest medicine 2009;30(4):769-82, ix.

Dave 2007

Dave RI, Tripathi UB, Parikh BJ, Patel KM, Shukla HK. Improvement in quality of life and symptom control with MYCOBACTERIUM W (CADI-05) in head and neck cancer. Journal of Clinical Oncology 2007;25(18S).

De Bruyn 2003

De Bruyn G., Garner P. Mycobacterium vaccae immunotherapy for tuberculosis. Cochrane Database of Systematic Reviews January 2003.

Dheda2010

Dheda K, Schwander SK, Zhu B, van Zyl-Smit RN, Zhang Y. The immunology of tuberculosis: from bench to bedside. Respirology 2010 2010;15(3):433-50.

Fan 2007

Fan M, Chen X, Wang K, et al. Adjuvant effect of Mycobacterium vaccae on treatment of recurrent treated pulmonary tuberculosis: a meta-analysis. Chinese Journal of Evidence-Based Medicine 2007;7(6):449–55.

Ganju 1990

Ganju L, Mukherjee R, Batra HV, Talwar G. Immuno-blot analysis of antigens of Mycobacterium w: a candidate anti-leprosy vaccine using monoclonal antibodies and patient sera. Zentralblatt fur Bakteriologie : international journal of medical microbiology 1990;273(3):378-85.

Guleria 1993

Guleria I, Mukherjee R, Kaufmann SH. In vivo depletion of CD4 and CD8 T lymphocytes impairs Mycobacterium w vaccine-induced protection against M. tuberculosis in mice. Medical microbiology and immunology 1993;182(3):129-35.

Gupta 2008

Gupta S, Malhotra AK, Verma KK, Sharma VK. Intralesional immunotherapy with killed Mycobacterium w vaccine for the treatment of ano-genital warts: an open label pilot study. Journal of the European Academy of Dermatology and Venereology : JEADV 2008;22(9):1089-93. [PubMed: 18484970]

Gupta 2009

Gupta A, Geetha N, Mani J, Upadhyay P, Katoch VM, Natrajan M, et al. Immunogenicity and protective efficacy of "Mycobacterium w" against Mycobacterium tuberculosis in mice immunized with live versus heat-killed M. w by the aerosol or parenteral route. Infection and immunity 2009;77(1):223-31. [PubMed: 18981249]

Higgins 2008

Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions. Wiley-Blackwell, 2008.

Kaur 2002

Kaur I, Dogra S, Kumar B, Radotra BD. Combined 12-month WHO/MDT MB regimen and Mycobacterium w. vaccine in multi-bacillary leprosy: a follow-up of 136 patients. International journal of leprosy and other mycobacterial diseases: official organ of the International Leprosy Association 2002;70(3):174-81.

Khamar 2008

Khamar B.M., Modi R.I. Patent application: POLY TLR ANTAGONIST. www.wipo.int/pctdb/en/wo.jsp?wo2008062288 2008.

Kharkar 2002

Kharkar R. Immune recovery in HIV with Mycobacterium W. Journal of the Indian Medical Association 2002;100(9):578-9. [PubMed: 12455393]

Kumar 2004

Kumar V, Abbas AK. Robbins & Cotran Pathologic Basis of Disease. 7th edition. Saunders,

2004.

Kumar 2005

Kumar B, Sandhu K, Kaur I. Role of Mycobacterium w vaccine in the management of psoriasis. The British journal of dermatology 2005;152(2):380-2. [PubMed: 15727665]

Light 1972

Light RW, Macgregor MI, Luchsinger PC, Ball WC Jr. Pleural effusions: the diagnostic separation of transudates and exudates. Annals of internal medicine 1972;77(4):507-13. [PubMed: 4642731]

Malmstrom 2010

Malmstrom PU, Loskog AS, Lindqvist CA, Mangsbo SM, Fransson M, Wanders A, et al. AdCD40L Immunogene Therapy for Bladder Carcinoma - The First Phase I/IIa Trial. Clinical cancer research : an official journal of the American Association for Cancer Research 2010. [PubMed: 20448220]

Mitnick 2007

Mitnick C.D., Castro K.G., Harrington M., Sacks L.V., and Burman W. Randomized trials to optimise treatment of multi drug-resistant tuberculosis: The time for action is now. Plos. Medicine 2007;4(11):e292.

Murphy 2007

Murphy KM, Travers P, Walport. Janeways Immunobiology. 7th edition. 2007.

Murray 2004

Murray JF. A century of tuberculosis. American journal of respiratory and critical care medicine 2004;169(11):1181-6. [PubMed: 15161611]

Mustafa 1988

Mustafa AS. Identification of T-cell-activating recombinant antigens shared among three candidate anti leprosy vaccines, killed M. leprae, M. bovis BCG, and mycobacterium w. International journal of leprosy and other mycobacterial diseases : official organ of the International Leprosy Association 1988;56(2):265-73. [PubMed: 3045224]

Mwinga 2002

Mwinga A, Nunn A, Ngwira B, Chintu C, Warndorff D, Fine P, et al. Mycobacterium vaccae (SRL172) immunotherapy as an adjunct to standard antituberculosis treatment in HIV-infected adults with pulmonary tuberculosis: a randomised placebo-controlled trial. Lancet 2002;360(9339):1050-5. [PubMed: 12383985]

Nath 1998

Nath I. A vaccine for leprosy. Nature medicine 1998;4(5):548-50. [PubMed: 9585220]

Rath 2003

Rath N, Kar HK. Efficacy of intradermal heat-killed Mycobacterium w in psoriasis: a pilot study. International journal of dermatology 2003;42(9):756-7. [PubMed: 12956699]

Rieder 1996

Rieder HL. Sputum smear conversion during directly observed treatment for tuberculosis. Tubercle and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease 1996;77(2):124-9. [PubMed: 8762846]

Saini 2009

Saini V, Raghuvanshi S, Talwar GP, Ahmed N, Khurana JP, Hasnain SE, et al. Polyphasic taxonomic analysis establishes Mycobacterium indicus pranii as a distinct species. PloS one 2009;4(7):e6263.

Schulz 2010

Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. Obstetrics and gynaecology 2010;115(5):1063-70. [PubMed: 20410783]

Sharma 2000

Sharma P, Misra RS, Kar HK, Mukherjee A, Poricha D, Kaur H, et al. Mycobacterium w vaccine, a useful adjuvant to multidrug therapy in multi-bacillary leprosy: a report on hospital based immunotherapeutic clinical trials with a follow-up of 1-7 years after treatment. Leprosy review 2000;71(2):179-92.

Sharma 2005

Sharma P, Mukherjee R, Talwar G.P, Sarathchandra K.G, Walia R, Parida S K, Pandey R M, Rani R, Kar H, Mukherjee A, Katoch K, Benara S K, Singh T, Singh P. Immunoprophylactic effects of the anti-leprosy M w vaccine in household contacts of leprosy patients: clinical field trials with a follow up of 8-10 years. Leprosy review June 2005;Vol. 76(Issue 2):Pg. 127-43.

Singh 1991

Singh IG, Mukherjee R, Talwar GP. Resistance to intravenous inoculation of Mycobacterium tuberculosis H37Rv in mice of different inbred strains following immunization with a leprosy vaccine based on Mycobacterium w. Vaccine 1991;9(1):10-4.

Singh 1992

Singh IG, Mukherjee R, Talwar GP, Kaufmann SH. In vitro characterization of T cells from Mycobacterium w-vaccinated mice. Infection and immunity 1992;60(1):257-63.

Skinner 1997

Skinner MA, Yuan S, Prestidge R, Chuk D, Watson JD, Tan PL. Immunization with heat-killed Mycobacterium vaccae stimulates CD8+ cytotoxic T cells specific for macrophages infected with Mycobacterium tuberculosis. Infection and immunity 1997;65(11):4525-30.

Sur 2003

Sur PK, Dastidar AG. Role of Mycobacterium w as Adjuvant Treatment of Lung Cancer (Non-small Cell Lung Cancer). JIMA 2003;101(2).

WHO 2008

World Health Organisation (WHO). Anti-tuberculosis Drug Resistance in the World Report 2008. Fourth Global Report 2008.

WHO 2009

World Health Organisation (WHO). GlobalTuberculosisControl: A short update to the 2009 report. WHO Library Cataloguing-in-Publication Data 2009;(ISBN 978 92 4 159886 6).

Wilkinson 2009

Wilkinson KA, Seldon R, Meintjes G, Rangaka MX, Hanekom WA, Maartens G, et al. Dissection of regenerating T-Cell responses against tuberculosis in HIV-infected adults sensitized by Mycobacterium tuberculosis. American journal of respiratory and critical care medicine 2009;180(7):674-83. [PubMed: 19628776]

Zaheer 1995

Zaheer SA, Beena KR, Kar HK, Sharma AK, Misra RS, Mukherjee A, et al. Addition of immunotherapy with Mycobacterium w vaccine to multi-drug therapy benefits multi-bacillary leprosy patients. Vaccine 1995;13(12):1102-10.

TABLES

CHARACTERISTICS OF INCLUDED STUDIES

LUHADIA 2004

Methods	Randomised placebo-controlled trial set in Udaipur, India Sequence generation: no information provided Allocation concealment: no information provided Blinding: single-blind
Participants	200 Sputum smear positive PTB participants CATEGORY I (new diagnosis) Total: 100 participants Mean age: 36 years Mean weight: 36.7 kg *M w* arm: 50 participants Control arm: 50 participants CATEGORY II (retreatment) Total: 100 participants Mean age: 35 years Mean weight: 37.7 kg *M w* arm: 50 participants Control arm: 50 participants
Interventions	Intervention: 0.1 ml of *M w* administered intradermally on days 0, 15, 30, and 60; and then two monthly until TB treatment is complete Control: placebo - 0.1 ml of saline administered intradermally (as above) Other: all participants received standard TB treatment as per WHO guidelines
Outcomes	Sputum conversion (on days 15, 30, 60, 120, and 120+) Adverse events (including death and skin reactions) Other clinical (weight gain) and radiological improvements
Notes	This paper was presented as a poster presentation at the National Conference on Pulmonary Diseases (NAPCON) in 2004 There is limited data as regards the methodology of the trial The authors were contacted in this regard, but no reply was received

RISK OF BIAS TABLE

Item	Judgement	Description
Adequate sequence generation?	Unclear	No information provided Paper states that it is a randomised controlled trial, but does not provide any details
Allocation concealment?	Unclear	No information provided
Blinding? (Primary outcome: Sputum conversion)	Yes	Single-blind Even though there is no detail provided about the blinding, the primary outcome of sputum negativity is a laboratory-assessed outcome that is unlikely to be influenced by whether or not the participant or clinician knows which treatment is being administered
Blinding? (Secondary outcome: Adverse events)	No	Single-blind For the secondary outcomes (adverse events, clinical improvement and radiological resolution), single blinding is insufficient. Both the participant and the clinician should be blinded to the treatment allocation
Incomplete outcome data addressed?	Yes	No missing data
Free of selective reporting?	Yes	Study protocol is not available but it is clear that the presented reports include all expected outcomes
Free of other bias?	No	Performance bias. Only single-blind (it is not specified if the laboratory technician, clinician or the participant were blinded) May influence the evaluation of clinical outcomes such as adverse reactions, clinical improvement and radiological resolution

PARIKH 2006

Methods	Randomised, placebo-controlled trial set in India Primary aim was to evaluate if the addition of *M w* to standard TB chemotherapy would reduce the time to intercostal tube drainage (ICTD) removal in participants with TB hydropneumothoraces Sequence generation: no information provided Allocation concealment: no information provided Blinding: double-blind
Participants	34 Participants with TB hydropneumothoraces diagnosed using Light's criteria* 4 Smear positive PTB participants enrolled into *M w* arm 3 Smear positive PTB participants enrolled into control arm Limited baseline data provided

Interventions	Intervention: *M w* 0.2 ml on day zero, followed by 0.1 ml on days 15, 30, 60, 120, 180, and until completion of TB chemotherapy Control: placebo administered as above All patients received standard TB treatment as per WHO guidelines Category I: rifafour, isoniazid, pyrazinamide and ethambutol (RHZE) for two months; and rifampicin and isoniazid for four months Category II: streptomycin plus RHEZ (SRHEZ) for two months; RHEZ for one month; and HER for five months
Outcomes	Resolution of hydropneumothorax and removal of ICTD Sputum conversion Weight gain
Notes	This paper was presented as a poster presentation at the TB Vaccines for the World Conference in 2006 Limited data is provided as regards the methodology of the trial The authors were contacted in this regard, but no reply was received According to Light's criteria (Light 1972), a pleural effusion is likely exudative if at least one of the following exists: • The ratio of pleural fluid protein to serum protein is greater than 0.5; • The ratio of pleural fluid LDH to serum LDH is greater than 0.6; or • Pleural fluid LDH is greater than 0.7 times the normal upper limit for serum

RISK OF BIAS TABLE

Item	Judgement	Description
Adequate sequence generation?	Unclear	No information provided
Allocation concealment?	Unclear	No information provided
Blinding? (Primary outcome: Sputum conversion)	Yes	Double-blind
Blinding? (Secondary outcome: Adverse events)	Unclear	Double-blind Even though it is not stated clearly, the only way there can be a double-blind is if a placebo was administered and neither the participant nor the clinician knew which treatment was being administered
Incomplete outcome data addressed?	Yes	No missing data
Free of selective reporting?	Yes	Study protocol is not available but it is clear that the presented reports include all expected outcomes
Free of other bias?	Unclear	Selection or referral bias: unusual collection of TB participants, with

		only four category I participants and 30 category II participants Clinical bias: assessment of severity of hydropneumothoraces and timing of ICTD removal were clinician dependant

PATEL 2002

Methods	Quasi-randomised controlled pilot study set in Ahmedabad, India Sequence generation: no information provided Allocation concealment: no information provided Blinding: single-blind
Participants	OVERALL 134 Consecutive smear positive PTB participants (102 males and 32 females; mean age 36.2 years (range 15 to 75 years)) *M w* arm: 69 Control arm: 65 CATEGORY I Total: 58 participants *M w* arm: 20 Control arm: 38 CATEGORY II Total: 76 participants *M w* arm: 49 Control arm: 27
Interventions	Intervention: 0.2 ml (0.1 ml in each deltoid) *M w* intradermal injection given on day zero, followed by 0.1 ml fortnightly for two months Control: no placebo given All participants received standard TB treatment as per WHO guidelines Category I: RHZE for two months and RH for four months Category II: SRHEZ for two months, RHEZ for one month, and HER for five months
Outcomes	Sputum conversion Adverse reactions
Notes	Inequality in number of male to female participants, and the ratio of category I to category II participants, give the impression that there was an error in randomisation.

RISK OF BIAS TABLE

Item	Judgement	Description
Adequate sequence generation?	No	No information has been provided as regards randomisation The unequal numbers of male to female participants makes one

		suspicious of the method of randomisation Failure to stratify or pre-specify the groups in terms of category I versus category II has resulted in unequal numbers of participants in comparative groups
Allocation concealment?	Unclear	No information provided
Blinding? (Primary outcome: Sputum conversion)	Yes	Single-blind Laboratory technician blinded to treatment allocation
Blinding? (Secondary outcome: Adverse events)	No	Single-blind Insufficient for the assessment of adverse events
Incomplete outcome data addressed?	Yes	No missing data
Free of selective reporting?	Yes	Study protocol is not available, but it is clear that the published reports include all expected outcomes
Free of other bias?	No	Selection or referral bias: more males than females, and more category II than category I participants

PATEL 2003

Methods	Follow-up of selected group (category II) from Patel 2002 pilot study Quasi-randomised controlled pilot study set in Ahmedabad, India Sequence generation: no information provided Allocation concealment: no information provided Blinding: single-blind
Participants	134 Consecutive smear positive PTB participants *M w* arm: 69 Control arm: 65 CATEGORY II Total: 76 participants *M w* arm: 49 Control arm: 27 No additional baseline data available
Interventions	Intervention: 0.2 ml (0.1 ml in each deltoid) *M w* intradermal injection given on day zero, followed by 0.1 ml fortnightly for two months Control: no placebo given All participants received standard TB treatment as per WHO guidelines Category II: SRHEZ for two months, RHEZ for one month, and HER for five months
Outcomes	Sputum conversion

Notes	Presented as a separate paper, but participants are from the Patel 2002 paper It is unclear whether category II group of participants was a prespecified subgroup

RISK OF BIAS TABLE

Item	Judgement	Description
Adequate sequence generation?	No	No information has been provided as regards randomisation The unequal numbers of male to female participants makes one suspicious of the method of randomisation Failure to stratify or prespecify groups in terms of category I versus category II has resulted in unequal numbers of participants in comparative groups
Allocation concealment?	Unclear	No information provided
Blinding? (Primary outcome: Sputum conversion)	Yes	Single-blind Laboratory technician blinded to treatment allocation
Blinding? (Secondary outcome: Adverse events)	No	Single-blind Insufficient for the assessment of adverse events
Incomplete outcome data addressed?	Yes	No missing data
Free of selective reporting?	No	Study protocol is not available The published reports do not include all expected outcomes
Free of other bias?	No	Selection or referral bias: more males than females Category II subgroup was not pre-specified

CHARACTERISTICS OF EXCLUDED STUDIES

CHADDA 2002

Reason for exclusion	Not a randomised controlled or quasi-randomised controlled trial

KATIYAR

Reason for exclusion	Not a randomised controlled or quasi-randomised controlled trial

KATOCH 2008

Reason for exclusion	Not a randomised controlled or quasi-randomised controlled trial

MARTHUR 2006

Reason for exclusion	Not a randomised controlled or quasi-randomised controlled trial

ZHOU 2002

Reason for exclusion	Intervention used was Bacillus Calmette Guerin (BCG), not *M w*

CHARACTERISTICS OF ONGOING STUDIES

NCT00265226

Study name	Efficacy and safety study of immunomodulator (*Mycobacterium w*) as an adjunct therapy in category-II pulmonary tuberculosis along with assessment of immunological parameters
Methods	In progress Interventional, treatment, randomised, double-blind (subject and investigator), placebo controlled, parallel assignment, safety and efficacy study
Participants	Category II PTB participants who meet the eligibility criteria
Interventions	Intervention: intradermal administration of *Mycobacterium w,* total of 6 doses given 0.2 ml at baseline and then 0.1 ml after interval of two weeks, up to eight weeks Control: placebo Category II TB chemotherapy according to guidelines
Outcomes	PRIMARY OUTCOME MEASURES The time to sputum conversion SECONDARY OUTCOME MEASURES Adverse reactions (assessment of safety) Participant's and physicians' global assessment of the clinical cure
Starting date	March 2005 until December 2010
Contact information	Surendra K Sharma, M.D., Ph.D sksharma@aiims.ac.in
Notes	On correspondence, Dr. Sharma had no preliminary data available

NCT00341328

Study name	Efficacy and safety of immunomodulator *(Mycobacterium w*) as an adjunct therapy in category I pulmonary tuberculosis and along with assessment of immunological parameters
Methods	In progress Treatment, randomised, double-blind (participant and investigator), placebo-controlled, parallel assignment, safety and efficacy study

Participants	INCLUSION CRITERIA • Participants of either sex aged between 18 to 60 yrs • Newly diagnosed PTB cases with at least two sputum samples that are positive on sputum microscopy • Participants willing to give informed consent EXCLUSION CRITERIA • Known hypersensitivity to category I TB chemotherapy • Known history of MDR and XDR TB (patients with *Mycobacterium tuberculosis* resistant to one or more drugs will be excluded) • Secondary immunodeficiency states: HIV, organ transplantation, diabetes mellitus, malignancy, treatment with corticosteroids • Hepatitis B and C positivity • Participants with known extrapulmonary TB • Currently receiving cytotoxic therapy, or having received it within the last three months • Pregnancy and lactation • Participants with a known seizure disorder • Participants with known symptomatic cardiac disease, such as arrhythmias or coronary artery disease • Participants with abnormal renal function • Participants with abnormal hepatic function (bilirubin = 1.5 mg/dl; AST, ALT, SAP more than 1.5 x ULN; PT = 1.3x control) • Participants with haematological abnormalities • Seriously ill and moribund patients with the complications of low lung reserve, marked tachypnoea, chronic cor pulmonale, congestive cardiac failure, BMI<15, and severe hypoalbuminaemia (< 2.5 g/dl) • Participants unlikely to survive for more than six months • Participants unable to comply with the treatment regimen • Participants with a history of alcohol or drug abuse
Interventions	Intervention: Intradermal injection of *Mycobacterium w* A total of 6 doses are given: 0.2 ml at baseline and then 0.1 ml after interval of two weeks up to eight weeks Control: placebo
Outcomes	PRIMARY OUTCOME MEASURES The time to sputum conversion SECONDARY OUTCOME MEASURES Adverse reactions Participant's and physicians' global assessment of clinical cure
Starting date	March 2007
Contact information	Surendra K Sharma, M.D., Ph.D sksharma@aiims.ac.in
Notes	Nil

NTC00810849

Study name	A pilot trial of adjunctive prednisolone and *Mycobacterium w* immunotherapy in

	tuberculous pericarditis
Methods	Interventional, treatment, randomised, double-blind (participant, caregiver, investigator, outcomes assessor), placebo controlled, factorial assignment, safety and efficacy study
Participants	INCLUSION CRITERIA Participants with a suspected tuberculous pericarditis will be eligible if they meet all three of the following criteria: • A confirmed pericardial effusion on echocardiography; • Evidence of definite* or probable** tuberculous pericarditis; and • Within one week of starting of anti-tuberculous treatment. EXCLUSION CRITERIA • Presence of an alternative cause of pericardial disease e.g. penetrating chest trauma in the preceding 12 months, or malignancy • Use of corticosteroids within the previous month • Hypersensitivity or allergy to the *Mycobacterium w* vaccine • Pregnancy • Age < 18 years
Interventions	PREDNISOLONE / PLACEBO Intervention: six-week tapering course of prednisolone Control: same number of identically-coated placebo tablets Prednisolone and placebo will be supplied as 5 mg identical tablets and given at a dosage of 120 mg/day in the first week, followed by 90 mg/day in the second week, 60 mg/day in the third week, 30 mg/day in the fourth week, 15 mg/day in the fifth week, and 5 mg/day in the sixth week *MYCOBACTERIUM w* / PLACEBO Intervention: five doses of 0.1 ml of *Mycobacterium w* intradermally (on enrolment, at two weeks, four weeks, six weeks, and three months) Control: Identical regiment of normal saline placebo injections
Outcomes	PRIMARY OUTCOME MEASURES Composite end-point of death, constriction, or cardiac tamponade requiring pericardial drainage SECONDARY OUTCOME MEASURES Safety of immuno-modulator treatment Long-term feasibility of conducting a multi-centre trial in Africa and India
Starting date	December 2008 to December 2012
Contact information	Professor Bongani Mayosi bongani.mayosi@uct.ac.za Dr. Mpiko Nstekhe mpiko.ntsekhe@uct.ac.za
Notes	The pilot phase of this study has been successfully completed, and the investigators have commenced the full study

