Author's response to reviews

Title: Circulating levels of Insulin-like Growth Factor-I (IGF-I) correlate with disease status in leprosy

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

I wish to thank the editors of BMC Infectious Diseases for giving us the opportunity to revise our manuscript based on the suggestions and constructive criticism provided by the reviewers. We have responded to all reviewer comments, point by point, and have revised the manuscript accordingly.

**Responses to comments of Reviewer #1**

**Major compulsory revisions:**

1. The results section of the abstract is confusing, e.g. lines 49-54, and should be revised
Response: Following the reviewer’s suggestion, the results section of the abstract was revised.

2. There are some concerns re patient selection:
   a. Are healthy staff appropriate controls?
      Response: This is an important question. The reviewer’s concern is probably related to the fact that healthy staff has been exposed to Mycobacterium leprae (ML) and might be latently infected. However, IGF-I serum levels in this group of individuals were in the expected normal range, similar to the levels observed in BT patients. Abnormal lower levels of IGFs were only observed in disseminated multibacillary infection as shown by the significant lower circulating IGF levels detected in LL and BL patients. Currently we are measuring serum IGF levels in healthy individuals with different degrees of exposure to ML to evaluate whether highly exposed individuals such as household contacts of multibacillary patients show lower levels when compared to the other groups.

   b. The male-female ratio for HC is not comparable to that for the patients.
      Response: The reviewer’s comment is pertinent. However, we believe that the differences in male-female ratios among groups did not influence the conclusions drawn in our study. The individuals included in our analysis were all over 18 years old. Several studies in the literature, including one performed on a Brazilian population to establish the circulating reference ranges for IGF-I/IGFBP-3 according to age and gender, have shown that these levels are influenced by gender at final childhood and puberty, but not at the adult phase (Rosario, Arq Bras Endocrinol Metab 2010, 54: 477; Brugts et al., J Clin Endocrin Metab 2008, 93: 2539).

   c. Were specimens for HC taken at the same time as those for the patients (years ago) or were they more recent? I.e., is there a bias for old vs new specimens?
      Response: The specimens of HC were taken at the time the study started, while those of the patients were older (frozen aliquots kept up to five years at -20C). Actually, the comparison of old with fresh serum samples was also our concern, since, as mentioned by the reviewer, this could introduce a bias in our study. This study started in 2005, when serum specimens of the HC individuals were collected. At the beginning of 2011, we
decided to measure again the IGF-I and IGFBP-3 levels in serum aliquots of the same HC individuals stored during 6 years in the freezer. The values obtained in the last measurement were quite similar to those observed in the year of serum collection (2005), showing that these proteins are very stable over time in serum.

d. What is known about diabetes or other endocrinopathies (e.g. thyroid) among patients? Were patients with diabetes excluded?

**Response:** The serum samples included in the present study were selected after careful evaluation of the recorded data of patients. Anthropometric measurement (body mass index, BMI) and biochemical analysis such as glucose, lipoprotein and low high-density, total cholesterol and triglycerides were among the parameters under consideration. Patients with diabetes, obesity or cholesterol disorders were excluded. Thyroid hormones were not measured. The criteria of inclusion in the study are now better described in the Methods section of the revised manuscript.

d. Nutritional status affects endocrine function – What is known about malnutrition or obesity among patients?

**Response:** As mentioned above, patients with obesity were excluded. Regarding the nutritional status, over 90% of our leprosy patients at FIOCRUZ/RJ are from the low socio-economic class, and consequently malnutrition must be frequent among all patients groups included in the study. Thus, we do not expect to see differences among groups in nutritional status that could explain the differences in IGF values observed among them.

3. Line 134 – T1R is not an acute inflammatory syndrome

**Response:** The reviewer is right and this mistake was corrected in the revised manuscript.

4. The findings during and post-treatment are very interesting, but the authors should avoid over-interpreting these because the numbers are small and many other factors have potentially changed – age, nutritional status, other illnesses, etc.

