Author's response to reviews

**Title:** Diagnosis and follow-up of treatment of latent tuberculosis; the utility of the QuantiFERON-TB Gold In-Tube assay in outpatients from a tuberculosis low-endemic country

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**Author's response to reviews:** see over
Dear Editor-In-Chief,

Please enclosed find our revised manuscript

“Diagnosis and follow-up of treatment of latent tuberculosis; the utility of the QuantiFERON-TB Gold In-Tube assay in outpatients from a tuberculosis low-endemic country”

We thank the three referees for a thoroughly review that has helped us to improve the paper. We think we have been able to address the specific issues raised by the reviewers and do the necessary changes. As suggested the title, abstract and statistics have been updated and the discussion and part of the results have been shortened.

This study is unique since it is longitudinal and reflects the clinical situation in our country. Many have studied interferon-gamma release assays (IGRA) in active tuberculosis and certain risk groups during the last few years. However, fewer studies have been performed in an outpatient clinical setting in a tuberculosis low-endemic country with high coverage of BCG vaccination including a wide range of individuals. Our data has contributed to new national guidelines for IGRA tests in latent tuberculosis. We have studied the effects of preventive therapy on QuantiFERON TB after 15 months and our data on the lack of effects of preventive therapy on IGRA tests are new. All together or study adds information to the growing amount of data concerning the usefulness and limitations of the IGRA tests.
Our main findings are:

1. Only a third of those with a positive tuberculin skin test were diagnosed as having tuberculosis infection using the QuantiFERON-TB test. The majority of those with tuberculin skin test > 15mm had a negative QuantiFERON-TB test and few risk factors.

2. Origin from and short time since stay in a tuberculosis endemic country, duration of tuberculosis exposure and previous tuberculosis were all associated with a positive QuantiFERON TB test. These groups should be targeted through screening and follow-up and there is a need to strengthen and simplify the co-ordinating activities.

3. After preventive therapy 87.5% and 84.6% still had a positive QuantiFERON-TB test at three and 15 months, respectively. Thus, the test could not be used to monitor the effect of therapy.

We indeed feel the paper is improved and hope you will consider this paper for publication in BMC Infectious Diseases. Please, see the attached comments to the reviewer's reports.

I confirm that all the authors of the manuscript have read and agreed to its content, that readily reproducible materials described in the manuscript will be freely available to any scientist wishing to use them for non-commercial purposes, and that we have ethical approval for any human experimentation. There are no conflicts of interest for any of the authors. I confirm that the manuscript is original, has not already been published in a journal and is not currently under consideration by another journal.

Yours sincerely,

Anne Margarita Dyrhol-Riise, MD, PhD
Review 1

Major Compulsory Revisions

1: Methods /Results: In the description of statistical methods and multivariable logistic regression, nothing about checking variables for interactions and correlations is mentioned. This needs to be checked and clearly stated and the results of the check mentioned.

We appreciate this comment. A priori, we considered the most important potential interactions in the model to be those between BCG vaccine status and other explanatory variables. We assessed all these potential interactions and did not find any significant interactions between BCG vaccine status and any of the other explanatory variables. This has been explained in the updated paper.

2: Methods: How was the questionnaire presented for people of different language backgrounds? Was e.g the questionnaire translated to different languages on paper, or did a translator translate from a Norwegian questionnaire and ask the question to the patients?

The questionnaire was written in Norwegian and English versions and a translator was used when the patient spoke another language.

Minor Essential Revisions

1: In the methods part some information about who was selected for the study is needed. Were everybody with the actual inclusion criteria asked? And corresponding in the results part, how many gave informed consent and how many refused? If possible, some information about those that refused could also be helpful.

All patients referred for the first evaluation to the TB outpatient clinic during the year 2006 were asked to be included in the study. Less than ten persons refused to participate and the main reason was unwillingness to draw extra blood. This has been stated more clearly in the updated paper.

2: Results of the logistic regression analysis should be reported both in the abstract and the main paper with odds ratios and confidence intervals, not with p-values.

We agree with the suggestion to present the logistic regression with OR and 95%CI instead of p-values and we have changed the paper accordingly. For the benefit of any reader who is accustomed to reading P-values, we have added an asterix (*) indicating significance at the level P<0.05 and a double asterix (**) indicating significance at the level P<0.001 in Table 5.

3: A separate paragraph with limitations of the study should be added.
This has been done in the reviewed manuscript under the last section in the discussion.

4: English translations of the titles in Reference 7 and 22 should be added.
This has been done in the revised manuscript.
**Discretionary revisions**

1: As latent TB prevalence but also new infection is supposed to be different in different age groups, age could be split into age groups (e.g. 0-14, 15-29--) in the logistic regression model to check if this gives an association to QFT results.

We had actually considered the same way of categorising age into groups and did so, but unfortunately we did not include an explanation of the age categorisation in the manuscript. In the revised manuscript we have included such an explanation in the methods section. Since the youngest was 9 years, age was categorised into 6 groups, 9-19, 20-29, 30-39, 40-49, 50-59, 60-87.

2: If one or two words to indicate that longitudinal measures of QFT have been done were added to the head line, this would give more information about the content of the paper and might increase the interest for the paper.

Thank you for the advice. We have changed the title.

3: Among the untreated, who were repeatedly tested? And how was the selection?

Everyone was planned to be retested, but due to problems with logistics and patients not showing up at controls only a limited available number of patients were tested. Thus, the patients tested were not selected.

4: Some description of characteristics of the treatment group could be added.

Please, see the updated manuscript where the treatment group is characterised.
Review II:

The main shortcoming of the manuscript is the multiple regression analysis, which I found confusing. For example, origin in a TB endemic country appears to be the strongest single factor in the univariate analysis, but in the multivariate analysis this is replaced by previous TB and parents from a TB endemic country without having travelled there. Although I am not a statistician, my understanding is that there are several approaches to such an analysis, and that they can sometimes lead to very different conclusions. I am not certain how robust the conclusions of this analysis might be. Lastly, I think the manuscript is rather lengthy given the modest amount of new data presented.

We appreciate these comments. The discussion and part of the results have been shortened. During the process of analysing the data we did discuss these issues, but we acknowledge that we have not provided enough details on this in the paper.

Upon revisiting the analysis, it also became obvious that there were too few observations in some of the categories, most notably, the category of persons born in Norway from immigrant parents from TB endemic countries. It would be interesting to describe in more detail this particular group, but the number of observations in this category is too small to draw any useful conclusions, and including this category weakened the whole model. We have thus decided to re-classify the study subjects in such a way that the categorical variable “origin/stay in TB endemic country” has been replaced by a 3-categories-variable currently categorised as 1) person from non-TB-endemic country, 2) person from non-TB-endemic country who has visited a TB endemic country and 3) person originating from TB endemic country. The second-generation immigrants in the previous variable are now classified as being persons originating from Norway.

Another issue with the previous multivariate analysis was that different variables to a certain extent measured the same biological effect, and to some extent there was too high degree of match between explanatory variables. Most notably, upon reviewing the multivariate model, we found that we had to remove the variable “duration of stay in TB endemic country” because it had a high degree of match with the variable expressing origin and stay in TB endemic country (i.e. currently categorised as 1) person from non-TB-endemic country, 2) person from non-TB-endemic country who has visited a TB endemic country and 3) person originating from TB endemic country). Removing the variable “duration of stay in TB endemic country” improved the model.

Furthermore, since observations with missing values are automatically removed from logistic regression models, only 389 of the total of 481 observations remained in the final multivariate model. The observation “duration of exposure” had a particularly high number of missing values (n=63) and was responsible for a large part of the missing values in the final model. To ascertain whether the loss of observations due to this particular variable affected the model, we performed the logistic regression on the final model with and without that variable, and found that excluding or including it did not significantly alter the findings in the model.

Lastly, in the revised manuscript, we have included information on how we considered the most important potential interactions in the model. We considered most important the potential interactions between BCG vaccine status and other explanatory variables due to the effect BCG vaccination could have on the risk of infection. We assessed all these potential interactions and did not find any significant interactions between BCG vaccine status and any of the other explanatory variables.
Review 3:

**Major Compulsory Revisions**

The title of the paper is misleading and needs revision:
A mixture of patients with active TB and possible latent TB were assessed including contact investigation.
The abstract should be improved
The text needs shortening, specifically the discussion is too long, particularly on risk factors for positive QFT-TB.

We thank for the review and have shortened and hopefully improved the paper, including title and abstract according to the comments raised by the referee. Further we have tried to address the issues raised by the referee. Concerning the mixture of various patients and risk factors we believe this reflects the clinical reality.

**Minor Essential Revisions**

**Abstract:**
Numbers with active TB, LTBI are not clearly reported. In results, all actual numbers together with percentages must be reported, e.g “the QFT-TB was positive in X/X (30.8%).”

These numbers have been included in the manuscript

**Introduction, p.3 quote:** “There is also no solid data concerning the usefulness of the IGRA tests to identify those individuals with LTBI who are at most risk for developing active disease and therefore most likely to benefit from preventive therapy [19].”