ANALYSES

1 SPUTUM CONVERSION

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
1.1 Sputum negative at Day 15	3	341	Risk Ratio (M-H, Random, 95% CI)	2.31 [1.75, 3.06]
1.1.1 Category I TB	3	158	Risk Ratio (M-H, Random, 95% CI)	2.25 [1.64, 3.09]
1.1.2 Category II TB	3	176	Risk Ratio (M-H, Random, 95% CI)	2.45 [1.33, 4.51]
1.1.3 Uncertain Category I or II TB	1	7	Risk Ratio (M-H, Random, 95% CI)	5.60 [0.39, 79.70]
1.2 Sputum negative at Day 30	3	341	Risk Ratio (M-H, Random, 95% CI)	1.83 [1.12, 2.98]
1.2.1 Category I TB	2	158	Risk Ratio (M-H, Random, 95% CI)	1.34 [1.07, 1.66]
1.2.2 Category II TB	2	176	Risk Ratio (M-H, Random, 95% CI)	2.28 [1.37, 3.77]
1.2.3 Uncertain Category I or II	1	7	Risk Ratio (M-H, Random, 95% CI)	7.20 [0.53, 97.83]
1.3 Sputum negative at Day 60	3	341	Risk Ratio (M-H, Random, 95% CI)	1.38 [0.99, 1.92]
1.3.1 Category I TB	2	158	Risk Ratio (M-H, Random, 95% CI)	1.17 [0.88, 1.56]
1.3.2 Category II TB	2	176	Risk Ratio (M-H, Random, 95% CI)	1.50 [1.10, 2.03]
1.3.3 Uncertain Category I and II TB	1	7	Risk Ratio (M-H, Random, 95% CI)	2.40 [0.66, 8.79]
1.4 Sputum negative at Day 120	4	183	Risk Ratio (M-H, Random, 95% CI)	1.35 [1.05, 1.72]
1.4.2 Category II TB	3	176	Risk Ratio (M-H, Random, 95% CI)	1.48 [1.15, 1.91]
1.4.3 Uncertain category I or II TB	1	7	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.62, 1.60]
1.5 Sputum negative after day 120	2	176	Risk Ratio (M-H, Random, 95% CI)	1.34 [1.11, 1.62]

1.5.2 Category II TB	2	176	Risk Ratio (M-H, Random, 95% CI)	1.34 [1.11, 1.62]

2 MORBIDITY AND MORTALITY

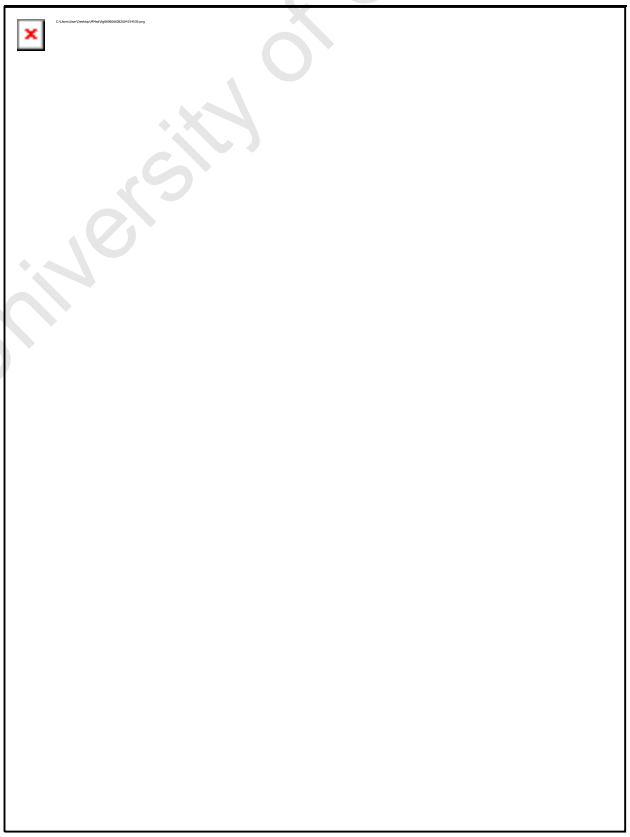
Outcome or Subgroup	**Studies**	**Participants**	**Statistical Method**	**Effect Estimate**
2.1 Mortality	1		Risk Ratio (M-H, Random, 95% CI)	No totals
2.2 Adverse events	1		Risk Ratio (M-H, Random, 95% CI)	No totals

GRAPHS AND FIGURES

FIGURE 2

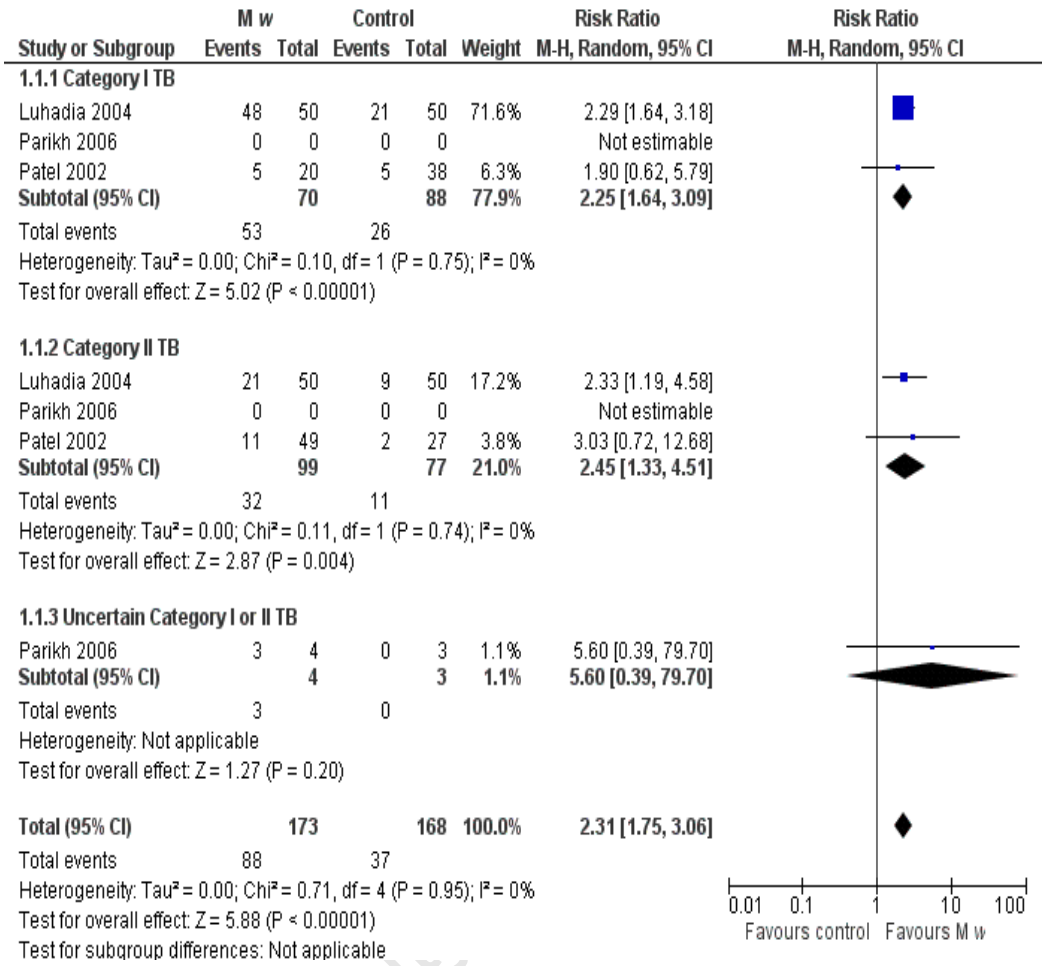
RISK OF BIAS GRAPH: AUTHORS' JUDGEMENTS REGARDING EACH RISK OF BIAS ITEM PRESENTED AS PERCENTAGES ACROSS ALL INCLUDED STUDIES.

FIGURE 3



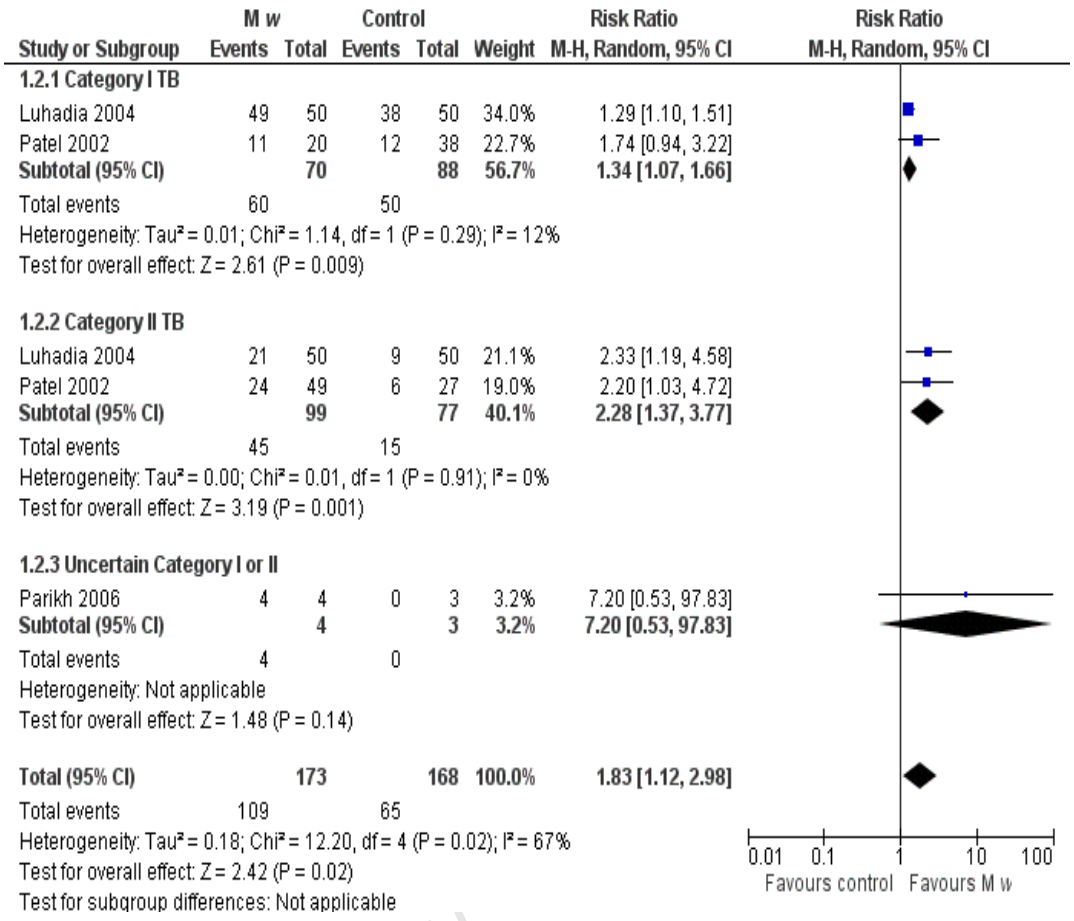
RISK OF BIAS SUMMARY: AUTHORS' JUDGMENTS REGARDING EACH RISK OF BIAS ITEM FOR EACH INCLUDED STUDY.

FIGURE 4 (ANALYSIS 1.1)



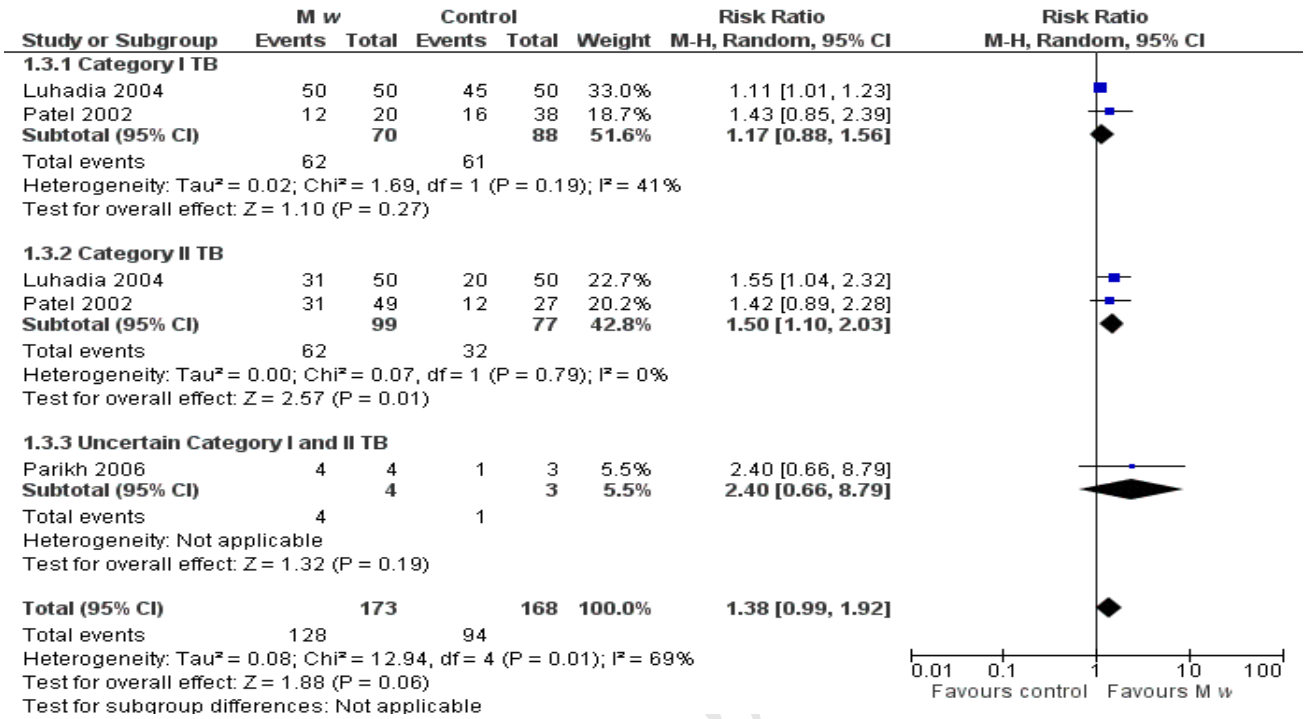
FOREST PLOT OF COMPARISON: 1 SPUTUM CONVERSION, OUTCOME: 1.1 SPUTUM NEGATIVE AT DAY 15.

FIGURE 5 (ANALYSIS 1.2)



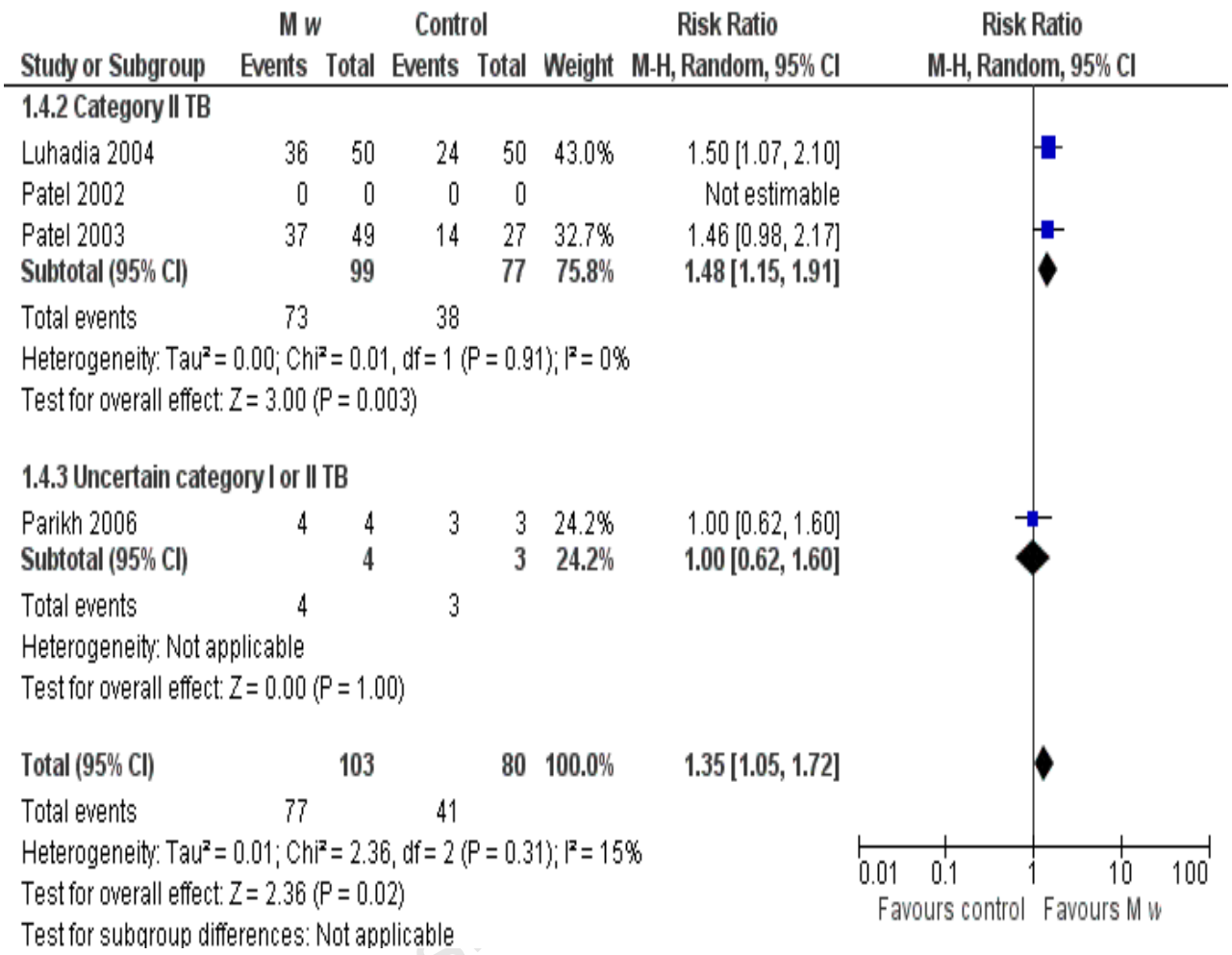
FOREST PLOT OF COMPARISON: 1 SPUTUM CONVERSION, OUTCOME: 1.2 SPUTUM NEGATIVE AT DAY 30.

FIGURE 6 (ANALYSIS 1.3)



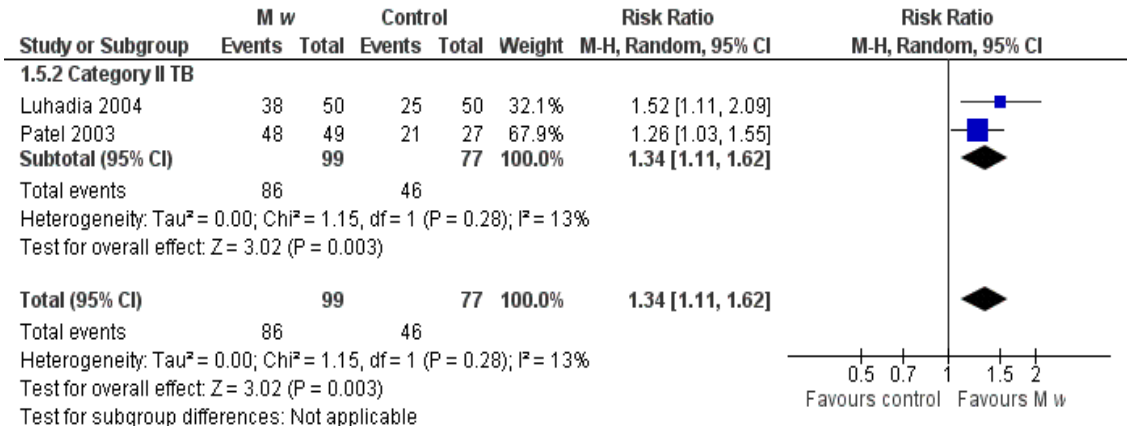
FOREST PLOT OF COMPARISON: 1 SPUTUM CONVERSION, OUTCOME: 1.3 SPUTUM NEGATIVE AT DAY 60.

FIGURE 7 (ANALYSIS 1.4)



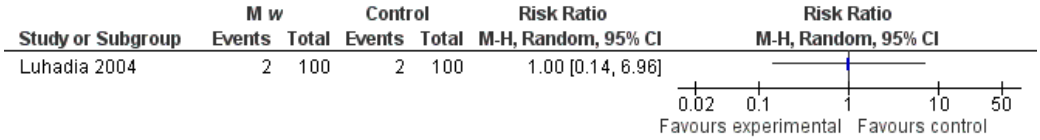
FOREST PLOT OF COMPARISON: 1 SPUTUM CONVERSION, OUTCOME: 1.4 SPUTUM NEGATIVE AT DAY 120.

FIGURE 8 (ANALYSIS 1.5)



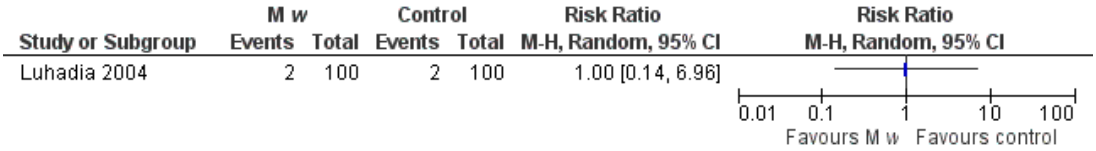
FOREST PLOT OF COMPARISON: 1 SPUTUM CONVERSION, OUTCOME: 1.5 SPUTUM NEGATIVE AFTER DAY 120.

FIGURE 9 (ANALYSIS 2.1)



FOREST PLOT OF COMPARISON: 2. MORBIDITY AND MORTALITY, OUTCOME: 2.1. MORTALITY.

FIGURE 10 (ANALYSIS 2.2)



FOREST PLOT OF COMPARISON: 2 MORBIDITY AND MORTALITY, OUTCOME: 2.2 ADVERSE EVENTS (ACCELERATED LOCAL SKIN REACTION).

APPENDICES

APPENDIX 1 DATA EXTRACTION FORM

DATA EXTRACTION SHEET

Date:

Reviewer ID:

Administrative details	
Study ID	
Trial Number	
Author(s)	
Publication details	
Year of Publication	
Number of studies in this paper	
Year in which study was concluded	
Other relevant papers cited	

Study Details	
Study Verification	
Study Design	
Type, duration and completeness of follow-up	
Country/ location of study	
Informed consent	
Ethics	

Participant details	
Setting / diagnosis	
Number	
Baseline characteristics	

Interventions (I) / Controls (C)	
I dosage / regimen	
Control	

Background treatment	

Risk of bias	**Judgement**	**Description**
Adequate sequence generation		
Allocation concealment		
Blinding		
Incomplete outcome data addressed?		
Free of selective reporting?		
Free of other bias?		

Primary Outcomes	**Overall PTB**	**Category I PTB**	**Category II PTB**
Sputum culture conversion **15 days**			
30 days			
60 days			
120 days			
120+ days			
Mortality			

Secondary outcomes	
Serious adverse reactions	
Adverse events related to the immunotherapy	

Additional notes	

APPENDIX 2 HIGGENS I^2

Higgins I^2 = Q-df / Q x 100%

Where

- Q =chi ^{2} statistic
- df = degrees of freedom

I^2 calculates the proportion of variation in the effect estimates that is due to heterogeneity rather than chance

Thresholds for interpreting I^2:

- 0 to 40 % = might not be important
- 30 to 60 % = moderate
- 50 to 90 % = substantial
- 75 to 100% = considerable

*From **"Analysing Data and Undertaking Meta-analyses":** Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions. Pages 277- 8.Wiley-Blackwell; 2008.*

COVER SHEET

Title	**Mycobacterium w immunotherapy for treating pulmonary tuberculosis – a systematic review**
Authors	Pandie S, Engel M, Mayosi B
Contribution of author(s)	SP was responsible for the development of the protocol, reviewing the abstracts, data extraction, analysis of results, interpretation of the findings, and writing the final report. OA was responsible for independently performing the literature search and extracting the data. BM was the project supervisor.
Issue Protocol first published	2009/12
Review first published	/
Date of most recent amendment	/
What's new	/
Date new studies sought but not found	Information not supplied
Date new studies found but not yet included/excluded	Information not supplied
Date new studies found and included/excluded	/
Date authors' conclusion section amended	/
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MYCOBACTERIUM *W* IMMUNOTHERAPY FOR TREATING PULMONARY TUBERCULOSIS – A SYSTEMATIC REVIEW

BY

SHAHEEN PANDIE

PNDSHA001

PART D: APPENDICES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

MASTERS OF MEDICINE IN MEDICINE

FACULTY OF HEALTH SCIENCES

UNIVERSITY OF CAPE TOWN

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14 May 2010

Adri Winkler

Manager of the Postgraduate Unit

Postgraduate Office

University of Cape Town

RE: Submission of Masters in Internal Medicine (M.Med)

This is to confirm that I, Shaheen Pandie, intend to submit my M.Med Dissertation in order to qualify for a December graduation.

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Please contact me if there are any queries.

Thanks

Shaheen Pandie

Signed by candidate

14 May 2010

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14 May 2010

Adri Winkler

Manager of the Postgraduate Unit

Postgraduate Office

University of Cape Town

RE: Format of Masters in Medicine (M.Med) Submission

My Masters in Medicine dissertation is a systematic review entitled:

MYCOBACTERIUM W IMMUNOTHERAPY FOR TREATING PULMONARY TUBERCULOSIS – A SYSTEMATIC REVIEW

The final document has been submitted in four parts:

Part A - Protocol for the systematic review

The protocol has been completed using The Cochrane Collaboration format. The original document was completed using Review Manager 5 (Rev.Man5). The document has not yet been officially published by The Cochrane Collaboration, and for this reason the printed document and the original document may differ slightly. The original Rev.Man5 protocol has been included in Part D as an additional appendix.

Part B – Literature review

The literature review focuses on both adjunctive therapies used in tuberculosis and Mycobacterium *w* immunotherapy itself. The Cochrane Collaboration format does not make allowance for such an extensive, comprehensive review. For this reason, Part B has been

completed in a general style fit for publication in most journals. The reference style chosen was for The Lancet Infectious Diseases. The Lancet Infectious Diseases "Information for Authors" has also been included in Part D as an appendix, even though this is not the primary journal for publication.

Part C – Manuscript

The final manuscript has been completed using The Cochrane Collaboration format. The original document and statistical analyses were completed using Review Manager 5 (Rev.Man5). The document has not yet been officially published by The Cochrane Collaboration, and for this reason the printed document and the original document may differ slightly. The original Rev.Man5 protocol has been included in Part D as an additional appendix.

Part D – Appendices

All correspondence, data sheets, resource material, style guides and original documentation have been included in Part D as appendices. Some of these appendices may also appear in Parts A to C.

Thanks

Shaheen Pandie

14 August 2010

Cochrane Style Guide 3.0

Updated October 2005

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About the Cochrane Style Guide

Preface

The Cochrane Style Guide helps authors and editors apply a consistent style across Cochrane Reviews and other documents prepared as part of The Cochrane Collaboration's efforts. Cochrane Reviews are prepared using The Cochrane Collaboration's software Review Manager and are published in the *Cochrane Database of Systematic Reviews* in *The Cochrane Library*. Published Cochrane Reviews are available as html (browsable) versions and PDF files. The visual presentation of the reviews differs in each version, and both differ from their visual presentation in Review Manager. Authors and editors need to keep this in mind when suggesting editorial or formatting changes, and are advised to become familiar with both the unpublished and published formats of reviews. This should ensure satisfaction with the published versions.

Editors

Harriet G MacLehose and Tracey Remmington.

Cochrane Style Guide Working Group

Alison Beamond (Epilepsy Group), Susanne Ebrahim (Metabolic and Endocrine Disorders Group), Luisa Fernandez (Oral Health Group), Lesley Gillespie (Bone, Joint and Muscle Trauma Group), Nikki Jahnke (Cystic Fibrosis and Genetic Disorders Group), Rachael Jowett (Epilepsy Group), Harriet G MacLehose (Infectious Diseases Group), Heather Maxwell (Peripheral Vascular Diseases Group), Laura Mellor (John Wiley & Sons, Ltd.), Tracey Remmington (Cystic Fibrosis and Genetic Disorders Group), Reive Robb (Infectious Diseases Group), Emma Tavender (Oral Health Group), and Sera Tort (Lung Cancer Group).

How to cite this version of the Cochrane Style Guide

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Internet address

www.cochrane.org/style/csg.htm

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Sources of support

Core funds of The Cochrane Collaboration support the Cochrane Style Guide Working Group.

What's new?

Corrections and changes in version 3.0 (October 2005)

Section	Change
Rationale for new version, version 3.0	Content extensively revised and formatted; table of contents revised and index added; streamlined content to exclude guidance on grammar, where appropriate; many sections restructured and relocated. Revised terms used throughout the Cochrane Style Guide to reflect changes in official Cochrane Collaboration guidance; this includes implementing terminology changes approved by The Cochrane Collaboration Steering Group, Providence, April 2005
About the Cochrane Style Guide	New section added to provide general information about the Cochrane Style Guide
Author contact details	Added guidance on correct format for entering into Review Manager or Archie[1]
Cochrane Style Guide references	Footnoted references; deleted general references
Computer software	New section added to help explain role of different types of software used by The Cochrane Collaboration and in *The Cochrane Library* (on Wiley InterScience)
Currency	Modified guidance
Lists	Modified guidance to allow different formats for ordered lists
Names: family	New section added; includes specific guidance for Chinese and Dutch names
Names: specific to The Cochrane Collaboration	Clarified which names officially start with 'The'; also clarified that 'in *The Cochrane Library*' is correct (not 'on *The Cochrane Library*').
P value	Clarified guidance
References	Included guidance on how to enter references for commonly used reference types into Review Manager; amended guidance for citing references in text of Cochrane Reviews
Removed UK [British] or USA [American] English and '-ize' and '-ise'	Put in English language: regional differences (changed to British and American English) with '-ize' as an example
Trade names changed to 'Brand and trade names'	To clarify that both use same format
Units and systems of measure	Included guidance about the International System of Units/Le Système International d'Unités (SI); moved relevant information to this section, such as standard unit abbreviations; this changes the guidance regarding the abbreviation for 'litre'

[1] archie.cochrane.org

A

Abbreviations and acronyms

Use abbreviations and acronyms only if they are widely known and not using them could make reading tedious. Write in full in the first instance and follow it immediately by the abbreviated version or acronym in brackets. If the review or document is long, it may be sensible to explain each abbreviation in each section of the text, such as the 'Background' and 'Discussion' in a Cochrane Review, in addition to the 'Abstract' and tables.

Abbreviations and acronyms should follow the style conventions in Table 1. Some terms, particularly statistical terms, are commonly abbreviated in Cochrane documents (Table 2), while others should be avoided (Table 3).

Table 1 Formatting abbreviations and acronyms

Guidance	✔	✘
Use upper-case letters to explain the abbreviation or acronym only if required by abbreviated term	World Health Organization (WHO) or angiotensin converting enzyme (ACE)	world health organization (WHO) or Angiotensin Converting Enzyme (ACE)
No full stops between letters of abbreviation or acronym, or at end of abbreviation version or acronym unless at end of a sentence	The Medical Research Council (MRC) funded the research.	The MRC funded the research. The MRC. funded the research. The M.R.C. funded the research.
Form plurals by adding 's'; no apostrophe ('s) needed unless used to indicate possession	The Review Group Co-ordinators (RGCs) met early in the morning. The Cochrane Review Group's (CRG's) decision …	The Review Group Co-ordinators (RGC's) met early in the morning. The CRG's were asked to provide information.
Bold may occasionally be used to indicate letters used to form an acronym or abbreviation	CRASH trial (**c**orticosteroid **r**andomisation **a**fter **s**ignificant **h**ead injury)	--
Avoid abbreviating terms that could be unclear to the general readership	the level of glycosylated haemoglobin	the level of Hb A1

Table 2 Commonly used abbreviations

Term	Abbreviation
absolute risk reduction	ARR
control group risk[2]	CGR
controlled clinical trial	CCT
confidence interval	CI
degrees of freedom (abbreviation is lower case)	df
mean difference[3]	MD
number needed to treat to harm[4]	NNTH
number needed to treat to benefit[5]	NNTB

[2] Formerly called control event rate; change approved by The Cochrane Collaboration Steering Group, April 2005, Item 13.7, www.cochrane.org/ccsg/ccsg_minutes_providence_april05.htm

[3] Formerly called weighted mean difference; change approved by The Cochrane Collaboration Steering Group, April 2005, Item 13.7, www.cochrane.org/ccsg/ccsg_minutes_providence_april05.htm

[4] Formerly called number needed to harm; see previous footnote.

[5] Formerly called number needed to treat; see previous footnote.

Term	Abbreviation
odds ratio	OR
Peto odds ratio	Peto OR
randomized controlled trial	RCT
risk difference	RD
risk ratio[6]	RR
standard deviation	SD
standard error	SE
standardized mean difference	SMD

Table 3 Abbreviations to avoid

Term	Abbreviation*
chemical names	Hg for mercury (for example)
Latin abbreviations used for dosing	qd, bd, bid, bds, tds, tid, etc
number	no.
versus	vs

*These might be appropriate for tables if footnoted

Abstract: Cochrane Review

Guidance on the format and content of Cochrane Review abstracts is available in the *Cochrane Handbook for Systematic Reviews of Interventions*.[7]

Active and passive voice

The active voice is preferable to the passive voice; see the examples in Table 4.

Table 4 Active and passive voice

Active voice	Passive voice
Two authors extracted data.	Data were extracted by two authors.
The editor will provide feedback.	Feedback will be provided by the editor.

Additional figure: Cochrane Review

Use the 'Guidelines for preparing Additional figures in Cochrane reviews'[8] to ensure the published figures are satisfactory, and appendix, 'Considerations and recommendations for figures in Cochrane reviews: Graphs of statistical data', of the *Cochrane Handbook for Systematic Reviews of Interventions*[9] for advice on which types of graphs to include.

[6] Formerly called relative risk; see previous footnote.
[7] www.cochrane.org/resources/handbook/index.htm; Section 3.
[8] www.cc-ims.net/RevMan/Additional_figures.pdf
[9] www.cochrane.org/resources/handbook/index.htm; Appendices.

And/or

Avoid using 'and/or' because it is not explicit. Try rephrasing the sentence; for example, 'fever and/or headache…' to 'fever or headache, or both…'.

Author contact details: Cochrane Review

Table 5 outlines the preferred Cochrane format for entering author contact details in Cochrane Reviews. A consistent format will help ensure there is only one record per person entered in Archie[10], the web interface of The Cochrane Collaboration's Information Management System (IMS).

Table 5 Entering author contact details in Review Manager and Archie

	Guidance	✔	✘
Telephone and fax number (international notation[11])	Separate groups of numbers using a space (not hyphens or full stops) Avoid using the trunk prefix '0' +[country code] [area code] [local number]	+44 151 123 4567	+44-151-123-4567 +44.151.123.4567 +44 0151 123 4567 +44 (0)151 123 4567
E-mail address (see footnote above)	Lower-case letters	myname@domain.org	Myname@domain.org
Web address (see footnote above)	Use without prefix http://	www.cochrane.org	http://www.cochrane.org
Name: prefix and suffix	Use open punctuation, ie without full stops	Mr or PhD	Mr. or Ph.D
	Use Dr or MD; Dr or PhD	Dr Jones Mr Jones, MD Dr Jones Mr Jones, PhD	Dr Jones, MD Dr Jones, MD Dr Jones, PhD Dr Jones, PhD
Name: middle initials	Avoid punctuation	David RA Jones	David R.A. Jones

C

Cochrane Review: content, structure, and format

Information on the content, structure, and format of Cochrane Reviews is available in the *Cochrane Handbook for Systematic Reviews of Interventions*.[12]

Common terms

See Table 6.

[10] archie.cochrane.org/

[11] Telecommunication Standardization Sector of the International Telecommunication Union (ITU-T). Recommendation E.123: Notation for national and international telephone numbers, e-mail addresses and Web addresses (02/2001).

[12] www.cochrane.org/resources/handbook/index.htm; Section 3.

Table 6 Common terms

✔	✘
care giver or caregiver (be consistent)	care-giver
Centers for Disease Control and Prevention (CDC)	Centers for Disease Control (*not* Center or Centre)
chi squared, chi-squared test (correct British English format)	chi squared test
chi square, chi-square test (correct American English format)	chi square test
cross-over study	cross over study
forest plot[13]	forrest plot
fixed-effect model	fixed effect model (no hyphen) or fixed effects model
follow up (noun or verb) or follow-up (adjective) The follow-up period was 10 weeks. Seven participants were lost to follow up.	--
handsearch We handsearched three journals. The handsearching process.	hand search or hand-search
health care (noun) or healthcare (adjective) The healthcare centre is nearby. (adjective) The health care was satisfactory. (noun)	--
intention-to-treat analysis	intention to treat analysis
internet or Internet (be consistent)	--
low-income, middle-income, and high-income countries Alternatives to 'developing countries' and 'developed countries' See the classifications of all countries according to their economies on the World Bank website[14]	--
multiple-drug resistance *and* multiple-drug resistant	multidrug resistance *and* multidrug resistant
number needed to treat to benefit	number-needed-to-treat-to-benefit
number needed to treat to harm	number-needed-to-treat-to-harm
online	on-line (hyphenated) or on line (two separate words)
participant or person; participants or people (preferred terms) Use participant or person instead of subject or patient, unless it changes the meaning of the text If trials are exclusively concerned with a single population, such as children or women, use children or women instead of participants	--
per cent[15]	percent
random-effects model	random effects model (no hyphen) or random-effect model (no 's' after 'effect')
regimen	regime
subgroup	sub-group or sub group
time point	timepoint (one word) or time-point (hyphenated)

[13] Lewis S, Clarke M. Forest plots: trying to see the wood and the trees. *BMJ*. 2001;322(7300):1479-80.

[14] www.worldbank.org/data/countryclass/countryclass.html; "Low-income and middle-income economies are sometimes referred to as developing economies. The use of the term is convenient; it is not intended to imply that all economies in the group are experiencing similar development or that other economies have reached a preferred or final stage of development. Classification by income does not necessarily reflect development status."

[15] Also see 'percentage sign' in 'Table 23 Punctuation: general guidance'.

✔	✘
website	web site (two words)
white (adjective) The white participants…	white (noun); avoid Caucasian unless there is a specific reason
world wide web or World Wide Web (be consistent)	--
World Health Organization	World Health Organisation

Computer software used to prepare and view Cochrane Reviews

Different types of software are available within The Cochrane Collaboration and in *The Cochrane Library* (on Wiley InterScience).

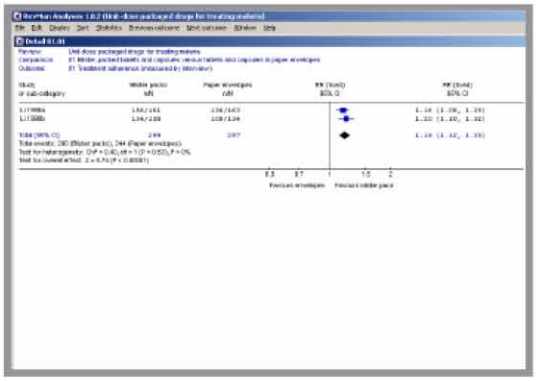
Review authors prepare Cochrane Reviews using **Review Manager**[16], which is often abbreviated to RevMan. Different versions are available, denoted by the version number. The first number (**4**.2.7) changes only when the format of a Cochrane Review changes, such as when new sections are added. A change in the second number (4.**2**.7) indicates changes to the interface or that new functionality has been added, or both. A change to the third number (4.2.**7**) refers to minor corrections with no new features, and it is not necessary to include this number in the citation when referencing the program.

RevMan Analyses (Figure 1) is Review Manager's statistical program that analyses data to produce meta-analysis graphs. (RevMan Analyses replaces **MetaView**, which was available in version 4.1).

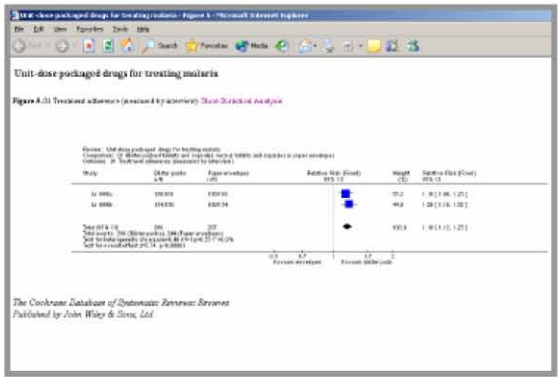
Statistical Analysis (Figure 1) is software run by Wiley[17] to display the graphs in the published version of a review and is unrelated to RevMan Analyses. Authors wishing to cite the program they used for statistical analyses should cite RevMan Analyses or MetaView as appropriate. Some reviews, however, contain sentences such as "the pooled results showed an overall benefit of the intervention, see MetaView graph". It is now incorrect to use MetaView in this context because a reader may not understand what the authors are referring to.

Figure 1 Screenshots of RevMan Analyses and Statistical Analysis

RevMan Analyses*



Statistical Analysis**



*Unpublished Review Manager version; **Published Wiley version

[16] www.cc-ims.net/RevMan

[17] Introduced in Issue 1, 2005 because this was the first issue of *The Cochrane Library* not published simultaneously on the Update Software website, which did use the MetaView program to display graphs.

Currency

Express currency as the currency abbreviation and amount (eg EUR 250), or as the currency symbol and amount (eg €250).[18]

D

Dates

Dates may be expressed in different ways, such as a specific date, a decade, or a century, as shown in Table 7.

There are regional differences in expressing date formats. Cochrane documents use the day (numeral), month (always in full), and year (numeral) with no additional punctuation. Use this format instead of seasons, which can be confusing to people in different parts of the world. Decades are always expressed as numerals, and century numbers may be expressed as numerals or written in full (eg 19th century or nineteenth century).

Table 7 Examples of date formats

✔	✘
1 May	May 1
1 May 2000	May 1 2000 or May 1, 2000
May 2000	--
7 November	7/11 (UK = 7 November; USA = 11 July)
1960s	1960's or '60s

E

eg

An abbreviation for 'for example' from the Latin '*exempli gratia*' that should only be used for lists within the text or in tables where 'for example' is inappropriate. Be consistent with your choice of punctuation (and use the same style for 'ie' and 'etc') (Table 8).

Table 8 Formatting styles for eg, ie, and etc

(e.g. men, women, children)	(i.e. men, women, children)	Style not applicable
(eg men, women, children)	(ie men, women, children)	(men, women, children, etc)
(eg, men, women, children)	(ie, men, women, children)	(men, women, children, etc.)

English language: regional differences

There are regional differences in the English language, and Cochrane Review Groups support both British and American English. However, the choice should be applied consistently within a single Cochrane Review or document. For example, the '-ize' suffix (eg randomize) is often associated with American English and '-ise' (eg randomise) with British English, when in fact '-ize' is also commonly used in British English.

[18] Some currency symbols are not available in Review Manager 4.2.

etc

Possible formatting styles are listed in Table 8. Use a comma before 'etc' if it follows more than one item in a list.

F

Feedback: Cochrane Review

No specific style guidelines available for this section. See the Feedback Management Advisory Group webpage[19] for further information.

Font effects: Cochrane Review

Two font effects are available for use in the text in Review Manager: subscript and superscript. Both have specific uses, as described in Table 9.

Table 9 Examples of subscript and superscript

	✔	✖
Subscript	Member of chemical group: vitamin D_3	vitamin D3
	Number of atoms: H_2O	H2O
Superscript	Mass number: ^{14}C	14C
	Meters squared: 12 m^2	12 m2

Font styles: Cochrane Review

Five font styles are available for use in the text in Review Manager: regular, **bold**, *italic*, ***bold italic***, and underline. These five styles (except underline) are used to create the different heading levels in Cochrane Reviews (see 'Headings: Cochrane Review'). Avoid using font styles other than 'regular' for emphasis; instead use an alternative sentence structure or intensify the adjectives and adverbs to achieve this.

Bold

Bold may be used to indicate letters used to form an acronym or abbreviation (see 'Abbreviations and acronyms').

Italic

Italic has other uses besides in headings (Table 10).

- Titles and subtitles of books and journals; if 'The' forms part of the title, it should be in upper-case and in italic.
- Genus and species names; the genus name starts with an upper-case letter, and the species name is all lower case.

There are also situations in which to avoid using italic.

