Author’s response to reviews

Title: Early and late mortality after malaria in young children in Papua, Indonesia

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Author’s response to reviews:

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Re: Early and late mortality after malaria in young children in Papua, Indonesia

Manuscript Reference Number: INFD-D-18-00771R1

18th February 2019

Dear Dr. Cecilia Devoto,

Thank you for allowing us to resubmit the revised manuscript.

The reviewers’ comments were very helpful in helping us improve the manuscript. We have made clarifications on the description of study methods and analysis as suggested.
Please find below our detailed response.
We hope that the manuscript is now ready for publication.

Yours sincerely,

Dr. Jeanne Rini Poespoprodjo, Paed, MSc, Ph.D

Response to reviewers’ comments

INFD-D-18-00771R1
Title: Early and late mortality after malaria in young children in Papua, Indonesia

Reviewer #1 (Sharon E Cox, PhD):

This manuscript reports an interesting analysis of a rare dataset from an area of high malaria transmission for both P. falciparum and vivax and high child mortality in Indonesian Papua. I congratulate the researchers on collecting and analysing this large important dataset in a challenging environment. However, although the analyses appear to be largely appropriate the description of the methodology is currently insufficient. The reporting of the results is sometimes repetitive and a little hard to follow, whilst the reasons for conducting some analyses or presentations of results and their interpretation is not always very clear.

I suggest that some of the tables and figures could be moved to supplementary material or removed.

We have revised the text to reduce repetition and improve clarity. Whilst we present six tables we believe that each is very important clarification of the accompanying text. We have reviewed again but believe that they would be better suited in the main text rather than supplementary material.

Major comments

A significant omission of the methods is that it is not made clear how survival status of the children was determined over the course of the observation period. No mention is made of any surveillance outside of the hospital. Therefore I am left to surmise that unless children re-presented to the hospital, they are assumed to be alive at month 12?
The reviewer is correct that our surveillance only extended to patients representing to the hospital since no community surveillance was undertaken. Survival analysis was undertaken until the time of the next recurrence of malaria or recorded death, with patients censored at the time of death, or if no death was recorded 12 months after the first presentation or the end of the study period (31st December 2013), whichever occurred earliest.

We assume that the majority of deaths would occur in the hospital, which was the only hospital and referral centre in the district providing free medical care for local tribes. Furthermore, we can be sure that deaths in primary centre is unlikely, since they don’t have inpatient facility, but we have no record of whether patients died at home. The lack of surveillance outside the hospital indeed raises potential attrition bias in patients after they were discharged from hospital. However early mortality will have been captured and we hypothesise that after discharge any attrition bias will be similar between patients with P. falciparum and P. vivax – hence the comparative mortality (the main focus of the analysis) remains valid.

We have revised the methods to make this clearer (Line 131-134) and in the discussion raise this again as part of the limitations (Lines 447-454).

Similarly, how were deaths detected? Was this limited to those that died during an admission?

See response above.

Although hospital data occurring within a 30 day period of a presentation were recoded as a single event (was this only done for the first presenting malaria episode, or for all presentations/episodes?), should children have been censored from the survival analysis (analysis of re-presentation) for a period after completion of treatment/admission to accurately assess PYO?

We consider malaria within 14 days (not 30 days) of first presentation as single event and define this as their “first malaria infection”. In the survival analysis, subsequent presentations within a similar 14 day period were also grouped as single episode of “recurrence/representation” and treated as an endpoint of interest.

However the period of observation was calculated from the time of the first episode of malaria, until death, or the end of a 12 month period or the end of the study (the 31st December 2013). This is now stated more clearly in the methods section.

Consider if including date of birth as the point of origin in the survival analysis might improve estimates of age stratified rates of recurrence and mortality. I believe this is called the Cnaan and Ryan approach?

The focus of the analysis is to compare the risks of early and late mortality rather than perinatal and
infant mortality per se. For this reason we commenced the survival analysis at presentation with malaria and, in view of the high perinatal mortality and congenital malaria in this area, we excluded patients less than one month of age. The all cause perinatal and infant mortality will be explored in a separate analysis.

The methods text also does not make it clear if all re-presentations at the hospital were included in the recurrent episodes analysis or ONLY re-presentations that were defined as malaria (which appears to be the case). The case definition of a presentation for malaria (first or subsequent cases) is also not clearly defined. Was this the presence of any parasites? Or was this based on the ICD-10 diagnosis of malaria (final, proximal or any recorded diagnosis).

