Author's response to reviews

Title: Early detection of secondary damage in ipsilateral thalamus after acute infarction at unilateral corona radiata by diffusion tensor imaging and magnetic resonance spectroscopy

Authors:

Chuo Li (lichuo0529@126.com)
Xueying Ling (lingxuey@163.com)
Sirun Liu (tlsr@jnu.edu.cn)
Anding Xu (andingxu@gmail.com)
Yusheng Zhang (yszhang100@yahoo.com.cn)
Shihui Xing (xingsh2006@yahoo.com.cn)
Zhong Pei (peizhong@mail.sysu.edu.cn)
Jinsheng Zeng (zengjs@pub.guangzhou.gd.cn)

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Author’s response to reviews: see over
Dear Prof Jorge Serrador and Miss Angelina Ilievska,

Thank you very much for providing us with a chance to revise our manuscript entitled “Early detection of secondary damage in ipsilateral thalamus after acute infarction at unilateral corona radiata by diffusion tensor imaging and magnetic resonance spectroscopy” (1253214881477765). We are grateful to your and the reviewer’s critical yet constructive comments and thoughtful suggestions. We have considered these comments and suggestions very carefully and have revised the manuscript accordingly. We have highlighted all changes with red underlines in the revised manuscript. Enclosed please find the revised manuscript and our point-to-point responses to you and the reviewer. The revised manuscript was corrected with a native English speaker. We believe that the revision has improved the manuscript, and look forward to your favorable consideration.

With best regards,

Sincerely yours,

Jinsheng Zeng, MD, PhD,

Department of Neurology and Stroke Centre,

The First Affiliated Hospital, Sun Yat-Sen University,

No. 58 Zhongshan Road 2, Guangzhou, 510080, China.
The following are our point-to-point responses to the reviewer’s comments.

**Responses to the Reviewer:**

**Comment:** The paper “early detection of secondary damage in ipsilateral thalamus after acute infarction…by DTI and MRS” is an interesting study that after revision should be suited for publication in BMC Neurology. 2D MRSI data must be presented in an alternative, more quantitative, manner, to demonstrate that the reported metabolite change (NAA/Cr decrease) reflects an NAA decrease rather than a Cr increase (this is certainly suggested by Fig.2!).

**Response:** We appreciate reviewer for comments and constructive suggestion. To demonstrate the NAA/Cr decrease reflects an NAA decrease rather than a Cr increase, we have replaced NAA/Cr and Cho/Cr with peak areas of NAA, Cho and Cr in thalamus included in all spectral maps.

**Comment:** The authors must assess the correlations between the NAA decrease and MD increases (FA decrease), etc. The results of that will greatly enhance the content of the discussion. Most probably NAA decrease is significantly correlated with MD increase, phenomena of neuronal degeneration. Presuming that more than a single MRS voxel fits in thalamus, it would be nicest if that correlation could be made on a voxel by voxel base using methodology as described in: Irwan R, et al.. Correlation of proton MR spectroscopy and diffusion tensor brain MR imaging. Magn Reson Imaging 2005;23:851-858. The results must be discussed in comparison with DTI/MRS correlations reported elsewhere

**Response:** We thank reviewer for the good suggestion. In the revised manuscript, the associations between MD, FA and peak area of NAA, Cho and Cr were assessed with Spearman correlation analysis. A significant negative correlation between MD and NAA at W1, W4, and W12 was found. It suggests that neuronal structure damage indicated by increased MD was approximately coincident with neuronal metabolism damage indicated by decreased NAA. This consistency of neuronal structure loss and dysfunction suggests a phenomenon of neuronal degeneration in the ipsilateral thalamus. Sijens PE and colleagues also reported a strong correlation between NAA and ADC in ppMS patients and controls( *Eur Radiol* 2005;15:1686-1693; *Magn Reson Imaging* 2005;23:851-858). Our finding is in agreement with their results. In addition, FA correlates positively with NAA at W1, but showed no correlations at W4 and W12, suggesting that FA cannot reflect secondary damage process indicated by increased MD and decreased NAA in the ipsilateral thalamus. Cho showed negative correlations with MD, suggesting a constructive role of Cho in the preservation of neuron structures (*Eur Radiol* 2005;15:1686-1693). It is in agreement with a previous study on healthy controls in which a negative correlation of MD with Cho was shown (*Magn Reson Imaging* 2005;23:851-858). Moreover, MD showed negative correlations to Cr suggests integrity of structure indicated by MD was associated with
neuronal energy use and storage indicated by Cr in thalamus (*Neurology* 2006;66:540-544).

We added the above content in the revised manuscript in Page 16 line 17-22, Page 17 line 1-11.

**Comment:** The English has to be corrected throughout the paper.

**Response:** Thank you for the suggestion, the paper was corrected by a native English speaker.

**Comment:** Abstract: The results description can be concensed without loss of content.

**Response:** Thank the reviewer for the suggestion. In the revised manuscript, we have deleted the numerical values shown in results and added “Spearman's rank correlation analysis revealed a significant negative correlation between MD and the peak area of NAA, Cho, and Cr at W1, W4, and W12 and a significant positive correlation of FA with NAA at W1.” in Page 2 line 21-22, Page 4 line 1.