- Non-English words that have become naturalized into English should be in 'regular' style.
- Punctuation around text in italic is in 'regular' style; for example, quotation marks, semicolons, and colons.

[19] www.cochrane.org/reviews/clibintro.htm#comcrit; changed from 'Criticisms Management Advisory Group' in 2005.

Table 10 Examples of italic

	✔	✘
Titles of books and journals	We looked through *Brain Injury*.	We looked through Brain Injury.
	We searched *The Cochrane Library* for a particular review.	We searched The Cochrane Library for a particular review.
Genus and species names	*Plasmodium falciparum*	Plasmodium falciparum
	Staphylococcus aureus	*Staphylococcus Aureus*
Words naturalized into the English language	in vitro	*in vitro*
	in vivo	*in vivo*
	a priori	*a priori*
	et al	*et al*

Underline

Avoid underlining words because underlined texts can be confused with internet hyperlinks.

G

Glossary

Terms commonly used in The Cochrane Collaboration and in Cochrane Reviews are defined in the 'Glossary of Cochrane Collaboration terms'.[20]

H

Headings: Cochrane Review

Five heading levels[21] are available for use within Review Manager (Table 11). Headings can be fixed or free. A fixed heading is one of the headings provided by Review Manager that cannot be altered. Free headings are inserted by the review author using the text formatting provided in Review Manager. Use sentence case (when only the first letter of the first word begins with an upper-case letter) for all free headings, and start the section text on next line.

Occasionally it is necessary to follow one heading immediately with a subheading. In this case, insert one clear line of text between the two headings.

[20] www.cochrane.org/resources/glossary.htm

[21] Approved by The Cochrane Collaboration Publishing Policy Group in October 2003.

Table 11 Heading levels available in Review Manager

Heading level	Description	Fixed or free
Heading one	(Note: Appears in upper-case letters in the published Cochrane Review)	Fixed (eg Background)
Heading two	Bold, sentence case (Note: Fixed headings appear as sentence case and bold letters in the published Cochrane Review (ie exactly the same as the free headings)	Fixed (eg Types of studies) or free
Heading three	Bold italic, sentence case	Free
Heading four	Italic, sentence case	Free
Heading five	Regular font style, sentence case	Free

In Cochrane Review tables

The heading levels available for the text of Cochrane Reviews are not available in the 'Additional' or 'Characteristics' tables. Instead use upper-case letters for a heading or subheading; there should be no space between the subheading and the text that follows (Table 12).

Table 12 Headings in Cochrane Review tables

✔	✘
MALARIA Malaria is endemic in some parts of the world.	MALARIA Malaria is endemic in some parts of the world.

Hyphens

Hyphens are used to link two or more word compounds used as adjectives, such as 'six-week interval' and 'four-dose regimen'. Be aware that hyphens can sometimes change the meaning of a word, such as 'unionised' (with a union) and 'un-ionised' (without ions).

I

ie

An abbreviation for 'that is' from the Latin '*id est*' that should only be used for lists within the text or in tables where 'that is' is inappropriate. Be consistent with your choice of punctuation (and use the same style for 'eg' and 'etc') (Table 8).

Indentation in Cochrane Reviews

Indentation is not supported in Review Manager. Do not attempt to arrange the layout of text using indentation because the layout will change during the publication process. This will affect the visual presentation of the review and result in an unsatisfactory published version.

L

Lists

There are different ways of formatting lists, depending on the best way to display the information. Be consistent in the choice of formatting and numbering within a single document. Lists may form parts of sentences within a paragraph (Table 13). Sometimes each item in a list is displayed on a separate line using numbers (numbered list), bullet points (bulleted list), or ordered (using numbers or letters, or both to display different levels within a list).

Within paragraphs

Table 13 Lists within paragraphs: general guidance

Guidance	Example
Separate each item with a comma	I decided to call Aika, Helen, Carolyn, Hasifa, and Christy.
Complex sentences: such as including several long phrases separated with commas, separate each point with a semicolon	The conference included topics such as learning how to prepare a protocol; search databases and trial registers; and draft a Methods section.

Note: The comma or semicolon before the "and" is optional (see 'Punctuation')

Numbered, bulleted, and ordered

Different styles are possible for these types of list. The style may depend upon using a 'platform phrase', which explains the type of items the list contains, and the choice of formatting, such as using standard numbering, bullet points, or an ordered approach (Table 14).

Table 14 Formatting lists

Guidance	Example
Platform phrase and items are one sentence	The programme aims to help you: (1) learn about systematic reviews; (2) develop your protocol; and (3) learn how to develop your search strategy.
	The programme aims to help you: • learn about systematic reviews; • develop your protocol; • learn how to develop your search strategy.
Platform phrase with a full stop before starting the list	The programme aims to help you with the following. 1. Learn about systematic reviews. 2. Develop your protocol. 3. Learn how to develop your search strategy.
Independent list with no platform phrase	(1) Australia (country) (a) South Australia (state) (i) Kangaroo Valley (town)
	1. Studies 1.1 Randomized controlled trials 1.2 Cohort studies

Note: Do not attempt to format the list by using the tab or several spaces to indent the items because the formatting changes during the publication process and the published result may look unsatisfactory. The "and" before the final item in the list is optional (see 'Punctuation').

N

Names

Brand and trade name

Both start with an upper-case letter; for example, Panadol (paracetamol drug) and Unilever (pharmaceutical company). Also see 'Pharmaceutical drugs'.

Country or region

The United Nations list of countries web page[22] is a useful resource for the correct spelling of country names; it also provides the official abbreviations for each country name. Ensure that you use the most appropriate name for a country (Table 15).

Table 15 Which country or regional name?

	✔	✘
New country or name change	Use the country name in use at the time when referring to the country Example: Thousands of people participated in the trial in the Kazakh Soviet Socialist Republic in 1976. Optional to put the current name in brackets Example: Thousands of people participated in the trial in the Kazakh Soviet Socialist Republic (now the Republic of Kazakhstan) in 1976.	Example: Thousands of people participated in the trial in the Republic of Kazakhstan in 1976.
Historical works	Contemporary name	Modern name
Common errors	British Columbia (province)	British Colombia (province)
	Cape Town	Capetown
	Colombia (country)	Columbia (country)
	former Soviet Union	Former Soviet Union
	former Yugoslavia	Former Yugoslavia
	Hong Kong	Hongkong
	Western Australia	West Australia
	sub-Saharan	subSaharan
Potentially ambiguous	Great Britain, UK, and British Isles Great Britain = England, Scotland, and Wales United Kingdom = Great Britain and Northern Ireland British Isles = United Kingdom and the Irish Republic	Not applicable
	Netherlands and Holland Holland = name of two provinces of the Netherlands – Noord-Holland and Zuid-Holland	
	Geographical areas that are part of the title or a political division start with an	

[22] www.un.org/Depts/Cartographic/english/geoname.pdf

	✔	✘
	upper case letter Western Australia; the West Geographical areas that are a general description start with a lower-case letter southern Scotland and the south of Scotland	

Family names

Some family names have specific formatting depending on their location in a reference. These are often regional differences. For consistency in Cochrane Collaboration documents, Chinese names should follow a Westernized style, that is, first name followed by the family name. Formatting of Dutch family names should follow the style in Table 16. It is advisable to seek confirmation from Cochrane Review authors before modifying.

Table 16 Dutch family names: general guidance

	Guidance	Example
First name (or initial) before the family name	van, de, der, and ter start with a lower-case letter	Danielle van der Windt or DAWM van der Windt,…
Only family name used, or initials after the last name	Van, De, Der, and Ter start with an upper-case letter	Van der Windt et al or Van der Windt, DAWM...

Genus and species

Genus name starts with an upper-case letter while the species name does not; both are in italic (Table 10).

Pharmaceutical drugs

Refer to pharmaceutical drugs using the recommended International Nonproprietary Name (rINN),[23] also known as the generic name, instead of the brand name. This system helps avoid confusion where common names for drugs differ around the world; for example, 'acetaminophen' is commonly used in the USA, but it is more commonly known as 'paracetamol' (also the rINN) in the UK. If needed, however, place the brand name in brackets after the rINN. An rINN should start with a lower-case letter, while brand names start with an upper-case letter. For example, the rINN for one type of antibiotic is 'ciprofloxacin'. This could be presented as 'ciprofloxacin' alone or 'ciprofloxacin (Ciproxin)' if essential, but not as 'Ciproxin' alone.

Useful resources for locating or checking the rINN are the *British National Formulary (BNF)*[24]; *Clinical Evidence* ('Drug nomenclature and brand names' web pages)[25], and the WHO Model Formulary (MF)[26].

[23] "International Nonproprietary Names (INN) facilitate the identification of pharmaceutical substances or active pharmaceutical ingredients. Each INN is a unique name that is globally recognized and is public property. A nonproprietary name is also known as a generic name." World Health Organization, Essential Drugs and Medicine Policy, International Nonproprietary Names; www.who.int/medicines/organization/qsm/activities/qualityassurance/inn/orginn.shtml

[24] www.bnf.org/; provides information on medicines prescribed in the UK.

[25] www.clinicalevidence.com/ceweb/resources/drug_names.jsp; lists the international name (rINN or proposed INN (pINN)), the common UK and USA names, and the UK and USA brand names.

[26] www.who.int/medicines/organization/par/formulary.shtml; provides comprehensive information on medicines in the WHO Model List of Essential Medicines.

Specific to The Cochrane Collaboration

See Table 17 for the correct spelling and formatting of names specific to The Cochrane Collaboration.

Table 17 Names specific to The Cochrane Collaboration

✔		✘	
author		reviewer	
Cochrane Center		Chinese Cochrane Centre	US Cochrane Centre
Chinese Cochrane Center	US Cochrane Center		
Cochrane Centre			
Australasian Cochrane Centre	Iberoamerican Cochrane Centre	Australasian Cochrane Center	Iberoamerican Cochrane Center
Brazilian Cochrane Centre	Italian Cochrane Centre	Brazilian Cochrane Center	Italian Cochrane Center
Canadian Cochrane Centre	Nordic Cochrane Centre	Canadian Cochrane Center	Nordic Cochrane Center
Dutch Cochrane Centre	South African Cochrane Centre	Dutch Cochrane Center	South African Cochrane Center
German Cochrane Centre	UK Cochrane Centre	German Cochrane Center	UK Cochrane Center
the Cochrane Central Register of Controlled Trials (CENTRAL)		The Cochrane Central Register of Controlled Trials (CENTRAL)	
the *Cochrane Database of Methodology Reviews*		*The Cochrane Database of Methodology Reviews*	
the *Cochrane Database of Systematic Reviews*		*The Cochrane Database of Systematic Reviews*	
The Cochrane Collaboration		the Cochrane Collaboration	
the Collaboration Use only when it is cumbersome to use the full name constantly in a single document. Remember to use 'The Cochrane Collaboration' in full the first time and inform your readers that you will refer to it as 'the Collaboration' from this point onwards.		The Collaboration	
The Cochrane Library …in *The Cochrane Library*		*the Cochrane Library* …on *The Cochrane Library*	
The Cochrane Manual		*the Cochrane Manual*	
the Cochrane Methodology Register		The Cochrane Methodology Register	
Cochrane Protocol or Cochrane protocol No policy on a particular format, only consistency within a single document			
Cochrane Review or Cochrane review No policy on a particular format, only consistency within a single document			
Cochrane Handbook for Systematic Reviews of Interventions[27]		*Cochrane Reviewers' Handbook*	
Cochrane Review Group[28]		Collaborative Review Group	
the Cochrane Style Guide		The Cochrane Style Guide	
Co-ordinating Editor		Coordinating Editor	
co-author		coauthor	
MeerKat		Meerkat	
ModMan		Modman	

[27] Title changed in version 4.2.4 from '*Cochrane Reviewers' Handbook*' to current format.
[28] Change approved by The Cochrane Collaboration Steering Group, April 2005, Item 13.7, www.cochrane.org/ccsg/ccsg_minutes_providence_april05.htm

✔	✘
Review Group Co-ordinator	Review Group Coordinator
Review Manager → RevMan (abbreviation)	Revman
Review Manager 4.1 or Review Manager 4.2	Review Manager version 4.1 or Review Manager version 4.2 or Review Manager 4.2.7 (or any third number because it indicates a small bug fix)
RevMan Analyses (only available from RevMan 4.2 and later versions)	RevMan Analysis
Statistical Analysis	Statistical Analyses
Trials Search Co-ordinator	Trial Search Co-ordinator

Numbers

The basic rule is to spell out in full numbers and ordered events less than 10 (Table 18). However, there are some exceptions (Table 19). Numbers between 1000 and 9999 should contain no punctuation. Numbers with five or more digits should include commas (not decimal points or full stops) (Table 20). Use 'from' and 'to' instead of a dash to describe a range of numbers (Table 21). Often judgement is needed to determine the best presentation for a set of numbers.

Table 18 Basic rule for numbers and ordered events less than 10: write in full

✔	✘
We sent the review to four referees.	We sent the review to 4 referees.
The 10 participants agreed.	The ten participants agreed.
The 25 studies are available.	The twenty-five studies are available.
Thirty-three adults and five children participated.	33 adults and 5 children participated.
Ninth	9th
112th	one hundred and twelfth

Table 19 Exceptions to basic rule for numbers and ordered events less than 10

Exception	Guidance	Example
Sentence contains numbers < 10 and ≥ 10	Acceptable to use only numerals	from 2 to 12 years
		from 5% to 25% of the number of participants
		There were between 9 and 15 people in the room.
Equations, numerical results, statistics	Numerals only	2/20
		OR 1.06 (95% CI 0.90 to 3.02)
Sentence starts with a number	Spell number	Eleven per cent of people…
		Twenty authors attended the workshop.
		Eight separate doses are described.
Number with a unit	Always use numerals	8 mg
		25 ml
Tables (also see 'Tables')	Numerals for all numbers including those < 10	--

Table 20 Numbers with five or more digits[29]

✔	✘
7677	7,677
10,000	10000
12,100	12.100

Table 21 Ranges of numbers

✔	✘
from three to nine participants	from three - nine participants
-12 to -4	-12 - -4
The risk ratio was 0.38 (95% CI 0.30 to 0.49)	The risk ratio was 0.38 (95% CI 0.30-0.49)
(MD -11.11 h; 95% CI -20.04 to -2.18)	(MD -11.11 h; 95% CI 20.04 - -2.18)
1% to 10%	1% - 10%
4 to 5 mg	4 - 5 mg

P

Plain language summary[30]: Cochrane Review

Information about the required structure and content of plain language summaries is provided in the *Cochrane Handbook for Systematic Reviews of Interventions*.[31]

Prefixes

General guidance on the use of prefixes is in Table 22.

Table 22 Prefixes: general guidance

Prefix	Guidance	Example
anti-	Use a hyphen with	
	• letters	anti-HBs
	• names	anti-Bitis-Echis-Naja serum
	• words beginning with 'i'	anti-icteric
	• two-word compounds used as adjectives	anti-gas gangrene serum
co-	Use a hyphen if the following word starts with the same vowel	co-ordinate, co-operation, co-opt coexist, comorbidity
inter-	No hyphen if following word starts with 'r'	interrelate
intra-	Use a hyphen if following word starts with 'a'	intra-abdominal, intra-acinar
meta-	Use a hyphen if following word starts with a vowel	meta-analysis, metastasis
micro-	Either joined to the word it modifies or uses a hyphen (it does not stand alone)	microbiology, microcirculation, microfilaria

[29] This is an exception to the style convention for SI units; *see* 'Units and systems of measure'.

[30] Formerly called synopsis; change approved by The Cochrane Collaboration Steering Group, April 2005, Item 13.7, www.cochrane.org/ccsg/ccsg_minutes_providence_april05.htm

[31] www.cochrane.org/resources/handbook/index.htm; Section 3.

Prefix	Guidance	Example
mini-	Either joined to the word it modifies or uses a hyphen (it does not stand alone)	minitracheostomy, mini-mental state examination
multi-	Either joined to the word it modifies or uses a hyphen (it does not stand alone)	multicentre, multi-agency
non-	Hyphenate if 'non' qualifies more than one word	non-insulin dependent, non-profit making
	Hyphen optional if qualifies one word	non-smoker, nonviolent
	No hyphen with Latin phrases	materia non medica, non sequitur
post-	One word unless following word starts with 't'	postgraduate, postorbital, post-treatment
pre-	Hyphen normally used when following word starts with 'e' or 'i'	pre-eclampsia, pre-embryo, pre-exist, pre-exposure, pre-install, pre-industrial
	Established combinations generally one word (except when the word begins with an 'e')	prearranged, prenatal, preoccupy, preschool, pre-empt
	In others the hyphen is not necessary, but is freely used if the compound is one made for the occasion (might be better to rewrite), or if any peculiarity in its form might prevent its elements from being instantly recognized	pre-medication, pre-tax, pre-war
re-	Use hyphen if following word starts with 'e'	re-edit, re-educate, re-establish, re-enter, re-enlist
	Rephrase when there would be confusion with another word	re-cover (cover again) and recover (get better)
self	All compound words with 'self' are two words	self limited
semi	Use a hyphen if following word starts with 'i'	semi-independent, semicolon
sub-	Use a hyphen if following word starts with 'b'	sub-basal, sub-breed (note: sub-Saharan is one exception)
un-	Words starting with 'un-' are generally one word	unnoticeable, unopened, unpaid, unpick
	Rephrase when there would be confusion with another word	unionised (with a union) and un-ionised (without ions)

Punctuation

General guidance on the use of punctuation is in Table 23.

Table 23 Punctuation: general guidance

Symbol	Guidance	Example
Ampersand (&)	Only use if part of a recognized trade name	Procter & Gamble
	Retain where it is used in the official journal title	Journal of Pain & Palliative Care Pharmacotherapy Annals of Nutrition & Metabolism
Brackets (parentheses)	Use round brackets for nested brackets	The standardized mean difference was -0.02 (95% confidence interval (CI) -0.13 to 0.08). They included five references (only two of these were references to full papers (Smith 2002; Taylor 2003)).
Colon	Follow with a lower-case letter unless it is followed by a complete sentence or proper noun	Review topic: cancer Review topic: HIV/AIDS

Symbol	Guidance	Example
Comma	Optional to use a comma before 'and' and 'or' in lists, but be consistent	I have read Cochrane Reviews about malaria, tuberculosis, and vaccines. I have read Cochrane Reviews about malaria, tuberculosis and vaccines.
	Use commas before 'and', 'or', and 'but' in two-phrase sentences (when these words join the two main clauses)	The reviews are written here, but they are available internationally. The reviews are sent here by post, or they are sent here electronically.
	Comma with 'which' and 'that' Use a comma with 'which', but if the sentence reads well with 'that' instead of 'which' then probably no comma needed	We produce Cochrane Reviews, which are relevant to healthcare workers. Note: the phrase after 'which' comments on the reviews We produce Cochrane Reviews that are relevant to healthcare workers. Note: the text after 'that' defines for whom the reviews are relevant
Full stop	Use one space after the full stop[32]	
Percentage sign	The percentage sign can be used in a block of text when it is used with a numeral	Less than 90% of the participants completed the study.
	Use 'per cent' instead of '%' when starting a sentence and when the number is written in full (ie not a numeral).	Three per cent of people
	No space between the number and percentage sign	Correct: 15%
Period	See full stop	
Quotation marks	Use double quotation marks for quoting within dialogue and when quoting text from a written source	In the study "12 participants experienced adverse effects" (Goodwin 1998).
	Use single quotation marks in all other instances and for referring to a specific section of a Cochrane Review if it is not a hyperlink	The 'standard' approach is to … The study details are in the 'Characteristics of included studies'.

R

References: entering and citing references in Cochrane Reviews

There are different types of references in Review Manager: those for included, excluded, ongoing studies, and studies awaiting assessment, and those for the other types of reference sources listed in the 'Other references' section. Each reference has a unique identifier used throughout the review and to link it to the text of the review; these are called 'study identifiers (study IDs)' for the study references and 'reference identifiers (reference IDs)' for the other references.

Entering references into Review Manager

References need to be entered into Review Manager using the designated fields (see Figure 2). Different reference types, such as journal articles and internet publications, need information in different fields.

[32] When publishers format Cochrane Reviews, one space is allocated after each full stop. This means if you use two spaces, they will be reduced to one.

Figure 2 Screenshot of Review Manager 4.2 reference fields

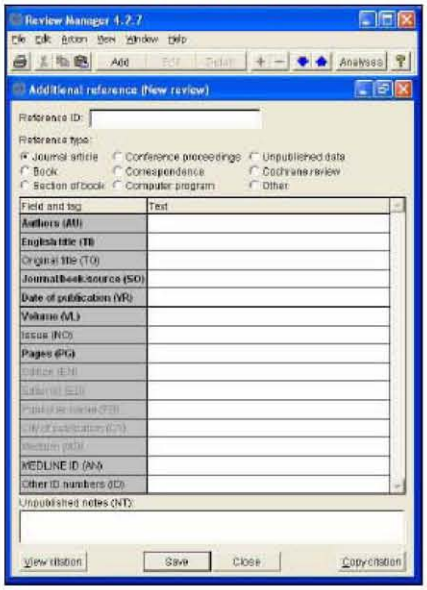


Table 24 has guidance on the correct way of entering data into the various references fields in Review Manager. Review Manager automatically inserts a full stop after each line in the reference, so ensure that there are no full stops at the end of each reference field.

Table 24 Entering references into Review Manager[33]

Field	Guidance	✔	✘
Study ID or Reference ID	Preferred Cochrane format uses last name of first author and year of publication; limited to 20 characters in Review Manager	Garner 2001	
	Two or more articles from the same author from the same year	Bushell 2000a, 2000b, 2000c	Bushell 2000 a, 2000 b, 2000 c (space between year and letter) Bushell 2000, 2000a, 2000c (no letter with year)
Authors	List the first six authors before using 'et al'; comma before 'et al' optional, but be consistent	Smith H, Tavender E, Klaes D, Hinds P, Remmington T, Sparkes V, et al Smith H, Tavender E, Klaes D, Hinds P, Remmington T, Sparkes V et al	
	No 'and' before the final author	Smith H, Tavender E, Klaes D, Hinds P	Smith H, Tavender E, Klaes D, and Hinds P
Article titles	First letter of the first word in	Antibiotics for treating	Antibiotics For Treating

[33] Reference details may be located through the following websites: Index Medicus Journal Abbreviations (ftp://nlmpubs.nlm.nih.gov/online/journals/ljiweb.pdf; PDF file of 3100+ journal titles, and their abbreviations, indexed in the National Library of Medicine's Index Medicus); Entrez Journals database (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Journals; database for searching journal titles of all journals in Entrez databases); NLM Locator Plus (locatorplus.gov/; search for book and journal titles in the United States National Library of Medicine database); and WHOLIS (dosei.who.int/; for World Health Organization documents).

Field	Guidance	✔	✖
	upper case; other words in lower case unless proper nouns or require an upper-case letter	infection The importance of vitamin A	Infection The importance of vitamin a
Journal article title	Include English translation *only* if provided by the journal or database		
Journal title	Write in full using title case (each substantive word starts with an upper-case letter)	*Journal of Pharmacy and Pharmacology*	*J Pharm Pharmacol*
	Journal titles sometimes change; use title current at time of publication		
	British Medical Journal (1857 to 1988) → BMJ (1988 to present)		
	Journal of the American Medical Association (1883 → 1960) → JAMA (1960 to present)		
Non-English language journals	Include English translation in square brackets after the original title *only* if translation provided by the journal or database	Zhonghua Yi Xue Za Zhi [Chinese Medical Journal]	Zhonghua Yi Xue Za Zhi (Chinese Medical Journal)
Page numbers	See examples	324-8, 556-60, 1093-8	324-28, 556-560, 1093-1098, and 1093-98

The correct formats for entering commonly used reference types, such as journal articles and book chapters, are given in Table 25. The complete list of reference types and instructions on how to enter them into Review Manager 4.2 is available in *RevMan User Guide*[34] and in RevMan Help. *The Cochrane Manual*[35] contains details on citing other products from The Cochrane Collaboration – *The Cochrane Library*, a module in *The Cochrane Library*, a Cochrane Review from the *Cochrane Database of Methodology Reviews*, and *The Cochrane Manual*.

Table 25 How to enter different reference types[36] into Review Manager 4.2

Reference type	Example
Standard journal article	
Reference type	Journal article
Authors (AU)	Osrin D, Vaidya A, Shrestha Y, Baniya RB, Manandhar DS, Adhikari RK, et al
English title (TI)	Effects of antenatal multiple micronutrient supplementation on birthweight and gestational duration in Nepal: double-blind, randomised controlled trial
Journal/book/source (SO)	Lancet
Date of publication (YR)	2005
Volume (VL)	365
Issue (NO)	9463

[34] www.cc-ims.net/RevMan/documentation.htm; Appendix E.
[35] www.cochrane.org/admin/manual.htm
[36] The Cochrane Style Guide Working Group identified, through consensus, that these were commonly used reference formats.

Reference type	Example
Pages (PG)	955-62
View citation	Osrin D, Vaidya A, Shrestha Y, Baniya RB, Manandhar DS, Adhikari RK, et al. Effects of antenatal multiple micronutrient supplementation on birthweight and gestational duration in Nepal: double-blind, randomised controlled trial. Lancet 2005;365(9463):955-62.
Journal articles in volume with supplement	
Reference type	Journal article
Authors (AU)	Bowman CM
English title (TI)	The long-term use of inhaled tobramycin in patients with cystic fibrosis
Journal/book/source (SO)	Journal of Cystic Fibrosis
Date of publication (YR)	2002
Volume (VL)	1 Suppl 2
Pages (PG)	194-8
View citation	Bowman CM. The long-term use of inhaled tobramycin in patients with cystic fibrosis. Journal of Cystic Fibrosis 2002; 1 Suppl 2:194-8.
Chapter in a book	
Reference type	Section of book
Authors (AU)	Weinstein L, Swartz MN
English title (TI)	Pathologic properties of invading microorganisms
Journal/book/source (SO)	Pathologic physiology: mechanisms of disease
Date of publication (YR)	1974
Edition	5th
Pages (PG)	457-72
Editor(s) (ED)	Sodeman WA Jr, Sodeman WA
Publisher name (PB)	Saunders
City of publication (CY)	Philadelphia
View citation	Weinstein L, Swartz MN. Pathologic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, editor(s). Pathologic physiology: mechanisms of disease. 5th edition. Philadelphia: Saunders, 1974:457-72.
Conference proceedings that do not use the same style as a journal article	
Reference type	Conference proceedings
Journal/book/source (SO)	Child abuse and neglect: a medical community response. Proceedings of the First AMA National Conference on Child Abuse and Neglect; 1984 Mar 30-31; Chicago
Date of publication (YR)	1985
Editor(s) (ED)	Vivian VL
Publisher name (PB)	American Medical Association

Reference type	Example
City of publication (CY)	Chicago
View citation	Vivian VL, editor(s). Child abuse and neglect: a medical community response. Proceedings of the First AMA National Conference on Child Abuse and Neglect; 1984 Mar 30-31; Chicago. Chicago: American Medical Association, 1985.
Paper in conference proceedings that does not use the same style as a journal article	
Reference type	Conference proceedings
Authors (AU)	Harley NH
English title (TI)	Comparing radon daughter dosimetric and risk models
Journal/book/source (SO)	Indoor air and human health. Proceedings of the Seventh Life Sciences Symposium; 1984 Oct 29-31; Knoxville (TN)
Date of publication (YR)	1985
Pages (PG)	69-78
Editor(s) (ED)	Gammage RB, Kaye SV
Publisher name (PB)	Lewis
City of publication (CY)	Chelsea (MI)
View citation	Harley NH. Comparing radon daughter dosimetric and risk models. In: Gammage RB, Kaye SV, editor(s). Indoor air and human health. Proceedings of the Seventh Life Sciences Symposium; 1984 Oct 29-31; Knoxville (TN). Chelsea (MI): Lewis, 1985:69-78.
Computer program	
Reference type	Computer program
English title (TI)	Review Manager (RevMan)
Date of publication (YR)	2003
Edition (EN)	4.2 for Windows
Publisher name (PB)	The Nordic Cochrane Centre, The Cochrane Collaboration
City of publication (CY)	Copenhagen
Medium (MD)	CD-ROM and Internet
View citation	Review Manager (RevMan) [Computer program]. Version 4.2 for Windows. Copenhagen: The Nordic Cochran Centre, The Cochrane Collaboration, 2003.
Publications on the Internet	
Reference type	Other
Authors (AU)	Royal College of Physicians of Edinburgh and UK Cochrane Centre
English title (TI)	Controlled trials from history
Journal/book/source (SO)	www.rcpe.ac.uk/cochrane/
Date of publication (YR)	(accessed 10 May 2000)
View citation	Royal College of Physicians of Edinburgh and UK Cochrane Centre. Controlled trials from history.

Reference type	Example
	www.rcpe.ac.uk/cochrane/ (accessed 10 May 2000).
Cochrane Review in the *Cochrane Database of Systematic Reviews*	
Reference type	Cochrane Review
Authors (AU)	Robinson PG, Deacon SA, Deery C, Heanue M, Walmsley AD, Worthington HV, et al
English title (TI)	Manual versus powered toothbrushing for oral health
Journal/book/source (SO)	Cochrane Database of Systematic Reviews
Date of publication (YR)	2005
Issue (NO)	2
View citation	Robinson PG, Deacon SA, Deery C, Heanue M, Walmsley AD, Worthington HV, et al. Manual versus powered toothbrushing for oral health. In: Cochrane Database of Systematic Reviews 2005, Issue 2.
Cochrane Handbook for Systematic Reviews of Interventions	
Reference type	Other
Authors (AU)	Higgins JPT, Green S, editors.
English title (TI)	Cochrane Handbook for Systematic Reviews of Interventions 4.2.5 [updated May 2005]
Journal/book/source (SO)	www.cochrane.org/resources/handbook/hbook.htm
Date of publication (YR)	(accessed 31 May 2005)
View citation	Higgins JPT, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions 4.2.5 [updated May 2005]. www.cochrane.org/resources/handbook/hbook.htm (accessed 31 May 2005).
Section of *Cochrane Handbook for Systematic Reviews of Interventions* with no section editors	
Reference type	Other
Authors (AU)	Higgins JPT, Green S, editors
English title (TI)	Formulating the problem. Cochrane Handbook for Systematic Reviews of Interventions 4.2.5 [updated May 2005]; Section 4
Journal/book/source (SO)	www.cochrane.org/resources/handbook/hbook.htm
Date of publication (YR)	(accessed 31 May 2005)
View citation	Higgins JPT, Green S, editors. Formulating the problem. Cochrane Handbook for Systematic Reviews of Interventions 4.2.5 [updated March 2005]; Section 4. www.cochrane.org/resources/handbook/hbook.htm (accessed 31 May 2005).
Section of *Cochrane Handbook for Systematic Reviews of Interventions* that has section editors listed	
Reference type	Other

Reference type	Example
Authors (AU)	Deeks JJ, Higgins JPT, Altman DG, editors. In: Higgins JPT, Green S, editors
English title (TI)	Analysing and presenting results. Cochrane Handbook for Systematic Reviews of Interventions 4.2.5 [updated May 2005]; Section 8
Journal/book/source (SO)	www.cochrane.org/resources/handbook/hbook.htm
Date of publication (YR)	(accessed 31 May 2005)
View citation	Deeks JJ, Higgins JPT, Altman DG, editors. In: Higgins JPT, Green S, editors. Analysing and presenting results.. Cochrane Handbook for Systematic Reviews of Interventions 4.2.5 [updated March 2005]; Section 8. www.cochrane.org/resources/handbook/hbook.htm (accessed 31 May 2005).

Citing references in the text of Cochrane Reviews

Guidance on inserting references in Cochrane Reviews is in Table 26.

Table 26 Citing references in the text of Cochrane Reviews

Guidance	✔	✘
Separate with a semicolon	Robb 2001; Smith 2000	Robb 2001, Smith 2000
List in alphabetical or chronological order, but be consistent within a single document[37]	**Alphabetical**: Bakri 1988a; Bakri 1988b; Davis 2003; Slinn 2001	**Alphabetical**: Bakri 1988a; Bakri 1988b; Slinn 2001; Davis 2003
	Chronological: Bakri 1988a; Bakri 1988b; Slinn 2001; Davis 2003	**Chronological**: Bakri 1988a; Bakri 1988b; Davis 2003; Slinn 2001
No 'and' before the final reference	Davis 2001; Omari 1988; Preston 1988; Slinn 2001	Davis 2001; Omari 1988; Preston 1988; and Slinn 2001
Can be used as part of a sentence or in round brackets within closest punctuation	The study was successful (Robeson 1990).	The study was successful [Robeson 1990].
	The study was successful (Griffin 1990); it confirmed previous findings (Howes 1995).	The study (Griffin 1990) was successful; it confirmed previous findings (Howes 1995).
	Bloggs 1991 reports the full details.	(Bloggs 1991) reports the full details.

Note: make sure identifier is linked to the reference list

S

Search methods

Citing databases and study registers

The preferred format for the following databases is all upper-case letters: MEDLINE, EMBASE, CENTRAL[38], OLDMEDLINE, and CINAHL (*not* CIN**HA**L). A number of databases use a mixture of lower-case and upper-case letters, for example, PsycLIT *(not* Psyc**h**LIT) and PsycINFO (*not* Psyc**h**INFO).

[37] Change in version 3.0 in response to new feedback; further discussion welcomed.

[38] The Cochrane Controlled Trials Register (CENTRAL/CCTR) was renamed the **Cochrane Central Register of Controlled Trials (CENTRAL)**. It can also be referred to as CENTRAL. This change was effective in *The Cochrane Library* from Issue 4, 2002.

Each Cochrane Review Group is responsible for preparing a register of studies in their area of expertise. The name of the register may vary, but it will follow one of the following formats depending on the Cochrane Review Group's choice: Cochrane [*insert name*] Group Specialized Register, Cochrane [*insert name*] Group Specialised Register, or Cochrane [*insert name*] Group Trials Register.

The databases and trials registers that are searched for studies for a Cochrane Review are listed in two sections: the 'Abstract' and the 'Search strategy for identification of studies'. The databases and registers must be listed in the following order: Cochrane [*insert name*] Group Specialized Register (or Specialised Register or Trials Register), CENTRAL, MEDLINE, EMBASE, and any other databases. The date range of each search must be listed with each database; for example, CENTRAL (*The Cochrane Library* year, issue number), and for most other databases, such as MEDLINE, it should be in the form 'MEDLINE (month year to month year)'.

When this section is published in *The Cochrane Library* the text 'See: [Cochrane Review Group name] search strategy' is inserted *automatically* by the publishers below the heading. This links to the Group's module in *The Cochrane Library* where the search methods for the Group's Trials Register are described. There is no need to include a line of text referring to the Cochrane Review Group's search strategy.

Search terms

Search terms consist of text words (preferred spelling is two words in Cochrane Reviews instead of 'textword') and controlled vocabulary. The preferred format for referring to the National Library of Medicine's controlled vocabulary used for indexing articles for MEDLINE (and PubMed) is MeSH (*not* MESH).[39]

Spacing

See 'Punctuation', 'Symbols', and 'Units and systems of measure' for relevant guidance on spacing.

Use one blank line between two paragraphs in a block of text. Avoid indicating a paragraph break using indentation because Review Manager does not support this type of formatting.

Statistical and mathematical presentation

General guidance on the presentation of statistical and mathematical values is in Table 27. Guidance on abbreviating statistical terms commonly used in Cochrane Reviews is in Table 2.

Table 27 Statistical and mathematical presentation: general guidance

	Guidance	✔	✘
Decimal places	Two decimal places unless number very small (eg 0.005) or the unit of measurement dictates otherwise	12.26 120/80 mm Hg	12.3
Decimal points	Use full stops, not commas	15.51	15,51
Mathematical formulae	Avoid building mathematical formulae spaced over two or more lines in the text of the review, as text formatting will change during publication process	2 = 10/5	$2 = \frac{10}{5}$
P value	Use an upper-case 'P' (no italic) No hyphen between the 'P' and the value Uncommon to state that a P value is 'less than' or 'more than', instead use P < 0.05	A P value of 0.05 was used as the cut-off value to determine statistical significance.	A p value of 0.05 was used as the cut-off value to determine statistical significance.

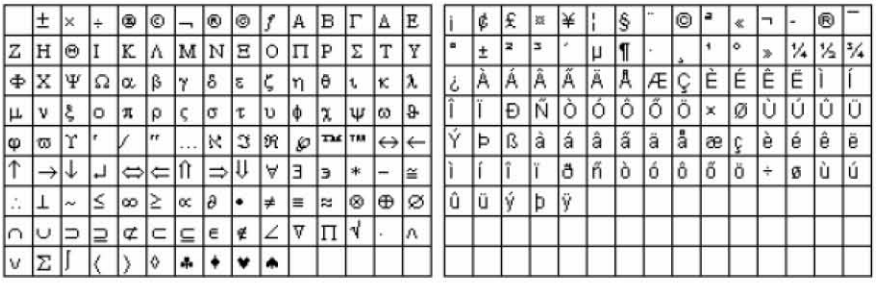
[39] www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=mesh

	Guidance	✓	✗
		There was a statistically significant intervention effect (P = 0.01).	There was a statistically significant intervention effect (P=0.01).
Sample and population sizes	For dichotomous outcomes, the RevMan Analyses and Statistical Analysis (see 'Computer software used to prepare and view Cochrane Reviews' for description) use the headings n/N within each intervention arm, where n denotes the number of events and N is the sample size. It is preferable to standardize the use of n/N for these where possible.	--	--
Summary statistic and confidence interval	Only use abbreviations for summary statistic (eg RR or MD) and confidence interval (CI) if already defined (see 'Abbreviations and acronyms')	The risk ratio (RR) was 0.38 (95% confidence interval (CI) 0.30 to 0.49)	--
	Separate summary statistic from its CI using a comma or semicolon if inside a single set of brackets	…was statistically significant (RR 0.09, 95% CI 0.02 to 0.38)	--
	Define the CI, eg 95% or 99%	(odds ratio 1.11, 95% CI 0.98 to 1.20)	(odds ratio 1.11, CI 0.98 to 1.20)
	Separate the CIs with 'to' instead of using a hyphen	(mean difference -11.11 hours; 95% CI -20.04 to -2.18)	(mean difference -11.11 hours; 95% CI -20.04 - -2.18)

Symbols and special characters

Many different symbols and special characters are available for use in Review Manager (see Figure 3).

Figure 3 Symbols and special characters available in Review Manager 4.2



Symbols, including those in Table 28, should be used in a block of text only if the descriptive version is cumbersome or inappropriate.

Table 28 Symbols: general usage

Symbol	Description	Spacing
+	plus, and	1 space either side
-	minus	1 space either side
/	per Use '/' instead of 'per' where it is otherwise cumbersome 10 mg/kg (*not* 10 mg per kg)	no spacing (10 g/L)

Symbol	Description	Spacing
<	less than (eg for percentages) fewer than (eg for people)	1 space either side
>	greater than more than	1 space either side
=	equals	1 space either side

T

Tables: Cochrane Review

There are two types of tables in Cochrane Reviews: 'Characteristics' tables and 'Additional' tables. The 'Characteristics' tables – of included studies, excluded studies, and ongoing studies – have a defined format, with specific column headings. Review Manager automatically generates the row headings based on the reference lists, which means it is not possible to insert additional column or row headings in the 'Characteristics' tables. 'Additional tables' allow authors to create tables for other types of information.

Formatting should be consistent within a single table. As with other parts of a Cochrane Review, the visual presentation of tables will change during the publication process. It is important not to use return key within a single block of text (eg in a single word if the word runs over two lines) because the text layout will also change during the publication process. Refer to the sections on 'Abbreviations and acronyms' and 'Numbers' for additional guidance.

Footnotes are a convenient way to define abbreviations and acronyms or display other explanatory notes. The 'Characteristics' tables have a specific footnotes section, unlike the 'Additional tables', which require a little imagination to include footnotes; for example, inserting an extra row at the bottom of the table and using one of the cells for the footnotes. Enter each footnote either on a new line or separate with a semicolon, remembering that all abbreviations and acronyms used in a table should be defined in the footnotes.

Table 29 Example of footnote formatting

Footnotes	Footnotes
DSS: dengue shock syndrome TB: tuberculosis	DSS: dengue shock syndrome; TB: tuberculosis

Some tables, such as the 'Characteristics' tables, may include blocks of text. Each block should start with an upper-case letter. It is optional to end each block with punctuation.

Tautology

Avoid using a tautology, which is "the saying of the same thing twice over in different words" (Pearsall 1998[40]) (Table 30).

Table 30 Example of a tautology

Tautology	✔
We excluded trials of children with a **history** of headaches **in the past**.	We excluded trials of children with a history of headaches.

[40] Pearsall J. *The new Oxford dictionary of English*. Oxford: Oxford University Press, 1998.

Tense: Cochrane Review

Write things you plan on doing in the future tense (such as in a protocol for a Cochrane Review) and things you have already done in the past tense (such as in a Cochrane Review).

Titles: Cochrane Review

Base the Cochrane Review title on the structure used for the vast majority of existing review titles in the *Cochrane Database of Systematic Reviews* (Table 31). If the review involves several interventions, start the title with 'Interventions' or more appropriate specific terms, such as pharmacotherapy, psychological therapy, physical therapies, or surgery. Rarely mention specific outcomes in the title because the title should reflect all the outcomes included in the review; if included, use a colon to separate the outcome from the main title.

Standardize titles by rarely using upper-case letters, avoiding the use of abbreviations, and avoiding superfluous elements (eg 'effects of', 'comparison of', 'a systematic review of'). Titles should not include unnecessary punctuation, such as a full stop at the end.

Table 31 Structure for Cochrane Review titles

Scenario	Structure	Example
Basic structure	[intervention] for [health problem]	Antibiotics for acute bronchitis
Comparing two active interventions	[intervention A] versus [intervention B] for [health problem]	Immediate versus delayed treatment for cervical intraepithelial neoplasia
Type of people being studied or location of intervention mentioned explicitly	[intervention] for [health problem] in [participant group/location]	Inhaled nitric oxide for respiratory failure in preterm infants
Not specifying a particular 'health problem' (eg 'Home versus hospital birth'), or if the intervention intends to influence a variety of problems (eg 'Prophylactic synthetic surfactant in preterm infants')	[intervention] in OR for [participant group/location]	Restricted versus liberal water intake in preterm infants
Sometimes you may need to specify that the intervention is for preventing, treating, or preventing and treating the health problem(s): If necessary, follow the word 'for' by 'preventing', 'treating', or 'preventing and treating'. This is better than using 'for the prevention of', etc.	--	Pool fencing for preventing drowning in children Amodiaquine for treating malaria Vitamin C for preventing and treating the common cold

U

Units and systems of measure

The International System of Units/Le Système International d'Unités (SI) is the modern metric system of measurement. This system is made up of SI base units (the foundation units) (eg metre), derived units (eg square metre), and non-SI units that are accepted for use within the SI (eg minute).

Table 32 lists SI units that are commonly used in Cochrane Reviews. The full list of units and further information on this System is available on the International Bureau of Weights and Measures (BIPM) website.[41]

[41] www1.bipm.org/en/si/

Table 32 Examples of commonly used units

Unit name	Symbol	Type
kilogram	kg	base unit
metre	m	base unit
second	s	base unit
cubic metre	m^3	derived unit
degree Celsius	°C	derived unit
metre per second	m/s	derived unit
square metre	m^2	derived unit
day	d	non-SI unit
degree	°	non-SI unit
hour	h	non-SI unit
litre*	l, L	non-SI unit
minute	min	non-SI unit
minute	'	non-SI unit
second	"	non-SI unit

*The BIPM adopted the symbol 'l' in 1879; it then adopted the alternative 'L' in 1979 in order to avoid the risk of confusion between the letter 'l' and the number '1'

Sometimes it is necessary to express units in quantities greater or smaller than the base unit. Table 33 contains the SI prefixes commonly used in Cochrane Reviews to derive such quantities.

Table 33 Prefixes for SI units

Factor	Name and symbol	Example
10^{-1}	deci (d)	decilitre (where 'litre' is the base unit)
10^{-2}	centi (c)	centimetre (where 'metre' is the base unit)
10^{-3}	milli (m)	millilitre
10^{-6}	micro (μ)	microlitre
10^{-9}	nano (n)	nanogram

SI units and their derivatives should follow the style conventions in Table 34. Unlike the 'Abbreviations and acronyms', it is not necessary to define the full unit name on first use.

Table 34 SI units[42]: general guidance

Guidance	✔	✘
Unit symbols are unaltered when plural	10 mg	10 mgs
Unit symbols are not followed by a full stop, except if followed by normal sentence punctuation	I added 60 mg of salt.	I added 60 mg. of salt.
It is clear to which unit symbol a numerical value belongs and which mathematical operation applies to the value of a quantity	20 ℃ to 30 ℃ or (20 to 30) ℃ 123 g ± 2 g or (123 ± 2) g	20 ℃-30 ℃ or 20 to 30 ℃ 123 ± 2 g

[42] These are a selection of style conventions from <physics.nist.gov/cuu/Units/rules.html> (which contains a comprehensive list) and <www1.bipm.org/en/si/>. Cochrane Reviews may deviate from some of the style conventions due to the nature of Cochrane Review production; for example, Cochrane Reviews use commas to separate digits into groups of three (eg 150,739) instead of thin, fixed spaces (150 739).

Guidance	✔	✖
Values of quantities use Arabic numerals and symbols for units	m = 5 kg the current was 15 A	m = five kilograms m = five kg the current was 15 amperes
One space between the numerical value and unit symbol,[43] even when the value is used in an adjectival sense	2 s a 25 kg sphere	2s a 25-kg sphere
Do not mix information with unit symbols or names	the water content is 20 mL/kg	20 mL H_2O/kg 20 mL of water/kg
Informal references to non-SI units, such as a historical quote using inches, are acceptable depending on the context	It took five hours to travel 10 miles in 1945.	It took five hours to travel 10 miles (16.09 km) in 1945.

Upper-case letters

Only use if there is a compelling reason not to use lower-case letters (such as the word being a proper noun).

V

Verbs: single or plural

Group nouns can use either a single or plural verb, but the choice should be consistent within a single Cochrane Review or document; for example, 'the government has...' or 'the government have…'.

[43] Except in the case of superscript units for plane angle (eg 'an angle of 2°3 ′ 4″ is correct).

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Information for Authors

The Lancet Infectious Diseases considers any original research contribution that advocates change in or illuminates infectious disease clinical practice and informative reviews on any topic connected with infectious diseases. Manuscripts must be solely the work of the author(s) listed, must not have been published elsewhere, and must not be under consideration by another journal. Because the journal has an international readership from a wide range of specialties, it is vital that articles should be written clearly, and should not assume a level of knowledge above that of, say, a reasonably well-read, recently qualified, doctor in training. One way to find out if your article is understandable to those reading outside their immediate field of interest is to show the manuscript to colleagues in other specialties. If they find it difficult to follow, so will a good proportion of the readership. Wherever possible, figures and good quality photographs (colour or black and white) should be used to supplement and enhance the text. Further details on the different sections of _The Lancet Infectious Diseases_, and how to submit to the journal, are provided below. If you require further clarification, the journal's editorial staff will be pleased to help.

How to submit your paper

The journal has an online submission and peer review website (EES). To submit a paper, visit http://ees.elsevier.com/thelancetid and follow the on-screen instructions. If you have not used EES before, you will need to register first. In EES, the corresponding author is the person who enters the manuscript details and uploads the submission files.

First submissions to _The Lancet Infectious Diseases_ should include: covering letter, manuscript, figures, conflicts of interest statements, acknowledgments, and personal communications. We encourage disclosure of correspondence from other journals and reviewers, if previously submitted, and we might contact relevant editors of such journals.

Should your paper be selected for further consideration, we will then ask you to send copies of the following documents: covering letter (signed by all authors), conflicts of interest statements, signed patient consent and permission to publish, acknowledgments (written consent of cited individual), personal communications (written consent of cited individual), and use of copyright-protected material (signed permission statements from original publishers).

To minimise delays in the peer-review process, we strongly advise that the signed copies of these documents are prepared before you submit your manuscript.

Submission categories

Reflection and Reaction

Commentaries may discuss articles in _The Lancet Infectious Diseases_ or in other journals. Most commentaries are commissioned, but unsolicited commentaries (no more than 700 words and 15 references) are also welcome. Unsolicited commentaries may be peer reviewed.

Correspondence

Letters should be written in response to previous content published in _The Lancet Infectious Diseases_, and must reach the journal within 8 weeks of publication of the original item. Length must not exceed 400 words, only one table or figure is permitted, and there should be no more than five references.

Newsdesk

Articles in this section are generally written by professional journalists. However, if you know of an event that might be of wider interest to the infectious diseases community please contact the Editor with your ideas.

Media Watch

Readers with an interest in contributing book, film, TV, or web reviews should contact the Editor. In general, these submissions should be between 350 and 400 words.

Reportage

These 1500–1750 word essays (with two or three pictures) are written in a journalistic style and provide a thorough overview of infectious diseases services in a particular country or region. Our aim is to draw attention to less developed countries, but reports from developed countries that receive little coverage are also welcome. As with the Newsdesk, these articles are usually written by commissioned professional journalists, but if you have an idea for inclusion please contact the Editor.

Formatting guidelines http://www.download.thelancet.com/flatcontentassets/authors/artwork-guidelines.pdf

Webmaterial guidelines http://www.download.thelancet.com/flatcontentassets/authors/webmaterial-guidelines.pdf

Articles

The Lancet Infectious Diseases prioritises reports of original research that is likely to change clinical practice or thinking about infectious diseases. We especially encourage submission of all types of clinical trial, whether phase 1, 2, 3, or 4. However, _The Lancet Infectious Diseases_ will only consider phase 1 trials if they report on a novel approach or unique indication where there is strong evidence of an unexpected beneficial or adverse response, or of a novel mechanism of action.

We encourage the registration of all interventional trials, whether early or late phase, in a primary register that participates in WHO's International Clinical Trial Registry Platform (see _Lancet_ 2007; 369: 1909–11). We also encourage full public disclosure of the minimum 20-item trial registration dataset at the time of registration and before recruitment of the first participant (see _Lancet_ 2006; 367: 1631–33). The registry must be independent of for-profit interest. In accordance with guidelines from the International Committee for Medical Journal Editors, of which _The Lancet Infectious Diseases_ is a member journal, papers can be submitted even if they contain results that have been posted in the same clinical trials registry in which the primary registration resides provided such results have only been presented in the form of a brief structured abstract (<500 words) or table (see _Lancet_ 2007; 369: 1909–11).

Reports of randomised trials must conform to the revised CONSORT guidelines and should be submitted with their protocols and a completed CONSORT checklist. We encourage all accepted articles to include a link to the full study protocol published on the authors' institutional website (see _Lancet_ 2009; 373: 992). For titles and abstracts, please adhere to the CONSORT guidelines on abstracts (see _Lancet_ 2010; 375: 1144–46). All reports of clinical

To find reporting guidelines, see http://www.equator-network.org

trials must include a summary of previous research findings and explain how this trial affects this summary. The relation between existing and new evidence should be illustrated by direct reference to an existing systematic review and meta-analysis; if neither exists, authors are encouraged to do their own or to describe the qualitative association between their research and previous findings (see *Lancet* 2005; **366**: 107–08). Cluster randomised trials should be reported according to extended CONSORT guidelines. Randomised trials reporting harms must be described according to extended CONSORT guidelines. All reports of randomised trials should include a section entitled 'Randomisation and masking' within the methods section. Studies of diagnostic accuracy should be reported according to STARD guidelines. Observational studies (cohort, case-control, or cross-sectional designs) must be reported according to the STROBE statement. Genetic association studies must be reported according to STREGA guidelines. Systematic reviews and meta-analyses must be reported according to PRISMA guidelines.

All articles should

- be up to 3000 words with 30 references
- include a semistructured summary with five paragraphs (Background, Methods, Findings, Interpretation, and Funding), not exceeding 250 words and reporting only primary outcomes if space is short. In EES, you will be asked to copy and paste this section at the "Submit Abstract" submission stage
- use the SI system of units and the recommended international non-proprietary name (rINN) for drug names. Ensure that the dose, route, and frequency of administration of any drug you mention are correct
- use gene names approved by the Human Gene Organisation. New gene sequences should be deposited in a public database (GenBank, EMBL, or DDBJ), and the accession number provided. Authors of microarray papers should include in their submission the information recommended by the MIAME guidelines. Authors should also submit their experimental details to one of the publicly available databases: ArrayExpress or GEO
- include any necessary additional data as part of your EES submission.

Human Gene Organisation http://www.gene.ucl.ac.uk/nomenclature

MIAME guidelines http://www.mged.org/Workgroups/MIAME/miame_checklist.html

Array and GEO http://www.ebi.ac.uk/arrayexpress http://www.ncbi.nlm.nih.gov/projects/geo

Meta-analyses See *Lancet* 2009; **374**: 1221–23 See http://www.equator-network.org

Meta-analysis

In general, these should follow the PRISMA guidelines. Manuscripts should be structured around five sections: summary, introduction, methods, results, and discussion. Aim for a maximum length of about 3000 words and 75 references. Meta-analyses should also contain a semistructured summary as described previously for Articles.

Review

Reviews may be commissioned or submitted unsolicited, although in the latter case it would be wise to send the Editor a one-page outline first (email ideditorial@lancet.com) to ensure that a review on the same subject has not already been commissioned. Manuscripts will be assessed in-house and those considered suitable will be peer reviewed before an editorial decision is made. Reviews should either be definitive overviews of a major topic connected with infectious diseases or updates of knowledge in a narrower field of current interest. The word count should be between 3000 and 5000 words, depending on the breadth of the topic. Reviews should begin with a summary of no more than 150 words, which briefly covers the content of the article. Use subheadings to break up the text in the main body of the article, and try to distribute references to the figures and tables evenly throughout the manuscript. References (maximum 150) should be chosen for their importance, ease of access, and for the "further reading" opportunities they provide. Following the references, authors should consider supplying a short list of useful websites where readers can find further information on the subject. *The Lancet Infectious Diseases* welcomes systematic reviews, which must be reported according to PRISMA guidelines.

When writing a literature review, complete transparency concerning the choice of material included is important. Reviews must therefore contain a brief section entitled Search strategy and selection criteria. This should state clearly the sources (databases, journals, or book reference lists, etc) of the material covered and the criteria used to include or exclude studies; for example, English language only or studies conducted after a specific date. Example:

> Data for this review were identified by searches of Medline, Current Contents, and references from relevant articles; numerous articles were identified through searches of the extensive files of the authors. Search terms were "hand hygiene", "handwashing", "hygienic handwash", "hand disinfection", "handrub", "cross infection", "epidemiology", "healthcare worker", and "behaviour". English and French language papers were reviewed.

Historical Review

These should follow the same rules and guidelines as for Reviews, but should cover the chronological developments in an important or interesting area of infectious diseases.

Personal View

These should be around 1500–3000 words in length, with a maximum of 75 references. These opinion pieces are thought-provoking essays on an infection-related subject and must be prepared in a similar way to a Review article. Unsolicited contributions are welcome, but it is best to contact the Editor before submission to ensure that the proposed topic is suitable for the journal.

Forum

These are a platform for discussing controversies in infectious diseases. Each Forum contains three or more 800 word opinion pieces written by infectious diseases specialists, researchers, nurses, patients, and others with an interest in the topic under discussion. Suggestions for topics from the journal's readers are very welcome.

Grand Round

These use a brief case report as the starting point for a review of the patient's diagnosis. Rather than rarity, we are looking for single cases that address common problems and evidence-based review of the implications of the case. Consent for publication must be obtained from the patient or next of kin before submission (see Clinical Picture, below). The case report part of the text should

be no longer than 800 words, the review part no longer than 3000 words, and up to 75 references are allowed.

Clinical Picture

These should feature interesting clinical photographs, accompanied by a brief text of up to 300 words. Illustration of a useful teaching point is more important than rarity. No references should be included. There should be no more than five authors; at least one should have been clinically responsible for the patient. Consent for publication in print and electronically must be obtained from the patient before submission or, if this is not possible, from their next of kin. For further details see below under Patients' consent and permission to publish.

Errata

Any substantial error in a published article will be corrected as soon as possible. Blame is not apportioned; the objective is to set the record straight. Please contact the editorial team if you spot a major error in the journal.

General submission guidelines

Covering letter

We ask that you upload a brief covering letter at the "Enter Comments" stage of the online submission process.

Authors and contributors

We ask all authors and all contributors (including medical writers and editors) to specify their individual contributions at the end of the text. *The Lancet Infectious Diseases* will not publish any articles unless we have the signatures of all authors. We suggest you use the author signature form and either upload the signed copy with your submission, or fax to +44 (0) 1865 853 017. In addition, please include written consent of any cited individual(s) noted in acknowledgments or personal communications.

Conflict of interest

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- The corresponding author should confirm that he or she had full access to all the data in the study and had final responsibility for the decision to submit for publication.
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Author signatures, contributions, and declarations
http://www.download.thelancet.com/flatcontentassets/authors/tlid-author-signatures.pdf

Conflicts of interest
Lancet 2001; **358:** 854–56
Lancet 2003; **361:** 8–9
Lancet 2004; **363:** 2–3

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DATA EXTRACTION SHEET

Date:

Reviewer ID:

Administrative details	
Study ID	
Trial Number	
Author(s)	
Publication details	
Year of Publication	
Number of studies in this paper	
Year in which study was concluded	
Other relevant papers cited	

Study Details	
Study Verification	
Study Design	
Type, duration and completeness of follow-up	
Country/ location of study	
Informed consent	
Ethics	

Participant details	
Setting / diagnosis	
Number	
Baseline characteristics	

Interventions (I) / Controls (C)	
I dosage / regimen	
Control	
Background treatment	

Risk of bias	**Judgment**	**Description**
Adequate sequence generation		
Allocation concealment		
Blinding		
Incomplete outcome data addressed		
Free of selective reporting		
Free of other bias		

Primary Outcomes	**Overall PTB**	**Category I PTB**	**Category II PTB**
Sputum culture conversion **15 days**			
30 days			
60 days			
120 days			
120+ days			
Mortality			

Secondary outcomes	
Serious adverse reactions	
Adverse events related to the immunotherapy	
Additional notes	.....

COVER SHEET

Title	**Mycobacterium w immunotherapy for treating pulmonary tuberculosis – a systematic review**
Authors	Pandie S, Engel M, Kerbelker Z, Mayosi BM
Contribution of author(s)	SP was responsible for the development of the protocol, reviewing the abstracts, data extraction, analysis of results, interpretation of the findings and writing the final report. ZK was responsible for independently performing the literature search and extracting the data. BM and ME were the project supervisors.
Issue Protocol first published	2009/12
Review first published	/
Date of most recent amendment	/
What's new	/
Date new studies sought but not found	Information not supplied
Date new studies found but not yet included/excluded	Information not supplied
Date new studies found and included/excluded	/
Date authors' conclusion section amended	/
Contact Address	Dr Shaheen Pandie E 17 Cardiac Clinic Groote Schuur Hospital Observatory Cape Town 7925 South Africa pandieshaheen@yahoo.com
DOI	/
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CHARACTERISTICS OF STUDIES

CHARACTERISTICS OF INCLUDED STUDIES

LUHADIA 2004

Methods	Randomised placebo-controlled trial set in Udaipur, India Sequence generation: no information provided Allocation concealment: no information provided Blinding: single-blind
Participants	200 Sputum smear positive pulmonary TB participants CATEGORY I (new diagnosis) Total: 100 participants Mean age: 36 years Mean weight: 36.7 kg *M w* arm: 50 participants Control arm: 50 participants CATEGORY II (retreatment) Total: 100 participants Mean age: 35 years Mean weight: 37.7 kg *M w* arm: 50 participants Control arm: 50 participants
Interventions	Intervention: 0.1 ml of *M w* administered intradermally on days 0, 15, 30, and 60; and then two monthly until TB treatment is complete Control: placebo of 0.1 ml of saline administered intradermally (as above) Other: all participants received standard TB treatment as per WHO guidelines
Outcomes	Sputum conversion (on days 15, 30, 60, 120, and 120+) Adverse events (including death and skin reactions) Other clinical (weight gain) and radiological improvements
Notes	This paper was presented as a poster presentation at the National Conference on Pulmonary Diseases (NAPCON) in 2004 There is limited data as regards the methodology of the trial The authors were contacted in this regard, but no reply was received

RISK OF BIAS TABLE

Item	Judgment	Description
Adequate sequence generation?	Unclear	No information provided Paper states that it is a randomised controlled trial, but does not provide any details
Allocation concealment?	Unclear	No information provided
Blinding? (Primary outcome: Sputum	Yes	Single-blind Even though there is no detail provided about the blinding, the primary outcome of sputum

conversion)		negativity is a laboratory-assessed outcome that is unlikely to be influenced by whether or not the participant or clinician knows which treatment is being administered
Blinding? (Secondary outcome: Adverse events)	No	Single-blind For the secondary outcomes (adverse events, clinical improvement and radiological resolution), single blinding is insufficient. Both the participant and the clinician should be blinded to the treatment allocation
Incomplete outcome data addressed?	Yes	No missing data
Free of selective reporting?	Yes	Study protocol is not available but it is clear that the presented reports include all expected outcomes
Free of other bias?	No	Performance bias. Only single-blind (it is not specified if the laboratory technician, clinician or the participant were blinded) May influence the evaluation of clinical outcomes such as adverse reactions, clinical improvement and radiological resolution

PARIKH 2006

Methods	Randomised placebo-controlled trial set in India Primary aim was to evaluate if the addition of *M w* to standard TB chemotherapy would reduce the time to intercostal tube drainage (ICTD) removal in participants with TB hydropneumothoraces Sequence generation: no information provided Allocation concealment: no information provided Blinding: double-blind
Participants	34 Participants with TB hydropneumothoraces diagnosed using Light's criteria* 4 Smear positive pulmonary TB patients enrolled into *M w* arm 3 Smear positive pulmonary TB patients enrolled into control arm Limited baseline data provided
Interventions	Intervention: *M w* 0.2 ml on day zero, followed by 0.1 ml on days 15, 30, 60, 120, 180, and until completion of TB chemotherapy Control: placebo administered as above All participants received standard TB treatment as per WHO guidelines Category I: rifafour, isoniazid, pyrazinamide and ethambutol (RHZE) for two months; and rifampicin and isoniazid for four months Category II: streptomycin plus RHEZ (SRHEZ) for two months; RHEZ for one month; and HER for five months
Outcomes	1. Resolution of hydropneumothorax and removal of ICTD 2. Sputum conversion 3. Weight gain
Notes	This paper was presented as a poster presentation at the TB Vaccines for the World Conference in 2006 Limited data is provided as regards the methodology of the trial The authors were contacted in this regard, but no reply was received *According to Light's criteria (Light 1972), a pleural effusion is likely exudative if at least one of the following exists: The ratio of pleural fluid protein to serum protein is greater than 0.5;

	The ratio of pleural fluid LDH to serum LDH is greater than 0.6; or Pleural fluid LDH greater than 0.7 times the normal upper limit for serum

RISK OF BIAS TABLE

Item	Judgment	Description
Adequate sequence generation?	Unclear	No information provided
Allocation concealment?	Unclear	No information provided
Blinding? (Primary outcome: Sputum conversion)	Yes	Double-blind
Blinding? (Secondary outcome: Adverse events)	Unclear	Double-blind Even though it is not stated clearly, the only way there can be a double blind is if a placebo was administered and neither the participant nor the clinician knew which treatment was being administered
Incomplete outcome data addressed?	Yes	No missing data
Free of selective reporting?	Yes	Study protocol is not available but it is clear that the presented reports include all expected outcomes
Free of other bias?	Unclear	Selection or referral bias: unusual collection of TB participants, with only four category I participants and 30 category II participants Clinical bias: assessment of severity of hydropneumothoraces and timing of ICTD removal were clinician dependant

PATEL 2002

Methods	Quasi-randomised controlled pilot study set in Ahmedabad, India Sequence generation: no information provided Allocation concealment: no information provided Blinding: single-blind
Participants	OVERALL 134 Consecutive smear positive pulmonary TB participants (102 males and 32 females; mean age 36.2 years (range 15 to 75 years)) *M w* arm: 69 Control arm: 65 CATEGORY I Total: 58 participants *M w* arm: 20 Control arm: 38 CATEGORY II Total: 76 participants *M w* arm: 49 Control arm: 27
Interventions	Intervention: 0.2 ml (0.1 ml in each deltoid) *M w* intradermal injection given at day zero,

	followed by 0.1 ml fortnightly for two months Control: no placebo given All participants received standard TB treatment as per WHO guidelines Category I: RHZE for two months and RH for four months Category II: SRHEZ for two months, RHEZ for one month, and HER for five months
Outcomes	1. Sputum conversion 2. Adverse reactions
Notes	Inequality in number of male to female participants and the ratio of category I to category II participants, give the impression that there was an error in randomisation.

