Author's response to reviews

Title: Allergy markers in active pulmonary tuberculosis at time of diagnosis and following chemotherapy

Authors:

Linda K Ellertsen (linda.ellertsen@gades.uib.no)
Dag G Storla (dgstorla@online.no)
Lien M Diep (Lm.diep@medisin.uio.no)
Karl A Brokstad (Karl.Brokstad@gades.uib.no)
Harald G Wiker (harald.wiker@gades.uib.no)
Geir Hetland (geir.hetland@ullevaal.no)

Version: 4 Date: 11 May 2009

Author's response to reviews: see over
Bergen 5.5.2009

We hereby submit the revised manuscript “Allergy markers in active pulmonary tuberculosis before and after chemotherapy by Linda K. Ellertsen, Dag G. Storla, Lien M. Diep, Karl A. Brokstad, Harald G. Wiker, Geir Hetland. We have answered the reviewers’ comments one by one below. We also made some additional corrections in the manuscript, and the list of these corrections is listed after the reply to the reviewers. A revised manuscript has now been submitted. We now hope the paper can be published in your journal in its present form.

Kindly regards,

Linda Kathrine Ellertsen
Reviewer's report
Title: Allergy markers in active pulmonary tuberculosis at time of diagnosis and following chemotherapy
Version: 1 Date: 29 April 2009
Reviewer: Christoph Grüber
Reviewer's report:

Major Essential Revisions

ABSTRACT
- Conclusion should be more balanced: Lines 62-66 are not really substantitated by the data of this study. Line 66 should read rather "The observed inverse association of allergic sensitization and reported BCG scar..." than "The observed reduction of allergic sensitization by BCG vaccination".
Answer: We agree and have corrected the conclusion in the abstract as recommended, line 67-69. We have also balanced the conclusion in both the abstract and the main manuscript.

RESULTS
Did you check for HIV infection as a potential confounder?
Answer: Unfortunately, we were not able to correct for HIV as a confounder. HIV as a potential confounder is added to the discussion; lines 288-289.

Minor Essential Revisions

ABSTRACT
- Line 1, correct "an inverse association".
Answer: Thanks

METHODS
- Line 126ff, reported BCG scar may overlap incomplete with BCG immunzation. Were vaccination cards checked?
Answer: No, vaccination cards were not checked, only visible BCG scar. It was difficult to retrieve vaccination cards from the participants in the study.

- Lines 170ff, calculations employing a more conventional cut-off for a negative IgE test should be offered as well.
Answer: Ok, this has been added to the results; lines 215-217 and 244-246

RESULTS
Lines 192-196 should be integrated into the Methods section.
Answer: Ok, originally this section was moved back and forth a couple of time before it was put under results. Now we have moved it under methods; lines 160-165

- Lines 202-217, Comparisons of read-outs after therapy and of controls should be added.
Answer: We have two time-point measurements from TB patients (before and after treatment). However, we have only one time-point measurement from healthy controls. According to statistical procedures it is not recommended to do that type of analyses, because one can not exclude the possibility the data among control will change during a time interval. Since we only have 1 time-point measurement for controls we did not perform comparison between controls and TB patients after therapy.

DISCUSSION
- Answers in questionnaires filled in by interviewers may reflect incompletely answers from study subjects.
Answer: Yes, we agree. In line 296-297 we have written that people in developing countries like Bangladesh may not be familiar with the term allergy. We compensated for some of this limitation by having questionnaires filled in by the field staff” In the revised version of the manuscript we have added a sentence that the results from the questionnaire must be read with caution (line 295-300). We believe that the specific IgE towards the inhalant allergens are the strongest results in the present study, and since the questionnaire support these results we decided to include them.

