# The effect of spatial registration algorithm on detection of white matter abnormalities in multiple sclerosis: a TBSS study.

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**INTRODUCTION:** Anatomical alignment in neuroimaging studies is of such importance that considerable effort is put into improving the registration used to establish spatial correspondence. Tract Based Spatial Statistics (TBSS) [1] is a popular analysis technique for the voxelwise comparison of diffusion characteristics (within the core of tracts) of cerebral white matter (WM) across subjects. TBSS solves in a clever way the main difficulties with VBM-style analysis of the diffusion measures, namely the exact alignment of the very fine WM structures and the amount of spatial smoothing to be used. By producing an alignment-invariant tract representation (the "skeleton"), by projecting the diffusion measures of interest (e.g.: fractional anisotropy, mean diffusivity, etc.) on this common structure, and by confining the statistical analysis to the skeleton, the TBSS approach allows a reliable comparison between group, being "guaranteed" that any data come from the same part of the same WM tract from each and every subject. Nevertheless, the TBSS analysis is considerably influenced by the nonlinear registration algorithm adopted, in terms of the produced skeleton layout and the projected diffusion measures, both conditioning the results of the statistical group comparison. In this study, we investigated how a TBSS analysis, aiming to compare a group of healthy subjects (HS) and a group of patients with relapsing remitting multiple sclerosis (RRMS) patients, is influenced by the spatial normalization algorithm.

**METHODS:** We recruited 37 patients diagnosed with RRMS [F/M=26/11; mean (SD) age=35.6 (8.4) years; median EDSS=2 (range: 0- 4.5)] and 39 HS [F/M=25/14; mean (SD) age=38.0 (13.4) years], age (p=0.35) and gender ($\chi^2$=0.327, p=0.57) matched to the RRMS group. All subjects underwent an MRI acquisition at 3.0T, including a diffusion weighted (DW) twice-refocused spin echo echo-planar imaging (SE EPI; TR=7s, TE=85ms, b factor=1000s/mm$^2$, isotropic resolution=2.3 mm$^3$), collecting seven images with no diffusion weighting (b=0) and 61 images with diffusion gradients applied along 61 non-collinear directions. DW images were processed (using FSL, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) to compute the fractional anisotropy (FA) maps in native space. Using FSL, we therefore performed a first TBSS analysis (TBSS-FNIRT), with the FSL-recommended settings:
1) FA maps in native space were warped to the FMRIB58_FA_1mm atlas (using the FSL-default nonlinear registration algorithm, FNIRT [2]);
2) mean_FA and mean_FA_skeleton were derived from mean of all subjects;
3) skeleton's threshold was set to 0.2.
Then, a voxel-wise group comparison was computed using the tool "randomise" (which performs permutation-based statistical analyses) with 2000 iterations and Threshold-Free Cluster Enhancement (TFCE; [3]) with 2D optimisation, covaring for age and gender. In evaluating the resultant statistical maps, we applied family-wise error (FWE) correction for multiple comparisons, and considered significant a p value less than 0.001. A second customized TBSS analysis (TBSS-ANTs) was therefore computed (on the same FA maps), replacing FNIRT with ANTs [4] in step 1, followed by otherwise identical pipeline as in the previous analysis; ANTs was set to perform a diffeomorphic transformation algorithm with cross-correlation similarity metric. Finally, residual FA-warped images were computed for both analysis, subtracting the mean of the warped FA maps coming from all subjects, to the FMRIB58_FA_1mm atlas.

**RESULTS:** As clearly shown by residual FA-warped images (see the left panel of fig.1), ANTs outperformed FNIRT in normalization precision, and this obviously affected the WM skeleton reconstruction. Comparing the FNIRT and ANTs skeletons (see upper part of right panel of fig.1), it appears that the ANTs skeleton includes more WM tracts, and this had evident impact on the group comparison results (see lower part of right panel of fig.1); for example, only the TBSS-ANTs analysis was able to detect a reduction of FA in RRMS patients in part of the anterior thalamic radiation (ATR) bilaterally, in virtue of the superior warping capability of ANTs, that allowed the skeletonization to reconstruct the ATR WM tract (see the white arrows in both panels of fig.1, that highlight this concept for the left ATR). In both analyses, no significant results were found when testing the FA reduction in HS subjects.