RISK OF BIAS TABLE

Item	Judgment	Description
Adequate sequence generation?	No	No information has been provided as regards randomisation The unequal numbers of male to female participants makes one suspicious of the method of randomisation Failure to stratify or prespecify the groups in terms of category I versus category II has resulted in unequal numbers of participants in comparative groups
Allocation concealment?	Unclear	No information provided
Blinding? (Primary outcome: Sputum conversion)	Yes	Single-blind Laboratory technician blinded to treatment allocation
Blinding? (Secondary outcome: Adverse events)	No	Single-blind Insufficient for the assessment of adverse events
Incomplete outcome data addressed?	Yes	No missing data
Free of selective reporting?	Yes	Study protocol is not available, but it is clear that the published reports include all expected outcomes
Free of other bias?	No	Selection or referral bias: more males than females, and more category II than category I participants

PATEL 2003

Methods	Follow-up of selected group (category II) from Patel 2002 pilot study Quasi-randomised controlled pilot study set in Ahmedabad, India Sequence generation: no information provided Allocation concealment: no information provided Blinding: single-blind
Participants	134 Consecutive smear positive pulmonary TB participants *M w* arm: 69 Control arm: 65 CATEGORY II Total: 76 participants *M w* arm: 49 Control arm: 27

	No additional baseline data available
Interventions	Intervention: 0.2 ml (0.1 ml in each deltoid) *M w* intradermal injection given on day zero, followed by 0.1 ml fortnightly for two months Control: no placebo given All participants received standard TB treatment as per WHO guidelines Category II: SRHEZ for two months, RHEZ for one month, and HER for five months
Outcomes	1. Sputum conversion
Notes	Presented as a separate paper; but participants are from the Patel 2002 paper It is unclear whether category II group of participants was a prespecified subgroup

RISK OF BIAS TABLE

Item	Judgment	Description
Adequate sequence generation?	No	No information has been provided as regards randomisation The unequal numbers of male to female participants makes one suspicious of the method of randomisation Failure to stratify or prespecify groups in terms of category I versus category II has resulted in unequal numbers of participants in comparative groups
Allocation concealment?	Unclear	No information provided
Blinding? (Primary outcome: Sputum conversion)	Yes	Single-blind Laboratory technician blinded to treatment allocation
Blinding? (Secondary outcome: Adverse events)	No	Single-blind Insufficient for the assessment of adverse events
Incomplete outcome data addressed?	Yes	No missing data
Free of selective reporting?	No	Study protocol is not available The published reports do not include all expected outcomes
Free of other bias?	No	Selection or referral bias: more males than females Category II subgroup was not prespecified

FOOTNOTES

CHARACTERISTICS OF EXCLUDED STUDIES

CHADDA

Reason for exclusion	Not a randomised controlled or quasi-randomised controlled trial

KATIYAR

Reason for exclusion	Not a randomised controlled or quasi-randomised controlled trial

KATOCH 2008

Reason for exclusion	Not a randomised controlled or quasi-randomised controlled trial

MARTHUR 2006

Reason for exclusion	Not a randomised controlled or quasi-randomised controlled trial

ZHOU 2002

Reason for exclusion	Intervention used was Bacillus Calmette Guerin (BCG), not *M w*

FOOTNOTES

CHARACTERISTICS OF STUDIES AWAITING CLASSIFICATION

FOOTNOTES

CHARACTERISTICS OF ONGOING STUDIES

NCT00265226

Study name	Efficacy and safety study of immunomodulator (*Mycobacterium w*) as an adjunct therapy in category-II pulmonary tuberculosis along with assessment of immunological parameters
Methods	In progress Interventional, treatment, randomised, double-blind (subject and investigator), placebo controlled, parallel assignment, safety and efficacy study
Participants	Category II pulmonary TB participants who meet the eligibility criteria
Interventions	Intervention: intradermal administration of *Mycobacterium w,* total of 6 doses given 0.2 ml at baseline and then 0.1 ml after interval of two weeks, up to eight weeks Control: placebo Category II TB chemotherapy according to guidelines
Outcomes	PRIMARY OUTCOME MEASURES The time to sputum conversion SECONDARY OUTCOME MEASURES Adverse reactions (assessment of safety) Participant's and physicians' global assessment of the clinical cure
Starting date	March 2005 until December 2010
Contact information	Surendra K Sharma, M.D., Ph.D sksharma@aiims.ac.in
Notes	On correspondence, Dr. Sharma had no preliminary data available

NCT00341328

Study name	Efficacy and safety of immunomodulator *(Mycobacterium w*) as an adjunct therapy in category I pulmonary tuberculosis and along with assessment of immunological parameters

Methods	In progress Treatment, randomised, double-blind (subject and investigator), placebo controlled, parallel assignment, safety and efficacy study
Participants	INCLUSION CRITERIA Participants of either sex aged between 18 to 60 yrs Newly diagnosed pulmonary TB cases with at least two sputum samples that are positive on sputum microscopy Participants willing to give informed consent EXCLUSION CRITERIA Known hypersensitivity to category I TB chemotherapy Known history of MDR and XDR TB (patients with *Mycobacterium tuberculosis* resistant to one or more drugs will be excluded) Secondary immunodeficiency states: HIV, organ transplantation, diabetes mellitus, malignancy, treatment with corticosteroids Hepatitis B and C positivity Participants with known extrapulmonary TB Currently receiving cytotoxic therapy, or having received it within the last three months Pregnancy and lactation Participants with a known seizure disorder Participants with known symptomatic cardiac disease, such as arrhythmias or coronary artery disease Participants with abnormal renal function Participants with abnormal hepatic function (bilirubin = 1.5 mg/dl; AST, ALT, SAP more than 1.5 x ULN; PT = 1.3x control) Participants with haematological abnormalities Seriously ill and moribund patients with the complications of low lung reserve, marked tachypnoea, chronic cor pulmonale, congestive cardiac failure, BMI<15, and severe hypoalbuminaemia (< 2.5 g/dl) Participants unlikely to survive for more than six months Participants unable to comply with the treatment regimen Participants with a history of alcohol or drug abuse
Interventions	Intervention: Intradermal injection of *Mycobacterium w* A total of 6 doses are given: 0.2 ml at baseline and then 0.1 ml after interval of two weeks up to eight weeks Control: placebo
Outcomes	PRIMARY OUTCOME MEASURES The time to sputum conversion SECONDARY OUTCOME MEASURES Adverse reactions Participant's and physicians' global assessment of clinical cure
Starting date	March 2007
Contact information	Surendra K Sharma, M.D., Ph.D sksharma@aiims.ac.in
Notes	Nil

NTC00810849

Study name	A pilot trial of adjunctive prednisolone and *Mycobacterium w* immunotherapy in tuberculous pericarditis

Methods	Interventional, treatment, randomised, double-blind (participant, caregiver, investigator, outcomes assessor), placebo controlled, factorial assignment, safety and efficacy study
Participants	INCLUSION CRITERIA Participants with a suspected tuberculous pericarditis will be eligible if they meet all three of the following criteria: A confirmed pericardial effusion on echocardiography; Evidence of definite* or probable** tuberculous pericarditis; and Within one week of starting of anti-tuberculous treatment. EXCLUSION CRITERIA Presence of an alternative cause of pericardial disease e.g. penetrating chest trauma in the preceding 12 months, or malignancy Use of corticosteroids within the previous month Hypersensitivity or allergy to the *Mycobacterium w* vaccine Pregnancy Age < 18 years
Interventions	PREDNISOLONE / PLACEBO Intervention: six-week tapering course of prednisolone Control: same number of identically-coated placebo tablets Prednisolone and placebo will be supplied as 5 mg identical tablets and given at a dosage of 120 mg/day in the first week, followed by 90 mg/day in the second week, 60 mg/day in the third week, 30 mg/day in the fourth week, 15 mg/day in the fifth week, and 5 mg/day in the sixth week *MYCOBACTERIUM w* / PLACEBO Intervention: five doses of 0.1 ml of *Mycobacterium w* intradermally (on enrolment, at two weeks, four weeks, six weeks, and three months) Control: Identical regiment of normal saline placebo injections
Outcomes	PRIMARY OUTCOME MEASURES Composite end-point of death, constriction, or cardiac tamponade requiring pericardial drainage SECONDARY OUTCOME MEASURES Safety of immuno-modulator treatment Long-term feasibility of conducting a multi-centre trial in Africa and India
Starting date	December 2008 to December 2011
Contact information	Professor Bongani Mayosi bongani.mayosi@uct.ac.za Dr. Mpiko Nstekhe mpiko.ntsekhe@uct.ac.za
Notes	The pilot phase of this study has been successfully completed, and the investigators have commenced the full study

DATA AND ANALYSES

1 SPUTUM CONVERSION

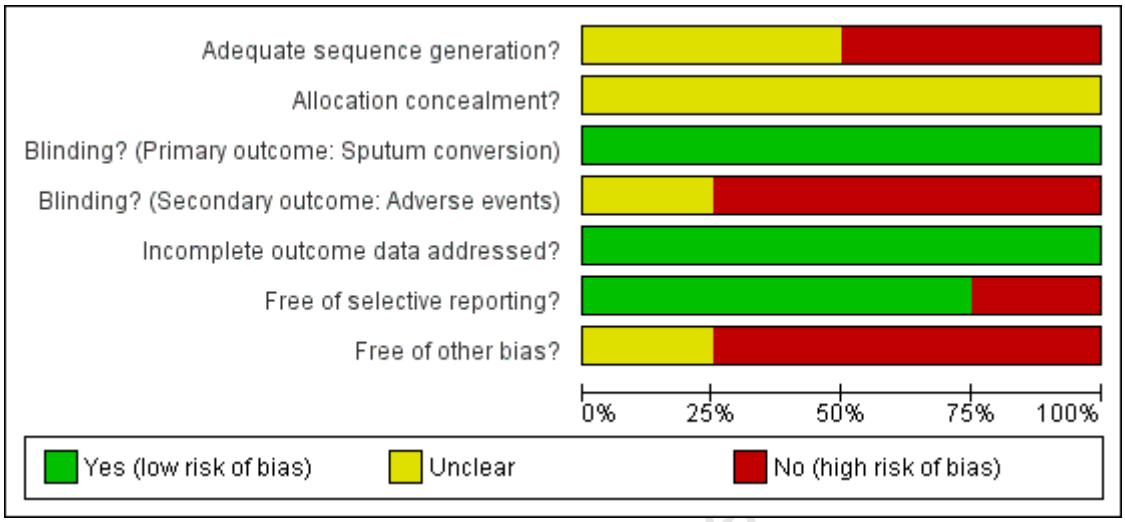
Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
1.1 Sputum negative at Day 15	3	341	Risk Ratio (M-H, Random, 95% CI)	2.31 [1.75, 3.06]
1.1.1 Category I TB	3	158	Risk Ratio (M-H, Random, 95% CI)	2.25 [1.64, 3.09]
1.1.2 Category II TB	3	176	Risk Ratio (M-H, Random, 95% CI)	2.45 [1.33, 4.51]
1.1.3 Uncertain Category I or II TB	1	7	Risk Ratio (M-H, Random, 95% CI)	5.60 [0.39, 79.70]
1.2 Sputum negative at Day 30	3	341	Risk Ratio (M-H, Random, 95% CI)	1.83 [1.12, 2.98]
1.2.1 Category I TB	2	158	Risk Ratio (M-H, Random, 95% CI)	1.34 [1.07, 1.66]
1.2.2 Category II TB	2	176	Risk Ratio (M-H, Random, 95% CI)	2.28 [1.37, 3.77]
1.2.3 Uncertain Category I or II	1	7	Risk Ratio (M-H, Random, 95% CI)	7.20 [0.53, 97.83]
1.3 Sputum negative at Day 60	3	341	Risk Ratio (M-H, Random, 95% CI)	1.38 [0.99, 1.92]
1.3.1 Category I TB	2	158	Risk Ratio (M-H, Random, 95% CI)	1.17 [0.88, 1.56]
1.3.2 Category II TB	2	176	Risk Ratio (M-H, Random, 95% CI)	1.50 [1.10, 2.03]
1.3.3 Uncertain Category I and II TB	1	7	Risk Ratio (M-H, Random, 95% CI)	2.40 [0.66, 8.79]
1.4 Sputum negative at Day 120	4	183	Risk Ratio (M-H, Random, 95% CI)	1.35 [1.05, 1.72]
1.4.2 Category II TB	3	176	Risk Ratio (M-H, Random, 95% CI)	1.48 [1.15, 1.91]
1.4.3 Uncertain category I or II TB	1	7	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.62, 1.60]
1.5 Sputum negative after day 120	2	176	Risk Ratio (M-H, Random, 95% CI)	1.34 [1.11, 1.62]
1.5.2 Category II TB	2	176	Risk Ratio (M-H, Random, 95% CI)	1.34 [1.11, 1.62]

2 MORBIDITY AND MORTALITY

Outcome or Subgroup	Studies	Participants	Statistical Method	Effect Estimate
2.1 Mortality	1		Risk Ratio (M-H, Random, 95% CI)	No totals
2.2 Adverse events	1		Risk Ratio (M-H, Random, 95% CI)	No totals

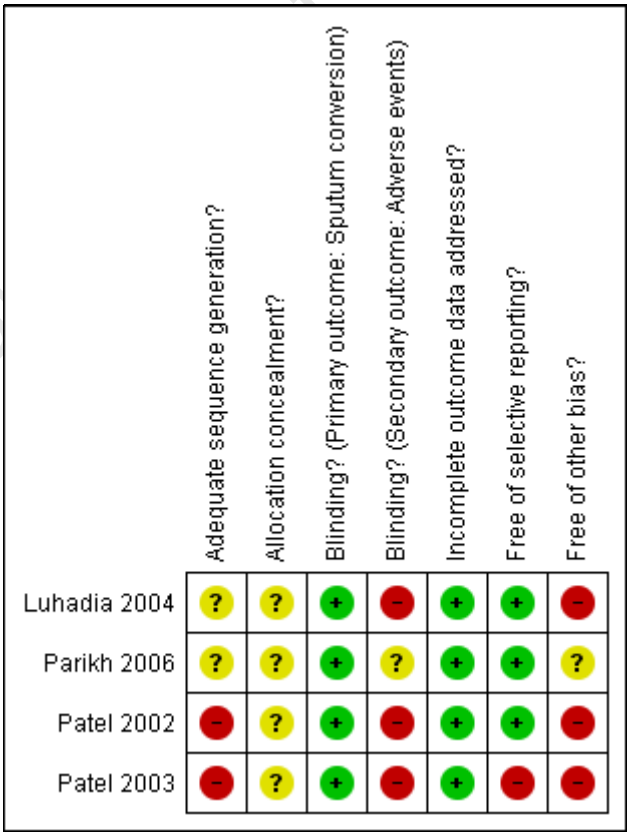
FIGURES

FIGURE 2



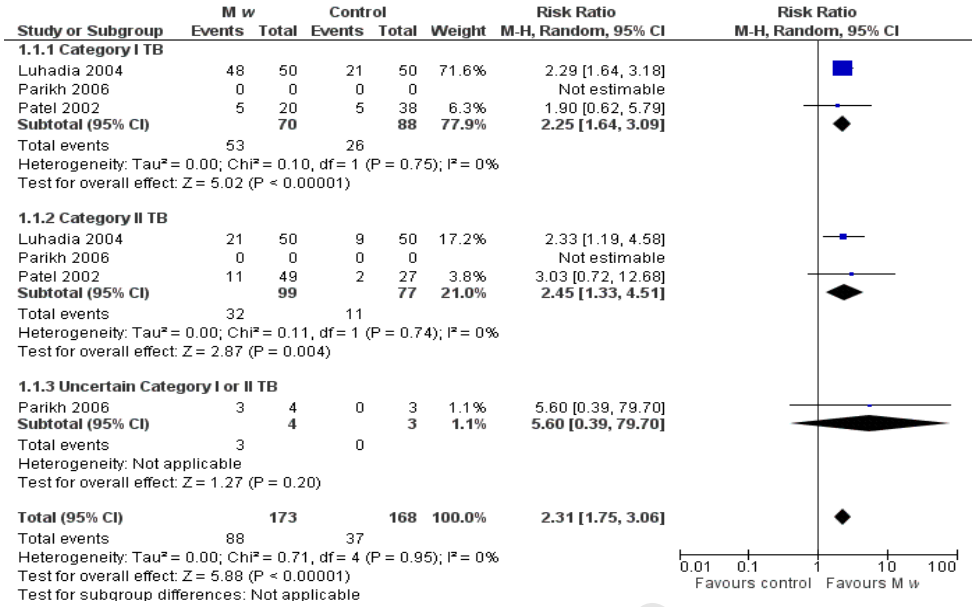
Risk of bias graph: authors' judgements regarding each risk of bias item presented as percentages across all included studies.

FIGURE 3



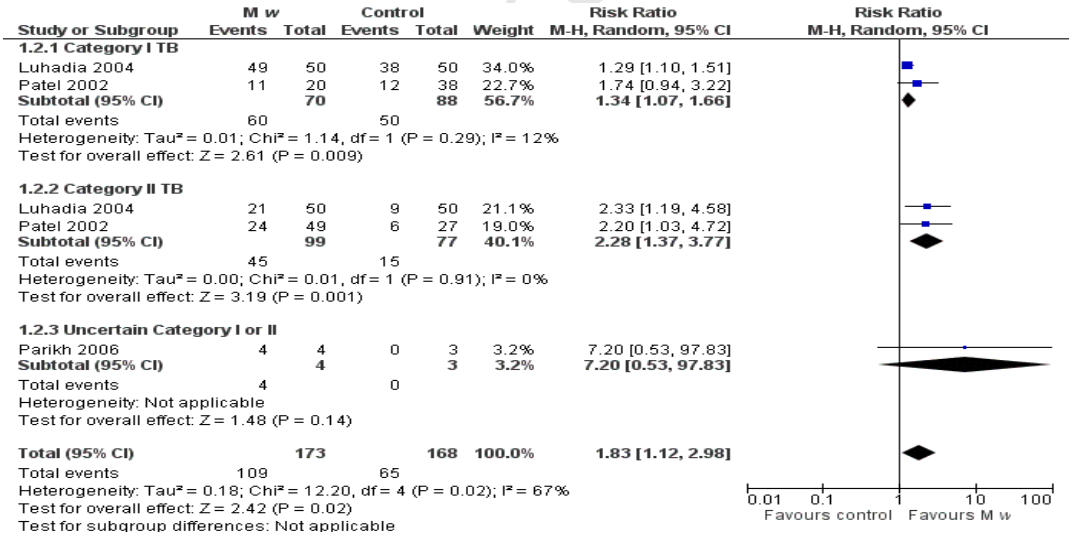
Risk of bias summary: authors' judgements regarding each risk of bias item for each included study.

FIGURE 4 (ANALYSIS 1.1)



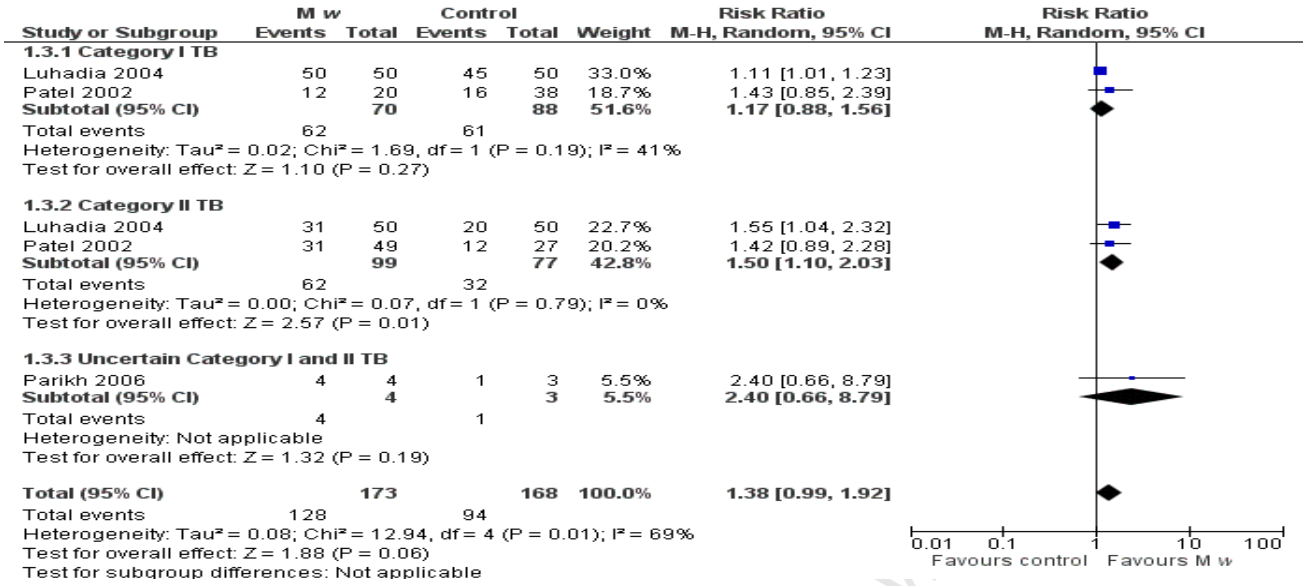
Forest plot of comparison: 1 Sputum Conversion, outcome: 1.1 Sputum negative at Day 15.

FIGURE 5 (ANALYSIS 1.2)



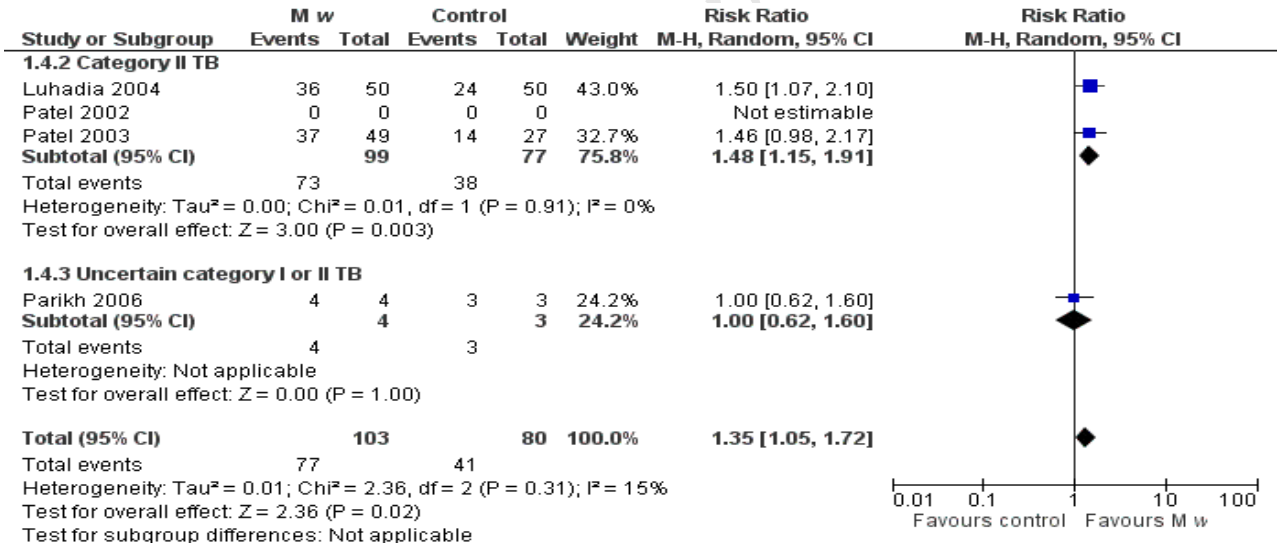
Forest plot of comparison: 1 Sputum Conversion, outcome: 1.2 Sputum negative at Day 30.

FIGURE 6 (ANALYSIS 1.3)



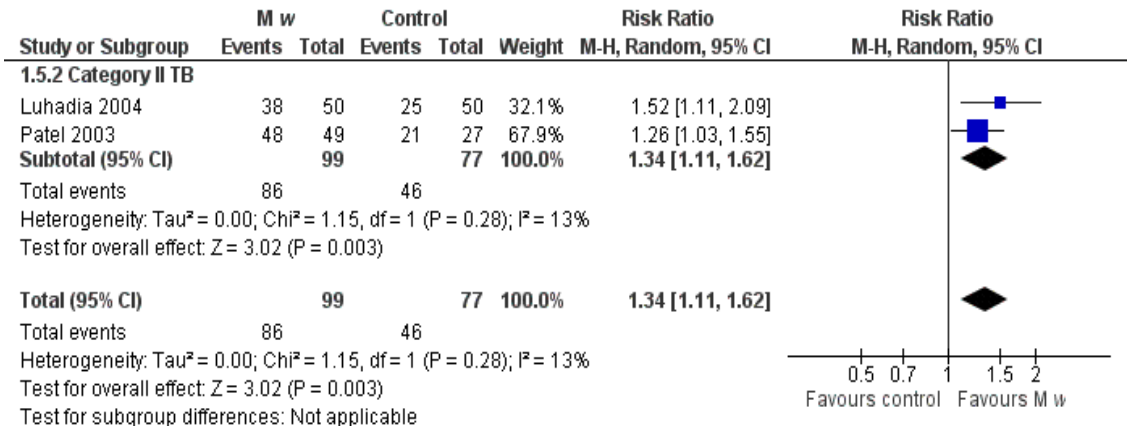
Forest plot of comparison: 1 Sputum Conversion, outcome: 1.3 Sputum negative at Day 60.

