Reviewer's report

Title: A Prospective Study of Improvements in Endothelial Activation Biomarkers, Including Plasma Angiopoietin-1 and Angiopoietin-2, in Kenyan Women Initiating Antiretroviral Therapy

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Reviewer: Sascha David

Reviewer's report:

This is an interesting report investigating the longitudinal course of a panel of endothelial biomarkers (with a focus in angiopoietins) in HIV infected women after treatment initiation.

MAJOR COMPULSATORY REVISION

1. The question how circulating biomarkers (i.e. the Angpt/Tie2 family) change after HIV treatment has not been addressed before. However, the biological relevance behind such a question is unclear. Problematic with the here presented data is the fact that “normal” Angpt2 levels in black women have never been reported to my knowledge, so that we can only assume that a baseline of 1.5 ng/mL before treatment is indeed elevated. As a matter of fact, healthy Caucasians are known to have circulating Angpt-2 values about 1 ng/mL (not so much lower than the HIV infected women in this cohort to start with).

2. Another general problem is the general (lack of) usefulness of their findings. Part of this study seems really exciting as it identified Angpt-2 as a predictor of mortality (although interpretation is limited by the very low mortality rate of only 5.3%), but most of the other data do not add useful knowledge to our current concepts with respect to patient care and monitoring strategies. In order to establish a biomarker for clinical use (and that’s the major purpose of a biomarker) it has to compete with or even outperform the current gold-standards. In other words, if circulating Angpt-2 behaves similar than HIV RNA levels after ART, what novelty does this add? The authors need to find a way to demonstrate that adding endothelial biomarkers to the current state-of-the-art protocol would indeed improve monitoring skills. I agree with them that therefore a clear endothelial outcome measure would be highly desirable.

3. All methods are well described and statistical analysis has been performed thoroughly. Unfortunately, the used assay from R&D system (Duoset ELISA) is absolutely for analysis of PLASMA Angpt-2 levels. This assay has never been evaluated for plasma samples and is exclusively recommended for endothelial cell supernatants. We work since many years with angiopoietins using the Quantikine kit (also R&D systems) and have systematically compared this DuoSet Kit with Quantikine and did not find transferable results at all.
Also, I have never seen a paper on Angpt’s in which certain individuals had levels that were undetectable, as you did? Your statement on page 9 that ‘ANG-2 levels are often below the limit of detection” is wrong. The published assay sensitivity is as low as 21.3 pg/mL. Frankly speaking, I do have major concerns regarding all reported Angpt-2 levels.

MINOR ESSENTIAL REVISIONS

1. If Angpt-2 indeed is a biomarker of endothelial activation in HIV why do Angpt-2 levels not correlate with baseline HIV RNA but the do so after ART? In other vascular pathologies it has been consistently demonstrated that Angpt-2 is associated with the severity of underlying diseases (e.g. vasculitis, sepsis, etc). And why do other markers (ICAM/VCAM) correlate with HIV-RNA at baseline?

2. Another way to establish a link between Angpt2 and severity of HIV infection might be bia stratifying it by HIV stages.

3. In the introduction you state that Angpt-1 is widely expressed in human EC. That's incorrect. Angpt-1 is produced from vascular supporting cells such as pericytes or VSMCs.

4. Why have only women been included in this study?

5. P-values in the figure are missing.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests