PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Comparison of routine prenatal iron prophylaxis and screening and treatment for anemia: pregnancy results and preliminary birth results from a pragmatic randomized controlled trial (PROFEG) in Maputo, Mozambique
AUTHORS	Nwaru, Bright; Parkkali, Saara; Abacassamo, Fatima; Salomé, Graca; Augusto, Orvalho; Regushevskaya, Elena; Dgedge, Martinho; Sousa, Cesar; Cliff, Julie; Chilundo, Baltazar; hemminki, elina

VERSION 1 - REVIEW

REVIEWER	Sharon Cox Lecturer MRC International Nutrition Group London School of Hygiene & Tropical Medicine, UK I have no competing interests to declare.
REVIEW RETURNED	14-Sep-2012

THE STUDY	Specific objectives of the trial. The conclusion is stated as “routine treatment did not confer advantages over screening and treatment for anaemia regarding maternal and child health”. However the emphasis given in this statement is rather different to that given in the justification for the trial provided in the introduction, which emphasises the possible risk of routine supplementation. No mention is made of what possible advantages there may be for routine supplementation compared to screening for anaemia. For example, that anemia is the end-stage of iron deficiency and screening for anaemia will therefore miss a proportion of iron deficient women and also fail to prevent iron deficiency. Finally, routine iron supplementation is more often used for pragmatic reasons than for assumed health benefits. Introduction Is there evidence of iron supplementation and risk of infection specific to pregnancy - references are not specific to pregnancy . Background information on settings and location of the trial. Information is missing regarding: • Malaria transmission intensity/seasonality • Use of anti-malarial prophylaxis • Routine practice regarding testing of haemoglobin at ante-natal clinics • Use of ARVS by women who were HIV-positive in and outside of

	the trial environment • Routine HIV testing at clinics Nawaru article needs to be submitted as a supporting document. Sample size It is not clearly described what criteria were used to determine the final sample size. 2,000 women in each group provided what power for each of the primary outcomes?. Was the 2,000 determine to include a proportion for incomplete data/loss to follow up. What baseline estimates were used and their sources for pre-term delivery and low birthweight? Consider including the different assumptions in a supplementary table. Intervention • Why was 400ug of folic acid given compared to 1mg folic acid in the two groups? • Were the tablets combined iron and folate or separate? Determination of outcomes Can more information be provided regarding how health centres measured gestation and birth weight at delivery. It is not reported if there were any trial-related efforts to change normal practice in these centres, although it would appear that there was not. This is relevant because it will affect the precision of the measures and therefore how likely relatively small effects may be observed. Statistical analysis No mention is made in the description of the statistical analyses or in the results of the gestational ages at the different clinic visits and therefore the time periods between them. Although there were similar proportions of women attending the clinic once, twice, thrice etc, I suggest that the mean gestational ages at baseline and subsequent clinic visits should be included in Figure 1.
RESULTS & CONCLUSIONS	Results Mean gestational age at recruitment is reported in Table 1 to be around 10 weeks for all groups. The exclusion criteria stated on p7 includes gestational age less than 12 weeks. How is this conflict accounted for? Discussion To provide context and assess the relative advantages and disadvantages of the two treatments, it would be useful to know the relative effects on haemoglobin at delivery. Is this information available? If so, it can and probably should be the subject of a different report, but worth mentioning its relevance here. Do the results answer the research questions? As the authors acknowledge, there is significant incomplete data. Although I understand the pressure to publish, it is not clear why the report should not wait until the much talked about further data are available. If the authors do not expect them to make any material difference, perhaps a sensitivity analysis could be included of different scenarios of the missing data?

	Tables and Figures Table 1. • Needs consistent labelling and inclusion of percentages Table 2 • Typo in the p-value for Duration of gestation? Currently reads 0.056!?

