Author's response to reviews

Title: Time-to-infection by Plasmodium falciparum is largely determined by random factors.

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Author's response to reviews: see over
Dr Sabina Alam
Editor
BMC Medicine.

Dear Dr Alam,

Re: MS: 1826501646138864 – resubmission

Thank you for your e-mail and reviews of manuscript MS: 1826501646138864 - “Time-to-infection by Plasmodium falciparum is largely determined by random factors”. We have revised the manuscript in response to the reviews and attach it for resubmission as an article for BMC Medicine.

The reviewers’ comments were very supportive of the approach and analysis, and suggested minor changes to the manuscript. We have responded to the comments individually in the attached sheets.

Thank you in advance for your consideration of this manuscript, and we look forward to its rapid acceptance at BMC Medicine.

Sincerely,

Miles Davenport, MB BS, D.Phil
Reviewer - Maude:

This was an interesting study which combined clinical data and modelling. However the methodology used some assumptions for which I could not find adequate justification. Additionally, the clinical data appeared to be somewhat under-powered and it is not clear that the conclusion was fully warranted in view of these limitations.

Major Compulsory Revisions

1. There were some inadequacies in the description of the method for determining PMR. Why assume a parasitaemia at the microscopy detection threshold at first PCR detection? Why assume PCR detection threshold 1 week prior? Why can only a maximum PMR be estimated for those who are PCR positive but don’t become microscopy positive? These should be clarified.

We thank the reviewer for identifying where the descriptions of the methods may have been a little too brief. We have now significantly clarified and expanded this section of methods.

2. It was assumed that PMR is normally distributed. Why should this be the case? Did the authors have data to show this?

There are two issues here. Firstly, whether the PMR was normally distributed, and secondly, whether the exact form of the distribution is important. Importantly, if the underlying PMR is normally distributed, we will only observe a truncated normal distribution (because we can’t observe very slow growing infections). We note that studies of subjects challenged in mosquito (Bejon et al, JID v919, p619) and blood stage infection (our own unpublished data with J McCarthy, Queensland Institute of Medical Research) suggest a normal distribution of growth rates. Moreover, we note that the major point we are making is that, given the observed range of differences in growth rate, the differences in growth rate make only a minor contribution to the delays to detection. Thus, whether the shape of the distribution is normal or some different distribution will have little effect. We have added a comment on this point to the methods section.

3. Time-to-detection and parasite growth rate appeared to be discussed as if they are independent measures but surely they are interdependent? Further explanation of this is needed.

The link between PMR and time-to-detection is one of the major issues addressed in the manuscript. Of course PMR will affect time taken from the liver stage until detection in the blood stage. However, as we illustrate in our study, in many circumstances any differences in this time are hard to detect, because of the random time-to-infection. We have included a discussion of this issue at the end of the first section of the results.

4. The power of the clinical part of this study was limited by the number of patients and 1 week gaps between blood samples to determine time to infection. With a larger number of patients and smaller intervals between
samples, the conclusions may have been different. The authors should acknowledge these limitations in the discussion. How was power calculated when planning this study? Out of the 197 individuals, how many were included? In the figures, there appear to be data from somewhat fewer than this? As children and adults were analysed separately, it should be stated how many individuals were in each group to give an indication of power to detect differences.

The reviewer suggests a better discussion of the power of such analyses to identify differences. It is indeed correct that with a sufficiently (and likely prohibitively) large sample, one can detect extremely minor differences in growth rate. However, a major message of the study is to point out some of the difficulties in time-to-infection studies, and to highlight in what circumstances it may be more or less feasible. In the initial cohort study the importance of random time-to-infection was not factored in, and the analysis we have performed was done post-hoc. The arguments we present later in the manuscript are not dependent on the power of the original study – we use a simulation approach to illustrate that this is a major issue for all studies.

As suggested, we have added details of the number of subjects included in the various analyses to the figures. We have also added a note at the start of the modeling section in the results to indicate that the experimental study represents only one snapshot of the issue, and that we use the modeling to illustrate the problem more generally, and how it might affect other study designs.

Minor Essential Revisions

1. **Figure 4. What were the units for parasite multiplication rate?**

   We have added this to figure 4.

2. **There are some grammatical errors that should be corrected e.g. “Using the mode the sensitive...”**

   Corrected.

