Title: Long-term mortality in HIV patients virally suppressed for more than three years with incomplete CD4 recovery: A cohort study.

Authors:

Frederik N Engsig (fren74@gmail.com)
Jan Gerstoft (jan.gerstoft@rh.regionh.dk)
Gitte Kronborg (gkronborg@dadlnet.dk)
Carsten S Larsen (Carsten.Schade.Larsen@vest.rm.dk)
Gitte Pedersen (gip@rn.dk)
Birgit T Røge (roege@dadlnet.dk)
Janne Jensen (Janne.Jensen@slb.regionsyddanmark.dk)
Lars N Nielsen (lnn@noh.regionh.dk)
Niels Obel (niels.obel@rh.regionh.dk)

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Author's response to reviews: see over
To the editor in chief.

Thank you very much for reviewing our article: “Long-term mortality in HIV patients virally suppressed for more than three years with incomplete CD4 recovery: A cohort study”.

We have revised the manuscript according to the comments of reviewers and editor and now resubmit the manuscript. Answers to the questions raised by the referees are answered below (in italics). Changes in the manuscript, tables, e.g. are marked with yellow color.

We hope that our answers and corrections are sufficient to have the paper published in your journal.

Yours sincerely,

Frederik N Engsig
Editorial requests:

- We recommend that you copyedit the paper to improve the style of written English. If this is not possible, you may need to use a professional copyediting service. Examples are those provided by the Manuscript Presentation Service (www.biomedes.co.uk), International Science Editing (http://www.internationalscienceediting.com/) and English Manager Science Editing (http://www.sciencemanager.com/). BioMed Central has no first-hand experience of these companies and can take no responsibility for the quality of their service.

  Answer: The paper has now been copyedited.

- Please clarify whether approval was obtained for the use of the data used in this study or whether the data is publically available. Please be sure to provide a statement within the Methods section of the manuscript.

  Answer: This has now been clarified under Methods, page 9, first paragraph which has been changed to: “The study was approved by the Danish Data Protection Agency (Denmark has no Institutional review boards). Data in the database is not publicly available.

- Please include an Authors' contributions section before the Acknowledgements and Reference list.

  Answer: This has now been added.
Answer to reviewer 1:

- Major Compulsory Revisions

Methods-

1. It is not clear if HCV status is obtained on all individuals or only if they have a positive test for HCV ab or HCV RNA. Please clarify.

   Answer: As noted under Methods, last paragraph page 7, chronic HCV infection was defined as one positive PCR for HCV RNA. All patients in The Danish HIV Cohort Study are intended tested for HCV IgG yearly and if the patient is antibody positive a PCR for HCV RNA is performed. The following sentence has been included in the method section (page 7, first paragraph: “Patients are intended tested yearly for hepatitis C antibodies, and if positive are tested for hepatitis C RNA.”)

Results-

1. Type of ARV regimen is not included. It is important for the reader to know the types of regimens used so as to understand whether these results are relevant to their population.

   Answer: Last HAART regimen prior to index date is now included in Appendix table 1.

2. Is it possible that drugs used in the regimen are associated with poor CD4 recovery (e.g., ZDV)? If so, it would be important to assess the relation between regimen and outcome group status.
Answer: Although we do have data on treatment history, time updated analysis on zidovudine treatment is beyond the scope of this study. Instead we have estimated the number of patients included in the study who had ever been treated with zidovudine prior to index date (54 (98%) INRs vs. 219 (93%) IRs, $P = 0.465$) and this has now been added in Table 1.

Discussion-

1. It is not quite accurate to say that by excluding those without virological suppression excludes non-adherers. It is possible to suboptimally adhere and still remain suppressed.

   Answer: Correct, the sentence in the discussion section has been deleted.

2. It is unclear what the authors mean by saying that including only good adherers eliminates potential bias introduced by risk factors for HCV infection, and substance use. HCV infection was presumably measured in all subjects. Did the investigators have access to substance use data? If so, it would be interesting to see. If not, this statement is inappropriate.

   Answer: We do not have access to data on substance use but only to route of infection (IDU). The sentence in Discussion, page 13, paragraph 2 has been deleted!

3. The Discussion is quite long and would benefit from reorganization and more
narrow targeting to the context and implication of the findings.

Answer: The Discussion has now been revised and shortened.

Figures-
1. Figure 3 is confusing as drawn and does not provide variability around estimates. A histogram with confidence bars for each group at each time point would accommodate this and should replace this figure. Alternatively, the figure adds relatively little to the overall point and could be deleted entirely.