FIGURE 7 (ANALYSIS 1.4)



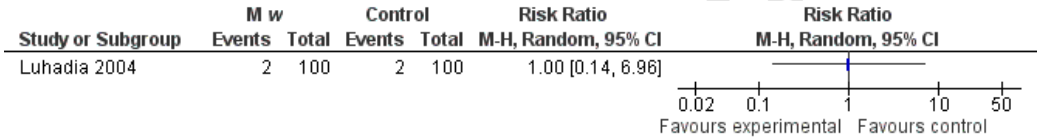
Forest plot of comparison: 1 Sputum Conversion, outcome: 1.4 Sputum negative at Day 120.

FIGURE 8 (ANALYSIS 1.5)



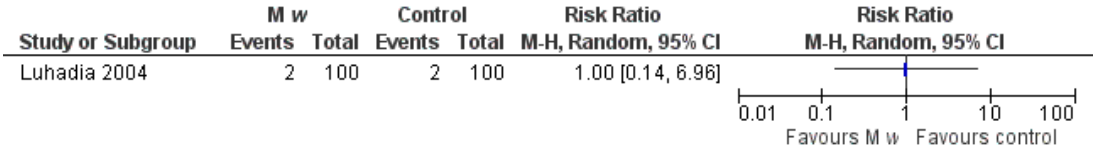
Forest plot of comparison: 1 Sputum Conversion, outcome: 1.5 Sputum negative after day 120.

FIGURE 9 (ANALYSIS 2.1)



Forest plot of comparison: 2 Morbidity and Mortality, outcome: 2.1 Mortality.

FIGURE10 (ANALYSIS 2.2)



Forest plot of comparison: 2 Morbidity and Mortality, outcome: 2.2 adverse events (Accelerated Local Skin Reaction)

MYCOBACTERIUM *W* ADJUVANT IMMUNOTHERAPY IN PULMONARY TUBERCULOSIS

DATA EXTRACTION SHEET

Effect of an Immunomodulator Containing Mycobacterium W on Sputum Conversion in Pulmonary Tuberculosis

Naresh Patel, MM Deshpande, Maya Shah

Study Verification	Meets inclusion criteria
Methods	Pilot study
	Included 134 consecutive participants (sputum positive pulmonary tuberculosis – new(fresh) cases as well as retreatment cases
	Randomised to usual TB treatment plus Mycobacterium w, or TB treatment only
	M w administered on day 1, then fortnightly. Not specified for how long.
	Sputum collected before treatment, and then at Days 15, 30, 45 and 60 for examination. Laboratory examiner was blinded to type and duration of treatments.
Participants	134 consecutive sputum positive patients
	69 randomised to M w (21= 1+sputum positive, 13= 2+, 35=3+)
	65 randomised to usual therapy (24=1+, 11=2+, 30=3+)
Interventions	69 received standard TB Rx and M w. 0.2ml loading dose (0.1ml in each deltoid). Then 0.1ml fortnightly.
	65 of control group received standard TB treatment. No placebo vaccine.
Outcomes and results	M w treatment group had a significantly faster sputum conversion rate at day 15, 30, and 45 for all groups of sputum positivity, and for both new (fresh) and retreatment participants. The sputum conversion rate achieved at day 60 in control was achieved at day 30 in M w group for sputum that was 1+. M w pre-poned sputum conversion by at least 30 days.
	No major adverse events reported. Skin induration and ulceration was self-limiting.

	CAT I cure rates: Mw 100% 19/19 Control 94.4% 34/36 CAT II cure rates: Mw 48/49 97.9% Control 21/27 77.7%
Notes	No comment about hard outcomes: all cause mortality or TB death. No placebo vaccine given. No comparisons given for 2+ sputa Only graphs provided. No statistical tables. Only pilot study Small numbers More retreatment patients in the control group. Sub-group analysis done after initial randomisation. Duration of vaccination not stated No comment about compliance (drop outs or drop ins).

Data table

	1+		3+		Retreatment		Overall	
	M w	**Control**	**Mw**	**Control**	**Mw**	**Control**	**Mw**	**Control**
Number	21	24	35	30	49	27	69	65
Day 15	57%	22%	13%	6%	22%	8%	21%	12%
Day 30	78%	34%	35%	10%	49%	21%	43%	28%
Day 45	92%	44%	35%	13%	56%	32%	50%	32%
Day 60	99%	78%	43%	17%	62%	43%	57%	43%

Sputum conversion: CAT-I vs. CAT-II

Days	CAT I		CAT II	
	Mw	Control	Mw	Control
Day 15	25%	13.1%	22.4%	7.4%
Day 30	55%	31.5%	48.9%	22.2%
Day 45	60%	34.2%	55.1%	33.3%
Day 60	60%	42.1%	63.2%	44.4%
Day 90	95%	84.2%	71.4%	48.1%
Day 120			75.5%	51.8%

RISK OF BIAS

Item	Judgment	Description
Adequate sequence generation	Unsure	Details not provided. Authors did not respond to correspondence
Allocation concealment	Unsure	
Blinding	Yes - Single	Laboratory technician was blinded to treatment type and duration
		Participants not blinded because no placebo
		Because no clinical outcomes measured no blinding of participants or clinical examiners
Incomplete outcome data addressed?	Yes	All allocated participants were accounted for, for the full duration of the study.
Free of selective reporting?	No	No comments made about 2+ sputum group. Actual values and statistical tables, as well as statistical methods of analysis not provided.
Free of other bias?	Unsure	

OUTCOMES

Primary	
All-cause mortality	Not assessed
Death from tuberculosis	Not assessed
Sputum culture negative at 2 months and at the end of anti-tuberculous chemotherapy	Yes
Rate of resolution of pleural effusion or other effusive forms of extrapulmonary tuberculosis.	Not applicable

Secondary outcomes	
Serious adverse reactions, i.e. fatal, life threatening or requiring hospitalisation	None
Adverse events related to the immunotherapy	Only self limiting events reported
Systemic adverse events, e.g. fever	None
Immunological adverse events, e.g. increase in HIV viral load	Not assessed

MYCOBACTERIUM *W* ADJUVANT IMMUNOTHERAPY IN PULMONARY TUBERCULOSIS

DATA EXTRACTION SHEET

Improved Cure Rates in Pulmonary Tuberculosis Category II (Retreatment) with Mycobacterium w

Naresh Patel, SB Tripathi

Study Verification	Meets Criteria (Sub-group analysis)
Methods	Post-Hoc analysis
	Records review of TB Category II (retreatment) participants that were originally enrolled into Mw versus control group (previous study mentioned)
	Participants randomised to Mw vaccine all received 0.2ml Mw (0.1ml in each deltoid) at day 1, then 0.1ml fortnightly for 2 months. No placebo given to control group.
	Sputum analysis for bacterial load were analysed at 3.5 and 8 months (for sputum negative participants) and at 4, 6 and 9 months (for sputum positive participants). Laboratory technician was blinded to treatment type and duration.
Participants	Follow-up of retreatment cases (1 month of TB treatment previously taken: Defaulters, previous TB,
	Total number of retreatment cases: 76
	Number in the Mw group: 49 (12=1+, 17=2+,20=3+)
	Number in control group: 27 (11=1+, 2=2+, 14=3+)
Interventions	Participants randomised to Mw vaccine all received 0.2ml Mw (0.1ml in each deltoid) at day 1, then 0.1ml fortnightly for 2 months. No placebo given to control group.
	All participants completed treatments.

Outcomes and Results	Sputum clearance at 3 months, 4 months, and 2 months post intensive phase (5 or 6 months depending on whether intensive therapy was 3 or 4 months) were all statistically significantly better in the Mw group. Mw also had statistically significantly lower treatment failure rate. No major adverse events were reported.
Notes	No comment about hard outcomes: all cause mortality or TB death. No placebo vaccine given. No comparisons given for 2+ sputa Only graphs provided. No statistical tables or methods of analysis provided. Large number of total cohort turned out to be retreatment patients (76/134 (56.7%)). This is unusual for enrolling consecutive patients. Would expect the rates of new (fresh cases) to be equal to or more than retreatment cases. Likely secondary to referral bias or selection bias. Possibly a reflection of too few participants enrolled. Post hoc analysis of non a priori non specified sub group. Unequal numbers in the two comparative groups. Much fewer participants in the control group and very small numbers, makes the statistical value of the data questionable and non-generalisable.

Data table

	M w	**Control**
Retreatment Number	49	27
Sputum negative		
3 months	35 (71.4%)	13(48%)
4 months	37 (75%)	14 (51.4%)
2 months post intensive phase	48(97.9%)	21 (77.7%)
Overall	48 (97.9%)	21 (77.7%)
Treatment Failure	1 (2.0%)	6 (22.2%)

RISK OF BIAS

Item	Judgment	Description
Adequate sequence generation	No	Post hoc analysis of a non- a prior (non-pre-specified) subgroup
Allocation concealment	Unsure	Not specified
Blinding	Yes - single	Laboratory technician was blinded to type and duration of therapy. Clinical reviewers were not blinded to Mw therapy because no placebo was given.
Incomplete outcome data addressed?	N/A	All participants completed treatment
Free of selective reporting?	No	Subgroup analysis No reporting on 2+ sputum conversion No formal reporting of adverse events. Because no placebo used, reporting of adverse events would apply only to Mw group.
Free of other bias?		Unsure

OUTCOMES

Primary	
All-cause mortality	Not assessed
Death from tuberculosis	Not assessed
Sputum culture negative at 2 months and at the end of anti-tuberculous chemotherapy	Yes
Rate of resolution of pleural effusion or other effusive forms of extrapulmonary tuberculosis.	Not applicable

Secondary outcomes	
Serious adverse reactions, i.e. fatal, life threatening or requiring hospitalisation	None reported
Adverse events related to the immunotherapy	None reported
Systemic adverse events, e.g. fever	None reported
Immunological adverse events, e.g. increase in HIV viral load	Not Applicable

DATA EXTRACTION SHEET

Role of Mw Vaccine in management of Hydropneumothorax as an adjuvant to ATT (Anti Tuberculosis Therapy) with ICTD (Intercostal Tube Drainage). Paper presented at: TBV 2006 - TB Vaccines for the World, 2006; Vienna,

Austria.

Parikh H, Shah N, Tewari T, Parswani JP, Maseeh A.

Study Verification	Yes
Methods	Randomised, double blind, placebo-controlled, comparative clinical trial.
	CAT I (new diagnosis) and CAT II (Retreatment) were treated as per WHO guidelines
	Both smear + and smear – patients were included
	Participants received Mw intradermally 0.2ml on day 0 followed by 0.1ml on days 15,30,60,120, 180 till the end of ATT
	Primary aim was to evaluate if the addition of M w would reduce the time to ICTD removal
	Light's criteria was used to detect exudative effusions
	Distinction between TB and bacterial effusions was on the basis of lymphocyte/neutrophil ratio, ADA, LDH levels
	Participants with bacterial infections were treated accordingly
Participants	Both CAT I and CAT II TB patients were included
	Both smear positive and smear negative patients were included
	Diagnosis of TB exudative effusions was made using Light's criteria
	Bacterial effusions were excluded
	Total of 18 participants were randomised to Mw + ATT + ICTD(4/18 smear positive, 14/18 smear negative, 2/18 CAT I, 16/18 CAT II)
	Total of 16 participants were randomised to ATT + ICTD (control arm) (3/16 smear positive, 13/16 smear negative, 2/16 CAT I, 14/16 CAT II)

Interventions	Participants received Mw intradermally 0.2ml on day 0 followed by 0.1ml on days 15,30,60,120, 180 till the end of ATT CAT I patients received RHZE (2 months) + RH(4months). CAT II patients (smear +ve & smear – ve) received SHREZ (2 months) + HREZ (1months)+ HER (5 months).
Outcomes and Results	M w arm had faster removal of ICTD with 72% removal within 22 days vs. 37.5% in the control arm Student's t-Test used to compare number of days taken for removal of ICTD between 2 groups: Group A had a faster (15.1+- 8.58 days) removal as compared to control group B (43.9 +- 31.6 days) with p<0.001 Sputum conversion was faster in group A (18.8 +- 7.5 days) vs. Group B 96.7+-40.4 days) p<0.012 Also demonstrated faster weight gain in Group A vs. Group B
Notes	No comment about mortality Very small study Unreliable sputum conversion analysis because so few participants were sputum positive to start with (7/34) Unusually high number of CAT II patients (?selection bias, referral bias) No comment about adverse reactions to Mw Indications or evaluation of effusion resolution not stipulated Labeled as a double blind study, but no mention of a placebo vaccine given to control group

Data Tables

Number of Participants	Total	Smear Positive	Smear Negative	CAT I	CAT II
Group A Mw/ATT/ICTD	18	4	14	2	16
Group B	16	3	13	2	14

ATT/ICTD					
Total	34	7	27	4	30

Days to ICTD Removal	Group A: Number of pt	Group B: Number of pt
0-7	3	1
8-14	4	2
15-21	6	3
22-30	4	3
31-60	1	3
61-90	0	2
91-120	0	2

RISK OF BIAS

Item	Judgment	Description
Adequate sequence generation	Unsure	Not detailed in paper
Allocation concealment	Unsure	Not detailed in paper
Blinding	Double-blind? Inadequate	No placebo vaccine given. Unlike in other studies were primary outcome was laboratory determined (sputum smears), decisions with regard to when ICTD needs to be removed in clinically determined. If participants did not receive a placebo vaccine then examiners and participants would know whether or M w was administered
Incomplete outcome data addressed?	N/A	
Free of selective reporting?	Yes	No exclusions in the study
Free of other bias?	No	Clinician bias ?Referral bias

Selection bias

OUTCOMES

Primary
All-cause mortality
Death from tuberculosis
Sputum culture negative at 2 months and at the end of anti-tuberculous chemotherapy
Rate of resolution of pleural effusion or other effusive forms of extrapulmonary tuberculosis.

Secondary outcomes
Serious adverse reactions, i.e. fatal, life threatening or requiring hospitalization
Adverse events related to the immunotherapy
Systemic adverse events, e.g. fever
Immunological adverse events, e.g. increase in HIV viral load

DATA EXTRACTION SHEET

Mycobacterium w as an adjuvant to chemotherapy in management of pulmonary tuberculosis

Dr. Luhadia S., Dr. Saugat R., Dr. Joshi V.

Study Verification	Meets inclusion criteria
Methods	Placebo controlled, single blind study 200 participants (100 New TB / Category I) (100 Retreatment / Category II) randomised to Mw or placebo plus DOTS therapy for TB 0.1 ml Mw or saline placebo given intra-dermally on day 0, 15, 30, 60 and then 2 monthly until treatment was complete TB was diagnosed on sputum examination Participants were followed up at 0, 15, 30, 60, 120 days and at the end. Sputum smears were done at each visit. X-rays were taken at 0, 60, 120 days and at the end.
Participants	Category I and Category II TB sputum smear positive. Category I (50 Mw: 3+=32, 2+=5, 1+=13) (50 placebo: 3+=28, 2+=9, 1+=13) Category II (50 Mw: 3+=34, 2+=6, 1+=10) (50 placebo: 3+=30, 2+=9, 1+=11)
Interventions	0.1 ml Mw or saline placebo given intradermally on day 0, 15, 30, 60 and then 2 monthly until treatment was complete
Outcomes and Results	<u>Death:</u> Category I Mw = nil Control=nil Category II Mw=2 Control=2 <u>Sputum conversion</u>: Mw group had significantly faster sputum conversion rates in

	both Category I and II participants versus placebo. Decreased time to sputum conversion by at least 45 days in both groups. <u>Cure:</u> Cure rate was improved by 25% in category II participants <u>Other:</u> Mw group also had improved weight gain, clinical improvement and radiological resolution <u>Adverse reactions</u>: No systemic adverse reactions seen with Mw Localised reaction documented in 2 Cat II participants receiving Mw *not stated number of participants that developed "Normal Local Reaction"= pustule 4-5 days, ulcer 7-10 days, scab 1 month
Notes	No comment on type of randomisation Unusual (but not impossible) to get exact numbers (50/50/50/50) across the 4 groups The nature of the single blind not stated. SURELY BOTH THE EXAMINER AND THE PATIENT would be blinded in a placebo control study. No comment about whether the lab tech were blinded or not. No details of type of statistical analysis provided. Excluded "Normal Local reaction" as an adverse event Small numbers Difficult to make a comment about mortality (but this was not a Primary objective) because so few events No comment about compliance (drop-outs / drop-ins) , missed doses etc.

RISK OF BIAS

Item	Judgment	Description
Adequate sequence generation	Unsure	Information not provided. Author did not respond to correspondence
Allocation concealment	Unsure	
Blinding	Yes	Single blind
Incomplete outcome data addressed?	No	Not discussed in paper (assumption is that all data was complete)
Free of selective reporting?	Yes	
Free of other bias?	Yes	

OUTCOMES

Primary	
All-cause mortality	Yes. Too few events to make a statistical assessment
Death from tuberculosis	Not specified
Sputum culture negative at 2 months and at the end of anti-tuberculous chemotherapy	Yes: Benefit
Rate of resolution of pleural effusion or other effusive forms of extrapulmonary tuberculosis.	No directly addressed. Radiological improvement was noted

Secondary outcomes		
Serious adverse reactions, i.e. fatal, life threatening or requiring hospitalization	Nil	
Adverse events related to the immunotherapy	Yes	2 = accelerated local reaction
Systemic adverse events, e.g. fever	Nil	
Immunological adverse events, e.g. increase in HIV viral load	Not measured	

Table 1 - Characteristics of Patients Studied

Parameters		Cat I		Cat II	
		M.w.	Control	M.w.	Control
		n=50	n=50	n=50	n=50
Average Age (Years)		36	36	33	37
Sex	M	38	36	39	40
	F	38	36	39	40
Average Wt. (Kg)	M	41	41	42	41
	F	34	31	34	34
Radiological Extent	Mild	10	9	5	3
	Moderate	33	31	11	14
	Severe	7	10	34	33
Grading of Sputum	+3	32	28	34	30
	+2	5	9	6	9
	+1	13	13	10	11

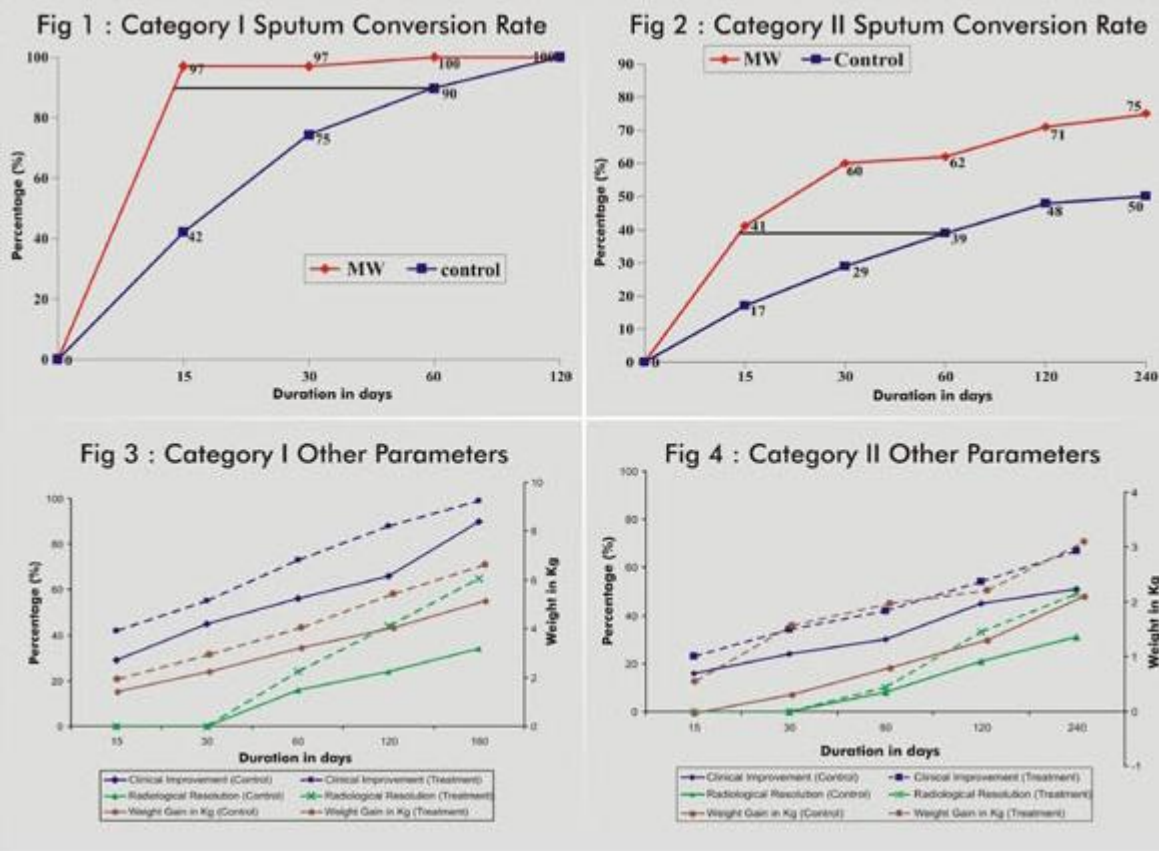
Table 2 - Death and Adverse Reaction

	Cat I	Cat II
Death		
M.w. group	Nil	2
Control group	Nil	2
Adverse Reactions		
M.w. group	Nil	2*
Control group	Nil	Nil

* Local site accelerated reaction

Table 3 - Chronology of Normal Local Reaction

Pustule	4-5 days
Ulcer	7-10 days
Scab	One month



Data table

	New		Retreatment	
	Mw	**Control**	**Mw**	**Control**
Number	50	50	50	50
Day 15	97%	42%	41%	17%
Day 30	97%	75%	60%	29%
Day 60	100%	90%	62%	39%
Day120			71%	48%
Day320			75%	50%

	New		Retreatment	
	Mw	**Control**	**Mw**	**Control**
Number	50	50	50	50
Death	0	0	2	2
Adverse Reaction				
Accelerated local rxn	0	0	2	2