Author’s response to reviews

Title: Sub-clinical abnormalities detected by PET/MRI in household tuberculosis contacts

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Dear Editor,

Many thanks for a chance to revise this manuscript. Please fine below our itemised responses to the reviewers comments with reference made to the amendments in the manuscript (line numbers refer to the tracked change version).

Reviewer reports:
Hanif Esmail (Reviewer 2): I appreciate the opportunity to review the revised manuscript. The authors
have addressed some of the concerns. However, I still do not feel the conclusions are justified by the data presented and I do not feel that the methodology of the study is adequate as it lacks the appropriate controls or benchmarking to hard endpoints.

The authors assert that the abnormalities they identify (lymph node of >1cm in short nodule >6mm and any SUV max >0.95) relate to subclinical TB disease and imply that they may be more likely to progress to clinical disease. Although it has previously been shown that HIV infected persons with infiltrates and scars within the lung parenchyma primarily within the upper lobes of the lungs are associated with clinical progression, there is no evidence (that I am aware of) that LN uptake >0.95 especially in the absence of lymphadenopathy is associated with disease progression. Even if these abnormalities are as a consequence of contact with TB is it not possible that the lymph node activity just relates to antigen presentation within the lymph node and is part of the natural response to infection (i.e. may result in subsequent control of infection rather than disease progression). Vaccination is well known to result in increased lymph node activity and in the smaller study by Ghesani et al 80% of IGRA positive contacts had lymph node activity which correlated with background subtracted antigen specific IFN gamma levels. It is possible that these abnormalities within the lymph node may improve over time rather than worsen (I appreciate that 3 of the participants had repeat PET/MRI). Hence I still feel that to be of value and infer that the variety of abnormalities on the PET/MRI are all related to subclinical disease, that they would need to be benchmarked to clinical endpoints. This is especially important if the imaging modality is to be used for biomarker discovery in the future.

Response:
We accept that from this study we cannot determine whether the abnormalities shown are related to an increased risk of later clinical disease, or whether they may represent lower risk perhaps due to a more active early immune response. We have made the following changes to the text to remove any implied assertions that the changes may be related to higher risk of later progression:

- Abstract: Deleted reference to higher risk of reactivation and added that larger studies are needed to determine this
- Discussion: Added line 244: “We cannot determine from this study whether those who have early subclinical changes detected on PET-based imaging are at higher risk of later reactivation and progression to clinical disease.”
- Line 251: “Equally those with a more active early immune response may be effecting better clearance of the infection and may be at lower risk of later reactivation.”

However, the term “subclinical disease activity” is reasonable as this can be taken to include the component of an immune response (and it is the immune response that causes much of the pathology of TB anyway). We have modified the wording in the following ways to try to make it clear that we are not implying this is active mycobacterial replication:

- Title: Modified to read subclinical disease rather than subclinical tuberculosis.
- Abstract: Modified to report the number of abnormal scans in contacts with negative IGRA to better reflect the fact that IGRA and PET seem to be giving us different information about disease status.
- Discussion: Added line 238: “The abnormalities that we identified, especially the uptake of FDG in lymph nodes, most likely represent immune activity following mycobacterial antigen presentation because FDG (glucose) uptake by metabolically-active bacilli is likely small given the low bacillary burden in this early disease stage. Such immune activity is likely to be transient.”
The authors are essentially making the a priori assumption that all the PET/MRI abnormalities they describe in their household contacts relate TB. Even we assume that the abnormalities could relate to natural infection with Mtb or the subclinical disease process it is surprising that there is no relationship with IGRA, intensity of exposure to index case, frequency of exposure, estimated duration of exposure or smear status of index case. These are all well known risk factors for both infection and disease risk in household contacts. The authors do not really explore this in the discussion. It may be that some of the abnormalities they identify are associated with future TB risk but does not appear that the sample size is adequate to investigate this in detail. A control group would allow for a more accurate assessment of where the threshold for the abnormalities should be set.

Response:

We have added further clarification to the discussion to clarify our rationale for why these abnormalities relate to TB:

- Added: Line 195: “Given the frequency and nature of the abnormalities seen and the previous smaller study reporting a similar finding, we believe that these findings are related to the documented recent household TB exposure rather than being incidental findings unrelated to the exposure.”

The limitations of IGRA are well known, including the fact that it relies on a response to a standard exogenous antigen and does not tell us anything about current infection. We have addressed this in the discussion already and added further clarification as follows:

- Line 274: “The lack of concordance between the two investigations may be a chance finding due to the small sample size, but might also reflect the difference between the standardised antigenic stimulation with IGRA.”

We had previously just shown P values for the exposure factors that one might have expected to show a higher prevalence of abnormal scans, but not the Odds Ratios. We have now added the Odds Ratios which do show higher rates with some risk factors, although not significant. The lack of significance is likely because the sample size is small and assessment of most of these factors is subjective and imprecise (relying in some cases on patient recall); thus one cannot place much weight on the absence of any statistically-significant associations.

We have made the following changes to make this clear.
- Results: Line 171 – 179: Analyses performed using 2-sided Fishers exact with odds ratios reported.
- Discussion: Line 222: “In addition, we found that abnormal scan findings appeared to be more common in those with exposure ≥90 days as would be expected if they were related to TB exposure. These associations were not significant, as expected due to the imprecision of the assessments and small sample size (studies showing such associations typically have sample sizes of many thousands of participants [10]).”

As for the concern about the lack of a control group, this would need a very large sample size to allow a robust comparison of prevalence of abnormalities in a control group with our TB contacts group. PET/MRI scans are expensive (several thousand dollars each, in our institution) and are logistically complex for participants who have to take time off work, fast etc. It was simply not possible to scan a meaningful number of controls for financial or practical reasons. Nor do we think our ethics committee would approve this. We understand the concerns of the reviewer and accept that this is a theoretical limitation of the study, but there are numerous
reasons set out extensively in the discussion why we believe our interpretation that the abnormalities are related to TB exposure/infection is reasonable. We have discussed this point very carefully and thoroughly so that the reader can make up their own mind on this matter. To stifle the publication of these important data (a much larger study than the previous cohort – which also incidentally also did not have a control group) based on this theoretical concern would be a disservice to science. The data should be in the public domain, with the discussion and then readers can debate and make up their own minds.

Added:

• Line 198: “We did not scan a control population without household exposure to TB so we cannot prove that these same findings are not also present at a similar frequency in unexposed controls in our population, but believe this to be unlikely for the reasons set out below.”
• Line 203: “Although most of the lesions had relatively low SUVmax values and interpretation of these depends on the background rate of lesions with low-level uptake in the normal population, these experienced reviewers did not consider the scan findings to be typical of a normal population.”
• Line 256: “Although it is a limitation of the study that we did not scan a group of unexposed controls in our local population for comparison, a study powered to determine whether there is a significantly higher rate of these lesions in TB household contacts versus unexposed controls would require a much larger sample size, which was not possible due to financial and logistic constraints. Furthermore, we felt there may be ethical issues with subjecting healthy controls to radiation exposure, given the existing literature in healthy individuals which was available for comparison (as discussed above).”

ANIRBAN MUKHERJEE, M.D. (Reviewer 3): Acceptable paper with insights.

Yours sincerely,

Dr. James Molton, on behalf of all authors