Whilst all patients’ encounters at the hospital were recorded (including those with and without malaria), the risk analysis only pertains to representations with malaria or with any illness resulting in death. The case definition of malaria refers to patients in whom malaria was confirmed by blood film microscopy. Since hospital protocol dictates that any patient presenting with fever, a history of fever or severe illness are checked for malaria by microscopy this will have captured all symptomatic malaria episodes.

We have clarified these case definitions in line 103-112 and 130-131.

Minor comments

Abstract - line 25: typo? Should it be "…before either 30 days or within 30 to 365 days…"

The reviewer is correct. We have revised the wording accordingly (line 25-26).

Study site, p5: Can more information be provided to indicate the likely proportion of cases with active symptoms who were likely to present for care at the study hospital compared to primary health care clinics in the area?

Previous community surveillance suggests that during the study period between 40-60% patients with malaria sought treatment at the hospital. We have included the statement in line 82-83.

Methods p6 - were there any hospital guidelines/criteria for admission. Was blood transfusion available for severe anemia - is BT history available in the dataset?

Admissions were made according to the criteria outlined in the hospital guidelines. Blood transfusion is available 24 hours for 7 days. The hospital protocol dictates that anyone with haemoglobin concentration less than 5 gr/dl should have blood transfusion. Severely ill children with anaemia would receive blood transfusion. In view of this, we don’t include history of blood transfusion in the database.
Methods - describe that both inpatient and outpatient presentations were included, earlier in the text.

We have clarified this on line 109-111.

Methods/results - malnutrition. It says this was assessed by the attending physician, yet complete data is presented for a composite score of severe acute malnutrition (weight for height/length zscore<-3) or stunting (height for age z-score <-3). This seems very unlikely that physicians assessed weight and height in every admission??? Or, was the absence of recorded malnutrition assumed to be "normal?" Finally, I would separate out severe acute malnutrition from stunting, as they are likely to have quite different associations with risk of death between the short and longer term.

In line 135 and 137 we define malnutrition based on weight for age or height/length for weight. We did not include stunting diagnosis in the analysis, as height is not routinely measured. RSMM has two Paediatricians to ensure the quality of child care. Weight assessment is mandatory to all paediatric hospital presentations. Height measurement is only performed to those with clinically visible wasting child to assess the degree of malnutrition and include that in the diagnosis. In this case, we can assume that those without a diagnosis of malnutrition can be considered “normal”.

Statistical methods: More details should be provided of how multivariable models were developed and models compared. Was effect modification assessed (please see later comment). Description of the calculation of PAF is not included.

We have now included further details to clarify the statistical methods on the multivariable analysis and the calculation of PAF (lines 156-160 and 162-177).

Results.

Figure 1: could be improved - formatting and consistent inclusion of percentages.

Figure 1 has been revised as requested.

Table 1:
1. Suggest re-naming to include "…. Under 5 years old at first presentation…"
2. Include denominators for inpatient/outpatient for those with hgb data
3. Proportions of children in the first column for Hgb<7g/dl or <5 g/dL are incorrect. It is correct in the text on p9.
4. Results in the text are too repetitive of those presented in the table.
We have revised the table as requested.

P9-10 - description of pharmacy records for first presentations - consider describing by inpatient/outpatient status, which is probably your best indication of disease severity.

All hospital admissions were screened for malaria, hence oral antimalarial drugs were prescribed for both inpatients and outpatients. The key difference in prescribing relates to intravenous antimalarial drugs which was restricted to inpatients with severe disease and unable to tolerate oral medication. Further explanation on this assumption is presented in the discussion section in line 376-386.

Risk factors for recurrent malaria?
1. P10 lines 174 - 181. Please specify if referring to Fig 2A or 2B.
2. Line 176-177 - it is not entirely clear if this means that 63.9% of all re-presentations (regardless of species at first presentation) were due to P. vivax in infants … and 50.4% in young children.

We have clarified which figure the text refers to (Figure 2A). The paragraph refers to 63.9% of all representations with malaria were due to P. vivax in infants and young children. We have revised the text to make this clearer.

Fig 2A is this excluding first presentations on the x-axis?

Fig 2B is not referred to in the text?

Figure 2A refers to recurrences (as stated in the X axis) and as such excludes first presentation on the x-axis. The legend has been revised to make this clearer.

