Author's response to reviews

Title: Factors associated with ultrasound-aided detection of suboptimal fetal growth in a malaria-endemic area in Papua New Guinea

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Author's response to reviews: see over
Overall response:

We thank the reviewers for their thorough revision and insightful comments on the manuscript. We have endeavoured to address all points raised, yet were unable to make all changes as requested (specifically, some suggestions made by reviewer 1), for reasons outlined below. Nevertheless, we believe that the MS has substantially improved as a result of the reviewers’ comments and the resulting changes.

We hope that despite its shortcomings the reviewers and editorial board will agree that this is an interesting piece of research that presents useful data in the best possible way given the logistic and analytical circumstances.

Reviewer 1

This manuscript provides an important insight in the interplay of poor socioeconomical conditions, parasitic infections and fetal growth. This study could add a real genuine new information on the interplay of low socioeconomical environment, major proxy of maternal nutrition, parasitic infection and fetal growth. I hope that the authors who did an incredible enormous efforts for this study could accept the major work required by the revision that I am suggesting.

MAJOR COMPULSORY REPORTS

ABSTRACT

- Please state the aim of the study after the background

Response: We amended the background section of the abstract to read

Lines 45-48: Fetal growth restriction (FGR) is associated with increased infant mortality rates and ill-health in adulthood. Evaluation of fetal growth requires ultrasound. As a result, ultrasound-assisted evaluations of causes of FGR in malaria-endemic developing countries are rare. We aimed to determine factors associated with indicators of abnormal fetal growth in rural lowland Papua New Guinea (PNG).

- FGR and SGA are used as if interchangeable, please use FGR for low fetal weight gain and SGA for cross sectional data (estimated U/S weight vs newborn weight charts, or observed newborn weight vs newborn weight charts.)

Response: Defining FGR is challenging. In our minds, and for the purpose of this study, both SGA and low fetal weight gain are indicators of FGR. First, it is understood that a clinical diagnosis of FGR is complex, and in addition to fetal size measurements, it usually includes additional assessments such as Doppler assessments, liquor volumes etc. For the purpose of this study, we had to restrict this to assessment of fetal size and changes thereof. Second, it is agreed that a measurement of SGA does not necessarily mean the fetus is suffering growth restriction – the fetus may just be constitutionally small or could indeed have suffered, or be suffering, growth restriction. Similarly, a downward
deviation of fetal weight gain from its projected trajectory does not necessarily mean the fetus is suffering FGR – it may simply be explained by (ultrasound) measurement error, in particular during advancing gestation. Lastly, an international consensus as to the best definition for FGR is lacking.

As such we set out to examine at a number of possible indicators of FGR, principally SGA (one or more measurements) and low fetal weight gain (one or more interval/episode). Our study is not a diagnostic study per se, but rather it attempts to evaluate possible risk factors for FGR, or if you like, factors associated with diminished fetal growth or size, in a rather challenging research environment.

We have been careful to distinguish between SGA (weight below 10th centile) and FGR throughout (e.g. lines 98-101, introduction; lines 169-172, methods); if there are specific instances that require further modification please highlight them.

- This should be a special strength for the prospective work: longitudinal U/S measurements to identify low weight gain or progressive restriction of growth, instead of simple weight at birth,

Response: We have added to the following to the abstract:

Lines 49-50: Weights and growth of 671 ultrasound-dated singleton pregnancies (<24 gestational weeks) were prospectively monitored using estimated fetal weights and birthweights.

INTRODUCTION
METHODS/RESULTS

Line 168 to 175

- EFW/BW below the 10th centile does not allow to diagnose a FGR.

Response: We agree that SGA does not diagnose FGR, yet it may indicate FGR (as discussed above). Given its limitation we opted to present SGA data together with fetal weight gain data (for women with growth interval measurements).

