Author's response to reviews

Title: Differential in vivo expression of mycobacterial antigens in Mycobacterium tuberculosis infected lungs and lymph node tissues

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

Thank you for your valuable feedback. Following is the response to your comments.

Comment 1. The TB cases are not confirmed by culture or microbiology in all cases. Its a combination of "The diagnostic criteria were a combination of clinical, microbiological and histological criteria". This makes interpretation of data problematic.

Author’s response: The microscopy and culture is known to have low sensitivity for extrapulmonary tuberculosis and the diagnosis has to be based on a combination of criteria. Good response to treatment is a reliable criterion to confirm a case of tuberculosis where microscopy and culture is negative and clinical, biochemical and histological information gives strong suspicion for TB, which is the case in this study. Furthermore the cases were confirmed by using *M. tuberculosis* specific nested-PCR.

Comment 2. The negative controls have no evidence of lack of MTB infection or even disease - moreso since some had positive antigen staining.

Author’s response: Negative controls were obtained from the archives. There were 9 foreign-body granulomas of the skin having no clinical symptoms of tuberculosis, and 1 each from colon cancer, normal tonsillar tissue, and lung tissue from ischemic heart disease. Based on the clinical information, there was no reason to suspect latent or active tuberculosis in these cases and therefore these biopsies were not originally tested for tuberculosis. For the purpose of this study the cases were further confirmed to lack MTB infection by negative nested-PCR (and negative histology and ZN staining). The positive antigen staining in the negative controls was concluded as non-specific based on the quality and location of positive signals, which were different from the positive controls and it was possible to discriminate that by an expert pathologist. However we decided to include this information of non-specific staining as positive signals in order to highlight that which of the antigens/antibodies are better suited for use as a diagnostic test, where the interpretation should be more robust without the need of an expert in immunohistochemistry.

Comment 3. Cutoffs for positive and negative seem not very clear, including "observer error" as pointed out.

Author’s response: The observers error mentioned was in the cell count of positive cells and therefore a semi-quantitative data is presented instead of exact numbers/percentages. We did not use a cut-off of positive cells to define a positive case since extrapulmonary TB is usually paucibacillary. The staining in negative controls was instead discriminated as non-specific based on the quality and location of staining, for example diffuse weaker nuclear staining in negative controls while strong positive cytoplasmic staining in the positive controls is one example.