Author's response to reviews

Title: Temporal changes in haematocrit following artemisinin-based combination treatments of uncomplicated falciparum malaria in children

Authors:

Akintunde Sowunmi (akinsowunmi@hotmail.com)
Kazeem Akano (heycarnow@yahoo.com)
Adejumoke I Ayede (idayede@yahoo.com)
Godwin Ntadom (ntadomg@yahoo.com)
Bayo Fatunmbi (bayofat@gmail.com)
Temitope Aderoyeje (tope_forever@yahoo.com)
Elsie O Adewoye (elolade@yahoo.com)

Version: 3
Date: 3 April 2015

Author's response to reviews:

Department of Clinical Pharmacology
University College Hospital
Ibadan

1 April 2015

The Editor

BMC Infectious Diseases

Dear Editor

Revised MS: 4977010871594931 - Temporal changes in haematocrit following artemisinin-based combination treatments of uncomplicated falciparum malaria in children

Thank you very much for your comments and suggestions on our manuscript. The following are the responses to the comments of the reviewers and those of the editor, and the changes made in the revised manuscript.

Reviewer 1

In a recently submitted manuscript to one of the BMC Journals, in a larger number of patients (n = 1339), we presented, using multiple logistic regression model, the independent risk factors for early or late fall in haematocrit to anaemia level. We wish to crave the indulgence of both reviewer and editor not to replicate part of the data of a larger series in a relatively small series describing temporal
changes in haematocrit following artemisinin-based combination treatments (ACTs).

In the present revision, the factors associated with a fall in haematocrit from baseline >5 units on days 21 or day 28, or on both days have been presented in a supplementary Table T1. A sentence in the results section indicating that relatively high parasitaemia but not the dose of artesunate was significantly associated with a fall >5 units have been included in the results section.

The limitations of our study have been included in a penultimate paragraph in the discussion section.

Minor essential revision

Methods (patients)

1. Both treatments were given under supervision. Artemether-lumefantrine (AL) was not given with fatty meal. We have indicated, in the methods section, that “All doses of artesunate-amodiaquine were given under direct observed therapy as were the doses of artemether-lumefantrine given at 0, 8, 24 and 48 hours. Doses of artemether-lumefantrine at 36 and 60 hours were given by parents/guardians of the children at home and enquiries were made by telephone calls at the expected times of administration to confirm that the doses were actually given. Artemether-lumefantrine was not given with fatty meals.”

2. Yes, quality control, internal or external, was performed in 25% of all slides. In addition, discrepancy in microscope procedure was resolved by a senior member of the study. These have been included in the methods section.

3. The reference to Turbo Ken programme has been described. There is no link for Turbo Ken programme.

Methods (Kinetics of the disposition of deficit in haematocrit from 30%)

4. A reference has been cited for the relationship between haematocrit and haemoglobin. This has been included in the methods section as well as in the reference section. In addition, we assumed the relationship is exactly 3 and that haematocrit is equivalent to 3 x haemoglobin.

Results

5. Paragraph 2: the statement made in paragraph 2 is valid. The weight quoted in Table 2 was a typographical error. The correct weight is 16.0 kg; the P value remains unchanged. Additional statement showing that weights were similar in patterns 2 and 6 by post-hoc comparison has been included under the results section.

6. Paragraph 3: the statement has been modified to read as follows “A late fall in haematocrit which began after day 14 and persisted beyond 28 days...”.
7. Paragraph 3: the duration of the anaemia has been included in the results section. The mean duration of anaemia was 16.0 days (95%CI 12.8 – 19.2; range 14 – 21 days).

8. Paragraph 6: The value quoted for 95%CI has been corrected to 1 decimal point.

9. Paragraph 8: All values quoted in 2 decimal places have been corrected and changed to 1 decimal place throughout the text and the tables except the values of P.

Discussion

10. Paragraph 1: “patten” had been changed to “pattern”.

11. Paragraph 4: “#5 units” has been re-written consistently without space.

12. Paragraph 4: Please see responses above on risk factors.

13. Paragraph 5: A full stop has been included.

Reviewer 2

Discretionary revision:

1. Line numbering has been retained in title page.

Minor essential revisions

1. Delay in publication for the 4 year was due to the following reasons:

i. The study was part of a larger and longer study which stated in 2008 and data were analysed only after a predetermined number of patients were enrolled.

ii. Despite completion of the manuscript in early 2013, in-house revision of the manuscript took almost two years before the manuscript was finally submitted to BMC Infectious Diseases.

2. P. 2, line 27: “is” has been change to “are”.

3. P. 3, line 12: “the” has been excluded from the sentence.

4. P. 5, line 10: “treatment” has been included between “before” and “and”

5. P. 6 line 28: A reference has been provided. The reference is Bian and Bate, 2001.

6. P. 7 line 10-11: We agree with the comment of the reviewer. However, we have indicated in the original manuscript that either Mann Whitney U or Wilcoxon rank-sum test was used.
7. Figure 1: “n” has been added to each pattern.

Major compulsory revisions

1. Table 2a and corresponding results in text (p. 8): Children in pattern 6 weight significantly less but the weight quoted was a typographical error. The correct weight is 16.0 kg (see response to comments of Reviewer 1 on the same issue).

Actual percentage of children with hyperpyrexia has been included in Table 2a.

The ALL column is a summation of Patterns 1, 2 and 6. Since BMC Infectious Diseases has a wide readership, it would be essential for the readers to have a quick summary of the three patterns described at a glance. We will like to crave the indulgence of reviewer and editor to retain this column. We have included as a footnote that the ALL column represents the summary of data on Patterns 1, 2 and 6.

We have indicated in the statistical analysis part of the methods section that, differences in mean were compared by ANOVA and differences in proportion were compared by chi-square. The percentage of children asexual parasites >100,000µL⁻¹ has been included in the bracket to the actual number. The analysis of this section was by chi-square test which is a test of proportion. Geometric mean parasitaemia between Patterns 1, 2 and 6 was compared by Kruskal Wallis test. Although Pattern 6 had higher geometric mean, this was not significantly different from other patterns.

2. Figure 2 and corresponding result in text (p. 8): Each patient was followed up for a period of 6 weeks. Measurement at each point was the mean value of the patients on that visit day. Malaria attributable fall in haematocrit is the difference between haematocrit on day 28 or 35 and that at presentation (day 0) {see reference 12}. Total malaria attributable fall in haematocrit is the difference between haematocrit on days 28 – 42 and the lowest haematocrit recorded during the follow-up usually in the first few days following treatment. Drug attributable fall in haematocrit is the difference between total malaria attributable fall and malaria attributable fall in haematocrit. It is, usually, not expressed as fraction of baseline haematocrit (see reference 12 and 13) but as absolute value(s).

3. Table 3: All patients came from Pattern 3. Pattern 3 has been included in the legend to the Table. We crave the indulgence of the reviewer and the editor to retain this table because it contains detailed data on individual patients with late fall in haematocrit to anaemia level.

4. P. 9, line 6 – 22: The section has been rewritten to make it clearer in the following ways:
i. Fall in haematocrit is relative to baseline (i.e. at presentation before treatment was instituted).

ii. Data on day 7 have been included in the text as follows “Based on the number of patients evaluated per visit day, on day 7, 16 of 23 children and 62 of 87 children had >5 and <5 units fall in haematocrit following treatment. The difference in this proportion was not significant (#2 = 0.02; P = 0.92).”

iii. Evaluation was based on the number of patient per visit day. Evaluation of patients per visit day has been used in recently published study for the evaluation of anaemia after antimalarial treatments (see Sagara I., Piarroux R., Djimde A., Giorgi R., Kayentao K., Doumbo O.K., Gaudart J: Delayed anaemia assessment in patients treated with oral artemisinin derivatives for uncomplicated malaria: a pooled analysis of clinical trials data from Mali. Mal. J. 2014, 13:358).

iv. We have double- checked our P values. They are correct.

5. Table 5: Table 4 only showed data per visit day which will be more than the 248 children evaluated when summed together. Of the 248 children evaluated, 89 had falls in haematocrit on days 21 or 28, or on both days. This was the reason Table 5 was based on the number of patients. Based on our explanation, we will want to retain the table.

6. P. 10, line 7-9: The distribution of anaemia recovery time in patient with anaemia at presentation was normal. The distribution of anaemia recovery time when late anaemia occurred was not normal; we have used median values for comparison of anaemia recovery time. Anaemia recovery time was significantly longer when late fall in haematocrit to anaemia level occurred compared to early fall in haematocrit to anaemia level. This has been reflected in the results section.

7. Assuming non equal variance, the value of P has been corrected to read “0.003”.

8. P. 11 line 9: Figure 7 has been put under additional materials. It is now the new Figure S4

9. Bland-Altman states that 2 methods measuring the same parameters may correlate but may not necessarily agree. For the 2 methods to agree, the average of their values plotted against the difference of their values should not be significantly different from zero (that is, P <0.05). If they agree, the mean should be very close to zero (0). The limit of agreement is assumed ‘narrow’ if P is not significantly different from zero (0) (Conventionally, the limit of agreement is mean ± 1.96 SD). The plot should show 1.96 SD above and 1.96 SD below mean value.

Figure 7 of the original manuscript was mislabelled as A, A, C and D. This has been corrected in the revised manuscript as A, B, C and D. The missing figure B has now been included in the new Figure 4 of the revised version.
10. Limitations of the study have been included as a penultimate paragraph in the discussion section of the revised manuscript.

Editor’s comments

1. The background and rationale have been expanded and now include the multifactorial nature of malaria-associated anaemia and what is currently known about the effects of artemisinin derivatives on haematocrit or haemoglobin. In this respect, information presented later in the discussion has been brought forward.

The patients described in the current paper are not the same as those described by Oguche and others (2014) in the American Journal of Tropical Medicine and Hygiene. The paper by Oguche and others were in under-five year-olds from all geographical areas of Nigeria. The present study is in younger and older children from southwestern Nigeria. Additional information such as the relationship between fall in haematocrit and time-course and the characteristics of the children with different patterns of change has been included in the background section.

2. The sentence ‘in general.....in haematocrit or haemoglobin’ has been modified to avoid misinterpretation.

3. We have included in the methods section that children were considered to have late fall in haematocrit to anaemia level if at the time of fall and during the period of fall they had no concomitant illness and were parasite negative by microscopy and PCR.

4. Figures 3, 4, 6 and 7 of the old manuscript have been moved to supplement section as S1, S2, S3 and S4, respectively. Other figures have been retained and arranged in ascending order.

Other modifications made:

All changes made in text, tables or figures have been underlined.

New references have been included and arranged appropriately in the reference section.

We hope the revised manuscript is satisfactory.

With kind regards