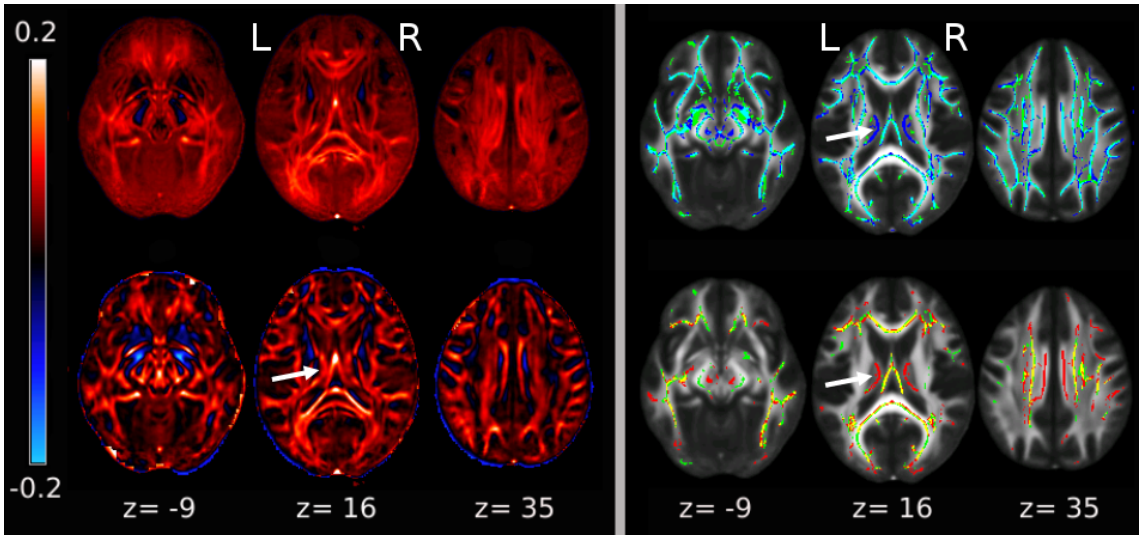


**FIGURE1. Left panel**. In the top row is shown the "residual ANTs image", obtained subtracting the mean of the ANTs-warped FA maps coming from all subjects, to the FMRIB58_FA_1mm atlas. In the bottom row is shown the "residual FNIRT image". **Right panel**. In the top row are shown the skeletons (threshold set to 0.2) obtained with the TBSS-ANTs (dark blue) and TBSS-FNIRT (green) analyses; the overlap regions are shown in sky blue. In the bottom row, are shown the skeletal voxels with a significant ($p < 0.001$, FWE corrected) reduction of FA in RRMS patients as assessed by the TBSS-ANTs (red) and TBSS-FNIRT analyses (in green); the overlap regions of the two statistics are shown in yellow. In both analyses, no significant results were found for the reversed contrast (HS<RRMS). The background image is the FMRIB58_FA_1mm atlas. **Left & right panels**. The white arrows, that point to the left side of thalamus, point out the influence of an incorrect normalization (left panel) on the reconstruction of the skeleton and subsequently on the group comparison results (right panel).

**DISCUSSION:** In this study we compared the results obtained repeating the same TBSS analyses (on the FA maps belonging to RRMS patients and HS) only changing the spatial normalization algorithms. In particular, we tested the differences in using FNIRT (available in FSL, and that carries out a cubic B-splines deformations warping) and ANTs, a software that implements a diffeomorphic transformations algorithm and that gave the best results in a direct comparison with other 13 nonlinear deformation software [5]. The TBSS analysis exploiting ANTs to warp the FA maps to the FA atlas, was able to skeletonize more WM tracts, and moreover different common reconstructed tracts were few voxels longer (i.e., see z=35 slice, in the upper part of right panel). This allowed the statistical comparison performed on the skeletonized data in the TBSS-ANTs analysis, to detect FA group differences in more brain regions, for example a reduction of FA in the ATR of RRMS patients, WM tract found to be largely impaired in multiple sclerosis, presenting the greatest percentage atrophy among the main WM tracts of the brain [6]. In conclusion, we found that, even though the TBSS algorithm manages in general efficaciously the main problems that would affect a VBM-style analysis of FA, nevertheless its results can be substantially improved using a more accurate spatial registration algorithm.

**References:** **1.** Smith SM et al, NeuroImage. 2006; 31:1487-1505; **2.** Andersson JL et al, FMRIB technical report TR07JA2 (available at: www.fmrib.ox.ac.uk/analysis/techrep); **3.** Smith SM and Nichols TE, Neuroimage. 2009; 44:83–98; **4.** Avants BB et al, NeuroImage. 2011; 54:2033-2044; **5.** Klein A et al, NeuroImage. 2009; 46: 786-802; **6.** Kezele IB et al, Multiple Sclerosis. 2008; 14:779–785