REVIEWER	Yana Vinogradova Research Statistician Department of Primary Care University of Nottingham United Kingdom No competing interests
REVIEW RETURNED	26-Sep-2012

THE STUDY	Are the patients representative...? The general reader might be not familiar with the Mozambique primary care health system and so not be clear about whether the participants derived from the general population and whether the findings are, therefore, generalisable. Nwaru's does describe the health system but it would be useful for this paper to say that any pregnant woman is eligible for prenatal care and therefore could be recruited into the study. Are the methods adequately described? The description of Interventions could do with reformulating. It appears to have been largely copied from Nwaru's paper with some loss and no improvements in clarity. For the Routine group the fact that the monthly supply was received over the whole period of pregnancy and at which points should be stated. The more complex intervention for the Selective group needs rewording to clarify how many tablets of each kind were given at each prenatal visit. As currently formulated, it is it is possible to understand the nature of the interventions, but only by expecting to make the reader make intelligent deductions. Are the statistical methods described? Were the risk ratios (in Table 4) adjusted for any baseline information?
RESULTS & CONCLUSIONS	The paper contains delivery outcomes, which were available for only just over half of the sample, and Table 1 demonstrates that the groups with available information were similar to those without. As the main purpose of this research is treating anaemia, however, it would be appropriate to present the levels of haemoglobin for the Selective group (as done in Nwaru's paper) but separately for the groups with and those without delivery data. According to Nwaru's paper there were only 32% of women in the Selective group who needed iron treatment at recruitment. As the groups appeared to have similar profiles, one might expect the same proportion to have anaemia in Routine group. Does this mean that about two thirds of women from Routine group might not have needed iron tables and that excessive levels of iron might have affected the outcomes? It would be interesting to have the authors' comments on this in the discussion.

VERSION 1 – AUTHOR RESPONSE

Reviewer: Sharon Cox

COMMENT

1. The conclusion is stated as “routine treatment did not confer advantages over screening and treatment for anaemia regarding maternal and child health”. However the emphasis given in this statement is rather different to that given in the justification for the trial provided in the introduction, which emphasises the possible risk of routine supplementation. No mention is made of what possible advantages there may be for routine supplementation compared to screening for anaemia. For example, that anemia is the end-stage of iron deficiency and screening for anaemia will therefore miss a proportion of iron deficient women and also fail to prevent iron deficiency. Finally, routine iron supplementation is more often used for pragmatic reasons than for assumed health benefits.

RESPONSE

We have now modified the Introduction section of the paper to make our purpose clearer. In our justification for the trial, with think that our statements are rather neutral with regards to the effects of routine iron prophylaxis, not emphasizing its benefits or harms. We have now added a sentence on feasibility into the end of the paper (Conclusions section).

COMMENT

2.Introduction. Is there evidence of iron supplementation and risk of infection specific to pregnancy - references are not specific to pregnancy .

RESPONSE

We have now modified the sentence to indicate that most information on the relation between iron and infections come from non-pregnant population (page 5).

COMMENT

3.Background information on settings and location of the trial. Information is missing regarding:

3a Malaria transmission intensity/seasonality.

RESPONSE

We have now provided the information on malaria situation of the study setting in the last part of section on Study design and population (pages 6-7).

COMMENT

3b Use of anti-malarial prophylaxis

RESPONSE

We have now added information on this in the last part of section on Study design and population (pages 6-7).

COMMENT

3c Routine practice regarding testing of haemoglobin at ante-natal clinics

RESPONSE

We have now added information on this in the last part of section on Study design and population (pages 6-7).

COMMENT

3d Use of ARVS by women who were HIV-positive in and outside of the trial environment

RESPONSE

We have now added information on this in the last part of section on Study design and population (page 7).

COMMENT

3e Routine HIV testing at clinics

RESPONSE

We have now added information on this in the last part of section on Study design and population (pages 6-7).

COMMENT

3f Nwaru article needs to be submitted as a supporting document.

RESPONSE

This has already been done.

COMMENT

4. Sample size. It is not clearly described what criteria were used to determine the final sample size. 2,000 women in each group provided what power for each of the primary outcomes?. Was the 2,000 determine to include a proportion for incomplete data/loss to follow up. What baseline estimates were used and their sources for pre-term delivery and low birthweight? Consider including the different assumptions in a supplementary table.

RESPONSE

We have now expanded the description of estimating sample size (pages 8-9).

