Reviewer’s report

Title: Gene expression profiling in blood from cerebral malaria patients and mild malaria patients living in Senegal

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Reviewer: Alister Craig

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The manuscript from Thiam et al joins a number of recent publications looking at transcriptional analysis of whole blood from patients (and mice) with malaria. The major finding from all of these, including this current paper, is that there are disease-specific signatures suggesting potential pathways for pathogenesis.

Comments

a. I do not claim to be an expert of the analysis of transcriptional data. However, I assume that the initial difficulties in finding statistically significant differentially expressed genes was due to the large variance often seen in patient samples. It would have been useful to see a PCA graph demonstrating the degree of variance in the individual samples to understand the degree of variation. Despite this, the authors have constructed a sensible approach using a multi-variate analysis that does identify a large number of genes differentiating between CM and MM/NCM patients (although the considerable differences in the CM gene expression patterns in the figures (e.g. Fig 3) are notable). It is interesting that the clustering is less able to differentiate between the two non-CM categories.

b. There is some confusion on the patient details, not helped by the loss of the footnotes from Table 1. What is the unit of age in this table? From the NCM and MM categories I would assume it is 'years' but the CM column would then suggest a recruitment range of 1-72 years. There does seem to be a wide range of ages in the recruitment, which probably underlies the lack of any association with age - too much variation. The use of the Glasgow score for categorising coma would also suggest that the patients are adults (the Blantyre Score is more appropriate for children). It would be helpful to have more clarity on this and, if the patients are predominantly paediatric, why the more standard WHO definition was not used for CM.

c. No information is provided about how the samples were processed (line 130). These details need to be added. It would also help to have more discussion about why the covariates of age and gender might be important. I'm assuming that leukocyte counts relate to yield of RNA, but as the amount of mRNA being input to the assay is standardised it would also be helpful to understand why this is a covariate.

d. The major issue with these studies is determining what is 'cause' and what is 'effect'. It is indisputable that malaria is a proinflammatory disease and so the identification of cytokine and immune pathways is unsurprising. However, direct measurements of important cytokines such as TNF and IFNgamma (line 315) have failed to show strong associations with CM specifically. Added to this is the uncertainty of how the murine experimental CM model matches human CM for different pathways.
Recent detailed histological examination has suggested that there may be some parallels between ECM and HCM, but the discussion in this paper skips between human and mouse observations without clearly identifying the source of the data, making it difficult to understand the direct relevance to human disease. An example of this is the complete dependence of ECM on CD8+ T-cells but a lack of evidence for their presence in vessels for HCM. This is not to discount a role for the ECM model, but rather to advise caution in bringing 'supportive data' from multiple sources to fit the transcriptional signatures without caveats.

e. Blood is a relatively simple sample to obtain from malaria patients, although I would not underestimate the infrastructure needed to do clinical studies. However, malaria pathology is probably focussed in the microvascular endothelium of the patient. Obtaining endothelial samples is very challenging (and for the brain, impossible other than post-mortem), so this and other analyses rely on the circulating cells reflecting the transcriptional patterns of the endothelium (and potentially resident immune cells) if they are to identify key pathogenic pathways rather than only biomarker signatures of disease. This adds another layer of caution to conclusions such as "the involvement of monocytes, macrophages, dendritic cells and B and T lymphocytes, and NK cells in malaria pathogenesis" (line 346). The call for further research is definitely warranted. the issue, given this list, is where would you start?

f. The final statement in the conclusion (line 350) was unusual and given the size of the human genetic studies on malaria pathology (e.g. MalariaGEN) it was surprising to see them described as "underpowered". Is this statement necessary for the paper? How are the final two sentences of the conclusion linked to the data in this paper?

Overall, this paper provides some interesting insights into biological expression signatures associated with CM. Whether these are part of the pathology of this syndrome remain to be explored, but the accumulating data on whole blood transcriptional patterns in malaria disease may provide a platform for meta-analyses if the harmonisation across studies is sufficient.

Minor corrections
Line 66 - diffuse encephalopathy
Line 155 - prior to anti-malarial treatment
Lines 255 & 256 - are the figure numbers correct?
Line 318 - histamine secreting basophils

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.
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