Author's response to reviews

Title: Cryptic Leishmania infantum infection in Italian HIV infected patients

Authors:

Claudia Colomba (claudia.colomba@libero.it)
Laura Saporito (laura.sapo@tin.it)
Fabrizio Vitale (fstefano.reale@izssicilia.it)
Stefano Reale (stefano.reale@izssicilia.it)
Giustina Vitale (vitale@unipa.it)
Alessandra Casuccio (casuccio@unipa.it)
Manlio Tolomeo (manliotolomeo@hotmail.com)
Daniela Maranto (dany.maranto@libero.it)
Raffaella Rubino (raffaellarubino@libero.it)
Paola Di Carlo (dicarlo19@libero.it)
Lucina Titone (titonel@unipa.it)

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

Please find herewith included the revised version of the manuscript entitled:

**Cryptic Leishmania infantum infection in Italian HIV infected patients.**

All the comments have been taken into due consideration and the text has been corrected accordingly.

We apologize for the mistakes and imprecision of the previous versions of our manuscript that has been the consequence of misunderstanding among researchers working in different fields. Our colleagues of the molecular biology lab reviewed the results of PCR leishmania and found that two samples, previously reported having a parasite load of 1 parasites/ml, had instead a parasite load of 0.24 and 0.12 parasites/ml respectively.

Due to these new data, univariate linear regression results changed as follows: a significant association for PCR levels was confirmed with plasma viral load (p=0.0001) but not with CD4 cells (p=0.061). For this reason, multivariate analysis was eliminated.

Results and interpretation of the data have been now submitted to a careful review with the statistician, who had not participate to drafting the discussion in the previous version of the manuscript. Consequently, the mention of a correlation with the parasitemia and CD4 counts has been eliminated.

Particularly, all the changes to the manuscript are reported below:

- Affiliation of dr. Casuccio has been changed with “Dipartimento di Neuroscienze Cliniche, Università di Palermo, Palermo, Italy”
• Mention of multivariate analysis has been removed from abstract, methods and results sections. Multivariate analysis has been eliminated because of new results of univariate analysis.

• The correlation with the parasitemia and CD4 counts has been eliminated from abstract, results, discussion and conclusion sections.

• In the abstract section, p value related to the association between PCR-Leishmania levels with plasma viral load has been mentioned.

• The methods section has been changed as follows: “We detected the limit of the sensitivity, by testing decimal serial dilutions of the standard DNA below to 1 parasite equivalent per ml. Reproducibility of the PCR Leishmania test was 100% at a level of DNA concentration corresponding to 0.1 parasites/ml, which was considered the threshold for positive samples.”

• The results section has been changed as follows: “L. infantum kDNA was detected by PCR in 24/145 (16.5%) asymptomatic patients. Parasitemia was detected also in one additional patient, who had been admitted for neurotoxoplasmosis, and during the hospitalization developed severe anemia and splenomegaly. For this reason he was treated with intravenous liposomal amphotericin B and was excluded by analysis. Parasitemia ranged between…”

• In the results section, the lower limit of leishmania parasitemia has been changed from 1 parasites/ml to 0.12 parasites/ml

• In the results section, the sentence about univariate analysis has been changed as follows: “Univariate regression analysis showed a significant association for PCR levels with plasma viral load (p=0.0001) but not with CD4 cells (p=0.061)”
• In the conclusions section, the following sentence has been included “Hence, as showed by regression analysis, the plasma viral load could be considered a good independent marker of a increase in the PCR parasitemia levels.”

• Table 1 has been modified with the new parasitemia values.

• Table 2 has been modified and its legend has been changed into “Univariate linear regression analysis”

• Figures 1 and 2 have been modified with the new parasitemia values.

The other authors and I thank you and the reviewers for the attention given to the manuscript. I hope to have given exhaustive responses and I look forward to your decision.

Regards,
Claudia Colomba

Dipartimento di Scienze per la Promozione della Salute, Sezione di Malattie Infettive

Università di Palermo

Via del Vespro 129, 90127 Palermo, Italy

claudia.colomba@libero.it

tel. +39 91 6554054    fax +39 91 6554050