Reviewer's report

Title: Lymphocyte transformation assay for C neoformans antigen is not reliable for detecting cellular impairment in patients with Neurocryptococcosis

Version: 1 Date: 27 March 2012

Reviewer: Bianca Bianco

Reviewer's report:

Dear Editor,

In the study performed by Rocha et al, the authors aimed to standardize a specific lymphocyte transformation assay to Cryptococcus neoformans, to explore different methods of analysis, and to propose statistical methods for conducting data analysis in healthy volunteers, poultry growers, and HIV-seronegative patients with neurocryptococcosis. Isolated PBMC were stimulated with C. neoformans antigen, C. albicans antigen, and pokeweed mitogen. For all antigens, the amount of 3H-thymidine incorporated was assessed, and the results were expressed as stimulation index (SI) and log SI, sensitivity, specificity, cut-off value (receiver operating characteristics curve). The results disclosed that the LTA have a low capacity with all the stimuli for classifying patients as responders and nonresponders. LTA stimulated by KAg from patients with NCC was not affected by TCD4+ cell count, and the intensity of response did not correlate with the clinical evolution of neurocryptococcosis. The authors concluded that response to lymphocyte transformation assay should be analyzed based on a normal range and using more than one stimulator; the use of a cut-off value to classify patients with neurocryptococcosis as responders or nonresponders is inadequate; statistical analysis should be based on the log transformation of SI; and a more purified antigen for evaluating specific response to C. neoformans is needed.

Overall it is an interesting study, but I have the following concerns and comments:

1. Abstract
Describe briefly the methods and statistical analysis in the abstract.
Avoid acronyms and abbreviations, and when to use them for the first time, spell out.
Describe how many patients there is in each group.

2. Material and Methods
In general, the methodology is well described. Nevertheless, insert city and country to “Amparo Maternal”.
Are the protocols approved by “Universidade Federal de São Paulo” and “Instituto Emílio Ribas” validated? Were the protocols published? If yes, please
quote the reference. Otherwise, using validated protocols.

4. Discussion

I advise authors to diminishing the discussion.

The results of the study should be discussed with recent data from literature. Moreover, the references are very old. The most recent reference is 2005. Discuss the importance of the study in the context of Neurocryptococciosis infection.

5. References

The references are old. There is no mention of reference newer than 2005. Update them.

6. Minor points

Write out the acronyms and abbreviations when first cited in the text. Example: SAg and KAg that were explained just in the discussion section.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this paper, either now or in the future?

NO

Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this paper, either now or in the future?

NO

Do you hold or are you currently applying for any patents relating to the content of the manuscript?

NO

Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

NO
Do you have any other financial competing interests?
NO
Do you have any non-financial competing interests in relation to this paper?
NO
I declare that I have no competing interests