Reviewer's report

Title: Genetic and biochemical markers of hydroxyurea therapeutic response in sickle cell anemia

Version: 2 Date: 4 March 2013

Reviewer: Umberto Moscato

Reviewer's report:

Introduction to the report

The authors evaluated in this study the effects of #S-haplotypes and Hb F levels on oxidative stress markers and their relationship with hydroxyurea (HU) treatment in sickle cell anemia (SCA) patients and they claim that, in Brazil, there have been no studies verifying the effect of #S-haplotypes on oxidative stress parameters.

In a group of 28 SCA patients, 13 treated with HU and 15 SCA patients not treated with HU the authors have identified haplotypes using molecular methodology (PCR-RFLP) and, for the biochemical parameters, using spectrophotometric methods and HPLC coupled to electrochemical detection.

The authors found the highest frequency of Bantu haplotype (48.2%) and Benin (32.1%) and then the presence of Cameroon haplotype in addition to 19.7% of atypical haplotypes. The authors state then that the protective effect of Hb F was confirmed in SCA patients because an increase of Hb F levels resulted in a 41.3% decrease on the lipid peroxidation levels using Pearson correlation (r = -0.74, p =0.01).

The authors conclude that SCA patients with Bantu haplotype showed the worst oxidative status and that, however, these patients also demonstrated a better response to the treatment with HU. Furthermore they affirm, based on this, that such treatment seems to have presented a "haplotype-dependent" pharmacological effect.

Discretionary Revisions

1) Would be useful, for the authors, use the symbology # or # or #, more correct, instead of the symbols < and > and =, even if the significance values achieved are much lower/higher than the limit indicated in the study.

Minor Essential Revisions

1) Abstract-Methods: in the phrase "treatment with HU in SCA patients," the authors should specify, before using the symbol encoded, the term hydroxyurea, and then use the abbreviation (HA).

2) Results second paragraph: authors should explain the meaning of encoding
“and three (10.8%) Atypical 2/Atípico2”, in particular, the term Atípico2.
3) Results last paragraph: authors should check if the decimal fraction of the number is all been properly marked with a dot or sometimes with a comma like this case.
4) References n. 59 and 60: authors should correct the erroneous symbols that appear in the two cited bibliographies (%: what it means, or it is a mistake?!)

Major Compulsory Revisions

1) Statistical Analysis:
a. The authors do not define the power of the study and therefore do not define the sample size under analysis in the study (on the basis of the power of the study), whereas if it was the only study in the Brazilian population, the level of significance of the study, the sample (number of subjects included, etc..), the stratification of the same, the range of significant age and gender, as well as the statistical analysis to be performed (parametric or non-parametric) should be chosen based on that parameter too.
b. In particular, according to the value of the statistical significance accepted, is not defined the level of alpha of the study and the number of cases and controls to be used in the study in question.
c. In fact, the use of parametric or non-parametric statistical analysis is not explained, however, is not defined the limit of only 28 subjects included in the study without explaining the limits which involve for the study such a limited number of subjects included or the motivation for the inclusion of only these subjects (between the other without a significant stratified analysis). It is also not defined clearly if the distribution is normal or not normal. In fact: though the authors use properly the Shapiro-Wilk test for this purpose, do not define the results of the same test, and therefore they do not define what data was correct to use with the parametric tests and with non-parametric tests……
d. Also the use of the Pearson correlation or the Spearman rank test should be explained in the grounds and within the limits expressed in the research in question.

Level of Interest
An article of importance in its field

Quality of Written English
Acceptable

Statistical Review
Review what is expressed in Major Compulsory Revisions…

Declaration of Competing Interests
I declare that I have no competing interests
Report on the study and conclusions on scientific Article

1) The question posed by the authors is well defined
2) The methods for the study are appropriate and fairly well described, except some aspects of statistical analysis that may affect the results and the findings of the research.
3) The data are sound although the number of enrolled in the study is just enough for a minimum statistical significance (but in other studies like this, the number of enrolled in the study is quite similar). It is important that the authors clarify the compulsory major revisions.
4) The manuscript complies with the relevant standards for reporting and data deposition, understanding some of the issues identified in this peer-review.
5) The discussion and conclusions are well balanced and adequately supported by the data, but it is important that the authors clarify the compulsory major revisions.
6) Study limitations were not well defined and described, in particular in relation to the claim of the authors that this is the only study on HA, beta-s haplotypes and Brazil's population (although other studies have not the same as the one in question, more "care" in this statement would have been more valuable…)
7) The authors do not seem to analyze all studies on the subject, particularly the most recent, whereas the cited articles from the years 2011 and 2012 are typical of some of the authors (self-cited) of the article under review (1. Silva DGH, Belini-Junior E, Torres LS, Ricci-Junior O, Lobo CLC, Bonini-Domingos CR et al.: Relationship between oxidative stress, glutathione S-transferase polymorphisms and hydroxyurea treatment in sickle cell anemia. Blood Cells Mol Dis 2011, 47: 23-28. 2. Belini-Junior E, Silva DGH, Torres LS, de Almeida EA, Cançado RD, Chiattone C et al.: Oxidative stress and antioxidant capacity in sickle cell anaemia patients receiving different treatments and medications for different periods of time. Ann Hematol 2012, 479-489).

In particular, the authors, do not cite recent studies in literature, of similar argument although not the same, also to clarify any limitations of the use of HA in patients with SCA and any side effects which, just as an example,:

• da Silva Rocha LB, Dias Elias DB, Barbosa MC, Bandeira IC, Gonçalves RP. DNA damage in leukocytes of sickle cell anemia patients is associated with hydroxyurea therapy and with HBB*S haplotype. Mutat Res. 2012 Dec 12; 749(1-2):48-52. Epub 2012 Aug 16.
8) The title and abstract convey what they have been found.
9) The paper writing is acceptable

Conclusions of Report

Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions (Statistical Analysis). In the event of a clear answer to the doubts expressed by data in MCR, the scientific paper is
interesting and acceptable for publication.

I attach the pdf with yellow highlighted some of the corrections to be performed.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.