**Response:** We are in agreement with the reviewer comment on regarding to the small sample numbers analyzed by this study. One justification for this small numbers is the rigorous criteria used for patient inclusion in the study as mentioned above. Moreover, since IGF-I levels vary according to age, potential differences in IGF-I levels due to age
differences were minimized by conducting a statistical analysis that included the age as a covariate, as mentioned in Methods section (Statistical analysis). The results obtained in the case of pre- and post-MDT were very consistent. All paired pre- and post-MDT serum samples taken from the same patient, although in small numbers, gave similar results.

5. Overall, the discussion is too long and often too speculative. The sample size is not great enough to reach too many conclusions, although the findings are interesting and provocative.

Response: Accordingly, the discussion section has been shortening as recommended by the reviewer.

6. Line275-6 – the findings may be over-interpreted here. They suggest that ML infection may have modulated IGF-I but do not “clearly indicate” this, due to the small sample size and retrospective nature of the study.

Response: As recommended by the reviewer, this sentence was changed in the revised manuscript as follows: “Moreover, recovery of IGF-I levels after treatment suggest that the low levels observed in these patients resulted from a direct down modulation by ML infection”.

Discretionary revisions:

7. It would be useful to have some sentence in the abstract that indicates why IGF-I was chosen instead of a myriad of other endocrine markers.

Response: As suggested by the reviewer the abstract was changed in the revised manuscript to turn this point clearer.

8. Some references are rather dated (e.g., 2, 4, 5, 19, 22, 23, 24) and for most of these there are more recent papers on the subject.

Response: As indicated by the reviewer, most of these references were placed by more recent ones.

9. Fig 2E (TNF declined during ENL in one patient – did this patient have any different pattern for IGF-I?

Response: Actually, the decrease of TNF-α levels during ENL in the patient mentioned was
also noticed by us at the time of data analysis. However, the IGF-I and IGFBP-3 serum levels in this patient followed the same pattern of the other patients.

10. Results, Line 226 ff and Fig 2: The suggestion that IGF-I levels at baseline might be predictive of future reaction is interesting but is certainly not proven by these preliminary findings in a retrospective study. Can the time variable be explored here? i.e., were increases in baseline RLL greater when the initial blood sample was taken closer to the time of subsequent reaction? Or is the sample is not large enough to evaluate this?

Response: This is an interesting question. Based on this comment, we re-analyzed our data dividing the RLL patients in two groups: those who developed RR at one year or less from the onset of treatment and those who underwent RR after one year of treatment. Both RLL groups presented similar IGF levels and, when compared individually to the LL NR group, both showed higher significant levels.

11. In the paragraph beginning on line 302, immunosuppression is invoked as an explanation. If so, then might some reduction If “LL suppression” also be associated with ENL?

Response: Yes. Actually, this is exactly what we were trying to explain in this paragraph. While lower levels of IGF-I/IGFBP-3 might be reflective of high immune-suppression levels, a controlled immune-inflammatory response, and high clinical stability in NR LL patients, higher levels of this hormone in R LL patients might indicate reduction of suppression and ENL development.

12. The paragraph starting at line 332 might be omitted or greatly condensed, since it discusses medical conditions quite unlike infection.

Response: Following the reviewer’s suggestion, this paragraph was removed.

13. Is there some literature on IGF-I levels in tuberculosis that might be enlightening?

Response: There is one paper by Del Rey et al. 2007 (Brain, Behavior, and Immunity 21:171) in which several cytokines and hormones, including IGF-I, were measured in male tuberculosis patients with mild, moderate, and advanced lung disease and in healthy controls. Although they found differences in several hormones according to disease status, plasma levels of IGF-I in TB patients did not differ from those of the controls. This
reference was included in the revised manuscript.

I hope the manuscript is now acceptable for publication. Thank you for your consideration.

Sincerely yours,

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