3. **The thresholds for microscopy and PCR detection and their margin of error should be stated early in the manuscript.**

   Added in methods.

4. **What was meant by “microscopy infection curves”?**

   We have changed this term to “distribution of time to detection of infection”
Reviewer - White:

Major Compulsory Revisions:

1. This approach combines two levels of organisation (population level transmission dynamics and individual level infection dynamics) in a very elegant mathematical framework. It is used to consider the implications for interpreting the time to infection with Plasmodium falciparum malaria derived from follow-up data. Two methods of detection are considered simultaneously: microscopy and PCR where the difference in the detection thresholds for within-host parasite loads is accounted for. It is my opinion that with some adjustments to the style and a little more consideration of the context that this research will be of interest to a very diverse readership.

2. The title “Time-to-infection by Plasmodium falciparum is largely determined by random factors” is supported by the analysis in the manuscript. However, I would like to know whether this is only the case for natural immunity or whether this is also the case for vaccine-induced immunity. Natural immunity is characterized very nicely as having no infection blocking action but rather acts to reduce parasite growth upon infection. This is a very nice result, but are the authors concluding that this is the case for vaccines as well?

3. The method demonstrates that immunity (assumed to increase with age) most likely manifests as a reduction the parasite multiplication rate upon infection rather than being protective against infection. This then differs significantly from the assumption that the RTS,S vaccine provides some protective efficacy against infection even if short-lived. Moreover, the design of the study from which the data are derived differs fundamentally from the phase III RTS,S vaccine trial design in that participants are sampled weekly regardless of symptoms in the time to infection study whereas in the RTS,S phase III vaccine trial, individuals are only sampled if they report a clinical case. With this key difference in mind, my first suggestion would be for the authors to find a way to use the time-to-infection study to simulate passive case detection and explore the implications of the analysis of this type of data. This would be possible if they have access to information on clinical cases which they could use retrospectively as they did with the PCR data. I think that such an analysis will highlight how typical vaccine trial designs for malaria fall short of providing sufficient information to understand the way in which both the vaccine-induced and natural immunity differ and interact with each other. This should be a caveat by which current trial data is interpreted and a contribution to the design of future trials of this and other malaria vaccines.

4. In the abstract the authors mention how important the identification of protective immune response is for vaccine development. I think that they could simulate a protective immune response, which is clearly not provided by natural immunity to demonstrate how the time to infection observations would change for detection by both microscopy and PCR. Many RTS,S vaccine trials demonstrate differences in time to infection (by both active and passive case detection) between the vaccine and control groups. It would be extremely useful if this approach were used to assist in the interpretation of such results.
The three comments above relate to similar issues. Firstly, what are the implications for vaccines (such as RTS,S) that are presumed to block infection, rather than slowing growth. Secondly, the implications for different study designs (such as passive case detection). We have added an additional paragraph to the discussion to cover these issues.

5. I have reproduced the model results using a simple set of ordinary differential equations in the following format:

\[
\begin{align*}
\frac{d}{dt}(\text{susceptible}[1..N,1..2]) &= -\lambda[i]\text{susceptible}[i,j] \\
\frac{d}{dt}(\text{infected}[1..N,1..2]) &= \lambda[i]\text{susceptible}[i,j] - \delta[i,j]\text{infected}[i,j] \\
\frac{d}{dt}(\text{detected}[1..N,1..2]) &= \delta[i,j]\text{infected}[i,j] \\
\text{out}[1..N,1..2] &= 1 - \text{detected}[i,j] \\
\delta[1..N,1..2] &= \logn(\text{PMR}[i])/(2*(\logn(\text{C}[i,j]) - \logn(\text{C0}))) \\
\lambda[1..N] &= \text{if time}<\tau \text{ then 0 else EIR*[i]/365}
\end{align*}
\]

with \(N=4\) age groups and 2 detection methods being microscopy and PCR. Then the function \(\delta\) relates the rate of detection to the average parasite multiplication rate PMR for each age group. The detection thresholds in each age group, \(i\), and for each detection method, \(j\), are given by \(C[i,j]\). The initial parasite load is given by \(C0\). The rate of infection is switched on after a lag time, \(\tau\). This makes me very confident that the results presented are sound. Some diagrams of the process being modelled along with the provision of an additional file showing the steps towards obtaining the equations in the manuscript would be useful for a more general reader.

