Author’s response to reviews

Title: Empirical treatment for tuberculosis in HIV: lessons from a cohort study of people living with HIV treated in Recife, Brazil

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Version: 7 Date: 27 January 2014

Author’s response to reviews: see over
Dear Editor

We are pleased to send you the revised version of our manuscript, undoubtedly improved by the reviewers’ relevant suggestions. Please, do not hesitate to contact us if you would like any further explanation.

Your sincerely,

Maria de Fátima Albuquerque

Reviewer's report

Title: Empirical treatment for tuberculosis in HIV: lessons from a cohort study of people living with HIV treated in two referral centers in Recife, Brazil

Authors:
Maria de Fátima Pessoa Militão de Albuquerque, Isabella Coimbra, Joanna d’Arc Lyra Batista, Magda Maruza, Ricardo Arraes de Alencar Ximenes, Heloísa Lacerda Ramos, Demócrito de Barros Miranda Filho, Marcela Lopes Santos, Laura Cunha Rodrigues.

Mortality among people living with HIV (PLHIV) remains an important area of research. The authors have identified an interesting area with limited research that is worth publishing as this is presumably what happens in many settings especially in the developing world where TB diagnosis can be extensive in these harsh economic times.

Reviewer: Kennedy Otwombe

Date: 18th November 2013

Title: Empirical treatment for tuberculosis in HIV: lessons from a cohort study of people living with HIV treated in two referral centers in Recife, Brazil.

Authors:
Maria de Fátima Pessoa Militão de Albuquerque, Isabella Coimbra, Joanna d’Arc Lyra Batista, Magda Maruza, Ricardo Arraes de Alencar Ximenes, Heloísa Lacerda Ramos, Demócrito de Barros Miranda Filho, Marcela Lopes Santos, Laura Cunha Rodrigues

Mortality among people living with HIV (PLHIV) remains an important area of research. The authors have identified an interesting area with limited research that is worth publishing as this is presumably what happens in many settings especially in the developing world where TB diagnosis can be extensive in these harsh economic times.
Authors' Reply:
Thank you for the opportunity of improving our paper following your questions and suggestions.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Abstract: Add comma to this sentence “To identify factors associated with death we estimated Hazard Ratio (HR) in bivariate and multivariate Cox regression analysis.”

Authors’ Reply:
We have changed the text accordingly.

2. Results: HART should be “HAART”

Authors’ Reply:
Thank you, the required correction has been made.

3. Background paragraph 1: This sentence is unclear “Population surveys and autopsy studies have shown that many of those deaths are undiagnosed and therefore untreated tuberculosis (TB).”

Authors’ Reply:
We have rewritten the sentence: Population surveys and autopsy studies have demonstrated that many of these deaths are due to undiagnosed and therefore untreated tuberculosis (TB).


Authors’ Reply:
We have changed the references accordingly.

5. Background paragraph 2: is “empiric” not meant to be “empirical”.

Authors’ Reply:
We have corrected this word in the text.

6. Background paragraph 3: Make sentence clear “Here in a routine care setting we estimated the probability of survival of PLHIV with complaint of cough as the main symptom to suspect of TB as recommended by the Brazilian Ministry of Health”

Authors’ Reply:
We have rewritten the sentence to make it clearer:

“In accordance with recommendations by the Brazilian Ministry of Health, in a routine care setting in Recife we estimated the probable survival and mortality
rates of PLHIV with complaint of cough as the main symptom for suspected TB."  

7. Methods paragraph 1: “consecutive sample of individuals who referred cough to estimate the mortality rate and identify the factors associated with the mortality with emphasis on the empirical treatment for TB.”

Authors’ Reply:  
“...consecutive sample of individuals who reported a cough to estimate the probability of survival and mortality rates. We also identified the factors associated with death with emphasis on the empirical treatment for TB.

8. Methods paragraph 2: Where is the closing bracket for this sentence? “We excluded those with positive bacteriology (sputum smear microscopy or culture for M.tb because they are confirmed.”

Authors’ Reply:  
We have corrected the sentence:  
“We excluded those with positive bacteriology (sputum smear microscopy or culture for M.tb) because they were TB confirmed.”

