Author's response to reviews

Title: Diagnosis of tuberculosis in an Indian population by an indirect ELISA protocol based on detection of Antigen 85 complex: a prospective cohort study

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Version: 4 Date: 23 February 2007

Author's response to reviews: see over
Diagnosis of tuberculosis in an Indian population by an indirect ELISA protocol based
Title: on detection of Antigen 85 complex: a prospective cohort study

Reviewer: Karin Weldingh

Reply to Reviewer’s report:

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Page 3: It is written that TST is the only way to determine an asymptomatic infection. This is not correct, as two IFNg release assay for detection of Mtb. infection has been marketed recently which should be mentioned here.

Reply: Agree. The information regarding the IFNg assay for detection of Mtb. Infection is also included in the background section of the MS.

Page 5: Please indicate how much serum (and dilution) was applied on the SDS gels for western blotting.

Reply: As per the suggestion. The amount of serum was applied on the SDS gels for western blotting is mentioned in the western blotting section

Discussion:

Page 8: Were the patients really included prospectively or retrospectively? The way it is described in the material and method section, it seems as they were included retrospectively? Were the analyses performed blinded / double blinded?

Reply: Patients were included prospectively and the analyses were performed double blinded and the same is mentioned in the material and method section

Page 8: This study is not a clinical trial so please rephrase that sentence.

Reply: The sentence is corrected as per the comments

Tables: Please merge table 1 and table 2 into one table.

Reply: Table 1 and 2 merged in one table i.e Table 1 and the information regarding the same is also changed in the text (results section) and legend section
Figure 2: Please indicate the y-axis name.

Reply: Corrected

Reviewer’s report

Reviewer: Marcus B Conde

Reply to Reviewer’s report:

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Detailed information regarding the healthy volunteers‘ evaluation should be given. Were they asymptomatic and with normal chest X ray? TST results?

Reply: Information regarding the non-Tb and healthy groups is already mentioned in the study subject section. Both the groups with no signs of clinical impairment, asymptomatic and normal chest radiographs, were included as controls. However, we do not have data for TST results for the same.

2. The gold standard for TB diagnosis is culture positive and authors enrolled only 24 patients with TB diagnosis confirmed. One hundred and four patients of the TB group were cases with clinical diagnosis an instead microbiologic diagnosis. I understand this is an important group to be evaluated in a serologic test because they represent the group of patients without microbiologic diagnosis (not able to provide sputum, AFB negative in the sputum, etc). However, the diagnosis criteria for the group with clinical diagnosis must be very clearly explained and pointed in the MS (example: ...patients with improvement in clinical and radiographic abnormalities after three months of standard anti-tuberculosis treatment in the absence of therapy directed against other pathogens and absence of an alternative diagnosis were diagnosed as a case of active TB, etc, etc). Furthermore, in the actual MS I am not sure if this group should be kept because the very small number of asymptomatic controls. The N of asymptomatic control must be similar to the N of TB group. Methodologically, a study with 24 cases of TB (confirmed) and 68 controls (20 asymptomatic and 49 non-TB cases) makes more sense to me than a study with 128 TB cases and 69 controls (mainly when 104 of TB cases had no bacteriological diagnosis). I think the authors must seriously considered exclude these TB cases clinically diagnosed of the MS and work with a total sample size of 92 cases (24+68).

Reply: The main aim of the present study was to develop a assay which will detect group of TB patients who were negative for AFB and culture tests. The developed method using mAb to Ag 85 complex detects almost 78% suspected TB patients who were negative for AFB and culture tests. These patients were given anti Koch treatment (AKT) treatment and all of responded and improved within 2-3 weeks and we think this is the strong point of the MS. Secondly cases of abdominal TB who never show AFB and culture positive but later on positive by biopsy. In these cases, we have demonstrated
the presence of this antigen in those cases also. At present, we feel that excluding this group and working with only confirmed and control group will lower down the interest of MS.

However, the suggestion on details of the patients with improvement in clinical and radiographic abnormalities after three months of standard specific anti-tuberculosis treatment is well taken and the information of the patients with to AKT treatment is included in the MS however we do not have data of radiographic studies and that is why is not presented in the MS.

3. TB cases may be stratified according with AFB status

Reply: corrected

4. It was not clear to me how the normal value of the test was calculated and this must be clearly stated in the MS. Normally, authors use a ROC curve or use the mean of healthy control plus two SD.

Reply: A cut-off value of absorbance Ag 85 complex antigen for TB patients was calculated using the mean plus SD of the absorbance in the non-TB group.

5. Sensitivity and specificity of the test with the CI 95% should be presented in a table (maybe on table 1). I think that results of table 2 can be presented in the text and this table excluded. Figure 1 could be excluded too.

Reply: Table 2 is excluded from the MS as per the suggestion. However, we feel that figure 1 is important figure, which tells reader details regarding standardization procedure of Indirect ELISA prior to patients sampling so at present it is not excluded.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

First paragraph: I am not sure if culture for TB is frequently negative as stated. It is negative in about 20% of patients who provide sputum and have TB.

Reply: Agree.

Second paragraph of the background: TST is not the only way to determine asymptomatic infection. Please, mention ELISPOT, etc.

Reply: The information regarding the IFNg assay for detection of Mtb. Infection is also included in the background section of the MS.

Discretionary Revisions (which the author can choose to ignore)
Information regarding the BCG vaccination (and re-vaccination if any) policy in India should be provided.

Reply: The information is included in the text

Other Changes

- New reference is added at number 8 position and accordingly all the reference number in the text and in the reference section was also changed.
- Figure 3 was modified