We have added a schematic as suggested.

6. Throughout the manuscript, the authors should clarify which parameters and are fixed in each analysis and which are estimated directly from the data or from fitting models (and which models/equations were used in each case).

We have clarified in the manuscript which parameters are fixed, which are fitted and which results are estimated from model and from data.

7. The authors should consider the positive correlation between age and biting rate and therefore infection rate and how including this this relationship might affect their results.

One conclusion from our study is that between age groups one can indeed see a difference in time to infection, and measure differences in parasite growth rate (as we showed in our previous publications (references 5 and 10 of manuscript).

However, within a given age group (and particularly in children), time-to-infection often does not provide much information on growth rates of parasites. In figure 5 we explore how biting rate changes our ability to detect differences in growth rate, and find that higher biting rates make this easier. However, this effect requires fairly drastic changes in biting rate, which might be greater than that expected with age.

We have added a note in the discussion of the effects of different biting rates with
age.

8. The authors should consider also the relationship between age (and therefore blood volume) on detection thresholds for both microscopy and PCR. That is, for the same parasite load, the concentration of parasites in children would be higher than in adults leading to detection at lower absolute loads in children compared to adults.

The major effect of the blood volume of the individual is at the stage of parasite emergence from the liver, where a fixed number of merozoites is released into a variable blood volume. This was discussed in the methods referred to in our original publications on time-to-infection, but we had unfortunately not detailed that here (although it was done). We have now clarified this in the methods. We note that the detection thresholds are the same (one parasite per microliter is the same regardless of the individual’s blood volume), just the parasite density at the time of emergence from the liver (and thus time from liver emergence to reaching a given threshold) changes slightly.

9. In general I think that there are too many very busy figures. Some of these should be relegated to additional material for completeness and a select few very simplified versions should be used in the main paper to illustrate key results. For example, figure 5 A to E is not necessary. It would be much better to pick one age group and show 5 lines for increasing EIR on a single graph. Graphs F to J could be summarised by plotting EIR on the x-axis and the corresponding mean early and late PMR values on the y-axis. Same for K to O. It is still not clear to me what P to T is showing me. The green area that is changing is really small compared to the blue and red lines.

We have modified figure 5 as suggested.

Minor Essential Revisions:
10. The numbering of the equations should be corrected

Done.

11. Results first paragraph: it looks like the time to detection was derived directly from the data an then the rate of new infections was derived from fitting a simple exponential curve. Is that correct?

This is correct, and we have clarified this in the results.

12. Line 255 to 265: was the equation on line 186 used to estimate the PMR in each case? If so, please state this more clearly.

We have now stated in the results that equation (1) was used to estimate growth in each case.

13. Line 383 to 399: I think the authors are saying here that by fixing the infection rate at different assumed values will lead to different estimates of the PMR.
We apologize if this was unclear. We have rewritten this section to make it clear that changing the infection rate will change the ability of a time-to-infection study to identify differences in PMR (not that it will change the estimated PMR).

14. Figure 2: please state which values are derived from the data and which are estimated from the model (eg I think “delay in weeks” is derived from the data and PMR is estimated).

The reviewer is correct that PMR is estimated from the data, and we have now made this clear in the legend (and referred to the equation, as suggested in item 12 above).

15. Figure 5: do these graphs represent the predictions using microscopy?

Yes, this is now stated also in the legend.

16. Figure 5 F to O: is dashed late infection and solid early infection?

Yes, and we have now simplified this figure in the manuscript, and stated this more clearly in the legend.

17. Figure 4 C to F: It is not clear to me how PMR can be estimated separately for both PCR and microscopy as I understood from the first equation that this is calculated by comparing the time to infection within an individual as detected by each of these methods.

The reviewer is correct that the PMR distribution is the same for microscopy and PCR detection. However, when we sort individuals detected at different times (eg: first, middle or third tertile of time-to-detection), then the PMR of individuals in each tertile will vary depending on whether infection is detected by PMR or microscopy. We have altered the legend to make this clearer.

18. Line 579: change “control the” to “control than the”

done.
Reviewer - Smith:
The changes suggested in the PDF have been made within the revised version of the text.