Author’s response to reviews

Title: Comparison of simultaneous capillary and venous parasite density and genotyping results from children and adults with uncomplicated malaria: A prospective observational study in Uganda

Authors:
Aine Lehane (aine.lehane@yale.edu)
Moses Were (were.mwima@gmail.com)
Martina Wade (martina.wade@yale.edu)
Musleehat Hamadu (musleehathamadu@gmail.com)
Megan Cahill (megan.cahill@yale.edu)
Sylvia Kiconco (sylviakiconco@gmail.com)
Richard Kajubi (richardkajubi@yahoo.com)
Francesca Aweeka (fran.aweeka@ucsf.edu)
Norah Mwebaza (mwebno@yahoo.com)
Fangyong Li (fang-yong.li@yale.edu)
Sunil Parikh (sunil.parikh@yale.edu)

Version: 1 Date: 26 Apr 2019

Author’s response to reviews:

April 23, 2019

Re: Ms. No. INFD-D-18-01083

Dear Editor,

We appreciate the opportunity to revise and resubmit our manuscript entitled “Comparison of simultaneous capillary and venous parasitemia and genotyping results from children and adults with uncomplicated malaria: A prospective observational study in Uganda” as an Article in the
BMC Infectious Diseases. We appreciate the reviewer’s thoughtful critiques, and have addressed each point with tracked changes in the manuscript. We feel that the manuscript has been significantly improved, and all of the reviewers comments have been attended to.

Reviewer comments are stated, and the response to reviewers/edits follows.

Technical Comments:

1) Please clarify whether you obtained informed consent from (written or verbal) from adult study participants and clearly state this in your manuscript in the 'Ethics and consent to participate' subsection of the 'Declarations', as currently, the current written statement in this section is about the consent where the participants are children.

This was addressed in the Ethics section.

2) Please move your trial registration number and date after the abstract conclusions, and before the list of keywords.

The registration number was moved accordingly.

3) Under the heading "Funding", please declare the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

This detail was added to the funding section

4) Under the heading "Authors' contributions", please confirm whether all authors read and approved the final manuscript.

This detail was added to the author’s section

5) Under the heading "Ethics approval and consent to participate" if possible, please provide the ethics committee’s reference number.

Approval numbers were added accordingly.
Reviewer 2

1) The x axis for figure 3 is the same as figure 4. Also the range of 2.5 to 15% does not make sense to encapsulate all the parasitemias.

The x axis for Figure 3 is parasitemia and for Figure 4 is the number of strains. These are not the same. We have modified the x axis for figure 3 to point out that parasite densities are on a log10 scale. Please note that the range represents densities on a logarithmic scale, and therefore does capture the full range seen in our study.

2) Another small point is the MSP2 diversity as a function of venous parasite density. Is the greater diversity more apparent at higher parasite densities—say the range of 1-1,000, 1,000 to 10,000 and 10,000 to max

This is a good question. We did the analysis and MSP-2-based strain diversity was not statistically correlated with parasite density. A sentence was added to the results to reflect this (Results, section on complexity of infection).

3) The paper relied on blood smear for parasite density. Why not verify with semiquantitative PCR or qPCR which would also relate relative amounts of WBC counted.

The reviewer brings up a good point, and we would have optimally preferred to use semiquantitative PCR or qPCR. However, there is unfortunately insufficient DNA available to conduct such analyses. Our desire was to use these samples in the same manner

4) Also how much of the DBS was processed for DNA extraction and what percent was input into PCR. IE what was microL equivalents of input into PCR.

A single DBS was processed using a Qiagen kit and eluted into 100uL. 4 uL was then used in the 25uL PCR reaction. Details were added to the Methods/Genotyping section.

5) Minor italics for genus species in reference

Genus/species have been italicized in the references.
Reviewer 3

1) Line 98-99: although this sentence introduces the other studies on this question and although these are explored more in the discussion, perhaps you can add a little foreshadowing here? The way the current Intro reads it makes it sound like the prior literature on this topic is minimal. This was also recently explored for molecular diagnostics (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4350552/), which could also be commented on. Because the patients in this report were all positive, there's no need to compare the "sensitivity" of molecular diagnostics for venous vs. capillary blood in this dataset unless a quantitative endpoint was available and could be systematically compared for same volume samples applied to blood spot cards (additional molecular testing is not necessary to move this manuscript forward).

We have expanded on the prior studies in the intro, as requested. However, we did not include the reference mentioned above, as this study uses ONLY venous blood.

2) Replace parasitemia with "parasite density" for any parasite/volume results

This was changed throughout the manuscript including the abstract and tables.

3) I suggest changing Table 1, Figure 2-3 and Supplemental Table 1 comparisons to log10 para/uL-based data rather than linear scale data unless you have a good rationale for why 2- or 4-fold changes are meaningful

Our figures had been on a natural log scale and were changed to log10 parasites/uL scale. The tables were not log transformed, as such values are less meaningful to readers, and were maintained as geometric means. However, as noted in the methods, all statistical comparisons and p-values are based on analyses of log-transformed data.

