Author's response to reviews

Title: Early microstructural white matter changes in patients with HIV: a diffusion tensor imaging study

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Author’s response to reviews: see over
Dear Editor

Thank you for letting us resubmit a revised version of our manuscript “Early microstructural white matter changes in patients with HIV: a diffusion tensor imaging study?”

We would like to address in detail the issues raised by the reviewers. Changes in the manuscript are marked.

The following criticisms have been raised by reviewer 3.:

Major compulsory revisions:

1. The control group is age-distribution matched. There are only male patients, but the control group is mixed. There are several papers reporting on the differences in FA-values between man and women (e.g. Menzler et al. 2010, Neuroimage). I would suggest discussing this “mismatch” somehow and explaining, why there’s no impact on the result. (page 4)

Twenty-two patients (1 female, 21 males) originally participated in the study. However, two were excluded due to extensive intracerebral lesions on MRI scans. Gender was not an inclusion criteria.

Different to Mezler et al we employed an iterative multicontrast registration approach. In a cohort of 33 males and 30 females we couldn’t find any significant voxel-based FA difference between genders in regions discussed in the present paper. For example, the FA averaged over the whole WM for males is 0.3570 and for females 0.3567 with a standard deviation of about 5 % in each group [1]. Because of this lack of systematic FA difference between genders - also in other WM regions - we didn’t regard gender as a relevant factor for WM FA differences between groups - if the employed spatial registration approach is used. However, we found age-dependent influence on FA [2]. Therefore we considered only a match of age-distribution as relevant but didn’t matched the groups for gender. If it will be still considered as important by the reviewers, we will, of course, change the control group to a “gender matched” group.

We added In a previous study we could detect age dependent differences on FA but no differences between gender [1].

2. The “new finding” of similar regions with reduced FA values for patients with and without macroscopic lesions could be discussed in a little more detail (e.g. are there any DTI studies with similar patients or different regions of interest) because of this “interesting” new result in correlation with its possible benefits (page 11).

We agree that this is an important aspect. The problem is that literature about this topic is rare. To our knowledge there is no study focusing directly on this aspect.
We added Chang et al. found greater than age-related changes in brain diffusion of HIV patients after 1 year although no comparison was made with patients with visible lesions (Stebbins et al., 2007), (Chang et al., 2008). Chen et al. (2009) identified widespread abnormal regions in HIV patients with and without dementia, although a greater distribution was observed in patients with dementia [3]. Gongvatana identifying changes in white matter tracts associated with more advanced HIV infection [4].

Minor essential revisions:

3. The authors clearly motivate the use of diffusion tensor imaging as possible predictive method for development of clinical symptoms and according implications of treatment. The first goal (early detection of white matter alterations) is clearly motivated and stated. The second goal could be stated more precisely also in correlation to the first goal. (page 3-4)

We added The positive effect of HAART on HIV induced cerebral lesions has already been shown in conventional MRI [5].

4. Data acquisition is only described for diffusion tensor imaging. As also structural MRI was used for including/excluding patients for this study, acquisition parameters for these measurements should be declared somehow (if done for all patients in the same way). At least, the MRI system and field strength should be listed. (page 4)

We changed and added: 2.2. Magnetic resonance imaging
All MRIs have been acquired on a Philips Intera 3 T scanner (Best, The Netherlands). Structural images were acquired by 3D T1w (1.0 x 1.0 x 1.0 mm$^3$), T2w, and FLAIR sequences...128 x 128, 36 axial slices, voxel size.....

5. In section “Diffusion tensor Imaging”, VBS is used as abbreviation but not introduced before (page 4)

We changed this: voxel based statistics (VBS)

6. SPM is mentioned in section “DTI”, a reference should be inserted and the abbreviation should be introduced (is done later on page 6).

We added: to the statistical parametric mapping (SPM, http://www.fil.ion.ucl.ac.uk/spm/) EPI template
And deleted later on.

7. Results of all ROIs for patients vs. controls are presented, but the ROI “brain stem” is missing in this part for goal 1, FA values are mentioned in table 2 (page 7, top)
Results of the ROI brain stem is missing because it does not reached significance.

8. Line 5 after Figure 3: “who were not” should be replaced by “who not” (page 7)
9. “FA reduction” instead of “FA reduction increase” in Section 3.2. (page 8).

We changed it

10. A p-value is missing for comparison of HIV patient with neuropsychological evidence for HIV associated neurocognitive disorder and HIV patient without evidence showing a significant widespread FA reduction (page 8)

Sorry for that. We added the p-value had significant widespread FA reduction without preference of a specific region \( (p<0.01) \)

11. “FA reduction” instead of “FA-reduction” for uniformity (page 9, line 8)
12. “mean diffusivity” instead of “man diffusivity” (page 9, section 4.1, line 7)
13. “employs” instead of “employ” (page 9, section 4.1, line 8)
14. The abbreviation MD for mean diffusivity should be introduced (page 9)
15. “reach” instead of “reached” (page 9, section 4.2, line 10)
16. “HIV patients” instead of “HIV-patients” for uniformity (page 11, section 4.4, line 16)


17. In the conclusion lesions and depression are mentioned, but some concluding result according to neurocognitive impairment is missing.

We added Patients with HIV associated neurocognitive disorder show an overall FA reduction suggesting no uniform structural patterns between patients.

18. Table 1: in the legend with “ew” there a mistake in writing “medication”, and some abbreviations should be introduced like “HAART”, “CDC” and “CD4”, maybe also at least a reference for HIV infection classification should be given for values “B3, C2 and so on”. Furthermore there are 3 times “?” and several times “unknown” in this table. Maybe in the Methods part there should be explained why, or which impact this has.

We added HAART= Highly Active Anti-Retroviral Therapy; For classification see for example http://www.aids-ed.org/aidsetc?page=cg-205_hiv_classification
19. Figure 1: the left picture should be labeled with “A” the right one with “B” according to figure legend.
20. Figure 2: the subimages should be labeled with “1” to “5” according to the figure legend, or within the figure legend insert “(from left to right)”
21. Figure 3: in the Figure legend add something like “for all defined ROIs”. Additionally, the same scale for all results would be good.
22. Figure 5: in the figure legend, line 2, “9” should be written as “nine” for uniformity

We labelled the figures and added 20. from left to right
We added...of all priori defined ROIs.... We abstained from changing the scales due to optical resions. The diagrams would become very small with in part much free space per diagram and chanced “9” to nine.

Discretionary Revisions:
23. The period of data acquisition should be declared somehow (page 4)

We added between November 2004 and January 2006

24. It would be interesting to give information on the more “liberal” thresholds used for the figures, in relation to the “real” ones. (page 6)

For figures we used more liberal (uncorrected) thresholds (The color coded t-values represent significant \( p < 0.01 \) FA reductions in the patients compared to the controls (minimum cluster size: 100 voxel) to show the extent of WM alterations, but all illustrated clusters achieved significance at corrected statistical thresholds.

25. In the conclusion it could be added something like “DTI detects early microstructural WM alterations in contrast to structural imaging techniques in HIV patients” to underline its potential power.

We chanced the first sentence of the conclusion
DTI is more sensitive to detect early microstructural WM alterations in HIV patients than structural imaging techniques.
The following criticisms have been raised by reviewer 2:

1. In section 2.1 it seems a bit atypical to use an entirely male patient sample and include 4 females in the control group considering that there are studies, such as [1,2] which suggest a gender difference in FA values. A brief explanation in the discussion may be sufficient to explain it?

   This important issue was raised by reviewer 1, too. We answered to it in point 1 of reviewer 1.

2. In section 2.1 test scores for many of the examinations would be useful to the reader. Particularly interesting would be to present the reader with a table summing up details about neuropsychological and depression tests (and possibly their correlation with FA).

