Author's response to reviews

Title: Profile of blood cell abnormalities among antiretroviral therapy naive HIV patients attending the Yaounde University Teaching Hospital, Cameroon

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Author's response to reviews: see over
Dear BMC Hematology editorials,
All the comments and suggestions given by each reviewer to our manuscript entitled
“Profile of blood cell abnormalities among antiretroviral therapy naïve HIV patients attending the Yaounde University Teaching Hospital, Cameroon” are very important and valuable to make the paper scientifically sound. We the authors tried to correct and include all the comments given by each reviewer’s point-by-point.

With regards
Reviewer's report
Title: Profile of blood cell abnormalities of treatment-naive HIV patients in the Yaounde University Teaching Hospital, Cameroon.
Version: 2 Date: 14 April 2014
Reviewer: Carol Briggs

Reviewer's report:

Major Compulsory Revisions.

Table 1. Throughout the paper it is quoted that anaemia is the cause of mortality in HIV patients but Haemoglobin does not appear in the table!

Accepted. Two new tables were added. Hemoglobin figures in table two, and it was included in the results.

The most common used term for reticulocytes stained on a normal blood film is polychromasias not polychromatophilia. Where are the reticulocyte counts? It is stated they were done.

Accepted. The reticulocyte count was included in the new table 2 and polychromasia was changed.

Discussion. Racial differences in White Cell count and Neutrophil counts are not discussed and will influence results. Even more importantly any patient with Malaria will affect the results. These have to be excluded or a separate group and shown in results.

Accepted. Racial difference in leucocyte count was added.
We did not particularly check for hematological parasites, this comment was added in the limitations section of the study.

Accepted and done as per the given comment

Minor Essential Revisions.

This is well written and sums up which we know on a daily basis in the laboratory. I have some comments.

Abstract: Results section, qualitatively is written twice. Should one be qualitative?

Accepted and done as per the given comment.

The same sentence is written in the conclusion of the abstract but correctly.
Delete one.

Accepted and done as per the given comment.

It should be mentioned early on that a point of care analyser was used, I was wondering why manual reticulocytes were performed. Which HumaCount analyser was used for the study and you should state the manufacturer, with address.

Accepted. The method section was re-organized, on line 99 of the manuscript, HumaCount Plus automated blood analyser (Human GmbH, Weisbaden, Germany) is indicated. This analyser does not do reticulocytes count. Thus we do a manual reticulocyte count when we want to investigate the etiology of anemia in our center.

Methods. How long was it before the sample was analysed after venesection.

Accepted. All samples were processed within 2 hours of blood collection. This additional information has been included in the manuscript.

Hypochromasia is used as a term but later hypochromia, be consistent, the latter better.

Accepted and done as per comment.

How were the CD4 counts performed?

The participants were sent to the Chantal Biya International Reference Center (www.circb.ca) that is a research laboratory at the proximity of the hospital. They used the Becton Dickinson FACSCalibur system (Becton Dickinson, Singapore), which is an eight color flow cytometer to do the CD4 analysis. This is indicated on line 103 of the manuscript.

Line 100. How many of these variables were taken from the full blood count and how many from an examined blood film? No mention of blood films are mentioned in the manuscript.

Accepted. This section has been re-formulated. The variables taken from the blood film are on table 3 and the variables taken from the full blood count are on table 2. Blood films have been included in this corrected manuscript.

Results. Lines 112-113 belong in the methods section. to be excluded or a separate group and shown in results.

Accepted. The results section has been reformulated, with sub headings added for better clarity.

Much of the discussion a repeat of the results, first paragraph is all repeat. Can be shortened.
Accepted. The first part of the paragraph was deleted.

HAART is not defined.

Accepted. The definition of HAART was included in the manuscript.

