Reviewer's report

Title: Gastric and intestinal barrier impairment in African adults: a randomised controlled trial of micronutrient supplementation

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Reviewer: Henrik Friis

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The manuscript reports data based on analyses of material from a micronutrient trial among adults from a community in Lusaka, Zambia. The original cluster-randomised trial allocated households to supplementation with micronutrients or placebo for two years, and then crossed-over, and included 500 adults with or without HIV. There was no effect on the primary outcome, incidence of diarrhea, but a reduction in severe diarrhea and mortality. The current paper comprises several substudies, somehow nested in this trial, with potential intermediate outcomes. The strength of the paper is, that it has data rarely reported from HIV afflicted, low-income populations, and that it aims to explain the mechanisms behind the effects of micronutrient supplementation.

Major comments:
The description of the substudies and subgroups is not clear. In the Study design and Methods section, the authors refer to four sub-studies, but they also define sub-groups by the outcomes as gastric acid or translocation (permeability, LPS/anti-LPS, sTNFR55) sub-groups (in Table 2). 203 were included in the former and 87 in the latter. It is not clear what the criteria were for including some of the 500 individuals in the individual substudies? Furthermore, the timing of the measurements of these outcomes is also not clear? Fx, gastric pH was measured after 4 to 19 months, while other outcomes were measured after the cross-over.

Obviously, the study presented is not a straightforward RCT. The title needs to be changed to reflect this. This is important, because in a RCT confounder are eliminated or reduced through randomization, and the effects on a well-defined primary outcome is assessed in a reasonably well-defined study population. In this case, it is not obvious that the randomization used for the main trial resulted in baseline equivalence with respect to the substudies? For example, the group sizes are quite different for the translocation study, and there seem to be considerable age and sex differences. Lack of statistical difference between the groups (Table 2) does not rule of confounding. Besides, it is not clear if what is reported in the table (BMI, MUAC) is measured at baseline. Time of measurements should also be reported. Furthermore, the two subgroups seem to be different with respect to BMI? So, it may be more correct to consider this study to be observational, although nested within a RCT. Consequently, study limitations need to be discussed.
The multivariate analysis is not adequately described. Which variables were in the final model – was age and sex controlled for?

Minor comments:

Abstract:
It is not correct that the participants comprised all consenting adults in the community. In this study, only subgroups of those participated. Baseline information is lacking, eg HIV prevalence, etc.

Introduction:
The introduction seems to be very long, and contains several categorical statements, that are not justified. I do not agree that it is controversial that zinc supplementation reduces risk of diarrhea, and that nutrition is not important to immunity. Referring to a single reference is not a fair reflection of the evidence. The effect of a nutritional intervention will depend on the nutritional status of the population, and a lack of effect can there for be due to the fact that the population was not deficient. There are a whole range of trials showing that zinc supplementation does reduce incidence of diarrhea, and a single study not finding such effect does not change that fact. Similarly, there are plenty of laboratory animal studies showing that zinc depletion leads disappearance of the thymus and to impaired cell-mediated immunity.

Methods
Again, selection criteria, and criteria for the timing of the measurements should be explained.

Results
The number of patients recruited in the main trial should not be mentioned here. The description in of background characteristics could be elaborated on. While it is correct not to do significance testing based on baseline characteristics in a proper randomized trial, it may be appropriate here, if it is not based on a random process. But in the text, irrespective of the p-values, attention should be drawn to possible differences.

In Table 2 and 3, the numbers in the MM don’t match – 37 or 38?.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests