Author’s response to reviews

Title: ALA-D activity is a reliable marker for oxidative stress in bone marrow transplantation patients

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Version: 3 Date: 23 December 2008

Author’s response to reviews: see over
We are returning you our above mentioned manuscript that was carefully revised to incorporate the suggestions made by the reviewers. We have also include the informed consent in Methods. We hope that now our manuscript be more suitable for publication in BMC Cancer and we would like also to thank the reviewers for their suggestions that have contribute to increase the quality and clarity of our manuscript.

For your convenience, we have answered the reviewers queries in red just before them (see bellow).

Sincerely yours,

Thissiane Gonçalves
Reviewer's report
Title: ALA-D activity is a reliable marker for oxidative stress in bone marrow transplantation patients
Version: 2 Date: 5 November 2008
Reviewer: Piotr Dziegiel

Reviewer's report:
The paper submitted for a review pertains study of ALA-D activity as reliable marker for oxidative stress in bone marrow transplant patients. The study involves an interesting approach to the problem of oxidative stress after chemo and radiotherapy before bone marrow transplantation.
I would like to present the following remarks and suggestions for the authors:
1. In my opinion the language correction should be done. I suggest the title should be changed into “#-ALA-D activity is a reliable marker for oxidative stress in bone marrow transplant patients”
    OK, title was changed according to the reviewer’s suggestion.
2. Methods: Description of the patients treatment is complicated and should be placed into the table 1.
    OK, we have moved the text to legend of the table 1.
3. Methods: The abbreviation „HSCT“ should be explained.
    OK.
4. Methods: The abbreviation „MDA“ (malondialdehyde) should be explained.
    OK
5. Methods: #-ALA-D – there is lack of measurement unit.
    OK.
6. For estimate oxidative stress, should be made activity of GPx (Glutathione peroxidase) and glutathione reduced (GSH) and oxidized disulfide form (GSSG).
    We, agree with the reviewer, but unfortunately at the time of sampling we were unable to carried out such important derminations. In fact, we are planning in the future to carry out these type of investigation in another set of patients, but now we cannot perform such determinations because the samples used in the present investigation are no more available. Anyway, we have determine the NPSH group that is an reliable indicator of GSH levels.
    Summing up, in my opinion the paper should be thoroughly corrected and re-edited.
    OK, we have re-edited the entire manuscript and the language was carefully corrected by someone more fluent in the English language.

Level of interest: An article of importance in its field
Quality of written English: Not suitable for publication unless extensively edited
Statistical review: Yes, and I have assessed the statistics in my report.
Reviewer's report
Title: ALA-D activity is a reliable marker for oxidative stress in bone marrow transplantation patients
Version: 2 Date: 24 November 2008
Reviewer: Andrea Ferencz

Reviewer's report:
Page 4. "a condition known as oxidative stress, which can be associated with serious complications such as hepatic failure." Why did you mention hepatic failure? As our knowledge the small bowel is one of the most sensitive tissue to oxidative injury, mainly during intestinal transplantation.
Please cite one article from this field.
OK. We agree with the reviewer and we have changed slightly the text and have also cited a paper about oxidative stress in small bowel transplantation (Surgery volume: 132 issue: 5 pages: 877-884)

Level of interest: An exceptional article
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Reviewer’s report
Title: ALA-D activity is a reliable marker for oxidative stress in bone marrow transplantation patients
Version: 2 Date: 2 December 2008
Reviewer: Ana Coto-Montes

Reviewer’s report:
ALA-D activity is a reliable marker for oxidative stress in bone marrow transplantation patients
The manuscript study the possible role of ALA-D as an adequate biomarker of oxidative stress in autologous and allogeneic bone marrow transplantation patients. Likewise, the manuscript shows the increase of oxidative stress in these patients by several procedures.
This is a very interesting article in which is possible to find habitual procedures of basic research developed in a clinical problem. This way is not very usual but it has always very good results. And this article is an example, since it is simple, direct, easy to read but also with interesting conclusions. I think that it could be accepted with minor changes.

We would like to thank the reviewer for her kind words.
Following the points the journal proposes for reviewing an article:
The initial questions have been clearly exposed and they are enough for developing the study.
Mostly of methods were appropriated but I have some questions about them:
-I don’t understand why authors have studied the activity of CAT and not glutathione peroxidase (GSH-Px) when this enzyme is the most abundant in erythrocytes. The results that they have obtained are very good but it is necessary to take into account that some of superoxide anions that SOD produces can be neutralized by GSH-Px, therefore its activity would be very useful in order to know whether SOD and CAT-GSH-Px are adjusted or not.
We agree with the reviewer, but unfortunately during the period of sampling were not able to carried out this important determination. In fact, this is a limitation of our study, but in the future we are planning to do such important determinations (but this will take more than one year to be carried out). The change in thiol status after BMT reinforces the importance of quantify GPx.
-Tandem protein-thiol and non protein thiol is not usual and it is interesting because of the reductor role of thiol groups but explanation about its meaning in material and methods would clarify the reasons of this procedure.
OK. We have tried to clarify this aspect and we emphasized in the revised manuscript that thiol status (P-SH and NPSH) can give an idea about the redox state of the plasma/blood.
The results are clear but I have a few questions about:
-Why authors have included the characteristics of patients into results? I think that all these data have to be included into Material and Methods because they have described the population of the study without any transformation.
OK, we have moved this part to Material and Methods section.
-Data from antioxidant enzymes (SOD and CAT activities) should be present by graphics. This is the usual way and it allows to compare them.
OK. We have now presented the data for SOD and CAT as figures.
-In relation to ALA-D, the results that authors have offered are very clear and interesting because they sowed that the activity of this enzyme goes down after the treatment. However, decrease in activity could be due to general decline of
the heme route, which doesn’t imply ALA accumulation. To solve this question is only necessary to study the activity of ALA-S (6-aminolevulinic acid synthase). This enzyme is the rate-limiting enzyme of heme synthesis and its activity study could indicate the existence (or not) of ALA accumulation. This perspicacious observation of the reviewer is now briefly discussed in the revised manuscript. In fact, this is an very important observation and we will consider it in the future. Unfortunately, this had not occurred to us during this study and in other previous studies from our lab. In fact, though it can be seen as a trivial suggestion by someone, we consider it very important to our future studies (not only in the case of BMT).

The discussion is short but clear. However, the absence of any description of several problems of ALA accumulation could produce in patients is observed since this is an important prooxidant with huge effects in the organism as porphyria can shown.

OK, we have expanded the discussion about the possible detrimental effects of an increase in ALA concentration in the revised manuscript.

In general the article is acceptable although some changes, above cited, could improved the manuscript. Mostly of my comments are discretionary revisions and authors can decide to follow the indications or not. However, some additional information about ALA-S, its activity or reasons for avoiding to do it should be included.

OK. We have added to the revised manuscript the importance of determining ALA-S in the future. The reasons for not carry such experiments were two: 1) this did not occur to us and 2) the colorimetric method for ALA-S determination could be an additional problem to us (due to blood availability). However, in the future, we will plan an study to determine only ALA-D, ALA-S and GPx, in order to be able to deal with sample availability.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests