Reviewer's report

**Title:** Diffusion Tensor Changes Correlate with Lesion Volume in Right Cerebral Hemisphere Infarctions

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**Reviewer:** Lars T. Westlye

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Diffusion Tensor Changes Correlate with Lesion Volume in Right Cerebral Hemisphere Infarctions

Rossi et al. measured lesion volumes in 41 patients suffering from right hemisphere medial (MCA) and posterior cerebral anterior (PCA) infarcts in acute (within 24 hrs after symptom onset) and chronic (on average 13 months after infarct) phases using CT and T2-FLAIR, respectively. Lesion volumes at both time-points and the difference in volume between acute and chronic stages were correlated with diffusion tensor imaging (DTI) indices of water diffusion measured in the chronic phase within several regions of interest. The relations between lesion volumes and four neurological screening instruments, including the Barthel Index (BI), the National Institutes of Health Stroke Scale (NIHSS), the Rankin Scale (RS) and the Mini-mental state examination (MMSE), were also tested.

In general, the analyses yielded negative correlations between lesion volumes and fractional anisotropy (FA) and positive correlations between lesion volumes and mean diffusivity (MD). Specifically, the authors report a negative correlation between lesion volumes measured in the acute phase and FA in the internal capsule and the centrum semiovale in the right hemisphere (i.e. ipsilateral relative to the lesion) and in the thalamus and internal capsule in the left hemisphere. Lesion volume in the chronic phase correlated negatively with FA within the lesion and the anatomically corresponding area in the left hemisphere. Lesion volume in the acute phase correlated positively with MD in the splenium of the corpus callosum, in all sampled areas in the right hemisphere, as well as in the left cerebral peduncle, internal capsule and the corresponding contralateral area of the infarction. In the chronic phase, lesion volume correlated positively with all sampled areas in the right hemisphere. The authors also report a negative correlation between lesion volumes and the MMSE in the chronic phase, i.e. worse performance with larger lesion volumes.

The authors conclude from these findings that DTI analysis might be a good supplementary technique in analysing the damage to brain tissue in the chronic phase of stroke recovery.

This is a concise and generally well written manuscript which presents potentially interesting data pertaining to the sensitivity of DTI to primary or secondary tissue alterations after infarction. The dataset comprises longitudinal lesion volume data
from a relatively large sample of stroke patients, and the methods applied are presumably valid and sound.

DTI is known to be sensitive to subtle perturbations to the parenchyma not necessarily detected by neuroradiological examinations or conventional MRI, including T1- or T2-weighted images. Associations between lesion volume and DTI indices in the hemisphere opposite to the lesion might therefore be of particular interest, as detailed DTI analysis could suggest more distributed damage to the tissue than initial conventional MRI might indicate. DTI could therefore provide highly valuable information beyond mere lesion volume calculation, and thus inform diagnostic and prognostic assessments in a clinical setting.

Thus, I think the scope of the paper is suitable for BMC Medical Imaging. However, I have several concerns which, if addressed, could substantially strengthen the manuscript.

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

1) My main concern is related to how the authors deal with the large number of statistical tests performed. The lack of corrections for multiple comparisons seems remiss and undermines the interpretations and validity of the results. For example, Table 2 shows the results from 52 correlation analyses, but the authors still regard an uncorrected p < .05 as statistically significant. Post-hoc corrections (e.g. Bonferroni) should be performed to control for Type I errors. This should also be performed for all other analyses presented in the paper, including the correlations between lesion volume and clinical test data.

Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)

1) The paper would be much more compelling if the authors had provided one or more specific hypotheses guiding their methodological and statistical choices.

2) The authors present FA and MD data only. Increased MD could be explained by both increased longitudinal and/or increased radial diffusion, which each would imply distinct biological interpretations. The authors might want to include these additional measures in their analyses or (to keep the number of tests at a minimum) at the very least discuss possible limitations with FA and MD measures.