COMMENT

5.Intervention.5a Why was 400ug of folic acid given compared to 1mg folic acid in the two groups?

RESPONSE

The routine iron group received iron 60 mg and folic acid 400 µg combination in one tablet, while the selective iron group received, depending on the hemoglobin levels, 1 mg folic acid. The rationale for 1 mg folic acid in selective group was that pure folic acid was not licensed in Mozambique in 400 µg. This information is now added to the Interventions section of the paper (pages 9-10).

COMMENT

5b Were the tablets combined iron and folate or separate?

RESPONSE

The iron and folic acids tablets were combined tablets for the routine group. This information is now added to the Interventions section of the paper (pages 9-10).

COMMENT

6. Determination of outcomes. Can more information be provided regarding how health centres measured gestation and birth weight at delivery. It is not reported if there were any trial-related efforts to change normal practice in these centres, although it would appear that there was not. This is relevant because it will affect the precision of the measures and therefore how likely relatively small effects may be observed.

RESPONSE

We have now indicated from which kind of data source the main outcome measures came. Gestational age at birth was most complicated as we used several sources to determine it (please see the section on Data collection and follow-up, pages 10.). The measurement of the outcomes was done as routine and no changes were made as a result of the trial.

COMMENT

7. Statistical analysis. No mention is made in the description of the statistical analyses or in the results of the gestational ages at the different clinic visits and therefore the time periods between them. Although there were similar proportions of women attending the clinic once, twice, thrice etc, I suggest that the mean gestational ages at baseline and subsequent clinic visits should be included in Figure 1.

RESPONSE

We have now included this information in Figure 1

COMMENT

8. Results. Mean gestational age at recruitment is reported in Table 1 to be around 10 weeks for all groups. The exclusion criteria stated on p7 includes gestational age less than 12 weeks. How is this conflict accounted for?

RESPONSE

Thank you for this important comment. We have now rectified the problem with our calculation of the

gestational age at recruitment and corrected the figures in Table 1 and Figure 1.

COMMENT

9. Discussion. To provide context and assess the relative advantages and disadvantages of the two treatments, it would be useful to know the relative effects on haemoglobin at delivery. Is this information available? If so, it can and probably should be the subject of a different report, but worth mentioning its relevance here.

RESPONSE

We do not have information of hemoglobin at delivery. (It is not customary to meaure it in birth hospitals/ health centers)

COMMENT

10. Do the results answer the research questions? As the authors acknowledge, there is significant incomplete data. Although I understand the pressure to publish, it is not clear why the report should not wait until the much talked about further data are available. If the authors do not expect them to make any material difference, perhaps a sensitivity analysis could be included of different scenarios of the missing data?

RESPONSE

The reason to write this paper is not due to pressure to publish (i.e. to get publications), but to contribute to the current debate of the need of iron prophylaxis. As we argued there is very little data on this in malaria areas, and other researchers may be interested in our preliminary birth results. The final results will be much delayed due to problems we could not anticipate in the planning stage of the project). Furthermore, this paper reports the final results on pregnancy outcomes.

COMMENT

11. Tables and Figures. 11a Table 1. Needs consistent labelling and inclusion of percentages

RESPONSE

We have now revised the tables to be consistent with the Figure. Percentages are now presented as part of the labeling for Table 1.

COMMENT

11b. Table 2.Typo in the p-value for Duration of gestation? Currently reads 0.056!?

RESPONSE

The p-value for the difference between the iron groups in gestational age at birth (p=0.056) is correct, not a typo.

Reviewer: Yana Vinogradova

COMMENT

1. Are the patients representative...? The general reader might be not familiar with the Mozambique primary care health system and so not be clear about whether the participants derived from the general population and whether the findings are, therefore, generalisable. Nwaru's does describe the health system but it would be useful for this paper to say that any pregnant woman is eligible for prenatal care and therefore could be recruited into the study.

RESPONSE

We have now added a statement to describe this in the section, Study design and population (pages 6-7).

COMMENT

2a. Are the methods adequately described? The description of Interventions could do with reformulating. It appears to have been largely copied from Nwaru's paper with some loss and no improvements in clarity.