Despite its shortcoming it is worth pointing out at this point that SGA is very commonly used to as a proxy for FGR in epidemiological research from developing countries (rightly or wrongly!), including research into parasitic disease and nutrition and its impact on pregnancy [1-4]. We believe our study is strengthened by the presentation of SGA data together with other indicators of poor fetal growth, e.g. low fetal weight gain, and z-score analyses.

We have added the following the following to the introduction

Line 100: A number of studies evaluated factors associated with measuring small-for-gestational-age (fetal size/weight <10th centile of a given weight standard, SGA) [5], commonly used as an indicator of FGR in epidemiological studies, …
The problem of reference chart is critical also for simple assessment of SGA: Hadlock could not be used I suppose in such a different macroethnicity. This is not a limitation this is a main problem for the interpretation of results. The methods and the results as far as fetal weight, growth, newborn weight are quit confusing and biased by inappropriate reference charts.

Response: The choice of reference chart (Hadlock) was based on a number of factors. First, there is no weight reference chart for Melanesian populations. Use of adjusted Hadlock charts (‘ethnic’ adjustment done using birthweights of at least 100 uncomplicated singleton pregnancies at 40 gestational weeks) [6] underestimates SGA [7, 8]. Fetal weight charts derived from a sub-set of our women could not be used due to their self-referential nature and the bias pertaining to this [2]. Given the study did not set out to derive an exact diagnosis of FGR, but rather aimed to evaluate which factors negatively affect fetal growth, we ultimately opted for the Hadlock standard. It is likely that it overestimates SGA (already discussed as a limitation). However, it still allows for identification of factors that may cause some babies being smaller than others – the aim of the study was not to estimate prevalence.

If we look at U/S biometry we find very interesting data. The very interesting info is reported in TABLE 1 supplement. As expected, HC is not significantly different between SGA and AGA whereas AC is significantly lower. This is of relevant significant to the interpretation of findings.

I would suggest that longitudinal findings used to diagnose fetal growth restriction should be based on Abdominal Circumference Zed score and NOT on estimated weight Zed score, that is less sensitive to growth faltering in utero.

Response: We agree that AC may be more sensitive to detect growth faltering that EFW, and indeed we did look at using AC or \( \Delta \) AC rather then EFW/birthweight as the main outcome measure.

We opted against using AC for the following reasons:

1) More than 40% of size measurements used in the various analyses were collected at birth, but not all infants who had a birthweight measured had an AC done, due to logistic reasons. The use of AC would have further reduced our sample size and hence power to demonstrate clinically relevant associations.

2) Great efforts were made to ensure birthweights were measured to precision, given they were the primary outcome measure of the parent trial [9], yet AC measurements at birth were only done when possible and the data suggests that QC on these data was not as good as for birthweight, which was our primary study endpoint. Thus, while our AC data demonstrate possible relationships between measuring SGA in utero and outcomes at birth (previous Supp table 1, now table 2 as suggested) we were concerned about using measurements of AC at birth as a substantial focus of our analysis due to missing data and to lower data quality. Excluding AC measured at birth from analyses would have reduced the sample size to an extent that precludes a meaningful analysis of these data.

3) To date, most malaria in pregnancy ultrasound studies have used EFW as their
main outcome measures. Using the same allows for a level of comparability with these studies.

4) Malaria (in particular infection in early pregnancy) and undernutrition has been associated with reduced skeletal growth [10]. Given most infections in this cohort occurred/were detected in early pregnancy we felt an outcome measure capturing both soft tissue and bone growth is most appropriate. Several of these points have been added to the discussion

Lines 286-292: AC is an important screening tool for FGR [25, 26]. We used EFW rather than AC (or a combination of both) in our analyses for several reasons. First, it increases comparability of our findings with our malaria studies [13, 16]. Second, many AC measurements were measured postnatally and not by ultrasound; they were not subject to the same stringent QC that ultrasound and birth weight measures were. Third, malaria in early pregnancy and undernutrition have been associated with reduced skeletal growth [11].

- If you introduce a different criteria in the method then you can keep the two assessment together (cross sectional and longitudinal)

Response: We have clarified this in the methods section. This now reads:

Lines 169-172: Given the detection of FGR is challenging and not all infants measuring are SGA are growth-restricted we opted to use two indicators of FGR, SGA (cross-sectional assessment) and low fetal weight gain (longitudinal assessment). Suboptimal fetal growth was suspected upon detection of an EFW/BW below the 10th centile (SGA) of the Hadlock standard (10th centiles of ultrasound-estimated and birthweight-derived standards are similar at term) [11, 12], and/or observation of low fetal weight gain, defined as a change in weight z-score (Δz) below the 25th centile of the overall distribution of Δz, using weight measurements obtained >14 days apart.

- This could be criteria n° 1: fetuses who underwent longitudinal interrogations were classified as low growth gain according to the AC zed score.
- Criteria n° 2 for cross sectional assessments could be the AC as reported by the Intergrowth Study

Response: See above

Table 1 supplement is interesting too as I said before it contains clues to the interpretation of fetal growth. I suggest that it be included in the main manuscript, I would suggest that birthweight being a selecting criteria be moved in the indicator line and not among results, birth weight goes with gestational age and the two lines should be moved together up in the indicator line

Response: We have integrated this table into the main MS, as Table 2.

Given our main interest is in FGR (and SGA and poor weight gain are our available measures for this), we do not think that making birth weight and gestational age dependent variables for this table (and moving them to the top line) really fits with the thrust of our analysis, which aims to find other measures which are associated with risk of FGR, through the two stated mechanisms.
Assessment and clear classification of poor fetal growth (mixed longitudinal and cross-sectional data) is the key issue of the genuine new results of this work. I suggest that relationship with socio, biological variable should be reassessed after a more consistent classification of fetal growth.

Response: see above.

MINOR ESSENTIAL REVISIONS

INTRODUCTION
This is a secondary analysis performed on a cohort of women recruited and randomized to an interesting two anti-malaria drug regimens. Outcome are not based on unblinded data, and the two arms are presented as a single cohort. Assuming that the two regimens do not influence the outcome or that the two regimens together might represent the real life variability of different antimalarial regimens. I suggest that this should be briefly presented in the Introduction and should be commented as a limitation of the study in the discussion. This is even more needed since the main paper is not yet available as a published contribute.

Response: See also our response to reviewer 2. The main trial analysis has now been published and we have added this reference to the paper. In the trial found that the intervention (IPTp with sulphadoxine-pyrimethamine plus azithromycin) reduces the risk of LBW and increases mean birthweight [9]. Amongst the subset of women who had timely dating ultrasounds, it appeared that the intervention primarily prevented LBW by preventing preterm birth (RR 0.62, CI 0.43-0.89, p=0.010). In settings like PNG, malaria is believed primarily to cause LBW through FGR, but malaria was relatively uncommon in the trial, and infection prevalence fell sharply after provision of bed nets and the first antimalarial treatment at enrolment (see discussion). In the present analysis, type of malaria prevention was not associated with indicators of FGR, which may be explained by the low prevalence of malaria, the sharp fall in infections detected, and possibly because the intervention was not primarily operating to decrease FGR, but rather decreases preterm delivery. The ultrasound subset was derived by opportunistic sampling (all women were offered scans when the machine was available), and included less than 1/3 of all trial participants; it may not be representative of the broader population. For example, more women were scanned later in the study, but there was no significant difference in treatment efficacy by study period.

We have added the following to the end of the introduction:

Lines 123-124: In the trial, IPTp reduced LBW by 26%, and reduced preterm birth by 38% [17].

In the discussion we have added:

Lines 280-284: Fourth, we used opportunistic sampling of our trial cohort for ultrasound studies, which may have introduced unintentional bias. Fifth, the trial
setting (with different antimalarial regimes used) may have resulted in differential effects on parameters of interest.