CONCLUSION
- Conclusion should be more balanced (see remarks on Abstract).
Answer: A more balanced conclusion has been written; lines 350-353

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:
I declare that I have no competing interests.
Reviewer's report
Title: Allergy markers in active pulmonary tuberculosis at time of diagnosis and following chemotherapy
Version: 1 Date: 29 March 2009
Reviewer: Maarten O Hoekstra

Reviewer's report:
In a previous study Ellertsen et al found that allergic sensitization, measured by serum total and specific IgE, was increased in TB patients compared to healthy controls (HC). In this longitudinal study, Ellertsen et al. compared several items between TB patients and HC. They confirmed that serum total and specific IgE, IFN#, and IL6 were higher in the TB patients group. Secondly, they found that serum total and specific IgE, and IL6 were reduced after successful TB treatment, and lastly they reported that in the control group, the presence of a BCG scar was associated with a reduced risk of developing sensitization. The authors explain the increased sensitization in TB patients by stating that TB patients are more susceptible to sensitization than the HC or that allergy patients are more disposed to develop TB. Secondly, they conclude that the reduction in sensitization during TB treatment is caused by a down regulation of the TH2 response by dead or dying TB bacilli.

Major Compulsory Revisions
- Ad Methods (L102-105): The authors report more that 30% (71 vs 108) loss to follow-up in the TB patients with respect to the collection of blood after the end of the TB treatment. Were the remaining 71 still matched with the 216 HCs? If no, can the data still be extrapolated to the entire group of TB patients?
Answer: We are not quite sure if we understand this question. In line 112 we have added information on how the controls were matched. If one look at Table 1 one can see that the 3 groups (108 TB patients, 71 TB patients and 216 controls) are still matched. However, in this study we do not compare TB patients after completed treatment with controls. We only compare TB patients (n = 108) with controls, and we compare the read-outs from TB patients before treatment (n = 71) with read-outs from TB patients after treatment (n = 71). According to statistical procedures, one can not use the controls in two different comparisons. There are 2 time-intervals for the patients and only 1 for the controls. It is theoretically possible the read-outs from controls would change over time, and therefore they can not be used twice. We hope this will answer your question.

- Ad Methods (L111-118): were the controls not matched with TB patients with respect to atopy or atopic background (the presence of atopy in first degree family members)? “Reported allergy” was measured by questionnaires. Were these data in any way confirmed, i.e. by doctor’s data? Is it possible that the increased prevalence of “reported allergy” in the TB patients was due to selection bias? Can it be imagined that the reported lower degree of sensitization in the HC group was just caused by a lower prevalence of allergy (or atopy or atopic background) in the HCs, instead of just the absence of TB?
Answer: No information regarding the family members was known. Reported allergy was not confirmed by doctors. Yes, it is possible that the increased prevalence of reported allergy is due to a selection bias. We have already pointed out that the questionnaire had limitations. We have now included the selection bias problem and added a sentence that these results should be read with caution; lines 295-300. We
consider the specific IgE data as the most important data in this paper, and since the questionnaire supports the laboratory findings, we decided to include them.

- Did the authors consider that the down-regulatory effect on serum total and specific IgE, and IL6 after successful TB treatment could also be due to an immunomodulatory effect caused by the TB treatment?
- Have the authors checked whether besides IgE also serum concentrations of other antibodies were increased during TB disease (and a decrease during treatment)? In other words, whether there was general downregulation of antibody production, not restricted to IgE (ie the effect may have been not IgE-specific)?

Answer: In the submitted paper we had written: “There could be a general polyclonal stimulation of IgE, which could explain the increased levels of specific IgE. Specific IgE has been deleted. Thereby other antibodies are included as well; lines 291-294. Additionally, three possible explanations have been summed up in the end of the discussion, lines 339-348

Minor Essential Revisions
- I did not find any Methods section in the abstract. Is that according to BMC rules?

Answer: This paper was originally submitted to BMC Immunology and on behalf of us the editor transferred the paper to BMC Infectious Diseases for consideration. According to BMC Immunology’s rules they do not include method in the abstract. However, this is of course included now.

- Abstract, L64: remove the word “sensitized”, since there is no such thing as “non-allergic sensitization”

Answer: Since specific IgE does not necessarily mean that the patients have clinical symptoms, we wanted to use the term “allergic sensitisation” and not allergy.

- the conclusion in the Abstract should be put more cautious: ”may indicate” instead of ”indicate(s)” since based on the current study nothing can be said about the exact pathophysiology as an explanation of the findings.

Answer: We have changed the conclusion in the abstract and thereby balanced it, lines 65-69

- looking at the abstract, I would supply findings in the Results-section the same order as questions have been given in the Background-section. The same an be applied to the Conclusions-section. This is facilitating the reader to get through the Abstract.

Answer: Yes, this is a good idea; line 58-63.

- p10, L183: “skewed” instead of “skew”?

Answer: Ok, thanks see new line 190

- Table 1: On top of the second column should we read “TB patients after treatment” instead of “TB patients before and after treatment”?
- Table 1: is everybody in this region BCG vaccinated and if yes, according to which scheme?
Answer: Everybody is not vaccinated in this region. We noticed now that Table 1 is easy to misinterpret and therefore we change the heading of the groups.

- p11 and Table 2,3: it is not clear to me how the Phadiatop performed for different allergens yielded only one number? Were all results for different allergens summed up and divided by the number of allergens that yielded positive results?

Answer: The test is designed as a sandwich immunoassay. The coating consists of a mixture of relevant inhalant allergens, which is covalently coupled to the ImmunoCAP. The specific IgE in the patient’s serum binds to the allergens. Enzyme-labelled antibodies against IgE are added to form a complex. The complex is then incubated with a developing solution and after stopping the reaction, the fluorescence of the eluate is measured. The response of a serum sample is compared to a standard. The test has a cut-off value at 0.35 kU/L. The test sums up all the allergen-binding signals. The sum of low signal levels close to the detection limit (0.35 kU/L) may when measured with ImmunoCAP Phadiatop reach into the detectable range and thereby give a positive result. However, in our paper we have used a higher cut-off value in our analyses. I have now included also results with the conventional cut-off value (0.35 kU/L), because referee 1 asked for these results, line 215-217 and line 244-246.

- p11, 2nd paragraph: a strange sentence: "To increase the probability if clinical symptoms, … (etc)"? “of” instead of “if”? 

Answer: Yes, it should be of. Thanks!

- p12, L220-232: If the observed changes during TB treatment were due to a down-regulation of a TH2 effect, would the authors not have expected to have observed a decrease in IL4 or IL5? Why was IL13 not determined? If IL4 and IL5 were below the detection limit, was allergy really present?

Answer: IL-4 and IL-5 is generally low in serum and difficult to measure (even if the patients are allergic). Limitations due to degradation of cytokines is already written in the discussion, line 317-322. Yes, IL-13 would have been interesting to measure. We used in a kit in our study (including 10 different cytokines) and added 3 different cytokines to the kit, including IL-,4 which we chose over IL-13. The cytokines included were chosen after compromising between two different groups (Th1 and TH2) and costs.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests.
Other additional changes made in the manuscript:
Lines 105-106: “In addition, sera were collected from TB patients after completed treatment” have been changed to “In addition, sera were collected from a subgroup of 71 TB patients after completed treatment.”

Lines 112-113 Changed from: “They matched the TB patients on group level by gender, age, and socio-economic status” to “The controls were recruited to match the TB patients on group level by gender, age, and socio-economic status.

Additional information to the methods have been added: Line 150-153: Relevant cytokines were analysed by Luminex 100 multiplex array (Luminex Corporation, Austin, TX, USA) according to the manufacturer’s instructions” to “Cytokines were analysed by multiplex bead immunoassay (Luminex 100, Luminex Corporation, Austin, TX, USA) according to the manufacturer’s instructions and using kits from Biosource (Cat. No.; LHC 0001, LHC 0151, LHC 9121, LHC 0171, Invitrogen, Carlsbad, CA, USA)”.

Lines 140-141: IL-17 was missing in the method section. Only the abbreviation for TNFα was given in the paper. Therefore “tumour necrosis factor” were added as an explanation.

We have corrected all “IFN-γ” to “IFNγ” and all “TNF-α” to “TNFα”. Minor changes to Table 1 and 2 have been performed.

Lines 341-342: Competing interests have been added: “The authors declare that they have no competing interests”

Line 2: “as proposed by the hygiene hypothesis” has been deleted.