**Comment:** Halfway page 5: Dissimilar …. DTI: unclear sentence.

**Response:** We revised the sentence in Page 6 line 14-18.

**Comment:** P6, subjects: replace the two last sentences by: Controls were 12 healthy age-matched volunteers recruited over the same period examined once by DTI and
Response: Thank you very much for suggestion, we have replaced the last two sentences by “Controls were 12 healthy age-matched volunteers recruited over the same period examined once by DTI and MRS.” in Page 8 line 1-3.

Comment: P7, MRI protocol, end: How many of the 32x32 MRS voxels fitted in the left and right thalamus? An image with spectral map must be included in Figure 2.

Response: We appreciate reviewer for this constructive suggestion. MRS data is obtained from 3 adjacent voxels (0.75x0.75x1 cm$^3$ each) at each side of thalamus of every patient or control. The images with spectral map are shown in Figure 2A-B.

Comment: P8, All peak areas must be calculated relative to an internal reference, for instance the NAA signal in an unaffected brain area included in all spectral maps. This enables the reader to discriminate NAA changes from any change in Cr or Cho.

Response: We appreciate reviewer for this constructive suggestion. In this study, peak areas of NAA, Cho and Cr in thalamus were measured. DTI and MRS parameter values in the contralateral thalamus were stable during the observation period, and thus can be regarded as an internal reference to calculate asymmetry indices. Asymmetry indices (ipsilateral/contralateral to the ischemic lesion) of MD, FA values and of peak areas of NAA, Cho, Cr obtained in patients were calculated according to Hervé et al.’s method (J Neurol Neurosurg Psychiatry 2005;76:200-205). This enables us to discriminate NAA changes from any change in Cr or Cho, and to
exclude hypertension effect on MRI data.

We added the above content in the revised manuscript in Page 10 line 17-20, Page 12 line 17-22, Page 14 line 2-14.

**Comment:** Results (p9): Include the assessment of any correlations between DTI and MRS parameters (or changes in these parameters relative to the control values), preferably on a voxel by voxel base (see general comment) or else for the 12 patients and 3 time points.

**Response:** Thank the reviewer for the suggestion. The associations between MD, FA, and the peak area of NAA, Cho, and Cr were assessed with Spearman correlation analysis for the 12 patients at W1, W4, and W12. A significant negative correlation of MD with NAA, Cho, and Cr at W1, W4, and W12, and a significant positive correlation of FA with NAA at W1 were found. There was no significant correlation between FA and NAA at W4 and W12, between FA and Cho at W1, W4, and W12.

We added the above content in the revised manuscript in Page 10 line 20-22, Page 13 line 2-10.

**Comment:** Discussion: See general comments.

**Response:** The associations between MD, FA, and the peak area of NAA, Cho, and Cr were discussed in Discussion section in Page 16 line 17-22, Page 17 line 1-11.

**Comment:** Fig.2: include spectral map allowing for an assessment of data quality and
showing the thalamus structures. Are the somewhat noisy spectra shown those of a single 0.75x0.75x1cm³ voxel?

**Response:** We appreciate reviewer for this constructive suggestion. The spectral maps showing thalamus structures and voxel location are included in Figure 2. To reduce the noise, automated shimming and water suppression was performed. It was impossible to eliminate noise completely although we tried to reduce the noise to the lowest. All of the patients and controls were underwent MRI examination under the same condition, and the influence of noise may partly offset when comparing MRI parameters in ipsilateral thalamus with that in contralateral thalamus in patients, and in patients with that in controls.

**Comment:** Table 2: replace NAA/Cr and Cho/Cr by three lines for Cho, Cr and NAA.

**Response:** Thank you for pointing out the insufficiency. Table 2 was modified in the revised manuscript accordingly.
The following are our point-to-point responses to the editor’s comments.

**Responses to the editor:**

**Comment:** The authors did a good job of highlighting the cardiovascular risk factors in the patient population. Did the control groups have similar cardiovascular risk factors or where they free of risk factors? Since previous data has demonstrated that cerebral blood flow regulation is affected by both hypertension and diabetes, comparing to a control group with similar risk factors could provide important data on whether differences in values were due to the premorbid conditions prior to infarct.

**Response:** We appreciate editor for the comments. Among the patients, ten of 12 had hypertension, none had diabetes mellitus before admission. However, there was no hypertension or cardiovascular risk factors in controls. It is a limitation that no hypertension controls were paired with patients, so effects of hypertension could not be ruled out. However, stable DTI and MRS parameters value were shown in the contralateral thalamus during observation period, suggesting that changes of MD and NAA in the ipsilateral thalamus to the infarction were a result of stroke.

**Comment:** Data in Table 2 would be much clearer if displayed in bar graph format. In addition, were there any differences between sides in the control subjects?

**Response:** Thank you for the suggestion. In this study, asymmetry indices comparing MD, FA, and peak areas of NAA, Cho, and Cr in thalamus were evaluated to reduce inter-patient and inter-exam variability. By using this method, effects of hypertension or other factors on MRI data may be ruled out during observation period. So we added
asymmetry indices data in Table 3. Thus, data in Table 3 instead of Table 2 were displayed in a bar graph format shown in Figure 3. There were not significant differences for MD, FA, NAA, Cho and Cr between the left and right thalamus in the control subjects. The data for MD, FA, NAA, Cho and Cr in the left and right thalamus in controls were shown in the revised manuscript in Page 11 line 16-22.