9. Methods paragraph 5: Change quitted to quit in this sentence “(smokers at the time of the study or had quitted smoking less than six months before)”.

Authors’ Reply:  
We have rewritten the sentence in the manuscript.

10. Discussion paragraph 4: Put comma after study in this sentence “In our study 64.8% had a chest X-ray, of which one in three was abnormal.”

Authors’ Reply:  
This has been done.

11. Discussion: It is unusual for results to be presented in the discussion section. Ideally the discussion section discusses results in comparison to what is known. Remove all the HRs and ORs and report them in the results section.

Authors' Reply:  
We have removed the measurement values from the discussion section.

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached).

1. In the discussion section of this manuscript, there is no mention of limitations. What were the limitations of this study?

Authors' Reply:  
We have rewritten the last paragraph in the discussion section emphasizing the limitations of the study:
“One limitation of our study is that of observational studies to evaluate the effectiveness of empirical treatment for TB, which was initiated by physicians during routine care within the health services. Besides this, the empirical treatment for TB did not follow a well-defined protocol for screening TB, which would have ensured the early introduction of TB treatment, and also avoid the misdiagnosis of other opportunistic diseases that could present with cough. Furthermore, data on opportunistic diseases were collected only at baseline and not during the follow up.”

2. To what extent do the authors think that patient or system delay may have contributed to the high mortality rate experienced in those without the three characteristics strongly suggestive of TB?

Authors’ Reply:
Considering all the patients who were treated, the mean time from registration of cough until the initiation of TB treatment was 163 days. Those who started treatment for TB and died experienced a greater delay (183 days) compared with those who were treated and did not die (152), although the difference was not statistically significant (p= 0.4999).

We believe that the delay may have contributed to the high mortality rate in those without the three characteristics strongly suggestive of TB. Reanalyzing the data (we added these results on page 7), we verified that those who did not report a history of previous TB treatment (223 days) and those who had normal chest radiographies (309 days) presented a greater average period of time from registration of cough until TB treatment compared with those who reported a history of previous TB treatment (83 days), and those with abnormal chest radiographies (95 days), respectively, and the differences were statistically significant (p=0.0010). However, although the delay may have contributed, an alternative explanation would be that these patients did not have TB, just severe HIV disease, as indicated in the discussion section.

3. Literature on mortality in HIV-infected people suggests that high mortality rates are experienced in the early periods after treatment initiation. This study found high mortality rates in those (without three characteristics of TB) who received empirical treatment for TB. The authors further speculate that these high mortality rates may be as a result of TB or late presentation of TB. It would be helpful to discuss to what extent this highly reported mortality rate may be associated with use of HAART as opposed to TB treatment?

Authors’ Reply:
When considering only those patients without three characteristics of TB, the average periods of time of using HAART were similar for those who died and those who did not.

```
. ttest tempo_tarv if sugesttb==0, by(obito)
 Two-sample t test with equal variances

                        |     Obs  | Mean    | Std. Err. | Std. Dev. | [95% Conf. Interval] |
------------------------+---------+---------+-----------+-----------+---------------------|
                        | 0       | 164     | 2466.415  | 113.709   | 1456.186 2690.947 |
                        | 1       | 9       | 2168.556  | 355.582   | 1066.747 2988.53  |
                        | combined| 173     | 2450.919  | 109.3029  | 1437.655 2666.667 |
```
4. Literature suggests potential gender differences on the impact of HIV and TB. To what extent do your results differ by gender? Are the predictors of mortality in males and females different? If so, speculate why?

Authors' Reply:
Although the mortality rate is higher for men (5.4 per 100 py) compared with women (3.3 per 100 py), and gender is associated with death in the bivariate analysis, gender was not associated with death in the Cox multivariate regression analysis, as shown in Table 4.

. stsum, by(sexo)
  failure _d:  obito == 1
  analysis time _t:  tempo_ano
  id:  id_pesquisa

<table>
<thead>
<tr>
<th>sexo</th>
<th>time at risk</th>
<th>incidence rate</th>
<th>no. of subjects</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>866.7500015</td>
<td>0.0334583</td>
<td>327</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1202.220003</td>
<td>0.0540666</td>
<td>472</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>2068.970004</td>
<td>0.0454332</td>
<td>799</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

. stcox sexo
  failure _d:  obito == 1
  analysis time _t:  tempo_ano
  id:  id_pesquisa

Iteration 0:  log likelihood = -607.73167
Iteration 1:  log likelihood = -605.43642
Iteration 2:  log likelihood = -605.42996
Iteration 3:  log likelihood = -605.42996
Refining estimates:
Iteration 0:  log likelihood = -605.42996

Cox regression -- Breslow method for ties
No. of subjects = 799  Number of obs = 1225
No. of failures = 94  Time at risk = 2068.970004
Log likelihood = -605.42996  LR chi2(1) = 4.60
                         Prob > chi2 = 0.0319

| sexo  | Haz. Ratio  | Std. Err. | z    | P>|z| | [95% Conf. Interval] |
|-------|-------------|-----------|------|-----|----------------------|
| 1     | 1.59493     | 0.3561876 | 2.09 | 0.037| 1.029549 2.470792    |

Stratifying the regression Cox analysis by gender, we found that the predictors of mortality were similar for men and women.

. stcox i.tb_coorte i.cd41 i.anemia1 i.novoetilismo if sexo==0
  failure _d:  obito == 1
  analysis time _t:  tempo_tarv_ano
  id:  id_pesquisa

Iteration 0:  log likelihood = -58.955068
Iteration 1:  log likelihood = -43.205073
Iteration 2:  log likelihood = -39.251939
Iteration 3:  log likelihood = -37.94465
Iteration 4:  log likelihood = -37.9007
Iteration 5:  log likelihood = -37.9007
Iteration 6:  log likelihood = -37.897682
Iteration 7:  log likelihood = -37.896573
Iteration 8:  log likelihood = -37.896165
Iteration 9: log likelihood = -37.896015
Iteration 10: log likelihood = -37.895959
Iteration 11: log likelihood = -37.895939
Iteration 12: log likelihood = -37.895932
Iteration 13: log likelihood = -37.895929
Iteration 14: log likelihood = -37.895928
Iteration 15: log likelihood = -37.895927
Iteration 16: log likelihood = -37.895927
Iteration 17: log likelihood = -37.895927
Iteration 18: log likelihood = -37.895927
Iteration 19: log likelihood = -37.895927
Iteration 20: log likelihood = -37.895927
Iteration 21: log likelihood = -37.895927
Iteration 22: log likelihood = -37.895927
Iteration 23: log likelihood = -37.895927
Iteration 24: log likelihood = -37.895927
Iteration 25: log likelihood = -37.895927
Iteration 26: log likelihood = -37.895927
Iteration 27: log likelihood = -37.895927
Iteration 28: log likelihood = -37.895927
Iteration 29: log likelihood = -37.895927
Iteration 30: log likelihood = -37.895927
Iteration 31: log likelihood = -37.895927
Iteration 32: log likelihood = -37.895927
Iteration 33: log likelihood = -37.895927
Refining estimates:
Iteration 0: log likelihood = -37.895927
Iteration 1: log likelihood = -37.895927
Iteration 2: log likelihood = -37.895927

Cox regression -- no ties

No. of subjects = 214
No. of failures = 12
Time at risk = 1286.884931
Log likelihood = -37.895927

LR chi2(5) = 42.12
Prob > chi2 = 0.0000

|              | Haz. Ratio | Std. Err. | z    | P>|z|  | [95% Conf. Interval] |
|--------------|------------|-----------|------|------|-----------------------|
| 1.tb_coorte  | 3.001926   | 2.308374  | 1.43 | 0.153| .6650534 - 13.55013 |
| 1.cd41      | 22.50104   | 16.56085  | 4.23 | 0.000| 5.317594 - 95.21162 |
| 1.anemia1    | 3.250673   | 2.222449  | 1.72 | 0.085| .85117 - 12.41453   |
| novoetilismo |            |           |      |      |                       |
| 1            | 0.316178   | 0.3502838 | -1.04 | 0.299| .0360507 - 2.773006  |
| 2            | 1.16e-16   | 5.50e-09  | -0.00 | 1.000| 0                      |

. stcox 1.tb_coorte 1.cd41 1.anemia1 novoetilismo if sexo==1
failure _d: obito == 1
analysis time _t: tempo_tarv_an
Id: id_pesquisa

Iteration 0: log likelihood = -153.90495
Iteration 1: log likelihood = -143.6068
Iteration 2: log likelihood = -132.96435
Iteration 3: log likelihood = -125.48626
Iteration 4: log likelihood = -123.35483
Iteration 5: log likelihood = -123.35368
Iteration 6: log likelihood = -123.35368
Refining estimates:
Iteration 0: log likelihood = -123.35368

Cox regression -- no ties

No. of subjects = 276
No. of failures = 31
Time at risk = 1807.08767
Log likelihood = -123.35368

LR chi2(5) = 61.10
Prob > chi2 = 0.0000

|              | Haz. Ratio | Std. Err. | z    | P>|z|  | [95% Conf. Interval] |
|--------------|------------|-----------|------|------|-----------------------|
| 1.tb_coorte  | 3.403407   | 1.381217  | 2.52 | 0.012| .0787358 - 7.267158  |
| 1.cd41      | 4.997982   | 1.934901  | 2.60 | 0.010| 2.340256 - 10.67397 |
| 1.anemia1    | 3.701441   | 1.946137  | 1.93 | 0.052| 1.323923 - 10.43854 |
| novoetilismo |            |           |      |      |                       |
| 1            | 0.2192327  | 0.1377491 | -2.42| 0.016| .0639846 - 0.7511648 |
| 2            | 2.39204    | 1.356188  | -2.52| 0.012| .0787358 - 7.267158  |
Reviewer: Mohamed Janabi
Reviewer’s report:
Minor Essential Revisions

1. Citations of the references should be uniform the authors have used (i) the [ ] (ii) superscript see page 4.

Authors’ Reply:
Thank you for your suggestions and questions which have helped us to improve our paper. The references have been corrected according to the Journal’s recommendation.

2. Can the authors explain in their discussion the reason for starting ant-TB 6 months after the patient had complaint.

Authors’ Reply:
This was an observational cohort study and the time to start the TB treatment was a decision of the attending physician. The data analysis suggests it is probable that for those patients without the three characteristics strongly suggestive of TB, or those with severe HIV disease, or both, the treatment for presumptive TB was started as a final resource in patients more likely to die, and perhaps too late. An alternative explanation would be that these patients did not have TB, just severe HIV disease, as mentioned in the discussion section.

3. Were the physician blinded in about the study? If yes/no how could it affect the results? Can the authors state which anti-TB were used in this study Rifampicin based? and Which HAART was used EFV based because this could bring a different discussion all together.

Authors’ Reply:
The physicians were blinded with regard to the study’s objective, and it is unlikely that the initiation of treatment for presumptive TB was influenced by the research. At the time of data collection, TB treatment in Brazil was carried out with three drugs: Rifampicin + Isoniazide + Pirazinamide for two months followed by Rifampicin + Isoniazide for four months.

In Brazil, access to antiretroviral treatment is free and the first choice scheme recommended by the Ministry of Health is a combination of two Nucleoside Reverse Transcriptase Inhibitors (NRTIs) associated with Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs). The alternative regimen is the combination of two Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and a protease inhibitor reinforced with ritonavir (IP / r). Among the NNRTIs, efavirenz (EFZ) and nevirapine (NVP) are available in the public health system, but the decision to use them is taken by the attending physician.

In this cohort, 64.7% of the patients used efavirenz during the follow-up, however the use of EFZ was not significantly associated with death, as shown below.
. xi: stcox i.efz
  i.efz  _Iefz_0-2  (naturally coded; _Iefz_0 omitted)
  
 failure _d:  obito == 1
 analysis time _t:  tempo_ano
 id:  id_pesquisa

Iteration 0:   log likelihood = -614.34887
Iteration 1:   log likelihood = -613.50668
Iteration 2:   log likelihood = -613.50387
Iteration 3:   log likelihood = -613.50387
Refining estimates:
Iteration 0:   log likelihood = -613.50387

Cox regression -- Breslow method for ties

No. of subjects =          800                     Number of obs   =       800
No. of failures =           95
Time at risk    =  2070.300004
LR chi2(2)      =      1.69
Log likelihood  = -613.50387                     Prob > chi2     =    0.4296

------------------------------------------------------------------------------
   _t  | Haz. Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-----|-------------|------------------|-------|--------|-----------------------------
   _Iefz_1 |    .773317   .2815922  -0.71   0.480     .3787955    1.578739
   _Iefz_2 |   1.049733   .3443269   0.15   0.882     .5519158     1.99657
------------------------------------------------------------------------------

4. Authors view were patients having a Probable TB? or Confirmed TB needs to be mentioned.

Authors’ Reply:
All the 171 patients treated for TB in this cohort were diagnosed with presumptive TB, as they were either AFB sputum smear or M.tb culture negative or they did not perform the tests. The TB confirmed cases were excluded as explained in the methods section.

Major Compulsory

1. The study had two centers as shown in the title but the results don't pot ray that where the findings same or similar at the two sites? For instance Is the decision to start anti TB same at the two sites, mortality.

Authors’ Reply:
In the absence of a standardized protocol for the treatment of presumptive TB, at the time of data collection, the presumptive TB treatment was initiated on the decision of the attending physician.

We analysed the factors associated with the initiation of TB treatment by hospital and verified that the factors associated with starting treatment for presumptive TB were similar in the two referral centers for HIV, as shown below.

. xi:logistic i.tb_coorte i.anemia1 i.cd41 i.do i.bacteriologia i.historiatb if hospital==0
  i.tb_coorte  _Itb_coorte_0-1  (naturally coded; _Itb_coorte_0 omitted)
  i.anemia1   _Ianemia1_0-1    (naturally coded; _Ianemia1_0 omitted)
Moreover, in the multivariate Cox regression analysis of factors associated with death when we adjusted for hospital, the same variables remained in the model and hospital was not significantly associated with death.

. stcox i.novoetilismo i.anemia1 i.cd41 i.do i.bacteriologia i.historiatb if hospital==1

Cox regression -- Breslow method for ties
No. of subjects = 697                    Number of obs = 1067
No. of failures = 71                    Time at risk = 678540
Log likelihood = -373.66593                  LR chi2(8) = 150.96
                           Prob > chi2 = 0.0000

-------------------------------------------------------------------------------
| t | Hazard Ratio | Std. Err. | z  | P>|z| | [95% Conf. Interval] |
-------------------------------------------------------------------------------
novoetilismo        |        |
1 |       .426468 | .1750537 | 2.85 | 0.004 |
2 |       .9574992 | .3339723 | -2.08 | 0.038 |
1.anemia1          |        |
1.cd41            |        |
1 |       2.998511 | .9635646 | 2.85 | 0.004 |
2 |       5.372301 | 1.422345 | -2.08 | 0.038 |
-------------------------------------------------------------------------------
2. This is an observation study can it really answer the impact of an intervention e.g. empirical starting of anti-TB?

Authors’ Reply:
We have mentioned in the study’s conclusion that a cohort study was not sufficiently adequate to evaluate the impact of empirical treatment for TB on mortality of PLHIV. Even so, we believe that it is very important to reveal our results because, probably, this is a very common situation in many settings, especially in the developing world where the presumptive diagnosis of TB in PLHIV occurs extensively.

3. Are the authors aware of IRIS (Immune reconstitution syndrome) interesting! Could all the results see are do IRIS which the authors have completely ignored to discuss!

Authors’ Reply:
Thank you for this important comment. We are aware of IRIS and probably this condition could have caused death in some cases due to unmasked TB after the initiation of HAART. In fact, the mortality rate was higher for those who had used HAART for less than one year and this may be due to IRIS. However, the impact of this was probably diminished by the low number of patients taking HAART for less than a year, only 27 individuals. We included a comment on this in the discussion section.

4. Can the authors discuss in the discussion section the mortality seen why attribute it to anti-TB and not the anemia(41% of study subjects), low CD4 below 200, OIs?

Authors’ Reply:
To identify the predictive factors for death in this cohort we estimated Hazard Ratio (HR) in bivariate and multivariate Cox regression. The analysis found that mortality was associated with CD4 cells count <200 (HR=5.3; CI 95%: 3.2-9.0; p=0.000), with anemia (HR=3.0; CI 95%: 1.6-5.6; p=0.001) and with abnormal chest radiography (HR=2.4; CI 95%: 1.4-4.0; p=0.001). Mortality was also higher in those receiving empirical TB treatment (HR=2.4; CI 95%: 1.4-4.0;
p=0.002), but only in those with normal X-ray, no history of tuberculosis and no bacteriology request. The final Cox multivariate regression model of factors associated with death is shown in table 4. We discussed the role for all these factors in the manuscript.

**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.
Reviewer: Valeria Saraceni

Reviewer's report:

Major Compulsory Revisions
Although I found merit in your study, and the topic is quite interesting, I have some comments:

Authors' Reply:
We would like to thank you for the opportunity of improving our paper following your questions and suggestions.

1 – Are those without TB test different from those who tested negative? It would imply a misclassification bias in those who were tested and were negative as TB suspects.

Authors’ Reply:
In this cohort of patients, who complained of cough, 492 (61.5%) did not have AFB sputum smear or M. tb culture tests during the follow-up (54.4% did not have sputum), and they were similar to those who tested and were negative, as demonstrated in the table below. We have added this information to the results section.

Table

<table>
<thead>
<tr>
<th></th>
<th>AFB sputum smear or M. tb culture during follow up</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not performed</td>
<td>Negative</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>203 (62.0)</td>
<td>124 (37.9)</td>
</tr>
<tr>
<td>Male</td>
<td>289 (61.1)</td>
<td>184 (38.9)</td>
</tr>
<tr>
<td>Age groups &lt;40</td>
<td>267 (61.1)</td>
<td>170 (38.9)</td>
</tr>
<tr>
<td>≥40</td>
<td>225 (62.0)</td>
<td>138 (38.0)</td>
</tr>
<tr>
<td>CD4 ≥ 200</td>
<td>391 (62.4)</td>
<td>236 (37.6)</td>
</tr>
<tr>
<td>&lt;200</td>
<td>94 (56.3)</td>
<td>72 (43.4)</td>
</tr>
<tr>
<td>HAART (baseline) No</td>
<td>131 (61.2)</td>
<td>83 (38.8)</td>
</tr>
<tr>
<td>Yes</td>
<td>360 (61.5)</td>
<td>225 (38.5)</td>
</tr>
</tbody>
</table>

2 – If I understood it correctly from Tables 1 to 3, you’re referring directly to Hazard Ratios, and not comparing the 2 groups, those who started and those who didn’t start TB therapy. Direct comparison of the 2 groups by visualizing if they are comparable always makes it easier for the reader. I’d like to see the proportions of CD4<200, HAART at baseline, etc, and the homogeneity test. Table 5 gives a clue that those who died were sicker than those who didn’t.

Authors’ Reply:
In Tables 1, 2 and 3 the outcome studied is death. The tables show the crude association (HR) between death and the demographic, socioeconomic and
lifestyle variables; variables related to general health, as well as those related to HIV and TB, which were statistically significant at a p value ≤ 0.20.

In Table 5 the outcome is empirical treatment for TB, and the table shows the multivariate logistic regression analysis comparing those who did and did not receive empirical treatment for TB. Treated subjects were more likely to have characteristics suggestive of severe HIV disease (low CD4, opportunistic infections) and characteristics suggestive of tuberculosis (abnormal chest X-ray, history of TB treatment, bacteriology testing) and characteristics suggestive of both TB and severe HIV: reported weight-loss and anemia.

3 – Then I’d like to see the KM (Figure 2) depicting both groups (TB Therapy yes and no).

Authors’ Reply

We added to figure 2 the KM by TB therapy and CD4 with respective Log Rank tests.

4 – I guess that, once you’ve rejected your null hypothesis, you should look at time from TB therapy initiation and death. Another KM by CD4 category (<200 and >=200) would make it clearer to help explain your findings. I have a general idea that those who died had little time between starting TB therapy and death. As you’ve stated, the median time from cough to decide to initiate TB therapy was 6 months. This is a major point to be further explored.
Authors' Reply:
The KM curve with only the 171 individuals who initiated empirical treatment for TB, considering the time from the date of starting treatment until death or end of the study. Most of the death occurred during the first months after initiating the TB treatment, suggesting empirical treatment for TB may have been introduced too late to prevent death, especially among those patients who presented normal chest X-rays and did not report a history of previous TB treatment. As we have stated in the discussion section, mortality was not significantly higher in those empirically treated for TB, who had three characteristics suggestive of the disease (abnormal chest X-ray, history of TB treatment, AFB sputum smear or M.tb culture testing). On the other hand, in those without the three characteristics strongly suggestive of TB, mortality was 14 times higher in those receiving empirical treatment. This is consistent with the attending physician being more likely to introduce tuberculosis treatment as a final resource in patients more likely to die. This could either be because there was another cause for the cough or because of severe HIV disease, or both.
Below the KM by CD4 category (<200 cell count and ≥ 200 cell count)

The mean time from registering a cough until initiating TB treatment was 163 days for all 171 patients treated for TB. Those who died had a longer period of time (183 days) compared with those who did not die (152), although the difference was not statistically significant (p = 0.4999). The issue of delay in initiating treatment for TB was explored further in the discussion.

5 – In Conclusions, you’ve said: “Those with strong indications of advanced HIV diseases were more likely to receive empirical TB treatment (even if they had few indications of having tuberculosis); those receiving treatment in this group had a marked increase in mortality, likely to be a result of their advanced HIV disease, and possibly of other causes for the cough.” So HIV advanced disease is a confounder, and CD4<200 was a strong predictor of death and cough. Therefore, the doctors may have tried TB therapy as salvage therapy. I wonder if this group of patients who had a cough and died was composed of smokers, had PCP, was failing HAART. I guess you have in your cohort data about those questions that would elucidate your points better. A table by CD4 category and risk factors would help to check on that.

Authors’ Reply:
Although 40.4% of the individuals in this cohort reported being current smokers, smoking was not associated with death in the bivariate Cox regression analysis (Table 1). Unfortunately, we do not have information on PCP and irregularities on taking HAART.
In this cohort all the 800 individuals presented cough. The CD4 cell count was a predictor of death and it is related with severity of HIV disease. We performed the Cox regression multivariate analysis stratifying by CD4 cell count and verified that the final predictive models for death are similar as shown below. The statistical significance may not have been reached for some variables due to the lack of power.

**Final predictive model for death stratified by CD4 count**

```
. stcox i.novoetilismo i.anemia1 i.res_rx1 i.tb_coorte if cd41==0
  failure _d: obito == 1
  analysis time _t: tempo
  id: id_pesquisa
Iteration 0: log likelihood = -158.39809
Iteration 1: log likelihood = -148.49452
Iteration 2: log likelihood = -140.85633
Iteration 3: log likelihood = -137.93409
Iteration 4: log likelihood = -137.7844
Iteration 5: log likelihood = -137.7844
Refining estimates:
Iteration 0: log likelihood = -137.7844
Cox regression -- Breslow method for ties
No. of subjects =          597                     Number of obs   =       743
No. of failures =           26                     Time at risk    =       567608
Log likelihood = -137.7844
LR chi2(6)      =     41.23                     Prob > chi2     =    0.0000
|             | Haz. Ratio   Std. Err.    z    P>|z|     [95% Conf. Interval]
|----------------+------------------------+--------+----------+----------------------------------|
| novoetilismo   |                        |        |          +-----------------------------|
| 1              | .1597802    .1646868   -1.78  0.075     .0211925    1.204658
| 2              | 1.167889    .645452    2.02  0.044     .3953408    3.450097
| anemia1        | 3.932992    2.048697   1.92  0.056     1.416876    10.91728
| res_rx1        |                        |        |          +-----------------------------|
| 1              | 2.105119    1.057274   1.99  0.046     .7866283    5.633571
| 2              | 1.600749    .839138    1.88  0.061     .619247    4.53935
| tb_coorte      | 3.864163    1.688641   2.30  0.021     1.640892    9.099781
```

```
. stcox i.novoetilismo i.anemia1 i.res_rx1 i.tb_coorte if cd41==1
  failure _d: obito == 1
  analysis time _t: tempo
  id: id_pesquisa
Iteration 0: log likelihood = -207.32817
Iteration 1: log likelihood = -191.01945
Iteration 2: log likelihood = -190.38721
Iteration 3: log likelihood = -190.38721
Iteration 4: log likelihood = -190.38721
Refining estimates:
Iteration 0: log likelihood = -190.38721
Cox regression -- Breslow method for ties
No. of subjects =          220                     Number of obs   =       324
No. of failures =           45                     Time at risk    =       110932
Log likelihood = -190.38721
LR chi2(6)      =     33.88                     Prob > chi2     =    0.0000
|             | Haz. Ratio   Std. Err.    z    P>|z|     [95% Conf. Interval]
|----------------+------------------------+--------+----------+----------------------------------|
| novoetilismo   |                        |        |          +-----------------------------|
| 1              | .658842    .3000984    -2.20  0.027     .269814    1.608786
| 2              | .8693279   .3939672   -2.20  0.027     .269814    1.608786
```

6 – HIV patients have a greater chance to have disseminated TB, and no cough. How many of those patients were checked for disseminated TB? Especially those with lower CD4?

Authors’ Reply:
All the individuals enrolled in the cohort presented cough. Of 171 Patients treated for presumptive TB, 69% had pulmonary disease, 18.1% extrapulmonary. 4.1% pulmonary + extrapulmonary and 8.9% disseminated TB.

```
. tab forma_tb if tb==1
----------|-----------+-------------------+---------+---------
          1 |        118   69.01   69.01 (Pulmonar)
          2 |         31  18.13   87.13 (Extrapulmonary)
          3 |          7  4.09   91.23 (Pulmonar and extrapulmonary)
          4 |         15  8.77  100.00 (Disseminated)
          +-----------------------------------
           Total |        171  100.00
```

Considering only the 171 individuals who were treated empirically for TB and analyzing clinical form variable as pulmonary and others, we find no association between clinical form and death.

```
. tab tb
-----|-----------+-------------------+---------+---------
     0 |        118  69.01   69.01
     1 |         53  30.99  100.00
     +-----------------------------------
       Total |        171  100.00
```

```
.xi: stcox i.tb
 i.tb              _Itb_0-1  (naturally coded; _Itb_0 omitted)
     failure  _d: obito == 1
     analysis time  _t: tempo_ano
     id: id_pesquisa

Iteration 0:  log likelihood = -282.33316
Iteration 1:  log likelihood = -282.33109
Iteration 2:  log likelihood = -282.33109
Pressing omitted

Cox regression -- Breslow method for ties
No. of subjects =          171                     Number of obs   =       171
No. of failures =           59                     Time at risk    =  366.3100003
Log likelihood =  -282.33109  LR chi2(1) =       0.00
                     Prob > chi2 =     0.9487
                     
      _t | Haz. Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
----------|----------------------+-------------------+---------+---------+
     _Itb_1 |    .9821893   .2747226   -0.06   0.949     .5676889    1.699339
```
7 – You’ve mentioned “controlling for CD4” and in Table 3 it says as time-dependent. Yet again, on page 8, 4th paragraph, you’ve said “During the follow up, almost all (99.4%) had at least one CD4 cell count measurement and 73% used HAART at entry.” I wonder how many CD4 per patient do you have. And which CD4 was used to categorize the value? The one nearest to starting coughing?

Authors’ Reply:
Our analysis included the CD4 cell count variable as time-varying that was measured more than once and had different values during the follow-up. Thus, variations in the CD4 count with time were taken into account, allowing for the changes in the category of exposure (CD4 <200; CD4 ≥ 200) for each patient during the follow-up. The CD4 cell count value closest to recording the complaint of a cough was used to categorize the CD4 at baseline.

The mean CD4 cell count per patient was 11, and the median was 10 measures.

```
. sum cont, detail

 cont
------------------------------------------------------------
Percentiles   Smallest
1%            1              1
5%            2              1
10%           3              1     Obs                 777
25%           6              1     Sum of Wgt.         777
50%           10             Largest     Mean           11.18275
75%           15             Std. Dev.    7.179586
90%           21             Variance     51.54645
95%           25             Skewness     1.013079
99%           33             Kurtosis     4.026154
```