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: 3 Date: 30 Oct 2018

Reviewer: Kristen Stevenson

Reviewer's report:

Below are the questions I submitted previously that still have outstanding issues:

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. 1-3 transfusions/year vs. lack of transfusion and > 3 transfusions/year vs. lack of transfusion were the only two risk factors retained in univariate analysis and which were confirmed in multivariate analysis (logistic regression).

The other factors were not associated with a ferritin level≥ 500 (sex, hydrea intake, CRP> 6, CRP> 12, LDH> median, plasma free Hb> median, age> 5 years)

The variables that were tested need to be included in the methods section. The aOR needs to be reported as an OR b/c it is only a univariable model (only 1 factor is included in the final model it is not adjusted). The final multivariable model is the same as the univariable model.

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.

We have recalculated the p-value.
I don't see any differences in the p-values in Tables 1 or 2 that were reported from the last time until now. I also strongly recommend using a Fisher Exact test rather than a Chi-square test since the frequencies in some of the groups are <=5. I would re-test using a Fisher exact test and include this in the methods.

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). Table 1 gives the generalities. The proposal made is relevant, but the subgroup of nontransfused children has only 9 cases.

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).

The variables were categorical.

The way they are categorized needs to be included in the methods section.

Please provide a the number of transfusions categorized as 0, 1-3, >3 vs. <500 vs. >=500 in Table 2, so that the OR can be calculated and I can check it.

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

Yes

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

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

Yes

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