

PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form ([see an example](#)) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below. Some articles will have been accepted based in part or entirely on reviews undertaken for other BMJ Group journals. These will be reproduced where possible.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Autoantibody Prevalence in Active Tuberculosis : Reactive or Pathognomonic?
AUTHORS	Shen, Chieh-Yu; Hsieh, Song-Chou; Yu, Chia-Li; Wang, Jann-Yuan; Lee, Li-Na; Yu, Chong-Jen

VERSION 1 - REVIEW

REVIEWER	Hojoon Sohn, MPH, PhD Candidate McGill University Department of Epidemiology and Biostatistics And 51st Infantry Division in the Army of the Republic of Korea The reviewer do not have any competing interest.
REVIEW RETURNED	16-Mar-2013

THE STUDY	<p>Introduction:</p> <p>1. In introducing the subject of the research - autoantibodies present in active TB patients that are unique in autoimmune disease - the authors should focus on how the research came about to propose the research hypothesis. The introduction section in current form does not express this important issue and it's rather vaguely stated in a fact reporting format.</p> <p>2. Actual study aim is stated adequately in the article focus section, but not clearly stated and is rather confusing in the introduction. The reviewer considers that the introduction section should be more tightly written to clearly link the research topic and the hypothesis/aim.</p> <p>Methods</p> <p>1. Patient selection Out of 933 potentially eligible cases, the reviewer is curious as to why only 100 patients were enrolled in the study. What is the patient selection criteria? This is very important part of the study design, which gives the readers idea about representation of the study population. Current draft does not describe this part well.</p> <p>2. What is the definition of the exposure?</p> <p>Exposed: active TB - pulmonary or active TB or both?</p> <p>Non-exposed: Non-TB patients</p> <p>Please confirm. Furthermore, why were there four cases of</p>
------------------	--

	<p>extrapulmonary TB included in the cases? Couldn't there be some differences in presence of autoantibodies in patients of pulmonary and extrapulmonary TB? Otherwise, this can be done in statistical analysis.</p> <p>3. Selection of the controls</p> <p>Stating that healthy medical staff members is not enough because the focus of the research is to compare the prevalence of autoantobodies bewteen the control and the cases, which would give the researchers an idea about the link between chronic TB and diagnosis and/or symptoms of immunological diseases. The reviewer questions as to why medical staff members were chosen as controls? Furthermore, the reviewer wonders what was the reason for deciding 1:1 case to control ratio?</p> <p>The authors should take more caution in describing limitations of choosing medical staff as controls, which may lead to selection bias - couldn't medical staff may be more prone to have higher prevalence of specific autoantibodies that the authors are trying to assess and compare considering the nature of their work environment?</p> <p>The authors could also consider matching methods (e.g. individual or frequency matching based on distributions of clinical parameters (attributes) of the cases. Considering that the study population is small, matching may be much more efficient and economical method to control for confounding. More on this in the 'methods' comments.</p> <p>2. Measurement</p> <p>It is not so clear in this current draft as to what is the effect measure? Comparison of the proportion of the level of autoantibody presence? Generally, in case control studies, researchers take odds ratio to describe the effect of causal factor on the disease.</p> <p>Clinical parameters, symptoms, adverse event definitions all are well stated, but the authors do not state in any way how this information is relevant to their research purpose and analysis.</p> <p>3. Statistical analysis</p> <p>Couldn't the authors have done regression analysis instead of doing simple comparative statistical analysis? Logistic regression analysis should be done to control for potential confounding factors that are assessed from the clinical parameters, unless the aim of the research is to also discover potential confounding factors that are relevant to the researcher's hypothesis. Furthermore, as described earlier, if matching was(or will be) done, matched pair analysis (specific to matching technique) should be done to adequately control for factors that were matched. Nonetheless, the reviewer feels that the current analytic technique described in this draft is not adequate to study the aim of the author's research and its goals. Subsequently, the authors should comment on the study power in this section to be more complete about their statistical analysis and its limitations.</p> <p>4. English & General format of the manuscript</p>
--	--

	<p>The authors must get this draft revised by a native English speaker with a proficient knowledge in this specific research area. In current written form, it is very difficult to follow and understand the overall research goals and the focus of the study.</p>
RESULTS & CONCLUSIONS	<p>Results</p> <p>One of the most important part of an epidemiological study is the table of statistical description of the study population. This is usually represented as Table 1 in the result section. This table should describe distribution of specific studying characteristics of cases and controls and this gives the readers an idea of whether or not recruitment of study sample was done well. Furthermore, this also gives the readers an idea whether or not the authors have used adequate statistical and analytic technique to control for uneven distribution of key attributing factors. The author do not have this important table which describes statistical distribution of key attributing factors such as age, sex, history of the disease specific to TB as well as immunological diseases the authors are studying (specific to the autoantibodies described in the study) etc. Please refer to other published case control studies for more information on this table.</p> <p>As described earlier in the methods section, author's analytic technique is rather primitive, which only allows the readers to understand differences between cases and controls in simple comparative form, which does not address confounding factors that may be present on the causal pathway that the authors are studying. In this current form, we are assessing the results that can be significantly biased from the 'truth' which the authors are trying to study. Therefore, the reviewer recommends that the authors consider analytic technique that can adjust for various biases that can be present in this current case control study (e.g. regression or matched analyses).</p> <p>Discussion</p> <p>Page 13, line 30 - what does the author intend to suggest? Increased serum autoantibodies in active TB patients are not diagnostic indication for autoimmune disease? This question seem to be resolved by the statement in the following paragraph; however, the sentence should be written more clealy to convey the readers of author's conclusions.</p> <p>Page 15, line 7-21 is very confusing.</p>
GENERAL COMMENTS	<p>While current draft (form as well as the research method) is not adequate for publication, the reviewer sees the important aspect of this research topic. With the major revision to the author's methods (statistical) and the actual draft of the manuscript (to adequately and promptly describe the author's research), the reviewer believes that the authors can provide very important scientific finding that can show whether or not the elevation of autoantibodies in TB patients are pathognomonic. This can certainly allow the clinician to better diagnose and manage autoimmune diseases and its related symptoms in chronic TB patients. For this reason, the reviewer recommends major revision for this manuscript.</p>
REVIEWER	<p>Tsutomu Takeuchi, MD.PhD. Professor and chief of Rheumatology, Division of Rheumatology,</p>

	Department of Internal Medicine, School of Medicine, Keio University, Tokyo, JAPAN
	I have no conflicts of interest with this manuscript.
REVIEW RETURNED	26-Mar-2013

GENERAL COMMENTS	<p>This is a well structured article and includes interesting topics on the positive relationship between active tuberculosis and serum autoantibody especially anti-CL antibody(IgG) or anti Scl-70 antibody. Nonetheless, there are several concerns that should be addressed.</p> <ol style="list-style-type: none"> 1. In the patients and methods section, the authors described that 100 patients were enrolled in this study. How did the authors select these patients? 2. The authors described limitation in the sample number on page 4, how did the authors set it in this study? 3. In the table 1, percentage of anti-Scl-70 in healthy control seems to be quite high as compared with normal population you showed. Did the both experiments utilize the same measurement methods? How did the authors set the cut off points of anti Scl-70 antibody? 4. In the table 1, percentage of anti-CL IgM antibody in healthy control is quite high as compared with general understanding. Are detection methods really appropriate? It has been known that rheumatoid factor can interfere with some ELISA system. In the cohort, did the authors check any association between the titer of RF and those of other autoantibodies? These information would help readers to understand the concerns above. 5. On page 16, disease name of progressive systemic sclerosis should be systemic sclerosis. 6. The authors concluded that TB culture test is recommended in the patients with high serum autoantibody titer and without rheumatic symptoms in TB endemic area. How did the authors explain the clinical setting for checking autoantibodies without any rheumatic symptoms. <p>Before considering the manuscript is acceptable for publication, the authors should answer clearly for the above issues and STROBE statement on page 28 and 29.</p>
-------------------------	--

VERSION 1 – AUTHOR RESPONSE

Responses to Dr. Hojoon Sohn

1.Introduction: In introducing the subject of the research - autoantibodies present in active TB patients that are unique in autoimmune disease - the authors should focus on how the research came about to propose the research hypothesis. The introduction section in current form does not express this important issue and it's rather vaguely stated in a fact reporting format. Actual study aim is stated adequately in the article focus section, but not clearly stated and is rather confusing in the introduction. The reviewer considers that the introduction section should be more tightly written to clearly link the research topic and the hypothesis/aim.

