Reviewer's report

Title: Diagnostic utility of zinc protoporphyrin to detect iron deficiency in Kenyan pregnant women

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Reviewer: Bernard J Brabin

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Diagnostic utility of zinc protoporphyrin to detect iron deficiency in Kenyan pregnant women

This is an important analysis on the utility of ZPP variables in assessment of iron status in pregnant women. There are several points that should be addressed or clarified.

1. Abstract. The first sentence that ‘Zinc protoporphyrin (ZPP) indicates iron-deficient erythropoiesis.’ This is too blunt and unclear. A ZPP/H finding within the reference range is evidence of an adequate systemic iron supply. ZnPP formation in excess is a reflection of iron-zinc substrate competition for ferrochelatase and in turn of iron deficient erythropoiesis. This phrase should also be revised in the introduction. The abstract mentions cut-offs but no statement on which gave the highest predictive values is provided. No conclusion given on EP. Half the abstract conclusion relates to future research suggestions which are not developed in the discussion.

2. Introduction. This needs more focus. First sentence unclear and the text could be shorter. The text for lines 119 - 127 is relevant, but is probably better covered in the discussion.

3. Methods. It is necessary to know more detail about methods as there is only reference to an unpublished paper of the source trial paper. Were any participants receiving iron or folate supplements or antimalarials from antenatal clinics, as use of these may produce a rapid post-treatment reticulocytosis in infected, or iron deficient subjects. Were known HIV infected subjects on ARVs? Volume of blood sample collected should be mentioned. Although results on bilirubin are provided this assay is not mentioned in methods. Storage temperature for sera samples should be stated, and whether measures taken to protect stored sera from oxidation for folate assays? Bilirubin not mentioned in methods, although results presented. Why is placental malaria mentioned in malaria definitions as the study sample was antenatal? Malaria definitions should be based on enrolment malaria status. Details of the laboratory quality control procedures followed should be included (other than for ZPP which are provided). Specific details of the Ethical committees should be given in addition to the country of location.

4. Unclear why ferritin alone is used for definition of iron deficiency when other
important iron biomarker data is available (eg sTfR/log ferritin ratio). Did ferritin concentrations vary with gestational age? The sTfR/ratio may be more independent of these gestational age changes.

5. Line 255. Sentence on unbiased estimates of ID using ZPP cut-offs with reference to unpublished paper. Further detail of methods for sample selection is necessary. Was this the same population without inflammation?

6. Statistical methods. For duplicate samples was the mean value used? Information on CV% would be helpful. Which factors were included in the parsimonious model?

7. Results. The tables and figures are well presented. Examining the diagnostic performance of ZPP in a population from an area were infection was very common, and then excluding those with evidence of inflammation (based on CRP and AGP, malaria and HIV), is of considerable interest, but leads to selection of a sub-population which, by definition, experience less infection. ZPP diagnostic utility using adjusted ferritin cut-offs and including the whole population is also of considerable interest, as this is the real life situation. Justification for not completing this analysis should be discussed, and preferably this analysis should be done. Percentage of folate and B12 deficient subjects should be given. Plasma lactate dehydrogenase is mentioned as measured in methods, but no results are presented. As hemolysis is an important factor which could influence ZPP levels there is an opportunity to examine this confounder. There are some minor differences within table 2 (folate in multivariate analysis; HIV data provided for EP, but not ZPP). The statement that there seemed to be gestational changes in ZPP/EP should be supported by a statistical test.

8. Discussion. The statement that (line 410) ‘the diagnostic performance of ZPP in an unrestricted dataset would presumably have been even worse’ is reasonable, but the authors have the data to test this assumption (see above). Discussion of the rationale for using a single iron biomarker for iron deficiency definition would be helpful, especially as additional iron biomarker results were available, for comparison. The association of ZPP with folate concentration deserves some discussion. High ZPP was expected in the presence of bilirubin (line 392), but the opposite was observed (line 284). Because of the importance of hemolysis in populations living in malaria endemic areas this finding should be discussed. Does this imply bilirubin does not interfere with ZPP measurement? Is it possible that trial the drugs provided to subjects at enrolment influenced ZPP measurements?

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare I have no competing interests