Comment: This statement needs modification, Diel and coworkers (1) among others have examined this question and should be referenced.

The statement has been modified and the reference to Diel et al. has been included.

**Introduction:** The current (or previous?) BCG vaccination strategy/policy is unclear: please define “high coverage” and at which age BCG is given in Norway.

Norway has until 2009 BCG vaccinated the population at the age of fourteen. This has been clarified in the manuscript.

**Introduction page 4, line 5-9:** please delete “have” from all sentences.
This has been corrected

**Methods:**
“Most of the TST test were performed at least 3 months prior”: Why this delay?

The TST testing was performed in the primary health care system as part of screening according to national guidelines (contact investigations, immigrants and health care workers). There was a delay from TST testing to specialist evaluation when active TB was not suspected by the primary health care worker due to limitation of resources at the hospital.

*Please provide range of delay between TST and admission.*
Unfortunately we do not have access to detailed data, but delay was typically 3-5 months for those not suspicious of active TB.

Unclear if all participants underwent X-ray, clinical examination and induced sputum: If not all were investigated this way- please provide numbers. In most other similar settings it would be unusual to do a “induced” sputum- in most other countries ordinary sputum samples would be sufficient- is this the actual Norwegian policy?

All participants underwent both X-ray, clinical examination and induced sputum in this study and this has been stated more clearly in the paper. X-ray is mandatory for persons belonging to the screening groups defined by the national guidelines. Induced sputum is not performed at all national hospitals, but at the TB out-patient clinic at our hospital there is facilities and logistics for “induced sputum” sampling and thus this has become the method of choice.

Statistics:
This section is poorly written. Please delete the Stata specific command( xi:) and delete “ we did not use stepwise removal of factors” suffice to say that multivariate logistic analysis were used. According to table 5, legend there were missing data, how where these handled in the multivariate analysis?

We are grateful for this comment, and have now improved on the statistics section. Stata specific commands have been deleted, as they are not necessary for the understanding of the matter. Current trends in statistics suggest that stepwise removal of non-significant variables should no longer be used. However, we agree that this information need not be given since it is obvious from the presentation of the multivariate model in Table 5.

As part of rewriting and improving upon the statistical methods section, we have also included information on handling of missing values and assessment of potential interactions, and other issues that influence the multivariate model.

Upon revisiting the analysis, it also became obvious that there were too few observations in some of the categories, most notably, the category of persons born in Norway from immigrant parents from TB endemic countries. It would be interesting to describe in more detail this particular group, but the number of observations in this category is too small to draw any useful conclusions, and including this category weakened the whole model. We have thus decided to re-classify the study subjects in such a way that the categorical variable “origin/stay in TB endemic country” has been replaced by a 3-categories-variable currently categorized as 1) person from non-TB-endemic country, 2) person from non-TB-endemic country who has visited a TB endemic country and 3) person originating from TB endemic country. The second-generation immigrants in the previous variable are now classified as being persons originating from Norway.

Since observations with missing values are automatically removed from logistic regression models, only 389 of the total of 481 observations remained in the final multivariate model. The observation “duration of exposure” had a particularly high number of missing values (n=63) and was responsible for a large part of the missing values in the final model. To ascertain whether the loss of observations due to this particular variable affected the model, we performed the logistic regression on the final model with and without that variable, and found that excluding or including it did not significantly alter the findings in the model.
Lastly, in the revised manuscript, we have included information on how we considered the most important potential interactions in the model. We considered most important the potential interactions between BCG vaccine status and other explanatory variables due to the effect BCG vaccination could have on the risk of infection. We assessed all these potential interactions and did not find any significant interactions between BCG vaccine status and any of the other explanatory variables. Also see our answer on the comment to Table 5.

**Results, p 6:**

*Please provide actual numbers together with percentages for all findings. The number of patients with active TB and suspected LTBI needs to be stated clear and early in the results.*

This has been done as suggested in the first part of the result section.

*According to the text 54 had TB suspect thoracic X-ray findings, however according to table 2, only 19 had thoracic X-ray findings + possible 8 (previous TB disease)- please clarify.*

Table 1 shows the indications for referral to specialist. Thus, the 19 with thoracic X-ray findings had no other risk factor for TB than suspect thoracic X-ray findings (not part of screening). However, thoracic X-ray findings was also present in some of the persons examined for TB because they were immigrant or part of a contact investigation adding up to a total number of 54 with thoracic X-ray findings that needed evaluation by the specialist.