METHODS/RESULTS
Albendazole should be presented as an antihelmintc drug, this is not immediately clear to those who do not work with tropical medicine.

Response: We have added the following to the manuscript.

Line 144: Anaemia was treated with iron/folate supplements and the anthelmintic albendazole

In conclusion:

1. The aim should be clearly stated also in the abstract

Response: We have amended this.
Line 47-48: We aimed to determine factors associated with indicators of abnormal fetal growth in rural lowland Papua New Guinea (PNG).

2. The Ultrasound data, criteria of classification, reference charts should be completely readdressed

Response: see above. We hope that this response, and the changes made, will reassure that our approach to analysing and interpreting the data make intuitive sense in our particular context, and with our specific aim to identify potential risk factors for SGA and low fetal weight gain, our directly-measured outcomes of interest.

3. Socio biological data are unique, genuine and worth correlating with fetal growth in utero

6. The results and discussion should be rewritten after appropriate classification of fetal growth in utero

Response: see above.

Reviewer 2

This paper examines fetal growth restriction in a cohort of mothers in PNG, where malaria transmission occurs year round. The use of ultrasound measures of fetal growth was a novelty of this study, as ultrasound is not available in many low-income countries and study settings.

Minor Essential Revisions:

Abstract
The data demonstrated an improvement in fetal growth where intermittent preventative treatment for malaria was used. The conclusion states that maternal
nutritional interventions could improve fetal growth. It may be more appropriate to state that malarial treatments may improve fetal growth, or that in additional to maternal nutritional interventions, treatment for malaria may improve fetal growth.

**Response:** We have amended this to read

Lines 72-73: Macronutrient undernutrition and anaemia increased the risk of FGR. *Antenatal nutritional interventions and malaria prevention could improve fetal growth in PNG.*

**Introduction:**

Page 5, line 117: A concluding sentence could be added to this paragraph, perhaps indicating that there is little information about malaria infection in pregnancy outside of Africa.

**Response:** We have added the following to this paragraph.

Lines 115-117: Overall, the number of ultrasound studies evaluating the role of undernutrition and malaria as causes of suboptimal fetal growth in LICs is limited, in particular outside of sub-Saharan Africa and South East Asia.

Page 5, line 122: It is not clear that “intermittent preventative treatment in pregnancy” is related to malaria treatment, so this should be added.

**Response:** We have amended this to read

Line 122: We evaluated factors associated with FGR in fetuses of women co-enrolled in a randomised controlled trial (RCT) evaluating intermittent preventive treatment of *malaria* in pregnancy (IPTp) in Papua New Guinea (PNG) (NCT01136850) [9].

**Materials and Methods**

Page 5, line 126: the word “their” should be added before “first prenatal visit”.

**Response:** We have amended this accordingly.

Page 5, line 128: LBW is said to be “common” (17%) in this area, but this data is unpublished. Is this necessary to include here? Can the rate of low birth weight be given from the data itself in the results section (in addition to SGA and low weight gain)?

**Response:** The parent trial, which includes these data, has been published since submission of the present manuscript. We have added the relevant reference to this paper. We have also included the prevalence of low birthweight in the ultrasound cohort to the results section (see below).

Page 6, line 144: It is not clear to me what ITNs are – please provide a definition.
Response: We have spelt this out and removed the acronym.

Line 146: …and **insecticide-treated bed nets** were provided when available.

**Results**

- qPCR data (maternal blood) is not shown. Could this be included as a supplement? Did this data result in different diagnosis of malaria compared to the other methods used?

Response: We are currently evaluating the role of submicroscopic infections (qPCR) and impact on birthweight in the trial cohort. This article will include an evaluation of qPCR versus LM, and for placental samples, against placental histology. Given the small sample size in this ultrasound cohort we would prefer to publish the results of this study for the overall cohort. However, the current manuscript contains some indication as to diagnosis of infection in the cohort by diagnostic tool, and we have highlighted this further.

