Author's response to reviews

Title: Tolerability and safety of weekly primaquine against relapse of Plasmodium vivax in glucose-6-phosphate dehydrogenase deficient and normal Cambodians.

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Version: 3 Date: 29 May 2015

Author's response to reviews: see over
Dear Editor,

We would like to thank you for considering for publication in BMC MEDICINE of our manuscript “Tolerability and safety of weekly primaquine against relapse of Plasmodium vivax in glucose-6-phosphate dehydrogenase deficient and normal Cambodians.”

The distinguished reviewers have been thorough and done a good job and we wish to express our sincere thanks to them both. We have read carefully their comments and responded to each comment and, where necessary, have amended the manuscript. Please find below the detailed responses.

We now believe that the paper has been improved thanks to the two reviewers. Our paper provides important and new clinical and public health messages for a broad readership.

All authors have read and approved the re-submission of the manuscript. A potential publication of our article in your journal would be a great honour for our study group.

Yours faithfully,

Dr. Walter RJ (Bob) Taylor.

Mahidol Oxford Clinical Research unit (MORU),
Bangkok,
Thailand.
Reviewer's report

Title: Tolerability and safety of weekly primaquine against relapse of Plasmodium vivax in glucose-6-phosphate dehydrogenase deficient and normal Cambodians.

Version: 1 Date: 2 April 2015

Reviewer: Wuelton M Monteiro

Reviewer's report:

The manuscript presents important information regarding PQ tolerability and safety in patients presenting G6PD deficiency, concluding that PQ as antirelapse treatment should not be given without knowing the G6PD status of patients and should be given under medical supervision to G6PDd patients. In general, the study was well conducted, in methodological and ethical terms.

Minor comments:

1. Introduction:

- Add data about tafenoquine, highlighting the need of attention in case of introduction of this drug.

Reply 1
This reference has been added to the paper.

We have not added data about tafenoquine in the Introduction as few relevant data are available currently in the public domain as peer reviewed publications. However, we have added a note in the Discussion regarding the safety implications for tafenoquine which is being developed for restricted use.

The key to the safe use of primaquine and, in the future, tafenoquine as antirelapse treatment is the accurate diagnosis of G6PDd and identifying those with more severe G6PDd. Indeed, tafenoquine registration trials are excluding patients with enzyme activities < 70% of the population median (NCT02216123)[29]. In our setting, a patient with severe G6PDd who would be misclassified as G6PDn and who would receive the
appropriate primaquine antirelapse dose of 0.5 mg/kg/d (30 mg in an adult) would probably suffer severe AHA [12] [17] [23] [30].

Comment 2. Methodology:
- G6PD patients were kept in the hospital during all the study to care guarantee?

Reply 2
No. Patients were admitted for the first 72 hours – this was already in the Methods section.

3. Discussion:

Reply 3
This reference from Cuba has not been added to the paper. As a non Spanish speaker it would be very difficult to read and interpret.
Reviewer's report

Title: Tolerability and safety of weekly primaquine against relapse of Plasmodium vivax in glucose-6-phosphate dehydrogenase deficient and normal Cambodians.

Version: 1 Date: 4 April 2015

Reviewer: Alan Magill

Reviewer's report:

General Comments:
• This paper reports results of a well-designed and carefully conducted clinical trial utilizing a treatment regimen for acute vivax malaria of DHA-PIP on days D0, D1, and D2 combined with 0.75 mg / kg primaquine given weekly (1st dose on D0, then weekly X 8) in both G6PDd and G6PDn individuals in three sites in Cambodia.

• The results of the study are important and will inform the appropriate use of primaquine for the radical cure of P. vivax malaria.

• What the results of this paper clearly show are that severely G6PD deficient individuals, those with an enzyme activity of about 1 IU or less / gram of Hgb, cannot safely take a single dose of 0.75 mg / kg. This does not mean that the more moderate deficiency phenotypes cannot take that dose or that even a modified lower mg / kg dose of primaquine given either daily or weekly, cannot be taken by even severely deficient individuals. So the study aim as listed on the bottom of page 4, “generate quality evidence to address the question of whether weekly primaquine could be tolerated…” is not really the question answered by the data.