Answer: Figure 3 has now been deleted, but the main results can still be found in Results, page 11, last paragraph which now quotes: “80% of the INRs achieved a CD4 cell count > 200 cells/µL after six years of observation (i.e. more than nine years after starting HAART). However, only 20 (35.1%) of the INRs were still under observation six years after study inclusion. After six years of observation 95.5% of the patients alive still had fully suppressed VL”.

- Discretionary Revisions
1. INR is awkward and is typically used in the US to refer to function of the coagulation cascade. Consider avoiding use of abbreviations to describe the
groups.

*Answer: The abbreviations INR (immunological non-responders) seems to be an established abbreviation used in Journals like CID and AIDS f. ex in:*


Answer to reviewer 2:

Engsig et al. attempted to identify risk factors for inadequate CD4 cell recovery and examined long-term mortality in HIV patients virally suppressed for more than three years. The questions posed by authors are well defined. The title and abstract accurately convey what has been found in the paper.

Major Compulsory Revisions:

Page 14, the authors state that “Our results clearly indicate that excess mortality seen among INRs is mainly related to prolonged immunological suppression prior to successful HAART and IDU”. I am not sure which results the authors were referring to. If they refer to results presented in the second paragraph of page 10, then I don’t think these are sufficient for this claim. The lack of significance after excluding 44% of subjects would very well be due to small sample size. Results based on the subgroup of 128 patients should also be presented to give a complete picture.

Answer: The MRR in the subgroup of 128 patients with more than one year from first CD4 measurement ≤ 200 cells/µL to start of the virologically suppressed period was 3.9 (95% CI; 1.5 – 10.6) and after adjustment for age and gender it was 3.6 (95%CI; 1.3 – 9.7). This has now been added under Results, page 11, second paragraph.
Similarly for the analysis relating to IDU, the fact that after excluding IDU subjects, the mortality risk was not seen to be statistically significantly increased does not necessarily lead to the conclusion that “IDU did account for a part of the excess mortality among INRs” (page 14). One would inevitably expect a wider interval with reduced sample size.

Answer: IDU accounted only for 5.9% of the IRs and 12.7% of the INRs. We believe that a reduction of the overall adjusted MRR from 3.4 (95%CI; 1.4 – 8.0) to 1.8 (95%CI; 0.6 – 5.1) when excluding the IDUs is considerable and indicates that part of the excess mortality among the INRs can be attributed to IDU. Correspondingly MRR on exclusively IDUs was 12.7 (95%CI; 1.5 – 109.9) and adjusted 9.8 ((95%CI; 1.1 – 86.1). The latter result has now been added under Results, page 11, second paragraph.

Minor Essential Revisions:

1. Time from first CD4 count ≤ 200 cells/μL to start of the virologically suppressed period (> 1 year vs. # 1 year) was identified as a risk factor for inadequate CD4 cell recovery. Please explain why 1 year was chosen as the cut point.

Answer: We chose a cut point based on a median time from first CD4 cell count ≤ 200 to start of the suppressed period of 0.7 year (IQR; 0.3 – 2.2) among IRs and 1.5 years (IQR; 0.4 – 3.2) among the INRs.

Similarly for age, 40 years old was used as the cut point, please describe the rationale behind this choice.
Answer: We chose a cut point based on the median age, which was 37.6 years (IQR; 32.1 – 45.3) for the IRs and 42.6 years (IQR; 36.1 – 51.3) for the INRs.

2. page 3, the second sentence in “Background” is incomplete. Please revise.

Answer: We thank the reviewer. The sentence has now been corrected to: “We aimed to identify predictors for inadequate CD4 cell recovery and estimate mortality in patients with low CD4 count but otherwise successful HAART.”

3. page 13, at the beginning of the second paragraph, it states that “19% of the study patients did not respond adequately to HAART”. I am a bit confused by this statement. How was “respond adequately to HAART” defined? Which result does this statement refer to? I thought all subjects were virally suppressed for three years? Please clarify.

Answer: All subjects were virally suppressed for three years. We agree that the statement “19% of the study patients did not respond adequately to HAART” is imprecise. We were referring to the insufficient immunological response to HAART among the 55 INRs (18.9%). The sentence has now been rephrased to: “19% of the study patients did not have an adequate immunologic response to HAART.” (page 14, second paragraph).

