Reviewer’s report

Title: The duration of chemoprophylaxis against malaria after treatment with artemether-lumefantrine and artemether-lumefantrine and the effects of pfmdr1 86Y and pfcrt 76T: a metaanalysis of individual patient data

Version: 0 Date: 21 Oct 2019

Reviewer: Ian Hastings

Reviewer's report:

I'll identify myself as Ian Hastings. I think this is only fair given that I'm referring extensively to our own work and it allows the authors to complain about me to the Editors.

Good news is that I am going to recommend acceptance: they are a strong group, its an important question, and analysis of field data is an important contribution to the field.

Bad news is that I think the field data is flawed and that this might affect their estimates (see major point 1 below) resulting in under-estimated duration of prophylaxis post treatment.

Major point 1.
My main major reservation is that the authors take "PCR corrected" data at face value. The correction methodology used to classify a recurrent malaria (i.e. one that occurs during patient follow-up during the trial) as a recrudescence (drug failure) or a new infection is being viewed with increasing suspicion as it is likely to misclassify a significant proportion of recrudescence as new infections. Recrudescences typically start to occur before new infections so the danger (perhaps inevitability) is that some early recrudescence are falsely called as new infections and this would bias the duration of prophylaxis downwards to an unknow degree. We have a recent paper discussing this i.e.


Jones et al recently simulated this effect (with Felger) i.e.


Fig 5 from Jones et al shows simulated distributions of recrudesces and new infections with the former tending to occur earlier in the follow-up period. Figure 3 (top left panel) in the same paper suggests around 1/3 of the recrudescence are misclassified as new infections. Taken together this suggests that some early recrudesces will be misclassified as new infections and this will result in under-estimated duration of prophylaxis.

Note also that we (Jones et al) assumed correct dosage on a mg/Kg basis (in reality the heavier/older kids in the weight/age band get low doses of mg/Kg), also that adherence was perfect, that no-one
vomited after treatment, and so on. The presence of these factors in real trials all results in more early recrudescences which may be misclassified as new infections. We assume gold-standard capillary electrophoresis was used to measure marker length. Many labs use agarose gels which are awful: they are highly inaccurate and unlikely to identify the length matches between treatment and recurrent samples that are required to correctly identify a recrudescence, again increasing the risk of mis-identifying recrudesce as a new infection. So, in summary, there are likely to be many more early recrudescences in their clinical datasets than simulated in Jones at al, and more of these recrudescences are likely to be misclassified as new infections.

Furthermore, I have assumed "PCR correction" is by the standard WHO-MMV method based on msp1, msp2 and glurp genes. If any trials used microsatellites (often used in CDC-sponsored trials) then the problem of mistakenly clarifying recrudescences as new infections is even worse, see Plucinski MM, Morton L, Bushman M, Dimbu PR, Udhayakumar V. Robust algorithm for systematic classification of malaria late treatment failures as recrudescence or reinfection using microsatellite genotyping. Antimicrob Agents Chemother 2015; 59.

So a minor point is that the authors should state whether all the trials used the WHO-MMV method and markers.

That is a bit of a rant but I assume the Editors and authors will now understand my concerns i.e. there is strong likelihood that some recrudescences emerging soon after treatment will be erroneously classified as "new infections" so the chemoprophylaxis (protection against new infections) may appear much shorter than it really is.

I'm not really sure how to proceed or what to recommend. One options is to include a critical evaluation of the quality of the "corrected" data along the lines above (but much abbreviated) and simply conclude that, in the absence of any alternative, the "corrected" data just needs to be taken at face values but noting that this will result in under-estimated period of protection, but to an unknown extent. This caveat should also be in the abstract. But I'll leave it to the authors and Editor to decide how best to proceed.

Major point 2.
Throughout the ms they swap between EIR (i.e. number of infective bites per person) and the key parameter FOI (force of infection) which is the number of these bites that actually result in a patent infection. The criticality of EIR/FOI is recognised on line 239 i.e.

"Using this prior information on EIR was essential, otherwise a slow reinfection rate could be explained equally well by either a low EIR or a long drug prophylactic time.

Its not clear what the relationship is between EIR and FOI. I think the key is probably around line 225 where they say
"Pre-erythrocytic immunity , i.e. an immune response that reduces the proportion of infectious bites resulting in successful blood stage infections was computed for each individual according to their age, prior exposure and local EIR , using the same mathematical model referenced above (34)."
Readers aren't going to find ref 34 and dig out the relationship so they should provide their estimates e.g. on Table 1 or maybe in the text saying something like "Under this model an EIR of 10 equates to a FOI of ??, an EIR of 20 equates to FOI=??, EIR=50 equates to ???. Incidentally, how these estimates compare to the conversion estimated by others e.g.


So we need to see a few values of FOI so people can check whether they think the values are in the right ballpark; this is especially the case given that the evidence base to obtain FOI seems to be taking prevalence in MAP, using it to infer EIR, which is then used to infer FOI.

I am also confused about exactly how FOI was obtained.

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Line 42-45 of Discussion states
"our methods required that we estimate the EIR for each included trial site, which is only possible when sufficient numbers of reinfections are observed per site"

But on line 190 they say
"We used predictions of the FOI as prior values in our model, based on prevalence of infection in 2-10 year olds estimated by the Malaria Atlas Project (MAP) at the location and year in which each trial was carried out"

Table 2, 3, SI… fit EIR. Surely, they are fitting FOI? This is important as FOI does not scale with EIR. Figure S1 could also show FOI

So I think they need to be very clear about FOI. I assume it is the key parameter they are putting in the model (not EIR) and they need to provide some idea of what values they are using. It is important to provide a few markers to allow readers to switch between the scales.

Major point 3

Line 209... They assume it takes 3.5 days for a new infection emerging from the liver to become patent. This seems way too short. Conventional wisdom (i.e. used by both Liverpool and Bangkok; we borrowed their figures) is that 105 parasites emerge from the liver, and become microscopically detectable when they reach 108. Growth is usually measured as parasite multiplication rate PMR i.e. number of new merozoites released after the 48 hour cycle that successfully invade new merozoites. The usual assumption is PMR=10 so it takes 3 cycles i.e. 6 days to reach patency. Controlled human infections also seem to suggest a PMR of around 10 e.g. "A detailed analysis of parasitemia by Q-PCR revealed a ~10 fold difference in the parasite burden during the first blood stage growth cycle." Taken from:

The max number of daughter merozoites observed in a "pregnant" merozoite is around 30 so even if PMR=30 (which most people would find unrealistic) it still takes close to 6 days to reach patency. Note that this 6 days is a minimum as there may still be residuals drugs slowing growth, just not sufficient to completely kill off the new infection

It's potentially a major problem because a lot of people reading that figure of 3.5 days between liver emergence and patency are simply not going to believe it.
So they need discuss the likely impact of this assumption of 3.5 days. Worst case scenario is that all the duration of prophylaxis should have another 3 days subtracted (e.g. Figure 1 panel A) although that would make some individual values suspiciously short. Alternately, the impact may not be that bad: they state that follow-intervals may be quite long so presumably do a calculation along the lines 1-pt for patients staying infection-free in the 't' days between follow-up where p is the daily probability of getting infected. If this is true, a difference of 3.5 vs 6 days may not have much impact but I'm guessing.

(3.5 parasites cycle each of 48 hours = 7 days which is about right, so maybe this is all about confusion between days and cycles??).

Major point 4,
They need to tightly define what they mean by "duration of chemoprophylaxis". We thought long and hard about how to define it in in Kay and Hastings i.e.


We went around in circles for a significant amount of time, so we have a lot of sympathy as it's a tricky definition. Essentially its probabilistic. I suggest they look at the AL simulation of that paper (fig 2A), noting that we have since re-calibrated our AL model and now consider our estimates of AL prophylaxis too long (you can cite me as pers. comm on this if you wish).

So using the black line of that figure (no resistance present) we could say the period of prophylaxis ends on the day when there is a 50% probability of survival of any given parasite clone being able to infect a randomly selected human, or a 10% probability, or a 90% probability. The choice of definition is subjective, and would give different values, and there is no objective way to decide which is the correct measure. Its an arbitrary decision which is fine provided it is made explicit.

They could use a similar approach based on patient probabilities such that chemoprophylaxis is probabilistic e.g. by day x a patient may have a 10% chance of being permissive for a randomly inoculated infection, by day x+1 a 30% chance, by day x+2 a 60% probability etc and so on as the patient's drug levels drop and they can become infected by even the most drug-susceptible clones. This occurs because there is a range of drug sensitivity in the parasite population (typically 10 to 100 fold) so on day x, they can only be infected by the most resistant 10%, by day X+1, a greater proportion of parasites (30%) can infect that person as his/her drug levels continues to drop. This is what occurs in reality but I assume I am correct that this does not occur in their model because the underlying assumption is that all parasites have same level of drug sensitivity?? If so, this need to be made explicit.

So I'm guessing that, having made this explicit, they can then define the duration of prophylaxis along the lines of "the day at which the patient's drug level become permissive for a new infection to successfully establish" i.e. it is dichotomous (yes/no) rather than being probabilistic.

They should also discuss how not recognising variation in parasite clones' drug sensitivity (if that is the case) may affect their estimates.

The discussion also needs tightening up once this definition is given
e.g.
fig 1. I assume this is time until patient becomes permissive for a new infection to establish not observed times to patency. I'm pretty sure its the former but many readers only skim the abstract and graphs so it may be best to be clear in the figure legends.

Line 9 of discussion "In some locations AS-AQ provided up to an estimated 19 days of protection"… is that "19 days" the mean of the distribution or 95% centile, or the maximum and so on.

Other points.

Table 3. (age)2 and (age)3 are presumably squared and cubed values in a polynomial fit?? Or are they age groups and the subscripts '2' and '3' omitted??

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