Figure 2B: Is now referred in the associated text in line 242-244.

Table 2: consider including inpatient/outpatient as columns as well as rows?

The main outcome is representation with malaria by species and inpatient status (ie rows) comparing those patients coming in different species (columns). Hence we feel it would be confusing to add further columns with IP and OP status.

Table 3: What number of children/events are included in the multivariable analyses - as presumably this is much reduced due to the smaller numbers of data points for hgb. Consider reporting sensitivity analyses in supp material - to determine if the other risk factors look different in multivar analysis not limited to those with Hgb data. However, at least for this table - the HRs don't appear to be altered very much between the univariable and multivariable models, so in this case it is probably not an issue.
The multivariable analysis presented in Table 3 was actually undertaken excluding Hb7Gr to prevent losing 45% of the records, as correctly pointed out by the reviewer. However to clarify the models with and without Hb we now present two multiple models with and without Hb. The AHRs don’t change much.

We have also used a similar approach to Table 4.

Table 3 Odd footnote text - typo?

The typo has been corrected.

1. P14 lines 226 - 232. to help the reader, I suggest explaining what question you are asking in relation to the results being presented. Lines 226-232 appear to be reporting results within subgroups ..., why? Were you trying to assess if the effect of parasite species differed by malnutrition - in which case report the stratified analysis (as supplementary info if no indication it differed) - -

2. also note that investigating effect modification is not described in the statistical methods.

Malnutrition is an independent risk factor for early mortality (table 4). Thus to determine the relative effect of malaria species we stratified by infants with and without malnutrition as well as in the multivariable analysis. We have now added details on effect modification in the statistical methods (lines 156-160).

P17 lines 250-251: Risk of later mortality greater in infants than young children. This is touched on in the discussion - that this may be that older children at first presentation represent a survivor cohort.

In this area where children are exposed to malaria since birth, it is possible/likely that young children may represent a survivor cohort and thus younger children (infants) are at higher risk of mortality. This type of limitation is inherent to study using secondary data. We are unable to explore this possibility in the current study design but acknowledged the limitations in the discussion (line 458-469).

Table 5: footnote erroneously relates to multivariable analyses

We were unable to present a multivariable model due to very low numbers of late deaths in some subgroups. We have corrected the footnote accordingly and also stated why there is no multivariable model.

Results: consider including analysis of all-cause risk of re-presentations?
We considered this analysis which indeed would be of interest. However the issues of comorbidity and mortality are beyond the current analysis which is already dense. We would prefer to address this in a subsequent paper.

Results: I expect if the data were available you would have included them?- parasite density; Blood transfusion during admission; co-morbidities at first/subsequent presentations? However, the latter is reported for diagnosis at time of death - and so the presence of co-morbid pneumonia or diarrhea is presumably available?

The ICD diagnosis at each encounter is recorded, but we feel that this is less reliable than the more objective measures we present in our analysis (age, sex, Hb, and malaria diagnosis). Hence whilst we refer to the diagnosis at the time of death, we don’t include more detailed analysis on the comorbidities. Parasitaemia was not quantified.

Results: Are you able to assess if there is any indication that when low dose primaquine was included in anti-malarial treatment (pre 2006?) if it was effective in reducing recurrence of P.vivax? the way it is written in the methods it appears that once DHP became the first line treatment, low dose primaquine was no longer included, even if was known P.vivax. Is this correct?

The effectiveness of low and high dose primaquine has been addressed in a previous analysis. We found that the Unsupervised 14 day regimen had at best 12% reduction in recurrence (Douglas et al PlosMed 2017). Since effectiveness of both low and high dose primaquine were very low, we attribute this to poor adherence rather than dose related efficacy. We refer to these findings and have clarified further in line 123.

Discussion

Any comments on the opposite effects observed for ethnicity in recurrent malaria vs death analyses?

Its an interesting point. Both highland and lowland Papuans have lower socioeconomic status and are more vulnerable to recurrent malaria than non Papuan. Their higher immunity may protect them from early mortality. However late mortality maybe more associated with the cumulative effects of malnutrition and anaemia arising from recurrent malaria and are thus is higher in highlanders. The number of late deaths in lowlanders is low and the confidence too wide to infer significance.

I don't think the inherent limitations of the data and are adequately acknowledged and discussed- and potential effects on bias.

We acknowledge that this is important and have revised the discussion further to mention attrition biases of routine hospital data on illness and deaths and confounding effect of varying immunity.
Conclusions - line 408. I don't think that inclusion of sepsis (or the later comments about broad spectrum antibiotics) in the conclusions are warranted - given that no data is presented concerning the incidence of sepsis.

We have revised our final conclusions to focus on the need to prevent recurrent malaria and have deleted the sentences regarding further studies of antibiotics.

Reviewer #2:

GENERAL COMMENTS: This is a well written manuscript. The authors have provide novel analysis and exposes one aspect that may hinder the eradication major malaria parasites in Indonesia. In general, the work is sound, but it would benefit from a few of modifications.

REQUESTED REVISIONS:
1) The authors never mention explicitly why it is so important to distinguish and quantify malaria and delayed effects between species. I presume that the need to distinguish and quantify different species of malaria will vary depending on interventions including vector management. At least a paragraph or two on this should be added.

The two malaria species have very different pathogenicity. Falciparum is more pathogenic, killing by sequestration and early parasite induced haemolysis. The pathogenicity of P. vivax is likely more related to its propensity to recur. We have revised the introduction to highlight this (line 51-55). We have also added information on bed-nets coverage (line 77-78).

2) Since these are hospital-based records, how did the authors handle the incomplete routine case and death data? Also, was the focus on children <5 or children in general? Be specific on your cut-off.

See the response to reviewer #1 above on attrition bias. Since the focus is on comparative mortality, we assume these biases are similar for both species.

The focus of this study is children aged less than 5 years old presenting to the hospital. This is now clearly stated in methods.

3) Line 63-67. It is indicated that, generally, malaria cases declined between 2004 and 2013, but in the same period the proportion of infections due to P. vivax increased substantially. What caused this shift in changes?

This is an important question and will be addressed in a subsequent temporal analysis. However our explanation is that P. vivax form dormant liver stage (hypnozoites) that can causes relapses. The only drug available to clear hypnozoites is primaquine (which has to be taken for 14 days to achieve an
optimum parasite clearance). We have shown that unsupervised primaquine treatment is not effective in preventing recurrence (Douglas et al plosMed 20170). This makes controlling P. vivax cases is more challenging. With improved schizontocidal antimalarial drug, P. falciparum cases is declining, but P. vivax cases remains static.

We have revised the introduction to highlight this (line 51-55).

4) Line 259-263 show that children with any early recurrence of malaria had an increased risk of late mortality with P. vivax recurrence, but not with P. falciparum recurrence. This is great visual showing the correlation between the efforts made, and the decrease in malaria. What might have caused this delay? These needs to be explained?

This is central to our analysis. P. vivax infection is highlighted by increase incidence rate of malaria. Thus early P. vivax heralds a child likely to have multiple further attacks and cumulative anaemia. Conversely P. falciparum has far fewer recurrences. The attributable mortality with P. falciparum is therefore early and the relative proportion for P. vivax occurs later.

5) I presuppose there could be ways to prevent the disease in Papua and this was indeed what is lessening the deaths from malaria, wouldn't there be a slight delay in the effects due to these factors? And if so what factors contributes to the diminishing of malaria cases that may either lead to early or late deaths?

The reduction in malaria over the period of the study is indeed an important confounding factor. We will address malaria related mortality in a separate analysis, however the current analysis is more focused on the comparative mortality and morbidity of P. falciparum and P. vivax. For this reason all of the multivariable models are stratified by year to account for background changes in transmission intensity. We have now clarified this in the statistical methods (line 156-162).

Our conclusion of the high risk of recurrent malaria in children with P. vivax and the high delayed mortality associated with this, argues strongly for the use of primaquine radical cure to cure the acute asexual infection and the subsequent relapses from the liver hypnozoites.

6) Conclusion is too general. A more precise conclusion based on summery of the findings should be made, including its implication on malaria elimination in Indonesia.

We thank the reviewer for the suggestions. We have added a statement on malaria elimination in line 474-478.

ADDITIONAL REQUESTS/SUGGESTIONS:
Yes. If possible a Bayesian analysis to distinguish and quantify these immediate and delayed deaths, so
that a difference can be statistically presented.

We have considered a Bayesian analysis, and also correlation of incidence and subsequent mortality, but this will require more detailed analysis including the influence of multiple events on an outcome. We believe that the current analysis is already very dense and that subsequent modelling would be better in a separate analysis.