Peer Review File

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Reviewer Comments

This work applies so-called tensor-valued diffusion encoding to estimate parameters related to the microstructure of breast tumours, before and after treatment. Results show that the parameters provided by the new method has a higher sensitivity to treatment-related effects. They are also sensitive to parameters obtained from histopathological analysis, such as the Ki-67 expression and the progeterone receptor level. The manuscript is clear, and I have mostly minor comments that the authors may want to consider.

Major comments

Reviewer's comments #1

The rationale for using methods more complex than ADC mapping should be made clearer. As far as I understand it, the challenge with ADC is that it is sensitive to the average of properties with the voxel. Thus, voxels with a uniform alteration of the microstructure appears similar to voxels with a heterogeneous alteration of it. DKI is a partial remedy, as the diffusional kurtosis is sensitive to heterogeneity, however, it is sensitive to heterogeneity in diffusion both between directions (e.g., anisotropy) and between environments with different diffusivities (e.g., isotropic dispersion). Diffusion tensor imaging (DTI) also adds information compared with ADC-mapping, that is, the fractional anisotropy (FA). However, that measure is sensitive to both anisotropy and orientation coherence. QTI with b-tensor encoding serves as a remedy to the problems of both DTI and DKI, as it separates effects of directional and isotropic heterogeneity. This should be communicated more clearly.

➔ We thank the reviewer for this request for clarification. There is evidence that ADC mapping overall reflects diffusion restriction by high tumor cell density, however, it does not reflect histopathological changes after neoadjuvant chemotherapy. In particular, it does not reflect tumor volume change, and cannot distinguish chemotherapy-related fibrosis or inflammatory cell aggregation from residual cancer. (references in revised manuscript 8, 9, 11, 13-15) To overcome the limitations of ADC mapping, analyses of fractional anisotropy (FA) provided from DTI can give us quantifies anisotropy. In the literature, there are few reports measuring FA in breast cancer after chemotherapy, but, unfortunately the previous studies revealed conflicting results as whether FA is helpful in distinguishing the malignant portion. (references in

revised manuscript 18-21, 48, 49) In contrast, the tensor-valued diffusion that was used in our study has the concept of kurtosis and diffusional variance are the same (the kurtosis of the propagator corresponds exactly to the variance of the distribution of diffusivities, and the name "MK" is used to connect it back to diffusional kurtosis imaging, by which it was inspired). However, the interpretation parameters in DKI cannot be lined to compatible features of the tissue microstructure yet. (references in revised manuscript 22-23) Our approach is based on a signal representation within which the parameters, e.g., MK_A and MK_I, can be interpreted from theory and by associating them to tissue features without making biophysical assumptions. This is analogous to how a reduced mean diffusivity is often interpreted as an indicator for high cell density. To clarify the issue that you have pointed out, we revised the content in the section of introduction with adding new references.

Reviewer's comments #2

The difference between diffusion methods should be communicated more clearly, in line with the reasoning above, both in the introduction, and on line 221: "Although conventional DWI with ADC using single diffusion encoding is widely used clinically, it is unclear whether it reflects detailed microstructural changes in breast tumors after NAC. Therefore, diffusion tensor invariants, such as DTI and DKI, have been suggested for evaluating post-NAC tumor response and predicting pathologic response (14,15)". It is not unclear – DTI is simply unable separate tissues with anisotropy + orientation dispersion from those that lack of anisotropy.

➔ We thank the reviewer for this important comment. We revised manuscript of both introduction and discussion section, describing the limitations of each diffusional invariant in more detail for the clarity.

Reviewer's comments #3

3.2. Comparisons were made for six different parameters for the same ROIs. Were any corrections for multiple comparisons made? I suggest a Bonferroni correction for six comparisons.

➔ Thank you for letting us know about the parts we haven't described in the manuscript. During analyses of our data, we used Bonferroni correction for multiple comparisons of QTI parameters between the subgroups. In this revision, we added it in the section of statistical analysis to clarify.

Reviewer's comments #4

The QTI parameters shows a relatively large response to the PR status. What microstructure differences between tumors could have caused this?

➔ We thank the reviewer for this valuable comment. In the previous reports, PR

negative cancers tend to show high-grade cancer and more commonly occur lymphovascular invasion, compared to PR positive cancers (2020, Fei et al, Clin Breast Cancer / 2005, Cui et al, J Clin Oncol) However, detailed histopathological and microstructural difference in PR negative cancers compared to PR positive cancers still remained unclear. Accordingly, we analyzed whether there was a statistically significant correlation between high nuclear grade cancer and PR status additionally, however, there was no significant correlation in our patient group. We thought that this insignificant result between the two groups regarding RP status in our study might be affected by small number of the included patients. Therefore, the subsequent study with larger number of patients should be required to analyze this factor and validate our study. Also, we added the number of each histologic grade of the tumor in the Table 1 to enhance the understanding.

Minor comments

Reviewer's comments #5

Row 79: To the best of our knowledge, only two recent studies -- cite them

➔ We thank the review for noticing this. We added citations of the two recent studies of Naranjo et al, and Cho et al.

Reviewer's comments #6

2.1. How were patients enrolled for the study? Was QTI data acquired in all breast tumor patients in this time window, and then a subset of these were selected for this study?

➔ We acquired QTI data in all breast cancer patients who performed breast MRI before and after neoadjuvant chemotherapy (NAC) in our hospital during the described period. But we excluded patients with radiologically complete remission on post-NAC MRI, and patients with insufficient diagnostic image quality due to artifacts.

Reviewer's comments #7

2.2. Was any in-plane acceleration technique used in the DWI and QTI scans?

➔ We did not apply any in-plane acceleration technique since this is not yet available.

Reviewer's comments #8

3.1. Tumor size was 44.0 pm 21.5 mm. How was the size measured? This appears not to be mentioned in the methods.

➔ The measurement of the tumor size was performed on the DCE T1-weighted images of both pre-NAC and post-NAC MRIs. The greatest dimension of the tumors was measured using a digital caliper. We added the description about the tumor size measurement in the image processing and analysis section.

Reviewer's comments #9

Figure 2. Can the histology slides be made available in higher resolution? Alternatively, please add zoom-ins illustrating the pleomorphic tumor cells with eccentric growth and the fibrosis appearing after treatment.

➔ We added x100 magnification slides of pre-NAC and post-NAC in the figure 2 (T and V).

Reviewer's comments #10

Line 228. Grammatical error: "Tumor response to NAC is strongly associated with the survival of those breast cancer.(38,39)"

➔ Thank you for the comment. We revised this sentence to "Tumor response to NAC is strongly associated with the survival in the patient with breast cancer."

Reviewer's comments #11

Line. 239. This sentence is hard to understand: "During the study period, we estimated that pre-NAC MKI had a negative correlation with tumor size changes after NAC based on a previous study that showed a positive correlation between MKI and tumor size.(23). 239. The reason for this discrepancy between observation and estimation remains unclear" – what is the difference between observation and estimation? Perhaps hypothesized is the correct word?

➔ We appreciate this comment and agree that clarification of the term "estimation" is warranted. On the previous study (reference 31 in revised manuscript), there was a positive correlation between the MK_I and the breast tumor size. A potential explanation suggested was that large tumors were more likely to contain poorly perfused regions with variable cell density than small tumors due to aggressive cell growth or necrosis. Based on the explanation, we hypothesized that pre-NAC MKI had a negative correlation with tumor size changes after NAC, due to decreased heterogeneity of the pleomorphic tumor cells response to the chemotherapy. We change the term "estimated" to hypothesized following your suggestion.

Thank you for the valuable comments on our paper.