Author's response to reviews

Title: Impact of Gender on Response to Highly Active Antiretroviral Therapy in HIV-1 Infected Patients: A nationwide population-based cohort study

Authors:

Kristina Thorsteinsson (kristina.thorsteinsson@gmail.com)
Steen Ladelund (steen.ladelund@hvh.regionh.dk)
Søren Jensen-Fangel (soejense@rm.dk)
Isik Somuncu Johansen (isik@dadlnet.dk)
Terese L Katzenstein (terese.katzenstein@rh.regionh.dk)
Gitte Pedersen (gitte.pedersen@dadlnet.dk)
Merete Storgaard (m.storgaard@dadlnet.dk)
Niels Obel (niels.obel@rh.regionh.dk)
Anne-Mette Lebech (lebech@dadlnet.dk)

Version: 3 Date: 3 October 2012

Author's response to reviews: see over
Dear Editor-in-Chief,

Thank you for having reviewed our paper, which presents findings from the Danish HIV Cohort Study, and for giving us the opportunity to revise our paper.

We have read all the valuable comments carefully and hope that we have addressed the reviewers’ concerns satisfactorily.

In summary, we believe the manuscript has benefited from the review process and we hope you will find it suitable for publication in the revised form. We are of course willing to make further changes if necessary.

Should you have questions or concerns regarding the manuscript, please do not hesitate to contact me.

Sincerely yours,

Kristina Thorsteinsson, MD, PhD-student
Department of Infectious Diseases
Hvidovre Hospital, Copenhagen University Hospital
Kettegaards Allé 30
2650 Hvidovre
Denmark
phone: (+45) 38 62 60 54
fax: (+45) 36 47 49 79
E-mail: kristina.thorsteinsson@gmail.com
Reviewer #1:

1) Why having excluded HCV-infected patients and IVDU? The explanations provided by the authors are not entirely satisfactory; they could have been taken into account by either adjustment or by introducing a term of interaction.

Reply: “In the present study we used a nationwide, population based cohort of heterosexual infected individuals to estimate gender differences in initiation of HAART regarding timing, regimen and modifications. Moreover, we aimed to estimate the effectiveness of HAART by means of viral load and CD4 count in genders in a setting with free access to HAART and healthcare”. We think there is strong evidence of a worse outcome in HIV-infected IDU’s not directly related to HIV infection (PloS One 2011;6(7):e22698, Addiction 2010 Mar;105(3):529-35, AIDS 2010;24:1537-48, Clin Infect Dis 2006 May 15;42(10):1481-7). Therefore in these patients the issue of gender/sex is difficult to address with so many possible confounders that could affect the course of disease. HCV coinfection patients were excluded since a large proportion of these patients is expected to be de facto IDU’s.

Change in manuscript:
Page 13, line 382-384: “Furthermore, due to concerns about residual confounding when adjusting for IDU in the model, we chose to perform restricted analyses with exclusion of this group of patients”.

2) Page 4, the description of HAART looks confusing, please reformulate.

Reply: The paragraph has been rewritten.

Change in manuscript:
Page 7-8, line 192-197: HAART was defined as a combination of antiretroviral treatment with at least three drugs, including at least one non-nucleoside reverse-transcriptase inhibitor (NNRTI) or a protease inhibitor (PI), and/or abacavir, or a treatment regimen with a combination of a NNRTI and a boosted PI [1].

3) Tables should not have vertical lines.

Reply: The vertical lines have now been removed.

Change in manuscript:
The vertical lines have been removed.

Reviewer #2:

1) This paper addresses an interesting question and I agree with the Authors that its major strength is its derivation from a large and well followed cohort with adequate and important links to the other National registries which help avoiding duplicates and other bias which may be so frequent in observational cohort studies.
Nevertheless, the methods and results are would benefit by a rearrangement in order to be better presented and clarified.

As stated in the Introduction, the aim of this study was “to estimate the effectiveness of HAART by means of viral load and CD4 count in genders in a setting with free access to HAART and healthcare.” The primary endpoint of the analysis should then be the effectiveness, in particular response to HAART and modification of HAART, and they should be given higher relevance in both methods and results section. These should be the first endpoints to be thoroughly presented and discussed.

**Reply:** We agree that the effectiveness of HAART is our primary focus, but we chose to follow the logical time course, when we built up the article, so that we study initiation, adherence and then the effectiveness of HAART. This order is observed throughout the article.

**Change in manuscript:**
None.

2) An interesting subanalysis including the evaluation of different initial regimens is announced in the methods section but the results are not clearly showed or described in the text.

**Reply:** The comparison of the initial antiretroviral regimens is shown in Table 1, in the “Result section” (Page 12, line 322-326) and in the “Discussion” (Page 14, line 404-410).

**Change in manuscript:**
None.

3) The study population limited to heterosexually infected people strongly limits the extensibility of the results and the sample size, patients with intravenous drug use as risk factor and HCV coinfection should probably be included, eventually keeping the original set as a subanalysis.

The Authors state in the discussion that they “excluded intravenous drug users and HCVcoinfected patients, since studies repeatedly report worse clinical outcomes [29-31] and weaker immunological and virological responses in these patients [7,32].” Anyway, just because the aim of the study was set on effectiveness, it looks odd to purposely avoid the most weak subpopulations excluding it from the analysis.

They also state that they “decided to focus on HIV positive patients infected heterosexually to study patients living in the same social context [5]” but in reference 5 only men who have sex with men were excluded from the analysis, so probably the inclusion of IDU and HCV coinfected patients does not have a strong impact on this.

**Reply:** Please see point 1 (1st reviewer).
Change in manuscript:
Please see point 1 (1st reviewer).

4) Moreover, patients with HCV coinfection were excluded while those with HBV coinfection were not, please justify. Finally, HBV coinfection has an impact on the decision to start antiretroviral therapy just as pregnancy or acute infection but it seems HBV-coinfected patients were not excluded from the analysis of time to HAART initiation, please comment on this.

Reply:
i) HBV coinfected patients differ from HCV coinfected patients. A study on HBV coinfected patients in the Danish HIV Cohort study show that only 9.6% of these are IDUs and their HBV coinfection has no impact on effectiveness of HAART (Omland LH et al. HIV Med 2008, 9: 300-306).

ii) We agree. All patients who started HAART before they were eligible were excluded from the initial analysis; thus also covering HBV-HIV coinfected patients initiating HAART due to treatment of hepatitis B. This information has now been added to the manuscript.

Change in manuscript:
Page 9, line 232-235: “The incentive for initiation of HAART during pregnancy, acute HIV infection and in HBV coinfection is different than in patients starting HAART due to an impaired immune system, i.e. low CD4+ count. In the analysis of time from eligibility to HAART initiation we therefore excluded patients initiating HAART before they were eligible for therapy.”

5) The paragraph would benefit by a reorganization; some definitions (in particular the first two paragraphs) are part of the description of your statistical analysis and could be moved to the “Statistical analysis” section.

Please leave/move all the “general” definitions (virological failure, undetectable viral load –which is currently included in the “statistical analysis” section - acute infection, definition of therapeutic regimens, reasons to start HAART, viroimmunological parameters and HBV coinfection, definition of “response to HAART”) in this section and move definitions of endpoints and criteria for the statistical analysis in the specific section.

Reply: We agree that these sections would benefit by a reorganization and the following two paragraphs have been moved from “Definitions” to “Statistical analyses”.

i) A patient was considered eligible for therapy the day they fulfilled one of the above mentioned national criteria concerning CD4+ count, HIV RNA or AIDS.

ii) The incentive for initiation of HAART during pregnancy, acute HIV infection and in HBV coinfection is different than in patients starting HAART due to an impaired immune system, i.e. low CD4+ count. In the analysis of time from eligibility to
HAART initiation we therefore excluded patients initiating HAART before they were eligible for therapy. Information on initiation of HAART due to non-AIDS defining HIV related disease was not available in the DHCS and therefore not assessed in the analysis of being eligible for start of HAART. When studying the actual number of patients initiating HAART during acute HIV infection this was defined as a patient with acute HIV infection initiating therapy during the first 3 months after HIV diagnosis.

Furthermore a definition of undetectable viral load and virological failure was moved/added to “Definitions”.

Change in manuscript:
Page 9, line 229-230: “A patient was considered eligible for therapy the day they fulfilled one of the above mentioned national criteria concerning CD4+ count, HIV RNA or AIDS”.

