Reviewer's report

Title: Microvascular obstruction and endothelial activation contribute independently to the pathophysiology of severe falciparum malaria in adults.

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Reviewer: Alister Craig

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The paper by Hanson et al describes the relationship of a range of clinical parameters, including microvascular obstruction and endothelial activation, with severe malaria in adults. The study is well-designed and has been described clearly and accurately. Their main findings are that this study supports previous work (with smaller clinical groups) showing independent associations between vessel blockage and endothelial inflammation with disease, but no link between these observations. The discussion provides a good balance with the rigour that an extensive and detailed study such as this provides but indicating potential weaknesses in the arguments. I have only a few comments (which should be seen as discretionary revisions) to add to the paper:

1. There are a couple of issues in claiming that microvascular obstruction and endothelial activation are independent variables; (i) people may read the title of the paper as meaning that these two phenotypes are not linked at all. I do not believe that the authors meant to imply this and indeed they say that endothelial activation could contribute to vessel blockage, for example, through upregulation of PRBC adhesion receptors; (ii) the measurements made for microvascular blockage and endothelial activation are different in character, with the former being a localized phenomenon (i.e. what is actually happening in the vessel itself) and the latter being a measurement of a systemic condition rather than localized endothelial activation. Measuring plasma biomarkers of activation is understandable as although it is possible to investigate endothelial cell activation, this is far from easy and requires significant intervention to obtain samples (rather than just taking a blood sample). I do not think that this detracts from the overall message of the paper but it should be flagged to remove any confusion.

2. The authors chose to record “severe malaria” as a single variable but this covers a fairly broad spectrum of pathology, which may not have a single aetiology. The balance between having enough numbers to drive ‘significance’ of a study against the need to reduce noise and favour ‘specificity’ is a difficult one and, as above, I do not disagree with the approach taken by the authors. I would only note, for the unwary, that severe malaria is not the same as cerebral malaria. This is done in detail in the methods section where the clinical definitions are well-described but readers may skip this section.

3. Microvascular obstruction in the rectal vessels may not reflect the situation in another organ, such as the brain. Of note are two major differences in the microvascular endothelia of the gut and brain, with the latter lacking
Weibel-Palade Bodies (which may affect Ang-2 release) and CD36 expression. In some studies PRBC adhesion to CD36 has been linked to uncomplicated malaria and it is possible that gut provides a ‘reservoir’ for cytoadherence that is less pathogenic than in other organs (although this is just speculation). The finding that microvascular obstruction, presumably due to sequestration, in the rectal mucosa is associated with severe malaria suggests that these endothelial compartments share at least some properties in terms of pathology.

4. Is an increase in white cell count (Table 1) associated with death in severe malaria commonly seen? A comment on this finding would be useful, even if only to say that the preliminary significant difference was lost on further analysis.

5. I am not a statistician so it is with some trepidation that I comment on this area. The multivariable regression analysis does not find a significant (p < 0.05) association between microvascular obstruction and Ang-2 concentration (a marker of endothelial activation) but with a p-value = 0.06 this does fall into a ‘zone’ that some statisticians define as “equivocal” or “borderline significance” (usually when p < 0.1 but > 0.05). So, along similar lines from my comment above, I wonder whether the message from the paper that these two properties are not associated is portrayed a bit too strongly.

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.