Reply to this general comment
We take a more optimistic view regarding what the data show. We feel that they show there is risk in prescribing the current dose of weekly primaquine and that this risk was transient in those G6PDd patients who had substantial fractional drops in haemoglobin. We do not know the benefits of the weekly regimen in Cambodia (or anywhere else in our region) which is why we call for piloting a strategy of G6PDd testing using the G6PD rapid diagnostic test, managing patients according to the result, and adequate follow up to assess its efficacy and safety.
At the start of the study, we wanted to know if the weekly regimen could be given without the need to pretest for G6PDd i.e. whether its tolerability would be acceptable in our setting. The small numbers in the study make it very difficult to say whether it is safe at a population level. We cannot now change the aim of the study retrospectively. Never the less, we have clarified this sentence to read:

**Given this fear and the paucity of data on 0.75 mg/kg of weekly primaquine, we assessed the tolerability of this primaquine regimen in Cambodian G6PD variants to ascertain whether weekly primaquine could be given without testing for G6PDd.**

- Cambodia appears to be rather unique in that a single severely deficient phenotype and genotype (Viangchan) makes up of most of the deficient individuals, at least in the study sites.

**Reply to this general comment**
We agree that Viangchan is the dominant variant in Cambodia, as has been reported by others using much larger sample sizes.

- The real contribution in this paper is the carefully monitored outcomes in the 18 individuals with severe G6PDd and could alone be the focus of the report.

**Reply to this general comment**
The thrust of this paper was its clinical and public health implications for Cambodia and other counties with similar G6PDd variants. We are planning a second paper that will examine in more detail the haemoglobin dynamics.

**Major Compulsory Revisions (these items must be addressed in a revised manuscript):**

**Comment 1.** Although the authors are clear on page 3 that primaquine causes a dose dependent AHA, this key fact is not always carried through the manuscript. Bottom of page 4 states the study aim was to generate evidence of safety and tolerability of weekly PQ, the issue is really about the safe dose of PQ for severely deficient phenotypes. In the manuscript ref No. 20 (Everett et al.), the fifteen G6PDd individuals easily tolerated the 15 mg daily dose without any clinical or laboratory sequelae. There was a statistically significant but clinically insignificant decline in hematocrit on day 7. All of the individuals where G6PD was characterized were the G6PDd Mahidol variant with 4-11% of normal enzyme activity (quantitative enzyme activity in IU / gm Hgb was not reported.) A
mg / kg dose was not presented in Everett et al. but one can estimate about some variation around a 0.20 mg /kg daily dose (15 mg in a 70 kg person), so the daily dose in this group is much lower and the enzyme activity is higher than in the current population. Therefore it appears that the 15 mg dose was on the margin of safety so it should be no surprise that a higher dose of 0.75 mg / kg used on the study would cause more toxicity. The statement on page 13 of the discussion, “A G6PDd patient receiving daily primaquine is likely to suffer AHA” is not correct and is in opposition to the dose dependent nature of the problem and as demonstrated in the Everett paper. An A- phenotype can easily take 15 mg (base) daily X 14 days w/o medical supervision or worry as was demonstrated in the 1950s.

Reply 1
We have noted the criticism and have tightened up the paper accordingly. We only managed to recruit 18 patients and most of them had G6PD activities < 1 U/g Hb. The enzyme activity range is smaller compared to previous studies so most of our patients were at the severe end of the G6PD spectrum.

Everett treated healthy Airmen with very healthy, starting haemoglobin concentrations; they did not have malaria and were given 15 mg of daily primaquine, half the recommended dose for ‘tropical’ vivax. Although they are not representative of patients we see in Cambodia, the data generated are still useful.

We have clarified this in the Discussion:

The key to the safe use of primaquine and, in the future, tafenoquine as antirelapse treatment is the accurate diagnosis of G6PDd and identifying those with more severe G6PDd. Indeed, tafenoquine registration trials are excluding patients with enzyme activities < 70% of the population median (NCT02216123) [29]. In our setting, a patient with severe G6PDd who would be misclassified as G6PDn and who would receive the appropriate primaquine antirelapse dose of 0.5 mg/kg/d (30 mg in an adult) would probably suffer severe AHA [12] [17] [23] [30]. Currently in Cambodia, G6PDd testing is laboratory-based but the wider availability of a promising and robust point-of-care rapid diagnostic test (RDT) [31] capable of detecting patients with G6PD enzyme activities < 30% (< 3.6 U/gHb) of the Cambodian median (i.e. those at the lower end of the G6PD activity spectrum) would open up the option of G6PDd testing by village malaria workers (VMWs), referring the RDT diagnosed G6PDd patients for medical supervision and treating the other patients in the community. Such a strategy should be piloted to assess its feasibility, VMW acceptability, cost, efficacy and safety.
Comment 2. Page 12, the first paragraph of the discussion is misleading as written. What the data show is that a severely deficient individual defined as about 1 or less IU / gm of Hgb) cannot take a single dose of 0.75 mg / kg of PQ safely. This is not the same as the weekly regimen. The sentence could be rewritten to insert the words “severely deficient as defined by enzyme activity less than 1 IU / gm Hgb” instead of “G6PDd”. The definition of G6PDd in this paper is < 7.2 IU / gm Hgb. We are pretty confident that the higher class III variants can take this regimen (Alving, Bull WHO 1960) so as written it would not be correct.

Reply 2
Our view on the suitability of the weekly regimen is more positive. As mentioned above there is risk with this regimen so it must be given supervised and prior testing for G6PD must be done.

We have amended the sentence to give greater clarity, as follows:

These results preclude the use of unsupervised weekly primaquine in settings where severe G6PDd is present and mandate prior testing for G6PDd.

Comment 3. The definition of G6PD deficiency as a percentage of normal is not well standardized as what is a population normal distribution? Most importantly the key piece of information is not an arbitrary label of enzyme activity that is designated as G6PDd, but rather can the individual safely take the dose of primaquine offered. A severely deficient individual cannot safely take a single dose of 0.75 mg / kg but a moderately deficient individual (3-5 IU / gm Hgb) could (likely) safely take a single dose of 0.75 mg / kg on a weekly schedule, but less likely to be able to take a full 14 day course of 0.50 mg / kg daily X 14 days. The authors should clearly state that these results apply to severely deficient individuals only at the dose given and cannot extrapolate more broadly to “G6PDd”.

The authors (Page 6) state the population median was 12 IU / gm Hgb from ref 18 and the deficient calculation was 60% of this median, thus anyone with an enzyme activity of < 7.2 IU / gm of HGB would be classified as deficient.

However the study population was skewed to the most severely deficient individuals (< 1 IU / GM Hgb). As shown in Fig. 2, the distribution of G6PD activity is 1) very broad, from < 1 to > 18 IU / gm Hgb and also 2) very bimodal with a break around 5-6 IU / gm Hgb.
Reply 3
We agree that the current system (classes I to V) for classifying definition of G6PDd is not standardised. Nevertheless, we have used it so that readers can compare more easily our findings with those of others. We would be happy to drop the class I to V classification in this paper, if the reviewers feel strongly about this.

Interestingly, the G6PD Evidence Review Group of the WHO is now talking in terms of classifying individuals as G6PD deficient, G6PD intermediate, and G6PD normal. This is better but the merits of this new classification is beyond the scope of the current paper.

In Table 1, G6PDd and G6PD normals is based on the genotype. It so happens that the G6PD activities were all low even in the three heterozygote females.

We have amended the paragraph on Definitions as follows:

**G6PD enzyme activity and G6PD status**

G6PD enzyme activity was classified I to V according to the measured G6PD activity expressed as a proportion of population median [21]. G6PD status was determined by the results of G6PD genotype as either wild type, G6PDd hemizygote male, G6PDd homozygous female or G6PDd heterozygous female. For all G6PD deficient patients, DNA…………………………..

We do not share the view that severe G6PD Viangchan patients cannot take this regimen, 17/18 patients with Hbs > 10 g/dL tolerated it well despite substantial drops in Hb in some of them. However, we must be cautious in extrapolating these results more widely. This explains our call for a pilot implementation and more data in patients with Hb concentrations < 8 g/dL. We have acknowledged that the collected are from essentially one G6PDd variant and have flagged this as a study limitation.

One patient was transfused and his decline in Hb may well have been related to taking cimetidine and ciprofloxacin; this was not declared when he was enrolled. However, we still consider this a risk because in real life patients will be on other drugs and have warned clinicians to be wary of concomitant drugs and primaquine.

We have underlined in the Limitations part of the Discussion the severe nature of the G6PD we treated:

This study had limitations. The total number of G6PDd patients was only 18 who mostly had the Viangchan variant; their measured enzyme activities were low (median < 1 U/g Hb), placing them at the severe end of the G6PD spectrum.

With the amended first paragraph of the Discussion:
These results preclude the use of unsupervised weekly primaquine in settings where severe G6PDd is present and mandate prior testing for G6PDd.

and an amended Conclusion:

This is the first study to evaluate weekly primaquine in vivax infected patients with low/very low G6PD enzyme activities. In our setting, primaquine as antirelapse treatment should not be given without knowing the G6PD status of patients and should be given under medical supervision to those found to be G6PDd.

we believe that the tone of the paper has shifted in accordance with the reviewer’s comments regarding severe G6PDd.

4. Page 9 and Figure 1, Fig.1 as currently exist is incomplete. The screening failures box only refers to Pailin. Anlong Venh screening failures are not defined. 242 were screened in Anlong Venh and only 10 were enrolled. What happened to the other 232? Of these 10, 9 were deficient. This seems very odd. Same for Veal Veng, Pursat.

We have clarified how patients were recruited in the Methods section and added a footnote to Figure 2.

Because the sample size requirement for G6PD normals was met in Pailin, we only recruited patients at the other two sites if the FST result showed they were G6PDd.

Figure 1. Trial profile*.

* G6PD status was determined initially using the fluorescent spot test (FST). At Anlong Venh and Veal Veng, only FST diagnosed G6PDd patients were recruited. Final G6PD status shown here is based on G6PD enzyme activity and G6PD genotype.

Comment 5. Page 12, comment that Aspirin is a known hemolytic drug is not supported by the literature. In Abeyraratne et al. (ref No. 27), there is no data on use of ASA in G6PDd rather only a reference to a Table that was derived from previously published reviews. Many drugs have been falsely implicated in causing AHA in G6PDd because many drugs are used in treating sick people who have g6PDd and are hemolyzing for many reasons. Only carefully performed studies where G6PDd RBCs are labeled and then followed in individuals can determined true drug effect. The world’s expert in this area, Beutler lists ASA as probably safe in G6PDd. (Blood 2008 111(1): 16-24.) The reference to aspirin is not supported by the literature, adds nothing to the data being presented, and thus should be deleted.
Reply 5. We have removed the sentence regarding aspirin. We agree with the general comments made about drugs with alleged haemolytic potential in G6PDd. Interestingly, aspirin may be unsuitable in congenital non spherotic haemolytic anaemia (Beutler 1998). Data form Hong Kong (n=1) found a reduced red cell half life following a large dose of aspirin (Chan et al BMJ 1975 2 1227).

Minor Essential revisions (items to consider in a revised manuscript):

Comment 1. Page 6, list the manufacturer, brand etc. of the urine pregnancy test used.

Reply 1 We have added in the name and country of the pregnancy tests used (Biotest™, Selangor, Malaysia).

Comment 2. Page 6, who did and who read the urine color grading chart (manuscript ref No. 22)? The patient, a study staff member? Routinely on day X or only when a patient reported a change in color?

Reply 2 (vi) urine colour graded 1-10 by the research team using a colour chart [25]; urine colour was graded every time patients passed urine as inpatients and if they were able to produce a urine sample at the follow up visits.

Comment 3. Assuring drug quality
a. Duo Cotexin (Holley) is not a drug approved by a stringent regulatory authority (FDA, EMA, etc.) and is not WHO pre-qualified (http://apps.who.int/prequal/query/ProductRegistry.aspx). What steps and what criteria did the study team take to assure drug quality of the lot used in the study?

b. Primaquine manufactured by the Government Pharmaceutical Organization, Thailand is not approved by a stringent regulatory authority (FDA, EMA, etc.) and is not WHO pre-qualified. What steps and what criteria did the study team take to assure drug quality of the lot used in the study?

Reply 3
The WHO Cambodia office sent Holey-Cotec DHAPP for analysis as part of routine testing before Sigma Tau DHAPP became available in Cambodia. Primaquine was not sent for QC prestudy. The Thai GPO follows Thai GMP and the GPO has a good system of internal drug QC. Please see the attached pdf file of a report by WHO SEARO.
We used dihydroartemisinin piperazine (DHAPP) that was produced by Holley-Cotec, Beijing, under the brand name Duo-Cotecxin™. Before being distributed in the health system, samples from new batches of Duo-Cotecxin™ were sent for analysis to an independent laboratory by the WHO Cambodia office and found to be satisfactory. DHAPP was dosed................................... Primaquine was obtained from the Government Pharmaceutical Organisation, Thailand, and was not sent for analysis.

Comment 4. The outcomes listed on page 7-8 seem intuitive and reasonable but where did they come from? Were these protocol specified criteria based on expert opinion and experience or some other source(s)? Of the 6 listed, 1-5 are laboratory biomarkers and only No. 6 is a clinical endpoint.

Reply 4.
The endpoints we chose were discussed and agreed upon by the research team. This has been added in the paper.

The primary outcome was patients completing eight primaquine doses i.e. not having primaquine stopped because of primaquine toxicity which was defined, by research team consensus, as any one of:.....................

This paper has increased our knowledge of haemolysis and we would use different endpoints in a future study. Moreover, we see the possible development of haematological “danger signs” that would trigger closer follow up / clinical care / stopping primaquine. This is something we will discuss in the next paper.

Comment 5. Page 8, suggest listing the reference and version of the NIH common terminology criteria for adverse events (CTCAE) used in the study. This is rather complex area and most malaria studies only use selected or modified criteria as CTCAE was developed by NCI for cancer chemotherapy.

Reply 5
We used the NIH DAIDS toxicity table 2004. We have added 2004 in brackets.

AEs and serious (S) AEs were defined and graded according to the US NIH Division of AIDS toxicity table (2004).

Comment 6. Page 9, the paragraph starting with “Baseline demographic… is a bit confusing. The comment that there was one patient with wild type genome (as defined by the six PCR sites determined) and a low G6PD activity due to delayed measurement does not make sense. Also a mention of “Four G6PDd patients
had missing…” is followed by 13 had class II and five with class II. There are usually temporal genotype – phenotype mismatches when the two tests are done in acutely ill hemolyzing patients. In this setting it is not important to know the genotype, but rather the phenotype. Does the patient in front of you have enough enzyme activity to safety take the dose of PQ that is being given.

Reply 6

We agree there are genotype phenotype discrepancies that could result from acute haemolysis; the latter would tend to increase the G6PD activity at presentation and decline subsequently with disease recovery. We did not experience this. Rather we had to tackle the issue of low G6PD enzyme activities and wild type PCR results. We have been back to the database and clarified this paragraph. There were 4 such patients. Three had later G6PD results that were much higher than baseline and one patient had no later results. The G6PD activities were measured several days after the blood was taken and so the G6PD activity is likely to have declined. We feel these baseline results are not reliable so have excluded them from Table 2.

Four PCR-determined G6PD wild type patients had low G6PD enzyme activities that were probably due to delayed measurement; in three patients, the baseline values were inconsistent with later G6PD activity values and in one there were no other G6PD activity values. All values have been excluded from Table2.

Regarding the G6PDd patients, there were two missing G6PD values on Day 0, not four as originally written. This has been corrected. The missing values were excluded from Table 2 but we substituted G6PD enzyme values measured on D7 and D56 to be able to categorise them using the class I to V system. In our small series we did not see much change in G6PD activity over time (for the next paper) so we feel justified in substituting these later values purely for the sake of classifying them.

Two G6PDd had missing baseline enzyme activity values (also excluded from Table 2) but were classified by class using post D0 G6PD enzyme activity results. Of the 18 G6PDd patients, 13 were class II (1 - < 10% population median of 12 U/g Hb) and five were class III (≥ 10 - 60%) G6PDd.

Comment 7. Figure 2, it would be useful to the reader to clearly indicate the G6PPDd line (7.2) as defined by the authors on the graph.

Reply 7

We have chosen to leave the graph as it is. The classification of G6PDd is changing as alluded to above and is not so straight forward. It would be wise to wait for the WHO
ERG report. Furthermore, the classification of G6PD warrants a thoughtful review paper.

Comment 8. Page 11, the fractional fall in Hgb on D7 compared to baseline and its relationship to enzyme activity seems quite important and would warrant a figure.

Reply 8
We have not added a Figure of enzyme activity and the fractional change on D7 because the association was weak - the coefficient of variation is about 8%: ... and was associated weakly with G6PD enzyme activity at baseline (p=0.013), for a coefficient of variation of ~8%.

We have also amended slightly the statistics paragraph:

The relationship between the fractional fall in Hb on D7 vs. baseline and: (i) the mg/kg dose of primaquine was assessed by Spearman rho test (skewed data), and (ii) the baseline G6PD enzyme activity by Pearson’s correlation coefficient (transforming the G6PD data to become normally distributed).

Comment 9. Page 11 & table 5, I find the presentation in table 5 confusing. I am used to seeing and thinking about methemoglobin saturation is expressed as the percentage of hemoglobin in the methemoglobin state; as in 1-2% is normal. I am not able to interpret table 5. On page 10 the authors state that no methHb > 4.9% was seen. I understand that. At the dose and regimen used, methemoglobinemia is not an issue. I am not sure Table 5 adds any value or maybe place in supplementary materials.

Reply 9
We have removed Table 5.

