Reviewer's report

Title: Increased deposition of C3b on red cells with low CR1 and CD55 in a malaria endemic region of western Kenya: Implications for the development of severe anaemia

Version: 1 Date: 9 March 2008

Reviewer: Jim Todd

Reviewer's report:

This is an interesting paper, trying to establish the role of different complement receptors and immune complexes in severe anaemia or cerebral malaria in young children. The study design is good, and it has been carried out in an appropriate way. I have a few comments mostly on the explanations given in the paper.

Major Compulsory Revisions

1. Background 3rd paragraph, page 5. The second sentence could better explain the role of CD55 and CD35 in the activation and binding of C3b molecules. This seems to be a general statement about their role, and it is not clear what the question is that will be answered by this paper. Some indication of this is found in the first paragraph of the discussion, but the rationale for those objectives should be given in the background.

2. How are C3b cells implicated in malarial infection along with other C3 convertases? This is further confused as the power calculation is given for differences in CR1, making the outcome for the paper unclear. This could be better explained, both in the rationale in the background and the methods where the power calculations are given.

3. Methods, 1st paragraph, page 6. The first sentence mentions 84 children from a previous study. It is by no means clear what role these children had in this work, apart from providing the basis for the age selection. I would suggest this is confusing, and that explicit reference to these children could be removed, both in methods and the first paragraph of the results.

4. Methods, Stats analysis, page 10. The ANOVA says that differences were compared across age groups, using independent t-tests. Yet in the results, no t-test results are given in the text, the ANOVA results are given in the figures, and the multiple regression gives results on the age (months), which is presumably a linear effect across age. I would suspect a linear effect for this analysis would be inappropriate, as the graphs do not suggest a linear relationship between C3b and age, or between haemoglobin and age.

Minor Essential Revisions
1. Methods, general. Where did this study get ethical approval? The only reference to ethical review was for the 84 children in the previous study.

2. Methods, 2nd paragraph, page 6. The power calculations are for CR1, which does not sit well with the title and research question raised. If the study really was powered for CR1 as the outcome, then it would be better to omit this from the paper. If there are relevant power calculations for haemoglobin or C3b then these should be given (even if calculated post-hoc).

3. Results, 1st paragraph. I would suggest removing the first paragraph, and figure 1, as these seem to have little relevance to the main results. Figure 1 has different age categories to those in the main analyses.

4. Results, 2nd paragraph, page 12. I would suggest taking the ANOVA results away from the graphs (figures). The ANOVA results are just the crude (uncorrected) analysis of the outcome by age. The p-values in Figure 4 seriously impede the visual understanding of the graph, and the multiple p-values are not clear. If these need to be presented then they should be included in the text. For the associations with C3b and haemoglobin, it would be best to include these as a separate row in Tables 3 & 4, showing the uncorrected effect of age. The it would be possible to see the effect of confounders by comparing the crude analysis with the adjusted analysis, adjusting for CR1, CD55 and malaria parasitemia.

5. Results, Multiple linear regression. A crude analysis could be given in the tables to allow the reader to see the effect of adjustment. It is extremely unlikely that the outcomes are directly and linearly related to age. It would be more useful if the multiple regression allowed for the effect of the age groups, which were defined and used in the sample selection in the first place.

6. The discussion is well argued, and they correctly allow the fact that increased C3b cells could be due to malaria infection, or the malaria treatment. In the last 2 sentences of the discussion TNF-alpha is introduced, which may be rational, but needs further explanation (or dropping from this paper).

Discretionary Revisions
1. Methods, 2nd paragraph, page 7. Were the subjects tested for HIV, and specific bacterial infections?

2. Methods, 3rd paragraph, page 7. Individuals were screened, and re-screened, and each time asked to come back after 2 weeks. How many times did this happen? Presumably these individuals are defined as 'malaria treated' in the analysis, but this is not made explicitly clear. Individuals who were repeatedly re-screened may be suffering from a complication, and might provide some bias in the analysis. Was any attempt made to re-analyse the data excluding the 10 individuals who were only included after the third screen?

3. Results, 3rd paragraph. What does the word 'discordant' mean in the second paragraph? If it means the age pattern differs, then this should be said. It may be
useful to show some graphs of the association between the 2 outcomes (haemoglobin and C3b) and the potential explanatory factors (CR1 and CD55).

4. The sentence explaining the “B” or coefficient, is not needed in a scientific journal, although the implications of the unit change may need to be discussed in the Discussion.

**Which journal?:** Appropriate or potentially appropriate for BMC Medicine: an article of importance in its field

**What next?:** Accept for publication in BMC Medicine after minor essential revisions

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests