Author's response to reviews

Title: Anaemia in HIV-infected children: Severity, types and effect on response to antiretroviral therapy.

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Version: 2 Date: 6 September 2012

Author's response to reviews:

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September 4, 2012

To The Editor in Chief
BMC Pediatrics,
Dear Sir/Madam,

Re: Submission of revised manuscript as Research Article

On behalf of my co-authors, I am pleased to submit a revised manuscript entitled “Anaemia in HIV-infected Children: Severity, Types and Effect on Response to HAART”.

We thank the reviewers and the editors for their thorough and thoughtful review. We have worked to improve the manuscript based on the reviewers’ comments. Our specific approach to each reviewer’s suggestions is detailed below.

Reviewer DCS
Scientifically- no definition of haemoglobin levels according to the age
In the abstract (results) anaemia is considered 11g/dl regardless of the age of the child
In the "Outcome measures" again no correlation with the ages of the patients (very broad 3 months-18 years)
Thank you for this important observation. While conducting the study, we defined anaemia according to WHO age ranges as quoted and we apologize for the oversight during manuscript writing.
The validity of the "post treatment score" calculated according to the percentage 10 and 15% as well as the "change score" -?
We appreciate this observation. The standard way of expressing or measuring
treatment success on HAART is by quantifying rise in CD4 counts and reduction in viral load. The scores referred to were created specifically for the purpose of the comparisons in this study and were not deliberately meant to be validated. We have re-written the relevant section to reflect the exact comparisons that were done as reflected later in the results section.

No iron studies as well as no exclusion of other possible causes to induce anaemia. No comparison to the levels of haemoglobin in the population (age related)

No RPI discussion (limited to reticulocyte production)

We appreciate these important observations. We have included a paragraph on limitations highlighting these specific weaknesses. We, however, feel that the data reinforce the observed association between anaemia and advancing HIV disease, and also raise important questions regarding the mechanism for the blunted response to HAART associated with anaemia.

The affirmation: The prevalence of anaemia in this group was lower than in other studies...) not explained by the higher mean age- any corrected age, any previous factors, etc.

We were concerned about the lower prevalence of anaemia in this study compared to previously published similar studies. Age was an important factor because when we consider the 145/257 children age 3-59 months, the prevalence of anaemia was 111/145 (76.5%) which is similar to the prevalence previously published in similar African settings (Eley et al, 2002; Adetifa et al 2006).

Reviewer AT

Page 6, Paragraph 1, Line 4: It must be rephrased as: Efavirenz replaced nevirapine...

Thank you for this observation. The error was corrected as recommended.

Page 9, paragraph 1: Baseline assessment: The % of patients with AIDS should be mentioned.

It is important to know the percentage of the population with AIDS and the association with anaemia

We appreciate this comment. The percentage of patients with AIDS (clinical stage IV) was merged with clinical stage III patients and addressed as late stage HIV disease to reflect the number of patients who needed ART as was relevant to the prospective cohort part of our study. A total of 79.1% of the anaemic children (117/148) were in late clinical stage and required ART (table 2).

Page 12, Line 1: It should be mentioned that another well studied cause of anaemia at least in adults is the presence of antibodies to endogenous erythropoietin Reference “Tsiakalos A, Kordossis T, Zikas PD, Kontos A, Kyriaki D, Sipsas NV. Circulating antibodies to endogenous erythropoietin and risk for HIV-1- related anaemia. Journal of Infection 2010; 60: 238-243.”

Thank you for this contribution. The study has been cited and the issue of lack of identifying the causes of anaemia was further highlighted in the limitations of the
study.
The authors should mention the proportion of the studied population that was infected with HIV-1 and HIV-2, respectively. Did they notice any significant differences between them?

We appreciate the observation but due to limited funding, we were unable to find out if the patients were infected with different strains of HIV. However we have indicated that other studies show HIV-1 is by far the predominant strain in this part of Africa.

Page 13, paragraph 3: The authors try to correlate virological response to anaemia in general. Firstly they do not distinguish the response to the various types of anaemia. Secondly they must consider that anaemia may not be the reason, of the worst virological response among the anaemic population but may be just an epiphenomenon.

Since malnutrition is a well-known cause of anaemia it is essential to measure parameters such as iron status, B12, folic acid. This is a major problem of the study, since we do not exactly know the extent that those parameters interact with anaemia.

Thank you for these observations. Our study did not have sufficient numbers to study the impact of type of anaemia on response to ART. This should be explored in larger studies.

Indeed anaemia is not the only factor to explain the outcome of successful treatment. Other factors include adherence to medications, severity of immune suppression, age and nutritional status. We were aware of this and conducted a multiple regression to adjust for these confounders and anaemia remained a significant predictor of virological response

Reviewer EM

The design is not clear. It appears the authors employed a hybrid of two designs. A cross sectional design to establish the prevalence, severity and types of anaemia among ART-naive HIV-infected children and a cohort to establish the effect of baseline anaemia on subsequent response to ART. It also not clear whether the cohort was prospective or retrospective. This should be clarified.

Thank you for this clarification; we have clarified in the design section that children eligible for initiation of ART from the cross-sectional evaluation were consecutively enrolled into a prospective observational HIV treatment cohort.

The study participants for the cohort design were not explicitly described.

The study participants for the cohort design were all the children who started and were followed up on ART.

It is not documented whether the variables such as viral load and CD4 met the assumptions for using the student t-test.

We appreciate the comment. We had sufficient numbers in our study and our variables met the assumptions for the t-test. We are grateful for this important observation and apologize for the omission.
Study limitations and their rebuttal were not explicitly articulated in the discussion. The study appears to have had power issues to establish the effect of baseline anaemia and subsequent response to ART. Only 98 participants were started on ART in time for the study, yet sample size estimates were 64 per group.

A paragraph of has been added to the discussion highlighting the limitations of this study including the fact that due to limited time, we were unable to achieve the sample size which may have affected the results in terms of the differences we intended to show. For this reason we would recommend a bigger cohort to make more affirmative conclusions.

Editorial comment/recommendation

Please address comments from the 3 reviewers. In addition, please edit the manuscript, which is too lengthy, especially the discussion, which should be edited and condensed while also adding a paragraph about the limitations of the study. Limitations to be addressed include the lack of determination of cause of anaemia and the assumption that anaemia is etiologic in the response to antiretrovirals rather than possibly just an associated phenomenon. The figures and tables should be reduced; specifically I would suggest eliminating Figures 1 and 2 since the information is in the text. Table 3 could also be eliminated as it does not offer additional information. In Table 1, I would suggest eliminating the mode for age, the parent situation and the WAZ (-2SD), WHZ (-2SD) and the combined mean CD4+%.

We appreciate the thorough and detailed review in quest to make this manuscript better. We have addressed the queries by the reviewers and the editor’s recommendations and we hope our manuscript will be considered for publication.

We thank you very much.

Yours faithfully,

Eunice Nyesigire Ruhinda
Corresponding Author.