Comment 10. Page 13, paragraph starting with in 2012..., consider adding the most recent (Jan 2015) WHO recommendation (http://www.who.int/malaria/publications/atoz/policy-brief-single-dose-primaquine-pf/en/) and the most recent WHO publication on 8AQ safety (http://www.who.int/malaria/publications/atoz/9789241506977/en/) as refs in addition to or as a replacement for ref No. 30. Also consider changing “would be well tolerated in G6PDd patients” to “would be well tolerated in the severely deficient G6PD patients in Cambodia” and consider changing “the epicenter of artemisinin resistance” to “the epicenter of artemisinin resistant P. falciparum malaria” for optimal clarity. It is extremely important to completely separate in the
eyes of the reader the dose of PQ for Pf radical cure and Pv radical cure. These are two entirely separate indications.

Reply 10
We have clarified the sentence regarding low dose primaquine for transmission blocking. We have also added the Ashley review of primaquine safety. The WHO 2015 update on low dose PQ is very similar to the paper by White et al, so we have kept the latter.

Additional comment (just for authors to note, no need to respond unless you want to):

Comment 1. Page 3, author’s state that increasing prevalence of CQ resistant vivax blood stage malaria complicates the treatment of vivax malaria. However, the combination of CQ plus an adequate dose of primaquine has been shown to be an effective cure for CQ resistant blood stage Pv. The complexity is that in many locations, Pv radical cure is not attempted or achieved. See

Reply 1
The cure rate of chloroquine resistant *P. vivax* is indeed increased by primaquine but we chose to leave this out.

Comment 2. In this study the authors chose to give the 1st primaquine on D0. In the Clyde paper (manuscript ref No. 16, WHO Bull, 1981), Dr. Clyde recommends giving the 1st PQ after the acute illness is resolving, for example on D4 after dosing of acute drugs. Do the authors think this would make any difference in safety or tolerability?

Reply 2
In our study, giving the primaquine with the first dose of ACT was well tolerated. We await the results of other studies before we can comment with confidence on Dr. Clyde’s thoughts. However, he may have made this suggestion because chloroquine has a greater tendency to cause vomiting than DHAPP (unavailable back then).

This is taken form Dr. Clyde’s review:

*It is important to consider at what stage during the course of the malaria primaquine should be administered. If possible, it should not be given during the acute stage of the*
disease. Apart from its inducing haemolysis, it adds to the intolerance of chloroquine and other blood schizontocides and may thus produce vomiting of both drugs. Also, it may have an immunosuppressive effect. A course of primaquine is best commenced, therefore, as soon as the severe symptoms begin to subside; in vivax malaria this is usually the day following completion of the course of chloroquine. However, under field conditions, where the patient is available for treatment only during the acute phase of illness, primaquine should be given immediately and continued for as long as possible.

The point about not giving primaquine during the acute stage is not referenced.

**Comment 3.** I would be curious to see the enzyme activity plotted against the D0 retic count. In Table 4, the range of D0 retic count in G6PDd is listed at 0.6 to 3.8. For example, did the individual with a retic count of 3.8% have an enzyme activity of < 1 IU / gm Hgb?

**Reply 3**
Interestingly, the G6PDd patient with the highest reticulocyte count on Day 0 had a G6PD activity exceeding 1 U/g Hb. There was no correlation between the reticulocyte count and G6PD activity in our small sample. We will be exploring the retic count in greater detail in the next paper.

**Comment 4.** Page 12, the authors nicely place the observed SAE in context of possible
The literature on DDI and drugs that might cause AHA in G6PDd is confusing and in many cases an association at best. The reality is that sick people take a variety of drugs and supplements and these may or may not cause AHA in G6PDd. Acute illness such as influenza, pneumococcal pneumonia, etc. are common causes of AHA in G6PDd. The severely deficient G6PDd simply have less margin for error. The highest quality evidence for a drug induced AHA came from the radionuclide studies done by Ernie Buetler on survival of labeled G6PDd RBCs into humans then exposing them to drugs. (nice summary in Beutler E. Glucose-6-phosphate dehydrogenase deficiency: a historical perspective. Blood. 2008 Jan 1;111(1):16-24). Attributions of AHA caused by drugs vary greatly in papers and textbooks often w/o listing the quality of evidence to support such listing. The case report (ref No. 25) is compelling and suggests ciprofloxacin might have contributed to the more significant AHA.

Reply 4
We are in agreement with the last comments. Fever is a well known cause of haemolysis in G6PDd and some thalassaemias. The taking of concomitant drugs is a risk that needs to be considered with primaquine and tafenoquine when these drugs are deployed.