Page 9, line 232-235: The incentive for initiation of HAART during pregnancy and acute HIV infection is different than in patients with an impaired immune system, therefore in the analysis of time to HAART initiation from eligibility, pregnancy and acute HIV infection were not assessed. Information on initiation of HAART due to non-AIDS defining HIV related disease was not available in the DHCS and therefore not assessed in the analysis of being eligible for start of HAART. When studying the actual number of patients initiating HAART during acute HIV infection this was defined as a patient with acute HIV infection initiating therapy during the first 3 months after HIV diagnosis.

Page 8, line 204-205: “Virological failure was defined as such if the physician treating the patients’ HIV infection had stated virological failure as the reason for modification in the medical file.”

Page 8, line 207-208: “Undetectable viral load was defined as a plasma HIV RNA load of <500 copies/mL, which was the highest level of sensitivity for testing in the observation period.”

6) Please clarify how you defined acute HIV infection since criteria for its definition may vary in literature.

Reply: The following sentence has been added in “Definitions”.

Change in manuscript:
Page 8, line 208-209: “We defined acute HIV infection as clinical seroconversion with a positive Western blot pattern”.

7) In the sensitivity analyses women initiating HAART due to pregnancy, women were excluded if date of HAART initiation was within the period of one year before the conception (estimated as 37 weeks before delivery) or during pregnancy
Please move to the “statistical analysis” section and clarify why you choose to extend the definition to a period as long as one year before the conception: this may exclude patients who did not pre-plan their pregnancy and did not really start HAART for pregnancy but just accidentally got pregnant shortly after starting from the analysis of time to HAART initiation.

Reply: The sentence regarding pregnancy has now been moved to “Statistical analysis”.

In Denmark HIV-positive women are offered free fertility treatment. Before a woman can enter treatment offers, her HIV infection needs to be under control with a fully suppressed viral load and therefore we chose a relatively long time-span.

Change in manuscript:
Page 10, line 274-276: “In the sensitivity analyses women initiating HAART due to pregnancy, women were excluded if date of HAART initiation was within the period of one year before the conception (estimated as 37 weeks before delivery) or during pregnancy.”

8) We categorized treatment modifications into 3 groups: virological failure
   Please define virological failure as its definition may vary in literature.

Reply: Please see point 5 (2nd reviewer).

Change in manuscript:
Please see point 5 (2nd reviewer).

9) Statistical analysis
   Please clearly define primary and secondary endpoints of your analysis coherently with the aim of the study as defined in the introduction (it looks like “Cohort 2” analysis best describe the efficacy endpoints and therefore the aim of the study).

Reply: To clarify which cohort that are used to study the different endpoints an explanatory sentence has been added to the description of the cohorts in the “Statistical analysis”.

Change in manuscript:
Page 8, line 222-223: “Cohort 1 is used in the analysis of time from eligibility for therapy to HAART initiation”.

Page 9, line 226-227: “Cohort 2 was studied in all other analyses, but timing of HAART initiation”.

10) As the aim of the study was defined as “effectiveness” and categories of treatment modification were identified, follow-up should be also censored at change of HAART regimen for virological failure, toxicity or for any of the other categories which were defined or these events should be evaluated during the whole follow-up time. Clinical progression (new AIDS-defining event) was not evaluated, most probably because
of the previously published paper on this issue (Ref. 28). Anyway, if you choose to evaluate only effectiveness, I feel it would be better to censor the follow-up at virological failure/loss of virological response or at the last available follow-up, excluding all clinical endpoints, while if you choose to also evaluate clinical endpoints, clinical progression (new AIDS defining events) should be also assessed.

Reply: The analyses of effectiveness of HAART (where we look at the CD4+ count and proportion of patients with a suppressed viral load) are cross sections over time. Censoring of patients with a new AIDS defining event would mean that these patient’s counts were not included in the analyses and since they most often continue HAART during treatment of their AIDS defining event, we believe their counts should still be included.

Change in manuscript:
None

11) Five initial HAART regimens were assessed for analysis in this study: i) 3 nucleoside reverse transcriptase inhibitors (NRTI’s), ii) 2 NRTI’s + efavirenz, iii) 2 NRTI’s + nevirapine, iv) 2 NRTI’s + PI/ritonavir or PI and v) other HAART regimen. There’s a great difference in potency, genetic barrier and efficacy of boosted and unboosted PI regimens, so they should not be evaluated together, more so if you choose to analyze separately Efavirenz and Nevirapine. A definition of the possible combination in the “other” HAART regimens would be useful.

Reply: The primary endpoint of this article is not to access differences of potency in the different HAART regimens.

Change in manuscript:
None.

12) Undetectable viral load during follow-up (defined as a plasma HIV RNA load of <500 copies/mL, which was the highest level of sensitivity for testing in the observation period). This should be moved to the “definition” section.

Moreover, even though it is correct that 500 copies/mL is the highest level of sensitivity in the study period if the definition of virological failure was based on this cut-off (to be clarified, as stated above) this definition could have contributed to underestimate the number of virological failures.

Reply: A definition of virological failure and of undetectable viral load has been added/moved to “Definitions”. Please see point 5 (2\textsuperscript{nd} reviewer).

As stated in point 5 (2\textsuperscript{nd} reviewer) data on virological failure is based on the discretion of the treating physician and therefore the viral load available at that time.

Please also see point 13 (2\textsuperscript{nd} reviewer).

Change in manuscript:
13) Results/Discussion
Only 5 (1.3%) women and 4 (1.5%) men switched HAART regimen because of virological failure and therefore this aspect was not further explored. This result is surprising, particularly if we consider that almost half of the study population has started HAART in the early HAART era, when virological failures were frequent and led to broad cross resistance. When we look at the proportion of patients with an undetectable viral load 1, 3 and 6 years after the initiation of HAART (page 8) the data seem to indicate higher proportions of patients with non suppressed viral load. The definition of virological suppression, virological failure and the highly selected patient population could have influenced this result.

Please discuss it thoroughly.

Reply: A comment on virological failure has been added in “Discussion”.

Change in manuscript:
Page 16, line 452-454: “Moreover, data on virological failure is based on the discretion of the physician and might therefore not reflect the actual number of virological failures.

14) Compared to other cohorts [9] where HIV RNA was undetectable in 48-79% of all patients a relatively high proportion of our patients (83% of women and 92% of men) had undetectable viral load after 1 year. In Ref. 9 the definition of “virological suppression was different (“Virological Suppression (<50 copies/ml), virological rebound (>500 copies/ml)”), so these results are difficult to compare.

Reply: A comment on this matter has been added.

Change in manuscript:
Page 14, line 395-396: “...though, different definitions of viral suppression make studies difficult to compare”.

15) Along with others [9], we found that women were less likely to receive a HAART regimen containing efavirenz. Since the publication of teratogenicity in animal studies [38] and of neural tube defects in infants exposed to efavirenz in their first trimester [39] guidelines have recommended avoiding this drug in women likely to conceive. Indeed, after excluding women who initiated HAART due to pregnancy from our analysis the initial HAART regimen did not differ significantly between genders. Presumably, in time this difference will even out, since prospective data from the Antiretroviral Pregnancy Registry cannot retrieve excess number of birth defects in infants exposed to efavirenz. Even though no significant increase in the prevalence of birth defects was observed in a Pregnancy Registry in South Africa (the reference is missing –and should be included- but I guess the Authors meant to cite Bera E, et al., AIDS. 2010 Jan 16;24(2):283-9), the Authors of this paper still conclude that “the limited number of first trimester EFV-exposed infants precludes..."
definitive conclusions on the teratogenicity or safety of EFV”. This paper was published in early 2010 and no guideline has changed the warnings against the use of EFV in the 1st trimester of pregnancy thereafter, so I’d suggest caution in such a statement as the one I evidenced in bold.

Reply: The following sentence has been deleted from the manuscript: “Presumably, in time this difference will even out, since prospective data from the Antiretroviral Pregnancy Registry cannot retrieve excess number of birth defects in infants exposed to efavirenz.”

Change in manuscript:
Please see above.

16) Regarding pharmacokinetics on HAART, data are limited, but current evidence suggests that gender differences exist [40]... Some have speculated that the higher rate of adverse effects in women are caused by a higher drug exposure in women [33]. PK is a very interesting and relevant topic to address when looking at gender differences in the efficacy of HAART. Anyway, as you couldn’t examine this aspect in your work (I guess data on PK should be increasing, but very limited and recent in the follow-up time of this study population), I would limit the extension of this paragraph and eventually add a short statement in the limitations, if you want to dwell on this subject.

Reply: In our study we look at gender differences in toxicity and we would therefore like to list pharmacokinetics as one of the possible explanations for gender differences reported.

Change in manuscript:
None.

17) MINOR ESSENTIAL REVISIONS
Introduction “gender differences on HIV therapies have been reported including (i) time of HAART initiation [1], (ii) adherence and toxicity to antiretroviral drugs [2-4], and (iii) virological and immunological response to HAART [2,5-9].”
Ref.2 states that “Data on drug response suggest similar response of treatment and similar outcomes in men and women, but female subjects appear to be more susceptible to adverse events related to antiretroviral treatment” while the slightly lower VL has been reported in untreated women, so it is pertinent for point (ii) regarding adherence and toxicity but not for point (iii) Ref. 6 states that “No differences were found between genders in terms of virological and immunological outcomes during long-term HAART. Nevertheless, a lower risk of clinical progression was reported among female patients with intermediate baseline viral load than in males”, so this should be considered as not pertinent unless you include the risk of clinical progression in your definition (and it still happens in specific conditions). Ref. 7 states that “The immunovirological response to treatment did not differ according to gender, but was better in homosexual patients than in patients in other categories. Injecting drug users had the weakest immunovirological responses.
Clinical outcome was not related to gender or to HIV transmission group so the difference in the response to cART is not related to gender but to the transmission risk factor and the reference seems not pertinent. Ref. 8 has been recently published (Soon GG, et al., AIDS Patient Care STDS. 2012 Aug;26(8):444-53. Epub 2012 Jun 26), so you can update it. In fact, this is in contradiction with a more correct statement following almost at the end of the introduction and citing almost the same studies ("Most studies report no gender-related differences in terms of virological and immunological response to HAART, however data are conflicting [2,5-8]")

Reply: Reference 8 has now been updated. Barber et al and Ko et al alone are used as references on virological and immunological response to HAART.

Change in manuscript:
References updated.

18) Most women with HIV in Europe and the US are of childbearing age [11,12]. As Ref. 11 is mostly related to the desire of childbearing than to the real prevalence of women of childbearing age in a population I would add a statement regarding the desire of pregnancy in the sentence to improve the pertinence of this citation.

Reply: A statement on the desire of pregnancy has been added.

Change in manuscript:
Page 5, line 116-117: “and the intention for childbearing is high in this population”.

19) Several cohorts studying gender differences mainly focus on surrogate markers of disease progression such as viral load and CD4 count [13,14], because of the significant reduction in mortality and rate of disease progression following HAART [15,16]. Both ref 13 and 14 also include the clinical outcome (hospital admissions, time to new AIDS and death).

Reply: The sentence has been modified.

Change in manuscript:
Page 5, line 121-123: Because of the significant reduction in mortality and rate of disease progression following HAART [15,16], surrogate markers of disease progression such as viral load and CD4 count have been introduced [13,14].

21) Our definition of virological response considered viral suppression as reduction to below 500 copies/mL, which might have underestimated the prognostic benefit of HAART. Please clarify this sentence.

Reply: The sentence has been deleted.

Change in manuscript:
22) Methods
   Setting
   Denmark has a population size of 5.5 million and an estimated HIV prevalence among adults of 0.09%.
   Please provide a reference for these data

   **Reply:** The references have now been provided.

   **Change in manuscript:**
   Page 6, line 139: “a population size of 5,6 million”.

   Page 6, line 140: “HIV prevalence among adults of 0.1%”.

23) National criteria for initiation of HAART are: (i) acute HIV-infection, (ii) HIV-related disease or an AIDS defining illness (ADI), (iii) pregnancy, (iv) until 1 May 2008 a CD4 count below 300 cells/\(\mu\)l and hereafter a CD4 count below 350 cells/\(\mu\)l and (v) until 31 December 2001 HIV-1 RNA > 100,000 copies/mL.
   Please provide a reference for these guidelines

   **Reply:** A reference has now been provided.

   **Change in manuscript:**
   Page 6, line 147: “[20]”.

24) Results
   Figure 2. Please detail the label of the Y axis

   **Reply:** The Y axis has been labelled.

   **Change in manuscript:**
   Please see above.

25) Figures 4 and 5. It is difficult to distinguish the lines representing the two genders, please use a clearer representation (the one used for figure 2 could be ok). Please add units (% for figure 4 and cells/\(\mu\)L for figure 5) to the Y axis.

   **Reply:** Figures 4 and 5 have been revised.

   **Change in manuscript:**
   Please see above.