Answer: Thank you for your comment. We revised the Introduction to emphasize the purpose of our study. Previous studies have shown that sera from patients with active TB contain autoantibodies that are unique to autoimmune diseases. However, little is known regarding their clinical significance (i.e., pathognomonic, reactive, or incidental) in TB patients and the necessity of corticosteroid therapy. When TB patients sometimes present with non-specific symptoms like fever, malaise, or weight loss, clinicians may simultaneously order mycobacterial studies and autoimmune serology. Results of the latter usually become available earlier than results of the former. Thus, some TB patients may first be put on systemic corticosteroid rather than anti-tuberculosis treatment, rendering further dissemination of *Mycobacterium tuberculosis* bacilli. This prospective study was conducted to evaluate the prevalence and dynamic changes of autoantibodies in patients with active TB. The clinical significance of autoantibodies in this special population was investigated.

2.Method: 1.Patient selection Out of 933 potentially eligible cases, the reviewer is curious as to why only 100 patients were enrolled in the study. What is the patient selection criteria? This is very important part of the study design, which gives the readers idea about representation of the study population. Current draft does not describe this part well.

Answer: The prevalence of anti-nuclear antibody in the general population varies, from 9.4% in Japan (Hayashi, N. *Mod Rheumatol* 2008;18:8) to 20% in Israel (Elkayam, O. *Int J Tuberc Lung Dis* 2007;11:306). The prevalence in TB patients is 33% in Israel (Elkayam, O. *Int J Tuberc Lung Dis* 2007;11:306). To have a power of 0.8 and an alpha error of 0.05 in a two-sided test where the proportions of event cases in the two independent samples are 33% and 20%, the calculated sample size is 83 for each. Therefore, we enrolled 100 TB patients and 100 control subjects in this study. The TB patients were new cases of culture-confirmed TB diagnosed at the National Taiwan University Hospital from January 2007 to December 2009. We added this information in the Methodology of the revised manuscript.

3.What is the definition of the exposure? Exposed: active TB - pulmonary or active TB or both? Non-exposed: Non-TB patients. Please confirm.

Answer: Exposure in this study is "active TB", which may be further classified into pulmonary or extra-pulmonary according to the site of involvement. The non-exposed group consisted of 100 health care workers. We added these descriptions in the Methodology of the revised manuscript.

4.Furthermore, why were there four cases of extra-pulmonary TB included in the cases? Couldn't there be some differences in presence of autoantibodies in patients of pulmonary and extra-pulmonary TB? Otherwise, this can be done in statistical analysis.

Answer: Thank you for your comment. Patients with either pulmonary or extra-pulmonary TB are all TB patients. They are different simply because the site of disease involvement. Because the aim of this study is to investigate the prevalence and clinical significance of autoantibodies during the TB disease process, all patients with active TB were enrolled, including both pulmonary and extra-pulmonary TB. We agree with the reviewer that there are probably some differences in the presence of autoantibodies between the pulmonary and extra-pulmonary subgroups. However, the differences were not statistically significant, most likely due to the small sample size of the extra-pulmonary subgroup.

5.Selection of the controls. Stating that healthy medical staff members is not enough because the

focus of the research is to compare the prevalence of autoantibodies between the control and the cases, which would give the researchers an idea about the link between chronic TB and diagnosis and/or symptoms of immunological diseases. The reviewer questions as to why medical staff members were chosen as controls? Furthermore, the reviewer wonders what was the reason for deciding 1:1 case to control ratio? The authors should take more caution in describing limitations of choosing medical staff as controls, which may lead to selection bias - couldn't medical staff may be more prone to have higher prevalence of specific autoantibodies that the authors are trying to assess and compare considering the nature of their work environment? The authors could also consider matching methods (e.g. individual or frequency matching based on distributions of clinical parameters (attributes) of the cases. Considering that the study population is small, matching may be much more efficient and economical method to control for confounding. More on this in the 'methods' comments.

Answer: Thank you for your critical comments. The natural course of TB begins with exposure, followed by infection and development of the disease. In this study, we aimed to evaluate the prevalence and clinical significance of autoantibodies during the TB disease process, not just the infection by *Mycobacterium tuberculosis*. We selected the medical staff as control because TB exposure and infection are more common in the medical staff than in the general population. The household contacts of TB patients may also have high TB exposure and infection. However, if the relatives of TB patients were used as control, the results might be confounded by similar environment and genetic components as the TB cases. We added these considerations in the Methodology of the revised manuscript.

