Author’s response to reviews

Title: Nitric oxide production in the exhaled air of patients with pulmonary tuberculosis in relation to HIV co-infection

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Author’s response to reviews: see over
Dear Editor,

Please find enclosed a thorough point by point response to the comments from the reviewers on the revised manuscript. We have also copyedited the paper in an effort to improve the style of the written English as well as checked carefully so that it conforms to the journal style.

With the best regards,

Thomas Schön MD PhD, on behalf of the authors.

Reviewer 1, Dr Malerba

In accordance with the valuable comments of this reviewer we discussed more thoroughly on the subgroup analysis of FeNO values in the HIV-/TB subgroups, but as suggested by the reviewer, this analysis was not included in the conclusions of the study and only as a part of the discussion section:

“Although the levels of FeNO in HIV+/TB patients did not differ from those of HIV-/TB patients there was a significantly higher proportion of patients with FeNO levels above 25 ppb in the HIV-/TB group compared to HIV+/TB patients. Interestingly, we found elevated levels of uNO in the subgroup of HIV-/TB patients with FeNO>25 ppb and this data indicates that the production of NO in response to TB could be heterogeneous with a subgroup of patients responding with an increased NO production that could be detected both by FeNO and urinary NO metabolites. However, there were no differences in sputum smear score, chest x-ray grade or BMI at treatment initiation with regards to FeNO levels that could support the hypothesis that the levels of FeNO could be associated to the severity of disease. Larger clinical studies including long term clinical follow up will be essential to test this hypothesis thoroughly.”

Moreover, the discussion section was again revised in an effort to increase clarity. As observed and suggested by the reviewer, the previously revised conclusions that was described in the paper was included in a revised version of the abstract.

“In both HIV- and HIV+/TB patients, low levels of exhaled NO compared to blood donors and household were observed. Future studies are needed to confirm whether low levels of exhaled NO could be a risk factor in acquiring TB and the relative importance of NO in human TB.”

Reviewer 2, Dr Wang

We are grateful to the comment on the subgroup of HIV-/TB patients with a FeNO level above 25 ppb. Following further analysis we found no significant difference in chest x-ray grading, body mass index or bacterial load with regards to FeNO levels but there was a trend for a increased urinary levels of NO in the FeNO>25 ppb-group. The result of this analysis was included in the results section:

“In the HIV-/TB patients with FeNO levels >25 ppb there were no differences with regards to sputum smear score, chest x-ray grade or BMI compared to the HIV-/TB patients with FeNO levels <25 ppb. Although not statistically significant, there was a trend for increased uNO levels in HIV-/TB patients with FeNO >25 ppb compared to those with FeNO <25 ppb (1684 vs. 1147 μM, p=0.140).”
This issue was also briefly discussed in relation to low NO production in the local lung as a risk factor for developing TB:

“Although the levels of FeNO in HIV+/TB patients did not differ from those of HIV-/TB patients there was a significantly higher proportion of patients with FeNO levels above 25 ppb in the HIV-/TB group compared to HIV+/TB patients. Interestingly, we found elevated levels of uNO in the subgroup of HIV-/TB patients with FeNO>25 ppb and this data indicates that the production of NO in response to TB could be heterogeneous with a subgroup of patients responding with an increased NO production that could be detected both by FeNO and urinary NO metabolites. However, there were no differences in sputum smear score, chest x-ray grade or BMI at treatment initiation with regards to FeNO levels that could support the hypothesis that the levels of FeNO could be associated to the severity of disease. Larger clinical studies including long term clinical follow up will be essential to test this hypothesis thoroughly.

... Future prospective follow up studies are needed to test the hypothesis that low NO production in a household contact could be a risk factor for developing TB and whether a high NO production on exposure could be protective against the disease.”

Reviewer 3. Dr Schluger

We agree that the study is largely observational as it is a cross sectional study and this was added to the abstract. Our hypothesis was that if nitric oxide is important in human TB we should be able to detect a difference in exhaled nitric oxide levels in between TB patients and controls and that this difference might be dependent on HIV status. Subsequently the aim as stated in the abstract was to investigate levels of FeNO in relation to clinical symptoms and urinary NO metabolites (uNO). We have now added some more background in the abstract about the link to our previous studies using L-arginine supplementation. We agree that further studies including longitudinal, interventional studies are needed to further substantiate these data and to clarify the role of NO in human TB and such efforts are underway.

1. The Gondar region in the north of Ethiopia is a high endemic area for tuberculosis and most likely the majority of the adult population has been exposed to tuberculosis several times and will acquire latent infection early in life. Because of this reason it is very difficult in such areas to determine if a healthy individual was recently or much earlier exposed to TB based on a PPD or IGRA-test. A medical history of recent TB exposure may be most important in relation to this issue and these control subjects were excluded from our study. Although not tested in our study, we find it unlikely that latent infection established from exposure several years back would influence the level of NO and this was not within the scope of our study. However, from our data we noticed that the highest levels of FeNO and uNO were found in household contacts to patients with active TB which could be due to a recent exposure and high level of antigen exposure. We also acknowledge that because of a high rate of ongoing transmission and exposure in the society, a recent TB exposure in some of the blood donors could be an explanation for the relatively high levels of NO in some individuals of this group which should be taken into consideration when comparing these data to populations in low endemic countries.
2. The HIV status of all the controls who were included in the study was known as they were all blood donors and 6 % (4/63) were HIV positive.

3. Although not studied in the present trial, we have previously observed a decrease in NO metabolites in Ethiopian HIV negative TB patients following treatment (Schön et al, Scand. Jour. Inf. Dis. 1999). As previously mentioned, we certainly agree that further studies including longitudinal, interventional studies are needed to further substantiate these data and to clarify the role of NO in human TB and such efforts are underway.

4. In the blood donors, the FeNO was measured on two consecutive days and there was a strong correlation (figure 2).