RESPONSE

We have now modified several sections of the methods part of the paper to make it clearer (see also our responses to Sharon Cox's comments).

COMMENT

2b. For the Routine group the fact that the monthly supply was received over the whole period of pregnancy and at which points should be stated.

RESPONSE

This has now been added in the Interventions section of the paper (pages 9-10).

COMMENT

2c. The more complex intervention for the Selective group needs rewording to clarify how many tablets of each kind were given at each prenatal visit. As currently formulated, it is it is possible to understand the nature of the interventions, but only by expecting to make the reader make intelligent deductions.

RESPONSE

The description of the intervention for the Selective group has now been revised (see the Interventions, pages 9-10) section.

COMMENT

3. Are the statistical methods described? Were the risk ratios (in Table 4) adjusted for any baseline information?

RESPONSE

They were not (as the baseline was similar in the two groups). This has now been added as a footnote to Table 4.

COMMENT

4. The paper contains delivery outcomes, which were available for only just over half of the sample, and Table 1 demonstrates that the groups with available information were similar to those without. As the main purpose of this research is treating anaemia, however, it would be appropriate to present the levels of haemoglobin for the Selective group (as done in Nwaru's paper) but separately for the groups with and those without delivery data.

RESPONSE

We have now added the variable (hemoglobin at recruitment in the selective group) to Table 1, and commented it in the Results section of the paper (page 13).

COMMENT

5. According to Nwaru's paper there were only 32% of women in the Selective group who needed iron treatment at recruitment. As the groups appeared to have similar profiles, one might expect the same proportion to have anaemia in Routine group. Does this mean that about two thirds of women from Routine group might not have needed iron tables and that excessive levels of iron might have affected the outcomes? It would be interesting to have the authors' comments on this in the discussion.

RESPONSE

The definition of anemia in Maputo context is difficult because it is not known what hemoglobin level is actually anemic. We used the <9 g/dL Hb cut-off while others have used other figures. Our cut-off was based on agreement with local researchers. We do not know at what level overload starts. We did not measure iron levels but only hemoglobin, and hemoglobin does not directly measure iron level in the body. Consequently, in the discussion of our results we have not gone into the potential mechanisms of the effect of prophylactic iron.

VERSION 2 – REVIEW

REVIEWER	Dr Sharon E Cox Lecturer MRC ING LSHTM I have no conflicts of interests to declare.
REVIEW RETURNED	02-Jan-2013

THE STUDY	Details of baseline assumptions used for the power calculations have not been included in a supplementary table as requested.
GENERAL COMMENTS	Most of the comments in my initial review have been addressed. However, it would have been much easier for the reviewer and more informative to have a additional document submitted outlining what changes had been made adn where with justifications and answers given to specific questions. This particularly relates to the two last comments/questions of my previous review.

VERSION 2 – AUTHOR RESPONSE

COMMENT

Details of baseline assumptions used for the power calculations have not been included in a supplementary table as requested.

RESPONSE
We are sorry for the oversight to the reviewer's earlier request. A table showing the baseline assumptions for the power calculations has now been included as an appendix, leaving its inclusion to the editor's discretion.

COMMENT
Most of the comments in my initial review have been addressed. However, it would have been much easier for the reviewer and more informative to have an additional document submitted outlining what changes had been made and where with justifications and answers given to specific questions. This particularly relates to the two last comments/questions of my previous review.

RESPONSE
In our previous response to the reviewer's comments, we did include a point-by-point letter in which we addressed the reviewer's questions. It is possible that the letter was somehow lost. We have here included it here at the end of this response. We note that the referred two last comments concerned the Tables and Figure, which the reviewer requested that we have consistent label for them. We implemented the requested changes using the track changes. As the changes were major in that most columns of the tables were re-organized, the tables became messy; consequently we removed the track changes from the tables. In this revised version we have highlighted (in RED) the sections of the tables and Figure that were affected by our revision: for the tables, the Selective and Routine iron columns were re-organized so that the Selective iron always comes before the Routine iron as it is in the Figure.