Lines 247-248: Forty-four percent (86/197) of infections were submicroscopic (detected by qPCR only).

- Could data on low birth weight (<2500 g) be presented?

Response: We added the following to the results section

Line 223: 91.1% (611/671) of BWs were eligible for inclusion: the prevalence of LBW was 15.7% (96/611).

**Discussion**

- A history of malaria infection was associated with an increased risk of SGA in an unadjusted model but not in the adjusted model. Undernutrition and primigravidity did not appear to be modifying this relationship - could the authors suggest any other possible factors which contributed?

Response: We believe the principal reasons for not observing an association between malaria and abnormal fetal growth in our cohort include: a) low prevalence of infection, in particular after receiving trial interventions b) high proportion of submicroscopic infections, whose role in fetal growth restriction is poorly understood and may be minor, c) one-third of infections were *P. vivax*, which appear to affect fetal growth to a lesser extent than *P. falciparum*, d) infections may have been missed as number of malaria screening visits was limited, e) episodes of FGR may have been missed, as the number of fetal weight measurements was limited, and f) adaptive (compensatory) processes after early effective treatment and subsequent protection from infection. We have added some additional information to the discussion paragraph pertaining to this:

Lines 315-320: Most infections were detected at enrolment. Although there is increasing evidence that malarial infection in early pregnancy can affect fetal growth [14], compensatory processes such as adaptive villous angiogenesis, and catch-up
growth, may have mitigated the deleterious effect on fetal growth of some these infections in the context of early treatment, insecticide-treated bed nets, and close clinical monitoring provided as part of the original trial [1, 15].

Page 11, line 280: potential confounders may not have been measured – can the authors expand on what these might be?

Response: We have reworded this to read:

Lines 283-284: Lastly, not all possible risk factors of FGR and potential confounders of the observed relationships may not have been measured and evaluated (e.g. HIV, helminth infection, micronutrient deficiencies).

Table 1:
Infant sex percentages for male (46.4%) and female (46.5%) do not add up to 100. The female percentage appears to be incorrect.

Response: We have corrected this typo.

Discretionary Revisions

Data from this study was part of an RCT evaluating intermittent preventative treatment of malaria in pregnancy. Did the randomisation group make any difference to the occurrence of low birth weight/SGA/low fetal growth rate?

Response: See also our response to Reviewer 1. The main trial analysis has now been published and we have added this reference to the paper. In the trial found that the intervention (IPTp with sulphadoxine-pyrimethamine plus azithromycin) reduces the risk of LBW and increases mean birthweight [9]. Amongst the subset of women who had timely dating ultrasounds, it appeared that the intervention primarily prevented LBW by preventing preterm birth (RR 0.62, CI 0.43-0.89, p=0.010). In settings like PNG, malaria is believed primarily to cause LBW through FGR, but malaria was relatively uncommon in the trial, and infection prevalence fell sharply after provision of bed nets and the first antimalarial treatment at enrolment (see discussion). In the present analysis, type of malaria prevention was not associated with indicators of FGR, which may be explained by the low prevalence of malaria, the sharp fall in infections detected, and possibly because the intervention was not primarily operating to decrease FGR, but rather decreases preterm delivery. The ultrasound subset was derived by opportunistic sampling (all women were offered scans when the machine was available), and included less than 1/3 of all trial participants; it may not be representative of the broader population. For example, more women were scanned later in the study, but there was no significant difference in treatment efficacy by study period.
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**Lines 280-283:** Fourth, we used opportunistic sampling of our trial cohort for ultrasound studies, which may have introduced unintentional bias. Fifth, the trial setting (with different antimalarial regimes used) may have resulted in differential effects on parameters of interest.

**Note to the Editor**

We have changed the format of Tables 3 and 4 to fully comply with BMC formatting guidelines (portrait format accepted only). No other changes were made to these tables.