4) I would suggest using the same terminology or abbreviations for Tables 2-3 (i.e., CC, VV, VC, etc or spelled out)

We have now removed the abbreviations in Table 3

5) There seems to be an error in the last line of Supplemental Table 1 (p=0.09???). Please adjust to use a consistent number of significant digits

Supplemental Table 1 was corrected. Thank you for catching this.
6) Figures 2-4 need clearly labeled X and Y axis labels and figures need to be higher quality.

The x and y axes were more clearly labeled and higher quality figures have been uploaded

7) There is an error in Figure 4 as presented since panels A and B appear identical.

Thank you for catching this. The figure has been rectified.

8) The Results section is a little difficult to read as is. Some of the results section currently magnifies differences in opposite directions without contextualizing the meaning or impact of such differences. After re-analysis on log10 scale, I would suspect that some of the differences would be less significant and the Results (no difference) could become easier to present.

We have extensively revised the discussion and added a summary table of previous results. We appreciate this comment and feel it has improved the manuscript. Please note, however, that all previous analyses were done on natural log-transformed data, so the p-values do not change if it is log10-transformed. Nonetheless, we have modified figures to log10 scale, and labeled this clearly.

9) This statement (Each compartment frequently detects strains that are not seen in other compartment) seems like the data should be shown, as this is one important outcome in the manuscript. Is this a function of volume of blood sampled?

We have quantified this data and added it to the manuscript as the last sentence in the results section. As noted above, this discrepancy in detection is not a function of the blood volume, and while overall more strains on average were seen at the time of diagnosis in venous blood, the differences in strains detected could be seen in either or both compartments.

10) Line 231-233: it's not clear to me that a 19% vs. 30% difference is significant, especially since the data not shown comment seems to indicate considerable variability upon additional testing.

A difference of 19% versus 30% recrudescence in a clinical trial of antimalarial efficacy would indeed be a dramatic difference. A failure rate of just 10% is enough to change guidelines.
11) The comparison of the outcome of this study to prior reports needs to take into consideration whether the data in prior reports was evaluated on a log10 or linear scale for quantitative comparisons between venous and capillary blood. Ref 21 = linear; Ref 22 at least partially log scale; I didn't find quantitative parasite density comparisons in the 1991 French-language manuscript in Ref 23 (though my French is rather elementary so please double-check). There's likely considerable variability in the methods of microscopy between the sites that generated these prior papers.

As the reviewer notes, prior studies differ in the level of detail as to how data was handled, both for log-transformation and microscopy methods. In our study, our sample size was large, and data showed a skewed distribution, necessitating log-transformation for statistical analysis. In addition, we followed WHO protocols for microscopy reading. Given the lack of detail presented in other papers, we are unable to make any firm conclusion on the impacts of these aspects on their conclusions. However, we have provided a supplementary table with important points (Supp Table 2).

12) Line 261-271: this is an excellent takeaway paragraph. Some other results sections could be simplified to adopt clear style. There's some sections of the Results that read as if these differences ARE significant AND important in the final analysis.

We appreciate this comment.

13) Suggest adding a summary Table to the Discussion that integrates the results of prior studies and this data (not really a meta analysis but at least a one-stop authoritative listing).

We have created a Supplementary Table 2 which summarizes the key points for all available studies we have located comparing aspects of malaria detection in capillary and venous blood.

14) Minor:

Line 40: spell out 3rd as third in the abstract

Line 91: Would probably be good to cite comparable DBS vs. venous blood literature in HIV (https://jcm.asm.org/content/52/5/1343; https://www.sciencedirect.com/science/article/pii/S1386653217301282)

Line 103: the placement of Ref 3 here is misleading. Suggest keeping sentence as is but deleting Ref.
Lines 116, 125, 236, etc. Be sure to consistently use P. falciparum after first use of Plasmodium falciparum.

Line 119-121: number of enrolled participants should be moved to "Results"

Line 125: anticoagulant for venous blood

Line 125: how was blood spotted onto DBS for capillary vs. venous blood? Microhematocrit tubes? Pipettes?

Line 132: Should be titled "Parasite densities"

Line 142: list volume of blood spotted onto on DBS

Line 143: list diameter of spots and number of spots need to be listed as input for QiaAMP extraction

Line 176: delete "of" in range of …

Line 178-179: Suggest change all instances of "parasitemia" to "parasite density" since para/volume is parasite density

Line 187 and 189: use consistent P or p in "p value" presentations (possible other instances elsewhere too)

Line 198: spell out 'doesn't'

Fig 1: fix the spell check red underlining for drug name

Line 330: delete UCSF abbreviation unless reusing this abbreviation

All minor comments were addressed aside from adding the HIV references, as these do not appear relevant to our study.

All authors have contributed significantly to this work and have seen and approved the content of this revised manuscript. Please do not hesitate to call us with any questions.

Sincerely,

Sunil Parikh, M.D., M.P.H.

Associate Professor