Reviewer's report

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

Version: 1
Date: 17 March 2015
Reviewer: Malcolm Edward Molyneux

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Major comments for attention

(I) The paper carries a declamatory title (“Microvascular obstruction and endothelial activation contribute independently to the pathophysiology….”). The title should be less emphatic, because as written it does not accurately reflect the findings reported: the word ‘contribute’ implies a causal role, where only associations or correlations have been shown; and independence is deduced from statistical relationships between microvascular obstruction and circulating plasma concentrations of Ang-2 and lactate, all of which, as surrogates of the mechanisms they are representing, are imprecise, especially when based on a single point in time. (In relation to microvascular obstruction, the authors mention on p.12 ‘the marked heterogeneity that is seen in the distribution of sequestration in different organs of the body’).

Unlike the title, the text (p.11) is appropriately tentative: ‘this finding is consistent with the hypothesis that the two processes make a separate contribution to disease severity’.

The same considerations apply to the last sentence of the abstract. The first sentence of the Discussion also makes greater claims than are warranted by the data from this study – the paper reports associations, not a ‘role’, in pathogenesis, and in the absence of control groups (non-malarial severe disease; non-severe malaria), the data do not demonstrate that the findings are peculiar to either malaria or its severe forms.

(II) There is no mention in this paper of the timing of the recordings of rectal microvascular blood flow in relation to the start of either supportive therapy (oxygen, fluids, blood transfusion, anticonvulsant drugs etc) or specific antiparasitic drugs. These measures might affect capillary perfusion. Nine patients were treated with quinine, a drug known to have effects on circulation – the timing of the OPS-recordings may be particularly important in these patients. An exclusion criterion was: having ‘received parenteral antimalarial treatment for >24h before enrolment’; this suggests that some patients may have been receiving such treatment for up to 24h before the OPS recordings – how many patients, and which parenteral drugs?

(III) It would be helpful to know whether rectal capillaries in the malaria patients would sometimes open and close variably (as described in sepsis) – or when a vessel was ‘blocked’, was it continuously obstructed? The latter situation would
particularly support the idea that the impaired flow is due to mechanical obstruction – eg by sequestered parasites – (although sequestered parasites may roll on, causing intermittent obstruction). It may be useful, to readers not familiar with this field, to point out that histopathology does not provide information about vessel blood flow, but only about what vessels contain – ‘blockage’ is a deduction, not an observation. A particular value of this study is that non-flow of blood is directly observed, and during life.

(IV) In the first group of patients, the video recordings of rectal vessels were read by 2 readers, and in the second group by 3. In the third (the largest) group the recordings were analyzed by only one reader. Is there a reason for this? How consistent were the various readers in their interpretations of the first two groups of patients? – were they so consistent that 2nd/3rd readings were considered unnecessary thereafter?

On p8 para 2, and p 10 para 1, controlling for ‘inter-rater variability’ is mentioned: how was this possible in the largest group for whom there was only one reader?

(V) More detail on the characteristics of the patients’ severe malaria syndromes would help us to understand the studied cases better. How many of the 52 patients with a single criterion of severity had each defining syndrome? And among the 90 with multi-organ dysfunction, how many had which combinations of organs affected? Was there any explanation for the remarkable disparity of case-fatality rates in the different study years (8.3%-60%)?

Minor points for attention.

(i) ‘Malaria transmission is seasonal at both sites.’ Still it would be helpful to know if there are any data from these sites as to the prevalence and densities of asymptomatic or ‘incidental’ parasitaemia in the population of the same age range and time of year as the patients. This could strengthen the evidence for P falciparum being the causative agent of the illnesses in the studied patients – all severe malaria syndromes can be mimicked by non-malarial diseases such as sepsis, encephalitides, etc.

(ii) page 5 line 6: ‘…coma and seizures and acidosis and hyperlactatemia were classified as a single criterion;…’. This may be confusing to readers, especially if their software fails to show italics. Better may be: ‘…coma and seizures were classified as a single criterion, as were acidosis and hyperlactatemia;…’

(iii) In p8 para 3 and the next paragraph, for some of the comparatives listed the comparator is not stated and is not obvious: for example ‘Admission plasma Ang-2 concentrations were significantly lower in patients with coma on admission’ – does this mean ‘than in controls’ or ‘than in patients without coma’? Please note that there are several other instances like this that need clarification.

(iv) p10 last sentence before Discussion: ‘Plasma Ang-2 had a stronger correlation with plasma lactate than [***] parasite biomass….’. It would clarify your meaning to add, at [***], either ‘with’ or ‘did’, to indicate which correlations you are referring to.

(v) p11 para 2 ‘…numerous obstructed capillaries, an appearance which exactly
replicates the histopathological findings from post-mortem cases’ – most of the quoted references and other studies show at least as much sequestration in small venules as in capillaries. It would be interesting if the authors could mention whether venular flow is ever impaired when capillary flow appears normal, in the rectal vasculature.

(vi) p 14 last para: ‘The inverse association between parasite biomass is hypothesized to result…’ There are obviously some words missing after ‘biomass’

(vii) p14 last sentence. Do you mean that deficiency of VEGF could provide another mechanism?

(viii) In all the figures it would be helpful to have the number of tests (n=) above or below each column of data pictured.

(ix) In 43 patients drawn from the previous study (ref 14), a mean of 50 vessels had been assessed per field for the original publication, but the criteria for the present study were that 20 should be assessed per field – which denominator was used for those patients in this report?

Minor point for discretionary consideration

(i) P11 line 4: ‘…relatively underpowered to assess its relative contribution…’

Relative to what in each case?

**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