   We agree that this would be interesting. But we did not want to go beyond the scope of the paper. Therefore, for details about the neuropsychological test we would like the reader to reffer to the literature [6-13].

3. In section 2.2, for the MRI measurements, it would be interesting to know the number of slices and their orientation.

   We added: TR=9.8 s / TE=95 ms, acquisition matrix: 128 x 128, 36 axial slices, voxel size: 1.8 x 1.8 x 3.6 mm³ (reconstructed to 0.89 x 0.89 x 3.6 mm³), 2 averages, scanning time 7:46 min).

4. In section 2.2, it is not entirely clear to me the steps involved in the optimized registration technique. A more exhaustive and clear explanation would help the reader to understand the methodology.

   For methodological details we referred to previous methodological paper describing the technique [14-16].

5. In section 2.2, five regions of interest are taken into consideration. It would be interesting to know how these ROIs were chosen and on which basis. Are they based on the authors' previous findings? Are they based on previous papers? On a pre-study?

   We defined regions which are relevant in the context of the disease orientated at the existing literature. These regions of interest (ROIs) were a priori defined to assess the specificity of regional FA alterations.

6. In section 3.1, Table 2 refers to FA values with an atypical representation (x1000). I would prefer to stay in line with the journal style which seem to adopt the original FA value between 0 and 1.

   We changed to the original FA values
7. In section 3.1, I would like to suggest the authors to include, as well as FA, also mean, axial, and radial diffusivity which give very useful and complementary information about white matter structure. This is especially useful when comparing results of other studies mentioned in this paper, in which mean diffusivity is taken into consideration (section 4.2).

We found no qualitative differences in the results between RD (radial diffusivity) and FA therefore we did not mentioned it. Wherever the MD gives additional information it is mentioned in the text.

8. In the abstract, the last line should probably read: “Furthermore, they suggest a biological rather *than* a reactive origin of depression in HIV patients.”

We added “than”

9. In section 2.1 the age range “range 26 to 56 years” is repeated twice.

We changed it,

10. Although commonly used in neurology papers, the terms CNS (section 2.1), CSF (section 3.1) and MD (section 4.2) should be defined.

We added opportunistic infections of the central nervous system (CNS); central spinal fluid (CSF) and mean diffusivity (MD)

11. In section 4.1, I believe that in the phrase: “There are additionally differences in the nature of WM abnormalities, given that some studies have preferentially observed man diffusivity alterations whilst other studies report multiple FA changes.” man should be substituted with main.

Sorry for the mistake. We changed it.

12. In section 4.4, I believe that “unnatural courses” should read “unnatural causes”.

Yes, we changed it.
The following criticism was raised by reviewer 1:

1) Results reported here using ROIs are not corrected for multiple comparisons. Authors should change the significant threshold according to the number of ROIs used in their analysis. Because 5 ROIs were used only results greater than 0.01 should be considered statistically significant.

Analysis of variance was done with Greenhous Geisser correction.

2) I could not find Table 1 reporting socio-demographic characteristics of the population anywhere. Could authors make sure that this table is included in the main manuscript please?

Because of the format it is an additional file. Sorry for that.

3) Could authors also report educational level for their subjects, please? Moreover, could they report p-values for sex distribution in the HIV patient and control groups please?

Sorry, we do not have the educational level for the subjects. For the sex distribution please see answer one to the criticism of reviewer 3.

4) Could authors explain the rationale behind ROIs selection, please?

Please see answer five to reviewer 2.

5) Could authors show scatterplots of the correlation between WM measures and laboratory test parameters, please? Moreover, could they specify whether a 1-tail or a 2-tails Persons test was used?

We did not want to go beyond the scope of the paper. Therefore we did not show the scatterplots in the paper. We used 2-tails Pearsons test.
6) Typo at page 9 of the manuscript, “4.1 WM abnormalities in patients with HIV”. Authors should change “man” with “mean”.

We did, thank you.
Reference List


