Reviewer’s report

Title: Induction and decay of functional complement-fixing antibodies by the RTS,S vaccine in children, and a negative impact of malaria exposure

Version: 0 Date: 28 Nov 2018

Reviewer: Adrian Luty

Reviewer's report:

The paper is very well written and brings important new information on antibody responses to RTS,S.

I have several points concerning methodological and other aspects:

1. The authors document the use throughout of serum (not plasma) samples from study participants. That being the case, and given the fact that they conducted assays designed to detect C1q binding by antibodies complexed with CSP components, one must assume that the serum samples were decomplemented (by heat treatment?) prior to use in such assays. There is no mention of such a step in the Methods section. This should be clarified.

2. More detail of the design of the assays of C1q binding should be provided. In the context of controls, for example, did the authors include wells in which C1q diluted in buffer was substituted with buffer alone? Also, they report using an 'in-house' rabbit anti-C1q IgG reagent. What quality control steps did they implement to confirm the sensitivity and specificity of this IgG?

3. In the context of the use of commercially-available reagents to detect IgG subclasses by ELISA in serum/plasma samples from sub-Saharan African populations, strenuous efforts have been made in the past to identify a panel of reagents that display minimal cross-reactivity specifically when used with African samples. The so-called 'Afro Immunoassay' project spearheaded those efforts roughly 15 years ago. Did the authors determine themselves that the reagents they used for this purpose from Thermo Fisher displayed appropriate characteristics, or did they have such information from others?

4. The serum samples used were derived from participants in one of the original Phase II efficacy studies of the RTS,S vaccine. That study included surveillance (active and/or passive) for the detection of infection and/or disease. Given the detailed parasitological/epidemiological information available, therefore, one wonders why the authors did not design the current study to include an assessment of, for example, antibody complement fixation capacity with respect to protection from one or other malariological measure?

5. In several respects the data presented in this article reprise some of those documented in the separate article recently published in BMC Medicine by Ubillos and colleagues i.e. assessments of Ig responses (including IgM & IgG subclasses) to the vaccine antigen in two populations with differing levels of exposure to infection with P. falciparum. The methodology used differed, however, in one important
respect. Quantification of antibodies in the published article relied on a protein-coupled bead-based approach whereas here the authors used a more conventional ELISA method. The authors should devote rather more discussion to the question of whether the results generated in common in the 2 studies are coherent and consistent. For example, one of the conclusions of the published article concerned the fact that children in an area of comparatively low transmission had, on average, a higher anti-vaccine IgG response post-vaccination at M3 than children in an area of higher transmission. This did not appear to be the case in the current study. The apparent difference in outcomes between the studies merits some discussion in the opinion of this reviewer. Readers may also speculate on whether one can rule out a possible influence of the adjuvant type used, since they differed between the two studies.

Modifying the manuscript to reflect these points should improve its clarity and overall quality.

**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.

No

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

No

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