Reviewer’s report

Title: Magnitude of Elevated iron stores and Risk associated in Steady state Sickle Cell Anemia Congolese children: a cross sectional study

Version: 2 Date: 14 Aug 2018

Reviewer: Kristen Stevenson

Reviewer's report:

The study seeks to estimate the prevalence of increased iron stores and identify risk factors in a cross-sectional study in Congolese Children.

1) Using the term "increased" tends to mean that a measurement within a patient increases from one time point to the next. In this context it only means high or elevated or excess iron store?

2) The cut-off that was used seems to be only be recommended based on recommended chelation >500 ng/ml. Did the authors also explore the WHO cut-off for risk of iron overload (>200 ng/ml)? The results of this may also be important with the association with risk factors.

3) In the table the >500 ng/ml cut-off is listed as >= 500 ng/ml, this needs to be consistent throughout the manuscript. Is it defined as greater than or greater than or equal to?

4) Normal blood serum iron levels are stated as between 10 and 30 umol/l however, it would be helpful to know what normal levels are in the same units (ng/ml) as reported for the above cut-offs (line 22 page 4).

5) In the statistical analysis section on page 4, the last sentence needs revised, to state "The risk factors associates with hyperferritinemia were investigated in univariate and multivariable logistic regression analysis and considered significant at the 5% level of significance (p<0.05)."

6) Although the methods state that logistic regression analysis was performed, the authors include no tables with the results from this analysis but only in the text report [aOR 6.17 (95%CI: 1.18-20.96)] for the probability of having a serum ferritin level >500 ng/ml for children transfused more than 3 times last year. Both results for the logistic regression should be stated in the abstract (>3 vs. 0 and 1-3 vs. 0). Usually the notation (aOR) would mean that an adjusted Odds ratio was being reported. It is not clear from the methods what other risk factors were adjusted for in this model? This needs to be stated in the methods and the results of univariate and multivariable (or adjusted) modeling would be best presented in a Table to
show which factors were explored and if they were in the model as continuous variables or categorical ones.

7) In Table 1, for number of transfusions, the % should be included so that the format is n (%). This is also required for Table 2 categorical variables, the n (%) should both be listed, not only the %. In Table 2, the heading is wrong for the definition >500 ng/ml it is listed as >=500 ng/ml.

8) The statistical methods section states that the p-value for the comparison for number of transfusion is a chi-square for testing for an association with sex, however, when I calculate the p-value for this comparison I have p=0.0167 not 0.243 reported in Table 1? Also in Table 2, for the chi-square test for transfusions <3/year% 43% vs. 73%, I get p=0.041? Please re-check all the chi-square tests.

9) It is not clear why Table 1 focuses on difference between sex, when there are no significant difference detected? Why not instead of sex include # of transfusions as the columns (0/yr, 1-3/yr, >3 /yr), since this is the covariate found to be most interesting in logistic regression modeling. It would be of interest to see what the median values of serum iron/ferritin were for each of these categories (like it is plotted in Figure 1) and for all the other lab values. Without these classification, I also cannot calculate the odds ratio reported in the manuscript from table 2, since it doesn't show the 3 categories (0/yr, 1-3/yr, >3/yr) by serum ferritin (<=500 vs. >500).

10) It is odd that the Hct % in Table 2 is exactly the same 22.4 in both categories, is this a typo? Please re-check.

11) Although the lab values are presented as continuous variables, WBC, Hct, serum iron etc. It may be of interest to categorize them into normal vs. non-normal (or different cuts based on the distribution) for children and then test these within logistic modeling. Were these explored categorically? It appears that CRP was using >=6 and >=12 to categorized. The way these variables were included in the model (categorized or continuous) should be shown in the logistic regression analysis tables that need to be provided (unless only 1 factor was found to be significant).

12) In the results section, it states with interquartile (IQ) 25 and 75 intervals, this should be change to read the interquartile range (IQR) was 6 to 13 years.

13) In the results section, it states that 38% received 1-3 transfusions and 12% had not been transfused, however 26/70 =37% and 7/70 is 13% based on Table 1.
14) The first line on page 5 had $\geq 500$ and $\geq 1000$ when that is not how these were defined
$\geq 500$ and $\geq 1000$.

15) The discussion states that multiple transfusions emerged as the main independent
determinant of increased iron stores. Was it the only factor found to be significant?

16) I would rephrase the conclusion section to read, "In SCA children, increased serum ferritin is
most strongly related to blood transfusion. In this study, approximately 1 in 5 children had
hyperferritinemia. Local physicians should......" and then end with a recommendation for
what physicians should do about it, summarizing the bottom paragraph on page 6.

17) Steady state (exclusion of children who were transfused, hospitalized or had major VOC
within the last two months page 4) should be defined in the methods.

18) Tshitol reported 35% of homozygous sickle cell children had ferritin levels above 300 ng/ml,
what is out rate with this cut-off? This should be mentioned if it was similar/different in the
discussion.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Unable to assess

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an
additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further
assessment in your comments to the editors.

I am able to assess the statistics

**Quality of written English**
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published
Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. 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 manuscript, either now or in the future?

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

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

4. 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?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal.