Author’s response to reviews

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

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Version: 3 Date: 05 Oct 2018

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Kristen Stevenson, MS Statistics (Reviewer 2): 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? We have taken into account the remark in the last version.

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.

In total, 47.8% of the children had ferritinemia ≥ 200 ng/ml. Comparing the number of transfusions with regard to this threshold, we get p = 0.055. We have maintained the threshold of 500 ng/ml (not 200) according to the objectives of our study.
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?

We have taken the remark into account. It is ≥ 500 ng / ml

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).

In this study, the results in ng / ml do not correspond to serum iron, but rather to ferritinemia.

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

We have taken the remark into account.

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)
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.

We have taken the remark into account.

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.

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 non-transfused children has only 9 cases.

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.

Analysis redone, the values of the median and interquartiles were confirmed. The database can be made available to you.

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.

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.

The correction was made.

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.

The correction was made.

14) The first line on page 5 had >=500 and >=1000 when that is not how these were defined >500 and >1000.

The correction was made.

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?

Effectively.

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.

We have taken the remark into account.

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.
We have taken the remark into account.

18) Tshilol 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.

41% of children had a ferritinemia ≥ 300 ng / dl. This threshold does not allow to indicate the iron chelation. To make it a point of the discussion might go beyond the scope of the study.