4. page 13, line -13, “Oppositely other studies” sounds a bit odd, please revise.
Answer: We thank reviewer. The sentence has now been revised and changed to “In contrast to other studies, we did not find a statistically significant association between IDU or chronic HCV infection and INR[3, 21]” (Discussion, page 14, third paragraph).

5. page 13, line -10, there is an extra “at” after “Nadir CD4 cell count”.

Answer: We thank reviewer. This has now been corrected.

6. page 14, line -8, are there words missing in “... development of e.g. cancer...”?

Answer: We thank reviewer. The sentence has been corrected to: “The increased mortality in patients with delayed initiation of HAART is well documented [27] and the contribution of persistent immunodeficiency to the development of e.g. cancer has also been observed by others [28].”

7. Figure 1, should “VL<51 copies/ml” be “VL<50 copies/ml” to be consistent with the text?

Answer: Thanks. This has now been corrected.

Discretionary Revisions:
I am curious to know why the authors did not assess whether time from first CD4 count ≤ 200 cells/μL to start of HAART as a risk factor, but assessed time from first CD4 count ≤ 200 cells/μL to start of virologically suppressed period. I did notice that in the discussion (page 14), the authors mentioned that “A longer period of immunological suppression prior to the three years of sustained viral suppression episode therefore is explained by delayed initiation of HAART and not poor compliance.” Why did the authors not assessing whether delayed initiation of HAART was a risk factor directly?

Answer:

*We choose to calculated time from first CD4 measurement ≤ 200 cells/μL to start of the suppressed period in order to get a more accurate estimate of time from first CD4 measurement ≤ 200 cells/μL to start of effective HAART. Also, we aimed to study the impact of a suppressed period as a surrogate marker of effective HAART.*

Age was identified as a risk factor. I wonder whether time since infection plays a role? That is, could it be that older people in the cohort having been infected longer? Could it be possible that it is not the absolute age, but longer time since infection to HAART initiation that led to inadequate CD4 recovery?

Answer: *Time since HIV infection include many problems, e.g.:*

1) *We only know time of HIV positive, not time of infection.*
2) Time from HIV positive to HAART is longer in those diagnosed before 1995, and thereby is strongly associated with pre-HAART HIV-diagnosis.

3) In case the patients have survived a long period with low CD4 count and no HAART it will mainly be in the pre-HAART period. This population will therefore potentially also benefit from some healthy survivor bias.

We therefore decided not to include this factor in our analysis.

It seems that it would be useful to use data from this cohort to address questions such as how delayed HAART or HAART initiation at various CD4 count can affect long-term mortality and/or other outcomes.

Answer:
We actually did partly perform these analyses. The KM above depicts the survival of 1)
Patients who do not reach a CD4 cell count above 200 after 3 years of viral suppression, 2)
Patients who reach a CD4 cell count between 200 and 350 after 3 years of viral suppression, 3)
Patients who reach a CD4 cell count between 350 and 500 after 3 years of viral suppression, 4)
Patients who reach a CD4 cell count above 500 after 3 years of viral suppression. As seen from
the KM the big difference is when patients do not reach a CD4 cell response above 200 after 3
years of viral suppression, which is why we pooled data on all patients with a response above 200
CD4 cells.

We have now included the following under Methods, page 7, last paragraph: “Mortality was also
stratified for more differentiated CD4 cell strata after 3 years of viral suppression (>200 cells/µL
and ≤ 350 cells/µL, > 350 cells/µL and ≤ 500 cells/µL and >500 cells/µL) but the prognosis differed
little for all strata above 200 cells/µL why we chose to pool all CD4 responses above 200 cells/µL
together.”
For the sensitive analysis where a cut-off value of 500 copies/ml for VL suppression was used, it would be helpful to report other key results parallel to when 50 copies/ml was used. For example, what are the risk factors identified in this case?

Answer: We have now identified risk factors for a cut-off value of 500 copies/ml for VL suppression and included the following under Results, page 11, last paragraph: “In the un-adjusted analysis age, Caucasian race, IDU, time from first CD4 cell count ≤ 200 cells/µL to start of the virologically suppressed period and CD4 cell count at start of the suppressed period were associated with immunological non-response. However, after having adjusted for potential confounders only age, IDU and CD4 cell count at start of the suppressed period remained associated with increased risk of being INR”. In the Discussion the following have been added: “However, when using a cut-off value of 500 copies/ml, IDU was associated with INR. This is probably due to the inclusion of a larger number of study patients with these characteristics when allowing for viral blips”, page 14, third paragraph.