Author's response to reviews

Title: Utility of CD4 cell counts for early prediction of virological failure during antiretroviral therapy in a resource-limited setting

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Author's response to reviews: see over
Dr. Anastasios Koutsos,
Assistant Editor,
BMC Infectious Diseases

Re: MS 6696955641799418. Utility of CD4 cell counts to predict early virological failure during antiretroviral therapy in a resource-limited setting

Dear Dr. Koutsos,
Thank you for your e-mail dated 14th April regarding the above manuscript. We are very grateful for the feedback from the reviewers and for your invitation to us to submit a revised version of this manuscript. We have now revised this paper and below we have itemised our responses to each of the reviewers’ comments as follows:

Reviewer: Luc Kestens.
We are grateful for these positive comments that the statistical analyses were thorough and that the findings were conclusive. No changes to the manuscript were requested.

Reviewer: Maria A Munoz-Fernandez
We are also grateful for the detailed comments provided by Dr. Munoz-Fernandez. Our responses to these are as follows:

1. Adherence. We agree entirely that treatment adherence is a key issue with regard to development of virological failure. However, the primary aim of this study was to determine the relationship between CD4 cell counts and development of virological failure irrespective of the underlying cause. Thus,
it was not an aim of the study to determine causes of virological failure. Although some adherence data were collected on this cohort, this was done inconsistently and was not incorporated into the main study database. No data on adherence have therefore been included in the Results. The methods section has been amended to reflect this and this minor limitation has also been included in the revised Discussion section (page 16).

2. **Follow-up.** With regard to the duration of follow-up, the median, interquartile range and range have now been given as requested on page 10 of the revised results section. Also, as requested, the numbers of patients followed up at 0, 1, 2, 3 etc years of ART is now stated in the Figure legend for Figure 1a (page 22).

3. **Treatment regimens.** All patients received combination antiretroviral therapy with a minimum of 3 drugs. As requested the numbers of patients receiving each type of regimen has now been included in the revised Results section on page 10. Details of the second-line regimen subsequently received by those with failure are not relevant as data were censored at the time virological failure developed. These have therefore not been included.

4. **Losses due to death during follow-up.** As requested we have now included the number of deaths in the revised Results section on page 10.

5. **CD8 counts.** CD8 counts were not measured in this cohort. In addition, analysis of CD4 cell count data is more useful, making these data more widely applicable in resource-limited settings where CD4 but not CD8 counts are generally measured.

6. **Route of HIV transmission.** Consistent with the epidemic across Africa, HIV was sexually acquired by all participants in this cohort. None acquired HIV by intravenous drug abuse or through medical procedures. As requested this information has now been included in the revised Results section on page 10.

7. **Wilcoxon test.** We used the Wilcoxon test because we were assessing matched-pairs of CD4 cell count and viral load values. Wilcoxon Matched Pairs test is the standard statistical technique to use for such types of analyses. We have checked through the paper to ensure that P values have been given for each of the statistical analyses.

8. **Pearson’s versus Spearman’s correlation coefficient.** The variables we assessed (CD4 cell count and viral load) were measured on an “interval scale” and not on an “ordinal scale”. The viral load measurements were transformed
to a log_{10} scale. The number of paired observations was very large (n=3756) and we estimated that the proportion of tied ranks within CD4 and viral load was large. As such use of the standard Pearson test is appropriately used.

9. **Figure 2.** The reviewer is correct that the symbols in Figure 2 do not represent individual patients but paired CD4-logVL observations, and that more than one data point may be included from each patient. Inclusion of all data points rather than using selected data provides a more valid evaluation of the overall relationship between CD4 cell counts and viral load and minimises bias as discussed on pages 13-14 of the revised discussion.

We carefully examined the relationships between viral load and CD4 cell counts in three different ways: absolute CD4 cell count, change in CD4 cell count from baseline and the CD4 cell slope (the current CD4 cell count trajectory at any given time-point). These three ways of examining CD4 cell counts are different and yet are clearly related. In this way we have provided a thorough analysis of the utility of this variable without making any judgements a priori as to which of the three is the most appropriate. This in no way constitutes any form of bias. From these analyses we found that the CD4 count slope was the most strongly correlated with viral load and was therefore the variable chosen for further examination in the ROC analysis in Figure 3.

The reviewer suggested that we might plot a new figure showing the mean CD4 cell count values over time or increases from basal values every 3 months comparing groups of patients with and without virological failure. We have done these plots and these confirm our findings, showing no difference at all between the two groups. Since mean values provide a very limited representation of group data we prefer to retain our original plots. We strongly believe these provide the clearest and most informative representation of the complete group data.

10. **Figures 2 D-F.** In these figures we examined whether the distributions of CD4 cell count data (absolute values, changes from baseline or CD4 count slopes) among patients at the time of virological failure differs from those distributions among all patients at all time-points when virological suppression was maintained. We realise that there was a clear error in the Figure Legend for Figure 2 and this may have caused confusion. The solid curve represents data from all patients in the cohort throughout follow-up at time-points when
virological suppression was maintained. The legend has been amended (page 22).

Again in this analysis, use of data from all time-points during virological suppression rather than data only from selected time-points provides the clearest way of showing the relationship between these variables and this minimises bias.

11. **Figure 3.** This figure further extends the findings of Figure 2. We found that of the 3 ways of examining CD4 cell counts, it was the CD4 count slope that best correlated with viral load (Figure 2C). In Figure 3 we extend this observation to further assess the sensitivity and specificity of CD4 count slopes in predicting virological failure. Multiple data points from each patient are included. The reviewer is correct in stating that the CD4 count slope for an individual patient may change multiple times during follow-up. This closely represents the reality of longitudinal monitoring of patients; in the clinical decision making process a clinician reviews longitudinal data collected over many clinic visits. Inclusion of all data points is therefore important, valid and eliminates the bias that would be inevitable were only selected data-points chosen to be examined.

12. **Minor comment: number of decimal digits in p values.** The convention we used in representing the p-values was to report two decimal points if the p-value was not significant and to truncate the value to the nearest possible figure if highly significant. However Figure A2 was not consistent with this rule and we therefore have changed the p-value to read <0.01. This has also been changed in 2nd paragraph, page 9.

We are grateful to the reviewers and for this opportunity to further strengthen this manuscript. We hope that the revised version is now suitable for publication. We look forward to your feedback in due course.

Yours sincerely,

Dr. Motasim Badri, Dr. Stephen D. Lawn, Prof. Robin Wood