6.Measurement. It is not so clear in this current draft as to what is the effect measure? Comparison of the proportion of the level of autoantibody presence? Generally, in case control studies, researchers take odds ratio to describe the effect of causal factor on the disease. Clinical parameters, symptoms, adverse event definitions all are well stated, but the authors do not state in any way how this information is relevant to their research purpose and analysis.

Answer: We apologize for this. The aim of the study is to evaluate the prevalence and clinical significance of autoantibodies in the TB disease process. The prevalence of autoantibodies in health care workers was described to give a simple picture of the normal population. In the cohort of TB patients, initial serum levels and dynamic changes of autoantibodies were measured. Together with clinical characteristics, symptoms, and radiographic findings, we evaluated the clinical significance of autoantibodies. Since our findings suggest that elevation of autoantibody levels is not pathognomonic in active TB patients, multivariate analysis for risk factors of the presence of autoantibody was not performed.

7.Statistical analysis. Couldn't the authors have done regression analysis instead of doing simple comparative statistical analysis? Logistic regression analysis should be done to control for potential confounding factors that are assessed from the clinical parameters, unless the aim of the research is to also discover potential confounding factors that are relevant to the researcher's hypothesis. Furthermore, as described earlier, if matching was (or will be) done, matched pair analysis (specific to matching technique) should be done to adequately control for factors that were matched. Nonetheless, the reviewer feels that the current analytic technique described in this draft is not adequate to study the aim of the author's research and its goals. Subsequently, the authors should comment on the study power in this section to be more complete about their statistical analysis and its limitations.

Answer: We apologize for this oversight. In this study, the prevalence of autoantibodies in health care workers (the control group) was described to give a simple picture of the normal population. We

evaluated the clinical significance of autoantibodies in TB patients based on the analysis of clinical characteristics, presenting symptoms, radiographic manifestations, and initial serum levels and dynamic changes of the autoantibodies. The results suggest that elevated levels of autoantibodies are not pathognomonic in active TB patients. As such, multivariate analysis comparing TB patients and controls was not performed. We revised the Introduction and Methodology sections to clarify our study objectives. Thank you for your comment.

8.English & General format of the manuscript. The authors must get this draft revised by a native English speaker with a proficient knowledge in this specific research area.

Answer: A native English speaking medical doctor reviewed and proofread our revised manuscript prior to this submission.

9.Result. One of the most important part of an epidemiological study is the table of statistical description of the study population. This is usually represented as Table 1 in the result section. This table should describe distribution of specific studying characteristics of cases and controls and this gives the readers an idea of whether or not recruitment of study sample was done well. Furthermore, this also gives the readers an idea whether or not the authors have used adequate statistical and analytic technique to control for uneven distribution of key attributing factors. The author do not have this important table which describes statistical distribution of key attributing factors such as age, sex, history of the disease specific to TB as well as immunological diseases the authors are studying (specific to the autoantibodies described in the study) etc. Please refer to other published case control studies for more information on this table. As described earlier in the methods section, author's analytic technique is rather primitive, which only allows the readers to understand differences between cases and controls in simple comparative form, which does not address confounding factors that may be present on the causal pathway that the authors are studying. In this current form, we are assessing the results that can be significantly biased from the 'truth' which the authors are trying to study. Therefore, the reviewer recommends that the authors consider analytic technique that can adjust for various biases that can be present in this current case control study (e.g. regression or matched analyses).

Answer: Thank you for your instructive comments. As we mentioned previously, the prevalence of autoantibodies in health care workers was reported to give a general picture of the normal population. The primary aim of this study is to evaluate the clinical significance of the presence of autoantibodies in TB patients. The clinical characteristics of TB patients, grouped according to the presence of autoantibodies, were summarized in Table 1 of the revised manuscript.

10.Discussion. Page 13, line 30 - what does the author intend to suggest? Increased serum autoantibodies in active TB patients are not diagnostic indication for autoimmune disease? This question seems to be resolved by the statement in the following paragraph; however, the sentence should be written more clearly to convey the readers of author's conclusions. Page 15, line 7-21 is very confusing.

Answer: Thank for your instructive comments. We revised the two paragraphs to make them more understandable.

Responses to Prof. Tsutomu Takeuchi

1. In the patients and methods section, the authors described that 100 patients were enrolled in this study. How did the authors select these patients?

Answer: Thank you for your comment. The prevalence of anti-nuclear antibody in the general population varies, from 9.4% in Japan (Hayashi, N. *Mod Rheumatol* 2008;18:8) to 20% in Israel (Elkayam, O. *Int J Tuberc Lung Dis* 2007;11:306). The prevalence in TB patients is 33% in Israel (Elkayam, O. *Int J Tuberc Lung Dis* 2007;11:306). To have a power of 0.8 and an alpha error of 0.05 in a two-sided test where the proportions of event cases in the two independent samples are 33% and 20%, the calculated sample size is 83 for each. Thus, we enrolled 100 TB patients and 100 control subjects. The TB patients were new cases of culture-confirmed TB diagnosed at the National Taiwan University Hospital from January 2007 to December 2009. We added this information in the Methodology of the revised manuscript.

2. The authors described limitation in the sample number on page 4, how did the authors set it in this study?

Answer: The sample size was calculated as mentioned above. The natural course of TB begins with exposure, followed by infection and development of disease. In this study, we aimed to evaluate the prevalence and clinical significance of autoantibodies during the TB disease process and not just infection by *Mycobacterium tuberculosis*. We selected medical staff as control because TB exposure and infection are more common in the medical staff than in the general population. However, as commented by another reviewer, medical staff, especially young nurses, may be more prone to higher prevalence of autoantibodies. In addition, age and sex are also potential confounders. Enrolling age- and sex-matched medical staff for TB patients is very difficult. Household contact of TB patients may also have high TB exposure and infection. If they are used as control, the results may also be confounded by similar environment and genetic components as the TB cases. The "Strengths and Limitations" of this study has been revised accordingly to reflect these considerations.

3. In the table 1, percentage of anti-Scl-70 in healthy control seems to be quite high as compared with normal population you showed. Did the both experiments utilize the same measurement methods? How did the authors set the cut off points of anti Scl-70 antibody?

Answer: Thank you for your reminder. Anti-Scl-70 antibody was measured using the MESACUP-2 TEST Scl-70 (MBL; recombinant Scl-70 protein, 24) in the reference and by the AtheNA Multi-Lyte® ANA-II Plus Test System in this study. The results were interpreted according to the manufacturers' instructions.

4. In the table 1, percentage of anti-CL IgM antibody in healthy control is quite high as compared with general understanding. Are detection methods really appropriate? It has been known that rheumatoid factor can interfere with some ELISA system. In the cohort, did the authors check any association between the titre of RF and those of other autoantibodies? These information would help readers to understand the concerns above.

Answer: In this study, serum level of ≥ 12.5 MPL was considered positive for anti-cardiolipin IgM. Among the 10 healthy controls with positive anti-cardiolipin IgM, eight had borderline titres that just passed the cut-off value. Two of them were positive for other autoantibodies (one for anti-RNP antibody and another for anti-histone antibody). Among the six TB patients with positive anti-cardiolipin IgM, four had borderline titres. One had positive anti-histone antibody. We added this information in the Results section. We thank the reviewer for the instructive comment and we

apologize that the results of rheumatoid factor are not available in this study.

5. On page 16, disease name of progressive systemic sclerosis should be systemic sclerosis.

Answer: Thank you. We revised this accordingly.

6. The authors concluded that TB culture test is recommended in the patients with high serum autoantibody titre and without rheumatic symptoms in TB endemic area. How did the authors explain the clinical setting for checking autoantibodies without any rheumatic symptoms?

Answer: Thank you for your comment. When TB patients present with non-specific symptoms like fever, malaise, or weight loss, clinicians may order mycobacterial studies and autoimmune serology at the same time. Results of the latter usually become available earlier than results of the former. As such, some TB patients may first be put on systemic corticosteroid rather than anti-tuberculosis treatment, rendering further dissemination of the Mycobacterium tuberculosis bacilli. We apologize for the misleading descriptions in the original manuscript. We revised our conclusions as follows: "In a TB endemic areas...mycobacterial studies should be performed in patients with elevated serum autoantibody titres but without the typical and multiple manifestations of autoimmune diseases."

VERSION 2 – REVIEW

REVIEWER	Tsutomu Takeuchi, MD.PhD. Professor of Rheumatology, Division of Rheumatology, Department of Internal Medicine, School of Medicine, Keio University, JAPAN. no conflict of interest.
REVIEW RETURNED	01-Jun-2013

GENERAL COMMENTS	Now this manuscript has been properly revised according to the reviewers' suggestions and can be acceptable for publication in BMJ open.
-------------------------	--