Reviewer’s report

Title: Diffusion Tensor Imaging in Neuropsychiatric Systemic Lupus Erythematosus

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Reviewer: Ping-Hong Yeh

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Summary. This MS reports results of DTI image analyses comparing neuropsychiatric systemic lupus erythematosus (NPSLE) subjects, SLE patients without NPSLE, and healthy controls. The NPSLE subjects exhibited a significant difference from the SLE subjects and healthy controls, distributed mainly in the body of corpus callosum, corona radiata, and forceps major in the brain.

Critique. The MS uses a relatively novel TBSS analytic approach towards diffusion imaging that provides a broad look at the integrity of white matter tracts in NPSLE. Certainly, the use of DTI complements other imaging analyses in NPSLE. Limitations in the study diminished enthusiasm somewhat.

- Major Compulsory Revisions

1. The DTI acquisition and other parameters needs to be clarified: What sequence was used for DTI? Single-shot EPI? Fat or water suppression What was the matrix size of acquisition? What was the b value? How many non-diffusion weighted images were acquired?
2. DTI post processing
   a. It is not clear how the two sets of DTI images were averaged. Any alignment was prescribed for correcting motion artifacts before averaging? What algorithm was used for tensor fitting? linear least square, non-linear or something else?
   b. TBSS method: Did the authors use the TBSS module in FSL package for the analysis? If so, what version of TBSS and FSL was used?
   c. For the FA TBSS analysis in FSL, each FA image was “non-linearly” warped to the template or the most representative FA image (target image) in the study population, and the transformation matrices were then applied to MD and other non-FA images. The text on page 6 stated “All subjects’ fractional anisotropy (FA) and mean diffusivity (MD) images are aligned to a target image, then the entire dataset is aligned into 1x1x1mm3 MNI152 space” did not reflect correctly the methods used in the TBSS module of FSL, unless the authors had modified the method. If that is the case, the methods need to be stated clearly.
   d. Similarly to “c”, only the mean FA image was “skeletonized” in the TBSS
module of FSL, and all the MD images are projected onto the original mean FA skeleton images, i.e. using the original FA, but not the MD, for finding the projection vectors. If the authors used the FSL package without modifying the methods of FSL toolboxes, the statement on page 6, “The mean FA or MD image is “skeletonized” to reflect common tracts across all subjects, and individual subject data are projected onto the skeleton.” would be incorrect and need to be rewritten.

e. What was thresholding value chosen for skeletonized FA?

3. Statistical analysis

a. What were the age ranges of each group, i.e. NPSLE, SLE, and healthy controls? The wide age range (18-60) with only 17-20 subjects/groups risks introducing artifacts. Even though there was no significantly different in age between three groups, the confounding effect introduced by age (which is a known confounder in DTI measures), and possible the gender, would needs to be considered in GLM model.

b. It is not clear in the statement “Our approach was to first compare NPSLE and SLE patients to controls, then acute NPSLE patients to controls, and finally acute NPSLE patients to SLE patients.” on page 6 whether 3 levels in the “group” predictor in a GLM analysis with post-hoc group comparisons was used, or a 2 levels (2 sample groups) GLM modeling, repeated in 3 separate models were applied for evaluating the three group differences. In addition, the degrees of freedom in GLM for FA or MD group comparisons need to be reported in the result session in order to justify the correct use of statistical models.

c. It is unclear whether the final statistical significance was referred from the parametric or non-parametric statistical analyses. First, the statements of three P values on page 6 are redundant. The statement of “voxelwise cross-subject statistical analyses are performed using the general linear model, with (FWE) corrections being made for multiple comparisons (p<.05). Additionally, we conducted 5000 Monte Carlo simulations to determine whether our results could have arisen by chance (p <.005)” indicates both parametric and non-parametric tests were used. However in the later sentence, “5000 Monte Carlo simulations to determine whether our results could have arisen by chance (p <.005) “ and “Only data that passed both FWE and Monte Carlo simulations and was significant at p < .01”, indicating a non-parametric permutation test using 5000 Monte Carlo simulations and only FWE-corrected P value less than 0.01 are accepted, or maybe something else. Furthermore, the “p <.005” is confusing and irrelevant. Secondly, it is not clear whether “voxel-based” or “cluster-based” thresholding was used for correcting for multiple comparisons in the permutation test. In the text, it states “Only data that passed both FWE and Monte Carlo simulations and was significant at p < .01”, which refers to the null distribution of the max voxel-wised permutation tests, but the legends in Table states “Voxels=significant continuous voxel cluster”, which implies cluster-based thresholding. If clustering was used after permutation test, then the clustering criteria need to be mentioned.

4. Results
a. Has the other tail of t-tests been evaluated as well, e.g. lower FA and higher MD in healthy controls compared to SLE patients and so on? If so, any significance?

b. The “anterior” corona radiata in Fig. 1 could be “superior” corona radiata, which can not be differentiated without performing tractography and fiber tracking.

c. Apparently, the significant clusters in Fig. 1 have been dilated, but it was not stated in the text..

5. Discussion

a. “Interestingly, there were no significant FA or MD differences observed between the 16 SLE patients without NPSLE and the twenty matched controls, many of whom had subcortical white matter and periventricular lesions” on page 8. Should the “many of whom had subcortical white matter and periventricular lesions” refer to healthy controls, but not SLE patients or vice versa? If so, the sample needs better description and justification.

b. The authors argue that the “none of these regions were identified on radiological scan to be regions of old infarct in NPSLE” by showing the examples of some proton density images from NPSLE. It has been shown that T2-weighted FLAIR imaging is one of the most sensitive image modality, in detecting the white matter hyperintensities (WMHs), and is superior to proton density imaging, in which WMHs might not be demonstrated. In fact, the WMHs shown in the supplement file are likely to cover the voxels of the body of corpus callosum and corona radiata, where DTI measures were different between NPSLE vs HC and SLE group. The easiest way to verify would be averaging all the transformed PDI images in the MNI template space and check if WMHs voxles match the clusters where DTI measures are different between groups.

c. The authors conclude that low FA and high MD in NPSLE is likely the manifestation of edema in acute NPSLE stage. In fact, it has been shown that cytotoxic edema in acute stage would restrict free water movement, and thus resulting in a decreased MD, and possibly an increased FA.

- Minor Essential Revisions

1. Font size mistakes: 1x1x1mm3 on page 6.

2. State the MNI coordinates for each cluster, i.e. MNI (x,y,z) = (-8, 5, 25), (-12, 6, 27) separately, on page 8.

3. “relatively young (<\=60) on page 8 in Discussion”, where (<\=60) should be changed to “less than 60 years old”

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:

I declare that I have no competing interests