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Response to letter to the editor: Comment on "Mutagenic damage among bronchiectasis patients attending in the pulmonology sector of a hospital in southern Brazil"

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Dear Editor,

We are grateful to have the opportunity to respond to the letter to the editor: "Comment on 'Mutagenic damage among bronchiectasis patients attending in the pulmonology sector of a hospital in southern Brazil'" and equally happy that the authors of the letter are interested in our study. The scientific debate that discusses theoretical and methodological aspects is capable of improving knowledge.

Regarding the content of the letter, de Moraes Malinverni et al.[1] comment on the limitations of using the staining technique with eosin-methylene blue according to Leishman. This point has already been discussed elsewhere[2], and we reinforce here that the study cited by the authors of the letter to the editor[3] did not mention staining using eosin-methylene blue according to Leishman. Furthermore, the study itself does not rule out the possibility of using simple staining techniques such as May-Grünwald. The technique employed by Votto Olmedo et al.[4] is an appropriate mixture for the differential visualization of both the cytoplasm and the cell nucleus and is recommended in the study by Korsakov et al.[5] for use in assessing mutagenicity in oral cells. For a complementary reading in relation to possible cellular artifacts, we recommend da Silva Júnior et al.[2] who already discuss these same aspects brought up here.

Regarding the second point discussed by the authors of the letter, the number of cells analyzed, we apologize to the readers because we did not include in the materials and methods section that 2,000 cells per patient were visualized and the results were expressed as the number of micronuclei in 1,000 cells. The way of describing the methodology was not clear and is certainly a point of confusion for readers. We appreciate the opportunity to clarify this point.

Considering the points highlighted by de Moraes Malinverni et al.[1], regarding the use of "lymphocytes as a result of systemic host response," we would like to highlight that the study was conducted in a hospital environment, including stable patients, where blood collection is not part of the routine consultations, and based on the high correlation of results obtained between oral mucosa and lymphocytes, previously described by Ceppi et al.[6], we chose to use a noninvasive method.

The final point of the letter concerns the investigation of cytotoxicity using metanuclear markers. In fact, it is a very interesting strategy, but it is beyond the scope of our study. In any case, cytotoxicity is always measured through simple techniques using Trypan blue, tetrazolium salts, or resazurine, where viability was always found to be above 90%.

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AUTHORS' CONTRIBUTIONS

FMRSJ: Conceptualization, Data curation, Writing – review & editing. **DFR**: Conceptualization, Data curation, Writing – review & editing. **DWVO**: Formal Analysis, Investigation. **KBM**: Formal Analysis, Investigation. **MMP**: Formal Analysis, Investigation. **CLFF**: Formal Analysis, Investigation.

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