3) The patients are in several paragraphs described as having “attention deficits”. In neuropsychological and cognitive terms, this is a vague and ambiguous description and should be operationalized or avoided. If the authors are referring to some kind of neglect or inattention, specific neuropsychological test data, screening instruments or clinical information supporting this interpretation/diagnosis should be presented. Further, the neurological findings presented in the Results section are restricted to aggregate measures across
subjects, with no descriptions of the psychometric properties of the tests or the clinical significance of the results. For example, MMSE is a widely used screening instrument for cognitive impairments related to neurodegeneration including Alzheimer’s disease. Ceiling effects in non-demented samples are expected (as probably seen here, with the lower inter-quartile range of 27/30). It would be interesting to know if the correlation between lesion volume and MMSE (if the authors are able to show that this correlation is not coincidental) is entirely driven by (the few?) patients scoring below some cut-off score and therefore might present cognitive impairments. Since the extreme scores on such screening instruments are of particular interest, please also report minimum and maximum scores.

4) Both FA and MD are highly sensitive to age. Given the relatively large age-span of the sample (57-75 years), the authors should include age as a covariate to exclude possible confounding effects of age on the relationships between lesion volume and DTI metrics.

5) Discussion, fifth paragraph: The authors claim that the negative correlation between the chronic lesion volume and the FA value can be “easily understood; in larger lesions, the tissue is destroyed more thoroughly, leading to smaller anisotropy.” This is an unnecessary simplification of a complicated issue, and I suggest it be removed or entirely reformulated. For instance, in the case of selective degeneration of one axonal pathway in areas of crossing fibres, one would expect to see an increase in FA rather than a decrease. FA is an index of the relative deviation between the eigenvalues of the diffusion tensor, and interpretations might therefore vary according to the underlying tissue and the nature of the neurobiological processes. This limitation to the interpretations of the FA index should be incorporated in the Discussion.

6) Discussion, sixth paragraph: 1) The correlations between DTI indices measured in the corpus callosum and lesion volume might be reiterated in the results section in addition to the mention in Table 3. 2) It is not entirely clear to me why the correlations between the acute lesion volume and MD measured in the splenium of the corpus callosum necessarily points to a Wallerian degeneration. Please clarify.

7) The authors relate the lateralization of the DTI measures to the infarcts only. While this could be the case, existing literature have pointed to significant lateralization of DTI metrics in specific white matter tracts, e.g. related to language. Although the lack of a control group in the present study precludes direct comparisons, the authors should discuss the possibility that some of the hemispheric differences might not be exclusively related to the infarct.

8) The section describing the DTI processing and analysis is on the short side. For example, did the authors correct for possible artefacts related to subject motion or eddy currents in the DTI data? How was FA and MD defined and calculated?

9) Did the DTI protocol vary between individuals? If it did, the possible
implications of this should be discussed and protocol should perhaps be included as a covariate or factor in the statistical models.

10) It is not entirely clear to me whether any of the ROIs were placed in close proximity to (or even within) the lesion in any of the patients. For example, patient 29 had a lesion in the internal capsule, which is one of the ROIs. How did the authors address issues concerning partial voluming in these cases?

11) The authors report a negative correlation between lesion volume and MMSE in the chronic phase. In addition to the issues concerning multiple comparisons (see above), the authors should explicitly test if a common effect of age could explain this association.

12) Title: The title might imply that the analysis included longitudinal DTI data, which is not the case. Perhaps replace “changes” with “imaging”?

13) It seems that the authors measured diffusion along 12 gradient directions (I guess this is what “number of excitations: 3 and 12 orthogonal axes” means). Although mathematically 6 directions is adequate for the calculation of the diffusion tensor, there is an increasing awareness in the imaging community that number of directions applied influences the reliability of the estimated tensor and its parameters. Indeed, Jones (2004) states that “at least 20 unique sampling orientations are necessary for a robust estimation of anisotropy, whereas at least 30 unique sampling orientations are required for a robust estimation of tensor-orientation and mean diffusivity”. Although I’m not convinced that the results would change a lot with a larger number of sampling directions, I think this important point needs to be addressed in the manuscript.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1) Table 2: All SDs are negative? Typo?

2) In the Methods section (first paragraph), the authors state that they studied 41 consecutive patients. However, in the rest of the manuscript, this number is reduced to 33. If this is not a typo, please clarify.

3) Abstract (Methods): For clarity, the authors might want to add “thrombolytic” before “therapy”.

4) Results (DTI-results): Please report the unity of the MD measures.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

I declare that I have no competing interests.