Reviewer's report
Title: Profile of blood cell abnormalities of treatment-naive HIV patients in the Yaounde University Teaching Hospital, Cameroon.
Version: 2
Date: 22 July 2014

Reviewer: Andrew Kerkhoff

Reviewer's report:
In the manuscript Paul and colleague report the frequency of blood dyscrasias among ART-naïve HIV patients attending a Cameroonian Hospital. While this study certainly has merit, I have several concerns as described below:

Major:

Methods:
- there is no description of eligibility criteria. What age range? Any excluding criteria?

Accepted. Line 89 has been modified as per the comment.

- statistics paragraph is poorly organized and should be rewritten with better flow (line 100-110)

Accepted and done as per the comment.

- I would suggest using the WHO definition of anaemia (<13 women and <12 men) to improve comparability between studies. Also using a simple cutoff ignores gender differences.

Accepted and done as per comment.

- statistical comparisons should be done using chi-squared tests as these are proportions. ANOVA is used to compare means across several groups and therefore this is inappropriate.

Accepted and done as per comment.
Results:

- In general the results can be shortened and made more concise with greater reference to the tables. It would also be very helpful if the organization of this section was made more clear through use of subheaders and paragraphs.

Accepted. The section has been totally reorganized with the addition of sub headers. Two tables have been added to facilitate comprehension.

- There should be a flow chart or a description as to how they arrived at 81 cases. How many patients were eligible or recruited?

Accepted and done as per comment.

- A patient characteristics table would be very useful. Age, sex, CD4 etc. Or at a minimum make sure median age, gender distribution and median CD4 count are described in text.

Accepted and done as per comment. Table 1 was added to the manuscript.

Discussion

- No strengths and limitations section. This is very important. What did you do well? Limitations would include small numbers, lack of information about drug exposures or nutritional deficiencies. No comparison to an HIV-uninfected comparison group from the same area to show what may be driven by local factors versus HIV-infection.

Accepted and done as per comment. Last paragraph of discussion.

- In general, the flow is broken by constantly referencing the authors of papers. Try to reduce the use of XX et al showed. State the findings and give the reference. This should improve readability.

Accepted and done as per comment.

- Line 156: there are several reasons and you should discuss them... One is the definition of anaemia that is used. Is it WHO (<13/<12) is it Hb<10, is it Hb<8? This greatly influences prevalence. Also consider local prevalence of malaria or hookworm. Variations in local nutrition patterns. Proportion of these studies that were women, etc... You mention many, but should state clearly that these likely contribute to differences between studies.

Accepted and done as per comment.

Line 159-160: Anaemia of chronic disease (ACD) does not cause an iron deficiency persay. It causes relative iron deficiency but not absolute iron deficiency through increased sequestration of iron by bone marrow macrophages and decreased gut
uptake. A microcytic anaemia in this context is likely due to iron deficiency secondary to poor nutritional intake, or in women, losses through menses. It however also may be due to thalassemia. Rarely ACD can cause a microcytic anaemia but is classically associated with a normochromic, normocytic anaemia.

Accepted. The comment linking ACD to microcytic anemia has been deleted.

-limitations?

Accepted. Limitations of the study have been included in the manuscript

Conclusion
This is too long. You are simply restating things that you’ve already stated. This should be 2-3 sentences with an easy take home message. What should the reader take away from this study?

Accepted and done as per the comment

Minor comments

Title page

For the title consider the following,” Profile of blood cell abnormalities from/among antiretroviral therapy naïve HIV patients at/attending the Yanounde University Teaching Hospital, Cameroon.

Accepted and done as per the comment.

Also, antiretroviral therapy naïve should be used instead of treatment naïve for improved clarity to readers.

Accepted and done as per the comment.

Abstract
Line 29- consider abnormal or irregular instead of deranged.

Accepted and done as per the comment.


Accepted and done as per the comment.

Line 30- either have been done/ or have been reported…
Accepted and done as per the comment.

Line 31- (therefore) this study aimed to

Accepted and done as per the comment.

Line 33- HIV immunological classes and clinical stages is not entirely clear. Consider saying by CD4 cell count and WHO clinical stage…

Accepted and done as per the comment.