*It is surprising that only one patient was HIV-tested and that routine HIV-test was not performed- this need a comment in the discussion as it is contradicts most international guidelines.*

We agree that the practice for testing TB patients routinely for HIV or vise versa has not been adequate in our country, but improvements has been done during the last couple of years. HIV testing was not included in the study protocol, and testing was decided by the clinician. However, we are no performing a comparable study in a known HIV positive population. As suggested by the referee this has been discussed in the paper.

**Results, p.7:**

*The sentence on the “index person” is confusing- who and why is this needed?*

The index person refers to the patient with active TB causing the contact investigation. This word had been changed to 'TB case' both in the text and in Table 3.

*The sentence on BCG vaccine status by questionnaire should be moved to the Method section. - the word “whereas” is used extremely often in the text, consider using another word.*

These corrections have been done

**Results, Active tuberculosis, page 8:**

*Induced sputum was obtained from all the participants and twelve had positive culture of M. tuberculosis and one of M. fortuitum. Two additional patients were regarded as active TB disease based on X-ray findings and one patient was diagnosed with glandular TB’ This adds to 15 ( 2+2+ 1; excluding the patient with M. fortuitum), however the text refers to 16 patients with active TB?’*
The patient with M. fortuitum was included in the 16 patients with active TB since the mycobacteria is recognized as an emergent pathogen, the patient had thoracic X-ray findings and anti-tuberculous therapy was started. This has been clarified in the revised paper and only 15 patients are regarded as active TB as suggested by the referee.

**Results, QFT-gold results, page 8 and table 4:**
Suggest that the authors produce a scatterplot of TST induration according to positive / negative QFT-TB.
We have made a scatterplot (Figure 1) as suggested.

**Results, QFT-gold results, page 9, line 10:** “moods” = modes?
The correction has been done

**Page 9. Predictive factors for a positive QuantiFERON-TB Gold test: This section can be shortened or perhaps omitted.**
The text has been shortened and changes made according to updated statistical analysis.

**Page 10, Results, QuantiFERON-TB Gold responses during prophylactic therapy Was prophylactic therapy only given to persons with positive QFT-TB tests?**

Prophylactic therapy was started in 57 persons with suspected latent TB. 56 of these were QFT-TB positive. The decision to treat was made by the clinician and the QFT-TB test was known at the time of decision. Only one QFT-TB negative person was treated (TST > 30 mm, previous long-term stay in a TB endemic country and planned for therapy with a TNF-alfa inhibitor). This has been clarified in the updated paper.

“Altogether 44 of the patients receiving prophylactic therapy were followed with repetitive QFT-TB tests. After three months 87.5% were still QFT-TB positive (35/40 tested) whereas after 15 months, one year after the end of therapy, 84.6% remained positive (22/26 tested).
” Comment: Perhaps a bit unclear if the dropout rate from testing did affect these findings-please provide numbers for how many which reverted from positive to negative between 3 and 15 months.

All patients with reversed QFT-TB test after three months were still negative at 15 months. None reverted from positive to negative between 3 and 15 months, although one additional person was negative at month 15, but no test was performed at three months. This has been clarified in the updated paper.

**Discussion:**
**Page 11: The risk factors for LTBI section needs shortening, particularly the discussion on Norwegian asylum seekers is irrelevant here and should be deleted, cannot see that the reported findings as such supports screening.**

This section has been shortened and the discussion on Norwegian asylum seekers deleted. Although this is an ongoing discussion in our country we agree that this might not be relevant in this context.

**Table 5:**
The result section does not provide adequate information to gauge the number of missing variables and how this affects the total multivariate analysis, it would be useful to know the
number of total persons included in the final multivariable model either in the footnote or in the result section.

We appreciate the suggestion regarding missing values and remaining observations in the logistic regression model. In the revised manuscript we have included the number of observations available for each variable and the total number of observations (no=389) included in the final logistic regression model. In the method section, we have also described how one particular variable (duration of exposure) accounts for the majority of missing values. We have run the logistic regression with and without this variable and have found that it does not significantly alter the odds ratio and confidence intervals for any of the other variables. This indicates that keeping this variable in the model does not negatively impact the model, but rather add relevant information. Please also see our reply to the comments on statistics.

*Please provide definition for duration of stay and recent stay (months or years?) directly in column 1 or in the footnote.*

Please see the definitions of the parameters in the footnote of table 5

*The 95% CI for previous TB, yes, multivariate must be wrong (213-91.98), please correct.*

This is a typing error. The correct figures are (2.02 – 66.73) and this has been corrected in the revised manuscript.