Line 35- don’t need to mention consenting patients in abstract

Accepted and done as per the comment.

Line 36- delete “then” and add for the (measurements of) full blood count

Accepted and done as per the comment.

Line 39- (Of) 81 cases reviewed, (n=?; 81.6%)

Accepted and done as per the comment.

Line 39- The second sentence doesn’t report any results from the study and can therefore be dropped.

Accepted and done as per the comment.

Line 43- I assume this is 86% of anemic patients. I would also consider using with low reticulocyte counts instead of aregenerative as not everyone knows what this means and you have not defined this.

Accepted and done as per the comment.

Line 43. Sentence starting “all blood cytopenias” should be reworded as it is awkward and p-value can be put into parenthesis.

Accepted and done as per the comment.

Line 47. You should delete “it is an important morbidity factor in HIV infection.” While this is true and it is also strongly associated with mortality, you did not assess this in this study and therefore should not comment on it. Second sentence of line 47 can be merged with the preceding sentence.

Accepted and done as per the comment.
Lines 48-50 can be combined into a single sentence to make for a stronger conclusion.

Accepted and done as per the comment.

Background:
Line 57: HIV disease is primary a deficiency in cellular response
Accepted and done as per the comment.

Line 65: should include drugs or pharmacological agents as a cause of cytopenias (especially AZT and TMP-SMX)
Accepted and done as per the comment.

Line 67: HIV-(related/associated) mortality. I would also list poor response to ART and then mortality as this is stronger.
Accepted and done as per the comment.

Line 71: this was not reported by Volberding et al, this is a review. Either don’t include author name here or cite original studies. There are several which have shown this.
Accepted. A relevant reference was included and the above was deleted.

Line 76: revealed that 95% of AIDS patients had abnormalities compared to 32% among healthy controls.
Accepted and done as per the comment.

Line 80: replace alike with similar
Accepted and done as per the comment.

Methods:
-line 90: reference should be moved to after the word disease on the following line
Accepted and done as per the comment.

-line 100: this should be a new paragraph with perhaps a subheading to denote that you are now talking about statistical analysis
Accepted and done as per the comment.

-line 109: this is out of place and should be moved to the beginning of the statistical analysis section. Also, you should define the reticulocyte value/cutoff that defines aregenerative.
Accepted and done as per the comment.

Results:
-line 113: can just list range 22-70 and make more succinct.
Accepted and done as per the comment.

-Lines 114-117 can merged and reference table 3. However this should then become table
Accepted. Two more tables were added to the manuscript.

-Line 127: remove extra comma before toxic neutrophils
Accepted and done as per the comment.

-Line 133: Much clearer to state,” Anaemia was prevalent regardless of CD4 count, however pancytopenia was much more common among those with advanced immunosuppression.
Accepted and done as per the comment.

-Lines 133 and 138: don’t need to state this table shows/revealed. Just state the findings and reference the table. Patients were more likely… (table 3). Accepted and done as per the comment.

Discussion
-Line 154: should reference original studies, especially from Africa, there are several. Harrison’s is not the best reference.
Accepted and done as per the comment.

-line 163: state more directly where instead of saying of a central nature, state that this indicates inappropriate bone marrow response.
Accepted and done as per the comment.

-I would move line 181 to combined with previous paragraph. This would allow a paragraph about red blood cell abnormalities starting line 173 to be more focused. Also these results are not necessarily discordant and depends on how big the studies were.
Accepted and done as per the comment.

-line 174: again, chronic infections tend to result in a normocytic, normochromic anaemia.
Accepted and done as per the comment.

-line 176: new results should not be reported in the discussion. Report these parasitic infections in table 1 or describe in results text.

Accepted. These results were deleted since we did not specifically search for blood parasites.

-line 177: macrocytosis is not associated with all ART. Its predominantly AZT and D4T.

Accepted and done as per the comment.

line 178: should mention folate and B12 deficiency as possible causes of megaloblastic anaemia but are unrelated to HIV itself

Accepted and done as per the comment.